Pathogenesis of type 2 diabetes and cardiovascular disease in South Asians

Effects of dietary interventions on
metabolism and cardiovascular function

Leontine Erica Henriëtte Bakker

ISBN: 978-94-6169-613-7

Cover design: Leontine Bakker en Optima Grafische Communicatie, Rotterdam, the Netherlands
Lay-out and print: Optima Grafische Communicatie, Rotterdam, the Netherlands

This thesis was partly financially supported by Roba Metals B.V., IJsselstein.
Publication of this thesis was financially supported by Boehringer Ingelheim B.V., ChipSoft B.V., Goodlife Healthcare B.V., Janssen-Cilag B.V., Novo Nordisk B.V., Rijnland Zorggroep, and is gratefully acknowledged.

Pathogenesis of type 2 diabetes and cardiovascular disease in South Asians

Effects of dietary interventions on
metabolism and cardiovascular function

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op woensdag 18 februari 2015
klokke 16.15 uur

door

Leontine Erica Henriëtte Bakker

geboren te Leiden
in 1981

PROMOTIECOMMISSIE

Promotores	Prof. dr. A.E. Meinders Prof. dr. H. Pijl
Co-promotor	Dr. I.M. Jazet
Overige leden:	Prof. dr. P.C.N. Rensen Dr. B.G.A. Guigas Prof. dr. H.J. Lamb Prof. dr. J.W.A. Smit (UMC St. Radboud, Nijmegen) Prof. dr. J.A. Romijn (AMC, Amsterdam)

CONTENTS

List of Abbreviations		7
Chapter 1	General introduction and outline of the thesis	11
PART 1	**TYPE 2 DIABETES MELLITUS**	29
Chapter 2	Pathogenesis of type 2 diabetes in South Asians	31
Chapter 3	Higher insulin and glucagon-like peptide-1 (GLP-1) levels in healthy, young South Asians as compared to white Caucasians during an oral glucose tolerance test	61
Chapter 4	A 5-day high fat high calorie diet impairs insulin sensitivity in healthy, young South Asian men but not in white Caucasian men	75
Chapter 5	Middle-aged overweight South Asian men exhibit a different metabolic adaptation to short-term caloric restriction compared to white Caucasians	99
Chapter 6	Brown adipose tissue: the body's own weapon against obesity?	123
Chapter 7	Brown adipose tissue volume in healthy lean South Asian adults compared with white Caucasians: a prospective, case-controlled observational study	135
PART 2	**CARDIOVASCULAR DISEASE**	155
Chapter 8	High prevalence of cardiovascular disease in South Asians: central role for brown adipose tissue?	157
Chapter 9	Functional and metabolic imaging of the cardiovascular system in young healthy South Asians compared with white Caucasians unveils early differences	175
Chapter 10	Cardiovascular flexibility in middle-aged overweight South Asians *vs.* white Caucasians: response to short-term caloric restriction	193
Chapter 11	South Asians exhibit disturbed HDL functionality as compared to white Caucasians	209
Chapter 12	Summary and conclusions	227
Chapter 13	Nederlandse samenvatting	249
List of publications		267
Curriculum Vitae		269

LIST OF ABBREVIATIONS

A	diastolic atrial contraction
ABCA1	ATP-binding cassette transporter A1
ACC	acetyl-CoA carboxylase
ADA	American Diabetes Association
AGEs	advanced glycation end products
AMPK	AMP-activated protein kinase
AO	aortic
AS160	Akt substrate of 160 kDa
AUC	area under the curve
BAT	brown adipose tissue
BIA	bioelectrical impedance analysis
BMI	body mass index
BMP7	bone morphogenetic protein 7
BSA	body surface area
CPT1A	carnitine palmitoyltransferase 1A
CRP	c-reactive protein
DEXA	dual-energy x-ray absorptiometry
DI_o	oral disposition index
DPP-IV	dipeptidyl peptidase IV
DSAT	deep subcutaneous adipose tissue
E	early diastolic filling phase
4E-BP1	eukaryotic translation initiation factor 4E-binding protein 1
EDM	end-diastolic mass
EDV	end-diastolic volume
E/Ea	estimate of left ventricular filling pressure
EF	ejection fraction
EGP	endogenous glucose production
EPC	endothelial progenitor cell
EPFR	early peak filling rate
ERK	extracellular signal-regulated kinase
ESV	end-systolic volume
ETC	electron transfer chain
FA	fatty acid

FASN	fatty acid synthase
FFA	free fatty acid
^{18}F-FDG	^{18}F-fluoro-deoxy-glucose
FTO	fat mass and obesity-associated gene
GLP-1	glucagon-like-peptide-1
GLUT4	glucose transporter protein 4
GS	glycogen synthase
GSK3	glycogen synthase kinase-3
HbA_{1c}	hemoglobin A_{1c} (glycosylated hemoglobin)
HDL	high density lipoprotein
HF	high fat
HFHCD	high fat high calorie diet
HIR	hepatic insulin resistance
^{1}H-MRS	proton magnetic resonance spectroscopy
HOMA %B	homeostasis model assessment of β-cell function
HOMA IR	homeostasis model assessment of insulin resistance
HOMA %S	homeostasis model assessment of insulin sensitivity
HTG	hepatic triglyceride content
IFN-γ	interferon γ
IGI	insulinogenic index
IL-6	interleukin 6
IMCL	intramyocellular lipid content
IQR	interquartile range
IRβ	insulin receptor β
IRS	insulin receptor substrate
IVGTT	intravenous glucose tolerance test
LBM	lean body mass
LDL	low density lipoprotein
LV	left ventricular
LVESWS	LV end systolic wall stress
MAPR	maximal mitochondrial ATP production rate
MCR_i	metabolic clearance rate of insulin
MR	magnetic resonance
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy

mtDNA	mitochondrial DNA copy number
mTOR	mammalian target of rapamycin
mTORC1	mammalian target of rapamycin complex 1
NAFLD	non-alcoholic fatty liver disease
nDNA	nuclear DNA copy number
NEFA	non-esterified fatty acid
NO	nitric oxide
NOGD	non-oxidative glucose disposal
NST	non-shivering thermogenesis
OGTT	oral glucose tolerance test
OXPHOS	oxidative phosphorylation
PCOS	polycystic ovary syndrome
PDH	pyruvate dehydrogenase enzyme complex
PDK	pyruvate dehydrogenase kinase
PET-CT	positron emission tomography and computed tomography scan
PI3K	phosphatidylinositol 3-kinase
PKB	protein kinase B (also known as Akt)
PPARα	peroxisome proliferator-activated receptor alpha
PPARγ	peroxisome proliferator-activated receptor gamma
PRAS40	proline-rich Akt substrate of 40kDa
PUFA	polyunsaturated fatty acids
PWV	pulse wave velocity
R_a	rate of glucose appearance
R_d	rate of glucose disappearance
REE	resting energy expenditure
ROI	region of interest
RQ	respiratory quotient
S6K1	ribosomal protein S6 kinase β1
SAT	subcutaneous adipose tissue
SD	standard deviation
SEM	standard error of the mean
SPIR	spectral inversion recovery
SSAT	superficial subcutaneous adipose tissue
SUV	standardized uptake value
SUV_{max}	maximum standardized uptake value in g/mL

SUV_{mean}	average standardized uptake value in g/mL
SV	stroke volume
TG	triglyceride(s)
TNF-α	tumor necrosis factor α
TSC	tuberous sclerosis complex
UCP-1	uncoupling protein 1
UK	United Kingdom
USA	United States of America
VAT	visceral adipose tissue
VCAM-1	vascular cell adhesion molecule-1
VLCD	very low calorie diet
$VO2_{max}$	measure of whole-body oxidative capacity
VOI	volume of interest
WHO	World Healthy Organization

1 General introduction and outline of the thesis

CONTENTS

Type 2 diabetes mellitus in South Asians

Cardiovascular disease in South Asians

Relevance of this thesis

Intermezzo: study population

Outline thesis

References

TYPE 2 DIABETES MELLITUS IN SOUTH ASIANS

Type 2 diabetes mellitus is a chronic, multifactorial disease characterized by variable degrees of insulin resistance at the skeletal muscle, adipose tissue and hepatic level, and impaired insulin secretion.[1-3] Type 2 diabetes is associated with reduced life expectancy, significant morbidity and mortality due to diabetes related complications affecting many organ systems, and diminished quality of life, imposing a tremendous burden on the individual with diabetes, but also on society and on the health care system.[4]

Epidemiology. Type 2 diabetes is one of the most common chronic diseases worldwide, and continues to increase in numbers and significance. The estimated prevalence of diabetes in 2011 was 366 million people worldwide, with an overall predicted increase in prevalence from 2011 to 2030 of 50.7%, leading to an estimated prevalence of 552 million people in 2030. 48% of the anticipated absolute global increase of 186 million people with diabetes is projected to occur in India and China alone. According to the World Health Organization (WHO), diabetes caused 1.3 million deaths in 2008 (mortality rate 2.3%). Worldwide, the global cost of diabetes in 2010 was estimated at nearly 500 billion US dollars, and it is estimated to rise to at least 745 billion US dollars in 2030, an increase of almost 50%, with developing countries, like India and China, increasingly taking on a much greater share of the expenses.[4]

There is a considerable geographic variation in the prevalence and incidence of type 2 diabetes, which is likely due to genetic, behavioural, and environmental factors, with a large impact of countries' income status. Diabetes prevalence also varies among different ethnic populations within a given country.[5] In this respect, people of South Asian descent stand out, since the rapid increase in the prevalence of type 2 diabetes is especially seen in this ethnic population (**Table 1**). South Asians originate from the

Table 1. Burden of type 2 diabetes in South Asians compared to white Caucasians.

Burden of type 2 diabetes in South Asians compared to white Caucasians	*References*
- 4-6 times higher prevalence rate	5-8
- Highest global number of T2DM patients in India with an estimated prevalence of up to 16.8% in urban areas	6;9-11
- Similar prevalence rates in South Asian migrants in the USA, Canada and various European countries	12-18
- Highest prevalence rate of all ethnic minorities in the Netherlands	19
- Age-standardized prevalence rate of T2DM is 26.7% *vs.* 5.5% in ethnic Dutch	8
- T2DM occurs at a younger age (approximately 10 years sooner)	8;14;20
- T2DM occurs at a lower BMI (22.6 kg/m2 *vs.* 30.0 kg/m2)	8;14;20-23
- Severity of T2DM is higher with an increased risk of complications	16;24-29

T2DM, type 2 diabetes mellitus

Indian subcontinent – India, Pakistan, Bangladesh, Nepal and Sri Lanka – and represent one fifth of the total world's population. Both native and migrant South Asians, such as the Surinamese South Asian population in the Netherlands, are at high risk of developing type 2 diabetes compared to white Caucasians.[5-8] India has currently the highest global number of diabetes patients, with an estimated prevalence of up to 16.8% in urban areas.[6;9-11] Similar prevalence rates have also been reported in migrants of South Asian descent in the United States of America (USA), Canada, and various European countries.[12-18] For example, in the United Kingdom (UK), diabetes prevalence in 35-60 year old South Asian males was 16% compared to only 4% among European whites.[13] In the Netherlands, South Asians (Dutch Hindostani – see Intermezzo) have the highest type 2 diabetes prevalence of all ethnic minorities living in the Netherlands.[19] In 2008, an age-standardized prevalence rate of type 2 diabetes of 26.7% for this group has been reported, compared to 5.5% in ethnic Dutch,[8] and to 10% globally (WHO).

Not only is the prevalence of type 2 diabetes four to six times higher in South Asians, it also occurs at a younger age[8;14;20] – around 10 years sooner – and lower body mass index (BMI)[8;14;20-23] compared to white Caucasians. In a recent study in 2011, BMI obesity cut-off points equivalent to 30.0 kg/m2 in white Caucasians were 22.6 kg/m2 for a glycaemia factor (combining fasting and 2 hour glucose and HbA_{1c}) in South Asian males.[22] Moreover, the severity of the disease is higher with an increased risk of cardiovascular and renal complications.[16;24-29]

Pathophysiology. The rapid increase in type 2 diabetes prevalence worldwide has been associated with a Western, obesogenic lifestyle.[30] South Asians, however, appear to have an exceptionally high susceptibility to develop type 2 diabetes in the context of the same environmental pressure when compared to other ethnicities.[5;7;8;21] Hence, a gene-environment interaction underlying this excess risk seems most likely: South Asians seem to have a high genetic susceptibility and enhanced interaction with environmental triggers such as an energy-rich diet and a sedentary lifestyle.

Risk factors to develop type 2 diabetes, such as visceral or abdominal fat distribution, a sedentary life style, and dietary factors, are highly present in South Asians. This is partly due to rapid nutrition, lifestyle, and socioeconomic transitions that are occurring in South Asians in the last decades. Unlike the gradual transitions in Western countries, these changes have happened rapidly in many lower-income countries, such as South Asian countries. However, although environmental factors such as urbanization are important, they cannot account for all the characteristics of the excess risk in South Asians, and genetic influences probably have an important role. For example, genetic makeup probably accounts for the disadvantageous body composition of South Asians with relatively thin extremities, i.e. low muscle mass, high proportion of total body fat, and prominent abdominal adiposity, i.e. increased visceral fat mass, the so called 'thin-

fat-phenotype'. Low lean body mass and abdominal adiposity are both associated with insulin resistance and the development of type 2 diabetes.[31;32]

Type 2 diabetes is characterized by insulin resistance and impaired insulin secretion. In South Asians, multiple studies have consistently shown higher insulin levels compared to other ethnic groups regardless of age, gender or BMI, suggesting a higher rate of insulin resistance in this population.[20;33-42] Already in neonates fasting insulin levels are markedly higher compared to white Caucasian neonates.[43;44] Hence, the predominant mechanism in this ethnicity seems to be insulin resistance rather than impaired insulin secretion. In **Chapter 2** we describe several possible mechanisms that may underlie or contribute to the increased insulin resistance observed in South Asians. Here, I will describe our main topics that we have investigated in this thesis.

Insulin signalling. Skeletal muscle accounts for 75-80% of whole-body insulin-stimulated glucose disposal.[45] Non-oxidative glucose disposal (NOGD) or glycogen synthesis, and oxidative glucose metabolism through glycolysis are the major pathways for glucose metabolism in skeletal muscle. In type 2 diabetes patients, the primary defect at the skeletal muscle level seems to reside in NOGD.[46-48] Surprisingly, in South Asians only two in-depth studies have been conducted so far, which obtained skeletal muscle biopsies to investigate mitochondrial function and the insulin signalling pathway.[38;49] In this thesis, we investigated insulin signalling in young adult and adult South Asians (**Chapters 4 and 5**). A depiction of the insulin signalling pathway aimed at increasing the rate of glucose transport is shown in **Figure 1**.

Energy/nutrient-sensing pathways. Given the high susceptibility of South Asians to develop type 2 diabetes despite a similar environmental pressure when compared to people of different origins, a possible explanation for this predisposition might be related to differences in the regulation of energy/nutrient-sensing pathways in metabolic tissues affecting whole-body substrate homeostasis. To date, however, these pathways have not been analysed in South Asians. Furthermore, in the context of energy/nutrient sensing, the effect of dietary intervention on insulin sensitivity has not been studied in this ethnicity. In this thesis, we investigated energy/nutrient-sensing pathways in adolescent and adult South Asians (**Chapters 4 and 5**).

mTOR. Among these pathways, the nutrient and energy-sensing protein kinase mammalian target of rapamycin (mTOR), which regulates cell growth according to nutrient availability and cellular energy status,[50] is of major importance. The mTOR kinase interacts with several proteins to form two distinct complexes named mTOR complex 1 (mTORC1) and mTOR complex 2, which differ in their molecular composition, regulation, sensitivity to rapamycin, and downstream targets.[50] mTORC1 responds to insulin and other growth factors, stress, oxygen and nutrient levels and controls key cellular

processes.[50;51] When active, mTORC1 promotes protein synthesis, cell growth and differentiation, and may inhibit insulin signalling by feedback regulation of the insulin receptor substrate 1 (IRS1). mTORC1 was also recently shown to play a crucial role in mitochondrial biogenesis and oxidative metabolism.[52-55] A simplified view of the mTORC1 signalling pathway at the skeletal muscle level is shown in **Figure 2**.

Brown adipose tissue. Another major hypothesis that we have investigated in this thesis is related to brown adipose tissue (BAT). In **Chapter 6** we give an overview of the anatomy, physiology and function of BAT. In short, BAT is a highly metabolically active tissue involved in facultative thermogenesis. In contrast to white adipose tissue, BAT takes up glucose and triglyceride-derived and free fatty acids from the plasma and subsequently burns fatty acids to generate heat through a process called mitochondrial uncoupling.[56] Only in 2009, cold-induced ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography-computed tomography (PET-CT) studies in humans have shown that BAT is still present and functional in adults.[57-59] It has been estimated that fully activated BAT in humans can contribute to up to 15-20% of total energy expenditure.[56] Intriguingly, obese individuals appear to have lower BAT volume and activity supporting the metabolic importance of BAT.[57-59] Therefore, stimulation of BAT is currently considered a potential preventive and therapeutic target in the combat against obesity and related diseases, such as dyslipidemia and type 2 diabetes. In this thesis, we hypothesize that a low BAT volume or activity might underlie the disadvantageous metabolic phenotype and susceptibility for type 2 diabetes in South Asians (**Chapter 7**). **Figure 3** shows the localization and a simplified view of the activation of BAT in human adults.

CARDIOVASCULAR DISEASE IN SOUTH ASIANS

Cardiovascular disease is common in the general population, affecting the majority of adults past the age of 60 years. Cardiovascular disease includes coronary heart disease, cerebrovascular disease, and diseases of the aorta and arteries including hypertension and peripheral vascular disease. Coronary heart diseases account for approximately one-third to one-half of the total cases of cardiovascular disease (WHO).

Epidemiology. Cardiovascular disease currently accounts for nearly half of noncommunicable diseases, principally cardiovascular disease, type 2 diabetes, cancer and chronic respiratory diseases, which have overtaken communicable diseases as the world's major disease burden. A rough estimated prevalence of cardiovascular disease in 2011 was 450 million people worldwide. Cardiovascular disease is the leading global cause of death, with an estimated number of 17.3 million deaths worldwide on an annual basis in 2012,

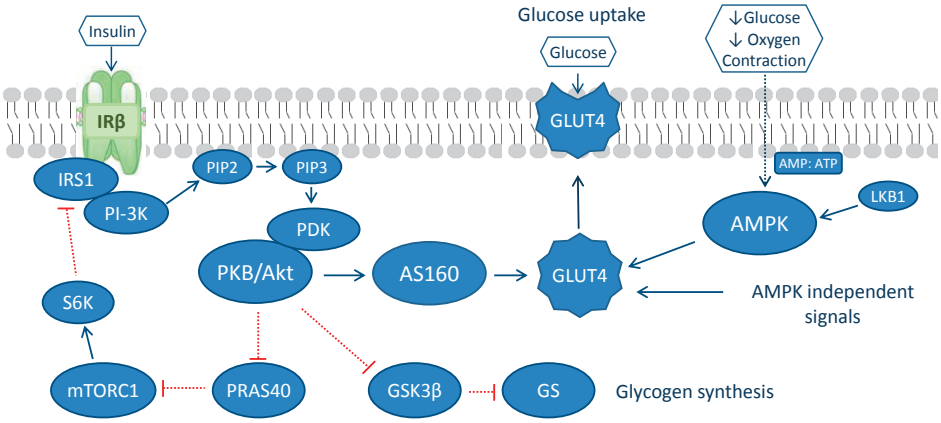


Figure 1. Insulin signalling pathway at the skeletal muscle level. Insulin binds at the cell membrane at the insulin receptor leading to phosphorylation of the receptor and insulin receptor substrate (IRS). Activated IRS-1 subsequently binds to phosphatidylinositol 3-kinase (PI3K), which is recruited to the cell membrane and converts phosphatidylinositols-4,5-biphosphate (PIP2) to phosphatidylinositols-3,4,5-triphosphate (PIP3). PIP3 attracts phosphatidylinositol-dependent protein kinase (PDK) and protein kinase B (PKB)/Akt to the cell membrane where Akt is activated by PKD-mediated phosphorylation and dissociated from the cell membrane to affect several metabolic processes. First, activated Akt leads to phosphorylation of Akt substrate 160 (AS160) that allows glucose transporter 4 (GLUT4) storage vesicles to translocate to the cell membrane. Insulin-independent pathways involved in GLUT-4 translocation involve adenosine monophosphate-activated kinase (AMPK)-dependent (contraction, hypoxia) and –independent pathways. Second, activated Akt inactivates glycogen synthase kinase 3 (GSK3), relieving the inhibitory action of GSK3 on glycogen synthase, and thus stimulating glycogen synthesis. Third, activated Akt phosphorylates the nuclear protein Proline-rich Akt substrate of 40 kDa (PRAS40), disrupting the interaction between mammalian target of rapamycin complex 1 (mTORC1) and PRAS40, thereby relieving the inhibitory action of PRAS40 on mTORC1 activity. A major mTORC1 substrate is S6 kinase 1 (S6K1). Activated S6K1 leads to enhanced phosphorylation of IRS-1, attenuating insulin-PI3K-Akt signalling.

a number that is expected to grow to 23.5 million by 2030, especially in low- and middle-income countries.[60] In 2010, the global cost of cardiovascular disease was estimated at 863 billion US dollars, and it is estimated to rise to 1.044 billion US dollars in 2030, an increase of 22%.[4]

People of South Asian origin have an increased risk of developing cardiovascular disease compared to white Caucasians (**Table 2**).[12] The age-standardized mortality rate from cardiovascular disease is around 50% higher for South Asians than for Caucasians.[26;61-64] Furthermore, the mean age of first acute myocardial infarction is approximately five years earlier in South Asians than in Caucasians.[65;66] Moreover, cardiovascular disease in this population is more aggressive and has higher mortality rates at younger ages.[12;26;61;65]

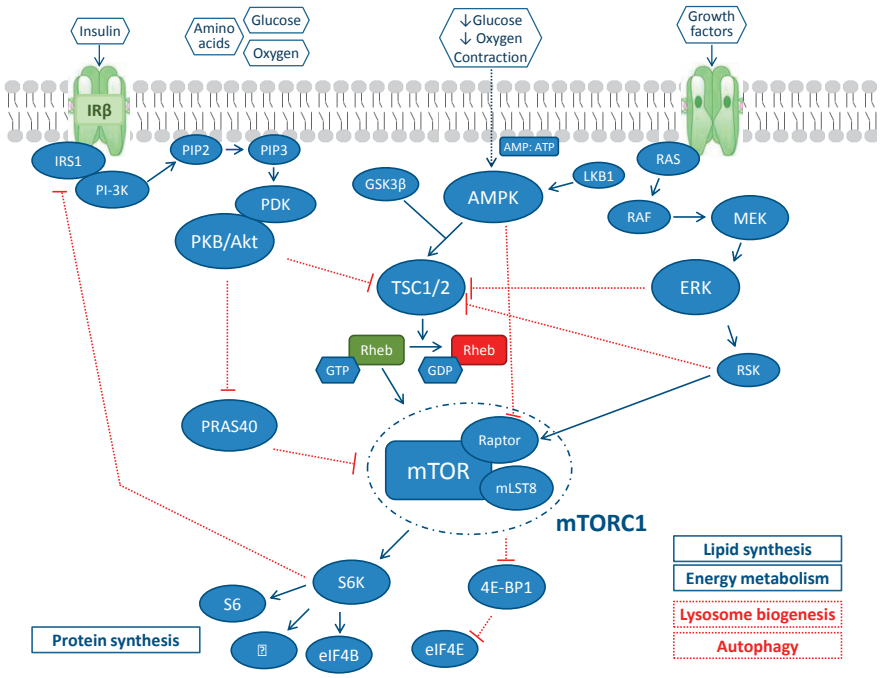


Figure 2. Simplified view of the mTORC1 signalling pathway at the skeletal muscle level. mTORC1 is composed of mTOR, mTOR-associated protein LST8 homologue (mLST8) and the regulatory-associated protein of mTOR (Raptor). mTORC1 responds to insulin and other growth factors, amino acids, stress, oxygen, and energy status. It is stimulated by the active, GTP-bound form of RHEB. Immediately upstream of RHEB is the TSC1/2 tumour suppressor complex, which contains a GTPase-activating protein (GAP) domain that converts RHEB to its inactive, GDP-bound form. Multiple upstream signalling inputs from e.g. PI3K–AKT, Ras–ERK–RSK, AMPK-GSK3β, LKB1–AMPK pathways either positively or negatively regulate mTORC1 signalling by inhibiting or activating, respectively, the ability of TSC2 to act as a GAP for RHEB. Kinases that inhibit the function of TSC2 towards RHEB and thus activate mTORC1 are AKT, ERK, and RSK. Conversely, AMPK- and GSK3-mediated phosphorylation events positively regulate TSC2 activity towards RHEB, resulting in inhibition of mTORC1. Furthermore, some of these kinases can modulate mTORC1 activity in a TSC2-independent manner. For example, AKT-mediated phosphorylation of the mTORC1 inhibitory factor PRAS40 and RSK-mediated phosphorylation of raptor contribute to mTORC1 activation, whereas AMPK-mediated phosphorylation of raptor results in the inhibition of mTORC1 signalling. When active, mTORC1 promotes protein synthesis, lipogenesis, and energy metabolism and inhibits autophagy and lysosome biogenesis. Furthermore, activated mTORC1 leads to enhanced phosphorylation of IRS-1, which serves as negative feedback to dampen the insulin response. Regarding protein synthesis, two major mTORC1 substrates are S6 kinase 1 (S6K1) and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1).

Table 2. Burden of cardiovascular disease in South Asians compared to white Caucasians.

Burden of cardiovascular disease in South Asians compared to white Caucasians	*References*
- Risk of CVD is at least two fold higher	12;26;61-64
- South Asians are affected at a younger age: mean age of first acute myocardial infarct ~5 years earlier	65;66
- CVD is more aggressive and has higher mortality rate at younger ages: age standardized mortality rate is ~50% higher	12;26;61;65

CVD, cardiovascular disease

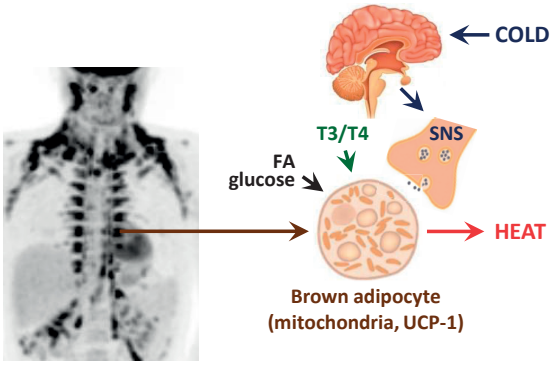


Figure 3. Localization and simplified view of activation of brown adipose tissue (BAT) in human adults. In adults, BAT is predominantly located in the supraclavicular and paravertebral regions (black areas on ^{18}F-FDG-PET-CT image on left side = ^{18}F-FDG uptake). In addition, individual brown fat cells lie scattered in other tissues, such as white adipose tissue and skeletal muscle, where they form a pool of ´peripheral brown adipocytes´, the so called 'beige adipocytes´ (not shown in this figure). BAT takes up glucose and triglyceride-derived fatty acids (FA) from the plasma and subsequently burns FA to generate heat by means of mitochondrial uncoupling, a process called thermogenesis. For this purpose, BAT contains high numbers of mitochondria that express uncoupling protein 1 (UCP-1), which uncouples respiration from ATP, in order to provide high oxidative capacity and is densely innervated by the sympathetic nervous system (SNS). The latter makes sure that BAT is rapidly activated in case of a cold environment, resulting in generation of heat. The thermogenic capacity of BAT is also dependent on the thyroid hormone axis (T3/T4). For a more detailed view on the activation of BAT see Figure 2 in Chapter 6.

Pathophysiology. The excess risk of cardiovascular disease in South Asians most likely reflects interactions between genetic susceptibility and environmental factors, such as changes secondary to urbanization and migration. Indeed, the risk of cardiovascular disease appears to increase as South Asians move from rural India to urban India to other countries.[67] With urbanization and migration to Western environments the consumption of energy rich diets markedly increases. In addition, energy expenditure decreases due to less physical activity, and exposure to stress increases. Acculturation is positively associated with coronary artery disease and type 2 diabetes in South Asian immigrants in the USA.[68] Thus, migration itself could be an aggravating factor in the high cardiovascular disease risk of migrant South Asians.

The major cause of cardiovascular disease is atherosclerosis, which is present many years before any clinical symptoms of cardiovascular disease become manifest, and which may be promoted by several risk factors. Several of these risk factors are highly present in South Asians.[27;62;66;69] As already mentioned, South Asians are known for their disadvantageous metabolic phenotype, consisting of insulin resistance, central obesity and dyslipidemia. However, after correction for these 'classical' risk factors, ethnicity remains an independent determinant of cardiovascular events.[12;27;62] Thus, residual risk is present sug-

gesting that additional 'non-classical' risk factors must play a role. This residual risk is most likely caused by genetic factors since it is present in both native and migrant South Asians. In **Chapter 8**, we review classical risk factors contributing to the increased cardiovascular risk of South Asians and discuss potential other non-classical factors that might elucidate the unexplained 'excess' risk for cardiovascular disease in this population.

RELEVANCE OF THIS THESIS

The high prevalence of type 2 diabetes and cardiovascular disease in South Asians, who comprise one fifth of the total world's population, poses a major health and socioeconomic burden worldwide. The underlying cause of this excess risk is still poorly understood. Remarkably, though, only few in-depth studies have been conducted to investigate the pathogenesis of type 2 diabetes and cardiovascular disease in South Asians. Therefore, this thesis aimed to gain more insight in the pathogenesis of, and to provide new leads for preventive strategies and treatment options for type 2 diabetes and cardiovascular disease in people of South Asian descent. For this purpose, sophisticated techniques such as a hyperinsulinemic euglycemic clamp with stable isotopes, indirect calorimetry, skeletal muscle biopsies, and magnetic resonance (MR)- and PET-CT-imaging were used, combined with short-term dietary interventions, in both young adult and adult subjects. A large cohort of South Asian immigrants, or Dutch Hindostani, lives in The Netherlands. The subjects who participated in the studies in this thesis are from this cohort, in addition to Dutch white Caucasian subjects who formed the control groups. See *Intermezzo: Study population* for more details on the study subjects.

INTERMEZZO: STUDY POPULATION

Caucasian subjects. The Caucasian subjects who participated in the studies described in this thesis are all of Dutch origin. Caucasian has multiple meanings. In this thesis Caucasian means of white or Western European origin. White Caucasian and Caucasian are used interchangeably throughout this text.

South Asian subjects. The South Asian subjects who participated in the studies included in this thesis are all Dutch Hindostani.

Etymology. Hindostan or Hindustan is derived from the Persian word Hindu, which is derived from the old Sanskrit name for the Indus River: Sindhu. Hence, originally, Hindu were the people in the land beyond the Indus River and Hindustan meant "land of the Indus". Hindostani or Hindustani are an ethnic group of South Asian origin in the Netherlands and Surinam. In Dutch they are called 'Hindostanen' or 'Hindoestanen', although some people say that 'Hindoestanen' refers to the religion (Hindu) and 'Hindostanen' to the ethnic group.

Migration history. Dutch Hindostani originally come from North India. After the abolition of slavery in 1862 in Dutch colonies, such as Surinam, the Dutch were allowed by the British in 1873 to recruit labourers in North India to Surinam. The recruitment of immigrants from North India was mainly done in one area: the United Provinces of Agra and Oudh, now known as the Indian states Uttar Pradesh and West Bihar. This area was known for its overpopulation, poverty and shortage of food and employment. The Dutch government chose this area hoping for more recruitment. South Asian migrants who were found physically fit after medical screening were selected. In total, around 34.000 South Asians migrated to Surinam, mainly to Nickerie and the districts Paramaribo, Wanica and Commewijne, and worked on the plantations (agriculture and sugar cane). After five years, they had the option to return to India with a free passage; around one third of the immigrants returned. Just before and just after the independence of Surinam in 1975 many Hindostani emigrated to the Netherlands.[70] The migration history of Dutch Hindostani is shown in **Figure 4**.

In 2010, around 123.000 – 147.000 Hindostani lived in the Netherlands (0.7-0.9% of the total population). The estimated number of Hindostani in Surinam was almost 147.000 in 2012 (27.4% of the total population); around 100.000 live elsewhere in the world. In the Netherlands, the largest group lives in The Hague with around 45.000 Hindostani; other cities are Almere (~7000), Rotterdam (~30.000), Amsterdam (~12.000) and Utrecht (~5000).[71-73]

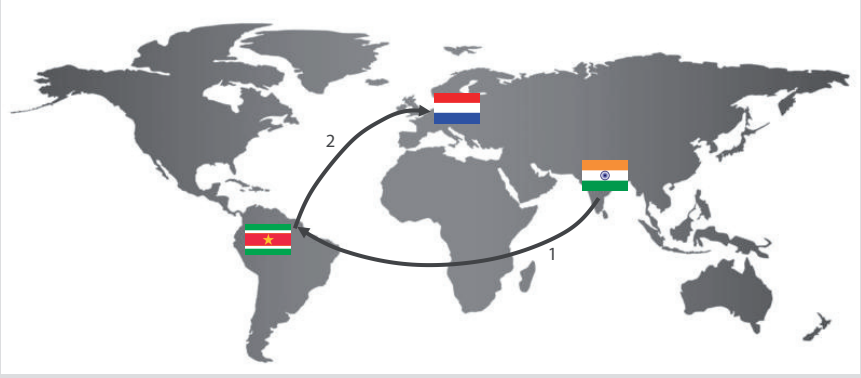


Figure 4. Migration history of Dutch Hindostani. The South Asian subjects who participated in the studies included in this thesis are Dutch Hindostani. They originally come from North India. After the abolition of slavery in 1862 in Dutch colonies, such as Surinam, the Dutch were allowed by the British in 1873 to recruit labourers in North India to Surinam (arrow 1). Just before and just after the independence of Surinam in 1975 many Hindostani emigrated to the Netherlands (arrow 2).

OUTLINE OF THE THESIS

The first part of this thesis focuses on the high prevalence of type 2 diabetes in South Asians. In **Chapter 2** we review the available literature on potential pathophysiological mechanisms responsible for the increased risk of type 2 diabetes in South Asians compared to white Caucasians, and we suggest several other areas of interest that should be explored to further investigate the increased risk for insulin resistance and type 2 diabetes.

Multiple studies have repeatedly shown that South Asians have higher insulin levels during an oral glucose test (OGTT) compared to white Caucasians. Therefore, in **Chapter 3,** we aimed to investigate if this increased insulin response causes reactive hypoglycemia, and if an increased glucagon-like-peptide-1 (GLP-1) response, which could contribute to the hyperinsulinemia, is present in young, healthy South Asian men, using a prolonged 6-hour 75-g OGTT.

In **Chapter 4** we compare the metabolic adaptation to a 5-day high-fat-high-calorie diet (HFHCD) between young, healthy, lean South Asian and white Caucasian men. In particular, we were interested whether differences in the activity of mTOR in skeletal muscle exist between the two ethnicities. Furthermore, hepatic and peripheral insulin sensitivity, substrate oxidation, abdominal fat distribution and skeletal muscle insulin signalling and mitochondrial respiratory-chain content were assessed. In **Chapter 5** we assessed the effect of caloric restriction through an 8-day very low calorie diet (VLCD) on skeletal muscle energy/nutrient-sensing pathways in middle-aged overweight South Asian and white Caucasian men. We hypothesized that differences in the regulation of energy/nutrient-sensing pathways in metabolic tissues may affect whole-body substrate metabolism, and ultimately contribute to the increased risk of type 2 diabetes in South Asians.

Recently, brown adipose tissue has emerged as a novel player in energy metabolism in humans by combusting fatty acids towards heat. In **Chapter 6** we give an overview of the anatomy, physiology and function of BAT and describe how BAT could be manipulated in order to increase energy expenditure and possibly induce weight loss. South Asians frequently exhibit a disadvantageous metabolic phenotype, consisting of central obesity, insulin resistance, and dyslipidemia. Since BAT is involved in total energy expenditure and clearance of serum triglycerides and glucose, we hypothesized that a low BAT volume and/or activity might underlie this disadvantageous metabolic phenotype and high susceptibility for type 2 diabetes in South Asians. Therefore, in **Chapter 7**, we investigated resting energy expenditure as well as BAT volume and activity in young healthy lean South Asian and white Caucasian men, using ventilated hoods and cold-induced ^{18}F-FDG-PET-CT-scans. In addition, we examined the effect of cold exposure on non-shivering thermogenesis, thermoregulation, and plasma lipid levels.

Part two of this thesis focuses on the high risk of developing cardiovascular disease in South Asians. In **Chapter 8**, we review potential factors, both classical 'metabolic' and non-classical 'inflammatory' factors, contributing to the increased cardiovascular risk of South Asians and propose a pathophysiological mechanism underlying the high prevalence of classical risk factors, i.e. the disadvantageous metabolic phenotype, in South Asians. Furthermore, we discuss novel therapeutic strategies based on recent insights.

Chapter 9 aimed to assess whether cardiac dimensions, cardiovascular function and myocardial triglyceride content differ between young, healthy South Asian and white Caucasian men, possibly contributing to the increased cardiovascular disease risk in South Asians. In addition, we hypothesized that possible differences in cardiovascular function between both ethnicities can be attributed to alterations in energy metabolism, including a differential fat distribution in South Asians. Therefore, we subjected the participants to a 5-day HFHCD. In **Chapter 10** we assessed whether metabolic and functional cardiovascular flexibility to caloric restriction differs between middle-aged, overweight South Asian and white Caucasian men.

Finally, numerous studies have consistently shown a strong inverse association between the level of high density lipoprotein (HDL)-cholesterol and cardiovascular risk. The cardiovascular protective effects of HDL have been attributed to several anti-atherogenic properties. Interestingly, multiple studies have repeatedly found lower HDL-cholesterol levels in South Asians compared to white Caucasians, even in South Asian neonates. Hence, a possible contributing factor to the high risk of developing cardiovascular disease in South Asians might be dysfunction of HDL. Therefore, **Chapter 11**, aimed to compare HDL function in South Asian and white Caucasian subjects.

The findings of this thesis are discussed in **Chapter 12**. This chapter also offers a perspective for future research.

REFERENCES

1. DeFronzo RA. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes* 1988;37(6):667-87.
2. Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. *Diabetologia* 2003;46(1):3-19.
3. Kahn SE, Cooper ME, del Prato S. Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. *Lancet* 2014;383(9922):1068-83.
4. Bloom DE, Cafiero ET, Jané-Llopis E, Abrahams-Gessel S, Bloom LR, Fathima S *et al*. The Global Economic Burden of Non-communicable Diseases. Geneva: World Economic Forum; Harvard School of Public Health; 2011.
5. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res Clin Pract* 2011;94(3):311-21.
6. Anjana RM, Pradeepa R, Deepa M, Datta M, Sudha V, Unnikrishnan R *et al*. Prevalence of diabetes and prediabetes (impaired fasting glucose and/or impaired glucose tolerance) in urban and rural India: phase I results of the Indian Council of Medical Research-INdia DIABetes (ICMR-INDIAB) study. *Diabetologia* 2011;54(12):3022-7.
7. Becker E, Boreham R, Chaudhury M, Craig R, Deverill C, Doyle M *et al*. Health Survey for England 2004. Volume 1. The health of minority ethnic groups. In: Sproston K, Mindell J, editors. *Cardiovascular disease and diabetes*. 1st ed. Leeds: The Information Centre; 2006. p. 63-94.
8. Bindraban NR, van Valkengoed IG, Mairuhu G, Holleman F, Hoekstra JB, Michels BP *et al*. Prevalence of diabetes mellitus and the performance of a risk score among Hindustani Surinamese, African Surinamese and ethnic Dutch: a cross-sectional population-based study. *BMC Public Health* 2008;8:271.
9. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 2010;87(1):4-14.
10. Gupta R, Misra A. Review: Type 2 diabetes in India: regional disparities. *The British Journal of Diabetes & Vascular Disease* 2007;7(1):12-6.
11. Katikireddi SV, Morling JR, Bhopal R. Is there a divergence in time trends in the prevalence of impaired glucose tolerance and diabetes? A systematic review in South Asian populations. *Int J Epidemiol* 2011;40(6):1542-53.
12. Anand SS, Yusuf S, Vuksan V, Devanesen S, Teo KK, Montague PA *et al*. Differences in risk factors, atherosclerosis, and cardiovascular disease between ethnic groups in Canada: the Study of Health Assessment and Risk in Ethnic groups (SHARE). *Lancet* 2000;356(9226):279-84.
13. Chambers JC, Eda S, Bassett P, Karim Y, Thompson SG, Gallimore JR *et al*. C-reactive protein, insulin resistance, central obesity, and coronary heart disease risk in Indian Asians from the United Kingdom compared with European whites. *Circulation* 2001;104(2):145-50.
14. Mukhopadhyay B, Forouhi NG, Fisher BM, Kesson CM, Sattar N. A comparison of glycaemic and metabolic control over time among South Asian and European patients with Type 2 diabetes: results from follow-up in a routine diabetes clinic. *Diabet Med* 2006;23(1):94-8.
15. Simmons D, Williams DR, Powell MJ. Prevalence of diabetes in a predominantly Asian community: preliminary findings of the Coventry diabetes study. *BMJ* 1989;298.
16. Barnett AH, Dixon AN, Bellary S, Hanif MW, O'Hare JP, Raymond NT *et al*. Type 2 diabetes and cardiovascular risk in the UK south Asian community. *Diabetologia* 2006;49(10):2234-46.
17. DECODE Study Group. Age- and sex-specific prevalences of diabetes and impaired glucose regulation in 13 European cohorts. *Diabetes Care* 2003;26(1):61-9.

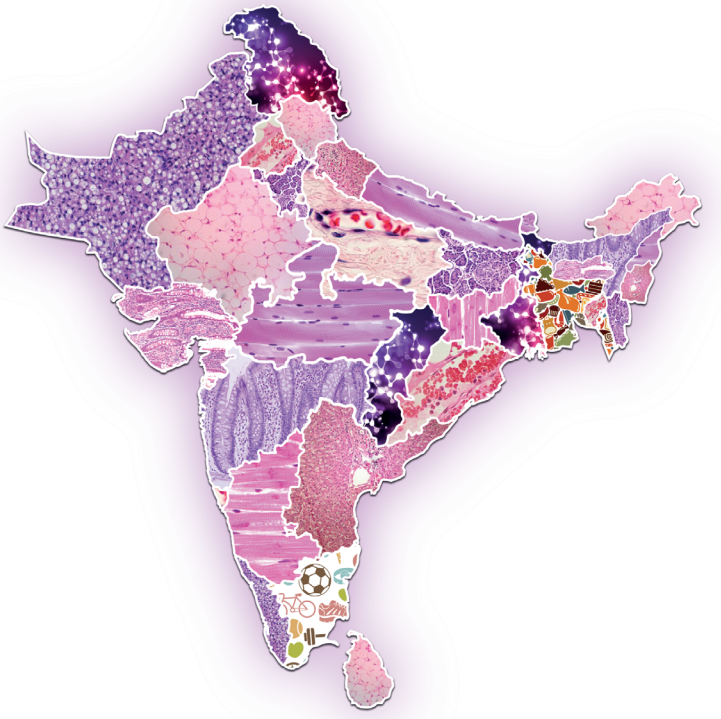
18. Mohanty SA, Woolhandler S, Himmelstein DU, Bor DH. Diabetes and cardiovascular disease among Asian Indians in the United States. *J Gen Intern Med* 2005;20(5):474-8.
19. Middelkoop BJ, Kesarlal-Sadhoeram SM, Ramsaransing GN, Struben HW. Diabetes mellitus among South Asian inhabitants of The Hague: high prevalence and an age-specific socioeconomic gradient. *Int J Epidemiol* 1999;28(6):1119-23.
20. UK Prospective Diabetes Study. XII: Differences between Asian, Afro-Caribbean and white Caucasian type 2 diabetic patients at diagnosis of diabetes. UK Prospective Diabetes Study Group. *Diabet Med* 1994;11(7):670-7.
21. Chiu M, Austin PC, Manuel DG, Shah BR, Tu JV. Deriving ethnic-specific BMI cutoff points for assessing diabetes risk. *Diabetes Care* 2011;34(8):1741-8.
22. Gray LJ, Yates T, Davies MJ, Brady E, Webb DR, Sattar N *et al*. Defining obesity cut-off points for migrant South Asians. *PLoS One* 2011;6(10):e26464.
23. Razak F, Anand SS, Shannon H, Vuksan V, Davis B, Jacobs R *et al*. Defining obesity cut points in a multiethnic population. *Circulation* 2007;115(16):2111-8.
24. Chandie Shaw PK, Vandenbroucke JP, Tjandra YI, Rosendaal FR, Rosman JB, Geerlings W *et al*. Increased end-stage diabetic nephropathy in Indo-Asian immigrants living in the Netherlands. *Diabetologia* 2002;45(3):337-41.
25. Chandie Shaw PK, Baboe F, van Es LA, van der Vijver JC, van de Ree MA, de Jonge N *et al*. South-Asian type 2 diabetic patients have higher incidence and faster progression of renal disease compared with Dutch-European diabetic patients. *Diabetes Care* 2006;29(6):1383-5.
26. Chaturvedi N, Fuller JH. Ethnic differences in mortality from cardiovascular disease in the UK: do they persist in people with diabetes? *J Epidemiol Community Health* 1996;50(2):137-9.
27. McKeigue PM, Ferrie JE, Pierpoint T, Marmot MG. Association of early-onset coronary heart disease in South Asian men with glucose intolerance and hyperinsulinemia. *Circulation* 1993;87(1):152-61.
28. Wilkinson P, Sayer J, Laji K, Grundy C, Marchant B, Kopelman P *et al*. Comparison of case fatality in south Asian and white patients after acute myocardial infarction: observational study. *BMJ* 1996;312(7042):1330-3.
29. Mather HM, Chaturvedi N, Kehely AM. Comparison of prevalence and risk factors for microalbuminuria in South Asians and Europeans with Type 2 diabetes mellitus. *Diabetic Medicine* 1998;15(8):672-7.
30. Overweight, obesity, and health risk. National Task Force on the Prevention and Treatment of Obesity. *Arch Intern Med* 2000;160(7):898-904.
31. Goossens GH. The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance. *Physiology & Behavior* 2008;94(2):206-18.
32. Srikanthan P, Karlamangla AS. Relative muscle mass is inversely associated with insulin resistance and prediabetes. Findings from the third National Health and Nutrition Examination Survey. *J Clin Endocrinol Metab* 2011;96(9):2898-903.
33. Banerji MA, Faridi N, Atluri R, Chaiken RL, Lebovitz HE. Body composition, visceral fat, leptin, and insulin resistance in Asian Indian men. *J Clin Endocrinol Metab* 1999;84(1):137-44.
34. Dickinson S, Colagiuri S, Faramus E, Petocz P, Brand-Miller JC. Postprandial hyperglycemia and insulin sensitivity differ among lean young adults of different ethnicities. *J Nutr* 2002;132(9):2574-9.
35. Liew CF, Seah ES, Yeo KP, Lee KO, Wise SD. Lean, nondiabetic Asian Indians have decreased insulin sensitivity and insulin clearance, and raised leptin compared to Caucasians and Chinese subjects. *Int J Obes Relat Metab Disord* 2003;27(7):784-9.

36. McKeigue PM, Marmot MG, Syndercombe Court YD, Cottier DE, Rahman S, Riemersma RA. Diabetes, hyperinsulinaemia, and coronary risk factors in Bangladeshis in east London. *Br Heart J* 1988;60(5):390-6.
37. McKeigue PM, Shah B, Marmot MG. Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. *Lancet* 1991;337(8738):382-6.
38. Nair KS, Bigelow ML, Asmann YW, Chow LS, Coenen-Schimke JM, Klaus KA *et al*. Asian Indians have enhanced skeletal muscle mitochondrial capacity to produce ATP in association with severe insulin resistance. *Diabetes* 2008;57(5):1166-75.
39. Petersen KF, Dufour S, Feng J, Befroy D, Dziura J, Dalla MC *et al*. Increased prevalence of insulin resistance and nonalcoholic fatty liver disease in Asian-Indian men. *Proc Natl Acad Sci U S A* 2006;103(48):18273-7.
40. Raji A, Seely EW, Arky RA, Simonson DC. Body fat distribution and insulin resistance in healthy Asian Indians and Caucasians. *J Clin Endocrinol Metab* 2001;86(11):5366-71.
41. Raji A, Gerhard-Herman MD, Warren M, Silverman SG, Raptopoulos V, Mantzoros CS *et al*. Insulin resistance and vascular dysfunction in nondiabetic Asian Indians. *J Clin Endocrinol Metab* 2004;89(8):3965-72.
42. Raji A, Gerhard-Herman MD, Williams JS, O'Connor ME, Simonson DC. Effect of pioglitazone on insulin sensitivity, vascular function and cardiovascular inflammatory markers in insulin-resistant non-diabetic Asian Indians. *Diabet Med* 2006;23(5):537-43.
43. Boon MR, Karamali NS, de Groot CJ, van Steijn L, Kanhai HH, van der Bent C *et al*. E-Selectin is Elevated in Cord Blood of South Asian Neonates Compared with Caucasian Neonates. *J Pediatr* 2011;160(5):844-8.
44. Yajnik CS, Lubree HG, Rege SS, Naik SS, Deshpande JA, Deshpande SS *et al*. Adiposity and hyperinsulinemia in Indians are present at birth. *J Clin Endocrinol Metab* 2002;87(12):5575-80.
45. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes* 1981;30(12):1000-7.
46. Shulman GI, Rothman DL, Jue T, Stein P, DeFronzo RA, Shulman RG. Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. *N Engl J Med* 1990;322(4):223-8.
47. Cline GW, Petersen KF, Krssak M, Shen J, Hundal RS, Trajanoski Z *et al*. Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes. *N Engl J Med* 1999;341(4):240-6.
48. Rothman DL, Shulman RG, Shulman GI. 31P nuclear magnetic resonance measurements of muscle glucose-6-phosphate. Evidence for reduced insulin-dependent muscle glucose transport or phosphorylation activity in non-insulin-dependent diabetes mellitus. *J Clin Invest* 1992;89(4):1069-75.
49. Hall LM, Moran CN, Milne GR, Wilson J, MacFarlane NG, Forouhi NG *et al*. Fat oxidation, fitness and skeletal muscle expression of oxidative/lipid metabolism genes in South Asians: implications for insulin resistance? *PLoS One* 2010;5(12):e14197.
50. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012;149(2):274-93.
51. Sengupta S, Peterson TR, Sabatini DM. Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. *Mol Cell* 2010;40(2):310-22.
52. Haruta T, Uno T, Kawahara J, Takano A, Egawa K, Sharma PM *et al*. A rapamycin-sensitive pathway down-regulates insulin signaling via phosphorylation and proteasomal degradation of insulin receptor substrate-1. *Mol Endocrinol* 2000;14(6):783-94.

53. Ricoult SJ, Manning BD. The multifaceted role of mTORC1 in the control of lipid metabolism. *EMBO Rep* 2013;14(3):242-51.
54. Takano A, Usui I, Haruta T, Kawahara J, Uno T, Iwata M *et al*. Mammalian target of rapamycin pathway regulates insulin signaling via subcellular redistribution of insulin receptor substrate 1 and integrates nutritional signals and metabolic signals of insulin. *Mol Cell Biol* 2001;21(15):5050-62.
55. Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M *et al*. Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature* 2004;431(7005):200-5.
56. van Marken Lichtenbelt WD, Schrauwen P. Implications of nonshivering thermogenesis for energy balance regulation in humans. *Am J Physiol Regul Integr Comp Physiol* 2011;301(2):R285-R296.
57. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB *et al*. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* 2009;360(15):1509-17.
58. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND *et al*. Cold-activated brown adipose tissue in healthy men. *N Engl J Med* 2009;360(15):1500-8.
59. Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T *et al*. Functional brown adipose tissue in healthy adults. *N Engl J Med* 2009;360(15):1518-25.
60. Laslett LJ, Alagona P, Jr., Clark BA, III, Drozda JP, Jr., Saldivar F, Wilson SR *et al*. The worldwide environment of cardiovascular disease: prevalence, diagnosis, therapy, and policy issues: a report from the American College of Cardiology. *J Am Coll Cardiol* 2012;60(25 Suppl):S1-49.
61. Balarajan R. Ethnic differences in mortality from ischaemic heart disease and cerebrovascular disease in England and Wales. *BMJ* 1991;302(6776):560-4.
62. Forouhi NG, Sattar N, Tillin T, McKeigue PM, Chaturvedi N. Do known risk factors explain the higher coronary heart disease mortality in South Asian compared with European men? Prospective follow-up of the Southall and Brent studies, UK. *Diabetologia* 2006;49(11):2580-8.
63. Turin TC, Shahana N, Wangchuk LZ, Specogna AV, Al Mamun M, Khan MA *et al*. Burden of Cardio- and Cerebro-vascular Disease and the Conventional Risk Factors in South Asian Population. *Global Health* 2005;8(2):121-30.
64. Wild SH, Fischbacher C, Brock A, Griffiths C, Bhopal R. Mortality from all causes and circulatory disease by country of birth in England and Wales 2001-2003. *J Public Health (Oxf)* 2007;29(2):191-8.
65. Enas EA, Yusuf S, Mehta JL. Prevalence of coronary artery disease in Asian Indians. *Am J Cardiol* 1992;70(9):945-9.
66. Joshi P, Islam S, Pais P, Reddy S, Dorairaj P, Kazmi K *et al*. Risk factors for early myocardial infarction in South Asians compared with individuals in other countries. *JAMA* 2007;297(3):286-94.
67. Gupta R, Gupta R, Agrawal A, Misra A, Guptha S, Pandey RM *et al*. Migrating husbands and changing cardiovascular risk factors in the wife: a cross sectional study in Asian Indian women. *J Epidemiol Community Health* 2012;66(10):881-9.
68. Dodani S, Dong L. Acculturation, coronary artery disease and carotid intima media thickness in South Asian immigrants—unique population with increased risk. *Ethn Dis* 2011;21(3):314-21.
69. Tziomalos K, Weerasinghe CN, Mikhailidis DP, Seifalian AM. Vascular risk factors in South Asians. *Int J Cardiol* 2008;128(1):5-16.
70. Chandie Shaw PK. Diabetic Nephropathy in Surinamese South Asian Subjects, Thesis 2007.
71. ACB Kenniscentrum. Factsheet Hindostanen in Nederland. 2011.
72. Choenni C, Harmsen C. Geboorteplaats en etnische samenstelling van Surinamers in Nederland. *Bevolkingstrends, 1e kwartaal 2007*. Centraal Bureau voor de Statistiek; 2007. p. 74-8.
73. Garssen MJ, Hoogenboezem J, Kerkhof AJFM. Zelfdoding onder Nederlandse Surinamers naar etniciteit. *Tijdschrift voor Psychiatrie* 2007;49(6):373-81.

PART 1

TYPE 2 DIABETES MELLITUS



2 Pathogenesis of type 2 diabetes in South Asians

Leontine E.H. Bakker*
Maria A. Sleddering*
Jan W. Schoones
A. Edo Meinders
Ingrid M. Jazet

*Authors contributed equally to manuscript

European Journal of Endocrinology 2013; 169(5): R99-R114

ABSTRACT

The risk of developing type 2 diabetes is exceptionally high among both native and migrant South Asians. Type 2 diabetes occurs more often and at a younger age and lower BMI, and the risk of coronary artery and cerebrovascular disease and renal complications is higher for this ethnicity compared to people of white Caucasian descent. The high prevalence of diabetes and its related complications in South Asians, which comprise one fifth of the total world's population, poses a major health and socioeconomic burden. The underlying cause of this excess risk, however, is still not completely understood. Therefore, gaining insight in the pathogenesis of type 2 diabetes in South Asians is of great importance. The predominant mechanism in this ethnicity seems to be insulin resistance rather than impairment in β-cell function. In this systematic review we describe several possible mechanisms that may underlie or contribute to the increased insulin resistance observed in South Asians.

INTRODUCTION

Worldwide the prevalence of type 2 diabetes increases, particularly in South Asian countries and especially in India, which currently has the highest global number of diabetes patients, with an estimated prevalence of up to 16.8% in urban areas.[1-4] Similar prevalence rates have also been reported in migrants of South Asian descent (India, Pakistan, Bangladesh, Nepal and Sri Lanka) in the United States of America (USA), Canada and various European countries.[5-8] In the Netherlands, South Asians mostly consist of Hindustani Surinamese who migrated from Surinam, a former Dutch colony in South America, and whose ancestors came from the Indian subcontinent about a century ago. Hindustani Surinamese have the highest type 2 diabetes prevalence of all ethnic minorities living in the Netherlands.[9] An age-standardized prevalence rate of type 2 diabetes of 26.7% for this group has been reported, compared to 5.5% in ethnic Dutch (**Table 1**).[10]

In addition to the increased prevalence, South Asians develop diabetes at a much younger age than white Caucasians and have an increased incidence of retinopathy, microalbuminuria and end-stage renal disease.[11-13] Furthermore, South Asians have an increased risk of developing coronary artery and cerebrovascular disease, and a 50% higher age-adjusted mortality rate from coronary heart disease.[8]

Uncovering the underlying mechanisms involved in the higher prevalence of type 2 diabetes in South Asians is very relevant, as they represent over 20% of the world's population. In this review we discuss the available literature on potential pathophysiological mechanisms responsible for the increased prevalence of type 2 diabetes in South Asians as compared to white Caucasians.

Table 1. Prevalence of T2DM in South Asians and white Caucasians

	Prevalence of T2DM	*References*
Rural India	3.0 - 8.3 %	2
Urban India	10.9 - 14.2 %	2
South Asians (Dutch)	26.7%	10
white Caucasians (Dutch)	5.5%	10

METHODS

The literature was searched using international databases: PubMed (1949 to July 2013), EMBASE (OVID-version, 1980 to July 2013), Web of Science (1945 to July 2013), and the Cochrane Library (1990 to July 2013). Terms used were 'South Asian' OR 'Indo Asian' combined with several keywords related to diabetes and its risk factors (i.e. type 2 diabetes mellitus, obesity, metabolic syndrome, insulin resistance, insulin secretion, body fat, liver fat, skeletal muscle, mitochondrial dysfunction, endothelial dysfunction, adipokines, in-

flammation). References were limited to studies in humans, written in English or Dutch. See the Supplementary Methods online for the complete literature search.

TYPE 2 DIABETES MELLITUS IN SOUTH ASIANS

Pathogenesis

Type 2 diabetes mellitus is a chronic, multifactorial disease characterized by a combination of insulin resistance and impaired insulin secretion (**Figure 1**).[14] The predominant mechanism, however, appears to be different in various ethnic groups.

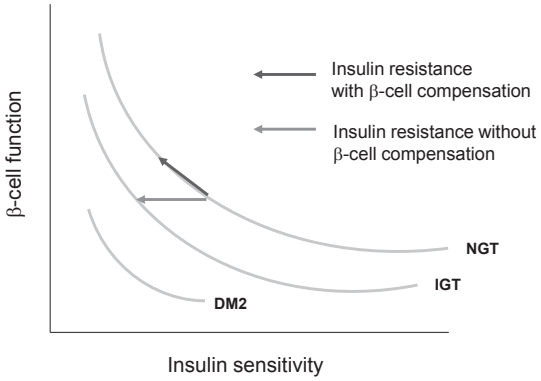


Figure 1. The relation between insulin sensitivity and β-cell function in type 2 diabetes. β-cell function adapts to insulin resistance in order to maintain glucose tolerance normal (derived from thesis I.M. Jazet, 2006, chapter 1, page 25).

Multiple studies have repeatedly shown that South Asians have higher fasting insulin concentrations compared to other ethnic groups regardless of age, gender or BMI, suggesting a higher rate of insulin resistance in this population.[15-25] Already in neonates fasting insulin levels are markedly higher compared to white Caucasian neonates,[26;27] and fasting insulin remains higher in school children[28;29]and teenagers.[30] In addition, studies with an oral glucose or meal tolerance test each show a higher serum insulin level after two hours and/or a higher insulin area under the curve with a normal glucose response in South Asians compared to different ethnicities.[16;17;19;20;23-25;28;31;32] The response to an insulin tolerance test is also worse in South Asians.[31;32] Moreover, hyperinsulinemic euglycemic clamp studies in men and women of all age groups and with a relatively normal BMI all show lower insulin sensitivity (up to almost 50%) in South Asians compared to different ethnic populations.[16;18;21;23-25;33-36] Thus, South Asians seem to resemble Pima Indians, in whom insulin resistance and hyperinsulinemia are also predominant findings starting from a young age.[37;38]

Insulin secretion or β-cell function has been investigated in fewer studies and with more inferior techniques (e.g. no hyperglycemic clamp studies have been performed) compared to insulin sensitivity in South Asians. In a large study of the UK Prospective Diabetes Study (UKPDS) in 5098 newly diagnosed type 2 diabetes patients (82% white Caucasians, 10% South Asians and 8% Afro-Caribbeans), β-cell function, measured with HOMA %B, was best in South Asians and worse in Afro-Caribbeans, while for insulin sensitivity, measured with HOMA %S, the opposite was true.[15] In another study, in which an intravenous glucose tolerance test (IVGTT) was performed in 17 healthy first degree relatives of patients with type 2 diabetes and 17 healthy controls with no family history of type 2 diabetes, insulin secretory defects prevailed in the European relatives (n=10), whereas insulin resistance was predominant in the South Asian relatives (n=7).[39] Similar results were found in a study in which an oral glucose tolerance test (OGTT) was performed in 260 middle-aged South Asians with different stages of glucose tolerance. They found that impaired glucose tolerance was not associated with a significant defect in insulin secretion, whereas insulin resistance was present already in an early stage of glucose intolerance, suggesting that insulin resistance might precede β-cell deficiency.[40] Another study found that Asian Indian men (n=21) had a ~30% increase in basal β-cell responsivity, measured by the oral C-peptide minimal model, compared to Caucasian men (n=71).[22] Although this increase in β-cell function was inadequate for their degree of insulin resistance as reflected by a lower disposition index, this compensatory increase suggests that β-cell dysfunction is not the main problem. Hence, impairment in insulin secretion does not seem the primary defect in the development of type 2 diabetes in South Asians, in contrast to other ethnicities, such as Japanese and Afro-Caribbeans.[15;41;42]

In the next sections we will describe several possible mechanisms that may contribute to the increased risk of type 2 diabetes, and in particular insulin resistance, in South Asians.

Evolutionary and developmental hypotheses

The excess risk of type 2 diabetes among South Asians has been attributed to several hypotheses (**Table 2**).

The thrifty *genotype* hypothesis states that predisposition to diabetes must have evolved as an adaptive trait in certain environmental situations that later turned disadvantageous due to changes in life style. According to Neel, the thrifty genotype helped survival in the "feast-or-famine days of hunting and gathering cultures", but has now turned detrimental in the modern era of "continuous feasting".[43] In line with the thrifty genotype hypothesis, other evolutionary theories, such as the adipose tissue overflow[44] (see "Body composition and fat distribution"), El Niño[45] and the variable disease selection[46] hypotheses, postulate that South Asians are particularly susceptible to central

Table 2. Evolutionary and developmental hypotheses explaining the excess risk of type 2 diabetes among South Asians.

Hypothesis	Description	Arguments pro/contra
Evolutionary hypotheses		
Thrifty genotype *Neel, 1962*	Predisposition to T2DM must have evolved as an adaptive trait in certain environmental situations that later turned disadvantageous due to changes in lifestyle	Does not explain why South Asians, in particular, are susceptible to central rather than peripheral obesity, or why central obesity is more important than generalized obesity in relation to T2DM.
Adipose tissue compartment *Sniderman, 2007*	The primary adipose tissue compartment is less developed in South Asians due to climatic influences, resulting in early expansion of the secondary adipose tissue compartments, especially in the face of excess energy intake, eventually leading to metabolic disturbances such as dysglycemia and dyslipidemia.	Explains why South Asians are particularly susceptible to central obesity, and why white Caucasians appear to be relatively protected from metabolic abnormalities and diabetes.
El Niño *Wells, 2007*	Susceptibility to central obesity and subsequently to insulin resistance and T2DM is due to nutritional influences. Chronic energy deficiency favours increased allocation to the visceral depot.	Explains why South Asians are particularly susceptible to central obesity. For many generations, South Asians have endured fluctuations of energy supply, associated in turn with global climate patterns (El Niño) and geographic circumstances.
Variable disease selection *Wells, 2009*	Susceptibility to central obesity and subsequently to insulin resistance and T2DM is due to infectious influences. Exposures to varying burdens of infectious disease may have been a selective pressure accounting for genetic ethnic variability in adipose tissue distribution.	Explains why South Asians are particularly susceptible to central obesity. Chronic exposures to endemic gastrointestinal diseases, including cholera, have been a long-term stress in South Asian populations.
Mitochondrial efficiency hypothesis *Bhopal 2009*	Energy producing efficiency of mitochondria enhanced the successful adaptation of South Asians to climatic (heat) and other nutritional exposures (periods of starvation). Instead of using energy to generate heat, South Asian' mitochondria are more likely to produce and subsequently store energy. This mitochondrial efficiency might be disadvantageous when adopting a new lifestyle with low physical activity and a high caloric diet.	Explains the tendency of South Asians to obesity *per se*, central obesity and adverse metabolic outcomes in our current environment, where food is abundant and physical activity is low. Integrates other hypotheses, and offers a biological mechanism (mitochondrial gene mutations).
Developmental hypotheses		
Thrifty phenotype *Hales & Barker, 2001*	An intrauterine disadvantageous environment induces thrifty mechanisms that sets the metabolism to cope with potential future food shortages, which is beneficial for early survival, but increases the risk of T2DM later in life in a nutrient rich environment. Based on strong association between low birth weight and increased risk of T2DM later in life, which is further increased by rapid weight gain in childhood.	Low birth weight and rapid weight gain are common in both native and migrant South Asian neonates. Does not explain why South Asians are susceptible to central rather than peripheral obesity, or why central obesity is more important than generalized obesity in relation to T2DM.

obesity and subsequently to insulin resistance and type 2 diabetes due to selective evolutionary pressures (e.g. climatic, nutritional, or infectious). Recently Bhopal and Rafnsson proposed the mitochondrial efficiency hypothesis: the energy producing efficiency of mitochondria enhanced the successful adaptation of South Asians to climatic (heat) and other nutritional exposures (periods of starvation). Instead of using energy to generate heat, South Asian mitochondria are therefore more likely to produce and subsequently store energy. This mitochondrial efficiency might be disadvantageous when adopting a new lifestyle with low physical activity and a high caloric diet, as is currently the case for South Asians.[47] The study of Nair *et al*. (discussed in "Skeletal muscle"), supports this hypothesis in that they found higher mitochondrial capacity for oxidative phosphorylation in both non-diabetic and diabetic South Asians compared to non-diabetic white Caucasians.[21]

Finally, according to the thrifty *phenotype* hypothesis, a developmental theory, there is a mismatch between intrauterine and adult life environments. An intrauterine disadvantageous environment (due to maternal malnutrition, maternal hyperglycemia, or other maternal/placental influences) induces thrifty mechanisms that sets the metabolism to cope with potential future food shortages, which is beneficial for early survival, but increases the risk of diabetes later in life in a nutrient rich environment.[48;49] This theory is based on the strong association between low birth weight and increased risk of type 2 diabetes later in life observed in a variety of ethnic populations.[50] Low birth weight is common in both native and migrant South Asian neonates.[27;51;52] The risk to develop type 2 diabetes is further increased by rapid weight gain (catch-up growth) in childhood. This applies particularly to countries going through a rapid nutritional transition or when migration takes place from less developed to developed countries, as is the case for both native and migrant South Asians. Interestingly, recent studies in rats showed that intrauterine growth restriction increases the susceptibility to high fat diet induced alterations of fat distribution, adipocyte size, lipid metabolism, and insulin-signalling pathways, supporting the thrifty phenotype hypothesis,[53] and resembling the problem in South Asians.

Although these hypotheses help explain better why South Asians are at an increased risk of developing insulin resistance and type 2 diabetes, they do not give an exact molecular mechanism, except the mitochondrial efficiency hypothesis.

Genetic factors

Type 2 diabetes is considered a polygenic disease that involves polymorphisms of several genes with a high gene-environment interaction.[54] Many loci associated with type 2 diabetes have been found in white Caucasians, however all variants found up till now have a modest effect size, with approximately twofold the lifetime prevalence rate of

type 2 diabetes in persons carrying two copies of the risk allele as compared to persons with none.[55]

Most loci found in white Caucasians have been verified in studies with South Asian subjects,[56-58] but few differences between the ethnic groups have been found and the differences are not all consistently shown. For example, Radha *et al*. found that in South Asians the Pro12Ala polymorphism of the peroxisome proliferator activator gamma (PPARγ) gene, which has a protective effect on type 2 diabetes development in white populations, is present at the same frequency in South Asians with and without diabetes and was not associated with a decreased risk of type 2 diabetes.[59] However, in a study in Asian Indian Sikhs they did see a protective effect of the polymorphism, suggesting that there might be differences between specific South Asian groups.[60] An interesting difference might lie in the fat-mass and obesity-associated (FTO) gene, which holds the strongest known obesity-susceptibility locus in Europeans. An association with type 2 diabetes has also been shown, but this seemed to be secondary to obesity. In South Asians however, the FTO polymorphism was found to be associated with type 2 diabetes independently of BMI,[61-63] implying that in South Asians there may be a different relationship between BMI and type 2 diabetes. However, associations between FTO and type 2 diabetes that were mediated by obesity have been found in South Asians as well[64] and in a study in North India none of the FTO variants was even associated with type 2 diabetes.[65] Two other recent studies in South Asians found polymorphisms of genes related to skeletal muscle; one associated with abdominal obesity and low lean body mass (*myostatin*)[66], and one contributing to type 2 diabetes susceptibility (*SCGC*)[67], which merit further investigation.

Thus, so far no clear genetic differences between white Caucasians and South Asians have been found. Interestingly, most loci associated with type 2 diabetes are related to impaired β-cell function and insulin secretion, which is not considered the primary defect in the South Asian population, as discussed before. Therefore, differences between the two ethnic groups on these loci is unlikely. However, an exceptionally high percentage of South Asians have a positive family history of type 2 diabetes, making it likely that genetic differences are somehow involved in the increased prevalence of TDM and insulin resistance in this ethnic group.

Diet and exercise

An unhealthy diet is a known risk factor for type 2 diabetes. Various studies have reported a number of dietary imbalances in South Asian diets associated with insulin resistance, such as high intake of total fat, saturated fatty acids, long chain ω-6 polyunsaturated fatty acids (PUFA), *trans*fatty acids, and carbohydrates, and low intake of monounsaturated fatty acids, long chain ω-3 PUFAs, fibre and several micronutrients (e.g. magnesium, calcium, vitamin D).[68-73] Furthermore, children and adolescents already

have a high intake of ω-6 PUFA and a low intake of ω-3 PUFA, which is correlated with fasting hyperinsulinemia.[74;75] However, supplementation of ω-3 PUFAs (fish oil) did not improve insulin sensitivity in South Asians.[69;76] Moreover, other studies even reported that South Asian diets are healthier compared to Caucasian diets (lower intake of fat).[73;77-79] Furthermore, different regional and religious South Asian communities in the United Kingdom (UK) all had a similar, markedly higher prevalence of diabetes compared to white Europeans, despite the known dietary, cultural and socioeconomic differences between these different South Asian communities. In addition, there were no discernible differences in the dietary customs of those with normal glucose tolerance, impaired glucose tolerance and newly diagnosed type 2 diabetes.[80;81] Lack of exercise is another risk factor for type 2 diabetes. The 2004 Health Survey for England data reported lower levels of physical activity in South Asian groups compared to the general UK population and other ethnic minority groups,[77] and other studies showed similar results in migrant and urban South Asians.[6;15;82-87] This low level of physical activity is already present in children and adolescents.[77;85;88-91]

Hence, although lifestyle factors will certainly play a role in the etiology of insulin resistance as they do in white Caucasians, there is no reason to assume that this role is any different between both ethnicities. This is strengthened by the fact that the excessive risk for type 2 diabetes applies to both native and migrant South Asians despite differences in lifestyle. Hence, South Asians seem to have an exceptionally high susceptibility to develop type 2 diabetes in the context of the same environmental pressure when compared to other ethnicities.

Body composition and fat distribution

South Asians develop insulin resistance and type 2 diabetes at lower ranges of BMI than white Caucasians. An equivalent incidence rate of type 2 diabetes is seen at a BMI of 24 kg/m^2 in South Asians compared to 30 kg/m^2 in Caucasian subjects.[92] Gray *et al*. even showed an equivalent level of dysglycemia at a BMI cut-off point of 22.6 kg/m^2 in South Asian males as compared to 30.0 kg/m^2 in white Caucasian males.[93] In addition, a cross-sectional study of 4633 9- to 10-year-old children of South Asian, black African-Caribbean and white European origin showed that South Asian children were more metabolically sensitive to adiposity as indicated by stronger positive associations between HOMA-IR and adiposity measures.[94] It has been proposed that an increase in total fat mass and an adverse pattern of fat distribution contributes to the higher risk of type 2 diabetes in South Asians at similar BMI levels. Therefore, it has been suggested that ethnic-specific BMI cut off values should be used for assessing diabetes risk in different populations.

Several studies have shown that South Asians have a higher percentage of body fat for comparable levels of BMI compared with white Caucasians and are therefore referred to as 'metabolically obese' (**Table 3**).[16;23;34;95-97] This is already apparent in children and

Table 3. Differences in body composition in South Asians *vs.* white Caucasians

South Asians *vs.* **white Caucasians**	*References*
Higher percentage of body fat	16;23;34;95-97
Thin fat phenotype in neonates	27;101-103
Increased abdominal adiposity	16;23;34;95;96
Increased VAT	23;96
Increased deep SAT, lower or similar superficial SAT	109;110
Decreased skeletal muscle mass/lean body mass	16;95;109;146

VAT: visceral adipose tissue; SAT: subcutaneous adipose tissue.

adolescents.[98-100] Also the distribution of fat differs between ethnicities. South Asian neonates exhibit the 'thin-fat phenotype', described as low muscle mass with preserved subscapular (central) fat[27;101;102] and this phenotype is retained in Surinam South Asian babies of the fourth to fifth generation after migration from India.[103] Modi *et al*. showed that South Asian neonates have significantly increased abdominal adiposity as compared to European babies[104] and this increase in abdominal adiposity has also been observed in adults in several other studies.[16;23;34;95;96] The 'thin-fat phenotype' is also apparent in pre-pubertal Indian children who have greater adiposity than white UK children despite significantly lower BMIs.[29]

It is currently unclear which of the abdominal adipose tissue compartments, visceral adipose tissue (VAT) or subcutaneous adipose tissue (SAT), has the most detrimental effect on insulin sensitivity.[44] Banerji *et al*. showed that South Asians have high amounts of VAT and that insulin resistance is correlated with total visceral and not subcutaneous abdominal adipose tissue volume.[16] Other studies also showed an association of VAT with diabetes[105] and cardiovascular risk factors in South Asians.[106;107] However, in a study of Raji *et al*., insulin sensitivity measured with a hyperinsulinemic euglycemic clamp in healthy South Asians and white Caucasians was inversely related with VAT as well as abdominal SAT and total abdominal adipose tissue.[23] This was however a small study including only 12 South Asian and 12 white Caucasian subjects. In another study in 171 South Asians, abdominal SAT was a better predictor of the metabolic syndrome. Also, SAT (and not VAT) was significantly correlated with insulin resistance, however insulin resistance was measured by HOMA and data were available for 46 patients only.[108] Furthermore, Chandalia *et al*. showed that insulin resistance was present in South Asians who had higher percentages of total body fat and abdominal SAT, but similar amounts of VAT as compared to white Caucasians.[34] However, these studies do not discriminate between superficial SAT (SSAT) and deep SAT (DSAT). It is believed that an increase in DSAT, similar to VAT, is associated with metabolic disturbances.[44] Sniderman *et al*. theorized in their 'overflow hypothesis' that SSAT is the primary adipose tissue compartment and DSAT and VAT are secondary compartments, which have adverse metabolic consequences.

They propose that South Asians have a less developed primary compartment, resulting in earlier expansion of the secondary compartment, thereby leading to the increased risk of type 2 diabetes and cardiovascular disease.[44] Studies showing that South Asians have higher levels of DSAT and lower or similar amounts of abdominal SSAT as compared to white Caucasians support this hypothesis.[109;110]

Thus, South Asians have higher total fat mass than white Caucasians. This fat is primarily stored in the visceral and deep subcutaneous compartments and correlates with insulin resistance. This might be due to different metabolic characteristics of adipocytes in this compartment as discussed below.

Adipose tissue dysfunction and inflammation

Not only the amount and distribution of body fat differs between South Asians and white Caucasians. It has been proposed that South Asians have abnormalities in adipocyte function as well (**Table 4**). Adipocytes serve as buffer for the daily influx of fat. When adipocytes are overloaded, for example in case of obesity, they become dysfunctional; the ability to store lipids is decreased.[111] Studies have shown that South Asians have significantly increased subcutaneous adipocyte size.[34;109] Hypertrophic adipocytes are thought of as dysfunctional and appear to be associated with insulin resistance in non-diabetic individuals independent of BMI and to be an independent predictor for the development of type 2 diabetes.[112;113] Furthermore, Balakrishnan *et al*. showed that South Asians not only have a higher fraction of very large adipocytes, but also exhibit a higher ratio of small-to-larger adipocytes, which is considered a defect in adipose tissue maturation, resulting in a decreased storage capacity of triglycerides.[114] Also, in a recent study, normoglycemic young South Asian men were shown to have increased expression of *col6a3* in SAT, which is known to reduce adipocyte maturation.[36]

Table 4. Differences in adipose tissue in South Asians *vs.* white Caucasians

South Asians *vs.* **white Caucasians**	*References*
Increased adipocyte size	34;109;114
Increased FFA release	115
Increased leptin	18;22;115;117-119
Decreased adiponectin	36;119;124;125
Increased IL-6 and TNF-alpha release	22;131
Increased CRP production	132;133

Adipocyte dysfunction was also shown in a study of Abate *et al*., demonstrating that non-diabetic South Asians have higher fasting levels of free fatty acids (FFAs) compared to white Caucasians, even when adjusted for body fat content, and fail to completely suppress plasma FFA concentration during hyperinsulinemia induced by an OGTT.[115]

This suggests that in healthy South Asians insulin is unable to sufficiently inhibit lipolysis, resulting in an excess efflux of FFA, which may play a role in the development of type 2 diabetes.

White adipose tissue not only has a function in the storage and release of FFAs, but is more and more recognized as an endocrine organ secreting several proteins called adipocytokines. Of those, leptin and adiponectin are the most frequently studied in relation to insulin resistance and type 2 diabetes. Leptin has an important role in food intake, energy expenditure and glucose metabolism. Leptin seems to have a glucose- and insulin-lowering and insulin-sensitising effect on the whole body level. Plasma leptin levels are positively correlated with plasma insulin, BMI and body fat content, therefore obesity reflects a state of leptin-resistance.[116] Plasma leptin levels were increased in South Asians as compared to Caucasian subjects in several studies[18;22;115;117-119] independent of overall or abdominal obesity. Leptin levels were found to be correlated with SAT and not with VAT.[16;120] However, in some of these studies a difference in fat mass was present between the two groups, or data on fat mass were not reported. Furthermore, in a recent study, no correlation was shown between leptin and insulin resistance in South Asians.[121]

In contrast to leptin, adiponectin is decreased in obesity, insulin resistance and type 2 diabetes.[122] Adiponectin is thought to exert insulin-sensitizing, antiatherogenic and anti-inflammatory effects.[123] South Asians exhibit lower levels of adiponectin compared to white Caucasians.[36;119;124] This is already apparent in babies of 3-6 months old.[125] Furthermore, lower adiponectin levels have been found in South Asians with impaired glucose tolerance and type 2 diabetes as compared to normal glucose tolerant South Asian individuals.[121;126] In addition, low adiponectin levels were found to be an independent predictor for type 2 diabetes development in South Asians.[127] However, another study showed no relation between adiponectin and insulin sensitivity in the South Asian group.[128]

Dysfunctional adipose tissue also produces pro-inflammatory cytokines, such as TNF-α and IL-6, leading to a chronic inflammatory state. Although not yet fully elucidated, it is hypothesized that activation of proinflammatory pathways in for example muscle, liver, and adipose tissue, leads to insulin resistance by inhibiting the insulin signalling cascade.[129;130] Middle-aged South Asian women were shown to exhibit significantly higher IL-6 levels than Europeans, however no ethnic difference in IL-6 was detected among men.[131] In young South Asian men however, Il-6 levels were found to be elevated as compared to white Caucasians.[22] In this study, TNF-α was elevated as well, yet this difference disappeared when correcting for insulin sensitivity. In addition, in comparison to white Caucasians, studies showed higher C-reactive protein (CRP) levels in South Asians, also suggesting a state of low grade inflammation.[132;133] The primary production site of CRP is the liver, and not adipose tissue. However, visceral fat is drained

by the portal vein to the liver and CRP production is induced by cytokines, such as IL-6.[129] In South Asians, visceral fat was positively associated with CRP levels, independent of total adiposity and was associated with fasting and 2-h insulin levels during an OGTT.[133]

In conclusion, dysfunctional adipose tissue and inflammation are likely to contribute to the South Asian phenotype of increased insulin resistance and type 2 diabetes. It is, however, difficult to determine the primary defect: adipocyte dysfunction leads to abnormalities in the insulin signalling pathway, or vice versa: abnormal insulin signalling results in adipocyte dysfunction. Abate *et al*.[115] proposed that it might be a vicious cycle starting with primary insulin resistance, leading to adipose tissue dysfunction, which is reflected by the increased secretion of FFAs and (adipo)cytokines. The high levels of circulating FFAs in turn can aggravate insulin resistance through the deposition of triglycerides in non-adipose tissues,[134] also called ectopic fat.

Ectopic fat

Insulin resistance and type 2 diabetes are associated with ectopic fat accumulation, i.e. the storage of triglycerides in nonadipose tissues such as the liver, heart, and skeletal muscle. Intracellular lipid deposition in these tissues is a consequence of oversupply of FFAs due to increased caloric intake, obesity, adipocyte dysfunction, increase in fatty acid transporters and/or impairment in mitochondrial lipid oxidation. The subsequent accumulation of intermediates of lipid metabolism, such as long-chain acyl-CoA, diacylglycerol, and ceramids, in these organs appears to disrupt normal metabolic processes, causing organ-specific dysfunction.[134]

Deposition of fat in the liver in the absence of excessive alcohol intake is referred to as nonalcoholic fatty liver disease (NAFLD), and is associated with hepatic insulin resistance.[135;136] This is due to a reduction in insulin-stimulated hepatic glucose uptake and decreased insulin suppressibility of hepatic glucose production, which both contribute to increased plasma glucose levels.[134] In South Asians, limited data have reported higher hepatic triglyceride content in comparison to white Caucasians, as measured by ^{1}H-MRS.[22;109] Petersen *et al*. showed that young healthy South Asian men (n=23) had a higher prevalence of insulin resistance, as assessed with an OGTT in combination with the insulin sensitivity index, which was associated with a ~2-fold increase in hepatic triglyceride content compared with Caucasian men (n=73).[22] Another study reported higher fat infiltration in the liver in adult South Asians (n=56) *vs.* white Caucasians (n=52).[109] These data suggest that South Asians appear to be predisposed to develop hepatic steatosis, associated with hepatic insulin resistance.

In non-athletic white Caucasians, intramyocellular lipid (IMCL) accumulation is associated with insulin resistance and type 2 diabetes, due to its toxic effects on insulin signalling.[137-139] In South Asians, IMCL content seems to be higher compared to white Caucasians.[22;140] However, in contrast to white Caucasians, no correlation between IMCL

and insulin resistance has been found in South Asians so far. [22;109;140-143] This suggests that IMCL is of less significance to skeletal muscle insulin sensitivity in South Asians as compared to Caucasians.

Role of skeletal muscle

Muscle glucose uptake accounts for 75-80% of whole-body insulin-stimulated glucose disposal.[144] Total body muscle mass (relative to body size) has been shown to exert an independent effect on insulin sensitivity and glucose disposal.[145] Several studies reported that skeletal muscle mass, or lean body mass, is lower in South Asians than in white Caucasians.[16;95;97;100;109;146] Furthermore, low muscle mass was associated with reduced insulin sensitivity in young, lean South Asian men.[147] In studies conducted at our research centre, we also found lower lean body mass in healthy young South Asian men compared to BMI-matched Caucasians, as measured by dual-energy x-ray absorptiometry (DEXA) scan (unpublished data).

In Caucasian type 2 diabetes patients the primary defect at the skeletal muscle level seems to reside in non-oxidative glucose disposal (NOGD), i.e. glycogen synthesis, due to impairments in insulin-stimulated GLUT-4 translocation leading to impaired glucose transport.[148-150] These impairments in the insulin signalling pathway seem to be induced by defects in mitochondrial fatty acid oxidation and/or increased delivery of fatty acids, leading to IMCL. IMCL, in turn, can impair insulin signal transduction.[134] Indeed, in type 2 diabetes patients a number of defects in the insulin signalling pathway have been found.[151] Furthermore, reduced mitochondrial density with reduced oxidative phosphorylation have been described in insulin-resistant offspring of patients with type 2 diabetes.[152] Moreover, maximal oxygen uptake, or VO_{2max} (a measure of whole-body oxidative capacity), is found to be a strong independent predictor of peripheral insulin sensitivity in white Caucasians,[153-155] and low cardiorespiratory fitness is associated with low skeletal muscle lipid oxidative capacity.[156] One might speculate, therefore, that the increased risk of insulin resistance and type 2 diabetes in South Asians might be, at least in part, explained by reduced skeletal muscle oxidative capacity.

In South Asians, several studies reported lower VO_{2max} values in South Asians compared to matched white Caucasians.[89;146;157] A recent study of Ghouri *et al.* confirmed this finding in middle-aged South Asian men without type 2 diabetes (n=87) compared to age- and BMI-matched European men (n=99) and, importantly, found that the lower cardiorespiratory fitness explained 68% of the ethnic difference in HOMA-IR.[97] Of note, the lower VO_{2max} could not be explained by their lower levels of physical activity, indicating that low physical fitness is an innate feature of the South Asian phenotype. However, so far only two relatively small in-depth studies have been performed in South Asians, in which skeletal muscle biopsies were obtained to find out more about the molecular mechanisms of the increased risk of insulin resistance and type 2 diabetes in this eth-

nicity. In a study of Nair *et al.* no impairment in mitochondrial function (measured as skeletal muscle mitochondrial capacity for oxidative phosphorylation (OXPHOS) as assessed by mitochondrial DNA copy number (mtDNA), OXPHOS gene transcripts, citrate synthase activity, and maximal mitochondrial ATP production rate (MAPR)) was found in 13 healthy, middle-aged South Asians living in the USA, even despite the finding that they were more insulin resistant than 13 age-, sex- and BMI-matched Northern European Americans. On the contrary: South Asians had even higher mitochondrial capacity for oxidative phosphorylation.[21] Hall *et al.* also reported that healthy, young, lean male South Asians (n=20) compared to age- and BMI-matched white Caucasians (n=20) did not exhibit lower expression of skeletal muscle oxidative and lipid metabolism genes, and mitochondrial DNA to nuclear DNA ratio (index of mitochondrial biogenesis) did not differ between groups. Gene expression of carnitine palmitoyltransferase 1A (CPT1A) and fatty acid synthase (FASN), both involved in lipid metabolism, was even higher in South Asians.[146] Consequently, both studies concluded that mitochondrial dysfunction did not account for the observed insulin resistance in South Asians. Importantly, Hall also showed that South Asians oxidized less fat during submaximal exercise, whereas the resting rate of fat oxidation did not differ between groups. This difference, however, was not reflected in reduced skeletal muscle expression of oxidative and lipid metabolism genes.[146] It should be noted, however, that these results are derived from only two relatively small studies in different age groups, and thus extrapolation of these results to the whole South Asian population should be done with caution.

The above-mentioned study of Hall *et al.* is the only study that compared skeletal muscle insulin signalling between both ethnicities.[146] Interestingly, this study showed that South Asians had reduced skeletal muscle protein expression of key insulin signalling proteins (phosphatidylinositol 3'-kinase p85 subunit (PI3K (p85)) and protein kinase B serine 473 phosphorylation (pPKB-Ser473)). Basal Ser473 phosphorylation of PKB was even 60% lower in South Asians, and was significantly correlated with whole-body insulin sensitivity. However, the expression of the insulin signalling proteins in hyperinsulinemic condition was assessed in response to maximal insulin stimulation via incubation for 10 minutes in the presence of 10 nM human soluble insulin, instead of using a hyperinsulinemic clamp. Hence, the meaning of this finding needs to be corroborated.

To summarize, South Asians have less skeletal muscle mass and seem to have lower cardiorespiratory fitness and reduced capacity for fat oxidation during submaximal exercise, all correlating with their reduced whole-body insulin sensitivity, which is not reflected in reduced expression of oxidative and lipid metabolism genes in skeletal muscle.[146] However, so far only two relatively small in-depth studies have been performed in South Asians, therefore these results should be interpreted with caution and more research is warranted.

Nitric oxide bioavailability: endothelial and HDL-cholesterol dysfunction

Apart from the aforementioned metabolic functions, insulin also stimulates the release of nitric oxide (NO) from endothelium, which leads to peripheral vasodilatation, increased capillary recruitment and increased blood flow. Subsequently, these hemodynamic actions increase the delivery of insulin to (underperfused) tissues and enhance the delivery of glucose and other substrates to skeletal muscle. It is thought that 25-40% of insulin-mediated glucose disposal is due to its hemodynamic effects.[158;159]

Several studies have demonstrated that South Asians have lower NO bioavailability compared to white Caucasians.[160;161] NO is mainly produced by the endothelium as a consequence of an interaction with high density lipoprotein (HDL)-cholesterol.[162;163] Thus, a diminished NO bioavailability might be caused by dysfunction of the endothelium and/or dysfunctional HDL. To what extent lower NO availability is present in South Asians as well as its cause, endothelial or HDL dysfunction or a combination of both, are yet unknown.

Endothelial dysfunction is defined as inadequate endothelial-mediated vasodilatation and is present in patients with obesity, dyslipidemia, diabetes and very early in individuals with (a high risk of) atherosclerosis. Insulin resistance and endothelial dysfunction are closely related. It has been shown that gluco- and lipotoxicity decrease NO availability.[158;159] In South Asians impairments in endothelial function have been demonstrated. Chambers *et al*. showed that endothelium-dependent dilatation (measured as brachial artery flow mediated dilatation) was reduced in South Asians living in the UK as compared to white Caucasians and this was confirmed by others.[161;164] In yet another study, although no difference in vasodilatation was observed after reactive hyperemia or sublingual nitroglycerin administration between the two ethnic groups, the increase in vasodilatation during hyperinsulinemia as compared to basal conditions was significantly lower in South Asians.[24] Signs of endothelial dysfunctions are already present early in life in South Asians. Din *et al*. showed that healthy, young South Asian men have increased arterial stiffness (reflected by an increased augmentation of radial artery pressure waveforms) compared to matched white Caucasians.[165] Interestingly, in cord blood of South Asian neonates an elevated level of E-selectin, a marker of endothelial dysfunction which has been shown to predict the occurrence of type 2 diabetes in adult women, was found, suggesting that endothelial dysfunction might already be present at birth.[26] Furthermore, it was shown that South Asians have lower circulating numbers of endothelial progenitor cells (EPCs) and EPC colony forming units, which may result in a reduced capacity for endothelial repair.[160;161] However, others did not find a difference in the EPC count between South Asian and Caucasian men with established atherosclerosis.[166]

Besides endothelial dysfunction, HDL dysfunction might also play a role in the decreased NO bioavailability observed in South Asians. Multiple studies have consis-

tently shown lower HDL-cholesterol levels in South Asians compared to white Caucasians.[19;20;23;33;99;132;167] Not only do they have lower levels of HDL-cholesterol, they also seem to have more small-dense dysfunctional HDL particles, which are thought to be proinflammatory and less protective compared to normal HDL particles..[168]

A diminished NO bioavailability in South Asians might thus be caused by both endothelial and HDL dysfunction, and might be a factor in the increased incidence of type 2 diabetes and cardiovascular disease in this ethnic group.

CONCLUSION & FUTURE DIRECTIONS

The risk of developing type 2 diabetes is exceptionally high among both native and migrant South Asians, comprising one fifth of the worlds' population. The disease develops about a decade earlier than in white Caucasians and South Asians also have an increased incidence of retinopathy, nephropathy and coronary artery and cerebrovascular disease. Even non-diabetic individuals have higher insulin levels compared to other ethnic groups regardless of age, gender or BMI. This points to an impairment in insulin sensitivity. Indeed, several studies have shown that the predominant mechanism leading to the increased risk of type 2 diabetes in South Asians seems to be insulin resistance rather than decreased β-cell function.

We have tried to review several pathogenetic factors that might underlie the increased and accelerated risk to develop insulin resistance and type 2 diabetes in South Asians, which is illustrated in **Figure 2**.

Given the strong familial clustering of type 2 diabetes in South Asians, one would assume distinctive genetic differences between white Caucasians and South Asians. However, the presence of polymorphisms associated with type 2 diabetes found thus far do not clearly differ between the two ethnicities. It might be that either the wrong loci have been investigated (i.e. South Asians have different polymorphisms), the sample sizes were too small, or that the increased risk is caused by epigenetic differences. We believe that genetics or epigenetics must play a role, despite the fact that this has not been confirmed yet.

South Asians unmistakably have a different body composition than white Caucasians with relatively thin extremities and increased abdominal adiposity, both in the visceral as well as in the deep subcutaneous compartments. Increased visceral and deep subcutaneous fat mass are associated with insulin resistance. Up till now, however, studies in South Asians show contradictory results with either an association of VAT with insulin resistance or of SAT with insulin resistance. Furthermore, South Asians appear to have dysfunctional adipocytes, leading to a decreased storage capacity for triglycerides

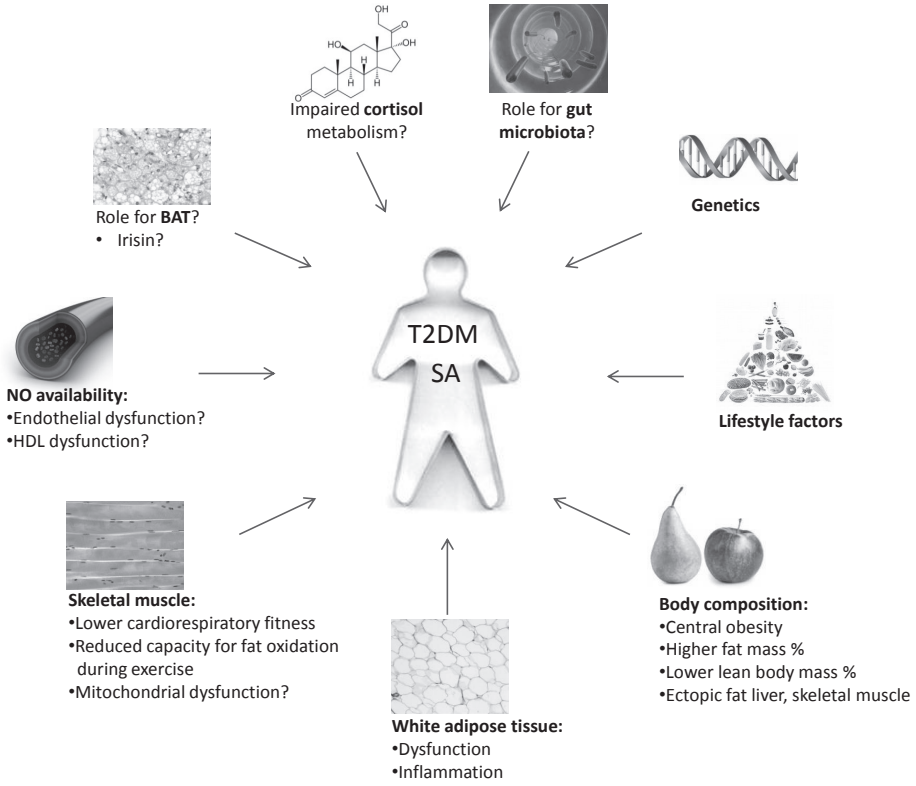


Figure 2. Potential pathophysiological mechanisms that may underlie or contribute to the increased risk of type 2 diabetes in South Asians as compared to white Caucasians.

and impaired release of FFA's, adipokines and pro-inflammatory cytokines, which are thought to disrupt the insulin signalling pathway.

Remarkably, as of yet no convincing differences in intracellular signalling cascades and enzymatic process involved in insulin signalling have been found between South Asians and Caucasians. However, so far only two relatively small studies obtained muscle biopsies and investigated mitochondrial function, and only one investigated the insulin signalling pathway. Some studies show differences in endothelial function, suggesting that perhaps impaired insulin-mediated capillary recruitment plays a role in the development of insulin resistance in South Asians. This would lead to diminished delivery of insulin to its site of action. Hence, perhaps the fact that no difference in insulin signalling was observed is a quantitative problem.

Differences in dietary habits do not seem to play an important role in the increased diabetes risk. The number of studies examining the effect of exercise are small but consistently show – self-reported – lower daily activity levels and lower cardiorespiratory

fitness (maximal oxygen uptake $VO2_{max}$) in South Asians, which appears to contribute to the increased level of insulin resistance. Further research should not only focus on duration and intensity of physical activity and exercise (endurance *vs.* strength) but also on the underlying cellular mechanisms.

We think there are several other areas of interest that should be explored in South Asians to further investigate the increased risk for insulin resistance and type 2 diabetes. Firstly, brown adipose tissue. Brown adipose tissue burns triglycerides and glucose to generate heat through a process called mitochondrial uncoupling[169]. Since brown adipose tissue is involved in around 20% of total energy expenditure[170] and clearance of serum triglycerides and glucose, it could play a role in the disturbed metabolic phenotype of South Asians. Secondly, and in light with the interest in brown adipose tissue, is Irisin. Irisin is a recently discovered myokine that increases with exercise and is, at least in rodents, involved in browning of white adipose tissue.[171] Given the fact that South Asians have lower muscle mass and lower physical activity levels the role of Irisin in insulin resistance and amount of brown adipose tissue should be further explored. Thirdly, the gut microbiota of South Asians might be quite different from white Caucasians. The gut microbiota of obese subjects appears to be different from that of lean subjects and is thought to be associated with insulin resistance.[172] Fourthly, the thin-fat phenotype might suggest differences in the hypothalamic-pituitary-adrenal axis with (tissue-specific) impaired cortisol metabolism.

As for now, we conclude that the strong genetic predisposition for type 2 diabetes in South Asians should be explained by as of yet undiscovered polymorphisms that negatively interact with environmental factors such as Western-type diet and low physical activity level. In addition, genetic makeup accounts for the disadvantageous body composition with low muscle mass and increased visceral fat mass. The ensuing effects on release of pro-inflammatory adipocytokines, myokines and FFAs disrupt cellular processes and induce insulin resistance.

REFERENCES

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diab Res Clin Pract* 2010;87(1):4-14.
2. Anjana RM, Pradeepa R, Deepa M, Datta M, Sudha V, Unnikrishnan R *et al*. Prevalence of diabetes and prediabetes (impaired fasting glucose and/or impaired glucose tolerance) in urban and rural India: phase I results of the Indian Council of Medical Research-INdia DIABetes (ICMR-INDIAB) study. *Diabetologia* 2011;54(12):3022-7.
3. Katikireddi SV, Morling JR, Bhopal R. Is there a divergence in time trends in the prevalence of impaired glucose tolerance and diabetes? A systematic review in South Asian populations. *Int J Epidemiol* 2011;40(6):1542-53.
4. Gupta R, Misra A. Review: Type 2 diabetes in India: regional disparities. *Br J Diab Vasc Dis* 2007;7(1):12-6.
5. Anand SS, Yusuf S, Vuksan V, Devanesen S, Teo KK, Montague PA *et al*. Differences in risk factors, atherosclerosis, and cardiovascular disease between ethnic groups in Canada: the Study of Health Assessment and Risk in Ethnic groups (SHARE). *Lancet* 2000;356(9226):279-84.
6. Mohanty SA, Woolhandler S, Himmelstein DU, Bor DH. Diabetes and cardiovascular disease among Asian Indians in the United States. *J Gen Intern Med* 2005;20(5):474-8.
7. DECODE Study Group. Age- and sex-specific prevalences of diabetes and impaired glucose regulation in 13 European cohorts. *Diabetes Care* 2003;26(1):61-9.
8. Barnett AH, Dixon AN, Bellary S, Hanif MW, O'Hare JP, Raymond NT *et al*. Type 2 diabetes and cardiovascular risk in the UK south Asian community. *Diabetologia* 2006;49(10):2234-46.
9. Middelkoop BJ, Kesarlal-Sadhoeram SM, Ramsaransing GN, Struben HW. Diabetes mellitus among South Asian inhabitants of The Hague: high prevalence and an age-specific socioeconomic gradient. *Int J Epidemiol* 1999;28(6):1119-23.
10. Bindraban NR, van Valkengoed IG, Mairuhu G, Holleman F, Hoekstra JB, Michels BP *et al*. Prevalence of diabetes mellitus and the performance of a risk score among Hindustani Surinamese, African Surinamese and ethnic Dutch: a cross-sectional population-based study. *BMC Public Health* 2008;8:271.
11. Chandie Shaw PK, Baboe F, van Es LA, van der Vijver JC, van de Ree MA, de Jonge N *et al*. South-Asian type 2 diabetic patients have higher incidence and faster progression of renal disease compared with Dutch-European diabetic patients. *Diabetes Care* 2006;29(6):1383-5.
12. Chandie Shaw PK, Vandenbroucke JP, Tjandra YI, Rosendaal FR, Rosman JB, Geerlings W *et al*. Increased end-stage diabetic nephropathy in Indo-Asian immigrants living in the Netherlands. *Diabetologia* 2002;45(3):337-41.
13. Mather HM, Chaturvedi N, Kehely AM. Comparison of prevalence and risk factors for microalbuminuria in South Asians and Europeans with Type 2 diabetes mellitus. *Diabet Med* 1998;15(8):672-7.
14. DeFronzo RA. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes* 1988;37(6):667-87.
15. UK Prospective Diabetes Study. XII: Differences between Asian, Afro-Caribbean and white Caucasian type 2 diabetic patients at diagnosis of diabetes. UK Prospective Diabetes Study Group. *Diabet Med* 1994;11(7):670-7.
16. Banerji MA, Faridi N, Atluri R, Chaiken RL, Lebovitz HE. Body composition, visceral fat, leptin, and insulin resistance in Asian Indian men. *J Clin Endocrinol Metab* 1999;84(1):137-44.
17. Dickinson S, Colagiuri S, Faramus E, Petocz P, Brand-Miller JC. Postprandial hyperglycemia and insulin sensitivity differ among lean young adults of different ethnicities. *J Nutr* 2002;132(9):2574-9.

18. Liew CF, Seah ES, Yeo KP, Lee KO, Wise SD. Lean, nondiabetic Asian Indians have decreased insulin sensitivity and insulin clearance, and raised leptin compared to Caucasians and Chinese subjects. *Int J Obes Relat Metab Disord* 2003;27(7):784-9.
19. McKeigue PM, Marmot MG, Syndercombe Court YD, Cottier DE, Rahman S, Riemersma RA. Diabetes, hyperinsulinaemia, and coronary risk factors in Bangladeshis in east London. *Br Heart J* 1988;60(5):390-6.
20. McKeigue PM, Shah B, Marmot MG. Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. *Lancet* 1991;337(8738):382-6.
21. Nair KS, Bigelow ML, Asmann YW, Chow LS, Coenen-Schimke JM, Klaus KA *et al*. Asian Indians have enhanced skeletal muscle mitochondrial capacity to produce ATP in association with severe insulin resistance. *Diabetes* 2008;57(5):1166-75.
22. Petersen KF, Dufour S, Feng J, Befroy D, Dziura J, Dalla MC *et al*. Increased prevalence of insulin resistance and nonalcoholic fatty liver disease in Asian-Indian men. *Proc Natl Acad Sci U S A* 2006;103(48):18273-7.
23. Raji A, Seely EW, Arky RA, Simonson DC. Body fat distribution and insulin resistance in healthy Asian Indians and Caucasians. *J Clin Endocrinol Metab* 2001;86(11):5366-71.
24. Raji A, Gerhard-Herman MD, Warren M, Silverman SG, Raptopoulos V, Mantzoros CS *et al*. Insulin resistance and vascular dysfunction in nondiabetic Asian Indians. *J Clin Endocrinol Metab* 2004;89(8):3965-72.
25. Raji A, Gerhard-Herman MD, Williams JS, O'Connor ME, Simonson DC. Effect of pioglitazone on insulin sensitivity, vascular function and cardiovascular inflammatory markers in insulin-resistant non-diabetic Asian Indians. *Diabet Med* 2006;23(5):537-43.
26. Boon MR, Karamali NS, de Groot CJ, van Steijn L, Kanhai HH, van der Bent C *et al*. E-Selectin is Elevated in Cord Blood of South Asian Neonates Compared with Caucasian Neonates. *J Pediatr* 2011;160(5):844-8.
27. Yajnik CS, Lubree HG, Rege SS, Naik SS, Deshpande JA, Deshpande SS *et al*. Adiposity and hyperinsulinemia in Indians are present at birth. *J Clin Endocrinol Metab* 2002;87(12):5575-80.
28. Whincup PH, Gilg JA, Papacosta O, Seymour C, Miller GJ, Alberti KG *et al*. Early evidence of ethnic differences in cardiovascular risk: cross sectional comparison of British South Asian and white children. *BMJ* 2002;324(7338):635.
29. Lakshmi S, Metcalf B, Joglekar C, Yajnik CS, Fall CH, Wilkin TJ. Differences in body composition and metabolic status between white U.K. and Asian Indian children (EarlyBird 24 and the Pune Maternal Nutrition Study). *Pediatr Obes* 2012;7(5):347-54.
30. Whincup PH, Gilg JA, Owen CG, Odoki K, Alberti KG, Cook DG. British South Asians aged 13-16 years have higher fasting glucose and insulin levels than Europeans. *Diabet Med* 2005;22(9):1275-7.
31. Cruz ML, Evans K, Frayn KN. Postprandial lipid metabolism and insulin sensitivity in young Northern Europeans, South Asians and Latin Americans in the UK. *Atherosclerosis* 2001;159(2):441-9.
32. Laws A, Jeppesen JL, Maheux PC, Schaaf P, Chen YD, Reaven GM. Resistance to insulin-stimulated glucose uptake and dyslipidemia in Asian Indians. *Arterioscler Thromb* 1994;14(6):917-22.
33. Chandalia M, Abate N, Garg A, Stray-Gundersen J, Grundy SM. Relationship between generalized and upper body obesity to insulin resistance in Asian Indian men. *J Clin Endocrinol Metab* 1999;84(7):2329-35.
34. Chandalia M, Lin P, Seenivasan T, Livingston EH, Snell PG, Grundy SM *et al*. Insulin resistance and body fat distribution in South Asian men compared to Caucasian men. *PLoS One* 2007;2(8):e812.
35. Sharp PS, Mohan V, Levy JC, Mather HM, Kohner EM. Insulin resistance in patients of Asian Indian and European origin with non-insulin dependent diabetes. *Horm Metab Res* 1987;19(2):84-5.

36. Munoz A, Abate N, Chandalia M. Adipose Tissue Collagen and Inflammation in Nonobese Asian Indian Men. *J Clin Endocrinol Metab* 2013.
37. Lillioja S, Mott DM, Howard BV, Bennett PH, Yki-Jarvinen H, Freymond D *et al*. Impaired glucose tolerance as a disorder of insulin action. Longitudinal and cross-sectional studies in Pima Indians. *N Engl J Med* 1988;318(19):1217-25.
38. Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E *et al*. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. *N Engl J Med* 1993;329(27):1988-92.
39. Gelding SV, Andres C, Niththyananthan R, Gray IP, Mather H, Johnston DG. Increased secretion of 32,33 split proinsulin after intravenous glucose in glucose-tolerant first-degree relatives of patients with non-insulin dependent diabetes of European, but not Asian, origin. *Clin Endocrinol (Oxf)* 1995;42(3):255-64.
40. Snehalatha C, Satyavani K, Sivasankari S, Vijay V, Ramachandran A. Insulin secretion and action in different stages of glucose tolerance in Asian Indians. *Diabet Med* 1999;16(5):408-14.
41. Matsumoto K, Miyake S, Yano M, Ueki Y, Yamaguchi Y, Akazawa S *et al*. Glucose tolerance, insulin secretion, and insulin sensitivity in nonobese and obese Japanese subjects. *Diabetes Care* 1997;20(10):1562-8.
42. Yoneda H, Ikegami H, Yamamoto Y, Yamato E, Cha T, Kawaguchi Y *et al*. Analysis of early-phase insulin responses in nonobese subjects with mild glucose intolerance. *Diabetes Care* 1992;15(11):1517-21.
43. Neel JV. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? *Am J Hum Genet* 1962;14:353-62.
44. Sniderman AD, Bhopal R, Prabhakaran D, Sarrafzadegan N, Tchernof A. Why might South Asians be so susceptible to central obesity and its atherogenic consequences? The adipose tissue overflow hypothesis. *Int J Epidemiol* 2007;36(1):220-5.
45. Wells JC. Commentary: Why are South Asians susceptible to central obesity?—the El Nino hypothesis. *Int J Epidemiol* 2007;36(1):226-7.
46. Wells JC. Ethnic variability in adiposity and cardiovascular risk: the variable disease selection hypothesis. *Int J Epidemiol* 2009;38(1):63-71.
47. Bhopal RS, Rafnsson SB. Could mitochondrial efficiency explain the susceptibility to adiposity, metabolic syndrome, diabetes and cardiovascular diseases in South Asian populations? *Int J Epidemiol* 2009;38(4):1072-81.
48. Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia* 1992;35(7):595-601.
49. Hales CN, Barker DJ. The thrifty phenotype hypothesis. *Br Med Bull* 2001;60:5-20.
50. Whincup PH, Kaye SJ, Owen CG, Huxley R, Cook DG, Anazawa S *et al*. Birth weight and risk of type 2 diabetes: a systematic review. *JAMA* 2008;300(24):2886-97.
51. Harding S, Rosato MG, Cruickshank JK. Lack of change in birthweights of infants by generational status among Indian, Pakistani, Bangladeshi, Black Caribbean, and Black African mothers in a British cohort study. *Int J Epidemiol* 2004;33(6):1279-85.
52. Norris SA, Osmond C, Gigante D, Kuzawa CW, Ramakrishnan L, Lee NR *et al*. Size at birth, weight gain in infancy and childhood, and adult diabetes risk in five low- or middle-income country birth cohorts. *Diabetes Care* 2012;35(1):72-9.
53. Rueda-Clausen CF, Dolinsky VW, Morton JS, Proctor SD, Dyck JR, Davidge ST. Hypoxia-induced intrauterine growth restriction increases the susceptibility of rats to high-fat diet-induced metabolic syndrome. *Diabetes* 2011;60(2):507-16.

54. Radha V, Mohan V. Genetic predisposition to type 2 diabetes among Asian Indians. *Indian J Med Res* 2007;125(3):259-74.
55. McCarthy MI. Genomics, Type 2 Diabetes, and Obesity. *N Engl J Med* 2010;363(24):2339-50.
56. Kooner JS, Saleheen D, Sim X, Sehmi J, Zhang W, Frossard P *et al*. Genome-wide association study in individuals of South Asian ancestry identifies six new type 2 diabetes susceptibility loci. *Nat Genet* 2011;43(10):984-9.
57. Rees SD, Hydrie MZ, Shera AS, Kumar S, O'Hare JP, Barnett AH *et al*. Replication of 13 genome-wide association (GWA)-validated risk variants for type 2 diabetes in Pakistani populations. *Diabetologia* 2011;54(6):1368-74.
58. Chauhan G, Spurgeon CJ, Tabassum R, Bhaskar S, Kulkarni SR, Mahajan A *et al*. Impact of common variants of PPARG, KCNJ11, TCF7L2, SLC30A8, HHEX, CDKN2A, IGF2BP2, and CDKAL1 on the risk of type 2 diabetes in 5,164 Indians. *Diabetes* 2010;59(8):2068-74.
59. Radha V, Vimaleswaran KS, Babu HN, Abate N, Chandalia M, Satija P *et al*. Role of genetic polymorphism peroxisome proliferator-activated receptor-gamma2 Pro12Ala on ethnic susceptibility to diabetes in South-Asian and Caucasian subjects: Evidence for heterogeneity. *Diabetes Care* 2006;29(5):1046-51.
60. Sanghera DK, Demirci FY, Been L, Ortega L, Ralhan S, Wander GS *et al*. PPARG and ADIPOQ gene polymorphisms increase type 2 diabetes mellitus risk in Asian Indian Sikhs: Pro12Ala still remains as the strongest predictor. *Metabolism* 2010;59(4):492-501.
61. Sanghera DK, Ortega L, Han S, Singh J, Ralhan SK, Wander GS *et al*. Impact of nine common type 2 diabetes risk polymorphisms in Asian Indian Sikhs: PPARG2 (Pro12Ala), IGF2BP2, TCF7L2 and FTO variants confer a significant risk. *BMC Med Genet* 2008;9:59.
62. Li H, Kilpelainen TO, Liu C, Zhu J, Liu Y, Hu C *et al*. Association of genetic variation in FTO with risk of obesity and type 2 diabetes with data from 96,551 East and South Asians. *Diabetologia* 2011.
63. Yajnik CS, Janipalli CS, Bhaskar S, Kulkarni SR, Freathy RM, Prakash S *et al*. FTO gene variants are strongly associated with type 2 diabetes in South Asian Indians. *Diabetologia* 2009;52(2):247-52.
64. Ramya K, Radha V, Ghosh S, Majumder PP, Mohan V. Genetic variations in the FTO gene are associated with type 2 diabetes and obesity in south Indians (CURES-79). *Diabetes Technol Ther* 2011;13(1):33-42.
65. Chauhan G, Tabassum R, Mahajan A, Dwivedi OP, Mahendran Y, Kaur I *et al*. Common variants of FTO and the risk of obesity and type 2 diabetes in Indians. *J Hum Genet* 2011;56(10):720-6.
66. Bhatt SP, Nigam P, Misra A, Guleria R, Luthra K, Jain SK *et al*. Association of the Myostatin gene with obesity, abdominal obesity and low lean body mass and in non-diabetic Asian Indians in north India. *PLoS One* 2012;7(8):e40977.
67. Saxena R, Saleheen D, Been LF, Garavito ML, Braun T, Bjonnes A *et al*. Genome-wide association study identifies a novel locus contributing to type 2 diabetes susceptibility in Sikhs of Punjabi origin from India. *Diabetes* 2013;62(5):1746-55.
68. Garduno-Diaz SD, Khokhar S. Prevalence, risk factors and complications associated with type 2 diabetes in migrant South Asians. *Diabetes Metab Res Rev* 2012;28(1):6-24.
69. Lovegrove JA, Lovegrove SS, Lesauvage SV, Brady LM, Saini N, Minihane AM *et al*. Moderate fish-oil supplementation reverses low-platelet, long-chain n-3 polyunsaturated fatty acid status and reduces plasma triacylglycerol concentrations in British Indo-Asians. *Am J Clin Nutr* 2004;79(6):974-82.
70. McKeigue PM, Marmot MG, Adelstein AM, Hunt SP, Shipley MJ, Butler SM *et al*. Diet and risk factors for coronary heart disease in Asians in northwest London. *Lancet* 1985;2(8464):1086-90.

71. Miller GJ, Kotecha S, Wilkinson WH, Wilkes H, Stirling Y, Sanders TA *et al*. Dietary and other characteristics relevant for coronary heart disease in men of Indian, West Indian and European descent in London. *Atherosclerosis* 1988;70(1-2):63-72.
72. Misra A, Khurana L, Isharwal S, Bhardwaj S. South Asian diets and insulin resistance. *Br J Nutr* 2009;101(4):465-73.
73. Sevak L, McKeigue PM, Marmot MG. Relationship of hyperinsulinemia to dietary intake in south Asian and European men. *Am J Clin Nutr* 1994;59(5):1069-74.
74. Donin AS, Nightingale CM, Owen CG, Rudnicka AR, McNamara MC, Prynne CJ *et al*. Nutritional composition of the diets of South Asian, black African-Caribbean and white European children in the United Kingdom: the Child Heart and Health Study in England (CHASE). *Br J Nutr* 2010;104(2):276-85.
75. Isharwal S, Arya S, Misra A, Wasir JS, Pandey RM, Rastogi K *et al*. Dietary nutrients and insulin resistance in urban Asian Indian adolescents and young adults. *Ann Nutr Metab* 2008;52(2):145-51.
76. Brady LM, Lovegrove SS, Lesauvage SV, Gower BA, Minihane AM, Williams CM *et al*. Increased n-6 polyunsaturated fatty acids do not attenuate the effects of long-chain n-3 polyunsaturated fatty acids on insulin sensitivity or triacylglycerol reduction in Indian Asians. *Am J Clin Nutr* 2004;79(6):983-91.
77. Becker E, Boreham R, Chaudhury M, Craig R, Deverill C, Doyle M *et al*. Health Survey for England 2004 Volume 1 The health of minority ethnic groups. The Information Centre; 2006.
78. Bowen L, Ebrahim S, De SB, Ness A, Kinra S, Bharathi AV *et al*. Dietary intake and rural-urban migration in India: a cross-sectional study. *PLoS One* 2011;6(6):e14822.
79. Leung G, Stanner S. Diets of minority ethnic groups in the UK: influence on chronic disease risk and implications for prevention. *Nutrition Bulletin* 2011;36:161-98.
80. Simmons D, Williams DR, Powell MJ. Prevalence of diabetes in different regional and religious south Asian communities in Coventry. *Diabet Med* 1992;9(5):428-31.
81. Simmons D, Williams R. Dietary practices among Europeans and different South Asian groups in Coventry. *Br J Nutr* 1997;78(1):5-14.
82. Admiraal WM, van Valkengoed IG, Munter JS Ld, Stronks K, Hoekstra JB, Holleman F. The association of physical inactivity with Type 2 diabetes among different ethnic groups. *Diabet Med* 2011;28(6):668-72.
83. Dhawan J, Bray CL. Asian Indians, coronary artery disease, and physical exercise. *Heart* 1997;78(6):550-4.
84. Fischbacher CM, Bhopal R, Unwin N, Walker M, White M, Alberti KG. Maternal transmission of type 2 diabetes varies by ethnic group: cross-sectional survey of Europeans and South Asians. *Diabetes Care* 2001;24(9):1685-6.
85. Fischbacher CM, Hunt S, Alexander L. How physically active are South Asians in the United Kingdom? A literature review. *Journal of Public Health* 2004;26(3):250-8.
86. Hayes L, White M, Unwin N, Bhopal R, Fischbacher C, Harland J *et al*. Patterns of physical activity and relationship with risk markers for cardiovascular disease and diabetes in Indian, Pakistani, Bangladeshi and European adults in a UK population. *J Public Health Med* 2002;24(3):170-8.
87. Lean ME, Han TS, Bush H, Anderson AS, Bradby H, Williams R. Ethnic differences in anthropometric and lifestyle measures related to coronary heart disease risk between South Asian, Italian and general-population British women living in the west of Scotland. *Int J Obes Relat Metab Disord* 2001;25(12):1800-5.
88. Bettiol H, Rona RJ, Chinn S. Variation in physical fitness between ethnic groups in nine year olds. *Int J Epidemiol* 1999;28(2):281-6.

89. Hardy CP, Eston RG. Aerobic fitness of Anglo-Saxon and Indian students. *Br J Sports Med* 1985;19(4):217-8.
90. Owen CG, Nightingale CM, Rudnicka AR, Cook DG, Ekelund U, Whincup PH. Ethnic and gender differences in physical activity levels among 9-10-year-old children of white European, South Asian and African-Caribbean origin: the Child Heart Health Study in England (CHASE Study). *Int J Epidemiol* 2009;38(4):1082-93.
91. Williams R, Shams M. Generational continuity and change in British Asian health and health behaviour. *J Epidemiol Community Health* 1998;52(9):558-63.
92. Chiu M, Austin PC, Manuel DG, Shah BR, Tu JV. Deriving ethnic-specific BMI cutoff points for assessing diabetes risk. *Diabetes Care* 2011;34(8):1741-8.
93. Gray LJ, Yates T, Davies MJ, Brady E, Webb DR, Sattar N *et al*. Defining Obesity Cut-Off Points for Migrant South Asians. *PLoS One* 2011;6(10):e26464.
94. Nightingale CM, Rudnicka AR, Owen CG, Wells JC, Sattar N, Cook DG *et al*. Influence of adiposity on insulin resistance and glycemia markers among U.K. Children of South Asian, black African-Caribbean, and white European origin: child heart and health study in England. *Diabetes Care* 2013;36(6):1712-9.
95. Rush EC, Freitas I, Plank LD. Body size, body composition and fat distribution: comparative analysis of European, Maori, Pacific Island and Asian Indian adults. *Br J Nutr* 2009;102(4):632-41.
96. Lear SA, Humphries KH, Kohli S, Chockalingam A, Frohlich JJ, Birmingham CL. Visceral adipose tissue accumulation differs according to ethnic background: results of the Multicultural Community Health Assessment Trial (M-CHAT). *Am J Clin Nutr* 2007;86(2):353-9.
97. Ghouri N, Purves D, McConnachie A, Wilson J, Gill JM, Sattar N. Lower cardiorespiratory fitness contributes to increased insulin resistance and fasting glycaemia in middle-aged South Asian compared with European men living in the UK. *Diabetologia* 2013.
98. Nightingale CM, Rudnicka AR, Owen CG, Cook DG, Whincup PH. Patterns of body size and adiposity among UK children of South Asian, black African-Caribbean and white European origin: Child Heart And health Study in England (CHASE Study). *Int J Epidemiol* 2011;40(1):33-44.
99. Ehtisham S, Crabtree N, Clark P, Shaw N, Barrett T. Ethnic differences in insulin resistance and body composition in United Kingdom adolescents. *J Clin Endocrinol Metab* 2005;90(7):3963-9.
100. Stanfield KM, Wells JC, Fewtrell MS, Frost C, Leon DA. Differences in body composition between infants of South Asian and European ancestry: the London Mother and Baby Study. *Int J Epidemiol* 2012;41(5):1409-18.
101. Yajnik CS, Fall CH, Coyaji KJ, Hirve SS, Rao S, Barker DJ *et al*. Neonatal anthropometry: the thin-fat Indian baby. The Pune Maternal Nutrition Study. *Int J Obes Relat Metab Disord* 2003;27(2):173-80.
102. Krishnaveni GV, Hill JC, Veena SR, Leary SD, Saperia J, Chachyamma KJ *et al*. Truncal adiposity is present at birth and in early childhood in South Indian children. *Indian Pediatr* 2005;42(6):527-38.
103. van Steijn L, Karamali NS, Kanhai HHH, Ariens GAM, Fall CHD, Yajnik CS *et al*. Neonatal anthropometry: thin-fat phenotype in fourth to fifth generation South Asian neonates in Surinam. *International Journal of Obesity* 2009;33(11):1326-9.
104. Modi N, Thomas EL, Uthaya SN, Umranikar S, Bell JD, Yajnik C. Whole body magnetic resonance imaging of healthy newborn infants demonstrates increased central adiposity in Asian Indians. *Pediatr Res* 2009;65(5):584-7.
105. Anjana M, Sandeep S, Deepa R, Vimaleswaran KS, Farooq S, Mohan V. Visceral and central abdominal fat and anthropometry in relation to diabetes in Asian Indians. *Diabetes Care* 2004;27(12):2948-53.

106. Indulekha K, Anjana RM, Surendar J, Mohan V. Association of visceral and subcutaneous fat with glucose intolerance, insulin resistance, adipocytokines and inflammatory markers in Asian Indians (CURES-113). *Clin Biochem* 2011;44(4):281-7.
107. Sandeep S, Gokulakrishnan K, Velmurugan K, Deepa M, Mohan V. Visceral & subcutaneous abdominal fat in relation to insulin resistance & metabolic syndrome in non-diabetic south Indians. *Indian J Med Res* 2010;131:629-35.
108. Goel K, Misra A, Vikram NK, Poddar P, Gupta N. Subcutaneous abdominal adipose tissue is associated with the metabolic syndrome in Asian Indians independent of intra-abdominal and total body fat. *Heart* 2010;96(8):579-83.
109. Anand SS, Tarnopolsky MA, Rashid S, Schulze KM, Desai D, Mente A *et al*. Adipocyte hypertrophy, fatty liver and metabolic risk factors in South Asians: the Molecular Study of Health and Risk in Ethnic Groups (mol-SHARE). *PLoS One* 2011;6(7):e22112.
110. Kohli S, Sniderman AD, Tchernof A, Lear SA. Ethnic-specific differences in abdominal subcutaneous adipose tissue compartments. *Obesity (Silver Spring)* 2010;18(11):2177-83.
111. Goossens GH. The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance. *Physiology & Behavior* 2008;94(2):206-18.
112. Lundgren M, Svensson M, Lindmark S, Renström F, Ruge T, Eriksson JW. Fat cell enlargement is an independent marker of insulin resistance and 'hyperleptinaemia'. *Diabetologia* 2007;50(3):625-33.
113. Weyer C, Foley JE, Bogardus C, Tataranni PA, Pratley RE. Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts Type II diabetes independent of insulin resistance. *Diabetologia* 2000;43(12):1498-506.
114. Balakrishnan P, Grundy SM, Islam A, Dunn F, Vega GL. Influence of upper and lower body adipose tissue on insulin sensitivity in South Asian men. *J Investig Med* 2012;60(7):999-1004.
115. Abate N, Chandalia M, Snell PG, Grundy SM. Adipose tissue metabolites and insulin resistance in nondiabetic Asian Indian men. *J Clin Endocrinol Metab* 2004;89(6):2750-5.
116. Jazet IM, Pijl H, Meinders AE. Adipose tissue as an endocrine organ: impact on insulin resistance. *Neth J Med* 2003;61(6):194-212.
117. Kalhan R, Puthawala K, Agarwal S, Amini SB, Kalhan SC. Altered lipid profile, leptin, insulin, and anthropometry in offspring of South Asian immigrants in the United States. *Metabolism* 2001;50(10):1197-202.
118. Lilja M, Rolandsson O, Shaw JE, Pauvaday V, Cameron AJ, Tuomilehto J *et al*. Higher leptin levels in Asian Indians than Creoles and Europids: a potential explanation for increased metabolic risk. *Int J Obes (Lond)* 2010;34(5):878-85.
119. Mente A, Razak F, Blankenberg S, Vuksan V, Davis AD, Miller R *et al*. Ethnic variation in adiponectin and leptin levels and their association with adiposity and insulin resistance. *Diabetes Care* 2010;33(7):1629-34.
120. Ramachandran A, Snehalatha C, Vijay V, Satyavani K, Latha E, Haffner SM. Plasma leptin in non-diabetic Asian Indians: association with abdominal adiposity. *Diabet Med* 1997;14(11):937-41.
121. Webb DR, Khunti K, Chatterjee S, Jarvis J, Davies MJ. Adipocytokine associations with insulin resistance in british South asians. *J Diabetes Res* 2013;2013:561016.
122. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE *et al*. Hypoadiponectinemia in Obesity and Type 2 Diabetes: Close Association with Insulin Resistance and Hyperinsulinemia. *Journal of Clinical Endocrinology & Metabolism* 2001;86(5):1930-5.
123. Goldstein BJ, Scalia R. Adiponectin: A Novel Adipokine Linking Adipocytes and Vascular Function. *Journal of Clinical Endocrinology & Metabolism* 2004;89(6):2563-8.

124. Valsamakis G, Chetty R, McTernan PG, Al-Daghri NM, Barnett AH, Kumar S. Fasting serum adiponectin concentration is reduced in Indo-Asian subjects and is related to HDL cholesterol. *Diabetes Obes Metab* 2003;5(2):131-5.
125. Bansal N, Anderson SG, Vyas A, Gemmell I, Charlton-Menys V, Oldroyd J *et al*. Adiponectin and lipid profiles compared with insulins in relation to early growth of British South Asian and European children: the Manchester children's growth and vascular health study. *J Clin Endocrinol Metab* 2011;96(8):2567-74.
126. Wasim H, Al-Daghri NM, Chetty R, McTernan PG, Barnett AH, Kumar S. Relationship of serum adiponectin and resistin to glucose intolerance and fat topography in South-Asians. *Cardiovasc Diabetol* 2006;5:10.
127. Snehalatha C, Mukesh B, Simon M, Viswanathan V, Haffner SM, Ramachandran A. Plasma adiponectin is an independent predictor of type 2 diabetes in Asian indians. *Diabetes Care* 2003;26(12):3226-9.
128. Martin M, Palaniappan LP, Kwan AC, Reaven GM, Reaven PD. Ethnic differences in the relationship between adiponectin and insulin sensitivity in South Asian and Caucasian women. *Diabetes Care* 2008;31(4):798-801.
129. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol* 2011;11(2):98-107.
130. Plomgaard P, Bouzakri K, Krogh-Madsen R, Mittendorfer B, Zierath JR, Pedersen BK. Tumor necrosis factor-alpha induces skeletal muscle insulin resistance in healthy human subjects via inhibition of Akt substrate 160 phosphorylation. *Diabetes* 2005;54(10):2939-45.
131. Peters MJ, Ghouri N, McKeigue P, Forouhi NG, Sattar N. Circulating IL-6 concentrations and associated anthropometric and metabolic parameters in South Asian men and women in comparison to European whites. *Cytokine* 2013;61(1):29-32.
132. Chambers JC, Eda S, Bassett P, Karim Y, Thompson SG, Gallimore JR *et al*. C-reactive protein, insulin resistance, central obesity, and coronary heart disease risk in Indian Asians from the United Kingdom compared with European whites. *Circulation* 2001;104(2):145-50.
133. Forouhi NG, Sattar N, McKeigue PM. Relation of C-reactive protein to body fat distribution and features of the metabolic syndrome in Europeans and South Asians. *Int J Obes Relat Metab Disord* 2001;25(9):1327-31.
134. Snel M, Jonker JT, Schoones J, Lamb H, de Roos A, Pijl H *et al*. Ectopic fat and insulin resistance: pathophysiology and effect of diet and lifestyle interventions. *Int J Endocrinol* 2012;2012:983814.
135. Korenblat KM, Fabbrini E, Mohammed BS, Klein S. Liver, muscle, and adipose tissue insulin action is directly related to intrahepatic triglyceride content in obese subjects. *Gastroenterology* 2008;134(5):1369-75.
136. Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, Goto T, Westerbacka J, Sovijarvi A *et al*. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J Clin Endocrinol Metab* 2002;87(7):3023-8.
137. Goodpaster BH, He J, Watkins S, Kelley DE. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metab* 2001;86(12):5755-61.
138. Krssak M, Falk PK, Dresner A, DiPietro L, Vogel SM, Rothman DL *et al*. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. *Diabetologia* 1999;42(1):113-6.

139. Jacob S, Machann J, Rett K, Brechtel K, Volk A, Renn W *et al*. Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetic subjects. *Diabetes* 1999;48(5):1113-9.
140. Forouhi NG, Jenkinson G, Thomas EL, Mullick S, Mierisova S, Bhonsle U *et al*. Relation of triglyceride stores in skeletal muscle cells to central obesity and insulin sensitivity in European and South Asian men. *Diabetologia* 1999;42(8):932-5.
141. Misra A, Sinha S, Kumar M, Jagannathan NR, Pandey RM. Proton magnetic resonance spectroscopy study of soleus muscle in non-obese healthy and Type 2 diabetic Asian Northern Indian males: high intramyocellular lipid content correlates with excess body fat and abdominal obesity. *Diabet Med* 2003;20(5):361-7.
142. Sinha S, Misra A, Rathi M, Kumar V, Pandey RM, Luthra K *et al*. Proton magnetic resonance spectroscopy and biochemical investigation of type 2 diabetes mellitus in Asian Indians: observation of high muscle lipids and C-reactive protein levels. *Magn Reson Imaging* 2009;27(1):94-100.
143. Sinha S, Rathi M, Misra A, Kumar V, Kumar M, Jagannathan NR. Sub-clinical inflammation and soleus muscle intra-myocellular lipids in healthy Asian Indian males. *Clinical Endocrinology* 2005;63:350-5.
144. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes* 1981;30(12):1000-7.
145. Srikanthan P, Karlamangla AS. Relative muscle mass is inversely associated with insulin resistance and prediabetes. Findings from the third National Health and Nutrition Examination Survey. *J Clin Endocrinol Metab* 2011;96(9):2898-903.
146. Hall LM, Moran CN, Milne GR, Wilson J, MacFarlane NG, Forouhi NG *et al*. Fat oxidation, fitness and skeletal muscle expression of oxidative/lipid metabolism genes in South Asians: implications for insulin resistance? *PLoS One* 2010;5(12):e14197.
147. Unni US, Ramakrishnan G, Raj T, Kishore RP, Thomas T, Vaz M *et al*. Muscle mass and functional correlates of insulin sensitivity in lean young Indian men. *Eur J Clin Nutr* 2009;63(10):1206-12.
148. Cline GW, Petersen KF, Krssak M, Shen J, Hundal RS, Trajanoski Z *et al*. Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes. *N Engl J Med* 1999;341(4):240-6.
149. Rothman DL, Shulman RG, Shulman GI. 31P nuclear magnetic resonance measurements of muscle glucose-6-phosphate. Evidence for reduced insulin-dependent muscle glucose transport or phosphorylation activity in non-insulin-dependent diabetes mellitus. *J Clin Invest* 1992;89(4):1069-75.
150. Shulman GI, Rothman DL, Jue T, Stein P, DeFronzo RA, Shulman RG. Quantitation of Muscle Glycogen Synthesis in Normal Subjects and Subjects with Non-Insulin-Dependent Diabetes by 13C Nuclear Magnetic Resonance Spectroscopy. *N Engl J Med* 1990;322(4):223-8.
151. Fröjdö S, Vidal H, Pirola L. Alterations of insulin signaling in type 2 diabetes: A review of the current evidence from humans. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 2009;1792(2):83-92.
152. Morino K, Petersen KF, Shulman GI. Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. *Diabetes* 2006;55 Suppl 2:S9-S15.
153. Bruce CR, Anderson MJ, Carey AL, Newman DG, Bonen A, Kriketos AD *et al*. Muscle oxidative capacity is a better predictor of insulin sensitivity than lipid status. *J Clin Endocrinol Metab* 2003;88(11):5444-51.

154. Nyholm B, Nielsen MF, Kristensen K, Nielsen S, Ostergard T, Pedersen SB *et al*. Evidence of increased visceral obesity and reduced physical fitness in healthy insulin-resistant first-degree relatives of type 2 diabetic patients. *Eur J Endocrinol* 2004;150(2):207-14.
155. Wei M, Gibbons LW, Mitchell TL, Kampert JB, Lee CD, Blair SN. The association between cardiorespiratory fitness and impaired fasting glucose and type 2 diabetes mellitus in men. *Ann Intern Med* 1999;130(2):89-96.
156. Venables MC, Achten J, Jeukendrup AE. Determinants of fat oxidation during exercise in healthy men and women: a cross-sectional study. *J Appl Physiol* 2005;98(1):160-7.
157. Davey GJG, Roberts JD, Patel S, Pierpoint T, Godsland IF, Davies B *et al*. Effects of exercise on insulin resistance in South Asians and Europeans. *Journal of Exercise Physiology Online* 2000;3(2):12-22.
158. Cersosimo E, DeFronzo RA. Insulin resistance and endothelial dysfunction: the road map to cardiovascular diseases. *Diabetes Metab Res Rev* 2006;22(6):423-36.
159. Kim J, Montagnani M, Koh KK, Quon MJ. Reciprocal Relationships Between Insulin Resistance and Endothelial Dysfunction. *Circulation* 2006;113(15):1888-904.
160. Cubbon RM, Murgatroyd SR, Ferguson C, Bowen TS, Rakobowchuk M, Baliga V *et al*. Human exercise-induced circulating progenitor cell mobilization is nitric oxide-dependent and is blunted in South Asian men. *Arterioscler Thromb Vasc Biol* 2010;30(4):878-84.
161. Murphy C, Kanaganayagam GS, Jiang B, Chowienczyk PJ, Zbinden R, Saha M *et al*. Vascular dysfunction and reduced circulating endothelial progenitor cells in young healthy UK South Asian men. *Arteriosclerosis, Thrombosis, and Vascular Biology* 2007;27(4):936-42.
162. Jin RC, Loscalzo J. Vascular Nitric Oxide: Formation and Function. *J Blood Med* 2010;2010(1):147-62.
163. Nofer JR, van der Giet M, Tolle M, Wolinska I, von Wnuck LK, Baba HA *et al*. HDL induces NO-dependent vasorelaxation via the lysophospholipid receptor S1P3. *J Clin Invest* 2004;113(4):569-81.
164. Chambers JC, McGregor A, Jean-Marie J, Kooner JS. Abnormalities of vascular endothelial function may contribute to increased coronary heart disease risk in UK Indian Asians. *Heart* 1999;81(5):501-4.
165. Din JN, Ashman OA, Aftab SM, Jubb AW, Newby DE, Flapan AD. Increased arterial stiffness in healthy young South Asian men. *Journal of Human Hypertension* 2006;20(2):163-5.
166. Hughes AD, Coady E, Raynor S, Mayet J, Wright AR, Shore AC *et al*. Reduced endothelial progenitor cells in European and South Asian men with atherosclerosis. *Eur J Clin Invest* 2007;37(1):35-41.
167. Ajjan R, Carter AM, Somani R, Kain K, Grant PJ. Ethnic differences in cardiovascular risk factors in healthy Caucasian and South Asian individuals with the metabolic syndrome. *J Thromb Haemost* 2007;5(4):754-60.
168. Bhalodkar NC, Blum S, Rana T, Bhalodkar A, Kitchappa R, Kim KS *et al*. Comparison of levels of large and small high-density lipoprotein cholesterol in Asian Indian men compared with Caucasian men in the Framingham Offspring Study. *Am J Cardiol* 2004;94(12):1561-3.
169. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev* 2004;84(1):277-359.
170. van Marken Lichtenbelt WD, Schrauwen P. Implications of nonshivering thermogenesis for energy balance regulation in humans. *Am J Physiol Regul Integr Comp Physiol* 2011;301(2):R285-R296.
171. Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC *et al*. A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 2012;481(7382):463-8.
172. Cani PD, Osto M, Geurts L, Everard A. Involvement of gut microbiota in the development of low-grade inflammation and type 2 diabetes associated with obesity. *Gut Microbes* 2012;3(4):279-88.

3 Higher insulin and glucagon-like peptide-1 (GLP-1) levels in healthy, young South Asians as compared to white Caucasians during an oral glucose tolerance test

Maria A. Sleddering
Leontine E.H. Bakker
Laura G.M. Janssen
A. Edo Meinders
Ingrid M. Jazet

Metabolism 2014; 63(2): 226-32

ABSTRACT

Objective: Higher insulin levels during an oral glucose test (OGTT) have consequently been shown in South Asians. We aimed to investigate if this increased insulin response causes reactive hypoglycemia later on, and if an increased glucagon-like-peptide-1 (GLP-1) response, which could contribute to the hyperinsulinemia, is present in this ethnic group.

Methods: A prolonged, 6-hour, 75-g OGTT was performed in healthy, young white Caucasian (n = 10) and South Asian (n = 8) men. The glucose, insulin and GLP-1 response was measured and indices of insulin sensitivity and beta-cell activity were calculated.

Results: Age (Caucasians 21.5±0.7 years *vs.* South Asians 21.4±0.7 years (mean±SEM) and body mass index (Caucasians 22.7±0.7 kg/m^2 *vs.* South Asians 22.1±0.8 kg/m^2) were comparable between the two groups. South Asian men were more insulin resistant, as indicated by a comparable glucose but significantly higher insulin response, and a significantly lower Matsuda index (Caucasians 8.7(8.6) *vs.* South Asians 3.2(19.2), median(IQR)). South Asians showed a higher GLP-1 response, as reflected by a higher area under the curve for GLP-1 (Caucasians 851 ± 100 mmol/L*h *vs.* South Asians 1235 ± 155 mmol/L*h). During the whole 6-hour period, no reactive hypoglycemia was observed.

Conclusion: Healthy, young South Asian men have higher insulin levels during an OGTT as compared to white Caucasians. This does not, however, lead to reactive hypoglycemia. The hyperinsulinemia is accompanied by increased levels of GLP-1. Whether this is an adaptive response to facilitate hyperinsulinemia to overcome insulin resistance or reflects a GLP-1 resistant state has yet to be elucidated.

INTRODUCTION

Among both native and migrant South Asians the risk of developing type 2 diabetes is exceptionally high. Furthermore, type 2 diabetes occurs at a younger age and lower BMI as compared to white Caucasians.[1-3] Also, long-term complications start earlier and run a more serious course. The predominant mechanism involved in the pathogenesis of type 2 diabetes in South Asians seems to be a decrease in insulin sensitivity.[4-6]

It has repeatedly been shown that South Asians, as compared to Caucasians, exhibit higher 2-hour insulin levels or a higher area under the curve (AUC) for insulin, with a normal glucose response, during an oral glucose or meal tolerance test (OGTT).[7-9] These higher insulin levels are considered a compensatory mechanism to overcome insulin resistance and maintain glucose tolerance. The hyperinsulinemia might be caused by a decreased insulin clearance,[5] but an increased β-cell response has been reported as well.[10]

Glucagon-like peptide-1 (GLP-1), an incretin secreted from the enteroendocrine L-cells in the gut in response to eating, is known to stimulate insulin secretion from pancreatic β-cells.[11] An increased GLP-1 response could therefore contribute to the glucose-stimulated hyperinsulinemia consequently seen in South Asians. However, whether GLP-1 levels are indeed higher in this ethnic group is currently unknown. Furthermore, not only the underlying mechanism, but also the consequences of the hyperinsulinemia in people of South Asian descent are not yet fully elucidated. It is, for instance, unknown if the increased insulin response in people of South Asian descent causes reactive hypoglycemia, a condition characterized by a drop in glucose levels 4-6 hours after a glucose load, which is considered a sign of early latent diabetes.[12-14] In the present study we therefore studied the glucose and insulin response during a prolonged 6-hour OGTT in healthy, young South Asian and white Caucasian men. Furthermore, GLP-1 levels were assessed to investigate whether an increased GLP-1 response is present in South Asians.

SUBJECTS AND METHODS

Subjects

Eighteen healthy, young men were included in the study (10 white Caucasians, 8 South Asians). Male subjects aged 18-25 years, with a BMI between 18.5 and 25 kg/m^2, and a positive family history of type 2 diabetes were eligible for enrolment. The South Asian subjects were all Hindustani Surinamese. In the Netherlands, almost all South Asians are Hindustani Surinamese, an ethnic group that has migrated from Surinam, a former Dutch colony in South America, and whose ancestors came from the Indian subcontinent. Seven of the South Asian subjects were born in the Netherlands. One was born in Surinam and migrated to the Netherlands at the age of eight. Exclusion criteria

were type 2 diabetes or any other chronic disease, smoking, use of medication known to influence glucose metabolism, and recent weight change. Subjects were recruited via advertisements placed online, in local media, and in public places. This study was approved by the Medical Ethical Committee of the Leiden University Medical Centre and performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all subjects before participation.

Oral glucose tolerance test

Following an initial screening visit, each subject was studied once. Subjects arrived at the research centre at 8.00 AM after an overnight fast. Anthropometric measurements were obtained and fat mass was assessed by bioelectrical impedance analysis (Bodystat® 1500, Bodystat Ltd., Douglas, Isle of Man, UK). After insertion of an intravenous catheter, two baseline blood samples were drawn (t = -15 and t = 0). Thereafter, subjects underwent a prolonged 75-g OGTT, with measurements of glucose and insulin at t = 15, 30, 60, 90, 120, 150, 180, 210, 240, 300, and 360 minutes. Samples for the measurement of GLP-1 were drawn at baseline and at t = 15, 30, 60, 90, 120, 150, and 180 minutes. Dipeptidyl peptidase IV (DPP-IV) inhibitor (10 µl/mL blood; Merck Millipore, Billerica, MA, USA) was added to these samples immediately. Blood samples were cooled on ice and centrifuged at 4 °C. Hereafter samples were distributed into aliquots and stored at −80 °C until analysis.

Assays

Serum glucose, total cholesterol, HDL-cholesterol and triglycerides were measured on a Modular P800 analyser (Roche, Almere, The Netherlands). LDL-cholesterol was calculated according to Friedewald's formula.[15] Serum insulin levels were analysed on an Immulite 2500 (Siemens, The Netherlands). Active GLP-1 was measured using a standardized ELISA kit (Meso Scale Diagnostics, Gaithersburg, MD, USA).

Statistical analysis and calculations

Results are expressed as mean ± standard error (SEM) or median and interquartile range (IQR) in case of non-normally distributed data. Baseline values for glucose, insulin and GLP-1 were calculated as the average of the two baseline measurements (t = -15 and t = 0). Reactive hypoglycemia was defined as a glucose level of 3 mmol/L or less between 3 and 6 hours after the oral glucose load. For type 2 diabetes patients on glucose lowering therapy usually a cut-off value for hypoglycemia of < 3.9 mmol/L is used. We chose a lower cut-off value for hypoglycemia suggested by Marks *et al*. (Hypoglycemia, 1987) that 3.0 mmol/L is an appropriate cut-off point for evaluating hypoglycemia in healthy (non-diabetic) volunteers, since 95% of blood glucose levels in healthy volunteers are above this level. AUC values were determined using the trapezoidal rule.[16] Incremental values are calculated by deducting the area below the baseline value from total AUCs.

Insulin sensitivity was estimated using the Matsuda index (glucose: mg/dL; insulin: mU/L).[17] Recently it was shown that the Matsuda index correlates highly with insulin sensitivity measured with a hyperinsulinemic clamp in South Asians and Caucasians.[18] The insulinogenic index (IGI; $\Delta I_{0-30}/\Delta G_{0-30}$) was used as a measurement of early insulin secretion (glucose: mmol/l insulin: pmol/L).[19] The oral disposition index (DI_o; ($\Delta I_{0-30}/\Delta G_{0-30}$)/fasting insulin) (glucose: mmol/L; insulin: mU/L) was used to provide an estimate of β-cell function relative to the prevailing level of insulin resistance.[20,21]

The independent Student's t-test was used for comparisons between the groups. A non-parametric test (Mann-Whitney U test) was applied when appropriate. A p-value of < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS for Windows (release 20.0, IBM, USA).

RESULTS

Anthropometric and laboratory measurements

Data on anthropometric and laboratory measurements are shown in **Table 1**. South Asians were significantly smaller and lighter compared to the Caucasian subjects. BMI, however, was comparable between the groups (Caucasians: 22.7 ± 0.7 *vs.* South Asians: 22.1 ± 0.8 kg/m^2). There were no significant differences in (percent of) fat mass, waist circumference, or fasting levels of glucose, insulin and lipids.

Table 1. Anthropometric and laboratory parameters in young, healthy white Caucasian and South Asian men.

	white Caucasians (n = 10)	South Asians (n = 8)
age (years)	21.5 ± 0.7	21.4 ± 0.7
height (m)	1.82 ± 0.01	1.72 ± 0.02
weight (kg)	76.0 ± 2.7	65.7 ± 2.8*
BMI (kg/m^2)	22.7 ± 0.7	22.1 ± 0.8
waist (cm)	81 ± 2.2	78 ± 2.1
fat mass (%)	14.9 ± 0.9	15.2 ± 1.5
fasting glucose (mmol/L)	4.9 ± 0.2	5.2 ± 0.1
fasting insulin (mU/L)	5.3 ± 1.5	9.5 ± 1.5
HOMA-IR	1.2 ± 0.4	2.2 ± 0.4
total cholesterol (mmol/L)	3.68 ± 0.26	3.90 ± 0.19
LDL-cholesterol (mmol/L)	2.10 ± 0.24	2.22 ± 0.12
triglycerides (mmol/L)	1.03 ± 0.13	0.92 ± 0.11
HDL-cholesterol (mmol/L)	1.10 ± 0.06	1.3 ± 0.06

Mean ± SEM. * $p < 0.05$ BMI: body mass index; HDL: high density lipoprotein; LDL: low density lipoprotein; HOMA-IR: homeostasis model of assessment insulin resistance

Prolonged oral glucose tolerance test

Time courses for glucose and insulin during the prolonged 75-g OGTT are shown in **Figure 1**. Insulin levels were significantly higher in the South Asian group at several time points. During the whole 6-hour period there were no differences in glucose levels between the groups and reactive hypoglycemia did not occur. The AUCs for glucose and insulin are depicted in **Figure 2**. The AUC_{360} for insulin was significantly higher in the South Asian group (Caucasians: 6.6 ± 0.9*10^3 *vs.* South Asians: 16.7 ± 4.2*10^3 mU/L*h;

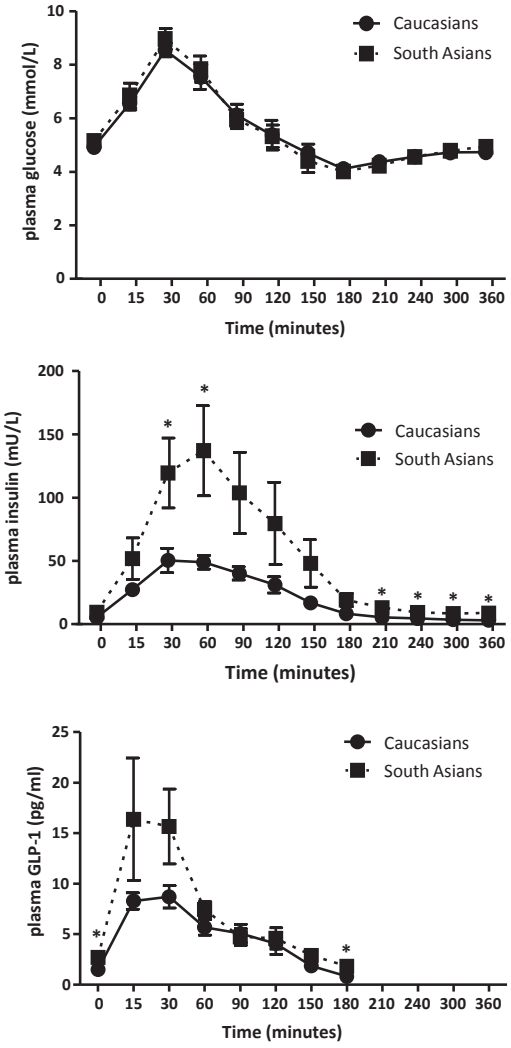


Figure 1. Time courses for plasma concentrations of glucose, insulin and glucagon-like peptide (GLP)-1 during an oral glucose tolerance test (OGTT) in healthy young white Caucasian and South Asian men. Data are mean ± SEM; * p<0.05.

p<0.05). South Asians were less insulin sensitive as reflected by a lower Matsuda index (**Table 2**). A compensatory increase in insulin secretion was observed in this group as shown by an increased IGI, although this was only borderline significant (p=0.051). β-cell function in relation to the level of insulin sensitivity, as assessed by the oral disposition index, did not differ between the two groups.

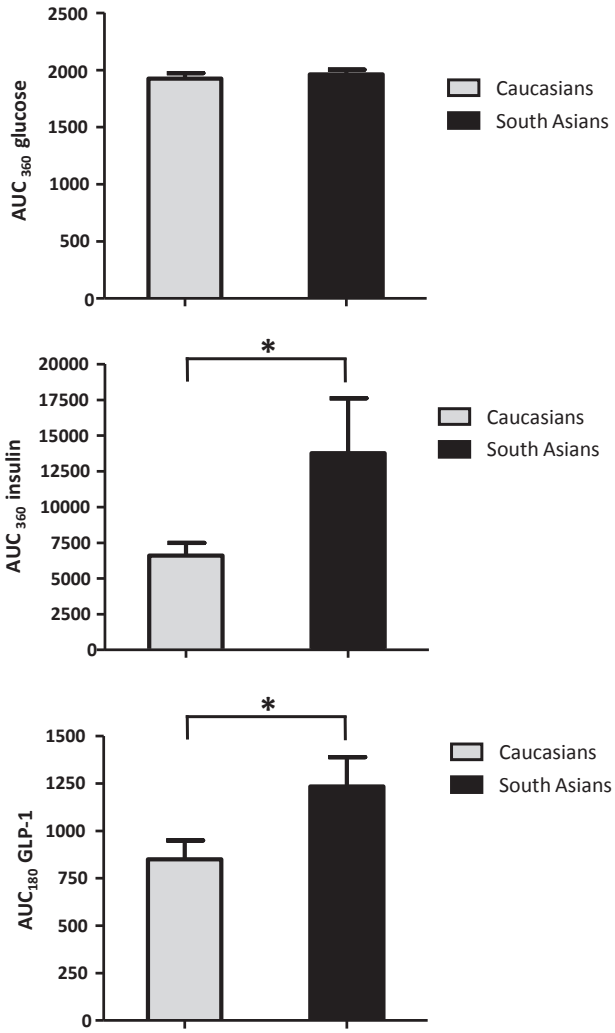


Figure 2. Area under the curve (AUC) for glucose, insulin and glucagon-like peptide (GLP)-1 during an oral glucose tolerance test (OGTT) in healthy, young white Caucasian and South Asian men. Data are mean ± SEM; * p<0.05.

Table 2. Glucose, insulin and GLP-1 indices during a 6-hour 75-g oral glucose tolerance test in young, healthy white Caucasian and South Asian men.

	white Caucasians (n = 10)	South Asians (n = 8)
peak glucose (mmol/L)	8.8 ± 0.30	9.1 ± 0.33
peak glucose time (min) (median, IQR)	30 (30)	30 (23)
peak insulin (mU/L)	59 ± 8.7	155 ± 39*
peak insulin time (min) (median, IQR)	60 (30)	60 (0)
peak GLP-1 (mmol/L)	9.4 ± 0.9	22.1 ± 6.2
peak GLP-1 time (min) (median, IQR)	30 (30)	30 (15)
AUC_{120} glucose (mmol/L * h)	818 ± 34	837 ± 32
AUC_{120} insulin (mU/L * h)	$4.7 \pm 0.6*10^3$	$12.0 \pm 3.0*10^3$*
AUC_{360} glucose (mmol/L * h)	1924 ± 47	1936 ± 48
AUC_{360} insulin (mU/L * h)	$6.6 \pm 0.9*10^3$	$16.7 \pm 4.2*10^3$*
AUC_{180} GLP-1 (mmol/L * h)	851 ± 100	1235 ± 155*
AUC_{180} incremental GLP-1 (mmol/L * h)	619 ± 94	851 ± 142
AUC_{120} glucose/AUC_{120} insulin (mmol/mU)	0.21 ± 0.33	0.13 ± 0.04
Matsuda index (median, IQR)	8.7 (8.6)	3.2 (19.2)*
IGI_{30} (pmol/mmol) (median, IQR)	83 (41)	175 (189)$^{p=0.051}$
DI_o (median, IQR)	3.1 (3.5)	2.4 (1.4)

Mean ± SEM, unless otherwise specified. * $p < 0.05$. GLP-1: glucagon-like peptide-1; AUC: area under the curve; IGI: insulinogenic index, DI_o: oral disposition index

GLP-1

The time course and AUC for GLP-1 during the OGTT are shown in **Figure 1 and 2**. The AUC_{180} for GLP-1 was higher in South Asian subjects compared to Caucasian subjects (Caucasians: 851 ± 100 *vs.* South Asians: 1235 ± 155 mmol/L*h; $p<0.05$). The incremental AUC_{180} was higher in South Asians as well, although this did not reach statistical difference (Caucasians: 619 ± 94 *vs.* South Asians: 851 ± 142 mmol/L*h; p=0.18). In univariate analysis, fat percentage or waist circumference did not significantly predict the GLP-1 AUC_{180} (p=0.852 and p=0.102). However when included in the model, they do alter the significance level of the between group difference in GLP-1 AUC_{180} (p=0.055 and p=0.103 instead of p=0.046). GLP-1 and insulin levels were highly correlated at several time points, especially GLP-1 at t = 15 min (with insulin at t = 15, 30, 90, 120, 150, 180, 210, 240, 300 min; $p<0.05$). The GLP-1 AUC_{180} showed a significant correlation with the insulin AUC_{120} and the insulin AUC_{360} (0.607, p=0.008, and 0.599, p=0.009), but not with the AUCs for glucose. Due to the small sample size, correlations could not be calculated for South Asians and Caucasians separately.

DISCUSSION

In this study we investigated the glucose, insulin and GLP-1 response during a prolonged OGTT in young, healthy South Asian men. We confirmed that young, healthy South Asian men are more insulin resistant, as reflected by higher insulin levels during an OGTT, than their white Caucasian counterparts. A novel finding is that these higher insulin levels are accompanied by increased levels of GLP-1. Also we demonstrated that no reactive hypoglycemia occurred in the South Asians.

Whether the hyperinsulinemia in South Asians is caused by reduced clearance or increased secretion of insulin is unclear. A decreased insulin clearance has been found in South Asians during a euglycemic hyperinsulinemic clamp by our group (*unpublished data*) and by others.[5] On the other hand, Petersen *et al*. showed an increase in β-cell response (estimated using the oral C-peptide minimal model) in South Asians as compared to Caucasians during a 2-hour, 75-g OGTT.[9] This increased β-cell response was, however, inadequate for their degree of insulin resistance as reflected by a lower disposition index. It is well known that to maintain glucose tolerance with declining insulin sensitivity, a proportionate increase in insulin output has to occur as a compensatory mechanism.[22] In our study the South Asian men were more insulin resistant, as shown by a comparable glucose but significantly higher insulin response and a decreased Matsuda index, and indeed showed an increased β-cell response, as reflected by a higher IGI. The oral disposition index did not differ between groups, suggesting that the increased insulin output was adequate for the level of insulin resistance.

In this study, we further explored a possible consequence of the glucose-stimulated hyperinsulinemia in South Asian men: reactive hypoglycemia. Despite the higher insulin levels, no reactive hypoglycemia was seen in this group. Reactive hypoglycemia 4-6 hours after a glucose load has been observed in obese subjects and is considered an early sign of diabetes.[12-14] Reactive hypoglycemia has also been found in young, lean women with polycystic ovary syndrome (PCOS), a condition known to be associated with insulin resistance and increased risk of diabetes development.[23] The fact that hypoglycemia did not occur in our study might be due to a high level of insulin resistance on a cellular level in the South Asian subjects, or by the induction of insulin resistance and increased hepatic glucose output by counter regulatory hormones, such as glucagon, catecholamines and cortisol, which were not measured in our study. Furthermore, because of the small sample size, it is possible that a difference was missed.

A novel finding is that the South Asians displayed a higher GLP-1 response to an oral glucose load, as reflected by an increased AUC for GLP-1. To our knowledge, this is the first study investigating the GLP-1 response in subjects of South Asian descent. GLP-1 is known to have several beneficial effects on glucose regulation. It stimulates endogenous insulin secretion in response to oral glucose or eating, suppresses glucagon secretion

resulting in a decreased hepatic glucose output, and is thought to exert extrapancreatic effects, since it improves glucose disposal and decreases endogenous glucose production independent of its release of islet hormones.[24-26] Therefore, we hypothesized that an increased GLP-1 response could possibly explain, or at least contribute to the higher insulin levels in South Asians, which may initially help overcome the insulin resistance in this ethnic group.

The precise role of GLP-1 in the pathogenesis of type 2 diabetes is currently unknown. It is well known that the incretin effect (i.e. the augmented insulin secretion in response to oral compared with intravenous isoglycemic administration of glucose) is diminished in type 2 diabetes patients.[27] It has been debated if this impaired incretin effect is caused by impaired GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) secretion or by a defective insulin secretory effect of these hormones ('incretin resistance'). Several studies showed a lower postprandial GLP-1 release in subjects with type 2 diabetes and insulin resistance, but a recent meta-analysis found that patients with type 2 diabetes on the whole do not exhibit reduced GLP-1 secretion in response to oral glucose or meal tests and that type 2 diabetes patients may even have higher GLP-1 peak levels.[28] Our data suggest that a state of insulin resistance leads to a higher GLP-1 response. Possibly, GLP-1 secretion changes during the progression from normal glucose tolerance to type 2 diabetes, which was also suggested by the authors of the aforementioned meta-analysis.[28] Early stages of type 2 diabetes may lead to compensatory increased GLP-1 secretion from intestinal L-cells, which is then followed by the exhaustion of these cells when the disease progresses. Indeed, a study by Theodorakis *et al*. in newly diagnosed type 2 diabetes patients showed an increase in late-phase (20-80 min) GLP-1 secretion after a 75-g OGTT, in parallel with rising plasma insulin levels. Furthermore, they found increased numbers of L-cells in the duodenum in this group.[29] However, in a study of Knop *et al*. insulin resistant, but normal glucose tolerant, obese subjects did not show an increased GLP-1 response. In this study, however, an oral glucose load of 50-g was used, instead of the 75-g OGTT in our study. Furthermore, these subjects already displayed signs of β-cell dysfunction, as shown by a decreased disposition index, indicating a more progressed state of insulin resistance.[30] In another study, insulin resistance induced in healthy, young men (using a 12 day intervention with prednisolone treatment, high-energy diet, and relative physical inactivity) led to higher fasting GLP-1 levels, but no difference in the GLP-1 response to an oral glucose load was found when comparing the baseline and insulin resistant state.[31] This might be due to the fact that the subjects in this study not only were insulin resistant, but also less glucose tolerant. Four of the 10 subjects even displayed impaired glucose tolerance or diabetes after the intervention, whereas all our subjects were normal glucose tolerant. Hence, glucotoxicity might have attenuated the GLP-1 response.

The increased GLP-1 response found in the South Asian subjects in our study on the other hand might also indicate a state of GLP-1 resistance. Although debated, evidence suggests that GLP-1 resistance is present in type 2 diabetes patients and their healthy offspring.[32] Furthermore, in the aforementioned study in which insulin resistance was induced in healthy subjects, although no alterations in the GLP-1 response were seen, a reduction in the incretin effect was shown.[31] In addition, the insulinotropic effect of GLP-1 was impaired, suggesting that incretin resistance was present and is a consequence of insulin resistance. We also showed higher fasting GLP-1 levels in the South Asian group. However, since the incretin effect and the direct insulinotropic action of GLP-1 were not assessed in our study, it remains to be elucidated whether the higher fasting GLP-1 levels and higher GLP-1 response in South Asians are due to a compensatory increased secretion or reflecting a GLP-1 resistant state. However, the peak GLP-1 levels preceded the peak insulin response and paralleled the increased β-cell activity (IGI), suggesting a direct relation between the increased GLP-1 response and the insulin secretion by the β-cell.

A limitation of our study is the small sample size. However, even with only 18 subjects, a difference in GLP-1 response was found. Further research is required to see whether these findings can be reproduced in larger samples. In univariate analysis, fat percentage and waist circumference did influence the significance level of the between group difference in GLP-1 AUC180. It can therefore not be excluded that differences in body composition, although not significantly different between the two groups, has influenced our findings on GLP-1 levels. In addition, we do not have data on nutritional intake and exercise. It is possible that differences in intake and physical activity influenced insulin resistance and GLP-1 secretion. However, it is unlikely that differences in behaviour solely explain the increased insulin sensitivity found in diverse groups of South Asians.[6] Furthermore, in a previous study of our group in a similar study population no differences were found in diet and exercise.[33] Hence, it seems unlikely that these factors have influenced our findings.

In conclusion, we confirmed that young, healthy South Asian men are more insulin resistant and have higher insulin levels during an OGTT than their white Caucasian counterparts. The higher insulin levels were accompanied by increased levels of GLP-1. No reactive hypoglycemia was observed in the South Asians despite the hyperinsulinemia. Whether this is an adaptive response to facilitate hyperinsulinemia to overcome insulin resistance or reflects a GLP-1 resistant state has yet to be elucidated.

REFERENCES

1. Anjana RM, Pradeepa R, Deepa M, Datta M, Sudha V, Unnikrishnan R *et al*. Prevalence of diabetes and prediabetes (impaired fasting glucose and/or impaired glucose tolerance) in urban and rural India: phase I results of the Indian Council of Medical Research-INdia DIABetes (ICMR-INDIAB) study. *Diabetologia* 2011;54(12):3022-7.
2. DECODE Study Group. Age- and sex-specific prevalences of diabetes and impaired glucose regulation in 13 European cohorts. *Diabetes Care* 2003;26:161-9.
3. Chiu M, Austin PC, Manuel DG, Shah BR, Tu JV. Deriving ethnic-specific BMI cutoff points for assessing diabetes risk. *Diabetes Care* 2011;34(8):1741-8.
4. Banerji MA, Faridi N, Atluri R, Chaiken RL, Lebovitz HE. Body composition, visceral fat, leptin, and insulin resistance in Asian Indian men. *J Clin Endocrinol Metab* 1999;84(1):137-44.
5. Liew CF, Seah ES, Yeo KP, Lee KO, Wise SD. Lean, nondiabetic Asian Indians have decreased insulin sensitivity and insulin clearance, and raised leptin compared to Caucasians and Chinese subjects. *Int J Obes Relat Metab Disord* 2003;27(7):784-9.
6. Bakker LE, Sleddering MA, Schoones JW, Meinders AE, Jazet IM. Pathogenesis of type 2 diabetes in South Asians. *Eur J Endocrinol* 2013;169(5):R99–114.
7. Laws A, Jeppesen JL, Maheux PC, Schaaf P, Chen YD, Reaven GM. Resistance to insulin-stimulated glucose uptake and dyslipidemia in Asian Indians. *Arterioscler Thromb* 1994;14 (6):917-22.
8. McKeigue PM, Shah B, Marmot MG. Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. *Lancet* 1991;337(8738):382-6.
9. Raji A, Gerhard-Herman MD, Warren M, Silverman SG, Raptopoulos V, Mantzoros CS *et al*. Insulin resistance and vascular dysfunction in nondiabetic Asian Indians. *J Clin Endocrinol Metab* 2004;89(8):3965-72.
10. Petersen KF, Dufour S, Feng J, Befroy D, Dziura J, Dalla MC *et al*. Increased prevalence of insulin resistance and nonalcoholic fatty liver disease in Asian-Indian men. *Proc Natl Acad Sci U S A* 2006;103(48):18273-7.
11. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 2011;368(9548):1696-705.
12. Faludi G, Bendersky G, Gerber P. Functional hypoglycemia in early latent diabetes. *Ann N Y Acad Sci* 1968;148(3):868-74.
13. Conn JW, Fajans SS, Seltzer HS. Spontaneous hypoglycemia as an early manifestation of diabetes mellitus. *Diabetes* 1956;5(6):437-42.
14. Anderson JW, Herman RH. Classification of reactive hypoglycemia. American Journal of Clinical *Nutrition* 1969;22(5):646-50.
15. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18(6):499-502.
16. Matthews JN, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. *BMJ* 1990;300(6719);230-5.
17. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 1999;22(9):1462-70.
18. Trikudanathan S, Raji A, Chamarthi B, Seely EW, Simonson DC. Comparison of insulin sensitivity measures in South Asians. *Metabolism* 2013;62(10):1448-54.
19. Tura A, Kautzky-Willer A, Pacini G. Insulinogenic indices from insulin and C-peptide: Comparison of beta-cell function from OGTT and IVGTT. *Diabetes Res Clin Pract* 2006;72(3):298-301.

20. Retnakaran R, Qi Y, Goran MI, Hamilton JK. Evaluation of proposed oral disposition index measures in relation to the actual disposition index. *Diabet Med* 2009;26(12):1198-203.
21. Utzschneider KM, Prigeon RL, Faulenbach MV, Tong J, Carr DB, Boyko EJ *et al*. Oral Disposition Index Predicts the Development of Future Diabetes Above and Beyond Fasting and 2-h Glucose Levels: Response to DeFronzo and Abdul-Ghani. *Diabetes Care* 2009;32(7):e87.
22. Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. *Diabetologia* 2003;46(1):3-19.
23. Altuntas Y, Bilir M, Ucak S, Gundogdu S. Reactive hypoglycemia in lean young women with PCOS and correlations with insulin sensitivity and with beta cell function. *Eur J Obstet Gynecol Reprod Biol* 2005;119(2):198-205.
24. Drucker DJ. The biology of incretin hormones. *Cell Metab* 2006;3(3):153-65.
25. Egan JM, Meneilly GS, Habener JF, Elahi D. Glucagon-Like Peptide-1 Augments Insulin-Mediated Glucose Uptake in the Obese State. *J Clin Endocrinol Metab* 2002;87(8):3768-73.
26. Prigeon RL, Quddusi S, Paty B, D'Alessio DA. Suppression of glucose production by GLP-1 independent of islet hormones: a novel extrapancreatic effect. *Am J Physiol Endocrinol Metab* 2003;285(4):E701-7.
27. Holst JJ, Knop FK, Vilsbøll T, Krarup T, Madsbad S. Loss of Incretin Effect Is a Specific, Important, and Early Characteristic of Type 2 Diabetes. *Diabetes Care* 2011;34(Suppl 2):S251-7.
28. Calanna S, Christensen M, Holst JJ, Laferrère B, Gluud LL, Vilsbøll T *et al*. Secretion of glucagon-like peptide-1 in patients with type 2 diabetes mellitus: systematic review and meta-analyses of clinical studies. *Diabetologia* 2013;56(5):965-72.
29. Theodorakis MJ, Carlson O, Michopoulos S, Doyle ME, Juhaszova M, Petraki K *et al*. Human duodenal enteroendocrine cells: source of both incretin peptides, GLP-1 and GIP. *Am J Physiol Endocrinol Metab* 2006;290(3):E550-9.
30. Knop FK, Aaboe K, Vilsbøll T, Vølund A, Holst JJ, Krarup T *et al*. Impaired incretin effect and fasting hyperglucagonaemia characterizing type 2 diabetic subjects are early signs of dysmetabolism in obesity. *Diabetes Obes Metab* 2012;14(6):500-10.
31. Hansen KB, Vilsbøll T, Bagger JI, Holst JJ, Knop FK. Reduced Glucose Tolerance and Insulin Resistance Induced by Steroid Treatment, Relative Physical Inactivity, and High-Calorie Diet Impairs the Incretin Effect in Healthy Subjects. *J Clin Endocrinol Metab* 2010;95(7):3309-17.
32. Herzberg-Schäfer S, Heni M, Stefan N, Häring HU, Fritsche A. Impairment of GLP1-induced insulin secretion: role of genetic background, insulin resistance and hyperglycaemia. *Diabetes Obes Metab* 2012;14(Suppl 3):85-90.
33. Bakker LE, van Schinkel LD, Guigas B, Streefland TC, Jonker JT, van Klinken JB *et al*. A 5-day high fat high calorie diet impairs insulin sensitivity in healthy, young South Asian men but not in Caucasian men. *Diabetes* 2014;63(1):248-58.

4 A 5-day high fat high calorie diet impairs insulin sensitivity in healthy, young South Asian men but not in white Caucasian men

Leontine E.H. Bakker
Linda D. van Schinkel
Bruno Guigas
Trea C.M. Streefland
Jacqueline T. Jonker
Jan B. van Klinken
Gerard C.M. van der Zon
Hildo J. Lamb
Johannes W.A. Smit
Hanno Pijl
A. Edo Meinders
Ingrid M. Jazet

Diabetes 2014; 63(1): 248-58

ABSTRACT

South Asians develop type 2 diabetes at a younger age and lower BMI compared to white Caucasians. The underlying cause is still poorly understood but might result from an innate inability to adapt to the Westernized diet. This study aimed to compare the metabolic adaptation to a high fat high calorie diet (HFHCD) between both ethnicities. Twelve healthy young lean male South Asians and 12 matched white Caucasians underwent a 2-step hyperinsulinemic-euglycemic clamp with skeletal muscle biopsies and indirect calorimetry before and after a 5-day HFHCD. Hepatic triglyceride content (HTG) and abdominal fat distribution were assessed using MRI/S. At baseline, South Asians had higher insulin clamp levels than Caucasians, indicating reduced insulin clearance rate. Despite the higher insulin levels, endogenous glucose production was comparable between groups, suggesting lower hepatic insulin sensitivity in South Asians. Furthermore, a 5-day HFHCD decreased insulin-stimulated (non-oxidative) glucose disposal rate only in South Asians. In skeletal muscle no significant differences were found between groups in insulin/mTOR-signalling, metabolic gene expression and mitochondrial respiratory-chain content. Furthermore, no differences in (mobilization of) HTG and abdominal fat were detected. We conclude that HFHC-feeding rapidly induces insulin resistance only in South Asians. Thus, distinct adaptation to "Western" food may partly explain their propensity to develop type 2 diabetes.

INTRODUCTION

The incidence of type 2 diabetes is increasing rapidly worldwide, especially in people of South Asian descent.[1] South Asians originate from the Indian subcontinent and represent one fifth of the world's population. Both native and migrant South Asians are at high risk of developing type 2 diabetes compared to Caucasians.[2-4] Not only is the prevalence of type 2 diabetes four to six times higher, it also occurs at a younger age and lower BMI.[4-6] Moreover, the risk of cardiovascular and renal complications is higher.[7-10] The underlying cause of this excess risk is still not completely understood, and only few in-depth studies have been conducted to investigate the pathogenesis of type 2 diabetes in South Asians.[11;12]

The observation that South Asians have high hepatic and intramyocellular lipid content compared to people of Caucasian descent[13;14] might suggest that South Asians have an impaired mitochondrial fatty acid beta-oxidation in either skeletal muscle and/or adipose tissue, resulting in ectopic fat deposition in peripheral tissues, eventually leading to insulin resistance and other metabolic dysfunctions.[15] South Asians may therefore be less capable to handle the Western-type high fat (HF)-diet as compared to Caucasians.

Interesting in this context are recent findings on the nutrient and energy-sensing mammalian target of rapamycin (mTOR)-pathway. The mTOR-pathway regulates cell growth according to cellular energy status and nutrient availability.[16] Activated mTOR complex 1 (mTORC1) controls key cellular processes, e.g. it inhibits insulin signalling[17] and plays a crucial role in the regulation of oxidative metabolism and mitochondrial biogenesis.[18-21] Importantly, mTORC1 also appears to promote lipid synthesis and storage, while inhibiting processes leading to lipid consumption.[22] Indeed, there is growing evidence that mTORC1 suppresses fatty acid beta-oxidation.[21;23;24] Therefore, we hypothesize that differences in mTOR activity between the two ethnicities may underlie or contribute to the increased risk of type 2 diabetes in South Asians.

The aim of this study was to investigate whether the metabolic adaptation to a 5-day high fat high calorie diet (HFHCD) is different between young healthy lean South Asian males and matched Caucasians. In particular, we were interested whether differences in the activity of mTOR in skeletal muscle exist between the two ethnicities, both at baseline and in response to the HFHCD. Furthermore, hepatic and peripheral insulin sensitivity, substrate oxidation, abdominal fat distribution and skeletal muscle insulin signalling and mitochondrial respiratory-chain content were assessed.

RESEARCH DESIGN AND METHODS

Subjects

Twelve Dutch South Asian and twelve Dutch Caucasian, lean (BMI < 25 kg/m^2) and healthy men, aged 19-25 years with a positive family history of type 2 diabetes were enrolled via local advertisements. Subjects underwent a medical screening including their medical history, a physical examination, blood chemistry tests and an oral glucose tolerance test to exclude individuals with type 2 diabetes according to the American Diabetes Association 2010 criteria. Other exclusion criteria were rigorous exercise, smoking and recent body weight change. The study was approved by the Medical Ethical Committee of the Leiden University Medical Centre and performed in accordance with the principles of the revised Declaration of Helsinki. All volunteers gave written informed consent before participation.

Study design

Subjects were studied before and after a 5-day HFHCD, consisting of the subject's regular diet supplemented with 375 mL of cream per day (=1275 kcal/day, 94% fat). At the end of the first study day, subjects received 15 125 mL cups of cream to take home. They were instructed to continue their regular diet and, on top of that, to consume three cups of cream per day, directly following a meal in order to make sure they could adhere to their regular dietary habits. In addition, they kept a food diary before and during the HFHCD to estimate normal dietary intake, to maximize compliance with the diet, and to check for compliance and compensation behaviour. Diaries were entered and analysed using a specialized internet application (http://www.dieetinzicht.nl, Dutch). Compliance was measured by asking to bring leftover cups, inquiring, analysing the food diaries and laboratory parameters. Subjects were instructed not to alter life style habits, and not to perform physical activity in the last 48 hours before the study days. Magnetic resonance (MR) studies were performed shortly before and on the fifth day of the HFHCD. Metabolic studies were performed one day before and one day after the diet.

MR studies

Abdominal fat depots were quantified with turbo spin echo MR-imaging using a 1.5 Tesla whole body MR-scanner (Gyroscan ACS-NT15; Philips, The Netherlands) four hours after the last meal.[25] During one breath hold, three transverse images were obtained at the level of L5. Volumes of visceral and subcutaneous fat depots were quantified using MASS analytical software (Medis, The Netherlands). The number of pixels were converted to cm^2 and multiplied by the slice thickness (10mm). Hepatic triglyceride content (HTG) was assessed by proton MR-spectroscopy (^{1}H-MRS)[26] A spectrum without water suppression, four averages, as internal standard was obtained, and 64 averages were collected with water suppression. The spectra were fitted using Java-based MR user interface soft-

ware (jMRUI version 2.2).[26] The percentage of hepatic triglyceride signals was calculated as: (signal amplitude hepatic triglycerides / signal amplitude water) x 100.

Metabolic studies

Anthropometric measurements, a 2-step hyperinsulinemic-euglycemic clamp with stable isotopes and indirect calorimetry were performed after an overnight fast. In addition, skeletal muscle biopsies were obtained. Fat and lean body mass (LBM) were assessed by bioelectrical impedance analysis (BIA; Bodystat® 1500, Bodystat Ltd., Douglas, UK).

Hyperinsulinemic-euglycemic clamp A 6-h 2-step hyperinsulinemic-euglycemic clamp was performed as described previously.[27] In short, a primed constant infusion of glucose tracer ([6,6-$^2H_2$]-glucose; 0.22 µmol/kg/min) was used to determine rates of glucose appearance (R_a) and disposal (R_d). At t=120 min (step 1) and t=240 min (step 2), a primed constant infusion of insulin (step 1: 10 mU/m^2/min, step 2: 40 mU/m^2/min) was started and glucose-20% enriched with 3% [6,6-$^2H_2$]-glucose was infused at a variable rate to maintain glucose level at 5.0 mmol/L. In basal state (t=0 min), at the end of the non-insulin stimulated period (t=95-115 min) and at the end of each step (t=210-240 min and t=330-360 min), blood samples were taken for determination of glucose, insulin, C-peptide, free fatty acids (FFAs), and [6,6-$^2H_2$]-glucose specific activity.

Indirect calorimetry Indirect calorimetry was performed with a ventilated hood (Oxycon Pro™, CareFusion, Germany) in basal condition and during both steps of the clamp.

Skeletal muscle biopsies Muscle biopsies from the *m. vastus lateralis* (~75-100 mg) were collected in basal and hyperinsulinemic condition (at 30 minutes of step 2) under localized anesthesia, using a modified Bergström needle.[28] Muscle samples were divided into two parts, snap-frozen in liquid nitrogen and stored at -80°C until further analysis.

Calculations

Glucose R_a and R_d were calculated as the tracer infusion rate divided by the tracer-to-tracee ratio.[29] Endogenous glucose production (EGP) was calculated as the difference between the rates of R_a and glucose infusion. R_d and EGP were adjusted for kilograms LBM. The metabolic clearance rate of insulin (MCR_i) was computed according to Elahi *et al*.[30] Resting energy expenditure (REE), respiratory quotient (RQ) and substrate oxidation rates were determined as described by Simonson and DeFronzo.[31] Non-oxidative glucose disposal (NOGD) was calculated by subtracting the glucose oxidation rate from R_d. The hepatic insulin resistance index (HIR) was calculated as the product of non-insulin stimulated EGP and fasting serum insulin concentration.[32] Glucose metabolic clearance rate (MCR_g) was calculated as the rate of disappearance of glucose (R_d) divided by the serum glucose concentration (average of steady-state measurements).[33]

Laboratory analysis

Fasting serum glucose and triglycerides were measured on a Modular P800 analyser (Roche, The Netherlands), serum insulin and C-peptide levels on an Immulite 2500 (Siemens, The Netherlands), HbA_{1c} on an HPLC machine Primus Ultra 2 (Kordia, The Netherlands), and plasma FFAs were determined by a colorimetric method (Wako Chemicals, Germany). Arterialized whole blood glucose levels during the clamp were measured by glucose dehydrogenase-NAD technique (Precision Xtra Blood Glucose Monitoring System, Abbott USA). [6,6-$^2H_2$]-glucose enrichment was measured in a single analytical run using gas chromatography-mass spectrometry as described previously.[34]

DNA/RNA isolation and real-time RT-PCR

Total RNA was isolated from skeletal muscle biopsies (~25-30 mg) using the phenol-chloroform extraction method (Tripure RNA Isolation reagent, Roche, Germany), treated with a DNAse kit according to the manufacturer instruction (TURBO DNAse, Life Technologies, The Netherlands), and quantified by NanoDrop. First-strand cDNA were synthesized from 1 µg total RNA using a Superscript first strand synthesis kit (Invitrogen, The Netherlands). Real-time PCR assays were performed using specific primers sets (sequences provided on request) and SYBR Green on a StepOne Plus Real-time PCR system (Applied Biosystems, USA). mRNA expression was normalized to ribosomal protein S18 (Rps18) and expressed as arbitrary units. Genomic DNA was extracted using the Qiagen Tissue and Blood Kit (Qiagen, Germany) and concentrations were measured spectrophotometrically (GeneQuant, GE Healthcare, Germany). Mitochondrial (mtDNA) and nuclear (nDNA) DNA copy numbers were quantified as described before[35] and the mtDNA-to-nDNA-ratio was used as an index of mitochondrial density. A complete overview of all analysed genes can be found in **Supplemental Table 1**.

Western Blot

Skeletal muscle biopsies (~30-45mg) were homogenized by Ultra-Turrax (22.000 rpm; 2x5sec) in a 6:1 (v/w) ratio of ice-cold buffer containing: 50mM HEPES (pH 7.6), 50mM NaF, 50mM KCl, 5mM NaPPi, 1mM EDTA, 1mM EGTA, 5mM β-GP, 1mM Na3VO4, 1mM DTT, 1% NP40 and protease inhibitors cocktail (Complete, Roche, The Netherlands). Western blots were performed using phospho-specific (Ser473-PKB, phospho-Akt substrate, Ser2448-mTOR, and Thr389-S6K from Cell Signalling; Thr246-PRAS40 from Biosource) or total primary antibodies (Tubulin, Akt1+2, Akt substrate of 160kDa, mTOR and S6K from Cell Signalling; PRAS40 from Biosource; MitoProfile OXPHOS from AbCam; IRβ from Santa Cruz).[36] Blots were quantified by densitometric analysis using Image J software (NIH USA).

Statistical analysis

Data are presented as mean±SEM when normally distributed or as median (IQR) when not normally distributed. A mixed effects model was applied to assess mean differences before and after the intervention within and between groups, and to determine differences in diet effect. Groups and intervention were modelled as fixed effects and the subject specific deviances from the group mean were modelled as random effects. Nonparametric tests (Wilcoxon signed-rank test within group, Mann-Whitney between groups) were performed when appropriate. Significance level was set at $p<0.05$. Statistical analyses were performed using SPSS for Windows version 20.0 (IBM, USA).

RESULTS

Clinical characteristics

BMI did not differ between groups (South Asians: 20.9±0.6 *vs.* Caucasians: 22.2±0.6 kg/m^2, p=0.11), but South Asian subjects were significantly shorter and lighter (**Table 1**). The percentage of fat mass was significantly higher in South Asians on both study days, and, consequently, the percentage of LBM was lower. Waist circumference did not differ between groups. Fasting glucose and insulin levels were similar at baseline, but were significantly higher in South Asians after the HFHCD. Fasting C-peptide levels increased significantly to a similar degree in both groups. HbA_{1c} was higher in South Asians, as was LDL-cholesterol (2.77 (1.69) *vs.* 1.84 (0.91) mmol/L, p=0.03).

Diet and exercise

The physical activity level was comparable between both ethnicities (**Supplemental Table 2**). The South Asian diet consisted of fewer calories per day (South Asians: 2170±102 *vs.* Caucasians: 2593±100 kcal, p=0.008), but corrected for bodyweight the amount of calories was similar (South Asians: 34±2 *vs.* Caucasians: 35±1 kcal/day/kg, p=0.91). Both ethnicities ate the same percentage of fat (~30%), carbohydrates (~50%) and proteins (~16%). Both groups complied well with the diet. Mean daily calorie intake was ~55% higher compared to their normal diet, and ~54% of energy was derived from fat (**Supplemental Table 2**).

Fat distribution

No differences were found between groups for visceral and subcutaneous fat volumes both at baseline and after the HFHCD. Furthermore, no diet effect was observed. HTG increased significantly after the diet in both groups, but no differences between groups were observed (**Table 1**).

Table 1. Clinical characteristics, body composition, and fasting plasma and serum levels before and after a 5-day HFHCD in healthy, young South Asian men and matched white Caucasians.

	white Caucasians		South Asians	
	before HFHCD	after HFHCD	before HFHCD	after HFHCD
Clinical characteristics				
age (years)	22.1 ± 0.6		22.2 ± 0.7	
length (m)	1.84 ± 0.01		1.74 ± 0.02**	
weight (kg)	75.1 ± 1.8	75.6 ± 1.8	63.2 ± 2.3**	63.7 ± 2.3$^{\dagger\,**}$
BMI (kg/m^2)	22.2 ± 0.6	22.4 ± 0.6	20.9 ± 0.6	21.0 ± 0.6†
waist (cm)	81.3 ± 2.2	82.0 ± 2.3	78.9 ± 2.2	79.5 ± 2.6
Body composition				
fat mass (%)	11.3 ± 0.9	11.3 ± 0.8	15.1 ± 0.9*	14.7 ± 0.8*
visceral fat (mL)	104 ± 14	111 ± 12	120 ± 19	125 ± 18
subcutaneous fat (mL)	348 ± 54	363 ± 59	442 ± 61	432 ± 54
hepatic TG content (%)	1.7 ± 0.4	4.5 ± 0.8††	1.3 ± 0.4	3.0 ± 0.5††
Fasting plasma and serum levels				
HbA_{1c} (%)	5.0 ± 0.1		5.2 ± 0.1*	
HbA_{1c} (mmol/mol)	31.2 ± 0.5		33.8 ± 0.6*	
glucose (mmol/L)	5.1 ± 0.1	5.2 ± 0.1	5.3 ± 0.1	5.5 ± 0.1$^{\dagger\dagger\,*}$
insulin (pmol/L)	34 (32)	49 (46)	49 (29)	73 (34)$^{\dagger\dagger\,**\,\ddagger\ddagger}$
C-peptide (nmol/L)	0.47 (0.15)	0.57 (0.28)†	0.48 (0.11)	0.61 (0.18)††
FFA (g/L)	0.131 ± 0.01	0.121 ± 0.01	0.144 ± 0.01	0.151 ± 0.01
TG (mmol/L)	0.79 (0.26)	0.75 (0.67)	1.01 (0.65)	1.12 (0.77)

Data are presented as mean ± SEM or median (IQR). BMI, body mass index. TG, triglyceride. FFA, free fatty acid. † p<0.05, †† p<0.005 within group *vs.* before diet. * p<0.05, ** p<0.005 *vs.* Caucasians. ‡ p<0.05, ‡‡ p<0.005 diet effect *vs.* Caucasians.

Endogenous glucose production and rate of glucose disposal

During the hyperinsulinemic-euglycemic clamp glucose concentrations were similar within and between groups for both steps (**Table 2**). Clamp insulin levels were significantly higher in South Asians compared to Caucasians before and after the HFHCD; no diet effect was observed. The MCR_i was significantly lower in South Asians on both study days. EGP in basal and insulin-stimulated conditions was similar for both groups, despite higher insulin levels in insulin-stimulated conditions in South Asians. Furthermore, no diet effect was observed. However, the calculated HIR index was higher in South Asians compared to Caucasians (p=0.065 before diet, p=0.002 after diet), and showed a significant increase after the diet only in South Asians (p diet effect = 0.008). Suppression of EGP by insulin was comparable between groups and was around 24% in step 1 and 42% in step 2. Insulin-stimulated R_d in step 1 was similar for both groups on both occasions. In step 2 R_d was higher in South Asians compared to Caucasians before the diet (South

Asians: 48.7±2.9 *vs.* Caucasians: 41.7±2.9 µmol/kg_{LBM}/min; p=0.003). However, when corrected for insulin level, this difference disappeared and was almost reversed (p=0.052). After the diet R_d decreased significantly in South Asians despite similar insulin levels,

Table 2. Metabolic parameters of a 2-step hyperinsulinemic-euglycemic clamp with stable isotopes before and after a 5-day HFHCD in healthy, young South Asian men and matched white Caucasians.

	white Caucasians		South Asians	
	before HFHCD	after HFHCD	before HFHCD	after HFHCD
Basal steady state				
average glucose (mmol/L)	5.1 ± 0.1	5.1 ± 0.1	5.2 ± 0.1	5.4 ± 0.1†
average insulin (pmol/L)	41 (26)	41 (27)	49 (36)	68 (45)$^{\dagger *}$
EGP = R_d (µmol kg_{LBM}^{-1} min^{-1})	16.3 ± 0.4	17.0 ± 0.3	17.5 ± 0.5	17.5 ± 0.4
HIR (µmol $pmol^{-1}$ kg_{LBM}^{-1} min^{-1} L^{-1})	562 (600)	760 (778)	763 (512)	1269 (520)$^{\dagger\dagger **\ddagger}$
MCR_g (mL kg_{LBM}^{-1} min^{-1})	3.2 ± 0.1	3.3 ± 0.1	3.4 ± 0.1	3.3 ± 0.1
Step 1				
average glucose (mmol/L)	5.1 ± 0.1	5.2 ± 0.1	5.0 ± 0.1	5.2 ± 0.1
average insulin (pmol/L)	83 ± 12	89 ± 11	116 ± 6**	126 ± 11**
average C-peptide (nmol/L)	0.26 (0.13)	0.27 (0.13)	0.22 (0.14)	0.29 (0.15)†
EGP (µmol kg_{LBM}^{-1} min^{-1})	12.6 ± 0.5	13.4 ± 0.4	12.8 ± 0.4	12.8 ± 0.4
suppression EGP (%)	-22.6 ± 1.8	-21.6 ± 1.6	-26.8 ± 1.3	-27.2 ± 1.0
R_d (µmol kg_{LBM}^{-1} min^{-1})	15.3 ± 0.8	17.4 ± 1.2	16.2 ± 0.9	15.3 ± 1.1
MCR_g (mL kg_{LBM}^{-1} min^{-1})	3.0 ± 0.2	3.4 ± 0.2	3.2 ± 0.2	3.0 ± 0.2
Step 2				
average glucose (mmol/L)	4.8 ± 0.1	4.8 ± 0.1	4.6 ± 0.1	4.6 ± 0.1
average insulin (pmol/L)	276 ± 19	285 ± 19	396 ± 15**	386 ± 21**
average C-peptide (nmol/L)	0.07 (0.12)	0.07 (0.09)	0.06 (0.12)	0.08 (0.08)†
EGP (µmol kg_{LBM}^{-1} min^{-1})	10.0 ± 0.7	10.2 ± 0.5	9.7 ± 0.7	9.6 ± 0.5
suppression EGP (%)	-38.9 ± 3.5	-39.8 ± 2.7	-43.9 ± 3.0	-45.7 ± 2.3
R_d (µmol kg_{LBM}^{-1} min^{-1})	41.7 ± 2.9	41.0 ± 2.8	48.7 ± 2.9**	39.0 ± 2.1$^{\dagger\dagger \ddagger\ddagger}$
R_d / insulin (µmol L^{-1} kg_{LBM}^{-1} min^{-1} mU^{-1})	1.14 ± 0.13	1.07 ± 0.12	0.87 ± 0.07	0.72 ± 0.05$^{\dagger *}$
MCR_i (mL m^{-2} min^{-1})	1076 (397)	1054 (270)	735 (70)**	771 (164)$^{\dagger **\ddagger}$
MCR_g (mL kg_{LBM}^{-1} min^{-1})	8.8 ± 0.7	8.7 ± 0.6	10.7 ± 0.8**	8.6 ± 0.5$^{\dagger\dagger \ddagger\ddagger}$

Data are presented as mean ± SEM or median (IQR). Due to hypoglycemia in the last part of step 2 of the clamp, two South Asian subjects on occasion 1 and one Caucasian subject on occasion 2 were excluded in the analysis of step 2. EGP=endogenous glucose production. R_d=rate of glucose disposal. HIR=hepatic insulin resistance. MCR_g=metabolic clearance rate of glucose. MCR_i=metabolic clearance rate of insulin. † p<0.05, †† p < 0.005 within group *vs.* before diet. * p<0.05, ** p<0.005 *vs.* Caucasians. ‡ p<0.05, ‡‡ p<0.005 diet effect *vs.* Caucasians.

whereas no diet effect was found in Caucasians (South Asians: 39.0±2.1 μmol/kg_{LBM}/min (p<0.001) *vs.* Caucasians: 41.0±2.8 μmol/kg_{LBM}/min (p=0.78); p diet effect = 0.002).

Glucose and lipid oxidation rates

REE, corrected for LBM, RQ, substrate oxidation rates and NOGD in basal condition and step 1 of the clamp were comparable for both groups before and after the HFHCD (**Table 3**). In step 2, however, glucose oxidation increased significantly in South Asians, whereas no diet effect was observed in Caucasians. Interestingly, NOGD in step 2 was significantly higher in South Asians compared to Caucasians at baseline (p<0.001), but decreased significantly after the HFHCD only in South Asians (South Asians: 34.4±4.0 *vs.* 19.3±2.0 μmol/kg_{LBM}/min (p<0.001), Caucasians: 24.1±2.1 *vs.* 23.8±1.6 μmol/kg_{LBM}/min (p=0.87); p diet effect < 0.001).

Table 3. Parameters for indirect calorimetry before and after a 5-day HFHCD in healthy, young South Asian men and matched Caucasians.

	white Caucasians		South Asians	
	before HFHCD	after HFHCD	before HFHCD	after HFHCD
Basal				
REE (kcal/day)	1469 ± 50	1523 ± 38	1220 ± 31**	1224 ± 22**
REE (kcal day^{-1} kg_{LBM}^{-1})	22.4 ± 0.7	22.7 ± 0.5	23.0 ± 0.9	22.8 ± 0.9
RQ	0.88 ± 0.01	0.87 ± 0.01	0.87 ± 0.02	0.89 ± 0.02
glucose oxidation (μmol kg_{LBM}^{-1} min^{-1})	14.3 ± 1.0	13.6 ± 1.1	13.9 ± 1.3	14.7 ± 1.5
lipid oxidation (μmol kg_{LBM}^{-1} min^{-1})	2.4 ± 0.3	2.7 ± 0.3	2.7 ± 0.4	2.4 ± 0.5
NOGD (μmol kg_{LBM}^{-1} min^{-1})	2.3 ± 0.7	3.7 ± 0.8	4.2 ± 1.2	3.5 ± 1.4
Step 1				
RQ	0.90 ± 0.02	0.91 ± 0.02	0.88 ± 0.02	0.90 ± 0.03
glucose oxidation (μmol kg_{LBM}^{-1} min^{-1})	16.2 ± 1.6	16.4 ± 1.6	14.3 ± 1.7	14.8 ± 1.5
lipid oxidation (μmol kg_{LBM}^{-1} min^{-1})	2.2 ± 0.5	1.9 ± 0.4	2.6 ± 0.5	2.3 ± 0.5
NOGD (μmol kg_{LBM}^{-1} min^{-1})	1.8 ± 0.9	2.8 ± 0.9	3.1 ± 1.2	2.5 ± 1.2
Step 2				
RQ	0.92 ± 0.02	0.93 ± 0.02	0.88 ± 0.02	0.95 ± 0.02†
glucose oxidation (μmol kg_{LBM}^{-1} min^{-1})	17.7 ± 1.5	18.2 ± 1.8	14.4 ± 1.2	19.2 ± 1.5†
lipid oxidation (μmol kg_{LBM}^{-1} min^{-1})	1.8 ± 0.4	1.6 ± 0.4	2.5 ± 0.4	1.4 ± 0.4
NOGD (μmol kg_{LBM}^{-1} min^{-1})	24.1 ± 2.1	23.8 ± 1.6	34.4 ± 4.0**	19.3 ± 2.0†† ‡‡

Data are presented as mean ± SEM. REE=resting energy expenditure. RQ=respiratory quotient. NOGD=non-oxidative glucose disposal rate. † p<0.05, †† p<0.005 within group *vs.* before diet. ** p<0.005 *vs.* Caucasians. ‡‡ p<0.005 diet effect *vs.* Caucasians.

Skeletal muscle signalling

The protein expression and phosphorylation state of key molecules involved in the insulin and mTOR signalling pathways were determined in basal condition and during the hyperinsulinemic-euglycemic clamp in skeletal muscle (**Figure 1**). A trend for a reduced IRβ expression was observed in South Asians. During hyperinsulinemia, the phosphorylation state of key proteins involved in the insulin/mTOR pathway (PKB, AS160, PRAS40, mTOR and S6K1) was significantly increased when compared to basal, as expected (**Figure 1**). No obvious differences were observed between groups whatever the conditions.

Skeletal muscle metabolic gene expression

The skeletal muscle expression of key metabolic genes involved in the regulation of glucose and fatty acid metabolism was determined (**Supplemental Table 1**).

At baseline, no significant differences between groups were observed in the transcript levels of all analysed genes. The HFHCD induced significant downregulation of SLC2A4, GSK3A, GYS1, AGL, PPP1R3A, PDK2, ACACA, PPARA and PPARD mRNA expression in Caucasian subjects, with a comparable response in South Asians. Only PKM2 was differentially affected in South Asians in response to the HFHCD.

Skeletal muscle mitochondrial respiratory-chain content

The protein expression of several mitochondrial respiratory chain complex subunits was determined (**Figure 2A**). Although at baseline no differences were observed between groups, the expression of respiratory chain complex 1 and 2 was significantly increased after the HFHCD only in Caucasians (**Figure 2B**). However, the complex 2-on-complex 1 ratio, as a measure of change in fat *vs.* glucose oxidation, was not significantly different between both ethnicities (**Figure 2C**). The mtDNA-on-nDNA-ratio was significantly lower in South Asians compared to Caucasians, but was not affected in response to the diet (**Figure 2D**). Of note, the mRNA expression of key genes involved in mitochondrial biogenesis and tricarboxylic acid cycle was not different between groups, whatever the conditions (**Supplemental Table 1**).

DISCUSSION

This is the first study in South Asians in which a 2-step hyperinsulinemic-euglycemic clamp with stable isotopes was performed to measure peripheral and hepatic insulin sensitivity, and the first one in this ethnicity which assessed the effect of HF-feeding on both insulin sensitivity and skeletal muscle insulin and mTOR signalling. Strikingly, a 5-day HFHCD was already sufficient to impair insulin-stimulated (non-oxidative) glucose disposal in South Asians, while such an effect was not observed in Caucasians.

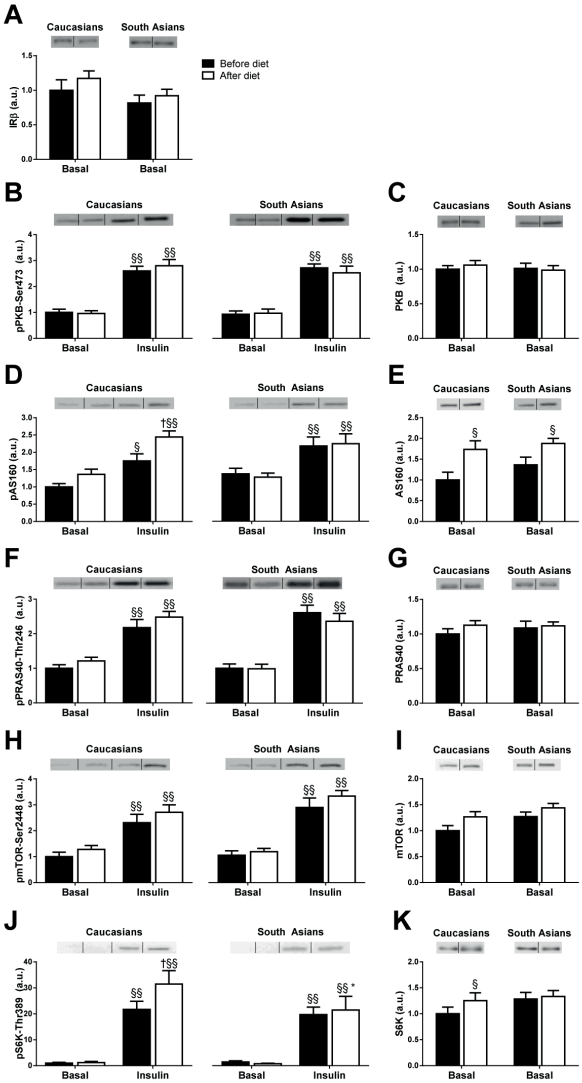


Figure 1. Insulin and mTOR signalling in skeletal muscle from healthy, young South Asian men and matched white Caucasians before (black bars) and after (white bars) a 5-day HFHCD. The protein expression of A. IRβ, B. Ser473-PKB, C. PKB, D. phospho-AS160, E. AS160, F. Thr246-PRAS40, G. PRAS40, H. Ser2448-mTOR, I. mTOR, J. Thr389-S6K, and K. S6K, were assessed by Western Blot. The phosphorylation state in basal and hyperinsulinemic (step 2) conditions (B, D, F, H, J), or the protein expression in basal conditions (A, C, E, G, I, K) are shown. Representative blots for one subject per group are shown. Results are normalized to Caucasian subjects (before diet, basal condition) and expressed as mean ± SEM. Due to a small amount of tissue two Caucasian subjects were excluded for Western Blot analysis. † $p<0.05$ within group *vs.* before diet. § $p<0.05$, §§ $p<0.005$ within groups *vs.* basal condition. * $p<0.05$ *vs.* Caucasians. IRβ, insulin receptor isoform β. PKB, protein kinase B. AS160, Akt substrate of 160 kDa. PRAS40, Proline rich Akt substrate of 40 kDa. mTOR, mammalian target of rapamycin. S6K1, ribosomal protein S6 kinase β1.

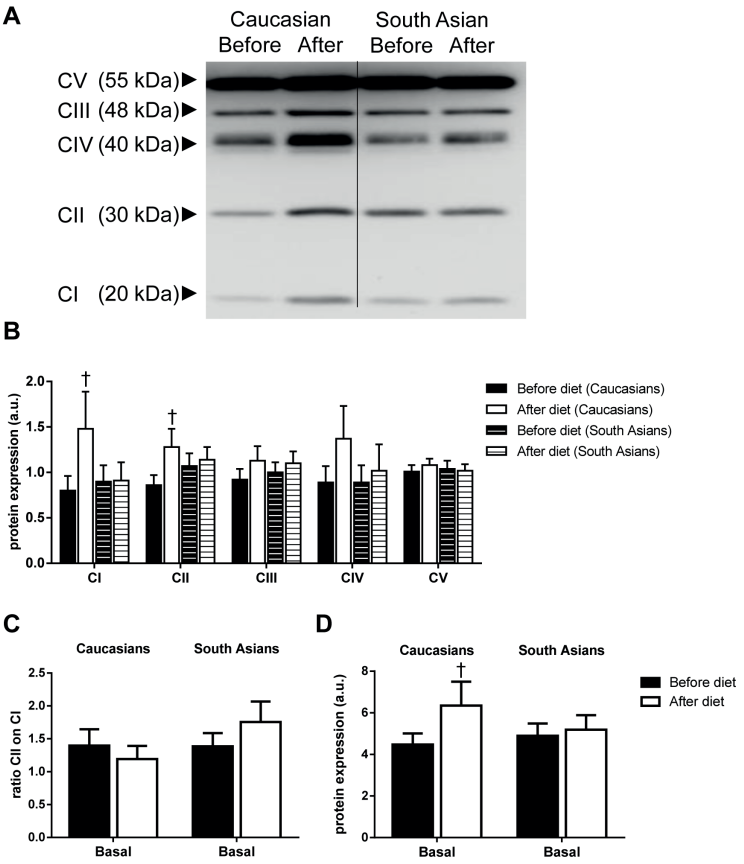


Figure 2. Protein expression of mitochondrial respiratory-chain subunits in skeletal muscle from healthy, young South Asian (striped bars) men and matched white Caucasians (closed bars) before (black bars) and after (white bars) a 5-day HFHCD. A. Representative blots for one subject per group. B. The expression of various mitochondrial-respiratory chain subunits (CI: NDUFB8, CII: SDHB, CIII: UQCRC2, CIV: MTCO1, CV: ATP5A) were assessed by Western Blot in basal condition. C. The respiratory-chain complex 2-on-complex 1 ratios were calculated. D. The mtDNA on nDNA ratio as assessed by qPCR in basal condition (n=7/12 (Caucasian/South Asian)). Results are normalized to Caucasian subjects (before diet) and expressed as mean ± SEM. Due to a small amount of tissue two Caucasian subjects were excluded for Western Blot analysis. † $p<0.05$ within group *vs.* before diet. * $p<0.05$ *vs.* Caucasians. CI–V, mitochondrial respiratory chain subunits I–V.

Baseline comparisons

In contrast to other studies, waist fat distribution and HTG did not significantly differ between both ethnicities.[13;14;37;38] In addition, we did not find higher fasting serum insulin levels,[14;37-41] nor lower peripheral insulin sensitivity in South Asians compared to Caucasians at baseline in both basal and insulin-stimulated conditions.[12;38;40-42] Instead, South Asians seemed to have even higher insulin-stimulated peripheral insulin sensitiv-

ity. However, insulin levels during the clamp were higher in South Asians on both study days, which is in line with other studies.[40-42] After correction for insulin levels, the difference in R_d between groups disappeared and was almost reversed. The higher insulin levels were presumably due to a lower MCR_i in South Asians, which has been shown before.[40] The lower MCR_i together with the higher HIR index in South Asians indicates lower hepatic insulin sensitivity both at baseline and after the diet.

The difference in above-mentioned findings compared to literature might be explained by the relatively young age, low BMI and sex (no females were included) of our subjects, geographical differences as reflected by dietary and/or other acculturation changes, and/or the small sample size (despite power calculation beforehand).

Response to a 5-day HFHCD

The mean daily calorie intake during the HFHCD was ~55% higher compared to their normal diet, and both groups reached ~54% of energy derived from fat compared to ~30% of their normal daily energy intake. HTG increased significantly after the diet in both groups, indicating good compliance to the diet, and consistent with a previous study in which young, healthy Caucasian males were subjected to a 3-day HF-diet.[26] In contrast, fasting glucose and insulin levels increased significantly only in South Asians. No effect of the diet on basal EGP or on the capacity of insulin to suppress EGP was observed in either group, although the HIR index, which corrects EGP for insulin level,[32] was significantly increased in South Asians only. Strikingly, insulin-stimulated R_d was significantly impaired after the diet in South Asians, whereas no diet effect was observed in Caucasians.

The response to a HF-diet on (skeletal muscle) insulin sensitivity in people of Caucasian descent is variable in the literature, depending on the percentage of fat and carbohydrates, duration of the diet, amount of calories (eucaloric or hypercaloric), effect on bodyweight, and method used to assess insulin sensitivity. In general, HF-diets of several hours up to 3 days induce whole-body insulin resistance,[43;44] whereas after HF-diets of several days up to 3 weeks usually no effect is seen on insulin sensitivity.[45-48] This difference in effect on insulin sensitivity might be attributed to a greater intramuscular lipid storage and/or use after several days, compensating for the increase in FFA availability induced by the HF-diet.[47]

The impairment in insulin-stimulated R_d after the diet in South Asians appears to be due to a decrease in NOGD, suggesting a defect in glycogen storage. Impaired non-oxidative glucose disposal is the main defect observed in patients with type 2 diabetes.[49] Interestingly, at baseline insulin-stimulated NOGD was significantly higher in South Asians compared to Caucasians, but this was possibly due to the higher insulin levels in South Asians. Because of the impairment in NOGD in South Asians after the diet, we also analysed proteins (Supplemental figure 1) and genes involved in glycolysis

and glycogen synthesis. However, no obvious differences were found between groups. The mRNA expression of GYS was significantly reduced in both groups after the diet (**Supplemental table 1**). Of note, in contrast to what was observed in South Asians in the present study, in several short-term HF-diet studies in Caucasians an increase in NOGD and a decrease in glucose oxidation was observed,[45;47;48;50] accompanied by an increase in skeletal muscle mRNA level of pyruvate dehydrogenase kinase 4 (PDK4) and a corresponding decrease in pyruvate dehydrogenase enzyme complex (PDH) in basal and insulin-stimulated conditions.[44;47;48] In the present study, PDK4 was not affected by the diet, and PDH was reduced only in South Asians (**Supplemental Table 1**). Therefore, it would have been interesting to determine skeletal muscle glycogen content. Further research is required to clarify the pathophysiological relevance of these apparent paradoxical findings in glycogen metabolism in South Asians.

The nutrient-sensing mTOR-pathway is mostly known for its regulating role in cellular proliferation and growth but it was also recently shown to be involved in key metabolic processes.[16] Therefore, it constitutes an interesting and relevant pathway to be investigated in the context of increased insulin resistance together with increased ectopic fat deposition in South Asians *vs.* Caucasians. Interestingly, mTORC1 appears to have negative effects on insulin signalling.[17] There are various mechanisms through which this negative feedback loop of mTORC1 on insulin signalling is initiated. When activated by mTORC1, downstream target S6K1 can suppress IRS-1 via direct phosphorylation of IRS1 on multiple serine residues, and via transcription repression of IRS1 gene expression. Additionally, mTORC1 directly interacts with IRS1 via raptor and phosphorylates IRS1 at Ser636/639. Furthermore, several biochemical and genetic studies have shown that mTORC1 plays a crucial role in the regulation of oxidative metabolism and mitochondrial biogenesis[18-21] as well as in lipid metabolism.[22] In particular, mTORC1 seems to suppress FA beta-oxidation.[21;23;24] Therefore, we hypothesized that differences in mTOR activity between the two ethnicities might underlie or contribute to the increased risk of insulin resistance and type 2 diabetes in South Asians. However, we did not find obvious differences in the mTOR-pathway between or within groups, neither at baseline nor after a 5-day HFHCD. Additionally, apart from a small difference in diet effect on respiratory chain complex subunits 1 and 2, we did not observe relevant differences in diet effect on skeletal muscle insulin signalling, mitochondrial density and expression of genes involved in oxidative phosphorylation and mitochondrial biogenesis that could explain the diet-induced impairment in insulin-stimulated R_d in South Asians, which is in line with a previous study in which young, healthy Caucasian males were subjected to a 5-day HFHCD.[46] The fact that we did not find obvious differences between groups might be explained by the relatively good health of our subjects and/or the small sample size. Of note, to confirm our findings on mitochondrial function other mitochondrial markers,

such as *ex vivo* determination of activities of mitochondrial respiratory-chain complexes and citrate synthase activity should be measured in future studies.

Only two other studies have been performed before in South Asians in which skeletal muscle biopsies were obtained to assess insulin signalling and/or mitochondrial function, and none assessed the mTOR-pathway. Nair *et al.* found no impairment in mitochondrial function in healthy, middle-aged South Asians, even despite the finding that they were more insulin resistant than matched Caucasians.[12] Correspondingly, Hall and colleagues reported that healthy, young, lean South Asian males did not exhibit lower expression of skeletal muscle oxidative and lipid metabolism genes compared to matched white Caucasians, and that mtDNA-to-nDNA-ratio, an index of mitochondrial content, did not significantly differ between groups, although a trend for a lower ratio in South Asians was observed.[11] Thus, both studies concluded that mitochondrial dysfunction did not account for the observed insulin resistance in South Asians, which is in line with our present findings concerning the effect of a HFHCD. Additionally, Hall's study showed that South Asians had reduced skeletal muscle protein expression of key insulin signalling proteins in the fasted state.[11] In that study, insulin sensitivity, as measured from the Matsuda insulin sensitivity index, was however significantly lower in South Asians. Thus, these subjects might have been more insulin resistant, explaining the reduced expression of insulin signalling proteins as compared to our study. Other possibilities for the different findings on insulin signalling are the larger group size in the study of Hall, and/or geographical differences as reflected by dietary and/or other acculturation changes.

Finally, we cannot exclude the possibility that white adipose tissue might have contributed to the diet-induced impairment in insulin-stimulated R_d in South Asians. Indeed, about 10-20% of whole-body glucose uptake occurs in white adipose tissue, which corresponds to the observed reduction in R_d in South Asians (mean percentage decrease: 20±5%).

In conclusion, we showed that a 5-day HFHCD is already sufficient to affect insulin-stimulated (non-oxidative) glucose disposal in healthy, young, lean South Asian males, whereas no diet effect was found in age- and BMI-matched Caucasians, suggesting that the propensity of South Asians to develop type 2 diabetes may be partly explained by the way they adapt to HF western food. The mTOR-pathway does not seem to be involved, at least in skeletal muscle. These findings might provide new leads for further investigation aimed to elucidate the pathogenesis of insulin resistance and type 2 diabetes in South Asians.

REFERENCES

1. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res Clin Pract* 2011;94(3):311-21.
2. Anjana RM, Pradeepa R, Deepa M, Datta M, Sudha V, Unnikrishnan R *et al*. Prevalence of diabetes and prediabetes (impaired fasting glucose and/or impaired glucose tolerance) in urban and rural India: phase I results of the Indian Council of Medical Research-INdia DIABetes (ICMR-INDIAB) study. *Diabetologia* 2011;54(12):3022-7.
3. Becker E, Boreham R, Chaudhury M, Craig R, Deverill C, Doyle M *et al*. Health Survey for England 2004. Volume 1. The health of minority ethnic groups. In: Sproston K, Mindell J, editors. *Cardiovascular disease and diabetes*. 1st ed. Leeds: The Information Centre; 2006. p. 63-94.
4. Bindraban NR, van Valkengoed IG, Mairuhu G, Holleman F, Hoekstra JB, Michels BP *et al*. Prevalence of diabetes mellitus and the performance of a risk score among Hindustani Surinamese, African Surinamese and ethnic Dutch: a cross-sectional population-based study. *BMC Public Health* 2008;8:271.
5. Chiu M, Austin PC, Manuel DG, Shah BR, Tu JV. Deriving ethnic-specific BMI cutoff points for assessing diabetes risk. *Diabetes Care* 2011;34(8):1741-8.
6. Simmons D, Williams DR, Powell MJ. Prevalence of diabetes in a predominantly Asian community: preliminary findings of the Coventry diabetes study. *BMJ* 1989;298.
7. Chandie Shaw PK, Baboe F, van Es LA, van der Vijver JC, van de Ree MA, de Jonge N *et al*. South-Asian type 2 diabetic patients have higher incidence and faster progression of renal disease compared with Dutch-European diabetic patients. *Diabetes Care* 2006;29(6):1383-5.
8. Chaturvedi N, Fuller JH. Ethnic differences in mortality from cardiovascular disease in the UK: do they persist in people with diabetes? *J Epidemiol Community Health* 1996;50(2):137-9.
9. McKeigue PM, Ferrie JE, Pierpoint T, Marmot MG. Association of early-onset coronary heart disease in South Asian men with glucose intolerance and hyperinsulinemia. *Circulation* 1993;87(1):152-61.
10. Wilkinson P, Sayer J, Laji K, Grundy C, Marchant B, Kopelman P *et al*. Comparison of case fatality in south Asian and white patients after acute myocardial infarction: observational study. *BMJ* 1996;312(7042):1330-3.
11. Hall LM, Moran CN, Milne GR, Wilson J, MacFarlane NG, Forouhi NG *et al*. Fat oxidation, fitness and skeletal muscle expression of oxidative/lipid metabolism genes in South Asians: implications for insulin resistance? *PLoS One* 2010;5(12):e14197.
12. Nair KS, Bigelow ML, Asmann YW, Chow LS, Coenen-Schimke JM, Klaus KA *et al*. Asian Indians have enhanced skeletal muscle mitochondrial capacity to produce ATP in association with severe insulin resistance. *Diabetes* 2008;57(5):1166-75.
13. Anand SS, Tarnopolsky MA, Rashid S, Schulze KM, Desai D, Mente A *et al*. Adipocyte hypertrophy, fatty liver and metabolic risk factors in South Asians: the Molecular Study of Health and Risk in Ethnic Groups (mol-SHARE). *PLoS One* 2011;6(7):e22112.
14. Petersen KF, Dufour S, Feng J, Befroy D, Dziura J, Dalla MC *et al*. Increased prevalence of insulin resistance and nonalcoholic fatty liver disease in Asian-Indian men. *Proc Natl Acad Sci U S A* 2006;103(48):18273-7.
15. Snel M, Jonker JT, Schoones J, Lamb H, de Roos A, Pijl H *et al*. Ectopic fat and insulin resistance: pathophysiology and effect of diet and lifestyle interventions. *Int J Endocrinol* 2012;2012:983814.
16. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012;149(2):274-93.

17. Copps KD, White MF. Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2. *Diabetologia* 2012;55(10):2565-82.
18. Cunningham JT, Rodgers JT, Arlow DH, Vazquez F, Mootha VK, Puigserver P. mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex. *Nature* 2007;450(7170):736-40.
19. Le Bacquer O, Petroulakis E, Paglialunga S, Poulin F, Richard D, Cianflone K *et al*. Elevated sensitivity to diet-induced obesity and insulin resistance in mice lacking 4E-BP1 and 4E-BP2. *J Clin Invest* 2007;117(2):387-96.
20. Schieke SM, Phillips D, McCoy JP, Jr., Aponte AM, Shen RF, Balaban RS *et al*. The mammalian target of rapamycin (mTOR) pathway regulates mitochondrial oxygen consumption and oxidative capacity. *J Biol Chem* 2006;281(37):27643-52.
21. Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M *et al*. Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature* 2004;431(7005):200-5.
22. Ricoult SJ, Manning BD. The multifaceted role of mTORC1 in the control of lipid metabolism. *EMBO Rep* 2013;14(3):242-51.
23. Peng T, Golub TR, Sabatini DM. The immunosuppressant rapamycin mimics a starvation-like signal distinct from amino acid and glucose deprivation. *Mol Cell Biol* 2002;22(15):5575-84.
24. Sipula IJ, Brown NF, Perdomo G. Rapamycin-mediated inhibition of mammalian target of rapamycin in skeletal muscle cells reduces glucose utilization and increases fatty acid oxidation. *Metabolism* 2006;55(12):1637-44.
25. Hammer S, van der Meer RW, Lamb HJ, de Boer HH, Bax JJ, de Roos A *et al*. Short-term flexibility of myocardial triglycerides and diastolic function in patients with type 2 diabetes mellitus. *Am J Physiol Endocrinol Metab* 2008;295(3):E714-E718.
26. van der Meer RW, Hammer S, Lamb HJ, Frolich M, Diamant M, Rijzewijk LJ *et al*. Effects of short-term high-fat, high-energy diet on hepatic and myocardial triglyceride content in healthy men. *J Clin Endocrinol Metab* 2008;93(7):2702-8.
27. Sleddering MA, Snel M, Streefland TC, Pijl H, Jazet IM. Short-term topiramate treatment does not improve insulin sensitivity or secretion in obese insulin-resistant women. *Eur J Endocrinol* 2012;167(6):839-45.
28. Bergstrom J. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. *Scand J Clin Lab Invest* 1975;35(7):609-16.
29. Steele R. Influences of glucose loading and of injected insulin on hepatic glucose output. *Ann N Y Acad Sci* 1959;82:420-30.
30. Elahi D, Nagulesparan M, Hershcopf RJ, Muller DC, Tobin JD, Blix PM *et al*. Feedback inhibition of insulin secretion by insulin: relation to the hyperinsulinemia of obesity. *N Engl J Med* 1982;306(20):1196-202.
31. Simonson DC, DeFronzo RA. Indirect calorimetry: methodological and interpretative problems. *Am J Physiol* 1990;258(3 Pt 1):E399-E412.
32. Gastaldelli A, Natali A, Vettor R, Corradini SG. Insulin resistance, adipose depots and gut: interactions and pathological implications. *Dig Liver Dis* 2010;42(5):310-9.
33. Ferrannini E, Mari A. How to measure insulin sensitivity. *J Hypertens* 1998;16(7):895-906.
34. Gastaldelli A, Coggan AR, Wolfe RR. Assessment of methods for improving tracer estimation of non-steady-state rate of appearance. *J Appl Physiol* 1999;87(5):1813-22.
35. Szuhai K, Ouweland J, Dirks R, Lemaitre M, Truffert J, Janssen G *et al*. Simultaneous A8344G heteroplasmy and mitochondrial DNA copy number quantification in myoclonus epilepsy and

ragged-red fibers (MERRF) syndrome by a multiplex molecular beacon based real-time fluorescence PCR. *Nucleic Acids Res* 2001;29(3):E13.

36. Wijngaarden MA, van der Zon GC, Willems van Dijk KW, Pijl H, Guigas B. Effects of prolonged fasting on AMPK signaling, gene expression and mitochondrial respiratory-chain content in skeletal muscle from lean and obese individuals. *Am J Physiol Endocrinol Metab* 2013.
37. Lear SA, Humphries KH, Kohli S, Chockalingam A, Frohlich JJ, Birmingham CL. Visceral adipose tissue accumulation differs according to ethnic background: results of the Multicultural Community Health Assessment Trial (M-CHAT). *Am J Clin Nutr* 2007;86(2):353-9.
38. Raji A, Seely EW, Arky RA, Simonson DC. Body fat distribution and insulin resistance in healthy Asian Indians and Caucasians. *J Clin Endocrinol Metab* 2001;86(11):5366-71.
39. Boon MR, Karamali NS, de Groot CJ, van Steijn L, Kanhai HH, van der Bent C *et al*. E-Selectin is Elevated in Cord Blood of South Asian Neonates Compared with Caucasian Neonates. *J Pediatr* 2011;160(5):844-8.
40. Liew CF, Seah ES, Yeo KP, Lee KO, Wise SD. Lean, nondiabetic Asian Indians have decreased insulin sensitivity and insulin clearance, and raised leptin compared to Caucasians and Chinese subjects. *Int J Obes Relat Metab Disord* 2003;27(7):784-9.
41. Raji A, Gerhard-Herman MD, Warren M, Silverman SG, Raptopoulos V, Mantzoros CS *et al*. Insulin resistance and vascular dysfunction in nondiabetic Asian Indians. *J Clin Endocrinol Metab* 2004;89(8):3965-72.
42. Chandalia M, Abate N, Garg A, Stray-Gundersen J, Grundy SM. Relationship between generalized and upper body obesity to insulin resistance in Asian Indian men. *J Clin Endocrinol Metab* 1999;84(7):2329-35.
43. Bachmann OP, Dahl DB, Brechtel K, Machann J, Haap M, Maier T *et al*. Effects of intravenous and dietary lipid challenge on intramyocellular lipid content and the relation with insulin sensitivity in humans. *Diabetes* 2001;50(11):2579-84.
44. Pehleman TL, Peters SJ, Heigenhauser GJ, Spriet LL. Enzymatic regulation of glucose disposal in human skeletal muscle after a high-fat, low-carbohydrate diet. *J Appl Physiol* 2005;98(1):100-7.
45. Bisschop PH, de MJ, Ackermans MT, Endert E, Pijl H, Kuipers F *et al*. Dietary fat content alters insulin-mediated glucose metabolism in healthy men. *Am J Clin Nutr* 2001;73(3):554-9.
46. Brons C, Jensen CB, Storgaard H, Hiscock NJ, White A, Appel JS *et al*. Impact of short-term high-fat feeding on glucose and insulin metabolism in young healthy men. *J Physiol* 2009;587(Pt 10):2387-97.
47. Chokkalingam K, Jewell K, Norton L, Littlewood J, van Loon LJ, Mansell P *et al*. High-fat/low-carbohydrate diet reduces insulin-stimulated carbohydrate oxidation but stimulates nonoxidative glucose disposal in humans: An important role for skeletal muscle pyruvate dehydrogenase kinase 4. *J Clin Endocrinol Metab* 2007;92(1):284-92.
48. Cutler DL, Gray CG, Park SW, Hickman MG, Bell JM, Kolterman OG. Low-carbohydrate di*et al*ters intracellular glucose metabolism but not overall glucose disposal in exercise-trained subjects. *Metabolism* 1995;44(10):1264-70.
49. Shulman GI, Rothman DL, Jue T, Stein P, DeFronzo RA, Shulman RG. Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. *N Engl J Med* 1990;322(4):223-8.
50. Bisschop PH, Ackermans MT, Endert E, Ruiter AF, Meijer AJ, Kuipers F *et al*. The effect of carbohydrate and fat variation in euenergetic diets on postabsorptive free fatty acid release. *Br J Nutr* 2002;87(6):555-9.

Supplemental Table 1. Overview of metabolic gene expression analysis in skeletal muscle from young, healthy South Asian men and matched white Caucasians before and after a 5-day HFHCD.

Gene name	Gene symbol	Entrez gene	white Caucasians		South Asians		Interaction p-value
			before	after	before	after	
Glucose Metabolism							
Glucose transport & phosphorylation							
Insulin receptor	INSR	3643	1.00 ± 0.13	0.89 ± 0.11	0.86 ± 0.16	0.77 ± 0.14	0.99
Akt substrate of 160 kDa (AS160)	TBC1D4	9882	1.00 (0.88)	0.67 (0.64)	1.05 (0.64)	0.80 (1.07)	0.44
TBC1D1	TBC1D1	23216	1.00 ± 0.15	0.88 ± 0.11	1.05 ± 0.13	**0.68 ± 0.11**†	0.27
Solute carrier family 2, member 1 (GLUT-1)	SLC2A1	6513	1.00 (1.68)	1.63 (2.79)	1.01 (0.46)	1.33 (1.17)	0.20
Solute carrier family 2, member 4 (GLUT-4)	SLC2A4	6517	1.00 ± 0.26	**0.61 ± 0.12**†	0.70 ± 0.14	0.48 ± 0.09	0.44
Hexokinase 1	HK1	3098	1.00 (0.87)	1.01 (0.89)	1.28 (1.16)	1.12 (0.71)	0.61
Hexokinase 2	HK2	3099	1.00 ± 0.22	1.30 ± 0.36	0.40 ± 0.07	0.83 ± 0.21	0.90
Glycolysis							
Phosphofructokinase	PFKM	5213	1.00 (0.44)	1.00 (0.78)	1.10 (0.58)	**0.67 (0.48)**†	0.10
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	2597	1.00 (0.21)	0.81 (0.13)	0.86 (0.12)	**0.76 (0.14)**†	0.75
Pyruvate kinase	PKM2	5315	1.00 ± 0.07	0.89 ± 0.06	1.18 ± 0.13	**0.73 ± 0.09**††‡	**0.04**
Glycogen metabolism							
Glycogen synthase kinase 3α	GSK3A	2931	1.00 ± 0.23	**0.71 ± 0.11**†	0.82 ± 0.14	0.66 ± 0.09	0.46
Glycogen synthase kinase 3β	GSK3B	2932	1.00 (0.74)	1.17 (1.05)	1.26 (0.67)	1.87 (1.82)	0.28
Glycogen synthase 1	GYS1	2997	1.00 ± 0.16	**0.73 ± 0.11**†	0.98 ± 0.14	**0.60 ± 0.09**†	0.65
Glycogen phosphorylase	PYGM	5837	1.00 ± 0.11	0.70 ± 0.07	0.98 ± 0.15	**0.55 ± 0.07**†	0.42
UDP-glucose pyrophosphorylase 2	UGP2	7360	1.00 (0.87)	0.84 (0.51)	0.69 (0.80)	0.40 (0.44)	0.80
Glycogen debranching enzyme	AGL	178	1.00 (0.41)	**0.61 (0.37)**†	1.30 (1.13)	0.78 (0.54)	0.61
Glycogen-branching enzyme	GBE1	2632	1.00 (0.16)	0.88 (0.36)	0.99 (0.15)	0.98 (0.44)	0.95
Phosphorylase kinase α1	PHKA1	5255	1.00 (0.88)	1.05 (0.66)	1.13 (0.80)	0.94 (1.09)	0.65
Phosphorylase kinase γ1	PHKG1	5260	1.00 (0.91)	0.82 (0.24)	1.03 (0.80)	0.71 (0.47)	0.81
Protein phosphatase 1, regulatory subunit 3A	PPP1R3A	5506	1.00 (0.47)	**0.69 (0.54)**†	1.17 (0.69)	0.68 (0.62)	0.56

Supplemental Table 1. Overview of metabolic gene expression analysis in skeletal muscle from young, healthy South Asian men and matched white Caucasians before and after a 5-day HFHCD. (continued)

Gene name	Gene symbol	Entrez gene	white Caucasians		South Asians		Interaction p-value
			before	after	before	after	
Fatty acid metabolism							
Transcription factors							
PPARα	PPARA	5465	1.00 (0.49)	**0.70 (0.22)**†	0.80 (0.49)	0.93 (0.64)	0.15
PPARδ	PPARD	5467	1.00 ± 0.21	**0.69 ± 0.10**†	0.83 ± 0.15	0.68 ± 0.09	0.51
PPARγ	PPARG	5468	1.00 (0.36)	1.21 (0.40)	0.83 (0.43)	0.89 (0.31)	0.48
Fatty acid transport, synthesis & oxidation							
Lipoprotein lipase	LPL	4023	1.00 (1.47)	0.63 (0.78)	0.58 (1.28)	0.36 (0.43)	0.91
Fatty acid translocase/CD36	CD36	948	1.00 ± 0.06	1.05 ± 0.07	0.89 ± 0.07	0.89 ± 0.16	0.83
Acetyl-CoA carboxylase α	ACACA	31	1.00 (1.02)	**0.82 (0.40)**†	1.09 (0.39)	0.97 (0.32)	0.17
Acetyl-CoA carboxylase β	ACACB	32	1.00 ± 0.21	0.95 ± 0.16	0.86 ± 0.11	1.00 ± 0.13	0.43
Mitochondrial fatty acid transport							
CPT 1A	CPT1A	1374	1.00 (0.92)	0.71 (0.85)	0.76 (0.25)	0.89 (0.70)	0.19
CPT 1B	CPT1B	1375	1.00 ± 0.10	1.11 ± 0.09	1.01 ± 0.10	0.90 ± 0.11	0.14
CPT 2	CPT2	1376	1.00 ± 0.13	0.91 ± 0.10	1.04 ± 0.11	1.17 ± 0.19	0.28
AMP-activated protein kinase subunits							
AMPK α1	PRKAA1	5562	1.00 ± 0.15	0.70 ± 0.09	0.85 ± 0.11	0.73 ± 0.12	0.35
AMPK α2	PRKAA2	5563	1.00 (0.42)	0.72 (0.63)	1.05 (0.84)	0.74 (0.58)	1.00
AMPK β1	PRKAB1	5564	1.00 ± 0.14	0.79 ± 0.06	0.77 ± 0.11	0.88 ± 0.15	0.12
AMPK β2	PRKAB2	5565	1.00 ± 0.21	0.61 ± 0.12	0.89 ± 0.19	0.93 ± 0.19	0.28
AMPK γ1	PRKAG1	5571	1.00 ± 0.19	0.94 ± 0.19	1.22 ± 0.21	**0.65 ± 0.09**†‡	**0.05**
AMPK γ2	PRKAG2	51422	1.00 (0.33)	0.65 (0.59)	0.66 (0.21)	**0.49 (0.24)***	0.56
AMPK γ3	PRKAG3	53632	1.00 ± 0.35	0.96 ± 0.32	1.00 ± 0.23	1.18 ± 0.26	0.75

Supplemental Table 1. Overview of metabolic gene expression analysis in skeletal muscle from young, healthy South Asian men and matched white Caucasians before and after a 5-day HFHCD. (continued)

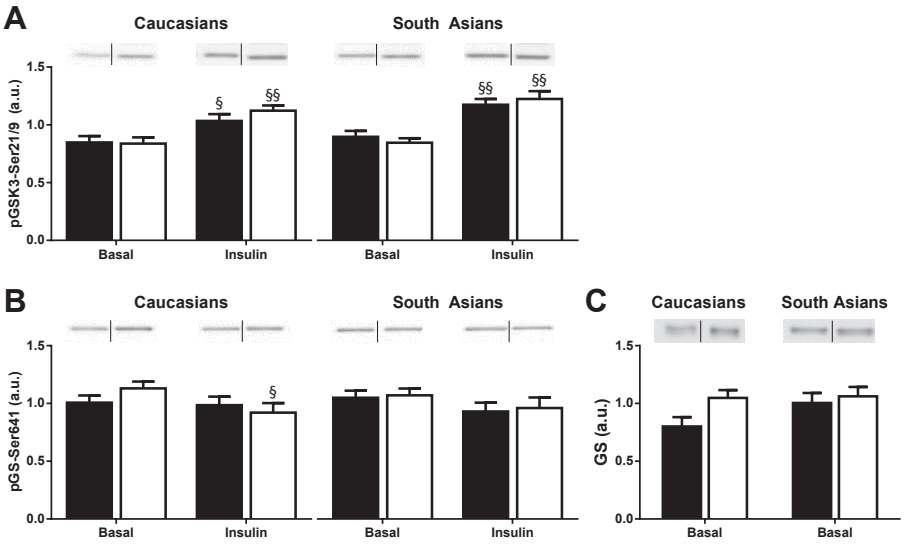
Gene name	Gene symbol	Entrez gene	white Caucasians		South Asians		Interaction p-value
			before	after	before	after	
Mitochondrial metabolism							
Mitochondrial biogenesis							
PPARα, coactivator 1α (PGC-1α)	PPARGC1A	10891	1.00 ± 0.16	**0.52 ± 0.09†**	0.73 ± 0.11	0.44 ± 0.09	0.35
PPARβ, coactivator 1b (PGC-1β)	PPARGC1B	133522	1.00 ± 0.16	0.92 ± 0.13	0.87 ± 0.16	0.62 ± 0.13	0.71
Transcription factor A	TFAM	7019	1.00 ± 0.09	0.80 ± 0.10	0.98 ± 0.14	**0.66 ± 0.12†**	0.48
Nuclear respiratory factor 1	NRF1	4899	1.00 ± 0.16	0.83 ± 0.12	0.98 ± 0.16	**0.71 ± 0.10†**	0.64
Nuclear factor (erythroid-derived 2)-like 2	NFE2L2	4780	1.00 (0.46)	0.83 (0.23)	0.80 (0.37)	0.78 (0.47)	0.85
Tricarboxylic acid cycle & electron transport chain							
Pyruvate dehydrogenase kinase 2	PDK2	5164	1.00 ± 0.12	**0.68 ± 0.13†**	0.87 ± 0.11	0.72 ± 0.06	0.23
Pyruvate dehydrogenase kinase 4	PDK4	5166	1.00 (1.14)	0.63 (0.94)	0.83 (1.65)	0.86 (0.52)	0.61
Pyruvate dehydrogenase α1	PDHA1	5160	1.00 ± 0.06	0.93 ± 0.06	1.04 ± 0.09	**0.75 ± 0.13†**	0.31
Pyruvate carboxylase	PC	5091	1.00 (0.38)	1.32 (0.39)	0.91 (0.37)	1.13 (0.48)	0.61
Citrate synthase	CS	1431	1.00 (0.52)	**0.70 (0.13)†**	0.94 (0.63)	0.59 (0.42)	0.52
ATPase, Ca++ transporting, cardiac muscle, fast twitch 1	ATP2A1	487	1.00 ± 0.06	0.79 ± 0.09	1.10 ± 0.12	**0.83 ± 0.05†**	0.25
Uncoupling protein 3	UCP3	7352	1.00 ± 0.10	0.94 ± 0.12	1.01 ± 0.16	0.88 ± 0.13	0.28
NADH dehydrogenase (ubiquinone) 1 β subcomplex, 8	NDUFB8	4714	1.00 (0.22)	0.94 (0.14)	0.94 (0.23)	0.94 (0.32)	0.25
Succinate dehydrogenase complex, subunit A	SDHA	6389	1.00 ± 0.06	1.02 ± 0.06	1.17 ± 0.06	**1.00 ± 0.07†**	0.06
Succinate dehydrogenase complex, subunit B	SDHB	6390	1.00 ± 0.04	**0.80 ± 0.06†**	0.92 ± 0.07	**0.72 ± 0.06†**	0.82
Ubiquinol-cytochrome c reductase core protein II	UQCRC2	7385	1.00 ± 0.09	0.88 ± 0.07	0.98 ± 0.11	0.64 ± 0.11	0.51
ATP synthase, H+ transporting, mitochondrial F1 complex, α subunit 1	ATP5A1	498	1.00 (0.30)	0.83 (0.27)	0.92 (0.13)	0.82 (0.28)	0.48

Data are presented as mean ± SEM or median (IQR). Due to a small amount of tissue one Caucasian and one South Asian subject were excluded for qPCR analysis. PPAR, Peroxisome proliferator-activated receptor. AMPK, AMP-activated protein kinase. CPT, Carnitine palmitoyltransferase. † p<0.05, †† p<0.005 within group *vs.* before diet. * p<0.05 between groups *vs.* Caucasians. ‡ p<0.05 diet effect *vs.* Caucasians (interaction p-value).

Supplemental Table 2. Activity level, normal dietary intake and intake during a 5-day HFHCD of healthy, young South Asian men and matched white Caucasians.

	white Caucasians	South Asians
Activity level		
exercise (min/week)	150 (203)	125 (210)
exercise (category)	2.5 (4)	2.5 (4)
activity factor	1.375 (0.22)	1.375 (0.26)
Normal diet		
total kcal per day	2593 ± 100	2170 ± 102 *
kcal per day per kg	34.6 ± 1.2	34.3 ± 2.0
fat (kcal/day)	835 ± 63	674 ± 60
carbohydrates (kcal/day)	1217 ± 47	1079 ± 48
protein (kcal/day)	404 ± 19	363 ± 35
fat (%)	31.6 ± 1.6	29.8 ± 1.9
carbohydrates (%)	47.6 ± 1.6	51.6 ± 2.1
protein (%)	15.8 ± 0.7	16.5 ± 1.0
Intake during a 5-day HFHCD		
total kcal per day	3824 ± 177	3453 ± 149
kcal per day per kg	51.1 ± 2.5	54.5 ± 2.9
fat (kcal/day)	2041 ± 92	1839 ± 62
carbohydrates (kcal/day)	1220 ± 81	1128 ± 65
protein (kcal/day)	439 ± 27	434 ± 31
fat (%)	54.0 ± 1.2	53.9 ± 1.2
carbohydrates (%)	31.6 ± 1.3	32.4 ± 0.9
protein (%)	11.4 ± 0.3	12.4 ± 0.6

Data are presented as mean ± SEM or median (IQR), n=12-11. Exercise categories: 0 = 0 minutes, 1 = 1-60 minutes, 2 = 61-120 minutes, 3 = 121-180 minutes, 4 = 181-240 minutes, 5 = 241-300 minutes. Activity factor according to the Harris-Benedict principle.



Supplemental Figure 1. Phosphorylation state of glycogen synthase kinase 3 and protein expression and phosphorylation state of glycogen synthase in skeletal muscle from healthy, young South Asian men and matched white Caucasians before (black bars) and after (white bars) a 5-day HFHCD. The protein expression of A. Ser21/9-GSK3, B. Ser641-GS, and C. GS were assessed by Western Blot. The phosphorylation state in basal and hyperinsulinemic (step 2) conditions (A, B), or the protein expression in basal conditions (C) are shown. Results are normalized to Caucasian subjects (before diet, basal condition) and expressed as mean ± SEM. Due to a small amount of tissue two Caucasian subjects were excluded for Western Blot analysis. § $p<0.05$, §§ $p<0.005$ within groups *vs.* basal condition. GS, glycogen synthase. GSK3, glycogen synthase kinase 3.

5 Middle-aged overweight South Asian men exhibit a different metabolic adaptation to short-term caloric restriction compared to white Caucasians

Leontine E.H. Bakker
Bruno Guigas
Linda D. van Schinkel
Gerard C.M. van der Zon
Trea C.M. Streefland
Jan B. van Klinken
Jacqueline T. Jonker
Hildo J. Lamb
Johannes W.A. Smit
Hanno Pijl
A. Edo Meinders
Ingrid M. Jazet

Diabetologia 2015; 58(1): 165-77

ABSTRACT

Aims. South Asians have a higher risk of developing type 2 diabetes than white Caucasians. The underlying cause is still poorly understood but might be related to differences in the regulation of energy/nutrient-sensing pathways in metabolic tissues and subsequent changes in whole-body substrate metabolism. In this study we investigated the whole-body and skeletal muscle metabolic adaptations to short-term caloric restriction in South Asian and white Caucasian volunteers.

Methods. 24 middle-aged overweight male South Asians and white Caucasians underwent a 2-step hyperinsulinemic-euglycemic clamp with skeletal muscle biopsies and indirect calorimetry before and after an 8-day very-low-calorie-diet. Abdominal fat distribution and hepatic triglyceride content (HTG) were assessed using MR-imaging/spectroscopy.

Results. South Asians had higher HTG than Caucasians, and exhibited elevated clamp insulin levels that likely reflect a lower insulin clearance rate. Despite higher insulin levels, endogenous glucose production rate was similar and (non-oxidative) glucose disposal rate (NOGD, R_d) was significantly lower in South Asians when compared to Caucasians, indicating impaired whole-body insulin sensitivity. Caloric restriction decreased abdominal fat mass and HTG in both groups. However, the caloric restriction induced shift from glucose towards lipid oxidation observed in Caucasians was impaired in South Asians, indicating whole-body metabolic inflexibility. Remarkably, although caloric restriction improved hepatic insulin sensitivity in both groups, R_d improved only in South Asians due to higher NOGD. At the molecular level, an increase in insulin-induced activation of the ERK-mTOR-S6K1 axis was found in South Asians, showing that skeletal muscle energy/nutrient-sensing pathways were differentially affected by caloric restriction.

Conclusions. We conclude that South Asians exhibit a different metabolic adaptation to short-term caloric restriction than white Caucasians.

INTRODUCTION

The rapid increase in type 2 diabetes prevalence worldwide has been associated with a Western, obesogenic lifestyle.[1] South Asians originating from the Indian sub-continent (India, Pakistan, Bangladesh, Nepal and Sri Lanka), who represent one fifth of the world's population, seem to have an exceptionally high susceptibility to develop the metabolic syndrome and type 2 diabetes in the context of the same environmental pressure when compared to other ethnicities.[2-5] A possible explanation for this excess risk might be related to differences in the regulation of energy/nutrient-sensing pathways in metabolic tissues thereby affecting whole-body substrate homeostasis.

Among these pathways, the nutrient and energy-sensing protein kinase mammalian target of rapamycin (mTOR), which regulates cell growth according to nutrient availability and cellular energy status,[6] is of major importance. The mTOR kinase interacts with several proteins to form two distinct complexes named mTOR complex 1 (mTORC1) and mTOR complex 2, which differ in their molecular composition, regulation, sensitivity to rapamycin, and downstream targets.[6] mTORC1 responds to insulin and other growth factors, stress, oxygen and nutrient levels and controls key cellular processes.[6] When active, mTORC1 promotes protein synthesis, cell growth and differentiation, and may inhibit insulin signalling by feedback regulation of the insulin receptor substrate 1 (IRS1). mTORC1 was also recently shown to play a crucial role in mitochondrial biogenesis and oxidative metabolism.[7-10] Caloric restriction reduces mTORC1 activity,[11] at least partly through activation of the AMP-activated protein kinase (AMPK), a key sensor of cellular energy status.[12;13]

We hypothesized that differences in the regulation of energy/nutrient-sensing pathways between people of South Asian and white Caucasian descent may affect whole-body glucose/lipid metabolism, and ultimately contribute to the increased risk of type 2 diabetes in South Asians. This study, therefore, assessed the effect of caloric restriction through an 8-day very low calorie diet (VLCD) on skeletal muscle energy/nutrient-sensing pathways, with a special focus on canonical insulin signalling and mTORC1 pathways, in both middle-aged overweight South Asian men and age- and BMI-matched white Caucasians.

METHODS

Participants

Twelve Dutch South Asian and twelve Dutch white Caucasian, overweight (BMI 25-30 kg/m^2) men, aged 40-50 years, with a waist circumference of >90 cm (South Asians) or >94 cm (Caucasians), and a positive family history for type 2 diabetes were enrolled via local advertisements. South Asian participants were all Dutch Hindostani, an ethnic group of South Asian origin composed of people who were all born in Surinam before moving to the

Netherlands. Participants underwent a medical screening including their medical history, a physical examination, blood chemistry tests and an oral glucose tolerance test (OGTT) to exclude individuals with type 2 diabetes according to the American Diabetes Association 2010 criteria. Other exclusion criteria were rigorous exercise, smoking and recent body weight change. The study was approved by the Medical Ethical Committee of the Leiden University Medical Centre and performed in accordance with the principles of the revised Declaration of Helsinki. Written informed consent was obtained from all volunteers prior to participation.

Study design

Participants were studied before and after an 8-day VLCD, consisting of three sachets of Modifast (Nutrition & Santé Benelux, Breda, The Netherlands) per day (~450 kcal/day, ~50g protein, 50-60g carbohydrates, 7g lipids and 15g dietary fibres). They were instructed not to alter life style habits, and not to perform physical activity in the last 48 hours before the study days. Magnetic resonance (MR) and metabolic studies were performed shortly before the start and on the eighth day of the diet, and one day before and one day after the VLCD, respectively.

MR studies

Abdominal fat depots were quantified with turbo spin echo MR-imaging using a 1.5 Tesla whole body MR-scanner (Gyroscan ACS-NT15, Philips, Best, The Netherlands) in postprandial state,[14] and hepatic triglyceride content (HTG) was assessed by proton MR-spectroscopy,[15], as described previously.[16]

Metabolic Studies

Participants underwent anthropometric measurements, a 7-h 2-step hyperinsulinemic-euglycemic clamp with stable isotopes, skeletal muscle biopsies and indirect calorimetry after an overnight fast, as described previously.[16] Body fat mass and lean body mass (LBM) were assessed by bioelectrical impedance analysis (BIA; Bodystat® 1500, Bodystat Ltd., UK). Laboratory analysis was done as described before.[16] Glucose appearance (R_a) and disposal (R_d), endogenous glucose production (EGP), metabolic clearance rate of insulin (MCR_i), hepatic insulin resistance index (HIR), resting energy expenditure (REE), respiratory quotient (RQ), substrate oxidation rates, and non-oxidative glucose disposal (NOGD) were calculated as described previously.[16] Metabolic flexibility was defined as the ability to increase lipid oxidation upon caloric restriction (change in fasting RQ in response to VLCD) and to switch from lipid to glucose oxidation upon insulin stimulation (change in RQ from the fasted to the insulin-stimulated state).[17]

DNA/RNA isolation and real-time RT-PCR

DNA/RNA isolation and real-time RT-PCR were done as previously described.[16] In short, total RNA was isolated from skeletal muscle biopsies. First-strand cDNA were synthe-

sized from 1 µg total RNA. Real-time PCR assays were performed using specific primers sets (sequences provided on request). mRNA expression was normalized to ribosomal protein S18 (Rps18) and expressed as arbitrary units. Genomic DNA was extracted for determination of mitochondrial (mtDNA) and nuclear (nDNA) DNA copy numbers.[18]

Western Blot

Skeletal muscle biopsies were homogenized as described previously.[16] Western blots were performed using phospho-specific (Ser473-PKB, phospho-Akt substrate, Thr202/Tyr204-ERK1/2, Thr1462-TSC2, Ser2448-mTOR, Thr389-S6K, Thr37/46-4EBP1, Ser21/9-GSK3, Ser641-GS, Thr172-AMPK, and Ser79-ACC from Cell Signaling; Thr246-PRAS40 from Biosource) or total primary antibodies (Tubulin, PKB, AS160, mTOR, S6K, AMPK, and ACC from Cell Signaling; IRβ and ERK1 from Santa Cruz; PRAS40 from Biosource; MitoProfile OXPHOS from AbCam).[19] Blots were quantified by densitometric analysis using Image J software (NIH, USA).

Statistical analysis

Data are presented as mean±SEM or as median (IQR) depending on normality distribution. A mixed effects model was applied to assess mean differences before and after the intervention within and between groups, and to determine differences in diet effect. Groups and intervention were modelled as fixed effects and the individual specific deviances from the group mean were modelled as random effects. Nonparametric tests (Wilcoxon signed-rank test within group, Mann-Whitney between groups) were performed when appropriate. $P<0.05$ was considered significant. Statistical analyses were performed using SPSS for Windows version 20.0 (IBM, USA).

RESULTS

Clinical characteristics

Mean age was 44.6±0.8 years. BMI did not differ between groups (South Asians: 28.4±0.4 *vs.* Caucasians: 28.1±0.5 kg/m^2, p=0.65), but South Asians tended to be lighter (p=0.055) and were significantly shorter (**Table 1**). The decreases in body weight (South Asians: -4.2±0.4% *vs.* Caucasians: -4.8±0.3%, p=0.14) and fat mass (South Asians: -3.7 (3.2)% *vs.* Caucasians: -5.3 (4.8)%, p=0.17) induced by caloric restriction were from the same extent in both groups. Fasting glucose, non-esterified fatty acids (NEFA) and triacylglycerol levels were comparable between groups, whereas HbA_{1c} and fasting and OGTT insulin levels were significantly higher in South Asians (**Table 1**). Caloric restriction induced a significant similar decrease in fasting serum glucose, insulin and triacylglycerol levels. Plasma NEFAs increased in both groups, but significantly less in South Asians.

Fat distribution

No differences between groups were observed for visceral and subcutaneous fat volumes (**Table 1**). However, HTG was significantly higher in South Asians at baseline. Caloric restriction led to a significant decrease in visceral and subcutaneous fat volumes and HTG in both South Asians and Caucasians (**Table 1**).

Table 1. Clinical and metabolic characteristics before and after an 8-day VLCD in middle-aged overweight South Asian men and matched white Caucasians.

	white Caucasians		South Asians	
	before VLCD	after VLCD	before VLCD	after VLCD
Clinical characteristics				
age (years)	44.3 ± 1.1		44.9 ± 0.9	
length (m)	1.81 ± 0.02		1.75 ± 0.01**	
weight (kg)	92.6 ± 2.5	88.2 ± 2.5††	86.7 ± 1.4	83.2 ± 1.6†† ‡
body mass index (kg/m^2)	28.1 ± 0.5	26.8 ± 0.5††	28.4 ± 0.4	27.3 ± 0.4††
waist (cm)	103 ± 1.8	100 ± 1.6††	101 ± 1.6	98 ± 1.5††
Body composition				
fat mass (%)	23.1 ± 0.6	21.8 ± 0.6††	23.8 ± 0.6	23.0 ± 0.6
lean body mass (kg)	71.1 ± 1.6	68.8 ± 1.6††	66.1 ± 1.2*	64.0 ± 1.3†† *
visceral fat (mL)	360 ± 37	301 ± 27††	359 ± 40	307 ± 33†
subcutaneous fat (mL)	791 (213)	779 (223)†	802 (321)	776 (261)
hepatic triglyceride content (%)	5.2 (3.0)	2.9 (1.2)†	9.3 (8.7)*	4.4 (9.1) †† ‡
Fasting plasma and serum levels				
HbA_{1c} (%, mmol/mol)	5.2 (0.5), 33.0 (6)		5.5 (0.1)*, 36.5 (1)*	
glucose (mmol/L)	5.3 ± 0.2	4.5 ± 0.2††	5.3 ± 0.1	4.5 ± 0.1††
insulin (mU/L)	12.8 (6.4)	5.5 (4.9) ††	16.6 (8.1)*	6.5 (7.2)††
C-peptide (nmol/L)	0.61 (0.28)	0.34 (0.30)††	0.75 (0.19)	0.40 (0.42)††
non-esterified fatty acids (mmol/L)	0.53 ± 0.03	1.36 ± 0.13††	0.58 ± 0.04	0.85 ± 0.06† ** ‡‡
triacylglycerol (mmol/L)	1.29 (2.48)	0.89 (0.18)††	1.78 (2.91)	0.91 (0.25)††
Oral glucose tolerance test				
2 hour insulin (mU/L)	45 ± 5.5		101 ± 17*	
glucose AUC (mmol/L * h)	959 ± 32		1027 ± 58	
insulin AUC (mU/L * h)	5.8 ± 0.5*10^3		11.4 ± 0.8*10^3**	

Data are presented as mean±SEM or median (IQR). AUC, area under the curve. † p<0.05, †† p<0.005 within group *vs.* before diet. * p<0.05, ** p<0.005 *vs.* Caucasians. ‡ p<0.05, ‡‡ p<0.005 diet effect *vs.* Caucasians.

EGP and glucose disposal

During hyperinsulinemic-euglycemic clamp, glucose concentrations were comparable between groups whereas insulin levels were significantly higher in South Asians and were accompanied by a lower MCR_i when compared to Caucasians (**Table 2**). Despite these higher insulin levels, EGP was similar between groups, indicating higher hepatic insulin resistance in South Asians. Furthermore, the insulin-stimulated R_d in step 2 was lower in South Asians, indicating reduced peripheral insulin sensitivity compared to Caucasians. In response to caloric restriction, hepatic insulin sensitivity was improved

Table 2. Metabolic parameters of a 2-step hyperinsulinemic-euglycemic clamp with [6,6-$^2H_2$]-glucose before and after an 8-day VLCD in middle-aged overweight South Asian men and matched white Caucasians.

	white Caucasians		South Asians	
	before VLCD	after VLCD	before VLCD	after VLCD
Basal steady state				
average glucose (mmol/L)	5.4 ± 0.2	4.3 ± 0.2††	5.2 ± 0.1	4.6 ± 0.1††‡
average insulin (mU/L)	8.3 (5.3)	2.2 (2.3)††	13.0 (6.5)*	4.0 (4.9)††
EGP = R_d (μmol kg_{LBM}^{-1} min^{-1})	16.1 ± 0.5	12.4 ± 0.4††	15.4 ± 0.6	12.1 ± 0.3††
HIR (μmol $pmol^{-1}$ kg_{LBM}^{-1} min^{-1} L^{-1})	1359 ± 139	504 ± 74††	1809 ± 151*	699 ± 131††
Step 1				
average glucose (mmol/L)	5.7 ± 0.1	5.5 ± 0.1	5.4 ± 0.1	5.3 ± 0.1
average insulin (mU/L)	15.7 ± 1.3	14.0 ± 1.3	21.8 ± 1.5*	18.0 ± 1.0
average C-peptide (nmol/L)	0.41 (0.25)	0.27 (0.12)††	0.61 (0.28)*	0.22 (0.30)††
average free fatty acids (mmol/L)	0.21 ± 0.02	0.45 ± 0.04††	0.23 ± 0.02	0.41 ± 0.05††
EGP (μmol kg_{LBM}^{-1} min^{-1})	11.1 ± 0.5	7.7 ± 0.3††	10.4 ± 0.3	7.5 ± 0.4††
R_d (μmol kg_{LBM}^{-1} min^{-1})	11.9 ± 0.4	12.7 ± 1.0	11.5 ± 0.3	11.8 ± 0.6
Step 2				
average glucose (mmol/L)	5.0 ± 0.1	5.0 ± 0.1	5.1 ± 0.1	5.0 ± 0.1
average insulin (mU/L)	53.1 ± 2.9	50.8 ± 2.1	66.1 ± 3.0**	60.5 ± 1.9†**
average C-peptide (nmol/L)	0.14 (0.19)	0.08 (0.04)†	0.24 (0.25)*	0.12 (0.08)††
average free fatty acids (mmol/L)	0.09 ± 0.01	0.26 ± 0.05††	0.10 ± 0.01	0.18 ± 0.03
EGP (μmol kg_{LBM}^{-1} min^{-1})	7.9 ± 0.5	6.0 ± 0.3††	6.8 ± 0.6	5.5 ± 0.5††
R_d (μmol kg_{LBM}^{-1} min^{-1})	37.7 ± 2.3	34.9 ± 2.1	30.0 ± 3.4**	34.5 ± 2.9†‡
R_d / insulin (μmol L^{-1} kg_{LBM}^{-1} min^{-1} mU^{-1})	0.75 ± 0.08	0.71 ± 0.06	0.46 ± 0.05**	0.56 ± 0.04
MCR_i (mL m^{-2} min^{-1})	840 ± 47	827 ± 34	697 ± 32*	694 ± 24*

Data are presented as mean±SEM or median (IQR). EGP=endogenous glucose production. R_d=rate of glucose disposal. HIR=hepatic insulin resistance. MCRi=metabolic clearance rate of insulin. † $p<0.05$, †† $p < 0.005$ within group *vs.* before diet. * $p<0.05$, ** $p<0.005$ *vs.* Caucasians. ‡ $p<0.05$, ‡‡ $p<0.005$ diet effect *vs.* Caucasians.

to a similar extent in both groups. However, while no diet effect was observed in Caucasians, R_d in step 2 was significantly increased after caloric restriction in South Asians despite a slight decrease in insulin levels. When corrected for steady state insulin levels, this effect in South Asians was only borderline significant (p=0.08).

REE, substrate oxidation and NOGD

At baseline, both REE (corrected for LBM) and substrate oxidation rates were comparable between groups (**Table 3**). During hyperinsulinemic-euglycemic clamp, insulin suppressed fat oxidation and stimulated glucose oxidation to a similar degree in both groups. NOGD in step 2 was significantly lower in South Asians, despite higher insulin levels. Caloric restriction led to a reduction in REE and a shift in substrate metabolism from glucose towards lipid oxidation. However, this shift was significantly blunted in South Asians compared to Caucasians. The effect of insulin on substrate oxidation rates

Table 3. Indirect calorimetry parameters before and after an 8-day VLCD in middle-aged overweight South Asian men and matched white Caucasians.

	white Caucasians		South Asians	
	before VLCD	after VLCD	before VLCD	after VLCD
Basal				
REE (kcal/day)	1592 ± 35	1435 ± 39††	1422 ± 30**	1291 ± 35$^{\dagger\dagger *}$
REE (kcal day^{-1} kg$_{LBM}^{-1}$)	22.5 ± 0.5	21.0 ± 0.7††	21.6 ± 0.6	20.2 ± 0.5††
RQ	0.83 ± 0.01	0.74 ± 0.01††	0.84 ± 0.01	0.81 ± 0.01$^{\dagger ** \ddagger}$
glucose oxidation (μmol kg$_{LBM}^{-1}$ min^{-1})	10.1 ± 1.1	3.1 ± 0.8††	10.9 ± 0.7	7.7 ± 0.8$^{\dagger ** \ddagger}$
lipid oxidation (μmol kg$_{LBM}^{-1}$ min^{-1})	3.3 ± 0.2	5.2 ± 0.5††	3.1 ± 0.2	3.6 ± 0.2$^{** \ddagger}$
NOGD (μmol kg$_{LBM}^{-1}$ min^{-1})	5.1 ± 0.6	10.1 ± 1.2†	4.5 ± 0.9	4.9 ± 0.6*
Step 1				
RQ	0.83 ± 0.01	0.76 ± 0.01††	0.85 ± 0.01	0.80 ± 0.01$^{\dagger\dagger **}$
glucose oxidation (μmol kg$_{LBM}^{-1}$ min^{-1})	11.0 ± 0.6	3.8 ± 0.6††	11.7 ± 1.0	7.5 ± 0.8$^{\dagger\dagger **}$
lipid oxidation (μmol kg$_{LBM}^{-1}$ min^{-1})	3.3 ± 0.3	4.7 ± 0.3††	3.0 ± 0.3	3.8 ± 0.2$^{\dagger *}$
NOGD (μmol kg$_{LBM}^{-1}$ min^{-1})	1.3 ± 0.4	8.7 ± 1.4††	1.3 ± 0.4	4.1 ± 0.9
Step 2				
RQ	0.86 ± 0.01	0.79 ± 0.01††	0.89 ± 0.01	0.82 ± 0.01††
glucose oxidation (μmol kg$_{LBM}^{-1}$ min^{-1})	12.9 ± 0.6	6.5 ± 1.0††	15.2 ± 1.2	9.1 ± 1.0$^{\dagger\dagger *}$
lipid oxidation (μmol kg$_{LBM}^{-1}$ min^{-1})	2.6 ± 0.1	4.3 ± 0.4††	2.1 ± 0.2	3.5 ± 0.3††
NOGD (μmol kg$_{LBM}^{-1}$ min^{-1})	23.6 ± 2.3	28.0 ± 2.6	14.8 ± 3.0**	25.3 ± 3.0††

Data are presented as mean±SEM. REE=resting energy expenditure. RQ=respiratory quotient. NOGD=non-oxidative glucose disposal rate. † p<0.05, †† p<0.005 within group *vs.* before diet. ** p<0.005 *vs.* Caucasians. ‡‡ p<0.005 diet effect *vs.* Caucasians.

was attenuated in South Asians after the diet compared to baseline, whereas it tended to improve in Caucasians (p diet effect *vs.* Caucasians =0.057 for both glucose and lipid oxidation). Caloric restriction induced a significant increase in NOGD in step 2 only in South Asians despite slightly reduced insulin levels in this group.

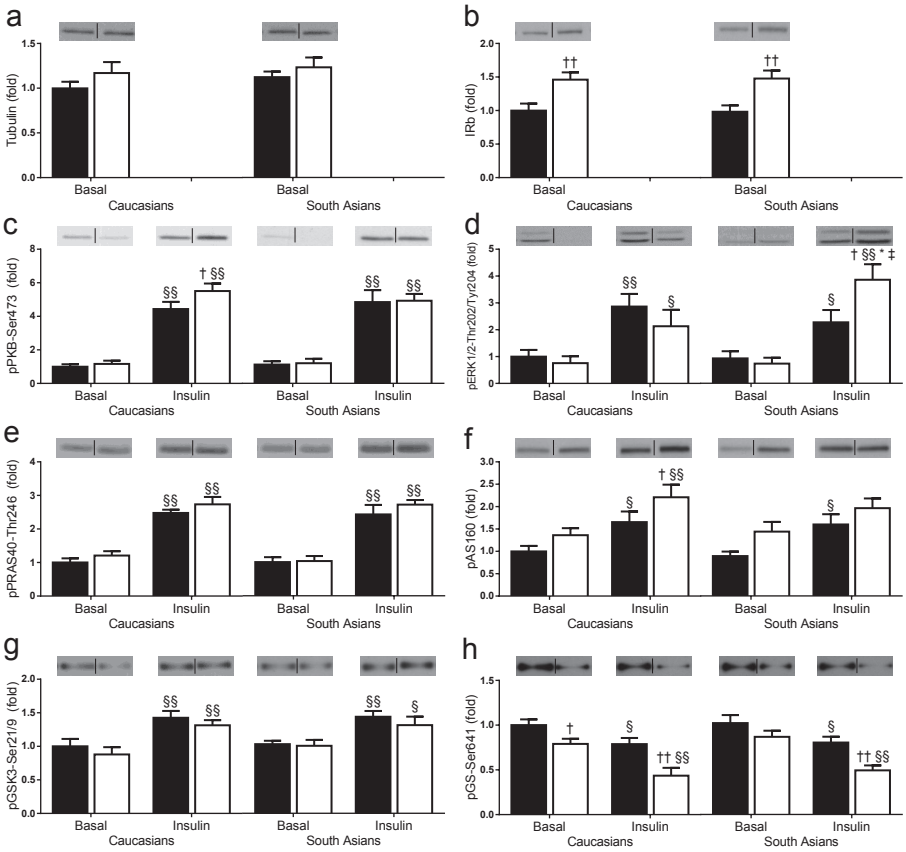


Figure 1. Effect of VLCD on insulin signalling pathways in skeletal muscle from South Asian and white Caucasian men in basal state and during a hyperinsulinemic-euglycemic clamp. The protein expression of tubulin (a), insulin receptor β (b) and the phosphorylation states of Ser473-PKB (c), Thr202/Tyr204-ERK (d), Thr256-PRAS40 (e), phospho-AS160 (f), Ser21/9-GSK3 (g) and Ser641-GS (h) were assessed by Western Blot in skeletal muscle from South Asian and white Caucasian volunteers before (black bars) and after (open bars) an 8-day VLCD in both basal and hyperinsulinemic states (step 2 of hyperinsulinemic-euglycemic clamp). Representative blots for one individual per group are shown. Results are normalized to Caucasian participants in basal state before VLCD and expressed as mean±SEM. † p<0.05, †† p<0.005 within group *vs.* before diet. § p<0.05, §§ p<0.005 within groups *vs.* basal condition. * p<0.05 *vs.* Caucasians. ‡ p<0.05 diet effect *vs.* Caucasians. IRβ, insulin receptor isoform β. PKB, protein kinase B. ERK, extracellular signal-regulated kinase. PRAS40, Proline rich Akt substrate of 40 kDa.AS160, Akt substrate of 160 kDa. GSK3, glycogen synthase kinase-3. GS, glycogen synthase.

Energy/nutrient-sensing signalling pathways in skeletal muscle

Insulin canonical pathway. Before diet, no significant differences between groups were observed in protein expression of IRβ and in both basal and insulin-induced phosphorylation of key proteins involved in the insulin canonical pathway (**Figure 1**). Caloric restriction induced a significant increase in protein expression of IRβ in South Asians and Caucasians (**Figure 1a**), whereas other proteins involved in insulin signalling were not affected (data not shown). The insulin-induced phosphorylation of PKB-Ser473, PRAS40-Thr246 and AS160 were slightly but significantly increased after caloric restriction in Caucasians but not in South Asians (**Figure 1c,e,f**). By contrast, insulin-induced phosphorylation of ERK-Thr202/Tyr204 was increased in South Asians, whereas it tended to decrease in Caucasians (**Figure 1d**). Finally, phosphorylation of GS-Ser641 was reduced in response to caloric restriction at both baseline and during clamp in both groups, suggesting enhanced insulin sensitivity that might promote skeletal muscle glycogen synthesis (**Figure 1g-h**).

mTOR signalling. At baseline, no significant differences between groups were observed in protein expression of mTOR and S6K1 (**Supplementary Figure 1**), nor in phosphorylation states of key upstream and downstream proteins involved in mTORC1 signalling, such as TSC2, S6K1 and 4EBP1 (**Figure 2**). Caloric restriction did not affect protein expression and phosphorylation states of TSC2, mTOR and S6K1 in basal condition, whereas 4E-BP1-Thr37/46 was slightly but significantly increased but only in Caucasians. Furthermore, except for 4E-BP1-Thr37/46 which was again significantly increased, the insulin-induced phosphorylation of most of the proteins involved in mTOR signalling

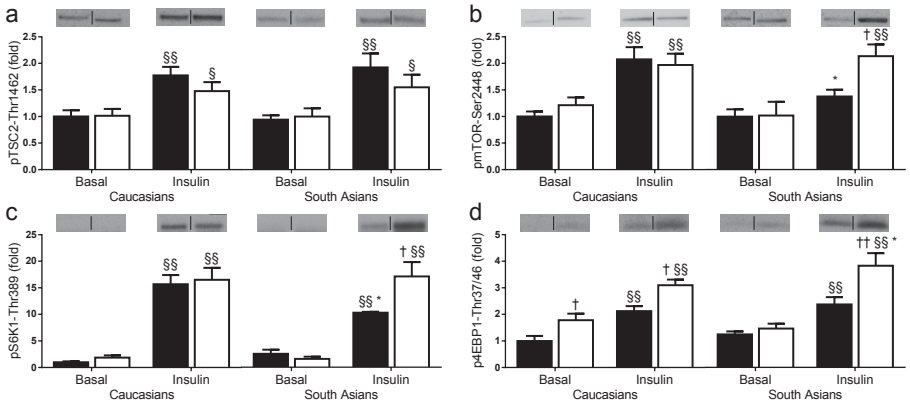


Figure 2. Effect of VLCD on mTOR signalling in skeletal muscle from South Asian and white Caucasian men in basal state and during a hyperinsulinemic-euglycemic clamp. The phosphorylation states of Thr1462-TSC2 (a), Ser2448-mTOR (b), Thr389-S6K1 (c) and Thr37/46-4EBP1 (d) were assessed by Western Blot in the same conditions as described in legend Figure 1. TSC2, tuberous sclerosis complex 2. mTOR, mammalian target of rapamycin. S6K1, ribosomal protein S6 kinase β1. 4EBP1, eukaryotic translation initiation factor 4E-binding protein 1.

was also not affected by caloric restriction in Caucasians. By contrast, the phosphorylation of mTOR-Ser2448, S6K1-Thr389 and 4E-BP1-Thr37/46 was significantly increased during hyperinsulinemia in South Asians, suggesting enhanced skeletal muscle mTORC1 activity in this condition.

AMPK pathway. Before diet, no differences were observed between groups in protein expression and phosphorylation states of AMPK and of its downstream target ACC in basal condition (**Supplementary Figure 1; Figure 3**). The phosphorylation state of AMPK-Thr172 was not affected by insulin in both groups, whereas a decrease in ACC-Ser79 phosphorylation was observed, but only in Caucasians. Caloric restriction did not affect protein expression of AMPKα and ACC while phosphorylation of AMPK-Thr172 Asians, but not in Caucasians. Phosphorylation of ACC-Ser79 was increased in both groups at basal state, whereas a higher degree of phosphorylation was only observed in Caucasians during hyperinsulinemic-euglycemic clamp.

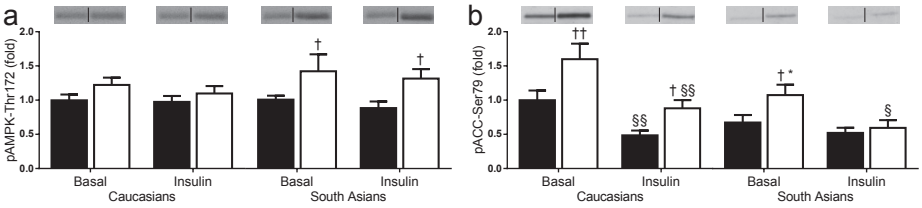


Figure 3. Effect of VLCD on AMPK signalling in skeletal muscle from South Asian and white Caucasian men in basal state and during a hyperinsulinemic-euglycemic clamp. The phosphorylation states of Thr172-AMPK (a) and Ser79-ACC (b) were assessed by Western Blot in the same conditions as described in legend Figure 1. AMPK, AMP-activated protein kinase. ACC, acetyl-CoA carboxylase.

Skeletal muscle mitochondrial respiratory-chain content

At baseline, the expression of mitochondrial respiratory-chain complex 2 was significantly cantly higher in South Asians. However, neither the complex-2-on-complex-1-ratio (**Figure 4b**) nor the mtDNA-to-nDNA-ratio (**Figure 4c**) was different between groups. Caloric restriction led to a significant increase in the expression of respiratory-chain complex 2 in Caucasians but not in South Asians. In both groups, the complex 2-on-complex 1 ratio was significantly higher after caloric restriction, suggesting a mitochondrial adaptation towards fat oxidation (**Figure 4b**). The mtDNA-on-nDNA-ratio was not affected by the diet (**Figure 4c**).

Skeletal muscle metabolic gene expression

The skeletal muscle expression of key metabolic genes involved in the regulation of glucose and FA metabolism in basal condition was assessed (**Supplementary Table 1**). At baseline, except for a trend for reduced INSR and SLC2A4 (GLUT4) expression in

South Asians no major differences were observed between groups. Caloric restriction induced downregulation of several genes involved in glycolysis (PFKM, PKM2), glycogen synthesis (UGP2, GBE1) and glycogen breakdown (PYGM) in both groups. Of note, no obvious differences were found in mRNA levels of key genes involved in mitochondrial biogenesis and tricarboxylic acid cycle whatever the conditions. Interestingly, PPARA was differentially affected by the diet, with a significant downregulation induced by caloric restriction in South Asians but no effect in Caucasians. In line with this, several PPARA target genes, such as CPT1a, ACAA2 and TXNIP, showed a similar pattern, although not always reaching a significant threshold. Furthermore, FABP3 and HMGCS2 were found to be significantly upregulated by caloric restriction only in Caucasians.

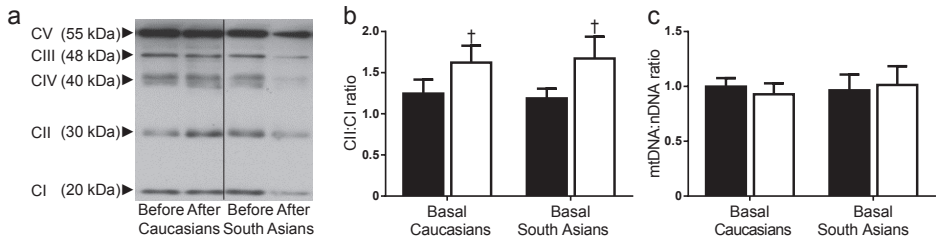


Figure 4. Effect of VLCD on protein expression of mitochondrial respiratory-chain subunits and mtDNA-to-nDNA ratio in skeletal muscle from South Asian and white Caucasian men in basal state and during a hyperinsulinemic-euglycemic clamp. The protein expression of various mitochondrial respiratory-chain subunits (a; CI: NDUFB8, CII: SDHB, CIII: UQCRC2, CIV: MTCO1, CV: ATP5A) were assessed by Western Blot in skeletal muscle from South Asian and white Caucasian volunteers in basal state before (black bars) and after (open bars) an 8-day VLCD. Representative blots for one individual per group are shown in (a). The respiratory-chain CII-on-CI ratio was calculated (b). The mtDNA-on-nDNA ratio was assessed in basal condition by qPCR (c). Results are normalised to Caucasian participants before VLCD and expressed as mean±SEM. † $p<0.05$ within group *vs.* before diet. CI–V, mitochondrial respiratory-chain subunits I–V.

DISCUSSION

South Asians have an exceptionally high risk to develop type 2 diabetes in the context of the same environmental pressure when compared to other ethnicities. A possible explanation for this excess risk might be related to differences in the regulation of energy/nutrient-sensing pathways. Intriguingly, the current study showed that middle-aged overweight South Asian men exhibited a different metabolic adaptation to an 8-day VLCD compared to age- and BMI-matched white Caucasians.

At baseline, South Asians were more insulin resistant compared to Caucasians, as indicated by higher insulin levels (both in fasted state and during OGTT), and lower hepatic and peripheral insulin sensitivity. In addition, HTG was significantly higher in

South Asians. Deposition of fat in the liver is often associated with hepatic insulin resistance[20] and previous studies also reported higher HTG and lower hepatic insulin resistance in South Asians when compared to Caucasians.[21;22] The reduced peripheral insulin sensitivity in South Asians appears to be due to a reduced rate of NOGD, suggesting an impairment in glycogen storage, one of the main defects also observed in patients with type 2 diabetes.[23] To analyse this further, we assessed the expression of key genes involved in glycolysis and glycogen synthesis in skeletal muscle but did not find relevant differences between ethnicities. As expected,[24] insulin promoted skeletal muscle GSK3-Ser21/9 phosphorylation and GS-Ser641 dephosphorylation, but no differences were observed between groups. Furthermore, caloric restriction similarly potentiated the insulin-induced dephosphorylation of GS-Ser641 in both South Asians and Caucasians, suggesting that skeletal muscle glycogen synthesis might be improved secondary to enhanced insulin sensitivity in this condition. However, the canonical insulin signalling pathway assessed by phosphorylation of PKB and its downstream targets PRAS40, which is partly involved in the control of glycogen synthesis through the GSK3-GS axis, was not found to be differently affected by caloric restriction. Of note, other upstream kinases than GSK3 were shown to phosphorylate GS on this specific residue,[25;26] and may therefore explain this apparent discrepancy.

In response to the 8-day VLCD, fasting glucose, insulin and triacylglycerol levels, and abdominal fat depots were reduced in both groups, with a concomitant increase in plasma NEFAs, reflecting increased lipolysis in adipose tissue. Furthermore, in line with previous studies HTG decreased in both groups,[27] with an accompanying improvement in hepatic insulin sensitivity.[28-30] Although, surprisingly, the changes in HTG were not correlated with the improvement in hepatic insulin sensitivity. In addition, Caucasians showed a classical switch from carbohydrate to lipid oxidation in response to caloric restriction together with an improved insulin effect on substrate oxidation rates, reflecting a clear improvement in metabolic flexibility.[28-30] Peripheral insulin sensitivity was not affected by the diet in Caucasians, in line with other short-term caloric restriction studies leading to minimal weight loss.[28-30] By contrast, peripheral insulin sensitivity was increased by caloric restriction in South Asians whereas the shift in whole-body substrate oxidation rates was found to be impaired, reflecting metabolic inflexibility. Although we do not have definitive explanation for this apparent dissociation, it is plausible that the metabolic inflexibility still present after caloric restriction in South Asians might result from impaired peripheral lipid metabolism despite better glucose R_d. Indeed, fatty acid oxidation rates, which are positively associated with plasma fatty acid levels, were reported to be an important determinant of metabolic flexibility.[31;32]

Remarkably, the skeletal muscle mTOR pathway was found to be differentially regulated in response to caloric restriction, with a higher activation of the mTORC1/S6K1 axis

upon insulin stimulation in South Asians when compared to Caucasians. Insulin, like many growth factors, induces activation of mTORC1 secondary to PKB and/or ERK1/2-mediated phosphorylation and inactivation of TSC1/2, the major upstream regulator of mTORC1.[33-35] Conversely, energy restriction leads to mTORC1 inhibition, partly due to TSC2 phosphorylation by AMPK on different regulatory residues than the ones targeted by PKB and ERK.[13] Finally, some of these kinases can also modulate mTORC1 activity independently of TSC2 by directly phosphorylating the mTOR catalytic subunit and/or some regulatory proteins of the mTORC1 complex, like Raptor and PRAS40.[12;36;37] In the present study, no diet effect was observed on PKB phosphorylation and its targeted residue on TSC2, and the AMPK activity was rather increased in South Asians after caloric restriction. Therefore, mTORC1 activation upon insulin-stimulation in South Asians most likely occurred via ERK, since phosphorylation of this kinase was significantly increased after caloric restriction when compared to Caucasians. Interestingly, insulin resistance was reported to be associated with defective insulin regulation of ERK signalling in skeletal muscle from women with polycystic ovary syndrome.[38] Thus, it is tempting to speculate that caloric restriction can restore an insulin-sensitive pathway involved in ERK regulation that was specifically impaired in South Asians. However, the exact underlying mechanism(s) still need to be clarified and we cannot rule out that other signalling pathways are also involved.

Besides its established role in regulating cell proliferation and growth, mTORC1 also promotes lipid synthesis and storage, while inhibiting fatty acid β-oxidation.[8] In the liver, this mTORC1-induced lipid partitioning has been shown to be mediated, at least partly, by inhibition of the transcription factor PPARα,[39] which is also a key regulator of skeletal muscle fatty acid oxidation.[40] Specifically, PPARα controls the transcription of genes involved in fatty acid uptake and mitochondrial import, as well as in β-oxidation and ketogenesis.[40] Interestingly, in the present study we observed a significant decrease in skeletal muscle PPARA gene expression in South Asians, but not in Caucasians after caloric restriction. In line with this, several PPARA target genes were also similarly downregulated in South Asians, including the fatty acid binding protein FABP3, which was previously reported to be positively associated with enhanced skeletal muscle oxidative capacity after caloric restriction.[41] Taken together, we might suggest that the higher insulin-stimulated mTORC1 activity observed after caloric restriction in South Asians may underlie a decrease in fatty acid oxidation secondary to inhibition of PPARα, ultimately resulting in impaired metabolic flexibility (**Figure 5**). Of note, mTORC1 was shown to control lipid metabolism in various other metabolic tissues.[8] Thus, it is conceivable that mTOR signalling can also be differently regulated in the adipose tissue from South Asians, thereby promoting the storage of NEFAs by inhibiting lipolysis.[8] This may be another explanation for the attenuated increase in plasma NEFAs in South Asians

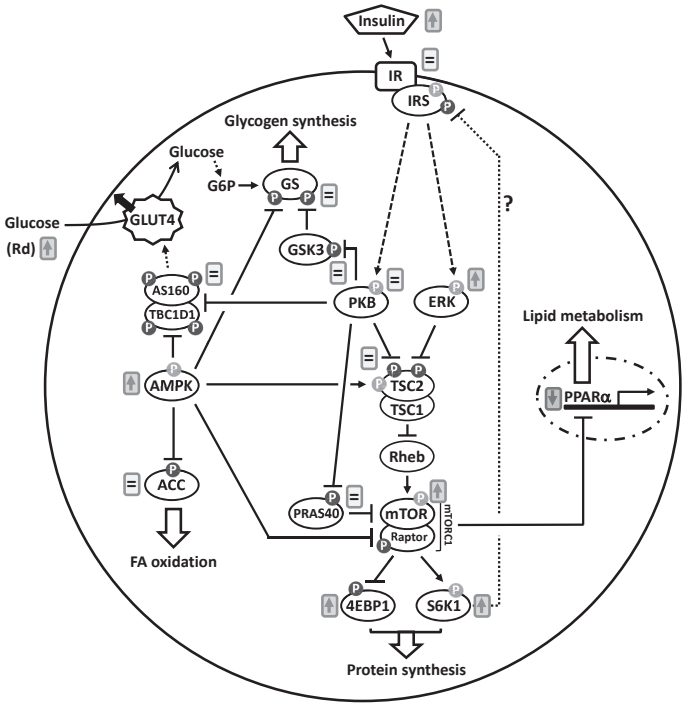


Figure 5. Proposed mechanism(s) underlying the different metabolic adaptations to short-term caloric restriction in South Asian compared to white Caucasian men. The differences in skeletal muscle response to caloric restriction between South Asians and white Caucasians during step 2 of the hyperinsulinemic-euglycemic clamp are shown on the various signalling pathways assessed. South Asians showed an increase in mTORC1 signalling upon insulin stimulation, which most likely occurred via ERK rather than PKB activation. This higher mTORC1 activation, by inhibiting PPARα and the transcriptional regulation of its target genes, may decrease fatty acid β-oxidation and contribute to the impaired metabolic flexibility observed in South Asians. Whole-body glucose disposal rate (R_d) was improved in South Asians, but not in Caucasians, after caloric restriction. This was associated with AMPK activation, which is known to promote skeletal muscle glucose uptake by an insulin-independent, but potentially additive, mechanism that remain to be clarified. FA, fatty acid; G6P, glucose 6-phosphate; Raptor, regulatory-associated protein of mTOR; TBC1D1, TBC1 domain family member 1. Dark grey phosphorylation (P) sites: inhibitory; light grey phosphorylation (P) sites: activating; down arrows: expression is decreased in South Asians; up arrows: expression (or level for insulin, rate for R_d) is increased in South Asians; = similar effect to energy restriction in South Asians and Caucasians.

compared to Caucasians, apart from suppressed fatty acid oxidation in skeletal muscle. Among other possible explanations, a lower proportion of slow-twitch type 1 oxidative muscle fibres in South Asians, rendering them less efficient for fatty acid oxidation,[42] or the fact that NEFAs are not directed towards oxidation but preferentially to storage into complex lipids, *i.e.* intramyocellular lipid content (IMCL), might be suggested. It would therefore be interesting to address these points by measuring skeletal muscle IMCL and muscle fibre type distribution in further experiments.

Finally, in addition to its role in lipid metabolism, mTORC1 is also known to modulate insulin sensitivity by phosphorylating IRS-1 on specific serine residues, resulting in a negative-feedback loop on the canonical insulin pathway. Enhanced mTORC1 activity in livers from obese insulin-resistant mice was indeed shown to promote S6K-mediated phosphorylation of IRS1, presumably on Ser636/639 [10] and/or Ser307.[43] Furthermore, S6K-mediated phosphorylation of IRS1 on Ser1101 was also reported to mediate hepatic and skeletal muscle insulin resistance in high-fat diet fed mice.[44] However, despite apparent enhanced mTORC1 activation upon insulin stimulation, R_d was paradoxically found to be improved after caloric restriction in South Asians in our condition. Unfortunately, we were not able to detect significant phosphorylation of IRS1 on both Ser636/639 and Ser307 using commercial antibodies (data not shown), although it is worth mentioning that the existence of this regulatory feedback loop and the exact IRS1 residue(s) involved are still a matter of debate, especially in human skeletal muscle (see [45] for review).

The improvement in R_d after caloric restriction in South Asians is apparently primarily accounted for by increased NOGD, suggesting that glycogen storage might be improved, although no change in the insulin-induced dephosphorylation of Ser641-GS was found. AMPK activation, which is known to promote skeletal muscle glucose uptake by an insulin-independent but additive mechanism increasing translocation of GLUT4 to the plasma membrane,[46] was significantly higher in South Asians than in Caucasians, suggesting that this kinase might be involved in the improved R_d in South Asians. Although the exact mechanism by which AMPK increases glucose uptake remains incompletely understood (see [47] for recent review), it apparently involves phosphorylation of the Rab GTPase-activating proteins AS160 (TBC1D4) and/or on TBC1D1 by the kinase, on residues different than the ones targeted by PKB.[48] However, activation of AMPK was also shown to decrease the rate of GLUT4 endocytosis both in human and rat muscle *in vitro*,[49] whereas insulin had opposite effects, showing that multiple steps in the control of glucose uptake can be differently regulated by insulin and AMPK-dependent pathways. Interestingly, a recent study has also reported that elevated glucose transport promoted by increased AMPK activity causes an accumulation of intracellular G6P leading to allosteric activation of GS and glycogen storage in skeletal muscle, independently of changes in GS phosphorylation, notably on Ser641.[50] Altogether, we might speculate that the higher AMPK activity observed during hyperinsulinemic-euglycemic clamp can underlie the improved NOGD in South Asians after caloric restriction, at least partly secondary to insulin-independent increase in glucose uptake. Further studies are required for clarifying this point, notably for measuring skeletal muscle glycogen content after caloric restriction in both South Asians and Caucasians.

Taken together, the signalling pathway analysis performed in skeletal muscle in the present study does not allow us to draw definitive conclusions on the mechanism(s) underlying the improvement in R_d observed after caloric restriction in South Asians, but not Caucasians. Additional in depth molecular investigations are therefore clearly required, not only in skeletal muscle but also in other organs involved in whole-body R_d, such as adipose tissue. In addition, as chronic mTORC1 activation is believed to contribute to the development of insulin resistance and type 2 diabetes,[8] it would be interesting to investigate the response to long-term caloric restriction on mTORC1 signalling and insulin sensitivity in South Asians compared to Caucasians. Of note, we have recently studied the effect of high fat feeding on energy/nutrient-sensing pathways in young, healthy lean South Asian and white Caucasian men and showed that this diet rapidly induced insulin resistance in South Asians, but had no effect on Caucasians.[16] However, in contrast to the present study, we did not observe differences in mTOR, AMPK or other energy/nutrient-sensing pathways, suggesting that differences in the regulation of these pathways may develop with age and in a more disadvantageous metabolic phenotype, *e.g.* in overweight individuals.

In conclusion, we showed that middle-aged overweight South Asian men exhibit a different metabolic adaptation to short-term caloric restriction compared to age- and BMI-matched Caucasians. Although metabolic flexibility was impaired after an 8-day VLCD, R_d was improved in South Asians in contrast to Caucasians, and was associated with an increase in insulin-induced activation of the skeletal muscle ERK-mTOR-S6K1 axis. Additional studies are required to expand these findings, which might provide new leads in our search to elucidate the pathogenesis of type 2 diabetes in South Asians.

REFERENCES

1. Overweight, obesity, and health risk. National Task Force on the Prevention and Treatment of Obesity. *Arch Intern Med* 2000;160(7):898-904.
2. Becker E, Boreham R, Chaudhury M, Craig R, Deverill C, Doyle M *et al*. Health Survey for England 2004 Volume 1 The health of minority ethnic groups. The Information Centre; 2006.
3. Bindraban NR, van Valkengoed IG, Mairuhu G, Holleman F, Hoekstra JB, Michels BP *et al*. Prevalence of diabetes mellitus and the performance of a risk score among Hindustani Surinamese, African Surinamese and ethnic Dutch: a cross-sectional population-based study. *BMC Public Health* 2008;8:271.
4. Chiu M, Austin PC, Manuel DG, Shah BR, Tu JV. Deriving ethnic-specific BMI cutoff points for assessing diabetes risk. *Diabetes Care* 2011;34(8):1741-8.
5. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res Clin Pract* 2011;94(3):311-21.
6. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012;149(2):274-93.
7. Haruta T, Uno T, Kawahara J, Takano A, Egawa K, Sharma PM *et al*. A rapamycin-sensitive pathway down-regulates insulin signaling via phosphorylation and proteasomal degradation of insulin receptor substrate-1. *Mol Endocrinol* 2000;14(6):783-94.
8. Ricoult SJ, Manning BD. The multifaceted role of mTORC1 in the control of lipid metabolism. *EMBO Rep* 2013;14(3):242-51.
9. Takano A, Usui I, Haruta T, Kawahara J, Uno T, Iwata M *et al*. Mammalian target of rapamycin pathway regulates insulin signaling via subcellular redistribution of insulin receptor substrate 1 and integrates nutritional signals and metabolic signals of insulin. *Mol Cell Biol* 2001;21(15):5050-62.
10. Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M *et al*. Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature* 2004;431(7005):200-5.
11. Sengupta S, Peterson TR, Sabatini DM. Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. *Mol Cell* 2010;40(2):310-22.
12. Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS *et al*. AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell* 2008;30(2):214-26.
13. Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. *Cell* 2003;115(5):577-90.
14. Hammer S, van der Meer RW, Lamb HJ, de Boer HH, Bax JJ, de Roos A *et al*. Short-term flexibility of myocardial triglycerides and diastolic function in patients with type 2 diabetes mellitus. *Am J Physiol Endocrinol Metab* 2008;295(3):E714-E718.
15. van der Meer RW, Hammer S, Lamb HJ, Frolich M, Diamant M, Rijzewijk LJ *et al*. Effects of short-term high-fat, high-energy diet on hepatic and myocardial triglyceride content in healthy men. *J Clin Endocrinol Metab* 2008;93(7):2702-8.
16. Bakker LE, van Schinkel LD, Guigas B, Streefland TC, Jonker JT, van Klinken JB *et al*. A 5-day high-fat, high-calorie diet impairs insulin sensitivity in healthy, young South Asian men but not in Caucasian men. *Diabetes* 2014;63(1):248-58.
17. Corpeleijn E, Saris WH, Blaak EE. Metabolic flexibility in the development of insulin resistance and type 2 diabetes: effects of lifestyle. *Obes Rev* 2009;10(2):178-93.
18. Szuhai K, Ouweland J, Dirks R, Lemaitre M, Truffert J, Janssen G *et al*. Simultaneous A8344G heteroplasmy and mitochondrial DNA copy number quantification in myoclonus epilepsy and ragged-red fibers (MERRF) syndrome by a multiplex molecular beacon based real-time fluorescence PCR. *Nucleic Acids Res* 2001;29(3):E13.

19. Wijngaarden MA, van der Zon GC, Willems van Dijk KW, Pijl H, Guigas B. Effects of prolonged fasting on AMPK signaling, gene expression and mitochondrial respiratory-chain content in skeletal muscle from lean and obese individuals. *Am J Physiol Endocrinol Metab* 2013.
20. Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, Goto T, Westerbacka J, Sovijarvi A *et al*. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J Clin Endocrinol Metab* 2002;87(7):3023-8.
21. Anand SS, Tarnopolsky MA, Rashid S, Schulze KM, Desai D, Mente A *et al*. Adipocyte hypertrophy, fatty liver and metabolic risk factors in South Asians: the Molecular Study of Health and Risk in Ethnic Groups (mol-SHARE). *PLoS One* 2011;6(7):e22112.
22. Petersen KF, Dufour S, Feng J, Befroy D, Dziura J, Dalla MC *et al*. Increased prevalence of insulin resistance and nonalcoholic fatty liver disease in Asian-Indian men. *Proc Natl Acad Sci U S A* 2006;103(48):18273-7.
23. Shulman GI, Rothman DL, Jue T, Stein P, DeFronzo RA, Shulman RG. Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. *N Engl J Med* 1990;322(4):223-8.
24. Jensen J, Ruge T, Lai YC, Svensson MK, Eriksson JW. Effects of adrenaline on whole-body glucose metabolism and insulin-mediated regulation of glycogen synthase and PKB phosphorylation in human skeletal muscle. *Metabolism* 2011;60(2):215-26.
25. Skurat AV, Dietrich AD. Phosphorylation of Ser640 in muscle glycogen synthase by DYRK family protein kinases. *J Biol Chem* 2004;279(4):2490-8.
26. Wilson WA, Skurat AV, Probst B, de Paoli-Roach A, Roach PJ, Rutter J. Control of mammalian glycogen synthase by PAS kinase. *Proc Natl Acad Sci U S A* 2005;102(46):16596-601.
27. van der Meer RW, Hammer S, Smit JW, Frolich M, Bax JJ, Diamant M *et al*. Short-term caloric restriction induces accumulation of myocardial triglycerides and decreases left ventricular diastolic function in healthy subjects. *Diabetes* 2007;56(12):2849-53.
28. Christiansen MP, Linfoot PA, Neese RA, Hellerstein MK. Effect of dietary energy restriction on glucose production and substrate utilization in type 2 diabetes. *Diabetes* 2000;49(10):1691-9.
29. Jazet IM, Pijl H, Frolich M, Romijn JA, Meinders AE. Two days of a very low calorie diet reduces endogenous glucose production in obese type 2 diabetic patients despite the withdrawal of blood glucose-lowering therapies including insulin. *Metabolism* 2005;54(6):705-12.
30. Markovic TP, Jenkins AB, Campbell LV, Furler SM, Kraegen EW, Chisholm DJ. The determinants of glycemic responses to diet restriction and weight loss in obesity and NIDDM. *Diabetes Care* 1998;21(5):687-94.
31. Galgani JE, Heilbronn LK, Azuma K, Kelley DE, Albu JB, Pi-Sunyer X *et al*. Metabolic flexibility in response to glucose is not impaired in people with type 2 diabetes after controlling for glucose disposal rate. *Diabetes* 2008;57(4):841-5.
32. van de Weijer T, Sparks LM, Phielix E, Meex RC, van Herpen NA, Hesselink MK *et al*. Relationships between mitochondrial function and metabolic flexibility in type 2 diabetes mellitus. *PLoS One* 2013;8(2):e51648.
33. Inoki K, Li Y, Xu T, Guan KL. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev* 2003;17(15):1829-34.
34. Inoki K, Li Y, Zhu T, Wu J, Guan KL. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Biol* 2002;4(9):648-57.

35. Ma L, Chen Z, Erdjument-Bromage H, Tempst P, Pandolfi PP. Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis. *Cell* 2005;121(2):179-93.
36. Carriere A, Romeo Y, Acosta-Jaquez HA, Moreau J, Bonneil E, Thibault P *et al*. ERK1/2 phosphorylate Raptor to promote Ras-dependent activation of mTOR complex 1 (mTORC1). *J Biol Chem* 2011;286(1):567-77.
37. Vander Haar E, Lee SI, Bandhakavi S, Griffin TJ, Kim DH. Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. *Nat Cell Biol* 2007;9(3):316-23.
38. Rajkhowa M, Brett S, Cuthbertson DJ, Lipina C, Ruiz-Alcaraz AJ, Thomas GE *et al*. Insulin resistance in polycystic ovary syndrome is associated with defective regulation of ERK1/2 by insulin in skeletal muscle in vivo. *Biochem J* 2009;418(3):665-71.
39. Sengupta S, Peterson TR, Laplante M, Oh S, Sabatini DM. mTORC1 controls fasting-induced ketogenesis and its modulation by ageing. *Nature* 2010;468(7327):1100-4.
40. Rakhshandehroo M, Knoch B, Muller M, Kersten S. Peroxisome proliferator-activated receptor alpha target genes. *PPAR Res* 2010;2010.
41. Blaak EE, Wagenmakers AJ, Glatz JF, Wolffenbuttel BH, Kemerink GJ, Langenberg CJ *et al*. Plasma FFA utilization and fatty acid-binding protein content are diminished in type 2 diabetic muscle. *Am J Physiol Endocrinol Metab* 2000;279(1):E146-E154.
42. Kelley DE, Goodpaster B, Wing RR, Simoneau JA. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *Am J Physiol* 1999;277(6 Pt 1):E1130-E1141.
43. Khamzina L, Veilleux A, Bergeron S, Marette A. Increased activation of the mammalian target of rapamycin pathway in liver and skeletal muscle of obese rats: possible involvement in obesity-linked insulin resistance. *Endocrinology* 2005;146(3):1473-81.
44. Tremblay F, Brule S, Hee US, Li Y, Masuda K, Roden M *et al*. Identification of IRS-1 Ser-1101 as a target of S6K1 in nutrient- and obesity-induced insulin resistance. *Proc Natl Acad Sci U S A* 2007;104(35):14056-61.
45. Copps KD, White MF. Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2. *Diabetologia* 2012;55(10):2565-82.
46. Hardie DG. Energy sensing by the AMP-activated protein kinase and its effects on muscle metabolism. *Proc Nutr Soc* 2011;70(1):92-9.
47. Richter EA, Hargreaves M. Exercise, GLUT4, and skeletal muscle glucose uptake. *Physiol Rev* 2013;93(3):993-1017.
48. Geraghty KM, Chen S, Harthill JE, Ibrahim AF, Toth R, Morrice NA *et al*. Regulation of multisite phosphorylation and 14-3-3 binding of AS160 in response to IGF-1, EGF, PMA and AICAR. *Biochem J* 2007;407(2):231-41.
49. Karlsson HK, Chibalin AV, Koistinen HA, Yang J, Koumanov F, Wallberg-Henriksson H *et al*. Kinetics of GLUT4 trafficking in rat and human skeletal muscle. *Diabetes* 2009;58(4):847-54.
50. Hunter RW, Treebak JT, Wojtaszewski JF, Sakamoto K. Molecular mechanism by which AMP-activated protein kinase activation promotes glycogen accumulation in muscle. *Diabetes* 2011;60(3):766-74.

Supplemental Table 1. Overview of metabolic gene expression analysis in skeletal muscle from middle-aged overweight South Asian men and matched white Caucasians before and after an 8-day VLCD.

Gene name	Gene symbol	Entrez gene	white Caucasians		South Asians		Interaction p-value
			before	after	before	after	
Glucose Metabolism							
Glucose transport & phosphorylation							
Insulin receptor	INSR	3643	1.00 ± 0.26	1.40 ± 0.32	0.59 ± 0.11	**0.81 ± 0.26†**	0.271
Akt substrate of 160 kDa (AS160)	TBC1D4	9882	1.00 (1.00)	1.31 (1.49)	**2.99 (3.07)***	1.75 (2.89)	0.295
Solute carrier family 2, member 1 (GLUT-1)	SLC2A1	6513	1.00 ± 0.19	0.54 ± 0.13	0.53 ± 0.13	**1.21 ± 0.26‡**	**0.045**
Solute carrier family 2, member 4 (GLUT-4)	SLC2A4	6517	1.00 ± 0.32	1.06 ± 0.31	0.60 ± 0.18	0.45 ± 0.12	0.659
Hexokinase 2	HK2	3099	1.00 (1.02)	**1.58 (1.62)†**	1.01 (0.97)	0.75 (0.48)	0.351
Glycolysis							
Phosphofructokinase	PFKM	5213	1.00 ± 0.18	**0.56 ± 0.11†**	1.12 ± 0.13	**0.44 ± 0.10†**	0.658
6-phosphofructo-2-kinase/fructose-2,6- bisphosphate 3	PFKFB3	5209	1.00 ± 0.25	1.85 ± 0.47	0.91 ± 0.27	**2.54 ± 0.71†**	0.257
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	2597	1.00 ± 0.14	0.95 ± 0.12	1.09 ± 0.18	**0.92 ± 0.12†**	0.104
Pyruvate kinase	PKM2	5315	1.00 ± 0.11	**0.64 ± 0.08††**	0.83 ± 0.11	**0.55 ± 0.06†**	0.824
Glycogen metabolism							
Glycogen synthase 1	GYS1	2997	1.00 ± 0.28	1.00 ± 0.23	0.62 ± 0.20	0.50 ± 0.14	0.419
Glycogen-branching enzyme	GBE1	2632	1.00 ± 0.14	**0.68 ± 0.10†**	1.16 ± 0.06	**0.68 ± 0.12†**	0.951
Glycogen phosphorylase	PYGM	5837	1.00 ± 0.13	0.69 ± 0.14	1.04 ± 0.15	**0.61 ± 0.10†**	0.395
UDP-glucose pyrophosphorylase 2	UGP2	7360	1.00 ± 0.21	0.44 ± 0.11	1.34 ± 0.26	**0.31 ± 0.12†**	0.460
Glycogen debranching enzyme	AGL	178	1.00 (1.46)	0.45 (0.56)	2.39 (2.63)	0.54 (2.32)	0.904
Phosphorylase kinase α1	PHKA1	5255	1.00 (0.66)	0.99 (0.36)	1.31 (0.45)	1.63 (1.57)	0.875
Protein phosphatase 1, regulatory subunit 3A	PPP1R3A	5506	1.00 (0.69)	0.78 (0.48)	1.81 (2.35)	0.93 (0.58)	0.395

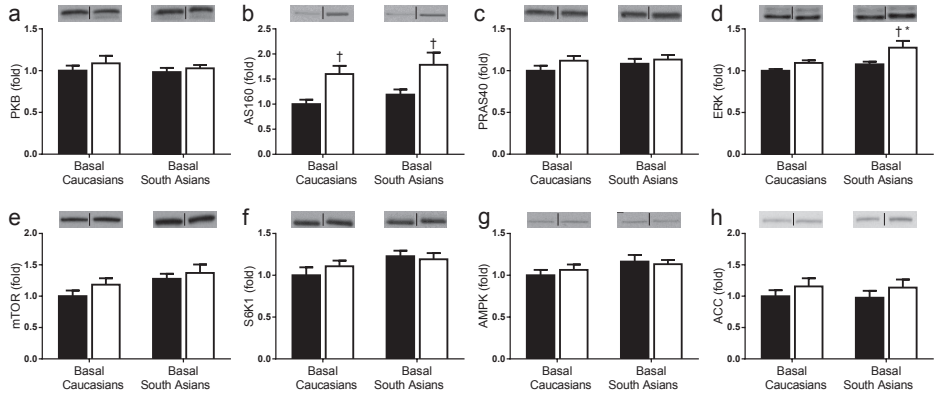
Supplemental Table 1. Overview of metabolic gene expression analysis in skeletal muscle from middle-aged overweight South Asian men and matched white Caucasians before and after an 8-day VLCD. (continued)

Gene name	Gene symbol	Entrez gene	white Caucasians		South Asians		Interaction p-value
			before	after	before	after	
Fatty acid metabolism							
Transcription factors							
PPARα	PPARA	5465	1.00 ± 0.13	0.94 ± 0.12	1.23 ± 0.09	**0.74 ± 0.14†‡**	**0.030**
PPARδ	PPARD	5467	1.00 (1.07)	1.40 (1.00)	0.99 (1.11)	1.64 (1.64)	0.395
Fatty acid uptake, synthesis and oxidation							
Lipoprotein lipase	LPL	4023	1.00 ± 0.20	0.95 ± 0.18	1.07 ± 0.18	0.87 ± 0.22	0.839
Fatty acid translocase/CD36	CD36	948	1.00 ± 0.14	0.82 ± 0.09	0.85 ± 0.13	0.63 ± 0.12	0.959
Fatty acid binding protein 3	FABP3	2170	1.00 ± 0.11	**2.04 ± 0.33††**	0.93 ± 0.08	**1.28 ± 0.21***	0.075
Acetyl-CoA carboxylase α	ACACA	31	1.00 (1.40)	1.71 (1.96)	1.21 (0.85)	0.96 (1.17)	0.600
Acetyl-CoA carboxylase β	ACACB	32	1.00 ± 0.19	**1.49 ± 0.29†**	0.60 ± 0.18	0.82 ± 0.28	0.728
Acetyl-Coenzyme A acyltransferase 2	ACAA2	10449	1.00 ± 0.13	0.89 ± 0.08	1.08 ± 0.11	0.65 ± 0.15	0.618
Thioredoxin-interacting protein	TXNIP	10628	1.00 (1.20)	1.11 (0.62)	**2.10 (1.11)***	0.90 (1.11)	0.651
3-hydroxy-3-methylglutaryl-CoA synthase 2	HMGCS2	3158	1.00 (3.35)	**6.92 (10.66)†**	0.72 (1.09)	**1.02 (1.61)*‡**	**0.028**
Mitochondrial fatty acid transport							
CPT 1A	CPT1A	1374	1.00 (0.87)	**2.76 (1.66)†**	1.20 (0.69)	**1.88 (0.64)†‡**	**0.046**
CPT 1B	CPT1B	1375	1.00 ± 0.11	1.05 ± 0.07	**0.77 ± 0.07***	**0.71 ± 0.10***	0.721
CPT 2	CPT2	1376	1.00 ± 0.16	1.18 ± 0.20	1.01 ± 0.12	1.00 ± 0.18	0.648

Supplemental Table 1. Overview of metabolic gene expression analysis in skeletal muscle from middle-aged overweight South Asian men and matched white Caucasians before and after an 8-day VLCD. (continued)

Gene name	Gene symbol	Entrez gene	white Caucasians		South Asians		Interaction p-value
			before	after	before	after	
AMP-activated protein kinase							
AMPK α1	PRKAA1	5562	1.00 (0.43)	1.03 (0.47)	1.63 (0.47)	1.13 (1.00)	0.408
AMPK α2	PRKAA2	5563	1.00 (0.60)	1.20 (0.73)	1.11 (0.48)	1.63 (1.07)	0.167
Mitochondrial metabolism							
Mitochondrial biogenesis							
PPARα, coactivator 1α (PGC-1α)	PPARGC1A	10891	1.00 ± 0.25	0.82 ± 0.14	1.00 ± 0.18	0.80 ± 0.12	0.983
PPARβ, coactivator 1b (PGC-1β)	PPARGC1B	133522	1.00 ± 0.26	0.68 ± 0.17	0.66 ± 0.16	0.34 ± 0.07	0.976
Transcription factor A	TFAM	7019	1.00 ± 0.16	0.63 ± 0.12	1.50 ± 0.23	**0.62 ± 0.18†**	0.368
Nuclear respiratory factor 1	NRF1	4899	1.00 (0.91)	0.68 (0.93)	0.83 (0.90)	0.43 (0.39)	0.702
Tricarboxylic acid cycle and UCP3							
Pyruvate carboxylase	PC	5091	1.00 ± 0.09	0.57 ± 0.11	0.82 ± 0.12	1.15 ± 0.21	0.270
Pyruvate dehydrogenase kinase 4	PDK4	5166	1.00 (2.14)	2.43 (3.60)	3.89 (3.75)	1.42 (1.54)	0.360
Citrate synthase	CS	1431	1.00 ± 0.25	**0.59 ± 0.12†**	1.03 ± 0.17	**0.35 ± 0.06†**	0.772
Uncoupling protein 3	UCP3	7352	1.00 (0.61)	1.49 (1.01)	0.94 (1.25)	2.04 (1.59)	0.766

Data are presented as mean±SEM or median (IQR). PPAR, Peroxisome proliferator-activated receptor. CPT, Carnitine palmitoyltransferase. AMPK, AMP-activated protein kinase. † p<0.05, †† p<0.005 within group *vs.* before diet. * p<0.05 between groups *vs.* Caucasians. ‡ p<0.05 diet effect *vs.* Caucasians (interaction p-value).



Supplemental Figure 1. Effect of VLCD on mTOR signalling in skeletal muscle from South Asian and white Caucasian men in basal state. The protein expression of PKB (a), AS160 (b), PRAS40 (c), ERK (d), mTOR (e), S6K (f), AMPK (g), and ACC (h), were assessed by Western Blot in skeletal muscle from South Asian and white Caucasian subjects before (black bars) and after an 8-day VLCD (open bars) in basal state. Representative blots for one subject per group are shown. Results are normalized to Caucasian subjects in basal state before VLCD and expressed as mean±SEM. † $p<0.05$ within group *vs.* before diet. * $p<0.05$ *vs.* Caucasians. AS160, Akt substrate of 160 kDa. mTOR, mammalian target of rapamycin. AMPK, AMP-activated protein kinase. ERK, extracellular signal-regulated kinase. PKB, protein kinase B protein kinase B. PRAS40, Proline rich Akt substrate of 40 kDa. S6K1, ribosomal protein S6 kinase β1. ACC, acetyl-CoA carboxylase.

6 Brown adipose tissue: the body's own weapon against obesity?

Leontine E.H. Bakker*
Mariëtte R. Boon*
A. Edo Meinders
Wouter D. van Marken Lichtenbelt
Patrick C.N. Rensen
Ingrid M. Jazet

*Authors contributed equally to manuscript

Translation of 'Bruin vet: een lichaamseigen middel in de strijd tegen obesitas?'
Nederlands Tijdschrift voor Geneeskunde 2013; 157(20): 958-64

ABSTRACT

Brown adipose tissue (BAT) dissipates energy stored in triglycerides as heat via the uncoupling protein UCP-1. It has recently been discovered that BAT is present and active in adults. BAT is situated predominantly around the aorta and in the supraclavicular area. BAT volume and activity are lower in individuals who are obese, suggesting that BAT significantly contributes to energy expenditure. Several pathological conditions that lead to activation of BAT, such as hyperthyroidism and pheochromocytoma, result in increased energy expenditure and in weight loss. Various ways in which BAT can be manipulated to increase expenditure of energy have been identified, e.g. exposure to cold, the use of so-called uncoupling agents or the administration of the hormone irisin. The activation of BAT could potentially be used to induce weight loss.

INTRODUCTION

Adipose tissue can be subdivided into 'white adipose tissue' and 'brown adipose tissue' (BAT). Until recently, it was thought that BAT disappears in adult life. However, modern techniques as ^{18}F-FDG-PET-CT-scans have proven otherwise. Interestingly, it has been discovered not long ago that BAT can be manipulated to increase energy expenditure.

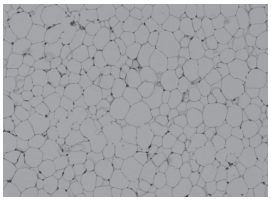
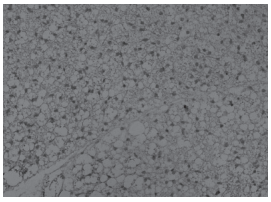
The function of white adipose tissue is to store triglycerides and to produce a large number of factors, the so called adipo(cyto)kines.[1] In contrast, BAT continuously burns triglycerides and glucose, thereby releasing energy as heat. This process is called thermogenesis. In neonates, thermogenesis contributes importantly to the maintenance of body temperature.

In this article we will give an overview of the anatomy, physiology and function of BAT and describe how BAT can be manipulated in order to increase energy expenditure and possibly induce weight loss.

Anatomy and origin of brown adipose tissue

BAT differs strongly from white adipose tissue in both volume and structure (**Table 1**). The total amount of BAT in an adult human is estimated to be 50-100 grams, while the amount of white adipose tissue is roughly 20% of total body weight. A white adipocyte consists of a big vacuole filled with triglycerides, surrounded by a thin rim of cytoplasm. The cytoplasm contains the nucleus and cell organelles, including a few mitochondria

Table 1 Differences between white and brown adipose tissue.

Characteristic	White adipose tissue	Brown adipose tissue
Microscopic image		
Amount in human body	12 – 35 kg	circa 50 – 100 g
Morphology	large cells filled with triglycerides few mitochondria	cells filled with small lipid droplets large number of mitochondria
Location	present throughout the whole body	in brown fat pads, predominantly in the subscapular area (neonates), and in the supra-clavicular region and along the aorta (adults); scattered as groups of cells in white adipose tissue and skeletal muscle
Function	storage of fat	conversion of triglycerides and glucose into heat

that provide for the formation of ATP, the main energy supplier of cells. In contrast, a brown adipocyte contains several small lipid droplets that are surrounded by a large number of mitochondria. The iron-containing proteins that are part of the respiratory chain inside the mitochondria give BAT its brownish colour.

Brown fat cells are present in two forms. On the one hand, they form brown fat pads, which are located in the subscapular area in neonates and along the aorta and in the supraclavicular region in adults.[2] In addition, individual brown fat cells lie scattered in other tissues, such as white adipose tissue and muscle, where they form a pool of ´peripheral brown adipocytes´. These cells are also called 'beige adipocytes´, since their phenotype lies between a white and brown fat cell.

The two types of brown fat cells have different origins (**Figure 1**). Brown adipocytes present in the brown fat pads originate from a Myf5 (myogenic factor 5)-positive precur-

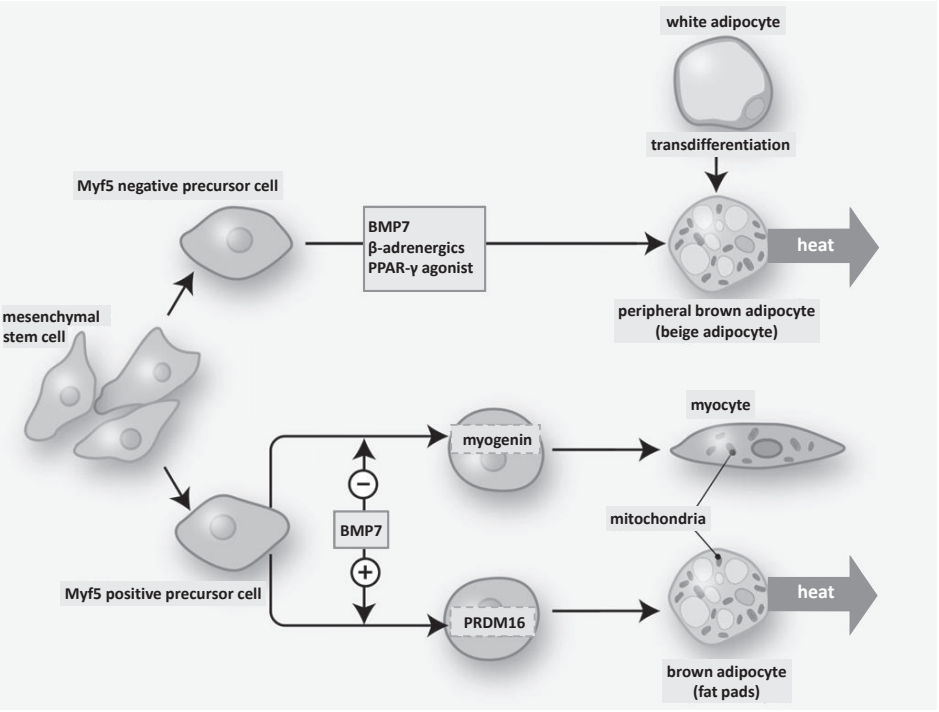


Figure 1. Differentiation of mesenchymal stem cells into brown adipocytes. Myf5-positive precursor cells can differentiate into skeletal muscle cells or brown fat cells, depending on the presence of BMP7. The skeletal muscle cells express myogenin, the brown fat cells PRDM16. This type of brown fat cell is present in fat pads. Myf5-negative precursor cells differentiate into peripheral brown fat cells ('beige fat cells') under influence of several stimuli, such as BMP7, β-adrenergics and PPAR-γ agonists. Peripheral brown fat cells can also originate from transdifferentiation of a white fat cell. (Myf5 = myogenic factor 5; BMP7 = 'bone morphogenetic protein-7'; PRDM16 = 'PR domain containing 16'; PPAR-γ = peroxisome proliferator-activated receptor-γ) (adaptation of a previously published figure).[3]

sor cell; this precursor cell can differentiate into both a brown fat cell and a skeletal muscle cell, depending on the presence of stimuli such as Bone Morphogenetic Protein 7 (BMP7). The peripheral brown fat cell – or beige fat cell – originates from a Myf5-negative precursor cell, as well as from transdifferentation of a white fat cell into a brown fat cell.[3;4] Several stimuli (such as BMP7, β-adrenergics and peroxisome proliferator-activated receptor-γ (PPAR-γ)-agonists) can activate the differentiation of Myf5-negative precursor cells into peripheral brown fat cells.[3] These stimuli are therefore considered interesting therapeutic targets.

Physiology of brown adipose tissue

In mitochondria ATP synthesis takes place. This process starts with the conversion of fatty acids and glucose into acetyl coenzyme A, that participates in the citric acid cycle in the mitochondrion. The cyclic acid cycle generates energy-rich compounds, such as

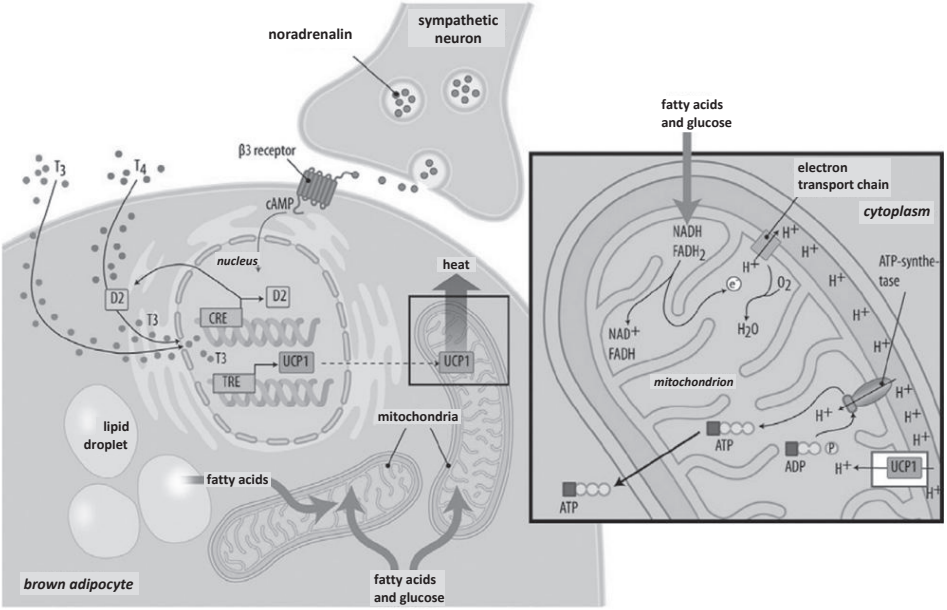


Figure 2. Activation of brown adipose tissue. In resting condition, the energy-rich compounds NADH en $FADH_2$ donate their energy in the form of electrons to the electron transport chain, thereby creating a gradient of H^+-ions across the inner mitochondrial membrane (see inset right). The energy stored in this gradient is used by the enzyme ATP synthetase to convert ADP into ATP. However, in BAT the protein UCP-1 uncouples the H^+-gradient of ATP-synthetase. This happens when a sympathetic nerve cell activates the β3-receptor of brown adipocytes by secretion of noradrenalin (left drawing), leading to the formation of cAMP and eventually to the production of UCP-1. Transport of H+ across the inner membrane via UCP-1 results in the production of heat instead of ATP. The active thyroid hormone T3 also leads to production of UCP-1 (cAMP = cyclisch AMP; UCP-1 = 'uncoupling protein 1'; T_3 = tri-iodothyronine; T_4 = thyroxine; D2 = type 2-deiodinase) (derived from a figure published elsewhere).[26]

NADH and $FADH_2$, which donate their electrons during oxidative phosphorylation to the electron transport chain, thereby creating a gradient of H^+-ions across the inner mitochondrial membrane (**Figure 2**). The energy stored in this gradient is used by the enzyme ATP synthetase to convert ADP into ATP.

The brown adipocyte is able to burn triglycerides and glucose via the process of ´uncoupling´, in which energy is released as heat instead of ATP, resulting in increased energy expenditure in the cell. A detailed description of the physiology of a brown fat cell is shown in **Figure 2**.

Every brown adipocyte is innervated by a sympathetic nerve. The most important stimulus of the sympathetic nervous system is cold; the sympathetic nerve is activated by the temperature centre in the brain, which receives input from sensory nerve fibres in the skin. Upon stimulation, the sympathetic nerve locally releases noradrenalin, which binds to β3-adrenergic receptors on the brown adipocyte. Activation of these receptors results in a cascade of intracellular reactions.

Uncoupling protein (UCP) First, the expression of the gene encoding for the uncoupling protein UCP-1 is induced via elevation of intracellular cyclic AMP. UCP-1 is unique for BAT. UCP-1 proteins are then incorporated into the inner membrane of mitochondria, forming pores. However, in the presence of UCP-1 the proton gradient is disturbed and the accumulated energy dissipates as heat rather than being converted into ATP. The greater the density of mitochondria (or the amount of UCP-1) in BAT, the more glucose and triglycerides will be burned and released as heat when BAT is activated.

Increased influx of substrate At the same time, sympathetic activation of BAT results in an increased amount and activity of the enzyme lipoprotein lipase (LPL), which cleaves fatty acids from triglycerides for uptake by BAT, and of GLUT-1, the glucose transporter that increases the uptake of glucose by BAT.[5] In this way sympathetic activation of BAT increases not only thermogenesis, but also the influx of the necessary substrate.

Thyroid hormone Remarkably, thyroid hormone is also involved in the activation of brown fat. After uptake into the brown fat cell and translocation to the nucleus, T3, the active thyroid hormone, binds to thyroid hormone responsive elements located on the promoter of the UCP-1 gene.[6] This leads to increased transcription of UCP-1 and consequently to increased conversion of triglycerides and glucose into heat.[5] Furthermore, T3 is able to stabilize the UCP-1-mRNA, thereby reducing its degradation in the cell.[5] During cold-induction, the activity of the enzyme type-2-deiodinase (D2) is increased in BAT, leading to locally increased amounts of T3. This is an additional and necessary mechanism to stimulate thermogenesis by BAT.

Presence of brown adipose tissue in adult humans

The primary function of BAT is the production of heat – nonshivering thermogenesis – to prevent a decrease in body temperature. In neonates this is particularly important,

since they have a relatively large body surface area and little capacity to shiver due to underdevelopment of their muscles.[5]

Although BAT remains present in large quantities in rodents and other mammals, in humans the amount of BAT declines fast after infancy. Until recently, it was even assumed that in adults BAT is almost completely absent; indeed, it is hardly necessary as in adults skeletal muscle contributes primarily to heat production. However, ^{18}F-FDG-PET-CT scans performed in wintertime in adults in the context of visualization of malignancy showed increased glucose uptake in locations corresponding to BAT. Like malignant cells, brown fat cells take up glucose at an increased rate due to their high metabolism. Biopsies from these areas indeed showed a very high expression of UCP-1, the unique marker for BAT.

Further research by Van Marken Lichtenbelt and others with ^{18}F-FDG-PET-CT scans showed that – following cold-induction – BAT is present in almost 100% of young adults (**Figure 3**).[2] However, recent genotyping of human brown fat biopsies showed that this brown fat resembles more the beige fat found in white fat depots in mice rather than the classical brown fat.[7] Whether beige fat in humans has the same physiological properties as the classical brown fat in mice remains to be determined in the coming years.

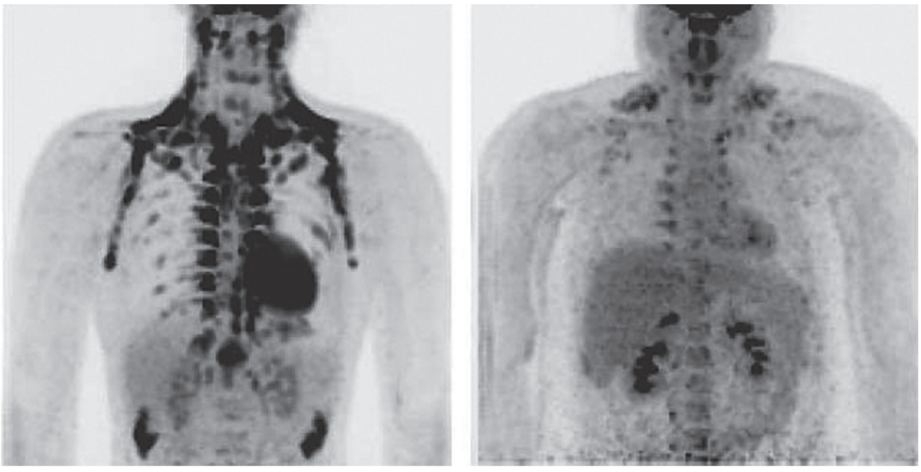


Figure 3. ^{18}F-fluordeoxyglucose (FDG)-PET-CT-uptake of brown adipose tissue (BAT) in adults. BAT can be visualized with a FDG-PET-CT-scan. To do this, a patient is exposed to cold (circa 16°C) during 2 hours to activate BAT. After 1 hour of cold-induction the radioactive tracer ^{18}F-FDG is injected intravenously. ^{18}F-FDG, a glucose analog, is taken up by organs that have a high glucose usage, especially the brain, heart and BAT. After 2 hours of cold-induction, and 1 hour after administration of the tracer, the uptake of ^{18}F-FDG is measured in circa 30 minutes using a low-dose CT-scan, immediately followed by a PET-scan. The CT-scan is used for localization of the FDG uptake sites. The activity and volume of BAT can be quantified by auto-contouring the areas in which FDG-uptake has taken place with a set threshold. Remarkable in this figure is the increased presence of BAT in a lean subject (left) compared to an obese subject (right) (figure is derived from a previous publication).[2]

Involvement of brown adipose tissue in pathology

Hibernomas and pheochromocytomas

In adults, the presence of BAT was already noticed in two clinical conditions: hibernomas and pheochromocytomas. Hibernomas are rare, benign tumours, named for their resemblance to BAT in animals that go into hibernation. They are clearly visible on ^{18}F-FDG-PET-CT scans, indicating that the tumour is metabolically active.[8] Pheochromocytomas, which are neuroendocrine tumours, secrete excessive amounts of noradrenalin, an important activator of BAT. Indeed, on ^{18}F-FDG-PET-CT scans in patients with this tumour, an increased volume and activity of BAT is seen. Moreover, after resection of the tumour FDG uptake decreases dramatically. The increased activity of BAT probably contributes significantly to the increased energy expenditure typical for this condition.[9]

Hyperthyroidism and hypothyroidism

In people with hyperthyroidism energy expenditure is increased, while this is decreased in hypothyroidism. In mice, thyroid hormone has been shown to be both directly – via the T3-receptor – and indirectly – via the sympathetic nervous system – involved in the activation of BAT.[10;11] The weight loss and excessive transpiration in hyperthyroidism, and the weight gain and reduced cold tolerance in hypothyroidism can therefore be (partly) attributed to an increased, respectively decreased activity of BAT.[5]

Obesity and type 2 diabetes mellitus

An interesting finding is that in adults the amount of BAT is inversely related to BMI and percentage of body fat.[2] More specifically: the volume and activity of BAT are inversely correlated with parameters of central obesity, such as visceral fat volume on CT-scan and waist circumference.[9] These findings suggest that obesity is associated with a low level of BAT activity. On the one hand, a reduced activity of BAT may predispose to obesity and obesity-related diseases such as type 2 diabetes mellitus by accumulation of triglycerides in the blood and subsequent storage in white adipose tissue, including ectopic fat depots such as skeletal muscle and the liver. Indeed, it has been shown in mice that excision of BAT results in hypertriglyceridemia and obesity.[12] Alternatively, insulation, due to the thick subcutaneous white fat layer in obese individuals, may be sufficient for the maintenance of body temperature, making active BAT redundant; low activity of BAT could then be the consequence of obesity.[13]

BAT burns both triglycerides and glucose. Therefore, BAT could also contribute to glucose homeostasis, particularly in resting conditions when glucose utilization by skeletal muscle is minimal. A low activity of BAT might, thus, not only predispose to type 2 diabetes via the aforementioned relation to obesity, but also via reduced glucose uptake at rest.[14]

Manipulating volume and activity of brown adipose tissue

The above-mentioned findings underscore that increasing the volume and activity of BAT is a promising target to increase total energy expenditure and consequently induce weight loss. Theoretically, BAT volume and activity can be increased in several ways. Generally, a distinction is made between methods that activate already present brown fat cells, and methods that stimulate the recruitment of new brown fat.

Activation of existing brown adipose tissue

BAT is strongly innervated by the sympathetic nervous system (**Figure 2**). This offers potential targets for intervention. Furthermore, the uncoupling phenomenon is a possible target.

Cold The most important activator of BAT via the sympathetic nervous system is cold. Several studies have shown a relation between the volume and activity of BAT and the outdoor temperature, with the highest activity during the coldest month of the year.[15] Therefore, the simplest method to activate BAT seems to be cold induction, for instance via creating a colder living or working environment. Whether this will actually affect BAT activity, and thus may induce weight loss, remains to be investigated.

Sympathicomimetics In addition to indirect activation of β_3-adrenergic receptors by cold, sympathicomimetics could also be used. In mouse models these seemed to be very successful.[16] Unfortunately, the expected weight-reducing effect failed to occur in humans. In addition, side effects – sometimes life threatening – were experienced.[17;18] So far, these agents are therefore not used for clinical purposes.

Uncouplers Increasing the uncoupling of ATP synthesis towards heat in BAT or other tissues, such as white adipose tissue or skeletal muscle (ectopic expression), might be an effective method to increase energy expenditure. Already in the thirties of the last century, the chemical uncoupler 2,4-dinitrophenol (DNP) was successfully used as an ingredient in diet pills. However, chemical uncouplers influence oxidative processes in all tissues, and not specifically in BAT. Due to serious side effects, like hyperthermia, this agent was therefore withdrawn from the market in 1938.[19] Current research now focuses on increasing (ectopic) expression of naturally occurring uncouplers, such as UCP-1.[20;21]

Recruitment of new brown adipose tissue

New BAT could be recruited by stimulating the differentiation of precursor cells of white adipocytes into brown adipocytes.

PPAR-γ agonists One of the key regulators in the differentiation of adipocytes is PPAR-γ. Animal studies have shown that PPAR-γ-agonists can recruit precursor cells of BAT and, in addition, can "brown" white adipocytes that thereby obtain UCP-1.[22] PPAR-γ-agonists are already used in the treatment of type 2 diabetes: the thiazolinediones (TZDs). Research has shown that the improvement in insulin sensitivity with TZDs is

partly due to an accelerated clearance of glucose in BAT.[23] Remarkably, though, use of TZDs leads to weight gain – partly due to fluid retention – and a different fat distribution, making their use as a weight loss agent less likely. Moreover, prescription of these agents is limited due to side effects such as heart failure and osteoporotic fractures.

BMP7 Another important regulator in the differentiation of BAT is BMP7. Recent studies in mice have shown that BMP7 is an effective agent to increase the amount of BAT – both in fat pads as peripherally – leading to an increase in energy expenditure and weight loss (Boon *et al*., unpublished data).[3;24]

Irisin Irisin is a recently discovered hormone that, in both mice and humans, is secreted by skeletal muscle during exercise. A recent study in mice demonstrated that exogenous administration of irisin induced 'browning' of subcutaneous white adipose tissue. This resulted in an increase in energy expenditure, a decrease in weight and an improvement in glucose tolerance.[25]

CONCLUSION

BAT burns triglycerides and glucose towards heat via the uncoupling protein UCP-1, and thus has a significant share in total energy expenditure. The recent observation of active BAT in adult humans might therefore offer new possibilities in the fight against obesity. Currently, various studies focus on activating BAT as a treatment strategy against obesity. In mouse models we seem to be close to success; the coming years will tell us whether BAT may be a novel therapeutic target organ in humans to combat obesity and related disorders such as type 2 diabetes.

REFERENCES

1. Jazet IM, Pijl H, Meinders AE. Adipose tissue as an endocrine organ: impact on insulin resistance. *Neth J Med* 2003;61(6):194-212.
2. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND *et al*. Cold-activated brown adipose tissue in healthy men. *N Engl J Med* 2009;360(15):1500-8.
3. Boon MR, van der Horst G, van der Pluijm G, Tamsma JT, Smit JW, Rensen PC. Bone morphogenetic protein 7: a broad-spectrum growth factor with multiple target therapeutic potency. *Cytokine Growth Factor Rev* 2011;22(4):221-9.
4. Fruhbeck G, Becerril S, Sainz N, Garrastachu P, Garcia-Velloso MJ. BAT: a new target for human obesity? *Trends Pharmacol Sci* 2009;30(8):387-96.
5. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev* 2004;84(1):277-359.
6. Rabelo R, Reyes C, Schifman A, Silva JE. Interactions among receptors, thyroid hormone response elements, and ligands in the regulation of the rat uncoupling protein gene expression by thyroid hormone. *Endocrinology* 1996;137(8):3478-87.
7. Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Giang AH *et al*. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* 2012;150(2):366-76.
8. Tsuchiya T, Osanai T, Ishikawa A, Kato N, Watanabe Y, Ogino T. Hibernomas show intense accumulation of FDG positron emission tomography. *J Comput Assist Tomogr* 2006;30(2):333-6.
9. Wang Q, Zhang M, Ning G, Gu W, Su T, Xu M *et al*. Brown adipose tissue in humans is activated by elevated plasma catecholamines levels and is inversely related to central obesity. *PLoS One* 2011;6(6):e21006.
10. Branco M, Ribeiro M, Negrao N, Bianco AC. 3,5,3'-Triiodothyronine actively stimulates UCP in brown fat under minimal sympathetic activity. *Am J Physiol* 1999;276(1 Pt 1):E179-E187.
11. Lopez M, Varela L, Vazquez MJ, Rodriguez-Cuenca S, Gonzalez CR, Velagapudi VR *et al*. Hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance. *Nat Med* 2010;16(9):1001-8.
12. Dulloo AG, Miller DS. Energy balance following sympathetic denervation of brown adipose tissue. *Can J Physiol Pharmacol* 1984;62(2):235-40.
13. Vijgen GH, Bouvy ND, Teule GJ, Brans B, Hoeks J, Schrauwen P *et al*. Increase in Brown Adipose Tissue Activity after Weight Loss in Morbidly Obese Subjects. *J Clin Endocrinol Metab* 2012.
14. Nedergaard J, Cannon B. The changed metabolic world with human brown adipose tissue: therapeutic visions. *Cell Metab* 2010;11(4):268-72.
15. Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J *et al*. High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. *Diabetes* 2009;58(7):1526-31.
16. Subramanian S, Vollmer RR. Sympathetic activation by fenfluramine depletes brown adipose tissue norepinephrine content in rats. *Pharmacol Biochem Behav* 2002;73(3):639-45.
17. Vosselman MJ, van der Lans AAJJ, Brans B, Wierts R, van Baak MA, Schrauwen P *et al*. Systemic β−Adrenergic stimulation of thermogenesis is not accompanied by brown adipose tissue activity in humans. *Diabetes* 2012;61.
18. Haller CA, Benowitz NL. Adverse cardiovascular and central nervous system events associated with dietary supplements containing ephedra alkaloids. *N Engl J Med* 2000;343(25):1833-8.
19. Harper JA, Dickinson K, Brand MD. Mitochondrial uncoupling as a target for drug development for the treatment of obesity. *Obes Rev* 2001;2(4):255-65.

20. Couplan E, Gelly C, Goubern M, Fleury C, Quesson B, Silberberg M *et al*. High level of uncoupling protein 1 expression in muscle of transgenic mice selectively affects muscles at rest and decreases their IIb fiber content. *J Biol Chem* 2002;277(45):43079-88.
21. Kopecky J, Rossmeisl M, Hodny Z, Syrovy I, Horakova M, Kolarova P. Reduction of dietary obesity in aP2-Ucp transgenic mice: mechanism and adipose tissue morphology. *Am J Physiol* 1996;270(5 Pt 1):E776-E786.
22. Petrovic N, Shabalina IG, Timmons JA, Cannon B, Nedergaard J. Thermogenically competent non-adrenergic recruitment in brown preadipocytes by a PPARgamma agonist. *Am J Physiol Endocrinol Metab* 2008;295(2):E287-E296.
23. Teruel T, Hernandez R, Rial E, Martin-Hidalgo A, Lorenzo M. Rosiglitazone up-regulates lipoprotein lipase, hormone-sensitive lipase and uncoupling protein-1, and down-regulates insulin-induced fatty acid synthase gene expression in brown adipocytes of Wistar rats. *Diabetologia* 2005;48(6):1180-8.
24. Schulz TJ, Huang TL, Tran TT, Zhang H, Townsend KL, Shadrach JL *et al*. Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat. *Proc Natl Acad Sci U S A* 2011;108(1):143-8.
25. Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC *et al*. A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 2012;481(7382):463-8.
26. Celi FS. Brown adipose tissue—when it pays to be inefficient. *N Engl J Med* 2009;360(15):1553-6.

7

Brown adipose tissue volume in healthy lean South Asian adults compared with white Caucasians: a prospective, case-controlled observational study

Leontine E.H. Bakker*
Mariëtte R. Boon*
Rianne A.D. van der Linden
Lenka Pereira Arias-Bouda
Jan B. van Klinken
Frits Smit
Hein J. Verberne
J. Wouter Jukema
Jouke T. Tamsma
Louis M. Havekes
Wouter D. van Marken Lichtenbelt
Ingrid M. Jazet#
Patrick C.N. Rensen#

*,#Authors contributed equally to manuscript

The Lancet Diabetes & Endocrinology 2014; 2(3): 210-7

ABSTRACT

Background. South Asians have an exceptionally high risk of developing type 2 diabetes mellitus compared to white Caucasians. Though the underlying cause is still poorly understood, it is assumed that an ethnic susceptibility towards a disturbed energy metabolism might be present. Brown adipose tissue (BAT) has emerged as an important player in energy metabolism by combusting fatty acids and glucose towards heat. We, therefore, hypothesized that a low total BAT activity might underlie the susceptibility for type 2 diabetes in South Asians.

Methods. BAT volume and activity were measured in healthy lean young adults (mean age 24.1±0.8 years) from South Asian (n=12) and white Caucasian (n=12) origin, matched for BMI, using cold-induced ^{18}F-FDG-PET-CT scans. Furthermore, resting energy expenditure (REE), non-shivering thermogenesis (NST) and serum parameters were assessed.

Findings. Thermoneutral REE was lower in South Asian compared to white Caucasian subjects (-32%, P=0.001). Upon cold exposure, the shiver temperature of South Asians was higher (+2.0°C, P=0.007). Furthermore, cold exposure significantly increased NST in white Caucasians (+20%, P<0.0001), but not in South Asians. Though the SUV_{max} and SUV_{mean} of ^{18}F-FDG in BAT did not differ, total BAT volume was markedly lower in South Asians (-34%, P=0.04). Taken the subjects together, BAT volume correlated positively with basal REE (β=0.44; P=0.04).

Interpretation. Healthy South Asian young adults have lower REE, NST, as well as lower BAT volume compared to matched white Caucasians. This might underlie their high susceptibility to develop metabolic disturbances, such as obesity and type 2 diabetes. Future studies should focus on developing novel strategies to increase BAT volume and activity.

INTRODUCTION

South Asians originate from the Indian sub-continent and represent one fifth of the world's population. The risk of developing type 2 diabetes and its related complications is exceptionally high among both native and migrant South Asians compared to people of white Caucasian descent, and is still rising.[1] Moreover, type 2 diabetes occurs at a younger age and lower BMI,[2,3] and the risk of diabetes related complications is higher.[4,5] The underlying cause of this excess risk is not completely understood, but might, involve their frequently present disadvantageous metabolic phenotype, consisting of central obesity, insulin resistance, and dyslipidemia.[6,7] It is commonly assumed that an ethnic susceptibility towards a disturbed energy homeostasis (e.g. lower oxidation of glucose and fatty acids by mitochondria) might underlie this phenotype.[8]

Recently, brown adipose tissue (BAT) has emerged as a novel player in energy homeostasis in humans.[9–12] In contrast to white adipose tissue, BAT burns triglycerides and glucose to generate heat through a process called mitochondrial uncoupling.[13] Interestingly, BAT volume and activity, as assessed after exposure to cold by ^{18}F-fluoro-deoxy-glucose (^{18}F-FDG) positron emission tomography and computed tomography (PET-CT) scans, are inversely related to BMI and percentage of body fat in adult humans, indicating an inverse relationship between BAT and obesity.[11,14,15] Besides a clear role for BAT in triglyceride metabolism[16] BAT is also thought to contribute to glucose homeostasis, particularly in resting conditions when glucose utilization by skeletal muscle is minimal.[17] Importantly, BAT appears to contribute to non-shivering thermogenesis (NST)[12,15] and it has been estimated that fully activated BAT in humans can contribute up to 15-20% of total energy expenditure.[13]

Thus, since BAT is involved in total energy expenditure and clearance of serum triglycerides and glucose thereby protecting against metabolic disturbances, we hypothesized that a low BAT volume or activity might underlie the disadvantageous metabolic phenotype and susceptibility for type 2 diabetes in South Asians. Therefore, we investigated resting energy expenditure (REE) as well as BAT volume and activity in young healthy lean South Asian males and matched white Caucasians (hereafter referred to as Caucasians), using ventilated hoods and cold-induced ^{18}F-FDG-PET-CT-scans. In addition, we examined the effect of cold exposure on NST, thermoregulation, and plasma lipid levels.

METHODS

Subjects

Twelve Dutch South Asian (subjects with two South Asian parents born in The Netherlands) and twelve Dutch Caucasian, lean (BMI <25 kg/m^2) and healthy males [age:

24.1±2.8 years] were enrolled via local advertisements. Subjects underwent a medical screening including their medical history, a physical examination, blood chemistry tests, and an OGTT to exclude individuals with type 2 diabetes according to the American Diabetes Association (ADA) 2010 criteria. Other exclusion criteria were rigorous exercise (>10 hours of exercise per week), smoking, and recent body weight change (>3 kg weight gain or loss within 3 months prior to the study). Subjects were matched for BMI by pairwise matching. The present study was approved by the Medical Ethical Committee of the Leiden University Medical Centre and performed in accordance with the principles of the revised Declaration of Helsinki. All volunteers gave written informed consent before participation.

Study design

The study was conducted in The Rijnland Hospital, Leiderdorp (The Netherlands). Subjects were studied in the morning after a 10-hour overnight fast and subjects were not allowed to exercise 24 hours prior to the study. Subjects wore standardized clothing, consisting of a T-shirt and boxer short. Body composition was determined by means of dual-energy x-ray absorptiometry (DEXA) (iDXA, GE Healthcare, UK). A cannula was inserted in the left antecubital vein for blood sampling and ^{18}F-FDG injection.

Details on the techniques used in the study are described in the supplementary technical appendix.

Cooling protocol. To activate BAT an individualized cooling protocol was applied, using two water perfused cooling mattresses (Blanketrol® III, Cincinatti Sub-Zero (CSZ) Products, Inc).[15] During the procedure subjects stayed in a clinical examination room. The protocol started with a baseline period of one hour in thermoneutral condition, after which subjects were exposed to mild cold. Since the onset temperature of shivering shows high interindividual variation (e.g. due to differences in body composition),[13] an individualized cooling protocol was used to ensure maximal NST, and thus a maximum level of BAT activity for each subject. Cooling started at 32°C and temperature was gradually decreased until shivering occurred. Temperature was then raised with 3-4°C and the cooling period of two hours was started (t_{cold}=0min). In case of shivering, temperature was raised with 1°C until shivering just stopped. Shivering was detected visually and by asking the subject. At the end of the first hour (t_{cold}=60min) of cooling ^{18}F-FDG was injected intravenously (2 MBq/kg). To exclude artefacts of muscle activity, subjects were instructed to lie still. Both in thermoneutral and cold-induced condition (t_{cold}=110min) venous blood was collected and indirect calorimetry was performed with a ventilated hood (Oxycon Pro™, CareFusion, Germany) (t_{cold}=80-110min). After the second hour (t_{cold}=120min) of cooling ^{18}F-FDG-PET-CT imaging was performed to quantify BAT.

^{18}F-FDG-PET-CT-scan. Imaging was performed on a PET-CT-scanner (Gemini TF PET-CT, Philips, The Netherlands) as described previously.[11] Imaging started with a low dose

CT-scan (effective dose 2 mSv), immediately followed by a PET-scan. The CT-scan was used for attenuation correction and localization of the ^{18}F-FDG uptake sites. Both image sets were reconstructed in transaxial, coronal, and sagittal images with a slice thickness of 4 mm. PET-CT images were interpreted blinded by both a nuclear medicine physician and a researcher using dedicated software (Hermes Hybrid Viewer™, Hermes Medical Solutions AB, Sweden). BAT activity and detectable BAT volume were quantified in the region of interest by autocontouring the BAT areas with a set threshold (SUV of 2.0 g/mL). One (Caucasian) subject developed hyperventilation following ^{18}F-FDG administration, and was therefore excluded from all cold-induced and BAT measurements.

Temperature registration. Core body temperature was measured continuously in the small intestine with the use of an ingestible telemetric capsule (Jonah™, BMedical, Australia). Core temperature measurement failed in two subjects (one South Asian and one Caucasian). Skin temperature was measured continually by wireless iButtons (iButtons®, Maxim, USA) placed at different positions on the skin.[18]

Calculations

Total detectable BAT volume is expressed in mL. BAT activity is expressed in standardized uptake values (SUV, the ratio of activity [kBq per mL] within the region of interest (ROI), and the injected activity [kBq] per bodyweight [g]). Both the maximum standardized uptake value (SUV_{max}) [g/mL] and the average standardized uptake value (SUV_{mean}) [g/mL] within the volume of interest (VOI) were determined. Energy expenditure, respiratory quotient (RQ) and substrate oxidation rates were sampled on a 1-minute basis and were determined as previously described.[19,20] Skin temperatures were measured according to the 14-point ISO method.[18] See supplementary technical appendix for detailed description.

Laboratory analysis

Serum triglyceride levels were determined using a commercially available kit (Roche Diagnostics, The Netherlands). Serum glucose and FFA levels were measured via enzymatic kits obtained via Instruchemie (Delfzijl, The Netherlands) and Wako Chemicals (Germany), respectively.

Statistical analysis

Data are presented as mean±SD when normally distributed or as median (IQR) when not normally distributed. A mixed effects model was applied to assess mean differences before and after cold exposure within and between groups, and to determine differences in the effect of cold exposure. Groups and intervention were modelled as fixed effects and the subject specific deviances from the group mean were modelled as random effects. Unpaired t-tests were used to compare baseline characteristics and BAT

parameters between groups. Nonparametric tests (Wilcoxon signed-rank test within group, Mann-Whitney between groups) were performed when appropriate. Correction of parameters for lean body mass (LBM) was performed by ANCOVA. To identify correlations between variables, linear regression analyses were performed. Significance level was set at P<0·05. Statistical analyses were performed using SPSS for Windows version 20·0 (IBM, USA).

RESULTS

Clinical characteristics

Mean age was 24.1±2.8 years (**Table 1**). BMI did not differ between groups (South Asians: 21.5±2.0 *vs.* Caucasians: 22.0±1.6 kg/m^2, p=0.50), but South Asians were shorter and lighter. The percentage of fat mass was higher in South Asians and, consequently, the percentage of LBM was lower. Additionally, the waist hip ratio was higher in South Asians.

Table 1. Clinical characteristics and body composition of healthy, young South Asian men and matched white Caucasians.

	white Caucasians (n=12)	South Asians (n=12)	p value
Clinical characteristics			
age (years)	24.6 ± 2.8	23.6 ± 2.8	0.390
length (m)	1.85 ± 0.04	1.74 ± 0.06	<0.0001
weight (kg)	75.1 ± 7.2	65.0 ± 8.5	0.005
body mass index (kg/m^2)	22.0 ± 1.6	21.5 ± 2.0	0.496
waist (cm)	84 ± 5.1	83 ± 7.7	0.804
hip (cm)	96 ± 3.5	89 ± 5.7	0.004
waist hip ratio	0.88 ± 0.04	0.93 ± 0.04	0.005
Body composition			
fat mass (%)	18.3 ± 5.0	23.9 ± 5.0	0.012
fat mass (kg)	13.9 ± 4.3	15.8 ± 4.6	0.306
lean body mass (%)	77.6 ± 4.8	72.1 ± 4.7	0.011
lean body mass (kg)	58.5 ± 6.0	46.8 ± 5.1	<0.0001
bone mineral content (%)	4.1 ± 0.2	4.0 ± 0.4	0.489
bone mineral mass (kg)	3.1 ± 0.3	2.6 ± 0.3	0.001

Data are presented as mean ± SD. P value *vs.* Caucasians based on an unpaired T-test

Table 2. Cardiovascular parameters and fasting serum levels in thermoneutral and cold-induced condition in healthy, young South Asian men and matched white Caucasians.

	white Caucasians (n=12)			South Asians (n=12)			
	TN	Cold-induced	p value†	TN	Cold-induced	p value†	p value*
Systolic BP (mmHg)							
Mean (SD)	135 ±11	143 ± 13	0.082	126 ± 18	125 ± 13‡	0.759	0.141
vs. TN (95% CI)		+6% (-1% to 13%)			+0% (-6% to 6%)		
Diastolic BP (mmHg)							
Mean (SD)	77 ± 6	84 ± 6	0.005	73 ± 9	79 ± 8	0.009	0.810
vs. TN (95% CI)		+10% (3% to 16%)			+9% (3% to 16%)		
Heart rate (bpm)							
Mean (SD)	64 ± 10	57 ± 10	0.017	62 ± 8	54 ± 8	0.003	0.620
vs. TN (95% CI)		-9% (-16% to -2%)			-12% (-19% to -5%)		
Glucose (mmol/L)							
Mean (SD)	4.17 ± 0.26	4.32 ± 0.29	0.213	4.30 ± 0.42	4.26 ± 0.58	0.698	0.244
vs. TN (95% CI)		+4% (-1% to 9%)			-1% (-7% to 5%)		
Triglycerides (mmol/L)							
Median (IQR)	0.78 (0.38)	0.92 (0.63)	0.014	0.77 (0.26)	0.90 (0.20)	0.003	0.624
vs. TN (95% CI)		+27% (6% to 48%)			+34% (13% to 56%)		
FFAs (mmol/L)							
Mean (SD)	0.66 ± 0.30	0.99 ± 0.29	<0.0001	0.88 ± 0.39	0.97 ± 0.37	0.099	0.005
vs. TN (95% CI)		+50% (34% to 66%)			+10% (-2% to 23%)		
FFAs (mmol/L/$kg_{fatmass}$)							
Mean (SD)	0.052 ± 0·035	0.078 ± 0.034	<0.0001	0.069 ±0.037	0.075 ± 0.035	0.099	0.005
vs. TN (95% CI)		+50% (34% to 66%)			+10% (-2% to 23%)		

† P value within group *vs.* thermoneutral condition. * P value cooling effect *vs.* Caucasians. ‡ P value of cold-induced systolic BP = 0.005 *vs.* Caucasians. All p values are based on a mixed model. BP, blood pressure; FFAs, free fatty acids; TN, thermoneutral.

Cardiovascular parameters

Cold exposure increased diastolic blood pressure and decreased heart rate in both groups (**Table 2**). Cold exposure tended to increase systolic blood pressure in Caucasians (+6%, 135±11 *vs.* 143±13 mmHg, p=0.08), but not in South Asians (+0%, 126±18 *vs.* 125±13 mmHg, p=0.76). Of note, cold-induced systolic blood pressure was significantly lower in South Asian compared to Caucasian subjects (125±13 *vs.* 143±13 mmHg, p=0.005).

Glucose and lipid levels

Fasting thermoneutral glucose and lipid levels were comparable between groups (**Table 2**). Cooling did not affect serum glucose levels, but markedly increased serum triglyceride levels in both groups. Of note, a significant cold-induced increase in serum FFA levels was present in Caucasian subjects (+50%, 0.66±0.30 *vs.* 0.99±0.29 mmol/L, p<0.0001), but not in South Asian subjects (+10%, 0.88±0.39 *vs.* 0.97±0.37 mmol/L, p=0.10). The ethnic difference in cold-induced FFA release was even more pronounced after dividing serum FFA levels by total fat mass, being the main source of serum FFA (**Table 2**).

Cold-induced thermogenesis

REE was 32% lower in South Asians compared to Caucasians (1279±123 *vs.* 1689±193 kcal/day, p=0.001) (**Table 3**). This difference was still apparent after correction for LBM, which was performed via ANCOVA (intercept 177±173 *vs.* 290±215 kcal/day, p=0.03, **Supplementary Figure 1**). During cold exposure, NST increased significantly in Cauca-

Table 3. Indirect calorimetry in thermoneutral and cold-induced condition in healthy, young South Asian men and matched white Caucasians.

	white Caucasians (n=12)			South Asians (n=12)			
	TN	Cold-induced	p value†	TN	Cold-induced	p value†	p value*
REE (kcal/day)							
Mean (SD)	1689 ± 193	2027 ± 471	0.001	1297 ± 123‡	1462 ± 127‡‡	0.072	0.186
vs. TN (95% CI)		+20% (9% to 31%)			+13% (-1% to 27%)		
Lipid ox (g/min)							
Mean (SD)	0.063 ±0.024	0.092 ±0.040	<0.0001	0.049 ±0.009	0.062 ±0.014$	0.072	0.119
vs. TN (95% CI)		+46% (24% to 70%)			+26% (-7% to 46%)		
Glucose ox (g/min)							
Mean (SD)	0.151 ± 0.057	0.141 ± 0.037	0.277	0.114 ±0.014$$	0.111 ±0.036	0.804	0.538
vs. TN (95% CI)		-6% (-23% to 12%)			-0·3% (-25% to 24%)		
Respiratory quotient							
Mean (SD)	0.85 ± 0.06	0.82 ± 0.04	0.030	0.84 ± 0.02	0.82 ± 0.04	0.128	0.577
vs. TN (95% CI)		-4% (-6% to -1%)			-2% (-6% to 1%)		

† P value within group *vs.* thermoneutral condition. * P value cooling effect *vs.* Caucasians. ‡ P value of thermoneutral REE = 0.001 *vs.* Caucasians. ‡‡ P value of cold-induced REE<0.0001 *vs.* Caucasians. $ P value of cold-induced lipid ox = 0.006 *vs.* Caucasians. $$ P value of thermoneutral glucose ox = 0.028 *vs.* Caucasians. All p values are based on a mixed model. ox, oxidation; REE, resting energy expenditure; TN, thermoneutral.

sians (+20%; 1689±193 *vs.* 2027±471 kcal/day, p<0.0001), but not in South Asians (+13%; 1297±123 *vs.* 1462±127 kcal/day, p=0.09) (**Supplementary Figure 2**). Furthermore, cold exposure significantly increased fat oxidation in Caucasians only (+46%, 0.063±0.024 *vs.* 0.092±0.040 g/min, p<0.0001), while glucose oxidation was not affected. In line with this, cold exposure significantly decreased RQ in Caucasians only (-0.03±0.01, p=0.03 *vs.* -0.02±0.01, p=0.13).

Core and skin temperature

Despite their increased fat mass percentage, the temperature at which shivering started was higher in South Asians than in Caucasians (10.9±1.8 *vs.* 8.9±1.5°C, p=0.007) (**Table 4**). Due to individual fine-tuning of the environmental temperature during NST, mean environmental temperature did not differ during the second half of cooling (19.8±2.5 *vs.* 18.7±2.2°C, p=0.27). Core temperature was not affected by cold exposure. Mean total, proximal and distal skin temperature markedly decreased to a similar extent in both groups. Consequently, core distal and core mean skin temperature gradients were significantly higher during cooling, indicating an insulative response in both South Asian and Caucasian subjects.

Table 4. Thermoregulation in thermoneutral and cold-induced condition in healthy, young South Asian men and matched white Caucasians.

	white Caucasians (n=12)			South Asians (n=12)			
	TN	Cold-induced	p value†	TN	Cold-induced	p value†	p value*
shiver temp (°C)		8.9 ±1.5			10.9 ±1.8		0.007
cooling temp (°C)		19.8± 2.5			18.7±2.2		0.271
core temp (°C)	36.8 ±0.2	36.6 ±0.3	0.130	36.6 ±0.3	36.8 ±0.2	0.128	0.034
mean skin temp (°C)	33.1 ±0.5	28.6 ±1.0	<0.0001	33.4 ±0.7	28.8 ±1.0	<0.0001	0.836
mean prox skin temp (°C)	34.4 ±0.4	30.8 ±1.0	<0.0001	34.4 ±0.4	31.0 ±1.1	<0.0001	0.535
mean dist skin temp (°C)	31.8 ±0.9	26.3 ±1.4	<0.0001	32.5 ±1.3	26.6 ±1.5	<0.0001	0.482
core mean skin temp gradient (°C)	3.6 ±0.4	7.9 ±0.9	<0.0001	3.2 ±1.0	8.1 ±1.1	<0.0001	0.643
core distal skin temp gradient (°C)	4.8 ±0.7	10.0 ±1.4	<0.0001	4.1 ±1.5	10.2 ±1.5	<0.0001	0.236

† P value within group *vs.* thermoneutral condition. * P value cooling effect *vs.* Caucasians or difference in shiver temp and cooling temp based on an unpaired T-test. All p values are based on a mixed model, except for shiver temp and cooling temp (unpaired T-test).dist, distal; prox, proximal; temp, temperature; TN, thermoneutral.

Brown adipose tissue volume and activity

In 96% (22/23) of the subjects active BAT was detected, as evidenced by ^{18}F-FDG uptake in the classical BAT regions (**Figure 1**).[14] The only (Caucasian) subject that lacked cold-induced BAT activity also exhibited the lowest REE when compared to all Caucasian subjects. SUV_{max} and SUV_{mean} in the VOI with metabolically active BAT did not differ

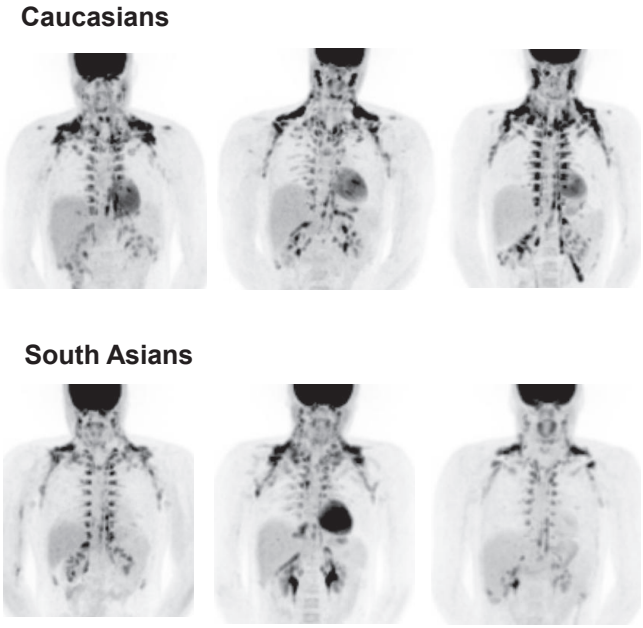


Figure 1. Brown adipose tissue activity in healthy young South Asian males and matched white Caucasians as assessed by PET-CT scan with ^{18}F-FDG. The images in the top row are from three representative Caucasian subjects, and the images in the bottom row from three representative South Asian subjects. FDG, ^{18}F-fluoro-deoxy-glucose. PET-CT, positron emission tomography and computed tomography.

between South Asian and Caucasian subjects (**Figure 2**). Intriguingly, detectable BAT volume was markedly lower in South Asians (-34%, 188±81 *vs.* 287±169 mL, p=0.04), which is also evident from **Figure 1**, depicting ^{18}F-FDG uptake in the upper body from three representative Caucasian and South Asian subjects.

Linear regression analysis showed a clear positive correlation between SUV_{max} and BAT volume (R^2=0.64, β=0.80, p<0.0001) (**Figure 3A**). Furthermore, thermoneutral serum FFA concentration correlated with BAT volume in Caucasian (R^2=0.49, β=0.70, p=0.02), but not in South Asian subjects (R^2=0.0009, β=0.03, p=0.97). Thermoneutral REE tended to correlate positively with BAT volume in both groups, although this correlation did not reach statistical significance per group. After pooling of all subjects, a clear positive correlation was evident between thermoneutral REE and BAT volume (R^2=0.19, β=0.44, p=0.04; **Figure 3B**), also after correction of REE for LBM (R^2=0.27, β=0.52, p=0.01; **Supplementary Figure 2**), strongly suggesting that BAT is involved in total energy metabolism.

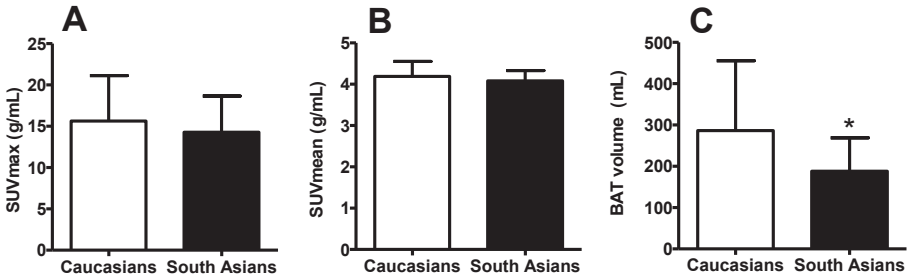


Figure 2. Brown adipose tissue (BAT) activity and volume in healthy young South Asian men and matched white Caucasians as assessed by quantifying ^{18}F-FDG-uptake on PET-CT scans. (A) SUV_{max}, the maximum standardized uptake value (B) SUV_{mean} [g/mL], the average standardized uptake value, and (C) total detectable BAT volume (mL). Results are expressed as mean± SD. * p<0·05 *vs.* Caucasians. BAT, brown adipose tissue. FDG, ^{18}F-fluoro-deoxy-glucose. PET-CT, positron emission tomography and computed tomography. SUV, standard uptake value.

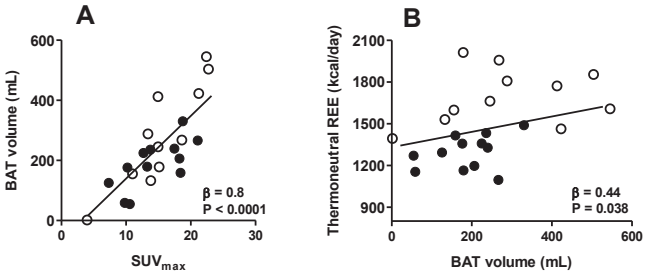


Figure 3. Correlations with brown adipose tissue (BAT). BAT volume in relation to SUV_{max} (A) and thermoneutral REE (B) in healthy young South Asian men (black circles) and matched white Caucasians (white circles). Correlations were determined by linear regression analysis. BAT, brown adipose tissue. REE, resting energy expenditure. SUV, standard uptake value.

DISCUSSION

In the present study, we demonstrate that healthy South Asian young adults have a lower REE compared to white Caucasians. Strikingly, we show that the detectable volume of metabolically active BAT, which has previously been shown to significantly contribute to energy metabolism,[11,14] is markedly lower in healthy South Asian subjects. These findings were corroborated by the higher shiver temperature and smaller cold-induced NST in South Asians compared to Caucasians.

We detected BAT in 96% (22/23) of the subjects, which corresponds to the numbers found in previous studies.[11,21] Moreover, as reported previously (reviewed in reference 13),[13,21] in Caucasian subjects cold exposure resulted in increased serum FFA levels, lipid oxidation, systolic blood pressure and NST, the latter aiming at preventing a drop

in core body temperature. In South Asians, all of these responses were reduced. Our previous study indicated that BAT, and not muscle, is responsible for NST via the process of mitochondrial uncoupling.[22] Intriguingly, lean subjects with detectable BAT activity have significantly higher NST than those without detectable BAT activity.[12] It is therefore tempting to speculate that the lower BAT volume might underlie the smaller increase in NST in South Asians, although we could not find a significant correlation between BAT volume and NST, as has been shown previously[12,15,23], albeit not consistently.[11,24]

Since cold-induced increases in lipolysis and systolic blood pressure are mediated by sympathetic activation, the lower response in South Asians may be due to a lower cold-induced sympathetic activation. We cannot rule out the possibility that this is due to the fact that South Asians were initially cooled at a somewhat higher initial environmental temperature, resulting in less sympathetic outflow. However, SUV_{max} and SUV_{mean} did not differ, suggesting that BAT could be equally stimulated in both groups by cold exposure. Thus, signs of lower sympathetic activation were only present in the white adipose tissue depot and the vasculature, and not in BAT. This can be explained given that sympathetic outflow neurons towards various organs derive from different brain regions.[25] A lower sympathetic response in South Asians may, at least in part, underlie their lower REE, as the reduced liberation of FFAs from white adipose tissue in plasma may have lowered the availability of FFAs for combustion by BAT, resulting in lower fat oxidation. Future studies would be needed to investigate a potentially different (organ-specific) sympathetic response in South Asians and the potential link with REE.

Strikingly, South Asians had a significantly higher shivering temperature upon cold exposure, despite their higher total percentage of fat mass. It has previously been shown that obese subjects have lower shivering temperatures compared to lean subjects due to better insulation.[15] However, the opposite occurred in South Asians. An impaired capacity of BAT to contribute to total heat production might underlie the accelerated action of the muscles to produce heat by shivering. Indeed, this is supported by a study of Ouellet *et al*[10] in which subjects with higher BAT volume experienced less shivering during cooling. However, we cannot exclude the possibility that the higher shivering temperature in South Asians could, at least in part, be influenced by their smaller body size and lean body mass.

It could be argued that the lower energy metabolism in South Asians may not be solely due to decreased BAT volume but also due to diminished oxidative metabolism in muscle. Of note, we recently obtained muscle biopsies from the same subjects before and after a short-term high-fat diet challenge, and did not observe differences in skeletal muscle insulin signalling and expression of genes involved in oxidative phosphorylation and mitochondrial biogenesis.[26] Furthermore, food intake and physical activity levels did not differ between South Asians and Caucasians.

It is interesting to speculate on possible mechanisms that could underlie the decreased BAT volume in South Asians. The fact that this is found already in healthy young adults without differences in the degree of ^{18}F-FDG uptake, as evidenced by equal SUV_{max} and SUV_{mean}, could point to a defect in BAT differentiation. However, ^{18}F-FDG uptake only represents glucose uptake by the tissue and not metabolism *per se*. Therefore, a potential dysfunction in oxidative metabolism in the tissue cannot be excluded and should be further investigated, for example with an ^{11}C-acetate tracer as previously described[10] or by studying BAT biopsies. The underlying cause of the lower BAT volume in South Asians may be genetic (i.e. blunted expression of signalling molecules involved in BAT differentiation), environmental (i.e. clothing behaviour or central heating setting), or a combination of the two, and is an interesting subject for future studies.

Our study is not without limitations. Although our group size is an accepted number conform several landmark BAT studies,[9,11] yielding sufficient power to identify differences in detectable BAT volume between South Asians and Caucasians, the numbers may be limited for interpreting certain correlations. Strengths of our study are the large number of measurements we performed next to ^{18}F-FDG PET-CT scans, such as indirect calorimetry and temperature records, and the use of a personalized cooling protocol with water perfused cooling mattresses, which results in maximal BAT activity and detectable BAT volume under non-shivering conditions.[13] The latter may well explain why in a recent study by Admiraal *et al*[27], in which all subjects were cooled in an air-cooled chamber with a stable temperature of 17°C, no difference in BAT volume could be identified in South Asian compared to Caucasian subjects. Since water has a higher heat transfer coefficient than air, water cooling results in more intense cooling of subjects and, likely, higher detectable BAT volume. Indeed, when comparing detectable BAT volume of Caucasian subjects between the two studies, BAT volume in the Admiraal study was markedly lower compared to the current study (16 *vs.* 287 mL). Thus, the less intense cooling protocol may have underestimated the BAT volume in their study subjects. Moreover, exposure of all subjects to a stable room temperature of 17°C instead of using a personalized cooling protocol might have led to relatively higher underestimation of BAT volume in Caucasian *vs.* South Asian subjects, as they have, according to our study, a markedly lower shiver temperature. A possible drawback of the use of a personalized cooling protocol may be that differences in environmental temperature may induce differences in BAT activity, since a steep relation exists between environmental temperature and thermogenesis.[28] This was, however, likely not the case in our study, since SUV_{max} and SUV_{mean} did not differ between the ethnic groups pointing to equal BAT activation.

This study may have major clinical impact. Untill now, little is known about the underlying mechanisms of the disadvantageous metabolic phenotype and the consequently high risk of type 2 diabetes in South Asians. Therefore, treatment options and, more

importantly, preventive strategies are unfocused and of limited efficacy in South Asians. Thus, increasing the volume or activity of BAT might be of great therapeutic potential in South Asians, resulting in increased clearance of glucose and fatty acids and increased total energy expenditure. We have recently shown that BAT can be recruited in humans following 10 days of cold intervention.[22] Future studies should be directed towards the efficacy of this strategy, as well as other options, such as medication, to increase BAT activity. These strategies might finally be used to improve the metabolic phenotype in South Asians.

In conclusion, this study shows that healthy, young South Asian subjects have lower BAT volume and lower REE compared to matched white Caucasians, possibly underlying their high susceptibility to develop metabolic disturbances, such as obesity and type 2 diabetes. Future studies should, therefore, be directed towards the development of novel strategies to increase BAT volume and activity.

REFERENCES

1. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res Clin Pract* 2011;94(3)**:**311-21.
2. Bindraban NR, van Valkengoed IG, Mairuhu G, Holleman F, Hoekstra JB, Michels BP *et al*. Prevalence of diabetes mellitus and the performance of a risk score among Hindustani Surinamese, African Surinamese and ethnic Dutch: a cross-sectional population-based study. *BMC Public Health* 2008;8:271.
3. Chiu M, Austin PC, Manuel DG, Shah BR, Tu JV. Deriving ethnic-specific BMI cutoff points for assessing diabetes risk. *Diabetes Care* 2011;34(8):1741-8.
4. Chandie Shaw PK, Baboe F, van Es LA, van der Vijver JC, van de Ree MA, de Jonge N *et al*. South-Asian type 2 diabetic patients have higher incidence and faster progression of renal disease compared with Dutch-European diabetic patients. *Diabetes Care* 2006;29(6):1383-5.
5. Chaturvedi N, Fuller JH. Ethnic differences in mortality from cardiovascular disease in the UK: do they persist in people with diabetes? *J Epidemiol Community Health* 1996;50(2):137-9.
6. McKeigue PM, Shah B, Marmot MG. Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. *Lancet* 1991;337(8738):382-6.
7. Boon MR, Karamali NS, de Groot CJ, van Steijn L, Kanhai HH, van der Bent C *et al*. E-Selectin is elevated in cord blood of South Asian neonates compared with Caucasian neonates. *J Pediatr* 2011;160(5):844-848.
8. Hall LML, Moran CN, Milne GR, Wilson J, MacFarlane NG, Forouhi NG *et al*. Fat oxidation, fitness and skeletal muscle expression of oxidative/lipid metabolism genes in South Asians: implications for insulin resistance? *PLoS One* 2010;5(12):e14197.
9. Cypess AM, Chen YC, Sze C, Wang K, English J, Chan O *et al*. Cold but not sympathomimetics activates human brown adipose tissue in vivo. *Proc Natl Acad Sci USA* 2012;109(25):10001-5.
10. Ouellet V, Labbe SM, Blondin DP, Phoenix S, Guérin B, Harnan F *et al*. Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. *J Clin Invest* 2012;122(2):545-52.
11. Van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND *et al*. Cold-activated brown adipose tissue in healthy men. *N Engl J Med* 2009;360(15):1500-8.
12. Yoneshiro T, Aita S, Matsushita M, Kameya T, Nakada K, Kawai Y *et al*. Brown adipose tissue, whole-body energy expenditure, and thermogenesis in healthy adult men. *Obesity* 2012;19(1):13-6.
13. Van Marken Lichtenbelt WD, Schrauwen P. Implications of nonshivering thermogenesis for energy balance regulation in humans. *Am J Physiol Regul Integr Comp Physiol* 2011;301(2):R285–96.
14. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB *et al*. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* 2009;360(15):1509-17.
15. Vijgen GH, Bouvy ND, Teule GJ, Brans B, Schrauwen P, van Marken Lichtenbelt WD. Brown adipose tissue in morbidly obese subjects. *PLoS One* 2011;6(2):e17247.
16. Bartelt A, Bruns OT, Reimer R, Hohenberg H, Ittrich H, Peldschus K *et al*. Brown adipose tissue controls triglyceride clearance. *Nat Med* 2011;17(2):200-5.
17. Stanford KI, Middelbeek RJ, Townsend KL, An D, Nygaard EB, Hitchcox KM *et al*. Brown adipose tissue regulates glucose homeostasis and insulin sensitivity. *J Clin Invest* 2013;123(1):215-23.
18. van Marken Lichtenbelt WD, Daanen HA, Wouters L, Fronczek R, Raymann RJ, Severens NM *et al*. Evaluation of wireless determination of skin temperature using iButtons. *Physiol Behav* 2006;88(4-5):489-97.

19. Simonson DC, DeFronzo RA. Indirect calorimetry: methodological and interpretative problems. *Am J Physiol* 1990;258(3 Pt 1):E399-412.
20. Feurer I, Mullen JL. Bedside measurement of resting energy expenditure and respiratory quotient via indirect calorimetry. *Nutr Clin Pract* 1986;1:43-9.
21. Vosselman MJ, Brans B, Van der Lans AA, Wierts R, van Baak MA, Mottaghy F *et al*. Brown adipose tissue activity after a high-calorie meal in humans. *Am J Clin Nutr* 2013;98(1):57-64.
22. Van der Lans AA, Hoeks J, Brans B, Vijgen GH, Visser MG, Vosselman MJ *et al*. Cold acclimation recruits brown fat and increases nonshivering thermogenesis. *J Clin Invest* 2013;123(8):3395-403.
23. Orava J, Nuutila P, Lidell M, Oikonen V, Noponen T, Viljanen T *et al*. Different metabolic responses of human brown adipose tissue to activation by cold and insulin. *Cell Metab* 2011;14(2):272-9.
24. Vosselman MJ, van der Lans AA, Brans B, Wierts R, van Baak MA, Schrauwen P *et al*. Systemic β-adrenergic stimulation of thermogenesis is not accompanied by brown adipose tissue activity in humans. *Diabetes* 2012;61(12):3106-13.
25. Llewellyn-Smith IJ. Anatomy of synaptic circuits controlling the activity of sympathetic preganglionic neurons. *J Chem Neuroanat* 2009;38(3):231-9.
26. Bakker LEH, van Schinkel LD, Guigas B, Streefland TC, Jonker JT, van Klinken JB *et al*. A 5-day high fat high calorie diet impairs insulin sensitivity in healthy, young South Asian men but not in Caucasian men. *Diabetes* 2014;63(1):248-58.
27. Admiraal WM, Verberne HJ, Karamat FA, Soeters MR, Hoekstra JB, Holleman F. Cold-induced activity of brown adipose tissue in young lean men of South-Asian and European origin. *Diabetologia* 2013;Epub.
28. Cannon B, Nedergaard J. Nonshivering thermogenesis and its adequate measurement in metabolic studies. *J Exp Biol* 2011;214(Pt 2):242-53.

SUPPLEMENTARY TECHNICAL APPENDIX

Cooling protocol

To activate BAT an individualized cooling protocol was applied.[1,2] Cooling was performed using two water perfused cooling mattresses (Blanketrol® III, Cincinatti Sub-Zero (CSZ) Products, Inc) to cool both the dorsal and ventral side of the body. During the procedure subjects stayed in a clinical examination room (temperature approx. 24°C) in a semi-supine position. The protocol started with a baseline period of one hour in thermoneutral condition (water temperature cooling mattresses 32°C), after which subjects were exposed to mild cold. Since the onset temperature of shivering shows a high interindividual variation (e.g. due to differences in body composition),[3,4] an individualized cooling protocol was used to ensure maximal non-shivering thermogenesis (NST), and thus a maximum level of BAT activity for each subject. Cooling started at a mattress temperature of 32°C and water temperature was gradually decreased until shivering occurred. In short, we first decreased the temperature with steps of 5°C every 5 minutes. After we reached a water temperature of 17°C, we decreased the temperature with 2°C every 10 minutes. When a water temperature of 11°C was reached (but not all subjects reached this temperature), we decreased temperature with 1°C every 10 minutes. This was continued until shivering occurred. Shivering was detected visually and by asking the subject if he experienced shivering. When the shivering temperature had been reached, the subject was warmed for 3 minutes with a warm blanket so that shivering stopped and then the subject was cooled with a water temperature that lay 3°C higher than the temperature at which shivering occurred. From that moment, the cooling period of two hours was started (t_{cold}=0min). In case of shivering, temperature was raised by steps of 1°C until shivering just stopped. In this manner NST was maximized for each individual without shivering. Also, just before administration of the FDG, we raised the temperature by 1°C to prevent occurrence of shivering during the FDG uptake period. At the end of the first hour (t_{cold}=60min) of cooling ^{18}F-FDG was injected intravenously (2 MBq/kg). To exclude the artefact of muscle activity, subjects were instructed to lay still. Both in thermoneutral and cold-induced condition (t_{cold}=110min) venous blood was collected, blood pressure was measured and indirect calorimetry was performed with a ventilated hood (Oxycon Pro™, CareFusion, Germany) (t_{cold}=80-110min). After the second hour (t_{cold}=120min) of cooling ^{18}F-FDG-PET-CT imaging was performed to quantify BAT.

^{18}F-FDG-PET-CT scan

Imaging was performed on a PET-CT-scanner (Gemini TF PET-CT, Philips, The Netherlands) after confirmation of the serum glucose level and the intravenous injection of 2 MBq/kg ^{18}F-FDG. Imaging was performed in three dimensional mode, with emission scans of 3 minutes per bed position in the upper part of the body where brown adipose tissue

is usually found (first seven bed positions) and scans of 30 seconds per bed position in the body area below. Imaging started with a low dose CT-scan (effective dose 2 mSv), immediately followed by a PET-scan. The CT-scan was used for attenuation correction and localization of the ^{18}F-FDG uptake sites. The resulting total radiation dose from the low-dose CT scan and the injected radioactive tracer was approximately 4.6 mSv, which is comparable to a standard CT-thorax. Both image sets were reconstructed in transaxial, coronal, and sagittal images with a slice thickness of 4 mm. PET-CT images were interpreted blinded by both a nuclear medicine physician and a researcher using dedicated software (Hermes Hybrid Viewer™, Hermes Medical Solutions AB, Sweden). BAT activity (measured in g/mL) and detectable BAT volume (measured in cubic centimetres) were quantified in anatomical regions of interest (i.e. the cervical, supraclavicular and superior mediastinal depots) using the auto contouring and region growing tool of the Hybrid Viewer. In these areas a SUV cut-off value for ^{18}F-FDG uptake indicating BAT was defined to be at least 2.0 g/mL.

Temperature registration

Core body temperature was measured continuously in the small intestine with the use of an ingestible telemetric capsule (Jonah™, BMedical, Australia) that recorded core temperature at 1-minute intervals. Skin temperature was measured continually by wireless iButtons (iButtons®, Maxim, USA).[5] An iButton contains a semiconductor temperature sensor, a computer chip with a real time clock and memory, and a battery. In total fourteen iButtons were attached to the skin with adhesive tape at the following ISO-defined locations: forehead, clavicular (left and right), sternal (left and right), supra umbilical, anterior thigh (left and right), lateral thigh (left and right), flat of the hand (left and right) and bow of the foot (left and right). The iButtons recorded skin temperature at 1-minute intervals.

Calculations

Total detectable BAT volume: In every slice, BAT size (measured in square centimetres) was quantified in the anatomical regions of interest (ROIs) using the auto contouring and region growing tool of the Hybrid Viewer. Detectable BAT volume (measured in cubic centimetres) was calculated by summing up the ROIs from the individual slices, establishing a volume of interest (VOI).

BAT activity: Within every region of interest, the Hybrid Viewer provides two measures of ^{18}F-FDG uptake, the maximal and mean standardized uptake value (SUV_{max} and SUV_{mean} respectively). The standardized uptake value (SUV) is defined as the ratio of activity [kBq per mL] within the region of interest (ROI) and the injected activity [kBq] per bodyweight [g] and is expressed in g/mL. For $SUV_{max,}$ the highest value in the VOI was taken. For SUV_{mean} the mean value within the VOI was determined.

Indirect calorimetry: Respiratory quotient (RQ) and substrate oxidation rates were sampled on a 1-minute basis and were determined as described by Simonson and DeFronzo.[6] Energy expenditure was calculated according to the Weir equation[7]. We used the following formula:

(1) Respiratory quotient (RQ) = VCO_2 / VO_2
(2) Lipid oxidation = $1.69*VO_2 – 1.69*VCO_2$
(3) Glucose oxidation = $4.57*VO_2 – 3.23*VCO_2$
(4) Resting energy expenditure = $(3.9*VO_2 + 1.1*VCO_2)*1.44$

In these equations, VCO_2 is the carbon dioxide production and VO_2 the oxygen consumption.

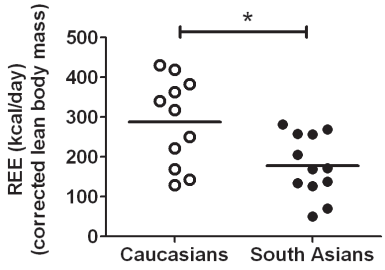
Skin temperature measurements: Distal skin temperature was calculated as the average temperature of hands and feet and proximal skin temperature as the weighted average temperature of claviculae, anterior thigh and umbilicus (Tprox=0.383*Tavg_thighs + 0.293*Tavg_clav + 0.324*Tavg_umbilicus) according to the equation of Van Marken Lichtenbelt *et al*[5], based on the formulas by Kräuchi *et al*[8] and Hardy *et al*[9]. Mean skin temperature was calculated as the average of distal and proximal skin temperature. Core mean skin temperature gradient was calculated as the difference between core and mean skin temperature, and core distal skin temperature gradient as the difference between core and distal skin temperature.

REFERENCES

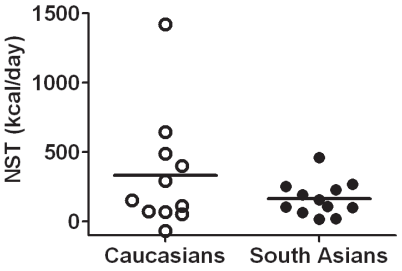
1. Van der Lans AA, Hoeks J, Brans B, Vijgen GH, Visser MG, Vosselman MJ *et al*. Cold acclimation recruits brown fat and increases nonshivering thermogenesis. *J Clin Invest* 2013;123(8):3395-403.
2. Vijgen GH, Bouvy ND, Teule GJ, Brans B, Schrauwen P, van Marken Lichtenbelt WD. Brown adipose tissue in morbidly obese subjects. *PLoS One* 2011;6(2):e17247.
3. Van Marken Lichtenbelt WD, Schrauwen P. Implications of nonshivering thermogenesis for energy balance regulation in humans. *Am J Physiol Regul Integr Comp Physiol* 2011;301(2):R285–96.
4. van Ooijen AM, van Marken Lichtenbelt WD, van Steenhoven AA, Westerterp KR. Seasonal changes in metabolic and temperature responses to cold air in humans. *Physiol Behav* 2004;82(2-3):545–53.
5. van Marken Lichtenbelt WD, Daanen HA, Wouters L, Fronczek R, Raymann RJ, Severens NM *et al*. Evaluation of wireless determination of skin temperature using iButtons. *Physiol Behav* 2006;88(4-5):489-97.
6. Simonson DC, DeFronzo RA. Indirect calorimetry: methodological and interpretative problems. *Am J Physiol* 1990;258(3 Pt 1):E399-412.
7. Feurer I, Mullen JL. Bedside measurement of resting energy expenditure and respiratory quotient via indirect calorimetry. *Nutr Clin Pract* 1986;1:43-9.

8. Kräuchi K, Cajochen C, Möri D, Graw P, Wirz-Justice A. Early evening melatonin and S-20098 advance circadian phase and nocturnal regulation of core body temperature. *Am J Physiol* 1997;272(4 Pt 2):R1178-88.
9. Hardy JD, Du Bois, EF. The technic of measuring radiation and convection. *J Nutr* 1938;15:461-75.

Supplemental figures



Supplemental figure 1. Resting energy expenditure (REE) after correction for lean body mass. REE in healthy young South Asian men (black circles) and matched white Caucasians (white circles) was corrected for lean body mass by ANCOVA. REE, resting energy expendtiure. *p = 0.03.



Supplemental figure 2. Individual responses of non-shivering thermogenesis in healthy young South Asian males and matched white Caucasians as assessed by indirect calorimetry. Non-shivering thermogenesis (NST) in healthy young South Asian males (black circles) and matched Caucasians (white circles). p = 0.186.

PART 2

CARDIOVASCULAR DISEASE

8 High prevalence of cardiovascular disease in South Asians: central role for brown adipose tissue?

Leontine E.H. Bakker*
Mariëtte R. Boon*
Rianne A.D. van der Linden
Antoinette F. van Ouwerkerk
Pauline L. de Goeje
Jacqueline Counotte
Ingrid M. Jazet#
Patrick C.N. Rensen#

*,# Authors contributed equally to manuscript

Accepted for publication in Crit Rev Clin Lab Sci

ABSTRACT

Cardiovascular disease is the leading cause of death in modern society. Interestingly, the risk of developing cardiovascular disease varies between different ethnical groups. A particularly high risk is faced by South Asians, representing over one fifth of the world's population. Here, we review potential factors contributing to the increased cardiovascular risk of the South Asian population and discuss novel therapeutic strategies based on recent insights. In South Asians, classical ('metabolic') risk factors associated with cardiovascular disease are highly prevalent and include central obesity, insulin resistance, type 2 diabetes and dyslipidemia. A contributing factor that may underlie the development of this disadvantageous metabolic phenotype is the presence of a lower amount of brown adipose tissue in South Asian subjects, resulting in lower energy expenditure and lower lipid oxidation and glucose uptake. As it has been established that the increased prevalence of classical risk factors in South Asians cannot fully explain their increased risk for cardiovascular disease, other non-classical risk factors must underlie this residual risk. In South Asians, the prevalence of 'inflammatory' risk factors including visceral adipose tissue inflammation, endothelial dysfunction and HDL dysfunction is higher compared to white Caucasians. We conclude that a potential novel therapy to lower cardiovascular disease risk in the South Asian population is to enhance brown adipose tissue volume or its activity in order to diminish classical risk factors. Furthermore, anti-inflammatory therapy may lower non-classical risk factors in this population and the combination of both strategies may be especially effective.

INTRODUCTION

The South Asian population originally descends from the Indian subcontinent (India, Pakistan, Bangladesh, Nepal and Sri Lanka) and comprises approximately 20% of the total world population. The burden and mortality of cardiovascular disease are significantly higher among both native and migrant South Asians in comparison to subjects of white Caucasian descent.[1-3] In addition, South Asian individuals are affected at a younger age and as a result India suffers the highest loss in potentially productive years of life due to cardiovascular deaths.[2;4;5] The exceptionally high cardiovascular disease risk in South Asians poses a major health and socioeconomic burden and gaining more insight in the pathogenesis of cardiovascular disease in this population is of great importance. Several studies show that in South Asians classical cardiovascular risk factors, including dyslipidemia, obesity, insulin resistance and type 2 diabetes are more prevalent. However, after correction for these classical risk factors, ethnicity remains an independent determinant of cardiovascular events.[1;4;6] Thus, residual risk is present suggesting that additional, non-classical, cardiovascular risk factors may play a role.

In this review, we discuss classical and non-classical risk factors for cardiovascular disease in subjects of South Asian origin and focus on potential pathophysiological pathways that might clarify the unexplained 'excess' risk for cardiovascular disease in this population. Furthermore, we discuss novel therapeutic strategies based on recent insights.

Epidemiology of cardiovascular disease in South Asians

The epidemiology of cardiovascular disease in South Asians has been studied extensively in countries with large South Asian immigrant populations. These studies consistently show that the risk of cardiovascular disease among South Asian immigrants is at least two-fold increased compared to native populations as well as to other immigrant groups. In Canada, the prevalence of cardiovascular disease among South Asian immigrants is 10.7%, compared to 5.4% and 2.4% for people from European and Chinese descent, respectively.[4] In the UK, South Asians show a 40-60% higher mortality rate from coronary heart disease compared to white Europeans.[1;6;7] Furthermore, in all of these studies South Asian immigrants were affected at a younger age than control groups. Importantly, cardiovascular disease risk is not solely increased in South Asian immigrants but also in native South Asian subjects as the age standardized mortality rate for cardiovascular disease is around 50% higher in South Asian countries as compared to Western countries.[3]

The higher risk of cardiovascular disease in South Asians most likely reflects interactions between genetic susceptibility and environmental factors, such as changes secondary to urbanization and migration. Indeed, the risk of cardiovascular disease

appears to increase as South Asians move from rural India to urban India to immigrant populations.[8] With urbanization and migration to Western environments the consumption of energy-rich diets markedly increases. In addition, energy expenditure decreases due to less physical activity, and exposure to stress increases. Acculturation is positively associated with coronary artery disease and type 2 diabetes in South Asian immigrants in the US.[9] Thus, migration itself could be an aggravating factor in the high cardiovascular disease risk of migrant South Asians. Most studies however, have not examined such variables, nor did they differentiate between first and second generation migration. We propose that future studies should include a more detailed migration history to examine what factors associated with migration may contribute to the increased cardiovascular disease risk of South Asians.

PATHOGENESIS OF CARDIOVASCULAR DISEASE

Initiation of atherosclerosis development

The major cause of cardiovascular disease is atherosclerosis, which is present many years before any clinical symptoms of cardiovascular disease become manifest, including ischemic heart disease, cerebrovascular accident and peripheral arterial occlusive disease. Atherosclerosis development starts with endothelial damage and dysfunction, often promoted by inflammatory mediators or shear stress induced by nonlaminar blood flow.[10] Endothelial activation is characterized by a proadhesion, proinflammatory, and procoagulatory milieu that favours all stages of atherogenesis. This results in enhanced recruitment of inflammatory leukocytes such as monocytes and T-lymphocytes towards the damaged site, and migration of monocytes into the subendothelial intima followed by transformation into macrophages. At the same time, low-density lipoprotein (LDL) particles may become oxidized (e.g. due to release of reactive oxygen species or cigarette smoke), resulting in accumulation of oxidized LDL within the vessel wall. Macrophages within the vessel wall engulf this oxidized LDL, and become lipid-laden foam cells.[11] What follows is an inflammatory status in which leukocytes and local endothelial cells excrete pro-inflammatory cytokines, including interferon γ (IFN-γ), tumour necrosis factor-α (TNF-α) and growth factors, further stimulating leukocyte recruitment, accumulation of macrophages as well as proliferation of smooth muscle cells in the vascular intima, which produce elastin and collagen.[12] This all sequentially leads to plaque formation, plaque expansion and formation of a fibrous cap. High-density lipoprotein (HDL) has been attributed several atheroprotective properties. Firstly, HDL stimulates cholesterol efflux from foam cells present in atherosclerotic plaques by acting as cholesterol acceptor and transporting cholesterol back to the liver for excretion into the bile.[13] Secondly, HDL prevents LDL from oxidation.[14-16] Thirdly, HDL has anti-inflammatory properties; during

the early phase of atherosclerosis development, HDL prevents leukocyte adhesion to endothelial cells by lowering expression of monocyte chemotactic protein 1 (MCP-1) and vascular cell adhesion molecule (VCAM-1) and by counteracting platelet-activating factor (PAF) induced adhesion of leukocytes.[13-15;17] Fourthly, HDL induces vasodilation through stimulation of nitric oxide (NO) release by endothelial cells.[18] This results in lower endothelial shear stress (*i.e.* improved endothelial function) and thereby lower initiation of atherosclerosis development. The vasodilating effect also increases delivery of insulin to tissues that take up glucose.

Classical and non-classical cardiovascular disease risk factors

From the above-mentioned pathophysiology it becomes clear that the development of atherosclerosis may be promoted by metabolic as well as inflammatory risk factors. Metabolic or 'classical' risk factors include dyslipidemia (marked by elevated LDL-cholesterol and triglycerides, and decreased HDL-cholesterol levels), hypertension (resulting in nonlaminar oscillatory blood flow), and smoking (resulting in endothelial dysfunction).[19] Furthermore, central obesity and insulin resistance are metabolic risk factors that are associated with increased cardiovascular disease risk.[20-22] In addition, although the precise mechanism is still under debate, inflammatory or 'non-classical' risk factors may contribute to development of cardiovascular disease. Among these are systemic inflammation (marked by elevated C-reactive protein and/or TNF-α levels), as well as HDL dysfunction and endothelial dysfunction which can both give rise to inflammation.[23]

Role of brown adipose tissue in whole-body metabolism

Interestingly, brown adipose tissue (BAT) recently emerged as a novel player in energy metabolism in humans. In contrast to white adipose tissue that stores excess triglycerides as fat, BAT takes up glucose and triglyceride-derived fatty acids (FA) from the plasma and subsequently burns FA to generate heat by means of mitochondrial uncoupling, a process called thermogenesis.[24] BAT is physiologically distinct from white adipose tissue as it contains high numbers of mitochondria in order to provide high oxidative capacity and is densely innervated by the sympathetic nervous system. The latter makes sure that BAT is rapidly activated in case of a cold environment, resulting in generation of heat. BAT was long thought to be present only in neonates, as they have minimal shiver capacity due to their underdeveloped muscles, and that with increasing age BAT would gradually disappear. Only in 2009 it has been discovered by means of cold-induced ^{18}F-fluorodeoxyglucose (FDG) PET-CT scans that BAT is still present and functional in adults,[25-27] and that it is mainly located in the supraclavicular and paravertebral regions.[28]

The major involvement of BAT in whole-body metabolism appeared from pre-clinical studies in rodents. Decreasing BAT activity or removal of BAT in mice markedly increased plasma glucose and triglyceride levels as well as the development of obesity and insulin

resistance.[29;30] Furthermore, in humans, BAT activity was found to be inversely related with BMI and fat mass.[26] Hence, a low BAT volume or activity may aggravate development of dyslipidemia, obesity and type 2 diabetes, i.e. classical metabolic risk factors.

Next, we will discuss the classical (metabolic) and non-classical (inflammatory) risk factors for cardiovascular disease in South Asian subjects, and speculate on underlying mechanisms.

Classical cardiovascular disease risk factors in South Asians

Dyslipidemia

Dyslipidemia often is one of the main risk factors of cardiovascular disease. South Asians have consistently been shown to have higher triglyceride levels.[7;31;32] Some studies also reported higher LDL-cholesterol levels in South Asian subjects compared to Caucasians.[4;33] Furthermore, multiple studies have consistently shown lower HDL-cholesterol levels in South Asians compared to Caucasians, even in South Asian neonates,[7;32;34-39] and the low levels of HDL-cholesterol are inversely related to cardiovascular risk.[40-45]

Obesity

South Asians have a disadvantageous fat distribution pattern with relatively thin extremities and increased abdominal adiposity.[46;47] Furthermore, at a similar level of BMI, body fat percentage is higher in South Asians compared to Caucasians.[46;48] South Asians also have a tendency for deposition of fat within cells of non-adipose tissues such as muscle and liver, so called "ectopic" sites. Petersen *et al*[49] showed that in young healthy lean South Asians hepatic triglyceride content was two-fold higher than in healthy lean Caucasians. This higher triglyceride content was associated with insulin resistance and increased levels of pro-inflammatory cytokines. We and others also reported higher fat infiltration in the liver in adult South Asians compared to white Caucasians (Bakker *et al*, submitted data, and others[50]). Storage of fat in these ectopic sites has a disruptive effect on glucose metabolism and it is now increasingly recognized that hepatic steatosis, besides abdominal obesity, may be causally related with hepatic insulin resistance, the metabolic syndrome, systemic inflammation and even cardiovascular disease.[49;51-53]

Insulin resistance

Insulin resistance and elevated fasting glucose levels are more prevalent in non-diabetic South Asians compared to non-diabetic white Caucasians,[6;31] possibly as a consequence of increased obesity and ectopic fat deposition. Most striking is the high rate of type 2 diabetes in South Asians. In 35-60 year old South Asian males living in the UK, diabetes prevalence was 16% compared with only 4% among European whites.[32] Other studies have reported an even higher prevalence of up to 25.4% for both South Asian men and

women.[54] Furthermore, the onset of diabetes is over 10 years earlier in South Asians,[54] and diabetes occurs at a lower BMI compared to Caucasians: the risk of developing type 2 diabetes for a South Asian with a BMI of 21 kg/m2 is comparable to the risk of a white Caucasian with a BMI of 30 kg/m2.[3;33] Finally, South Asians develop diabetes-related complications, such as diabetic nephropathy and retinopathy, more often.[55]

Thus, in South Asians a disadvantageous metabolic profile, including dyslipidemia, obesity insulin resistance, and type 2 diabetes is highly prevalent. Up to recently, little was known about the pathophysiological mechanism that underlies this metabolic phenotype, but it was hypothesized that this may be explained by a disturbed energy homeostasis (i.e. lower oxidation of glucose and fatty acids by mitochondria) in South Asians as they have recently been shown to exhibit reduced fat oxidation during sub-maximal exercise as compared to Caucasians.[56]

Brown adipose tissue: central role in classical cardiovascular disease risk factors?

Recently, we demonstrated that Dutch South Asians adolescents have a markedly lower resting energy expenditure (-32%) than BMI-matched Dutch white Caucasians as well as less cold-induced non-shivering thermogenesis, both of which are consistent with lower BAT function. Indeed, cold-induced ^{18}F-FDG PET-CT scans revealed that South Asian adolescents have a markedly lower BAT volume (-34%) in comparison to white Caucasians.[57] Interestingly, the BAT volume correlated positively with thermoneutral resting energy expenditure, indicating that BAT contributes to energy expenditure even under non-cold-induced conditions. Therefore, it is likely that the reduced BAT activity causally contributes to the adverse metabolic phenotype of South Asians including dyslipidemia, obesity, insulin resistance and type 2 diabetes.

It is interesting to speculate on possible mechanisms that underlie the decreased BAT volume in South Asians. The underlying cause may be genetic, i.e. blunted expression of signalling molecules involved in BAT differentiation, environmental, i.e. clothing behaviour, central heating setting or eating pattern, or a combination of the two. Several key molecules have been shown to be importantly involved in BAT differentiation in rodents, including bone morphogenetic protein 7 (BMP7)[58] and NO[59]. We have previously measured BMP7 levels in several cohorts of South Asian subjects including neonates, and we consistently found increased rather than decreased BMP7 levels compared to Caucasians (unpublished data). Thus, decreased BAT differentiation in South Asians does not seem to be caused by impaired BMP7 availability. NO has been recently linked to BAT, as mice that lack the enzyme NO synthase, crucial for catalysing the conversion of L-arginine to NO, have fewer and smaller mitochondria in BAT and lower energy expenditure leading to obesity.[59] Interestingly, South Asians have reduced bioavailability of NO in comparison to white Caucasians.[60] Thus, an inborn reduction in NO bioavailability

might underlie the lower BAT volume in South Asians and is an interesting subject for future studies.

Taken together, as BAT is an important player in triglyceride and glucose clearance as well as in total energy expenditure, a low BAT volume may well contribute to the high prevalence of classical cardiovascular disease risk factors in South Asians, including dyslipidemia, obesity, insulin resistance and type 2 diabetes.

Non-classical cardiovascular disease risk factors in South Asians

Visceral adipose tissue inflammation

As mentioned above, inflammation is a well-recognized key player in the pathogenesis of atherosclerosis and may, therefore, be considered a risk factor for cardiovascular disease.[61] Besides promoting initiation of atherosclerosis development through monocyte attraction, inflammation may lead to instability of the fibrous cap of the atherosclerotic plaque, resulting in rupture of the plaque and a subsequent cardiovascular event. C-reactive protein (CRP), which is synthesized by the liver in response to inflammatory factors released by macrophages and adipocytes,[62;63] is a sensitive marker of inflammation.[64] In a study of Chambers *et al*,[32] CRP levels were found to be significantly increased in South Asians compared with Caucasians even after adjustment for factors such as age, smoking and body mass index, suggesting a chronic state of low grade inflammation in this population. The difference in CRP levels was predominantly explained by greater central obesity and insulin resistance in South Asians. Visceral adipose tissue has been found to be a major source of cytokine release into the circulation.[32;65] Not only do South Asians have more visceral adipose tissue, their adipocytes appear to be more inflammatory as well. Several studies reported that South Asian visceral adipocytes release higher levels of pro-inflammatory cytokines, such as TNF-α and interleukin 6 (IL-6) in comparison to Caucasians,[49;66] which may contribute to increased cardiovascular disease risk. Indeed, a larger amount of visceral adipose tissue associates with the increased risk of cardiovascular disease in South Asians.[67] Of note, enhanced visceral adipose tissue inflammation may also be linked to the lower BAT volume in South Asian subjects, as TNF-α has been shown to induce brown adipocyte apoptosis and hamper BAT differentiation in pre-clinical models.[68]

HDL dysfunction

As described above, HDL has several anti-atherogenic properties and dysfunction of HDL may not only directly aggravate atherosclerosis development as a consequence of lower cholesterol uptake from the vascular wall, but also indirectly through induction of inflammation as well as endothelial dysfunction.

Recent evidence suggests that HDL functionality may be more importantly linked to cardiovascular disease than plasma HDL-cholesterol levels per se.[69;70] In trials that aimed at raising HDL-cholesterol levels with dalcetrapib or niacin on top of statin, no decrease in the occurrence of cardiovascular endpoints was observed compared to treatment with statins only.[71;72] In line with this, several studies showed that HDL is dysfunctional in patients with coronary atherosclerosis, in patients with an acute phase response after surgery, and in men with cardiovascular risk factors.[73-76]

Remarkably, little is known about HDL functionality in South Asians. To date only one cross-sectional, uncontrolled pilot study assessed the anti-oxidative capacity of HDL in South Asian immigrants living in the USA. Dysfunctional HDL was found in 50% of the participants, which was significantly correlated with carotid intima media thickness , a surrogate marker of atherosclerosis.[77] However, another ethnic control group was lacking in the study, so no statements could be made on the implication of this percentage for the South Asian population specifically. Future studies are needed to investigate whether the prevalence of HDL dysfunction is higher in South Asian compared to white Caucasian subjects, as this may contribute to the excess risk of cardiovascular disease in people of South Asian origin.

Endothelial activation

A hallmark of endothelial activation is a reduction in the bioavailability of endothelium-derived NO. An impaired NO-mediated vasodilatory response has been demonstrated in patients with cardiac risk factors or established atherosclerosis.[78;79] Furthermore, the degree of impairment is related to the severity and extent of coronary artery disease.[80] NO not only has vasodilating properties, but also anti-platelet, anti-proliferative, and anti-inflammatory properties.[81;82] Thus, there is a close link between inflammation, HDL function, and endothelial function; HDL dysfunction may result in inflammation and endothelial activation and furthermore, endothelial activation could induce inflammation, resulting in a negative feedback loop. In addition, the vasodilating effect of NO is related to insulin resistance and type 2 diabetes: vasodilatation increases delivery of insulin to tissues that take up glucose, and, vice versa, insulin stimulates the release of NO from the endothelium.[83;84] Moreover, as NO is important for BAT development, a link between diminished NO availability and BAT function and thus energy metabolism may also exist.

Interestingly, signs of endothelial activation in South Asians are already present upon birth. We previously found that levels of E-selectin, a marker of endothelial activation, are elevated in South Asian neonates compared with Caucasian neonates.[35] In line with this, several studies demonstrated reduced endothelium-dependent vasodilatation in South Asians compared to white Caucasians, pointing to lower NO bioavailability.[85;86] Of note, NO is mainly produced by the endothelium as a consequence of an interaction with HDL.[81;87] The reduced NO bioavailability in South Asians may thus be a consequence

of endothelial activation *per se* or of dysfunctional HDL. Since release of NO from endothelium is also stimulated by insulin, the highly prevalent insulin resistance in South Asians may also contribute to lower NO availability.[83;84]

Circulating endothelial progenitor cells (EPCs), mobilized from the bone marrow, have an important role in the repair and regeneration of the endothelium.[88-90] The number of circulating EPCs is lower in patients with established coronary artery disease, is predictive of future cardiovascular events, and is positively correlated with measures of endothelial function.[91;92] Intriguingly, South Asians have lower circulating numbers of EPCs compared to Caucasians, which may lead to a reduced capacity for endothelial repair.[60;85] Furthermore, exercise-induced EPC mobilization was reduced in South Asian men.[60] Interestingly, NO appears to be critical for EPC mobilization in response to exercise.[60] Hence, the reduced exercise mediated EPC mobilization in South Asians is likely secondary to their reduced NO bioavailability. Future studies should be directed at developing strategies that enhance EPC mobilization by augmenting NO bioavailability.

CONCLUSIONS AND FUTURE DIRECTIONS

South Asians are more liable to develop cardiovascular disease at an early age, and classical risk factors associated with cardiovascular disease, including dyslipidemia, central obesity and insulin resistance, are more prevalent in this population. However, these 'metabolic' risk factors seem to account for only part of the increased risk in South Asians. We propose that non-classical 'inflammatory' risk factors, i.e. higher levels of (visceral adipose tissue) inflammation, HDL dysfunction, and endothelial activation are involved in the residual cardiovascular disease risk in South Asians (see **Figure 1**).

The pathophysiological mechanism for the high prevalence of classical cardiovascular disease risk factors has not been fully established, but may be due to a lower BAT volume in South Asians. This offers a promising new target for preventive and therapeutic interventions as increasing BAT volume or activity will enhance energy expenditure and subsequent clearance of lipids and glucose from the circulation, resulting in improvement of the disadvantageous metabolic phenotype of South Asians and possibly reducing cardiovascular disease risk. Indeed, we recently showed that activation of BAT by ß3-adrenergic stimulation, in a human-like mouse model of lipoprotein metabolism and atherosclerosis, markedly diminished atherosclerosis development (Berbée *et al*, unpublished). Possible options for increasing BAT volume or activity are cold exposure or medication. BAT can be recruited in human subjects following cold exposure resulting in lowering of fat mass.[93;94] Furthermore, we recently showed in a pre-clinical setting that the commonly used anti-diabetic drug metformin activates BAT, which is responsible for its lipid-lowering effect.[95] Moreover, since South Asians have been shown to exhibit

lower NO bioavailability, BAT function may be improved by targeting the NO pathway. As NO also has anti-inflammatory properties and is involved in EPC mobilization, this strategy may also lower inflammation and improve endothelial function and thereby lower the 'residual' cardiovascular disease risk in South Asians.

Thus, future studies should investigate the efficacy of treatment strategies that target both classical as well as non-classical risk factors in the South Asian population to lower cardiovascular disease risk. A combination of therapies that increases BAT activity (i.e. via cold exposure or medical strategies) and lower inflammation may be especially effective.

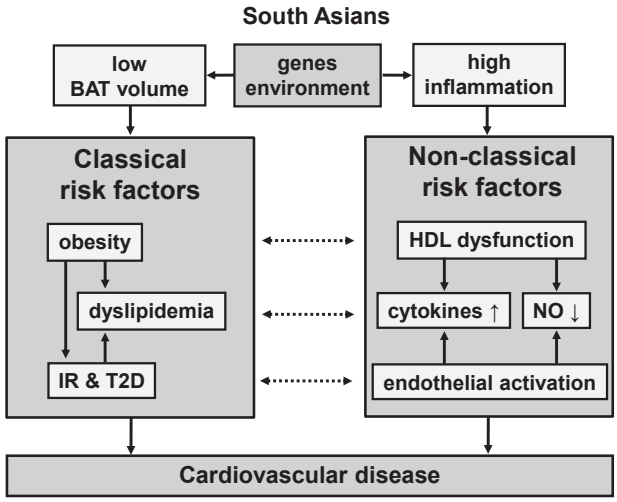


Figure 1. Proposed underlying mechanisms in the high cardiovascular risk in the South Asian population. Classical (metabolic) risk factors, i.e. dyslipidemia, obesity, insulin resistance (IR) and type 2 diabetes (T2D), are highly prevalent in the South Asian population. A low brown adipose tissue (BAT) volume might underlie this disadvantageous metabolic phenotype. In addition, non-classical (inflammatory) risk factors may contribute to the high cardiovascular disease risk in South Asians, such as HDL dysfunction, enhanced pro-inflammatory cytokine release and low nitric oxide (NO) availability, as well as endothelial cell activation. Visceral adipose tissue inflammation may link obesity and inflammation. The classical and non-classical risk factors are likely influenced by both genetic and environmental factors.

REFERENCES

1. Forouhi N, Sattar N, Tillin T, McKeigue P, Chaturvedi N. Do known risk factors explain the higher coronary heart disease mortality in South Asians compared with European men? Prospective follow-up of the Southall and Brent studies, UK. *Diabetologia* 2006;49(11):2580-8.
2. Srinath R, Shah B, Varghese C, Ramadoss A. Responding to the threat of chronic diseases in India. *Lancet* 2005;366(9498):1744-9.
3. Turin TC, Shahana N, Wangchuk LZ, Specogna AV, Al Mamun M, Khan MA *et al*. Burden of Cardio- and Cerebro-vascular Disease and the Conventional Risk Factors in South Asian Population. *Global Health* 2005;8(2):121-30.
4. Anand SS, Yusuf S, Vuksan V, Devanesen S, Teo KK, Montague PA *et al*. Differences in risk factors, atherosclerosis and cardiovascular disease between ethnic groups in Canada: the study of health assessment and risk in ethnic groups (SHARE). *Lancet* 2000;356:279-84.
5. Joshi P, Islam S, Pais P, Reddy S, Dorairaj P, Kazmi K *et al*. Risk factors for early myocardial infarction in South Asians compared with individuals in other countries. *JAMA* 2007;297(3):286-94.
6. McKeigue PM, Ferrie JE, Pierpoint T, Marmot MG. Association of early-onset coronary heart disease in South Asian men with glucose intolerance and hyperinsulinemia. *Circulation* 1993;87(1):152-61.
7. McKeigue PM, Shah B, Marmot MG. Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. *Lancet* 1991;337(8738):382-6.
8. Gupta R, Gupta R, Agrawal A, Misra A, Guptha S, Pandey RM *et al*. Migrating husbands and changing cardiovascular risk factors in the wife: a cross sectional study in Asian Indian women. *J Epidemiol Community Health* 2012;66(10):881-9.
9. Dodani S, Dong L. Acculturation, coronary artery disease and carotid intima media thickness in South Asian immigrants—unique population with increased risk. *Ethn Dis* 2011;21(3):314-21.
10. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atherosclerotic risk. *Arterioscler Thromb Vasc Biol* 2003;23(2):168-75.
11. Steinberg D. Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. *Nat Med* 2002;8(11):1211-7.
12. Hansson GK, Libby P. The immune response in atherosclerosis: a double-edged sword. *Nat Rev Immunol* 2006;6(7):508-19.
13. Barter PJ, Baker PW, Rye KA. Effect of high-density lipoproteins on the expression of adhesion molecules in endothelial cells. *Curr Opin Lipidol* 2002;13(3):285-8.
14. Navab M, Ananthramaiah GM, Reddy ST, Van Lenten BJ, Ansell BJ, Hama S *et al*. The double jeopardy of HDL. *Ann Med* 2005;37(3):173-8.
15. von Eckardstein A, Hersberger M, Rohrer L. Current understanding of the metabolism and biological actions of HDL. *Curr Opin Clin Nutr Metab Care* 2005;8(2):147-52.
16. Navab M, Hama SY, Cooke CJ, Anantharamaiah GM, Chaddha M, Jin L *et al*. Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: step 1. *J Lipid Res* 2000;41(9):1481-94.
17. Sugatani J, Miwa M, Komiyama Y, Ito S. High-density lipoprotein inhibits the synthesis of platelet-activating factor in human vascular endothelial cells. *J Lipid Mediat Cell Signal* 1996;13(1):73-88.
18. Annema W, von Eckardstein A. High-density lipoproteins. *Circ J* 2013;77(10):2432-48.
19. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002;106(25):3143-421.

20. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 1998;339(4):229-34.
21. Lee CD, Folsom AR, Pankow JS, Brancati FL. Cardiovascular events in diabetic and nondiabetic adults with or without history of myocardial infarction. *Circulation* 2004;109(7):855-60.
22. Malmberg K, Yusuf S, Gerstein HC, Brown J, Zhao F, Hunt D *et al*. Impact of diabetes on long-term prognosis in patients with unstable angina and non-Q-wave myocardial infarction: results of the OASIS (Organization to Assess Strategies for Ischemic Syndromes) Registry. *Circulation* 2000;102(9):1014-9.
23. Bloomgarden ZT. Inflammation, atherosclerosis, and aspects of insulin action. *Diabetes Care* 2005;28(9):2312-9.
24. Yoneshiro T, Aita S, Matsushita M, Kameya T, Nakada K, Kawai Y *et al*. Brown adipose tissue, whole-body energy expenditure, and thermogenesis in healthy adult men. *Obesity (Silver Spring)* 2011;19(1):13-6.
25. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB *et al*. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* 2009;360(15):1509-17.
26. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND *et al*. Cold-activated brown adipose tissue in healthy men. *N Engl J Med* 2009;360(15):1500-8.
27. Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T *et al*. Functional brown adipose tissue in healthy adults. *N Engl J Med* 2009;360(15):1518-25.
28. Enerback S. Human brown adipose tissue. *Cell Metab* 2010;11(4):248-52.
29. Connolly E, Morrisey RD, Carnie JA. The effect of interscapular brown adipose tissue removal on body-weight and cold response in the mouse. *Br J Nutr* 1982;47(3):653-8.
30. Dulloo AG, Miller DS. Energy balance following sympathetic denervation of brown adipose tissue. *Can J Physiol Pharmacol* 1984;62(2):235-40.
31. Tziomalos K, Weerasinghe CN, Mikhailidis DP, Seifalian AM. Vascular risk factors in South Asians. *Int J Cardiol* 2008;128(1):5-16.
32. Chambers JC, Eda S, Bassett P, Karim Y, Thompson SG, Gallimore JR *et al*. C-reactive protein, insulin resistance, central obesity, and coronary heart disease risk in Indian Asians from the United Kingdom compared with European whites. *Circulation* 2001;104(2):145-50.
33. Razak F, Anand SS, Shannon H, Vuksan V, Davis B, Jacobs R *et al*. Defining obesity cut points in a multiethnic population. *Circulation* 2007;115(16):2111-8.
34. Ajjan R, Carter AM, Somani R, Kain K, Grant PJ. Ethnic differences in cardiovascular risk factors in healthy Caucasian and South Asian individuals with the metabolic syndrome. *J Thromb Haemost* 2007;5(4):754-60.
35. Boon MR, Karamali NS, de Groot CJ, van Steijn L, Kanhai HH, van der Bent C *et al*. E-selectin is elevated in cord blood of South Asian neonates compared with Caucasian neonates. *J Pediatr* 2012;160(5):844-8.
36. Chandalia M, Abate N, Garg A, Stray-Gundersen J, Grundy SM. Relationship between generalized and upper body obesity to insulin resistance in Asian Indian men. *J Clin Endocrinol Metab* 1999;84(7):2329-35.
37. Ehtisham S, Crabtree N, Clark P, Shaw N, Barrett T. Ethnic differences in insulin resistance and body composition in United Kingdom adolescents. *J Clin Endocrinol Metab* 2005;90(7):3963-9.
38. McKeigue PM, Marmot MG, Syndercombe Court YD, Cottier DE, Rahman S, Riemersma RA. Diabetes, hyperinsulinaemia, and coronary risk factors in Bangladeshis in east London. *Br Heart J* 1988;60(5):390-6.

39. Raji A, Gerhard-Herman MD, Warren M, Silverman SG, Raptopoulos V, Mantzoros CS *et al*. Insulin resistance and vascular dysfunction in nondiabetic Asian Indians. *J Clin Endocrinol Metab* 2004;89(8):3965-72.
40. Assmann G, Schulte H, von Eckardstein A, Huang Y. High-density lipoprotein cholesterol as a predictor of coronary heart disease risk. The PROCAM experience and pathophysiological implications for reverse cholesterol transport. *Atherosclerosis* 1996;124 Suppl:S11-S20.
41. Barter P, Gotto AM, LaRosa JC, Maroni J, Szarek M, Grundy SM *et al*. HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. *N Engl J Med* 2007;357(13):1301-10.
42. Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A *et al*. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA* 2009;302(18):1993-2000.
43. Gordon DJ, Rifkind BM. High-density lipoprotein—the clinical implications of recent studies. *N Engl J Med* 1989;321(19):1311-6.
44. Sharrett AR, Ballantyne CM, Coady SA, Heiss G, Sorlie PD, Catellier D *et al*. Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: The Atherosclerosis Risk in Communities (ARIC) Study. *Circulation* 2001;104(10):1108-13.
45. Wilson PW, Abbott RD, Castelli WP. High density lipoprotein cholesterol and mortality. The Framingham Heart Study. *Arteriosclerosis* 1988;8(6):737-41.
46. Lear SA, Humphries KH, Kohli S, Birmingham CL. The use of BMI and waist circumference as surrogates of body fat differs by ethnicity. *Obesity (Silver Spring)* 2007;15(11):2817-24.
47. Raji A, Seely EW, Arky RA, Simonson DC. Body fat distribution and insulin resistance in healthy Asian Indians and Caucasians. *J Clin Endocrinol Metab* 2001;86(11):5366-71.
48. Chandalia M, Lin P, Seenivasan T, Livingston EH, Snell PG, Grundy SM *et al*. Insulin resistance and body fat distribution in South Asian men compared to Caucasian men. *PLoS One* 2007;2(8):e812.
49. Petersen KF, Dufour S, Feng J, Befroy D, Dziura J, Dalla MC *et al*. Increased prevalence of insulin resistance and nonalcoholic fatty liver disease in Asian-Indian men. *Proc Natl Acad Sci U S A* 2006;103(48):18273-7.
50. Anand SS, Tarnopolsky MA, Rashid S, Schulze KM, Desai D, Mente A *et al*. Adipocyte hypertrophy, fatty liver and metabolic risk factors in South Asians: the Molecular Study of Health and Risk in Ethnic Groups (mol-SHARE). *PLoS One* 2011;6(7):e22112.
51. Ndumele CE, Nasir K, Conceicao RD, Carvalho JA, Blumenthal RS, Santos RD. Hepatic steatosis, obesity, and the metabolic syndrome are independently and additively associated with increased systemic inflammation. *Arterioscler Thromb Vasc Biol* 2011;31(8):1927-32.
52. Targher G, Bertolini L, Padovani R, Rodella S, Tessari R, Zenari L *et al*. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care* 2007;30(5):1212-8.
53. Bajaj S, Nigam P, Luthra A, Pandey RM, Kondal D, Bhatt SP *et al*. A case-control study on insulin resistance, metabolic co-variates & prediction score in non-alcoholic fatty liver disease. *Indian J Med Res* 2009;129(3):285-92.
54. Mukhopadhyay B, Forouhi NG, Fisher BM, Kesson CM, Sattar N. A comparison of glycaemic and metabolic control over time among South Asian and European patients with Type 2 diabetes: results from follow-up in a routine diabetes clinic. *Diabet Med* 2006;23(1):94-8.
55. Chandie Shaw PK, Vandenbroucke JP, Tjandra YI, Rosendaal FR, Rosman JB, Geerlings W *et al*. Increased end-stage diabetic nephropathy in Indo-Asian immigrants living in the Netherlands. *Diabetologia* 2002;45(3):337-41.

56. Hall LM, Moran CN, Milne GR, Wilson J, MacFarlane NG, Forouhi NG *et al*. Fat oxidation, fitness and skeletal muscle expression of oxidative/lipid metabolism genes in South Asians: implications for insulin resistance? *PLoS One* 2010;5(12):e14197.
57. Bakker LE, Boon MR, van der Linden RA, Arias-Bouda LP, van Klinken JB, Smit F *et al*. Brown adipose tissue volume in healthy lean south Asian adults compared with white Caucasians: a prospective, case-controlled observational study. *Lancet Diabetes Endocrinol* 2014;2(3):210-7.
58. Tseng YH, Kokkotou E, Schulz TJ, Huang TL, Winnay JN, Taniguchi CM *et al*. New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 2008;454(7207):1000-4.
59. Nisoli E, Clementi E, Paolucci C, Cozzi V, Tonello C, Sciorati C *et al*. Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. *Science* 2003;299(5608):896-9.
60. Cubbon RM, Murgatroyd SR, Ferguson C, Bowen TS, Rakobowchuk M, Baliga V *et al*. Human exercise-induced circulating progenitor cell mobilization is nitric oxide-dependent and is blunted in South Asian men. *Arterioscler Thromb Vasc Biol* 2010;30(4):878-84.
61. van Diepen JA, Berbee JF, Havekes LM, Rensen PC. Interactions between inflammation and lipid metabolism: relevance for efficacy of anti-inflammatory drugs in the treatment of atherosclerosis. *Atherosclerosis* 2013;228(2):306-15.
62. Lau DC, Dhillon B, Yan H, Szmitko PE, Verma S. Adipokines: molecular links between obesity and atheroslcerosis. *Am J Physiol Heart Circ Physiol* 2005;288(5):H2031-H2041.
63. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest* 2003;111(12):1805-12.
64. Madjid M, Willerson JT. Inflammatory markers in coronary heart disease. *Br Med Bull* 2011;100:23-38.
65. Kissebah AH. Intra-abdominal fat: is it a major factor in developing diabetes and coronary artery disease? *Diabetes Res Clin Pract* 1996;30 Suppl:25-30.
66. Peters MJ, Ghouri N, McKeigue P, Forouhi NG, Sattar N. Circulating IL-6 concentrations and associated anthropometric and metabolic parameters in South Asian men and women in comparison to European whites. *Cytokine* 2013;61(1):29-32.
67. Lear SA, Chockalingam A, Kohli S, Richardson CG, Humphries KH. Elevation in cardiovascular disease risk in South Asians is mediated by differences in visceral adipose tissue. *Obesity (Silver Spring)* 2012;20(6):1293-300.
68. Nisoli E, Briscini L, Giordano A, Tonello C, Wiesbrock SM, Uysal KT *et al*. Tumor necrosis factor alpha mediates apoptosis of brown adipocytes and defective brown adipocyte function in obesity. *Proc Natl Acad Sci U S A* 2000;97(14):8033-8.
69. Corsetti JP, Gansevoort RT, Sparks CE, Dullaart RP. Inflammation reduces HDL protection against primary cardiac risk. *Eur J Clin Invest* 2010;40(6):483-9.
70. deGoma EM, deGoma RL, Rader DJ. Beyond high-density lipoprotein cholesterol levels evaluating high-density lipoprotein function as influenced by novel therapeutic approaches. *J Am Coll Cardiol* 2008;51(23):2199-211.
71. Sharma M. Combination therapy for dyslipidemia. *Curr Opin Cardiol* 2011;26(5):420-3.
72. Schwartz GG, Olsson AG, Abt M, Ballantyne CM, Barter PJ, Brumm J *et al*. Effects of dalcetrapib in patients with a recent acute coronary syndrome. *N Engl J Med* 2012;367(22):2089-99.
73. Ansell BJ, Navab M, Hama S, Kamranpour N, Fonarow G, Hough G *et al*. Inflammatory/antiinflammatory properties of high-density lipoprotein distinguish patients from control subjects better than high-density lipoprotein cholesterol levels and are favorably affected by simvastatin treatment. *Circulation* 2003;108(22):2751-6.

74. Navab M, Hama SY, Hough GP, Subbanagounder G, Reddy ST, Fogelman AM. A cell-free assay for detecting HDL that is dysfunctional in preventing the formation of or inactivating oxidized phospholipids. *J Lipid Res* 2001;42(8):1308-17.
75. Roberts CK, Ng C, Hama S, Eliseo AJ, Barnard RJ. Effect of a short-term diet and exercise intervention on inflammatory/anti-inflammatory properties of HDL in overweight/obese men with cardiovascular risk factors. *J Appl Physiol* 2006;101(6):1727-32.
76. Van Lenten BJ, Hama SY, de Beer FC, Stafforini DM, McIntyre TM, Prescott SM *et al*. Anti-inflammatory HDL becomes pro-inflammatory during the acute phase response. Loss of protective effect of HDL against LDL oxidation in aortic wall cell cocultures. *J Clin Invest* 1995;96(6):2758-67.
77. Dodani S, Kaur R, Reddy S, Reed GL, Navab M, George V. Can dysfunctional HDL explain high coronary artery disease risk in South Asians? *Int J Cardiol* 2008;129(1):125-32.
78. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID *et al*. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992;340(8828):1111-5.
79. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* 2000;101(16):1899-906.
80. Neunteufl T, Katzenschlager R, Hassan A, Klaar U, Schwarzacher S, Glogar D *et al*. Systemic endothelial dysfunction is related to the extent and severity of coronary artery disease. *Atherosclerosis* 1997;129(1):111-8.
81. Jin RC, Loscalzo J. Vascular Nitric Oxide: Formation and Function. *J Blood Med* 2010;2010(1):147-62.
82. Kawashima S. The two faces of endothelial nitric oxide synthase in the pathophysiology of atherosclerosis. *Endothelium* 2004;11(2):99-107.
83. Cersosimo E, DeFronzo RA. Insulin resistance and endothelial dysfunction: the road map to cardiovascular diseases. *Diabetes Metab Res Rev* 2006;22(6):423-36.
84. Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. *Circulation* 2006;113(15):1888-904.
85. Murphy C, Kanaganayagam GS, Jiang B, Chowienczyk PJ, Zbinden R, Saha M *et al*. Vascular dysfunction and reduced circulating endothelial progenitor cells in young healthy UK South Asian men. *Arterioscler Thromb Vasc Biol* 2007;27(4):936-42.
86. Chambers JC, McGregor A, Jean-Marie J, Kooner JS. Abnormalities of vascular endothelial function may contribute to increased coronary heart disease risk in UK Indian Asians. *Heart* 1999;81(5):501-4.
87. Nofer JR, van der Giet M, Tolle M, Wolinska I, von Wnuck LK, Baba HA *et al*. HDL induces NO-dependent vasorelaxation via the lysophospholipid receptor S1P3. *J Clin Invest* 2004;113(4):569-81.
88. Aicher A, Zeiher AM, Dimmeler S. Mobilizing endothelial progenitor cells. *Hypertension* 2005;45(3):321-5.
89. Griese DP, Ehsan A, Melo LG, Kong D, Zhang L, Mann MJ *et al*. Isolation and transplantation of autologous circulating endothelial cells into denuded vessels and prosthetic grafts: implications for cell-based vascular therapy. *Circulation* 2003;108(21):2710-5.
90. Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M *et al*. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. *Blood* 2000;95(3):952-8.

91. Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA *et al*. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 2003;348(7):593-600.
92. Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H *et al*. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 2001;89(1):E1-E7.
93. van der Lans AA, Hoeks J, Brans B, Vijgen GH, Visser MG, Vosselman MJ *et al*. Cold acclimation recruits human brown fat and increases nonshivering thermogenesis. *J Clin Invest* 2013;123(8):3395-403.
94. Yoneshiro T, Aita S, Matsushita M, Kayahara T, Kameya T, Kawai Y *et al*. Recruited brown adipose tissue as an antiobesity agent in humans. *J Clin Invest* 2013;123(8):3404-8.
95. Geerling JJ, Boon MR, van der Zon GC, van den Berg SA, van den Hoek AM, Lombes M *et al*. Metformin Lowers Plasma Triglycerides by Promoting VLDL-Triglyceride Clearance by Brown Adipose Tissue in Mice. *Diabetes* 2014;63(3):880-91.

9 Functional and metabolic imaging of the cardiovascular system in young healthy South Asians and white Caucasians unveils early differences

Leontine E.H. Bakker*
Linda D. van Schinkel*
Jacqueline T. Jonker
Albert de Roos
Hanno Pijl
A. Edo Meinders
Ingrid M. Jazet
Johannes W.A. Smit
Hildo J. Lamb

* Authors contributed equally to manuscript

Diabetes Care 2013; 36(10): e178-9

ABSTRACT

Background. South Asians have a higher risk of developing cardiovascular disease than white Caucasians. Since the mortality risk of cardiovascular disease associated with type 2 diabetes is higher in South Asians, the excess cardiovascular disease risk in this group might be due to inherent ethnicity-associated structural cardiac diseases and/or a higher cardiac susceptibility to metabolic disorders. Therefore, this study assessed whether cardiac dimensions and cardiovascular function differ between young South Asians and white Caucasians and whether there is a differential response to a high fat diet.

Methods. Cardiac dimensions and cardiovascular function were assessed using a 1.5T-MRI-scanner in 12 young, healthy South Asian and 12 matched white Caucasian men. Both groups were subjected to a 5-day high fat high calorie diet (HFHCD) to study cardiac response to metabolic stress.

Results. At baseline South Asians had lower left ventricular mass ($p<0.001$) and end-diastolic volume ($p<0.001$), indexed for body surface area, than Caucasians. Furthermore, differences in cardiac function profile were observed. E acceleration peak ($p=0.010$) and E deceleration peak ($p=0.005$) were lower in South Asians. Additionally, South Asians had lower acceleration ($p=0.001$) and deceleration peak flows ($p<0.001$) over the aorta. A 5-day HFHCD did not increase these differences. Finally, pulse wave velocity at baseline was higher in South Asians ($p=0.022$), which normalized after the diet.

Conclusions. Young, healthy South Asians have smaller cardiac dimensions and a different cardiovascular function profile than white Caucasians. A 5-day HFHCD did not increase these differences, suggesting these findings cannot be explained by a different metabolic response to dietary fat.

INTRODUCTION

People of South Asian descent, originating from the Indian subcontinent, represent one fifth of the world's population. South Asians are at an increased risk of developing cardiovascular disease compared to white Caucasians.[1] The age-standardized mortality rate from cardiovascular disease is around 50% higher for South Asians than for white Caucasians.[2-5] Furthermore, the mean age of first acute myocardial infarction is approximately five years earlier in South Asians than in Caucasians.[6;7] Moreover, cardiovascular disease in this population is more aggressive and has higher mortality rates at younger ages.[1-3;7]

The differences in cardiovascular disease prevalence and severity between both ethnicities cannot be explained by traditional risk factors, such as smoking, hypertension and cholesterol levels.[4] Since insulin resistance and type 2 diabetes mellitus are highly prevalent in South Asians[8;9] and the mortality risk of cardiovascular disease associated with type 2 diabetes is higher in this ethnicity compared to Caucasians,[4;10] the increased cardiovascular disease risk might be related to inherent ethnicity-associated structural cardiac features and/or a higher susceptibility to detrimental metabolic changes as reflected by ectopic fat deposition in organs such as the heart, liver and skeletal muscle.[11]

Little is known about differences in cardiovascular function between South Asians and Caucasians at a relatively young age. In a previous study, in which cardiac function was assessed with echocardiography, middle-aged South Asians had attenuated longitudinal left ventricular (LV) function, higher LV filling pressure and a greater degree of concentric remodelling compared to Caucasians.[12] Whether these findings are related to the increased cardiovascular disease risk, however, remains to be determined.

The aim of the present study was to assess whether differences in cardiac dimensions, cardiovascular function, and myocardial triglyceride (TG) content are present between young, healthy South Asians and matched Caucasians using Magnetic Resonance (MR) Imaging (MRI) and Spectroscopic (MRS) techniques. In addition, we measured abdominal fat distribution and hepatic TG content. We hypothesized that possible differences in cardiovascular function between South Asians and Caucasians can be attributed to a higher cardiac susceptibility to metabolic disorders in South Asians. In a previous study, short-term high fat feeding decreased diastolic function.[13] If the differences in cardiovascular function and dimensions in South Asians can indeed be attributed to a higher susceptibility to metabolic stress, a high fat high calorie diet (HFHCD) may have more profound effects on cardiovascular function in this ethnicity then in white Caucasians. Therefore, we subjected the participants to a 5-day HFHCD.

METHODS

Subjects

Twelve Dutch South Asian and twelve Dutch Caucasian healthy men matched for age (19-25 years) and BMI (<25 kg/m^2), with a positive family history for type 2 diabetes were enrolled. Exclusion criteria were: any significant chronic disease (including type 2 diabetes), use of medication known to influence glucose and/or lipid metabolism, smoking, recent weight change and general contraindications to MR-scanning. Subjects were recruited via advertisements in newspapers. The study was approved by the local ethics committee and performed in accordance with the principles of the revised Declaration of Helsinki. Written informed consent was obtained from all subjects.

Study design

The study consisted of 2 occasions separated by a 5-day HFHCD. The HFHCD consisted of the subject's regular diet, supplemented with 375 mL of cream per day (=1275 kcal/day, 94% fat), yielding to around 3775 kcal/day and 54% of fat. Subjects underwent MRI/MRS shortly before the start of the HFHCD and at the end of the 5th day of the diet. Participants were instructed not to alter lifestyle habits. Anthropometric measurements and blood samples were obtained on both occasions after a 10-hour overnight fast.

MR protocol

All measurements were performed on a 1.5-Tesla MR-scanner (Gyroscan ACS-NT15; Philips Medical Systems, The Netherlands) in supine position, and were made in post-prandial state (four hours after the last meal).

Left ventricular dimensions and function

Data were analysed blinded for ethnicity and study occasion.

The heart was imaged in short-axis orientation, using electrocardiographically gated breath-hold cine steady-state free-precession sequences as previously described.[14] Imaging parameters were: repetition time (TR) 3.4ms, echo time (TE) 1.7ms, flip angle (FA) 35º, field of view (FOV) 400×320mm, and slice thickness 10mm, no slice gap was used. Epicardial and endocardial left ventricular (LV) contours were manually drawn in the end-systolic and end-diastolic phases of the short-axis data, using validated MASS® software (Medis, Leiden, The Netherlands). LV end-diastolic volume (EDV), end-systolic volume (ESV), ejection fraction (EF), stroke volume (SV) and end-diastolic mass (EDM) were assessed. We divided LVEDM by LVEDV to obtain the LVEDM/LVEDV ratio (also known as concentricity). Volumes and mass were indexed (I) for body surface area (BSA).

We calculated LV end systolic wall stress (LVESWS) with the formula 0.133*systolic blood pressure*((3xESV/wall volume)+1).[15]

For assessment of LV diastolic function, transmitral flow was measured, using electrocardiographically gated gradient echo sequence with a velocity sensitivity of 100 cm/sec (TR 9.1ms, TE 1.0ms, FA 20°, slice thickness 8mm, FOV 350mm^2, matrix 256x256 pixels). Flow velocities in early diastole (E) and at atrial contraction (A) were measured

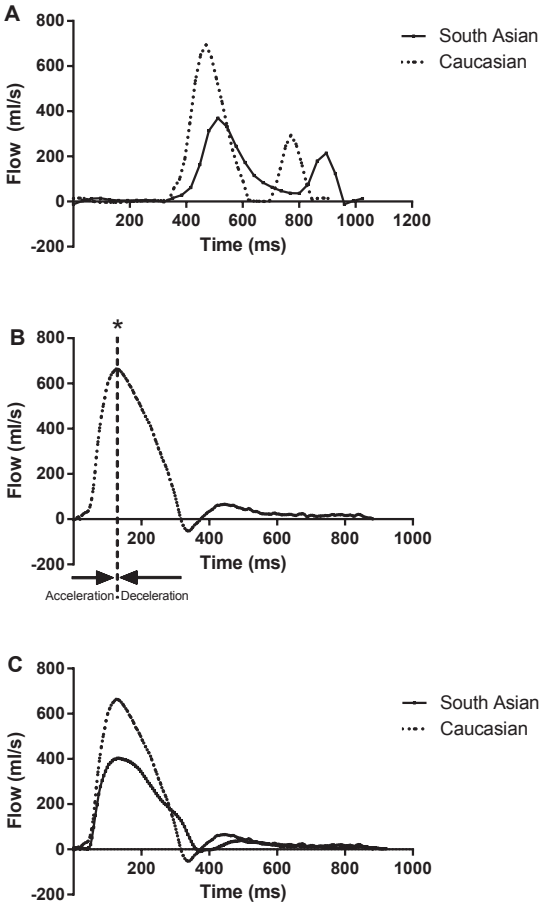


Figure 1. Panel A: Mitral valve flow curve. Panel B and C: Aortic flow curve.
Panel A shows an example of the flow through the mitral valve, representing diastolic cardiac function. The black line represents a South Asian subject, the dotted line a Caucasian subject. These curves suggest that cardiac relaxation is prolonged in South Asians.
Panel B shows how aortic flow parameters are assessed. * is the AO peak flow rate. Acceleration duration is the time between the beginning of the flow curve and the peak flow rate. The deceleration duration is the time between the peak flow rate and the end of the deceleration period. The acceleration peak is the peak slope (dy/dx) of the acceleration phase, the deceleration peak the peak slope (dy/dx) of the deceleration phase.
Panel C shows an example of flow velocity curve through the ascending aorta. The black line represents a typical curve of a South Asian subject, the dotted line of a Caucasian subject: the cardiac contraction is somewhat prolonged in South Asians.

and their peak flow ratio was calculated (E/A ratio) using FLOW® software (Medis, Leiden, The Netherlands). Furthermore the peak deceleration gradient of E, the E/A peak ratio and LV filling pressures E/Ea were determined.[15;16] Heart rate was monitored and stored during the transmitral flow measurements.

As a measurement of more subtle changes in systolic function of the heart, aortic flow curves were acquired, using electrocardiographically gated gradient echo sequence with a velocity sensitivity of 150 cm/sec (TR 5.0ms, TE 1.0ms, FA 20°, slice thickness 8mm, FOV 300mm^2, matrix 128x128 pixels). Flow velocities in the ascending aorta at the level of the pulmonary trunk were measured and calculated using FLOW® software (Medis, Leiden, The Netherlands). The peak slope of the acceleration (aortic (AO) acceleration peak) and deceleration (AO deceleration peak) of the aortic flow curve were calculated. Furthermore, AO duration, AO peak filling rate and AO deceleration duration were determined (**Figure 1B**).

Pulse Wave Velocity

Aortic PWV was determined for the evaluation of aortic stiffness, using a previously described protocol.[17] In short, a scout view of the aorta was performed. Next, a velocity encoded image perpendicular to the ascending aorta at the level of the pulmonary trunk, and at the level of the aortic bifurcation was assessed. This resulted in through-plane flow measurements of the ascending and descending aorta. Scan parameters were: TR 5.0ms, TE 1.0ms, flip angle 20°, FOV 300mm, 128×128 acquisition matrix, slice thickness 8mm, with maximal number of phases reconstructed ensuring high (6-10ms) effective temporal resolution. True temporal resolution is defined as 2 times TR = 10ms. PWV was calculated using the formula: $\Delta x/\Delta t$, where Δx describes the aortic path length between two measurement sites and Δt describes the transit time between the arrival of the PWV at three respective sites. The distance between the measurement sites was manually determined by drawing a poly-line in the centre of the aorta as defined in a double-oblique parasagittal aortic scout view, using the software package MASS®. Data were analysed using MASS® and FLOW® (Medis, Leiden, The Netherlands).

Myocardial and liver triglyceride content

MR spectroscopy (^{1}H-MRS) was used to quantify myocardial and hepatic TG content. Details on ^{1}H-MRS acquisition and post processing were published before.[18;19] In short, myocardial and hepatic ^{1}H-MR single voxel MR spectroscopic data were acquired using a point resolved spectroscopy sequence. For the heart an 8-mL voxel was positioned in the interventricular septum on four-chamber and short-axis images in end-systole, avoiding contamination from epicardial fat. Electrocardiographically triggering (only for myocardial spectra) and respiratory pencil beam navigator were used during acquisition.[18] For the liver, voxel sites were matched at both study occasions, avoiding

blood vessels and bile ducts. Main acquisition parameters for water suppressed spectra were: TE 26ms, TR 3000ms, 1,024 data points, spectral bandwidth 1,000-Hz, 128 averages. Acquisitions were performed with and without (TE 10000ms, 4 averages) water suppression, with myocardial TG expressed as percentage of the unsuppressed water signal. Hepatic ^{1}H-MRS was performed using the same acquisition parameters, except for 64 averages for the suppressed spectrum. Java-based MR user interface software (jMRUI v2.2, Leuven, Belgium) was used for fitting of the spectra.[19] The TG content was calculated as the amplitude of the (TG signal/amplitude of water signal)*100.

Visceral and subcutaneous fat

Abdominal visceral and subcutaneous fat volumes were imaged using a turbo spin echo imaging sequence.[20] During one breath-hold, three consecutive transversal slices of 10mm thickness were scanned at level of L5 (TR 168ms, TE 11ms, FA 90º). Volumes of visceral and subcutaneous fat depots were quantified using MASS® software (Medis, Leiden, The Netherlands). Visceral and subcutaneous fat areas of each individual slice were multiplied by the slice thickness to acquire a volume and the volumes of all three slices were summed.

Assays

Serum concentrations of glucose, total cholesterol, HDL and triglycerides were measured on a Modular P800 analyser (Roche, Netherlands), and serum insulin levels on an Immulite 2500 (Siemens, The Netherlands). HbA_{1c} was measured on an HPLC system (Kordia, The Netherlands). Plasma free fatty acids (FFAs) concentrations were measured by a commercial kit (Wako Chemicals, Germany).

Statistical analysis

Data are presented as mean ± SEM or median (interquartile range (IQR)). A mixed model was applied to assess mean differences before and after the intervention within and between groups, and to assess differences in diet effect. Nonparametric tests (Wilcoxon signed-rank test within group, Mann-Whitney between groups) were performed when appropriate. Significance level was set at $p < 0.05$. Statistical analyses were performed using SPSS for Windows version 20.0 (IBM, USA).

RESULTS

Clinical and metabolic characteristics

Mean age was 22.1 ± 0.4 years. BSA was lower in South Asians. As expected, BMI did not differ between groups (South Asians: 20.9 ± 0.6 kg/m^2 *vs.* Caucasians: 22.2 ± 0.6

kg/m^2 (p=0.11)), but South Asians were shorter and weighed less. After the HFHCD a very small increase in BMI and weight to a similar extent in both groups was observed. Waist circumference did not differ between groups. Blood pressure and heart rate were comparable between groups and did not change after the HFHCD (**Table 1**).

HbA_{1c} was higher in South Asians. Fasting glucose, insulin levels and HOMA-B, a measure for pancreas function, were comparable at baseline, but were significantly higher in South Asians after the diet. FFAs were comparable between groups and no diet effect was found. LDL-cholesterol was slightly higher in South Asians, whereas other lipid levels did not differ significantly (**Table 1**).

Table 1. Clinical and metabolic characteristics

	white Caucasians		South Asians	
	before HFHCD	after HFHCD	before HFHCD	after HFHCD
Clinical characteristics				
age (years)	22.1 ± 0.6		22.2 ± 0.7	
height (m)	1.84 ± 0.01		1.74 ± 0.02**	
weight (kg)	75.1 ± 1.8	75.6 ± 1.8	63.2 ± 2.3**	63.7 ± 2.3† **
BSA (m^2)	1.97 ± 0.02	1.98 ± 0.02	1.76 ± 0.04**	1.76 ± 0.04† **
BMI (kg/m^2)	22.2 ± 0.6	22.4 ± 0.6	20.9 ± 0.6	21.0 ± 0.6†
waist (cm)	81 ± 2	82 ± 2	79 ± 2	80 ± 3
systolic BP (mmHg)	135 ± 3	133 ± 3	129 ± 3	129 ± 3
diastolic BP (mmHg)	79 ± 3	80 ± 2	76.8 ± 2.3	76 ± 2
heart rate (bpm)	65 ± 2	64 ± 2	61 ± 2	66 ± 3
Metabolic characteristics				
HbA_{1c} (%)	5.02 ± 0.06		5.24 ± 0.05*	
HbA_{1c} (mmol/mol)	31.2 ± 0.5		33.8 ± 0.6*	
glucose (mmol/L)	5.09 ± 0.09	5.22 ± 0.07	5.26 ± 0.09	5.53 ± 0.08†† *
insulin (pmol/L)	16 (24)	30 (37)	30 (26)	52 (31)†† ** §§
HOMA-B%	52 ± 11	55 ± 10	65 ± 14	90 ± 11†† * §
free fatty acids (mmol/L)	0.46 ± 0.05	0.43 ± 0.04	0.51 ± 0.04	0.54 ± 0.05
triglycerides (mmol/L)	0.79 (0.26)	0.75 (0.67)	1.01 (0.65)	1.12 (0.77)
total cholesterol (mmol/L)	3.10 (1.80)		4.34 (2.21)	
HDL-cholesterol (mmol/L)	1.05 (0.35)		1.02 (0.38)	
LDL-cholesterol (mmol/L)	1.84 (0.91)		2.77 (1.69)*	
total cholesterol/HDL ratio	3.00 (0.80)		4.05 (2.48)	

Data are presented as mean ± SEM or median (IQR). BSA, body surface area; BMI, body mass index; BP, blood pressure. † p<0.05, †† p<0.05 within groups. * p<0.05, ** p<0.005 between groups. § p<0.05, §§ p<0.005 diet effect between groups.

Left ventricular dimensions and function

At baseline all cardiac left ventricular dimensions indexed for BSA, i.e. EDVI, ESVI, SVI, CI and EDMI, were lower in South Asians than in Caucasians (**Table 2**). EF did not differ between groups at baseline. In addition, LVESWS and LVEDM/LVEDV were comparable (**Table 2**).

Table 2. Cardiac dimensions and parameters of cardiovascular function assessed with MRI before and after a 5-day HFHCD.

	white Caucasians		South Asians	
	before HFHCD	after HFHCD	before HFHCD	after HFHCD
Cardiac dimensions and basic function				
LVEDMI (g/m^2)	62.2 ± 1.2	62.0 ± 1.5	50.7 ± 1.4**	50.0 ± 1.3**
EDVI (mL/m^2)	102.2 ± 3.0	102.7 ± 2.8	83.3 ± 3.4**	81.5 ± 2.9**
ESVI (mL/m^2)	42.8 ± 2.1	42.3 ± 2.4	33.9 ± 2.0*	33.1 ± 1.7**
SVI (mL/m^2)	59.4 ± 2.2	60.4 ± 1.3	49.3 ± 2.1**	48.3 ± 1.7**
CI (mL/min/m^2)	3.8 ± 0.3*10^3	3.8 ± 0.2*10^3	3.0 ± 0.1*10^3**	3.2 ± 0.2*10^3*
EF (%)	58.2 ± 1.5	59.1 ± 1.4	59.4 ± 1.4	59.4 ± 1.1
LVESWS (kN/m^2)	56.3 ± 1.4	55.4 ± 2.1	53.0 ± 1.8	52.4 ± 1.2
LVEDM/EDV (g/mL)	0.61 ± 0.02	0.61 ± 0.02	0.62 ± 0.02	0.62 ± 0.02
Systolic cardiac function				
AO peak filling rate (mL/s)	538 ± 17	549 ± 18	404 ± 17**	429 ± 14**
AO acceleration peak (mL/s^2·10^{-3})	13.1 ± 0.5	13.6 ± 0.7	10.6 ± 0.4**	12.6 ± 0.5†
AO acceleration duration (ms)	101 ± 3	99 ± 4	91 ± 2*	88 ± 3*
AO deceleration peak (mL/s^2·10^{-3})	-5.9 ± 0.3	-5.9 ± 0.4	-3.7 ± 0.2**	-3.9 ± 0.2**
AO deceleration duration (ms)	227 ± 6	231 ± 4	252 ± 4**	240 ± 5$^{\dagger\,\S}$
AO duration (ms)	328 ± 6	330 ± 5	343 ± 5	328 ± 6†
Diastolic cardiac function				
E peak filling rate (mL/s)	570 ± 20	571 ± 13	431 ± 18**	447 ± 14**
E acceleration peak (mL/s^{2}x10^{-3})	7.5 ± 0.5	7.3 ± 0.4	5.9 ± 0.3**	6.4 ± 0.3
E deceleration peak (mL/s^{2}x10^{-3})	-5.0 ± 0.3	-4.9 ± 0.4	-3.8 ± 0.2*	-4.1 ± 0.2
A peak filling rate (mL/s)	262 ± 10	266 ± 13	201 ± 9**	205 ± 10**
A acceleration peak (mL/s^{2}x10^{-3})	4.4 ± 0.3	4.8 ± 0.4	3.3 ± 0.2*	3.7 ± 0.3*
A deceleration peak (mL/s^{2}x10^{-3})	-4.6 ± 0.3	-4.7 ± 0.3	-3.7 ± 0.3*	-4.2 ± 0.5
E/A-peak ratio	2.2 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.2 ± 0.1
E/Ea	8.8 ± 1.0	8.6 ± 0.6	11.0 ± 1.3	10.4 ± 1.1
Pulse wave velocity				
PWV total aorta (m/s)	4.3 ± 0.1	4.4 ± 0.1	4.7 ± 0.1*	4.4 ± 0.1†

Data are mean ± SEM. HFHCD, high fat high calorie diet; LV, left ventricular; EDM, end diastolic mass; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume; CI, cardiac index; EF, ejection fraction; ESWS, end-systolic wall stress. I, indexed for body surface area; AO, Aortic; E, early diastolic wave; A, atrial diastolic wave; E/Ea, estimated left ventricular filling pressure; PWV, pulse wave velocity. † $p<0.05$ within groups. * $p<0.05$, ** $p<0.005$ between groups. § $p<0.05$ diet effect between groups.

Flow velocities through the ascending aorta were measured. Typical aortic flow curves of a South Asian versus a Caucasian subject are depicted in **Figure 1C**. The acceleration peak and duration were significantly lower in South Asians. Furthermore, the aortic peak flow rate, deceleration peak and duration were significantly lower in South Asians. After the HFHCD the acceleration peak and deceleration duration over the aorta significantly changed in South Asians, but not in Caucasians (**Table 2**).

Several parameters of diastolic cardiac function differed at baseline between both groups: E peak filling rate, E acceleration peak and E deceleration peak were significantly lower in South Asians as compared with Caucasians. In addition, the A peak filling rate, the A acceleration peak and A deceleration peak were significantly lower in South Asians (**Table 2**). E/A ratio and the estimated filling pressure E/Ea were the same in both groups and did not change after the HFHCD. Examples of mitral valve flow curves of a South Asian versus a Caucasian subject are depicted in **Figure 1A**.

Pulse Wave Velocity

The aortic PWV was significantly higher in South Asians than in Caucasians at baseline, 4.7±0.1 m/s *vs.* 4.3±0.1 m/s, p=0.022. After the HFHCD, PWV decreased significantly only in South Asians, and was no longer different from Caucasians

Fat distribution

Although South Asians tended to have more visceral and subcutaneous adipose tissue, differences were not significant between groups (**Table 3**). Also, the visceral/subcutaneous fat ratio did not differ between groups. Furthermore, no diet effect was observed. Additionally, there was no significant difference between groups in hepatic and myocardial TG content at baseline (**Table 3**). After the HFHCD hepatic TG content increased in both groups, whereas myocardial TG content did not change.

Table 3. Waist fat distribution and myocardial and hepatic triglyceride content assessed with MRI and MRS before and after a 5-day HFHCD.

	white Caucasians		South Asians	
	before HFHCD	after HFHCD	before HFHCD	after HFHCD
Visceral fat (mL)	104 ± 14	111 ± 12	120 ± 19	125 ± 18
Subcutaneous fat (mL)	348 ± 54	363 ± 59	442 ± 61	432 ± 54
Visceral / subcutaneous ratio	0.33 ± 0.04	0.36 ± 0.05	0.28 ± 0.03	0.29 ± 0.03
Total fat (mL)	453 ± 65	474 ± 70	563 ± 76	558 ± 71
Myocardial TG content (%)	0.34 ± 0.06	0.32 ± 0.03	0.33 ± 0.04	0.34 ± 0.08
Hepatic TG content (%)	1.7 ± 0.4	4.5 ± 0.8††	1.3 ± 0.4	3.0 ± 0.5††

Data are mean ± SEM. HFHCD, high fat high calorie diet; TG, triglyceride. †† p<0.005 within groups.

DISCUSSION

This study shows that young, healthy South Asians have smaller cardiac dimensions compared to age- and BMI-matched white Caucasians, even after correction for BSA. Furthermore, diastolic cardiac function in South Asians is different. In addition, although the EF, a gross parameter of systolic function, was comparable between both groups, more subtle parameters of systolic function were different. A 5-day HFHCD did not increase these differences. Finally, South Asians had a higher aortic PWV on baseline.

Cardiac dimensions

Little is known about differences in cardiac dimensions and cardiovascular function at a young age between South Asians and Caucasians. Previous studies showed smaller left heart volumes, i.e. LVEDV, LVESV, and LV mass in South Asians, which is in line with our results.[12;21] However, these studies were performed in older subjects (mean age ~50yr) using echocardiography, while in the current study young adults (mean age ~22yr) were included and MRI was used. One might suggest that the smaller cardiac dimensions observed in South Asians are a consequence of their overall smaller body size. However, adjustment for different parameters of body size such as BMI, BSA and lean body mass did not attenuate the observed differences in the present and other studies.

Left ventricular function

Besides smaller cardiac dimensions, we found a different diastolic and systolic functional profile between both ethnicities. Although the traditional parameters of diastolic function (E/A) and systolic function (EF) did not differ between both groups, more sensitive parameters were significantly different. E and A peak filling rate, and E and A acceleration peak were lower and E and A deceleration peak were higher in South Asians, suggesting that cardiac relaxation is prolonged in South Asians compared to Caucasians (**Figure 1A**). The E/Ea ratio, an estimation of LV filling pressure, was the same for both ethnicities, which is expected in two groups with comparable blood pressures. In concordance with the present study, Chahal *et al*. also found that E/A ratio did not differ between South Asians and Caucasians.[12] However, in contrast to the present study, they did find a higher E/Ea ratio in South Asians. This discrepancy could be due to differences in age of subjects and/or to different methods of cardiac assessment between the studies. In this study we assessed flow through the aorta ascendens at the level of the pulmonary trunk. This showed a flow profile difference between groups. The difference is similar to what we observed in the diastolic flow profile as described above: the cardiac contraction is somewhat prolonged in South Asians (**Figure 1C**).

LVESWS, which is considered to be an important determinant of cardiac function and myocardial oxygen demand, did not differ between South Asians and Caucasians.

This finding indicates that no pressure overload was present in either of the groups, which is compatible with the normal LV mass in both groups. Additionally, LVEDM/EDV, a measure for concentricity, was the same between both groups, suggesting there was no difference in LV concentric remodelling either.

Effect of HFHC diet

With respect to the increased risk of cardiovascular disease, an important notion is that insulin resistance and type 2 diabetes are also highly prevalent in South Asians.[8;9] Moreover, South Asians develop type 2 diabetes at a younger age and lower BMI compared to Caucasians,[22;23] suggesting South Asians are metabolically at a higher risk. The increased risk of cardiovascular disease might be related to the metabolic changes that occur with insulin resistance. Therefore, we hypothesized that alterations in energy metabolism between South Asians and Caucasians, including differential fat distribution, might be responsible for the higher risk of cardiovascular disease in South Asians. In this study, South Asians were already more insulin resistant at baseline compared to Caucasians, as reflected by a comparable glucose but higher insulin curve and area under the curve measured by an OGTT (data not shown). Since people with insulin resistance are known to have abnormal cardiac relaxation,[24] the prolonged cardiac relaxation observed in South Asians in this study might be due to their underlying insulin resistance.

To test whether possible differences in cardiovascular function between South Asians and Caucasians can be attributed to alterations in energy metabolism the effect of a 5-day HFHCD, inducing fat overload, on cardiovascular function was assessed. Previous studies showed that short-term dietary interventions can induce changes in cardiac function.[13;25] A short-term HFHCD, consisting of 800 mL cream per day, in 15 Caucasian healthy males (age 25.0±6.6yr), already decreased diastolic function after 3 days.[13] Therefore, we expected that if metabolic variations were the cause of differences in cardiovascular function, these differences would become more pronounced after a HFHCD.

However, although both insulin levels and HOMA-B% increased significantly only in South Asians, indicating they became even more insulin resistant, cardiovascular function did not deteriorate after the diet. Therefore, we did not find support for our hypothesis. It might be that the observed differences in cardiovascular function are innate and that these findings are simply representative of differing normal reference values in these two ethnic groups. Whether these findings are related to increased cardiovascular disease risk in South Asians is unclear.

After the HFHCD hepatic TG content significantly increased in both groups, indicating good dietary compliance of the volunteers. In contrast to accumulation of hepatic TG content, myocardial TGs did not increase after the diet in both groups. A possible explanation is that the liver acts as a buffer for excessive postprandial flux of FFAs and

TGs resulting in no net change in myocardial TG content. This is in line with results of the above mentioned study in Caucasian males who received a 3-day HFHCD.[13] However, in contrast to other studies, which observed higher hepatic TG in (young) healthy South Asians compared to Caucasians,[26;27] in the present study no differences were found between groups before and after the diet. Surprisingly, we did not find a significant difference in abdominal fat distribution between groups either, although South Asians tended to have more visceral and subcutaneous fat mass. Other studies did find significantly more abdominal fat mass in South Asians compared to Caucasians,[27-29] though not all studies reached significance.[30] These differences in (ectopic) fat distribution might be attributed to the relatively young age and low BMI of subjects in the present study compared to other studies. Possibly, the differences in body fat distribution become stronger with increasing age. Other explanations might be that we included only males, or that the group sizes were too small to reach significance.

Vascular function

PWV is a surrogate marker for arterial stiffness and is a powerful independent predictor of cardiovascular events.[31] The aortic PWV in this study was significantly higher in South Asians at baseline, indicating a stiffer aorta. Previous studies in older subjects also reported a higher PWV in South Asians than in Caucasians.[32;33] After the HFHCD the PWV decreased significantly in South Asians, but not in Caucasians. This difference in diet effect might be explained by the significant increase in insulin levels after the diet occurring only in South Asians. Insulin is known to acutely act as a vasodilator via stimulation of the vasculature to produce endothelial-derived vasodilator nitric oxide.[34;35] In contrast, long-term increased insulin levels, as present in insulin resistance and type 2 diabetes, can contribute to increased arterial wall thickness by direct and indirect trophic effects on smooth muscle cells.[36]

The strength of this study is that this is the first time that the response to a HFHCD on cardiovascular function was assessed in South Asians. Furthermore, cardiovascular function was extensively analysed. A possible limitation of this study is the small sample size, which might limit generalization potential. In addition, a 5-day HFHCD might not necessarily be of sufficient duration to already observe differences in cardiovascular function. However, previous studies showed that short-term dietary interventions can induce changes in cardiac function in young, healthy people.[13;25]

In conclusion, already at a young age, South Asians have smaller cardiac dimensions and different diastolic and systolic cardiac function profiles as compared to white Caucasians. To our knowledge, these differences in cardiac dimensions and function between healthy, lean South Asians and Caucasians of young age have not been described before.

Additionally, South Asians have higher aortic PWV, indicating increased arterial stiffness. Reduced insulin sensitivity and increased LDL-cholesterol might be causally related to the different cardiac function profiles in South Asians.[24;37] Whether these differences contribute to the higher incidence of cardiovascular disease in South Asians, however, remains to be determined. A 5-day HFHCD did not increase the observed functional cardiovascular differences between both groups, despite distinct metabolic effects of the diet. This might suggest that these findings cannot be explained by a different metabolic response to short-term dietary fat consumption between both ethnicities at young age. It is possible, however, that a longer HF-diet is needed to induce changes.

REFERENCES

1. Anand SS, Yusuf S, Vuksan V, Devanesen S, Teo KK, Montague PA *et al*. Differences in risk factors, atherosclerosis, and cardiovascular disease between ethnic groups in Canada: the Study of Health Assessment and Risk in Ethnic groups (SHARE). *Lancet* 2000;356(9226):279-84.
2. Balarajan R. Ethnic differences in mortality from ischaemic heart disease and cerebrovascular disease in England and Wales. *BMJ* 1991;302(6776):560-4.
3. Chaturvedi N, Fuller JH. Ethnic differences in mortality from cardiovascular disease in the UK: do they persist in people with diabetes? *J Epidemiol Community Health* 1996;50(2):137-9.
4. Forouhi NG, Sattar N, Tillin T, McKeigue PM, Chaturvedi N. Do known risk factors explain the higher coronary heart disease mortality in South Asian compared with European men? Prospective follow-up of the Southall and Brent studies, UK. *Diabetologia* 2006;49(11):2580-8.
5. Wild SH, Fischbacher C, Brock A, Griffiths C, Bhopal R. Mortality from all causes and circulatory disease by country of birth in England and Wales 2001-2003. *J Public Health (Oxf)* 2007;29(2):191-8.
6. Joshi P, Islam S, Pais P, Reddy S, Dorairaj P, Kazmi K *et al*. Risk factors for early myocardial infarction in South Asians compared with individuals in other countries. *JAMA* 2007;297(3):286-94.
7. Enas EA, Yusuf S, Mehta JL. Prevalence of coronary artery disease in Asian Indians. *Am J Cardiol* 1992;70(9):945-9.
8. Anjana RM, Pradeepa R, Deepa M, Datta M, Sudha V, Unnikrishnan R *et al*. Prevalence of diabetes and prediabetes (impaired fasting glucose and/or impaired glucose tolerance) in urban and rural India: phase I results of the Indian Council of Medical Research-INdia DIABetes (ICMR-INDIAB) study. *Diabetologia* 2011;54(12):3022-7.
9. Becker E, Boreham R, Chaudhury M, Craig R, Deverill C, Doyle M *et al*. Health Survey for England 2004 Volume 1 The health of minority ethnic groups. The Information Centre; 2006.
10. Wilkinson P, Sayer J, Laji K, Grundy C, Marchant B, Kopelman P *et al*. Comparison of case fatality in south Asian and white patients after acute myocardial infarction: observational study. *BMJ* 1996;312(7042):1330-3.
11. Snel M, Jonker JT, Schoones J, Lamb H, de Roos A, Pijl H *et al*. Ectopic fat and insulin resistance: pathophysiology and effect of diet and lifestyle interventions. *Int J Endocrinol* 2012;2012:983814.
12. Chahal NS, Lim TK, Jain P, Chambers JC, Kooner JS, Senior R. Ethnicity-related differences in left ventricular function, structure and geometry: a population study of UK Indian Asian and European white subjects. *Heart* 2010;96(6):466-71.
13. van der Meer RW, Hammer S, Lamb HJ, Frolich M, Diamant M, Rijzewijk LJ *et al*. Effects of short-term high-fat, high-energy diet on hepatic and myocardial triglyceride content in healthy men. *J Clin Endocrinol Metab* 2008;93(7):2702-8.
14. Lamb HJ, Doornbos J, van der Velde EA, Kruit MC, Reiber JH, de Roos A. Echo planar MRI of the heart on a standard system: validation of measurements of left ventricular function and mass. *J Comput Assist Tomogr* 1996;20(6):942-9.
15. Pattynama PM, Lamb HJ, van der Velde EA, van der Wall EE, de Roos A. Left ventricular measurements with cine and spin-echo MR imaging: a study of reproducibility with variance component analysis. *Radiology* 1993;187(1):261-8.
16. Paelinck BP, de Roos A, Bax JJ, Bosmans JM, van der Geest RJ, Dhondt D *et al*. Feasibility of tissue magnetic resonance imaging: a pilot study in comparison with tissue Doppler imaging and invasive measurement. *J Am Coll Cardiol* 2005;45(7):1109-16.

17. Grotenhuis HB, Westenberg JJ, Steendijk P, van Der Geest RJ, Ottenkamp J, Bax JJ *et al*. Validation and reproducibility of aortic pulse wave velocity as assessed with velocity-encoded MRI. *J Magn Reson Imaging* 2009;30(3):521-6.
18. van der Meer RW, Doornbos J, Kozerke S, Schar M, Bax JJ, Hammer S *et al*. Metabolic imaging of myocardial triglyceride content: reproducibility of 1H MR spectroscopy with respiratory navigator gating in volunteers. *Radiology* 2007;245(1):251-7.
19. Vanhamme L, van den Boogaart A, van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *J Magn Reson* 1997;129(1):35-43.
20. Rijzewijk LJ, van der Meer RW, Smit JW, Diamant M, Bax JJ, Hammer S *et al*. Myocardial steatosis is an independent predictor of diastolic dysfunction in type 2 diabetes mellitus. *J Am Coll Cardiol* 2008;52(22):1793-9.
21. Kumaran K, Fall CH, Martyn CN, Vijayakumar M, Stein CE, Shier R. Left ventricular mass and arterial compliance: relation to coronary heart disease and its risk factors in South Indian adults. *Int J Cardiol* 2002;83(1):1-9.
22. Gray LJ, Yates T, Davies MJ, Brady E, Webb DR, Sattar N *et al*. Defining obesity cut-off points for migrant South Asians. *PLoS One* 2011;6(10):e26464.
23. Gholap N, Davies M, Patel K, Sattar N, Khunti K. Type 2 diabetes and cardiovascular disease in South Asians. *Prim Care Diabetes* 2011;5(1):45-56.
24. Celentano A, Vaccaro O, Tammaro P, Galderisi M, Crivaro M, Oliviero M *et al*. Early abnormalities of cardiac function in non-insulin-dependent diabetes mellitus and impaired glucose tolerance. *Am J Cardiol* 1995;76(16):1173-6.
25. van der Meer RW, Hammer S, Smit JW, Frolich M, Bax JJ, Diamant M *et al*. Short-term caloric restriction induces accumulation of myocardial triglycerides and decreases left ventricular diastolic function in healthy subjects. *Diabetes* 2007;56(12):2849-53.
26. Petersen KF, Dufour S, Feng J, Befroy D, Dziura J, Dalla MC *et al*. Increased prevalence of insulin resistance and nonalcoholic fatty liver disease in Asian-Indian men. *Proc Natl Acad Sci U S A* 2006;103(48):18273-7.
27. Anand SS, Tarnopolsky MA, Rashid S, Schulze KM, Desai D, Mente A *et al*. Adipocyte hypertrophy, fatty liver and metabolic risk factors in South Asians: the Molecular Study of Health and Risk in Ethnic Groups (mol-SHARE). *PLoS One* 2011;6(7):e22112.
28. Raji A, Seely EW, Arky RA, Simonson DC. Body fat distribution and insulin resistance in healthy Asian Indians and Caucasians. *J Clin Endocrinol Metab* 2001;86(11):5366-71.
29. Lear SA, Humphries KH, Kohli S, Chockalingam A, Frohlich JJ, Birmingham CL. Visceral adipose tissue accumulation differs according to ethnic background: results of the Multicultural Community Health Assessment Trial (M-CHAT). *Am J Clin Nutr* 2007;86(2):353-9.
30. Raji A, Gerhard-Herman MD, Warren M, Silverman SG, Raptopoulos V, Mantzoros CS *et al*. Insulin resistance and vascular dysfunction in nondiabetic Asian Indians. *J Clin Endocrinol Metab* 2004;89(8):3965-72.
31. Mitchell GF, Hwang SJ, Vasan RS, Larson MG, Pencina MJ, Hamburg NM *et al*. Arterial stiffness and cardiovascular events: the Framingham Heart Study. *Circulation* 2010;121(4):505-11.
32. Rezai MR, Wallace AM, Sattar N, Finn JD, Wu FC, Cruickshank JK. Ethnic differences in aortic pulse wave velocity occur in the descending aorta and may be related to vitamin D. *Hypertension* 2011;58(2):247-53.
33. Webb DR, Khunti K, Lacy P, Gray LJ, Mostafa S, Talbot D *et al*. Conduit vessel stiffness in British south Asians of Indian descent relates to 25-hydroxyvitamin D status. *J Hypertens* 2012;30(8):1588-96.

34. Schnyder B, Pittet M, Durand J, Schnyder-Candrian S. Rapid effects of glucose on the insulin signaling of endothelial NO generation and epithelial Na transport. *Am J Physiol Endocrinol Metab* 2002;282(1):E87-E94.
35. Westerbacka J, Wilkinson I, Cockcroft J, Utriainen T, Vehkavaara S, Yki-Jarvinen H. Diminished wave reflection in the aorta. A novel physiological action of insulin on large blood vessels. *Hypertension* 1999;33(5):1118-22.
36. Stapleton PA, James ME, Goodwill AG, Frisbee JC. Obesity and vascular dysfunction. *Pathophysiology* 2008;15(2):79-89.
37. Rietzschel ER, Langlois M, De Buyzere ML, Segers P, de Bacquer D, Bekaert S *et al*. Oxidized low-density lipoprotein cholesterol is associated with decreases in cardiac function independent of vascular alterations. *Hypertension* 2008;52(3):535-41.

10 Cardiovascular flexibility in middle-aged overweight South Asians *vs.* white Caucasians: response to short-term caloric restriction

Leontine E.H. Bakker*
Linda D. van Schinkel*
Jacqueline T. Jonker
Albert de Roos
Hanno Pijl
A. Edo Meinders
Ingrid M. Jazet
Hildo J. Lamb
Johannes W.A. Smit

* Authors contributed equally to manuscript

Accepted for publication in Nutr Metab Cardiovasc Dis

ABSTRACT

Background. South Asians have a higher risk of developing cardiovascular disease than white Caucasians. The underlying cause is unknown, but might be related to higher cardiac susceptibility to metabolic disorders. Short-term caloric restriction can be used as a metabolic stress test to study cardiac flexibility. We assessed whether metabolic and functional cardiovascular flexibility to caloric restriction differs between South Asians and white Caucasians.

Methods. Cardiovascular function and myocardial triglycerides were assessed using a 1.5T-MRI/S-scanner in 12 middle-aged overweight male South Asians and 12 matched white Caucasians before and after an 8-day very low calorie diet (VLCD).

Results. At baseline South Asians were more insulin resistant than Caucasians. Cardiac dimensions were smaller, despite correction for body surface area, and PWV in the distal aorta was higher in South Asians. Systolic and diastolic function, myocardial triglycerides and pericardial fat did not differ significantly between groups. After the VLCD body weight reduced on average with 4.0±0.2 kg. Myocardial triglycerides increased in both ethnicities with 69±18%, and diastolic function decreased although this was not significant in South Asians. However, pericardial fat and PWV in the proximal and total aorta were reduced in Caucasians only.

Conclusions. Myocardial triglyceride stores in middle-aged overweight and insulin resistant South Asians are as flexible and amenable to therapeutic intervention by caloric restriction as age-, sex- and BMI-matched but less insulin resistant white Caucasians. However, paracardial fat volume and PWV showed a differential effect in response to an 8-day VLCD in favour of Caucasians.

INTRODUCTION

People of South Asian descent are at an increased risk of developing cardiovascular disease compared to white Caucasians. The age-standardized mortality rate from cardiovascular disease is approximately 50% higher for South Asians.[1-3] Furthermore, cardiovascular disease in South Asians is more aggressive and has higher mortality rates at younger ages.[1;2;4] The mean age of first acute myocardial infarction is around five years earlier than in Caucasians.[5]

Traditional risk factors, such as smoking, hypertension and cholesterol levels, do not seem to account for the excess risk for cardiovascular disease in South Asians.[3] Major contributing factors to the high prevalence of cardiovascular disease in South Asians are insulin resistance and type 2 diabetes, also highly prevalent in this group. Mortality risk of cardiovascular disease associated with diabetes is higher in South Asians compared to Caucasians,[3] which might suggest that South Asians have a higher cardiac susceptibility to metabolic disorders. Since South Asians represent one fifth of the world's population, the increased risk for cardiovascular disease and type 2 diabetes in this ethnicity poses a major burden on the health care system. Therefore, we aimed to gain more insight in the underlying cause of the increased susceptibility of South Asians to develop cardiovascular disease compared to white Caucasians, and, more specifically, in the interrelationship between metabolic disorders and cardiac function.

We have shown previously that short-term caloric restriction can be used as a metabolic stress test to induce a short-term physiological increase of plasma free fatty acid (FFA) levels, which enables us to study the flexibility of myocardial triglyceride (TG) content and cardiac function, as assessed by magnetic resonance (MR) techniques.[6-9] Surprisingly, so far no studies have been published on the effect of caloric restriction on cardiovascular function in South Asians.

Given the high risk of cardiovascular disease in South Asians, we hypothesize that cardiovascular function in middle-aged overweight South Asians is impaired compared to Caucasians. Furthermore, we hypothesize that the metabolic and functional cardiovascular flexibility in response to caloric restriction is compromised in people of South Asian descent. Therefore, we subjected middle-aged, overweight South Asians and age-, sex- and BMI-matched white Caucasians to an 8-day very low calorie diet (VLCD) and studied cardiac function and myocardial TG content using MR techniques. In addition, we studied aortic pulse wave velocity (PWV), a cardiovascular risk indicator.

METHODS

Study population

Eligible participants were men of Dutch South Asian origin (n=12) or Dutch white Caucasian origin (n=12), aged 40-50 year, with BMI between 25 and 30 kg/m^2, waist circumference >90 cm (South Asians) or >94 cm (Caucasians), and a positive family history for type 2 diabetes (at least 1 (grand)parent and 1 other family member with type 2 diabetes). Subjects were recruited between October 2010 and May 2012 via local advertisements, and underwent a medical screening including a physical examination, blood chemistry tests and an oral glucose tolerance test (OGTT) to exclude type 2 diabetes. Other exclusion criteria were: cardiovascular disease, any significant chronic disease, use of medication known to influence glucose and/or lipid metabolism, smoking, recent weight change, and general contraindications to MR scanning. The study was approved by the local ethics committee and performed in accordance with the principles of the revised Declaration of Helsinki. Subjects gave written informed consent prior to participation.

Study design

In this prospective, non-randomized clinical intervention study, participants were studied on 2 study days after a 10-hour fast, separated by an 8-day VLCD. The VLCD consisted of three sachets of Modifast® (Nutrition & Santé Benelux, Breda, The Netherlands) per day (~450 kcal/day; ~50 g protein, 50-60 g carbohydrates, ~7 g lipids and ~15 g dietary fibres). MR studies were performed shortly before the start and at the end of the 8th day of the diet. Subjects were instructed not to alter life style habits. Anthropometric measurements were performed according to WHO recommendations. Body fat was assessed by bioelectrical impedance analysis (Bodystat® 1500). Blood pressure was measured with a vital function monitor (Philips Sure Signs VS3). A 75-gram 2-hour OGTT was performed on the screening day. Total areas under the curve (AUC) for glucose and insulin were determined using the linear trapezoidal rule. The Matsuda index was used as a measure for insulin sensitivity.[10]

MR protocol

Measurements were performed using a 1.5-Tesla whole-body MR-scanner (Gyroscan ACS-NT15; Philips Medical Systems, The Netherlands) in postprandial state (four hours after last meal).

Myocardial triglyceride content

MR spectroscopy (^{1}H-MRS) was used to quantify myocardial TG content as described before.[11] In summary, an 8-mL voxel was placed in the interventricular septum on four-chamber and short-axis images at end-systole. Electrocardiographic triggering (for myo-

cardial spectra) and respiratory pencil beam navigator were used during acquisition.[11] Acquisitions were performed with and without water suppression, with myocardial TG expressed as percentage of the unsuppressed water signal.

Pericardial fat quantification

As described before,[12] to quantify the pericardial fat volume, the heart was imaged using electrocardiographically-gated breath-holds with a multi shot turbo spin echo sequence in a four-chamber view orientation. Water was suppressed using Spectral Inversion Recovery (SPIR). Contours were drawn around both pericardial fat layers surrounding the ventricles and atria using MASS® software (Medis, Leiden, The Netherlands) (**Figure 1**). The number of pixels were converted to square centimetres and multiplied by the slice thickness to obtain volume.

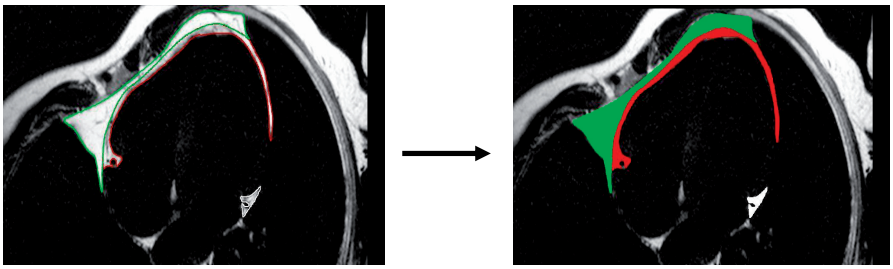


Figure 1. Quantification of the pericardial fat layer. This figure shows the quantification of the pericardial fat layer, which can be divided in an epicardial (red) and paracardial (green) fat layer.

Left ventricular dimensions and function

Data were analysed blinded for ethnicity and study occasion. As previously described, the entire heart was imaged in short-axis orientation, using electrocardiographically-gated breath-hold cine steady-state free-precession sequences.[13] Left ventricular (LV) epicardial and endocardial contours were manually drawn in the end-systolic and end-diastolic phases of the short-axis images, using validated MASS® software. LV end-diastolic volume (EDV), end-systolic volume (ESV), ejection fraction (EF), stroke volume (SV) and end-diastolic mass (EDM) were calculated.

Furthermore, an electrocardiographically-gated gradient-echo sequence with velocity encoding (100 cm/sec) was performed to measure transmitral blood, for the determination of LV diastolic function. Analysis was performed by using FLOW® software (Medis, Leiden, The Netherlands). The early filling phase (E) and the atrial contraction (A) were analysed and their peak flow ratio was calculated (E/A ratio). Additionally, the peak deceleration gradient of E and LV filling pressures (E/Ea) were assessed.[14;15] Heart rate was monitored and stored during the transmitral flow measurements.

Pulse Wave Velocity

To evaluate the aortic stiffness, aortic PWV was determined, using a previously described protocol.[16] Shortly, a scout view of the aorta was performed. Subsequently, a velocity encoded image perpendicular to the ascending aorta at the level of the pulmonary trunk, at the level of the aorta crossing the diaphragm and at the level of the aortic bifurcation was assessed (**Figure 2**). This resulted in through-plane flow measurements of the ascending and descending aorta. PWV was calculated using the formula: $\Delta x/\Delta t$, where Δx is the aortic path length between two measurement sites and Δt is the time delay between the arrivals of the foot of the pulse wave at the respective measurements site. The distance between the measurement sites was determined manually using MASS®. Data were analysed using MASS® and FLOW®.

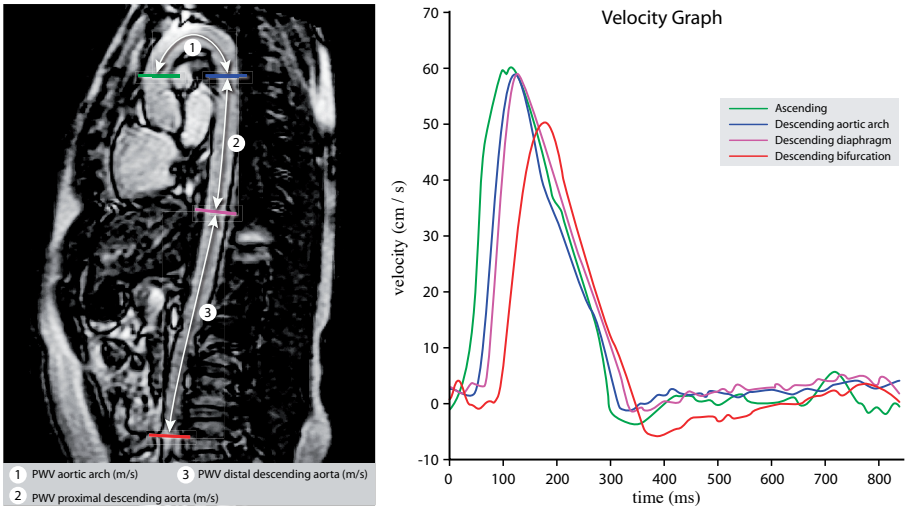


Figure 2. Aortic PWV determination with MRI. The left panel shows a double-oblique parasagittal image of the aorta. The coloured lines represent the acquisition planes for velocity-encoded MRI which are positioned perpendicular to the aorta. 1 is the path length of the aortic arch, 2 of the proximal descending aorta and 3 of the distal descending aorta, determined along the centreline of the aorta. The right panel shows the velocity-time curves for the four different measurement sites in the aorta.

Assays

Serum concentrations of glucose, total cholesterol, HDL and TG were measured on a Modular P800 analyser (Roche, The Netherlands), serum insulin levels on an Immulite 2500 (Siemens, The Netherlands), HbA_{1c} on an HPLC system (Kordia, The Netherlands), and plasma FFAs by a commercial kit (Wako Chemicals, Germany).

Statistical analysis

Data are presented as mean±SEM or median (interquartile range (IQR)). A mixed effects model was applied to assess mean differences within and between groups, and to determine differences in diet effect. Groups and intervention were modelled as fixed effects and the subject specific deviances from the group mean were modelled as random effects. Nonparametric tests were performed when appropriate (related-samples Wilcoxon Signed Rank Test (within group), independent-samples Mann-Whitney U Test (between-group)). Significance level was set at $p<0.05$ (two-sided). Statistical analyses were performed using SPSS for Windows version 20.0 (IBM, USA).

RESULTS

Clinical and metabolic characteristics

Baseline Mean age was 44.6±0.8 year. Body surface area (BSA) was significantly lower in South Asians compared to Caucasians. However, BMI (28.3±0.3 kg/m^2), waist and hip circumference and percentage of fat mass were comparable between groups. The same was true for blood pressure and heart rate. Both ethnicities had similar fasting glucose levels, but insulin levels (both fasting and during OGTT) were higher and Matsuda index was lower in South Asians. Fasting FFAs, TGs and cholesterol levels did not significantly differ between groups (**Table 1**).

Effect of VLCD Anthropometric measurements were significantly reduced after the diet in both groups. The mean reduction in body weight for both groups was 4.0±0.2 kg, of which approximately 50% was fat mass. BMI decreased on average with 1.28±0.07 kg/m^2. Systolic and diastolic blood pressure were reduced in both ethnicities. The heart rate was not affected. In both groups, fasting glucose, insulin, TG and total cholesterol levels decreased significantly, while FFAs increased in response to the VLCD (**Table 1**).

Myocardial TG content

Baseline No differences in myocardial TG content were found between both groups at baseline (**Table 2, Figure 3**).

Effect of VLCD Myocardial TG content increased in both ethnicities, although in Caucasians this did not reach significance ($p=0.067$). The percentage of myocardial TG increase, however, was comparable between groups (69±18%, $p=0.868$) (**Table 2, Figure 3 and 4**).

Pericardial fat distribution

Baseline There were no differences in pericardial, epicardial or paracardial fat volumes between groups at baseline (**Table 2, Figure 3**).

Table 1. Clinical and metabolic characteristics.

	white Caucasians		South Asians	
	before VLCD	after VLCD	before VLCD	after VLCD
Clinical characteristics				
age (years)	44.3 ± 1.1		44.9 ± 0.9	
height (m)	1.81 ± 0.02		1.75 ± 0.01**	
weight (kg)	92.6 ± 2.5	88.2 ± 2.5††	86.7 ± 1.4	83.2 ± 1.6††‡
BSA (m^2)	2.14 ± 0.04	2.09 ± 0.04††	2.02 ± 0.02*	1.99 ± 0.02††*‡
BMI (kg/m^2)	28.1 ± 0.5	26.8 ± 0.5††	28.4 ± 0.4	27.3 ± 0.4††
waist (cm)	103 ± 2	100 ± 2††	99 ± 2	97 ± 1††
hip (cm)	102 ± 1	100 ± 1	99 ± 2	97 ± 1†
WHR	1.01 ± 0.01	0.99 ± 0.01†	1.02 ± 0.01	1.01 ± 0.01
fat mass (%)	23.1 ± 0.6	21.8 ± 0.6††	23.8 ± 0.6	23.0 ± 0.6††
systolic BP (mmHg)	130 ± 3	118 ± 2††	136 ± 3	124 ± 3††
diastolic BP (mmHg)	85 ± 3	77 ± 3††	90 ± 4	85 ± 3††
heart rate (bpm)	64 ± 3	61 ± 2	70 ± 3	65 ± 3
Metabolic characteristics				
free fatty acids (mmol/L)	0.53 ± 0.03	1.36 ± 0.13†	0.58 ± 0.04	0.85 ± 0.06†*‡
triglycerides (mmol/L)	1.29 (2.48)	0.89 (0.18) †	1.78 (2.91)	0.91 (0.25)†
total cholesterol (mmol/L)	5.56 ± 0.24	4.72 ± 0.33†	5.74 ± 0.28	5.13 ± 0.26†
HDL-cholesterol (mmol/L)	1.09 ± 0.08	0.99 ± 0.06	1.00 ± 0.07	0.95 ± 0.05
LDL-cholesterol (mmol/L)	3.54 ± 0.28	3.42 ± 0.37	3.58 ± 0.25	3.77 ± 0.24
total cholesterol/HDL ratio	5.57 ± 0.59	5.02 ± 0.54	6.05 ± 0.48	5.49 ± 0.35
HbA_{1c} (mmol/mol, %)	33.0 (6), 5.20 (0.5)		36.5 (1)*, 5.45 (0.1) *	
glucose (mmol/L)	5.33 ± 0.20	4.45 ± 0.22††	5.30 ± 0.11	4.51 ± 0.14††
insulin (mU/L)	6.0 (3.0)	1.7 (3.7)††	8.5 (2.5)**	2.3 (4.7)††
Oral glucose tolerance test				
2 hour insulin (mU/L)	31 (29)		77 (76)*	
glucose AUC (mmol/L * h)	959 ± 32		1027 ± 58	
insulin AUC (mU/L * h)	4477 ± 586		8790 ± 711**	
Matsuda index	7.1 ± 1.3		3.9 ± 0.6*	

Data are presented as mean ± SEM or median (IQR). VLCD, very low calorie diet. BSA, body surface area; BMI, body mass index; WHR, waist hip ratio; BP, blood pressure; AUC, total area under the curve. † $p<0.05$, †† $p<0.005$ within group *vs.* before diet. * $p<0.05$, ** $p<0.005$ *vs.* Caucasians. ‡ $p<0.05$, ‡‡ $p<0.005$ diet effect *vs.* Caucasians.

Table 2. Cardiac dimensions, parameters of cardiovascular function, pericardial fat distribution and myocardial triglyceride content assessed with MRI and MRS before and after an 8-day VLCD.

	white Caucasians		South Asians	
	before VLCD	after VLCD	before VLCD	after VLCD
Cardiac dimensions and basic function				
LVEDMI (g/m^2)	51 ± 2	48 ± 2††	52 ± 1	50 ± 1†
EDVI (mL/m^2)	87 ± 3	83 ± 2†	74 ± 3**	72 ± 3*
ESVI (mL/m^2)	34 ± 1	32 ± 1	29 ± 1*	27 ± 1*
SVI (mL/m^2)	53 ± 2	51 ± 2	46 ± 2*	45 ± 2*
CI ($mL/min/m^2$)	3.3 ± 0.1*10^3	3.1 ± 0.1*$10^{3\dagger}$	3.2 ± 0.2*10^{3}*	2.9 ± 0.1*$10^{3\dagger}$*
EF (%)	61 ± 2	62 ± 1	62 ± 1	63 ± 1
Diastolic cardiac function				
E peak filling rate (mL/s)	549 ± 28	477 ± 26††	493 ± 26	445 ± 22†
E acceleration peak (mL/s^2x10^{-3})	8.4 ± 0.6	7.0 ± 0.5††	7.5 ± 0.5	6.4 ± 0.3
E deceleration peak (mL/s^2x10^{-3})	-4.7 ± 0.3	-3.7 ± 0.2††	-4.9 ± 0.3	-4.1 ± 0.3†
E deceleration mean (mL/s^2x10^{-3})	-3.2 ± 0.2	-2.5 ± 0.2††	-3.4 ± 0.3	-2.8 ± 0.3†
A peak filling rate (mL/s)	392 ± 18	364 ± 20	365 ± 17	360 ± 10
E/A-peak ratio	1.43 ± 0.10	1.34 ± 0.09†	1.37 ± 0.08	1.24 ± 0.07
E/Ea	9.4 ± 0.7	8.3 ± 1.0	9.8 ± 0.8	7.2 ± 0.8†
Pulse wave velocity				
PWV aortic arch (m/s)	5.6 (0.9)	5.3 (1.1)	5.7 (1.3)	5.7 (0.9)
PWV proximal aorta (m/s)	6.7 (2.4)	5.2 (1.4)†	7.2 (3.7)	7.1 (2.6)**
PWV distal aorta (m/s)	5.0 (0.5)	4.9 (1.7)	5.5 (1.2)*	5.2 (1.0)
PWV total aorta (m/s)	5.5 (1.0)	5.2 (0.4)†	6.1 (0.9)	5.8 (0.9)*
Fat distribution				
Epicardial fat (mL)	3.1 (1.8)	3.6 (1.4)	3.3 (1.4)	3.0 (1.0)
Paracardial fat (mL)	4.8 (3.7)	3.7 (3.0)††	3.5 (2.1)	2.7 (2.6)‡
Pericardial fat (mL)	7.6 (4.0)	6.6 (3.5)†	6.7 (3.0)	6.0 (4.0)
Myocardial TG content (%)	0.56 ± 0.08	0.74 ± 0.08	0.59 ± 0.08	0.98 ± 0.13††

Data are mean ± SEM or median (IQR). VLCD, very low calorie diet; LV, left ventricular; EDM, end diastolic mass; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume; CI, cardiac index; EF, ejection fraction; ESWS, end-systolic wall stress. I, indexed for body surface area; E, early diastolic wave; A, atrial diastolic wave; E/Ea, estimated left ventricular filling pressure; PWV, pulse wave velocity; TG, triglyceride. † p<0.05, †† p<0.005 within group *vs.* before diet. * p<0.05, ** p<0.005 *vs.* Caucasians. ‡ p<0.05, ‡‡ p<0.005 diet effect *vs.* Caucasians.

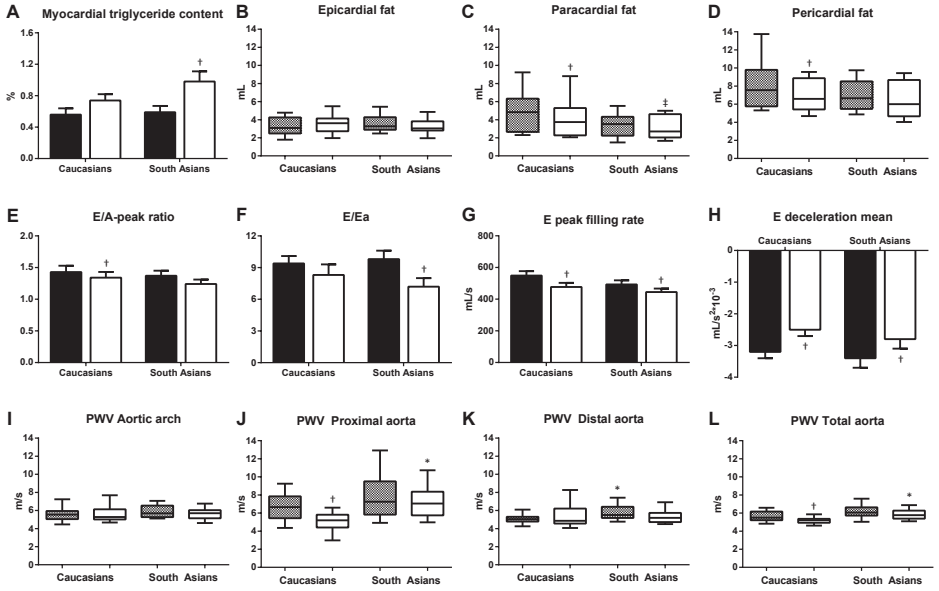


Figure 3. Overview of main results in middle-aged overweight South Asian and white Caucasian men before (dark bars) and after (open bars) an 8-day VLCD. A: Myocardial triglyceride content. B-D: pericardial fat. E-H: variables of diastolic cardiac function. I-L: pulse wave velocity (PWV). Data are expressed as mean ± SEM (A, E-H) or as median (IQR) in a box-whiskerplot (B-D, I-L). † p<0.05 within group *vs.* before diet. * p<0.05 *vs.* Caucasians. ‡ p<0.05 diet effect *vs.* Caucasians.

Effect of VLCD The pericardial and paracardial fat volumes decreased significantly in Caucasians in response to the VLCD (p=0.003 and p=0.050, respectively), whereas no significant changes occurred in South Asians (**Table 2, Figure 3**).

Left ventricular dimensions and function

Baseline Despite correction for BSA EDV, ESV and SV were significantly lower in South Asians. EF was on average 61±4%, and was comparable between ethnicities (p=0.808). There were no significant differences in diastolic cardiac function, as reflected in the E/A ratio (p=0.168) and the E/Ea ratio (p=0.088) (**Table 2, Figure 3**).

Effect of VLCD LV mass, indexed for BSA, decreased slightly in both groups after the diet. EDV reduced in Caucasians, however no significant change occurred in South Asians. The cardiac index reduced equally in both ethnicities. The E/A ratio reduced significantly in Caucasians, whereas no significant diet effect was observed in South Asians. In contrast, the VLCD did not induce significant changes in the E/Ea ratio in Caucasians, while in South Asians E/Ea decreased significantly. The early peak filling rate (EPFR) and early deceleration mean showed a significant decrease in both groups in response to the VLCD (**Table 2, Figure 3**).

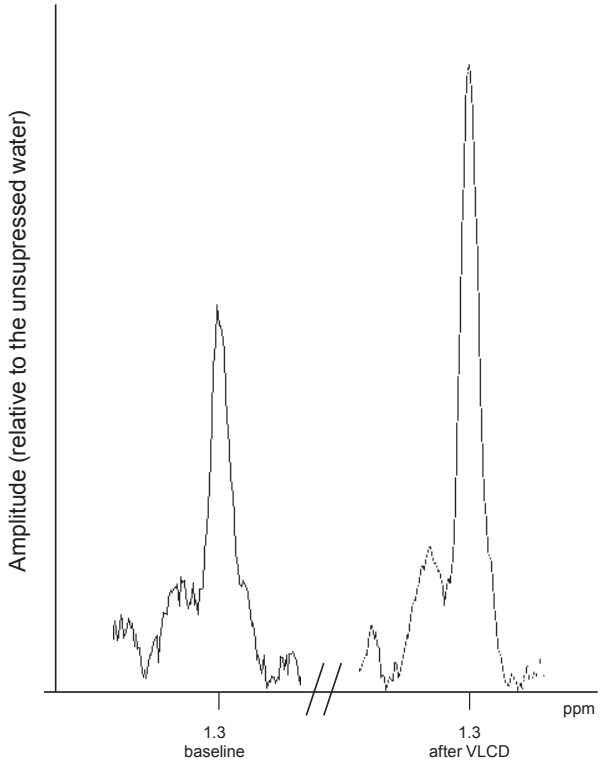


Figure 4. Myocardial spectra. Example of typical myocardial spectra of one subject before and after an 8-day VLCD.

Pulse wave velocity

Baseline PWV in the distal segment of the aorta was significantly higher in South Asians compared to Caucasians. Furthermore, PWV in the total aorta tended to be higher in South Asians, however this did not reach statistical significance (p=0.068) (**Table 2, Figure 3**).

Effect of VLCD After the VLCD, PWV in the proximal descending part of the aorta and PWV of the total aorta were significantly reduced in Caucasians. In contrast, no diet effect on PWV in any of the segments of the aorta was observed in South Asians (**Table 2, Figure 3**).

DISCUSSION

South Asians have a higher risk of developing cardiovascular disease than white Caucasians. Additionally, the risk of cardiac complications in subjects with insulin resistance

and type 2 diabetes is higher in this population, indicating they are metabolically more at risk. Previous studies in healthy subjects and obese patients with type 2 diabetes with and without cardiovascular disease of Caucasian descent demonstrated metabolic and functional flexibility of the heart in response to both short- and long-term caloric restriction. To date, however, it was unknown if caloric restriction in South Asians has comparable effects. This study showed that an 8-day VLCD increased myocardial TG content to a similar degree in middle-age overweight South Asians as compared to age-, sex- and BMI-matched but less insulin resistant Caucasians, indicating comparable flexibility of the heart. Paracardial fat volume and PWV, however, showed a differential effect in response to caloric restriction in favour of Caucasians.

Myocardial TG content

At baseline, South Asians were more insulin resistant, indicated by higher insulin levels (both in fasted condition and during OGTT) and lower Matsuda index (**Table 1**). Studies in animals and humans have demonstrated that increased myocardial TG content in insulin resistance is associated with impaired myocardial function.[17-19] Paradoxically, however, the increase in myocardial TG observed after a short-term VLCD is a sign of preserved metabolic flexibility of the heart. Given the high risk on cardiovascular disease and diabetes in South Asians, we hypothesized, therefore, that the flexibility of the heart to adjust myocardial TG content in response to caloric restriction was diminished in South Asians compared to Caucasians. Surprisingly, however, an 8-day VLCD increased myocardial TG similarly in both groups. Thus, South Asians showed a similar physiological flexibility of myocardial lipid metabolism as Caucasians.

Previous short-term VLCD studies have shown that the increase in myocardial TG is the net result of increased uptake of FFAs in cardiomyocytes in relation to oxidative FFA requirements. This increased uptake is due to an increased release of FFAs from the adipose tissue into the circulation, which is caused by increased lipolysis of TG in adipose tissue in response to caloric restriction.[7;9] Indeed, in the present study FFAs were significantly increased after the diet in both ethnicities, and waist fat was significantly reduced (data not shown), indicating increased lipolysis in the adipose tissue.

Pericardial fat

Pericardial fat, the layer of fat surrounding the heart, can be divided in an epicardial and paracardial layer. Both fat layers are metabolically different. Whereas epicardial fat has been shown to be a source of several inflammatory mediators, paracardial fat seems to have a greater importance in mechanical restriction, which exerts an unfavourable effect on the coronary vasculature.[20] In the present study, pericardial fat distribution was similar between groups at baseline. However, pericardial fat decreased significantly in Caucasians in response to the dietary intervention, mainly due to a reduction in the

paracardial fat layer, whereas in South Asians no significant diet effect was observed. Since the paracardial fat layer has been found to be a predictor of cardiovascular disease, the decrease in this specific fat compartment in Caucasians probably conveys reduced cardiovascular risk.[21]

Cardiac dimensions and function

Cardiac dimensions were smaller in South Asians compared to Caucasians, despite correction for BSA. This is in line with other studies, which showed smaller left heart volumes in middle-aged South Asians,[22;23] using echocardiography. In a recent study in healthy young adults, we showed that these smaller cardiac dimensions are already present at a young age.[24] No major effects of the diet on cardiac dimensions were observed.

Cardiac systolic function, reflected as the LV ejection fraction, was normal (~62%) and comparable in both groups. Systolic function was not affected by the diet, which is in line with previous VLCD studies.[7;9;25]

Diastolic cardiac function, reflected as the E/A ratio, decreased after the diet as expected from previous studies.[7;9;26] The reduction, however, was only significant in Caucasians. This difference in diet effect might be attributed to a decrease in filling pressure (E/Ea ratio) in South Asians. In addition, other parameters for diastolic function did decrease in both groups. The decrease in diastolic function can probably be explained by changes in elastic properties of the LV. In animal models, TG accumulation in cardiomyocytes is directly related to impaired cardiac function via complex mechanisms involving fatty acid derivatives.[17] An alternative explanation may be that changes in plasma FFAs, induced by caloric restriction, change the calcium homeostasis in the myocardium, thereby influencing LV diastolic function.[27]

Pulse wave velocity

The PWV is a powerful independent predictor of cardiovascular events.[28] In the present study, PWV in the distal aorta was significantly higher in South Asians compared to Caucasians at baseline, indicating a stiffer aorta. This is in line with other studies that showed a higher PWV in middle-aged South Asians compared to Caucasians.[29;30] In addition, we have shown recently that PWV is already higher in healthy young South Asians.[24] It is known that insulin resistance and diabetes compromise aortic elastic function. Although the precise underlying mechanisms remain unclear, it is known that long-term increased insulin levels can contribute to increased arterial wall thickness, and thereby to increased arterial stiffening, by direct and indirect trophic effects on smooth muscle cells.[31] In the present study, South Asians were more insulin resistant than Caucasian subjects – as reflected in higher insulin levels (both fasting and during OGTT) – which might explain the higher PWV observed in South Asians.

The PWV responded differentially to an 8-day VLCD, consisting of a reduction in proximal and total PWV in Caucasians, whereas no diet effect was observed in South Asians, suggesting that large arteries are less flexible in South Asians in response to caloric restriction. This might be due to the, probably long-term existing, higher insulin resistance observed in South Asians which may have induced irreversible changes in the arterial wall according to the aforementioned mechanism.

Strengths of this study are that this is the first time the response to a VLCD on cardiovascular function was assessed and the first time myocardial and pericardial TG content were measured in South Asians. We used an extreme intervention (8-day VLCD) to be able to detect differences between ethnicities. Furthermore, we matched on BMI in order to gain more insight in the pathophysiological mechanism behind the increased risk of South Asians to develop insulin resistance and type 2 diabetes at lower ranges of BMI than Caucasians. A possible limitation is the relatively small sample size, which might limit generalization potential. However, subjects were their own controls, which increases power to detect relevant differences.

In conclusion, this study proves that myocardial TG stores in middle-aged overweight and insulin resistant South Asians are as flexible and amenable to therapeutic intervention by caloric restriction as age-, sex- and BMI-matched but less insulin resistant Caucasians. However, paracardial fat volume and PWV showed a differential effect in response to an 8-day VLCD in favour of Caucasians.

REFERENCES

1. Balarajan R. Ethnic differences in mortality from ischaemic heart disease and cerebrovascular disease in England and Wales. *BMJ* 1991;302(6776):560-4.
2. Chaturvedi N, Fuller JH. Ethnic differences in mortality from cardiovascular disease in the UK: do they persist in people with diabetes? *J Epidemiol Community Health* 1996;50(2):137-9.
3. Forouhi NG, Sattar N, Tillin T, McKeigue PM, Chaturvedi N. Do known risk factors explain the higher coronary heart disease mortality in South Asian compared with European men? Prospective follow-up of the Southall and Brent studies, UK. *Diabetologia* 2006;49(11):2580-8.
4. Anand SS, Yusuf S, Vuksan V, Devanesen S, Teo KK, Montague PA *et al*. Differences in risk factors, atherosclerosis, and cardiovascular disease between ethnic groups in Canada: the Study of Health Assessment and Risk in Ethnic groups (SHARE). *Lancet* 2000;356(9226):279-84.
5. Joshi P, Islam S, Pais P, Reddy S, Dorairaj P, Kazmi K *et al*. Risk factors for early myocardial infarction in South Asians compared with individuals in other countries. *JAMA* 2007;297(3):286-94.
6. Hammer S, van der Meer RW, Lamb HJ, Schar M, de Roos A, Smit JW *et al*. Progressive caloric restriction induces dose-dependent changes in myocardial triglyceride content and diastolic function in healthy men. *J Clin Endocrinol Metab* 2008;93(2):497-503.
7. van der Meer RW, Hammer S, Smit JW, Frolich M, Bax JJ, Diamant M *et al*. Short-term caloric restriction induces accumulation of myocardial triglycerides and decreases left ventricular diastolic function in healthy subjects. *Diabetes* 2007;56(12):2849-53.
8. Hammer S, Snel M, Lamb HJ, Jazet IM, van der Meer RW, Pijl H *et al*. Prolonged caloric restriction in obese patients with type 2 diabetes mellitus decreases myocardial triglyceride content and improves myocardial function. *J Am Coll Cardiol* 2008;52(12):1006-12.
9. Hammer S, van der Meer RW, Lamb HJ, de Boer HH, Bax JJ, de Roos A *et al*. Short-term flexibility of myocardial triglycerides and diastolic function in patients with type 2 diabetes mellitus. *Am J Physiol Endocrinol Metab* 2008;295(3):E714-E718.
10. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 1999;22(9):1462-70.
11. van der Meer RW, Doornbos J, Kozerke S, Schar M, Bax JJ, Hammer S *et al*. Metabolic imaging of myocardial triglyceride content: reproducibility of 1H MR spectroscopy with respiratory navigator gating in volunteers. *Radiology* 2007;245(1):251-7.
12. Jonker JT, de Mol P, de Vries ST, Widya RL, Hammer S, van Schinkel LD *et al*. Exercise and type 2 diabetes mellitus: changes in tissue-specific fat distribution and cardiac function. *Radiology* 2013;269(2):434-42.
13. Lamb HJ, Doornbos J, van der Velde EA, Kruit MC, Reiber JH, de Roos A. Echo planar MRI of the heart on a standard system: validation of measurements of left ventricular function and mass. *J Comput Assist Tomogr* 1996;20(6):942-9.
14. Pattynama PM, Lamb HJ, van der Velde EA, van der Wall EE, de Roos A. Left ventricular measurements with cine and spin-echo MR imaging: a study of reproducibility with variance component analysis. *Radiology* 1993;187(1):261-8.
15. Paelinck BP, de Roos A, Bax JJ, Bosmans JM, van der Geest RJ, Dhondt D *et al*. Feasibility of tissue magnetic resonance imaging: a pilot study in comparison with tissue Doppler imaging and invasive measurement. *J Am Coll Cardiol* 2005;45(7):1109-16.
16. Grotenhuis HB, Westenberg JJ, Steendijk P, van der Geest RJ, Ottenkamp J, Bax JJ *et al*. Validation and reproducibility of aortic pulse wave velocity as assessed with velocity-encoded MRI. *J Magn Reson Imaging* 2009;30(3):521-6.

17. Christoffersen C, Bollano E, Lindegaard ML, Bartels ED, Goetze JP, Andersen CB *et al*. Cardiac lipid accumulation associated with diastolic dysfunction in obese mice. *Endocrinology* 2003;144(8):3483-90.
18. McGavock JM, Lingvay I, Zib I, Tillery T, Salas N, Unger R *et al*. Cardiac steatosis in diabetes mellitus: a 1H-magnetic resonance spectroscopy study. *Circulation* 2007;116(10):1170-5.
19. Szczepaniak LS, Dobbins RL, Metzger GJ, Sartoni-D'Ambrosia G, Arbique D, Vongpatanasin W *et al*. Myocardial triglycerides and systolic function in humans: in vivo evaluation by localized proton spectroscopy and cardiac imaging. *Magn Reson Med* 2003;49(3):417-23.
20. van der Meer RW, Lamb HJ, Smit JW, de Roos A. MR imaging evaluation of cardiovascular risk in metabolic syndrome. *Radiology* 2012;264(1):21-37.
21. Sicari R, Sironi AM, Petz R, Frassi F, Chubuchny V, De Marchi D *et al*. Pericardial rather than epicardial fat is a cardiometabolic risk marker: an MRI *vs.* echo study. *J Am Soc Echocardiogr* 2011;24(10):1156-62.
22. Chahal NS, Lim TK, Jain P, Chambers JC, Kooner JS, Senior R. Ethnicity-related differences in left ventricular function, structure and geometry: a population study of UK Indian Asian and European white subjects. *Heart* 2010;96(6):466-71.
23. Kumaran K, Fall CH, Martyn CN, Vijayakumar M, Stein CE, Shier R. Left ventricular mass and arterial compliance: relation to coronary heart disease and its risk factors in South Indian adults. *Int J Cardiol* 2002;83(1):1-9.
24. van Schinkel LD, Bakker LE, Jonker JT, de Roos A, Pijl H, Meinders AE *et al*. Functional and metabolic imaging of the cardiovascular system in young healthy South Asians and Caucasians unveils early differences. *Diabetes Care* 2013;36(10):e178-e179.
25. Hammer S, Snel M, Lamb HJ, Jazet IM, van der Meer RW, Pijl H *et al*. Prolonged caloric restriction in obese patients with type 2 diabetes mellitus decreases myocardial triglyceride content and improves myocardial function. *J Am Coll Cardiol* 2008;52(12):1006-12.
26. Hammer S, van der Meer RW, Lamb HJ, Schar M, de Roos A, Smit JW *et al*. Progressive caloric restriction induces dose-dependent changes in myocardial triglyceride content and diastolic function in healthy men. *J Clin Endocrinol Metab* 2008;93(2):497-503.
27. Huang JM, Xian H, Bacaner M. Long-chain fatty acids activate calcium channels in ventricular myocytes. *Proc Natl Acad Sci U S A* 1992;89(14):6452-6.
28. Mitchell GF, Hwang SJ, Vasan RS, Larson MG, Pencina MJ, Hamburg NM *et al*. Arterial stiffness and cardiovascular events: the Framingham Heart Study. *Circulation* 2010;121(4):505-11.
29. Rezai MR, Wallace AM, Sattar N, Finn JD, Wu FC, Cruickshank JK. Ethnic differences in aortic pulse wave velocity occur in the descending aorta and may be related to vitamin D. *Hypertension* 2011;58(2):247-53.
30. Webb DR, Khunti K, Lacy P, Gray LJ, Mostafa S, Talbot D *et al*. Conduit vessel stiffness in British south Asians of Indian descent relates to 25-hydroxyvitamin D status. *J Hypertens* 2012;30(8):1588-96.
31. Stapleton PA, James ME, Goodwill AG, Frisbee JC. Obesity and vascular dysfunction. *Pathophysiology* 2008;15(2):79-89.

11 South Asians exhibit disturbed HDL functionality as compared to white Caucasians

Leontine E.H. Bakker*
Mariëtte R. Boon*
Wijtske Annema
Arne Dikkers
Huub J. van Eyk
J. Wouter Jukema
Louis M. Havekes
A. Edo Meinders
Ingrid M. Jazet#
Uwe J.F. Tietge#
Patrick C.N. Rensen#

*,# Authors contributed equally to manuscript

Submitted

ABSTRACT

Objective. South Asians have an exceptionally high risk of developing cardiovascular disease compared to white Caucasians. A contributing factor might be dysfunction of high density lipoprotein (HDL). This study aimed to compare HDL function in neonates, adolescents and adults of both ethnicities.

Methods. HDL functionality with respect to cholesterol efflux, anti-oxidation and anti-inflammation was determined using fasting plasma samples from South Asian and white Caucasian neonates (n=14 each), young adult healthy men (n=12 each, 18-25y), and adult, overweight men (n=12 each, 40-50y). Young adults were subjected to a 5-day high fat high calorie diet (HFHCD) and adults to an 8-day very low calorie diet (VLCD).

Results. Anti-oxidative capacity was lower in South Asian adults before VLCD (18.1±2.6 *vs.* 24.2±2.2%, p=0.077) and after VLCD (16.4±2.4 *vs.* 27.6±2.7%, p=0.003). Anti-inflammatory capacity was reduced in South Asian neonates (22.9±0.7 *vs.* 35.9±1.9%, p<0.00001), and was negatively affected by an 8-day VLCD only in South Asian adults (-12.2±4.3%, p=0.005). Cholesterol efflux capacity was increased in response to HFHCD in the young adult groups (South Asians: +6.3±2.9%, p=0.073, Caucasians: +11.8±3.4%, p=0.002) and decreased after VLCD in the adult groups (South Asians: -10.3±2.4%, p<0.001, Caucasians: -13.7±1.9%, p<0.0001)).

Conclusions. South Asians exhibit disturbed age-dependent function of HDL compared to white Caucasians, which may conceivably contribute to their excess risk of cardiovascular disease.

INTRODUCTION

The burden and mortality of cardiovascular disease are significantly increased among both native and migrant South Asians compared to people of white Caucasian descent. The age-standardized mortality rate from cardiovascular disease is around 50% higher for South Asians.[1-4] In addition, cardiovascular disease is more aggressive and has higher mortality rates at younger ages in South Asians.[2;5-8] The underlying mechanism of this excess risk is still poorly understood. Traditional risk factors such as smoking, hypertension, and type 2 diabetes seem to account for only part of the excess risk in South Asians.[3;9-11] Thus, additional factors likely play a role. One of these factors may be dysfunction of high density lipoprotein (HDL).

Numerous clinical and epidemiological studies have consistently shown a strong inverse association between the level of HDL-cholesterol and cardiovascular risk.[12-16] The cardiovascular protective effects of HDL have been attributed to several anti-atherogenic functional properties, including: HDL (i) prevents LDL oxidation, (ii) is anti-inflammatory, and (iii) stimulates cholesterol efflux from macrophage foam cells.[17-19] Of note, recent evidence suggests that HDL functionality might be affected independent of changes in plasma HDL-cholesterol level.[20;21] In trials that aimed at raising HDL-cholesterol levels with niacin or dalcetrapib on top of LDL lowering, no decrease in the occurrence of cardiovascular endpoints was observed compared to LDL lowering therapy only.[22;23] This suggests that simply raising HDL-cholesterol levels is not sufficient to lower cardiovascular disease risk and that HDL functionality may thus be more importantly linked to cardiovascular disease than plasma HDL-cholesterol concentrations. Indeed, previous studies showed that HDL is dysfunctional in people with (increased risk of) coronary atherosclerosis.[24-26]

HDL functionality is not only related to the cardiovascular health status. A 3-week high-fibre-low-fat diet and exercise intervention converted HDL from pro- to anti-inflammatory.[27] Furthermore, data from our study group demonstrated a decrease in cholesterol efflux capacity of HDL after very low calorie diet (VLCD)-induced weight loss.[28]

Interestingly, multiple studies have found lower HDL-cholesterol levels in South Asians compared to white Caucasians, even in South Asian neonates.[29-32] In addition, South Asians seem to have relatively more small sized HDL particles, which are associated with decreased cardiac protection compared to normal sized HDL particles.[33-35] Remarkably, little is known about HDL functionality in South Asians. Therefore, this study aimed to compare HDL function in South Asian and white Caucasian subjects. In particular, we were interested in the following questions: (i) is HDL function impaired in middle-aged overweight South Asians compared to matched white Caucasians?, (ii) if so, is this dysfunction already present in young healthy subjects or even at birth?, and (iii) is the effect of short-term dietary intervention on HDL function different between South

Asians and Caucasians? To address these questions, we determined three key biological functions of HDL, namely its ability to induce cholesterol efflux, anti-oxidative and anti-inflammatory properties, in three groups of South Asian and white Caucasian neonates, young healthy men (young adults) and middle-aged overweight men (adults). The young adults and adults were subjected to a 5-day high-fat-high-calorie diet (HFHCD) and an 8-day VLCD, respectively.

MATERIAL AND METHODS

Subjects

Neonates. Umbilical venous cord blood was collected from 28 neonates (14 South Asian and 14 Caucasian neonates) as described previously.[36] All neonates were born between January 1st, 2006, and January 1st, 2009, and were examined after their mothers had given informed consent and approval had been obtained by the local ethics committee. Live-born singleton babies with 4 South Asian grandparents or 4 Caucasian grandparents were included. Pregnancies complicated by pre-eclampsia were excluded. Gestational age was based on last menstrual period or ultrasound scanning (before 12 weeks). Birth weights were taken from the medical records. Immediately after delivery, 20 mL of cord blood was collected from the umbilical vein according to standard protocols. South Asian and Caucasian neonates were matched for HDL-cholesterol level.

Young adults and adults. 24 young healthy Dutch males (young adults; age 18-25y, BMI <25 kg/m^2) and 24 middle-aged overweight Dutch males (adults; age 40-50y, BMI 25-30 kg/m^2) were enrolled via local advertisements. Subjects were of South Asian (n=12 for each group) or Caucasian (n=12 for each group) origin. They underwent a medical screening including their medical history, a physical examination, blood chemistry tests and an oral glucose tolerance test to exclude individuals with type 2 diabetes. Other exclusion criteria were cardiovascular disease, any significant chronic disease, use of medication known to influence lipid metabolism, rigorous exercise, smoking and recent body weight change. The study was approved by the Medical Ethical Committee of the Leiden University Medical Centre and performed in accordance with the principles of the revised Declaration of Helsinki. Written informed consent was obtained from all subjects prior to participation.

Dietary interventions in young adults and adults

The young adults were subjected to a 5-day HFHCD, consisting of the subject's regular diet, supplemented with 375 mL of cream per day (1275 kcal/day, 94% fat), yielding to approx. 3775 kcal/day and 54% of fat. The adults received an 8-day VLCD, consisting of three sachets of Modifast per day (approx. 450 kcal/day, 50 g protein, 50-60 g carbohy-

drates, 7 g lipids and 15 g dietary fibres). Subjects were instructed not to alter life style habits, and not to perform physical activity in the last 48 h before the study days. One day before and one day after the dietary interventions blood samples were obtained from all subjects after a 10h overnight fast.

Laboratory analyses

Fasting plasma total cholesterol, triglycerides and glucose were measured on a Modular P800 analyser (Roche, Almere, The Netherlands) using commercially available enzymatic kits from Roche Molecular Biochemicals (total cholesterol, triglycerides) and Instruchemie, Delfzijl, The Netherlands (glucose). HDL-cholesterol levels were determined after precipitating the apoB-containing lipoproteins as described previously.[37] LDL-cholesterol was calculated according to Friedewald's formula.[38] Serum insulin levels were analysed using a commercially available chemiluminescence immunometric assay on an Immulite 2500 analyser (Siemens Healthcare Medical Diagnostics, Germany).

HDL function measurements

ApoB-containing lipoproteins were precipitated by adding 100 µL 36% polyethylene glycol (PEG 6000, Sigma, St. Louis, MO, USA) in 10 mM HEPES (pH = 8.0) to 200 µL plasma. Subsequently, samples were incubated for 30 min on ice and centrifuged for 30 min at 2200 g.[39] The HDL-containing supernatant was collected, kept on ice, and used within the same week for HDL function assays.

Cholesterol efflux capacity. To determine HDL-mediated cholesterol efflux, THP-1 human monocytes (ATCC via LGC Promochem, Teddington, UK) were differentiated into macrophages in the presence of 100 nM phorbol myristate acetate.[40] Differentiated THP-1 macrophages were then loaded with 50 µg/mL acetylated LDL and 1 µCi/mL [^{3}H]cholesterol (Perkin Elmer, Boston, MA, USA) for 24 h. Next, macrophages were equilibrated for 18 h in RPMI 1640 medium containing 2% bovine serum albumin.[40] Thereafter, 2% apoB-depleted plasma was added to the macrophages. After 5 h, effluxed radioactivity was determined by liquid scintillation counting (Packard 1600CA Tri-Carb, Packard, Meriden, CT, USA). Then the plates were washed two times with PBS and cells were lysed with 0.1 M NaOH (30 min incubation at room temperature). Subsequently, the radioactivity within the cells was counted. Efflux per well is expressed as the percentage of radioactivity released into the medium related to the total initial dose of radioactivity. To correct for nonspecific efflux, we subtracted values obtained from control cells without added apoB-depleted plasma.

Anti-oxidative capacity. The anti-oxidative properties of HDL were assessed by measuring the capacity of HDL from the respective groups to inhibit the oxidation of native LDL using a previously published method.[41] LDL was isolated from plasma of a fasted healthy male donor by density gradient ultracentrifugation ($1.019 < d < 1.063$

g/mL).[42] LDL (100 mg/dL cholesterol) was then oxidized with 5 mM 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) for 24 h at 37°C either in the presence of 2% apoB-depleted plasma or an equal volume of precipitation reagent in PBS as a control. Protein was precipitated with 10% trichloroacetic acid. Then, the accumulation of thiobarbituric acid reactive substances (TBARS) as a measure of oxidative modification was determined by incubating the samples for 10 min at 99°C and measuring the fluorescence at 485 nm excitation and 545 nm emission using 1,1,3,3-tetramethoxypropane as a standard as published previously.[42] The anti-oxidative capacity of HDL was expressed as the amount of TBARS accumulating in the samples relative to control LDL oxidized in the absence of HDL.

Anti-inflammatory capacity. Anti-inflammatory properties of HDL were assessed using human umbilical vein endothelial cells (HUVECs, provided by the Endothelial Cell Core Facility of the UMCG) isolated and cultured as described previously.[43] HUVECs were pre-incubated with 2% apoB-depleted plasma or an equal volume of precipitation reagent in PBS as a control for 1 h. Then, 10 ng/mL tumor necrosis factor α (TNF-α; R&D systems, Abingdon, UK) was added. After an additional incubation for 8 h, total RNA was isolated using Trizol (Invitrogen, Carlsbad, CA, USA) and quantified with a NanoDrop ND-100 UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). One µg of total RNA was reverse transcribed into cDNA (Invitrogen). Real-time quantitative PCR was performed on an ABI-Prism 7700 (Applied Biosystems) sequence detector.[44] Vascular cell adhesion molecule-1 (VCAM-1) mRNA expression was calculated relative to the expression of the housekeeping gene cyclophilin.

Statistical analysis

Data are presented as mean ± SEM when normally distributed or as median (IQR) when not normally distributed. A mixed effects model was applied to assess mean differences before and after the intervention within and between groups, and to determine differences in diet effect. Groups and intervention were modelled as fixed effects and the subject specific deviances from the group mean were modelled as random effects. Nonparametric tests (Wilcoxon signed-rank test within group, Mann-Whitney between groups) were performed when appropriate. Significance level was set at $p<0.05$. Statistical analyses were performed using SPSS for Windows version 20.0 (IBM, USA).

RESULTS

Clinical characteristics

Clinical characteristics are shown in **Table 1**. In all groups, HDL-cholesterol levels were comparable between South Asian and Caucasian subjects.

Table 1. Clinical and metabolic characteristics in South Asian and white Caucasian neonates, and in young adult healthy and adult overweight South Asian and white Caucasian men before and after a 5-day HFHCD and 8-day VLCD, respectively.

	Neonates		Group 18 – 25 years				Group 40 – 50 years			
	white Caucasians	South Asians	white Caucasians		South Asians		white Caucasians		South Asians	
			before HFHCD	after HFHCD	before HFHCD	after HFHCD	before VLCD	after VLCD	before VLCD	after VLCD
Clinical characteristics										
(Gestational) age (days or years)	277 ± 3	279 ± 2	22.1 ± 0.6		22.2 ± 0.7		44.3 ± 1.1		44.9 ± 0.9	
length (m)			1.84 ± 0.01		1.74 ± 0.02**		1.81 ± 0.02		1.75 ± 0.01**	
weight (kg)	3.47 ± 0.13	3.15 ± 0.12	75.1 ± 1.8	75.6 ± 1.8	63.2 ± 2.3**	63.7 ± 2.3$^{\dagger **}$	92.6 ± 2.5	88.2 ± 2.5††	86.7 ± 1.4	83.2 ± 1.6$^{\dagger\dagger \ddagger}$
body mass index (kg/m^2)			22.2 ± 0.6	22.4 ± 0.6	20.9 ± 0.6	21.0 ± 0.6†	28.1 ± 0.5	26.8 ± 0.5††	28.4 ± 0.4	27.3 ± 0.4††
waist circumference (cm)			81.3 ± 2.2	82.0 ± 2.3	78.9 ± 2.2	79.5 ± 2.6	103 ± 1.8	100 ± 1.6††	101 ± 1.6	98 ± 1.5††
Fasting plasma and serum levels										
glucose (mmol/L)	4.7 ± 0.4	4.7 ± 0.3	5.1 ± 0.1	5.2 ± 0.1	5.3 ± 0.1	5.5 ± 0.1$^{\dagger\dagger *}$	5.3 ± 0.2	4.5 ± 0.2††	5.3 ± 0.1	4.5 ± 0.1††
insulin (mU/L)	4.4 (3.9)	7.9 (6.7)*	4.8 (4.5)	7.0 (6.6)	7.1 (4.2)	10.6 (4.9)$^{\dagger\dagger **\ddagger\ddagger}$	12.8 (6.4)	5.5 (4.9)††	16.6 (8.1)*	6.5 (7.2)††
triglycerides (mmol/L)	0.29 (0.13)	0.29 (0.33)	0.79 (0.26)	0.75 (0.67)	0.98 (0.75)	1.08 (0.70)	1.29 (2.48)	0.89 (0.18)††	1.78 (2.91)	0.91 (0.25)††
total cholesterol (mmol/L)	1.48 ± 0.09	1.55 ± 0.09	3.75 ± 0.19	4.25 ± 0.22††	4.60 ± 0.30*	4.79 ± 0.24	5.56 ± 0.24	4.72 ± 0.33††	5.74 ± 0.28	5.13 ± 0.26††
HDL-cholesterol (mmol/L)	0.98 ± 0.04	0.94 ± 0.04	1.18 ± 0.05	1.31 ± 0.06†	1.26 ± 0.08	1.36 ± 0.07	1.09 ± 0.08	0.99 ± 0.06	1.00 ± 0.07	0.95 ± 0.05
LDL-cholesterol (mmol/L)	0.37 ± 0.07	0.43 ± 0.07	2.21 ± 0.21	2.55 ± 0.22††	2.83 ± 0.24	2.95 ± 0.20	3.54 ± 0.28	3.42 ± 0.37	3.58 ± 0.25	3.77 ± 0.24

Data are presented as mean ± SEM or median (IQR). HFHCD, 5-day high fat high calorie diet. VLCD, 8-day very low calorie diet. † $p<0.05$, †† $p<0.005$ within group *vs.* before diet. * $p<0.05$, ** $p<0.005$ *vs.* Caucasians. ‡ $p<0.05$, ‡‡ $p<0.005$ diet effect *vs.* Caucasians.

Neonates. Mean gestational age was 278 ± 2 days and did not differ between groups. Birth weight tended to be lower in South Asian neonates (p=0.078). Glucose levels were comparable between groups, but insulin levels were significantly higher in South Asian neonates (p=0.044). No significant differences were observed in lipid levels.

Young adults. Mean age was 22.1 ± 0.4 years. BMI did not differ between groups (p=0.11 (baseline)). Fasting glucose, insulin, triglyceride, HDL-cholesterol and phospholipid levels were comparable at baseline, while total cholesterol levels were significantly higher (p=0.021) and LDL-cholesterol tended (p=0.055) to be higher in South Asians. After the HFHCD, anthropometric parameters were unchanged. Fasting glucose and insulin levels were significantly increased only in South Asians. The HFHCD induced a significant increase in total cholesterol, HDL-cholesterol and LDL-cholesterol levels in Caucasians. In South Asians only HDL-cholesterol showed a tendency towards an increase (p=0.060). Phospholipids increased significantly to a similar degree in both groups (data not shown).

Adults. Mean age was 44.6 ± 0.8 years. BMI did not differ between groups (p=0.65 (baseline)). Fasting glucose and lipid levels were comparable between groups, whereas insulin levels were significantly higher in South Asians (p=0.002). After the VLCD, anthropometric parameters were significantly reduced in both groups. The mean reduction in body weight was 4.0±0.2 kg. Furthermore, caloric restriction induced a comparable, significant decrease in fasting plasma glucose, insulin, triglyceride, total cholesterol and phospholipid levels in both South Asians and Caucasians. HDL-cholesterol and LDL-cholesterol levels were not affected by the diet.

Of note, HDL-cholesterol levels were significantly lower in adult South Asians *vs.* young adult South Asians (p=0.027). Furthermore, HDL-cholesterol was significantly lower at neonatal age compared to young adult age for both South Asian (p=0.001 and p=0.040) and Caucasian (p=0.005 and p=0.017) subjects.

HDL functionality

The functionalities of HDL in Caucasians and South Asians with respect to inducing cholesterol efflux and anti-oxidative and anti-inflammatory activity are depicted in **Figure 1**.

Cholesterol efflux capacity is affected by dietary intervention. The ability of HDL to elicit cholesterol efflux from macrophages did not differ between ethnicities for all ages, both at baseline and after the dietary interventions. However, the HFHCD led to a significant increase in cholesterol efflux ability in Caucasian young adults (+11.8 ± 3.4%, p=0.002), while in South Asian young adults there was a tendency towards an increase (+6.3 ± 2.9%, p=0.073). On the other hand, the VLCD in the adult group resulted in a significantly lower cholesterol efflux capacity for both ethnicities (South Asians: -10.3 ± 2.4% (p<0.001) *vs.* Caucasians: -13.7 ± 1.9% (p<0.0001)). Cholesterol efflux capacity was comparable for all three age groups for both ethnicities.

Anti-oxidative capacity is impaired in adult South Asians. The ability of HDL to prevent oxidation of LDL was comparable between groups at neonatal and young adult age. However, this ability was markedly impaired in adult South Asian subjects compared to Caucasians (before VLCD: 18.1 ± 2.6 *vs.* 24.2 ± 2.2%, p=0.077; after VLCD: 16.4 ± 2.4 *vs.* 27.6 ± 2.7%, p=0.003). Anti-oxidative capacity was not affected by dietary intervention. Of note, anti-oxidative capacity was significantly lower in adult South Asians compared to young adult South Asians (p<0.005), while for Caucasian subjects anti-oxidative capacity was comparable between both age groups (p=0.160). Furthermore, anti-oxidative capacity was markedly lower at neonatal age compared to young adult and adult age for both South Asian (*vs.* young adults: p<0.001, *vs.* adults: p=0.048) and Caucasian (*vs.* young adults: p<0.001; *vs.* adults: p<0.001) subjects.

Anti-inflammatory capacity is impaired in South Asian neonates and is negatively affected by an 8-day VLCD in South Asian adults only. The anti-inflammatory capacity of HDL was significantly lower in South Asian compared to Caucasian neonates (22.9 ± 0.7 *vs.* 35.9 ± 1.9%, p<0.00001), a difference that was not present at young adult and adult age, at least at baseline. A 5-day HFHCD had no impact on anti-inflammatory capacity. Remarkably, though, anti-inflammatory capacity was negatively affected by an 8-day VLCD in adult South Asians only (-12.2 ± 4.3% (p=0.005) *vs.* +0.2 ± 4.2% (p=0.984)), resulting in a significantly lower anti-inflammatory capacity compared to adult Caucasians after the diet (23.0 ± 1.6% *vs.* 29.8 ± 2.3%, p=0.024). Anti-inflammatory capacity was significantly higher in Caucasian neonates compared to Caucasian adults (p=0.044). No other significant differences were observed for anti-inflammatory capacity at different ages.

Cholesterol efflux capacity positively correlates with HDL-cholesterol and phospholipid levels.

Linear regression analysis showed a clear positive correlation between cholesterol efflux capacity and HDL-cholesterol levels for all ages at baseline (neonates: R^2=0.451, β=0.672, p<0.0001; young adults: R^2=0.286, β=0.534, p=0.009; adults: R^2=0.201, β=0.448, p=0.032; **Figure 2**). The same was true for cholesterol efflux capacity and HDL-phospholipid levels (neonates: R^2=0.277, β=0.527, p=0.004; young adults: R^2=0.293, β=0.541, p=0.008; adults: R^2=0.176, β=0.419, p=0.047; data not shown). In addition, cholesterol efflux capacity was positively correlated with HDL-cholesterol after the diet (young adults: R^2=0.456, β=0.676, p=0.0004; adults: R^2=0.307, β=0.554, p=0.006) and for diet effect (delta HDL-cholesterol; young adults: R^2=0.395, β=0.629, p=0.001; adults: R^2=0.240, β=0.489, p=0.018). Of note, correlations were pooled for South Asians and Caucasians. Similar correlations were observed per group, although these did not always reach statistical significance, likely due to the limited number of subjects.

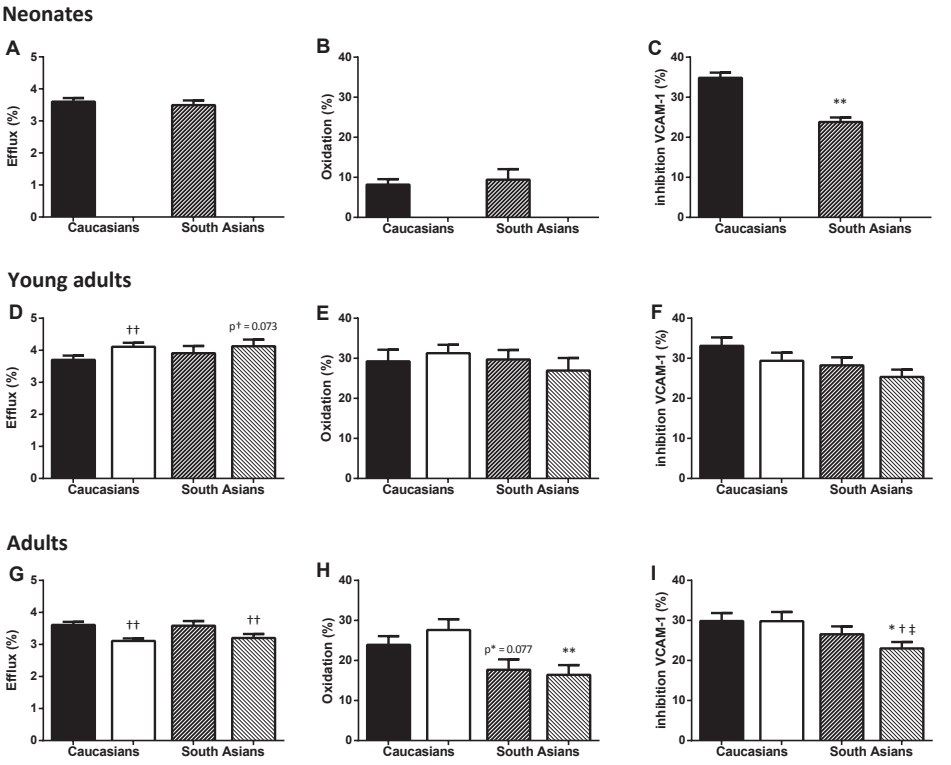


Figure 1. HDL functionality in South Asian (striped black) and white Caucasian (solid black) neonates, and in young adult healthy and adult overweight South Asian and white Caucasian men before (striped black and solid black, respectively) and after (striped white and solid white, respectively) a 5-day HFHCD and 8-day VLCD, respectively. HDL function was determined as A/D/G) inducing cholesterol efflux from cholesterol-laden THP-1 cells, B/E/H) protection against oxidation of LDL, and C/F/I) protection of HUVECs against TNFα-induced inflammation as determined by VCAM-1 expression. Assays were performed as detailed in methods. Data are presented as mean ± SEM. † p<0.05, †† p<0.005 within group *vs.* before diet. * p<0.05, ** p<0.005 *vs.* Caucasians. ‡ p<0.05 diet effect *vs.* Caucasians.

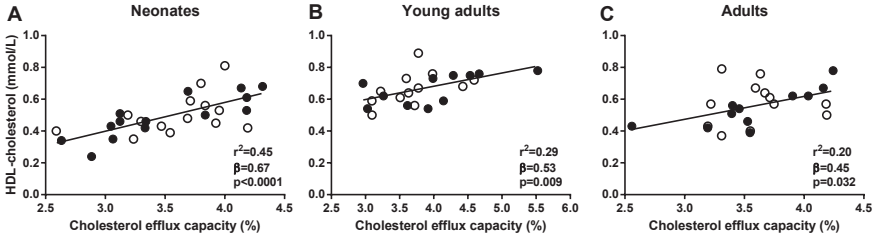


Figure 2. Correlations of cholesterol efflux capacity with HDL-cholesterol. Cholesterol efflux capacity in relation to HDL-cholesterol levels in (A) South Asian and white Caucasian neonates, and in (B) young adult healthy and (C) adult overweight South Asian and white Caucasian men, respectively. Correlations were determined by linear regression analysis. South Asian subjects are depicted in black circles and Caucasian subjects in white circles. HDL-cholesterol values in this figure are after precipitation.

Anti-oxidative and anti-inflammatory capacity did not correlate with HDL-cholesterol levels nor with HDL-phospholipid levels for all age groups (data not shown). As expected, HDL-cholesterol and HDL-phospholipid levels were positively correlated for all ages and all conditions (data not shown).

DISCUSSION

South Asians have an exceptionally high risk to develop cardiovascular disease compared to white Caucasians. A contributing factor might be dysfunctionality of HDL, which has been shown to be associated with cardiovascular disease in human studies. The current study showed that the ability of HDL to prevent oxidation of LDL was impaired in adult overweight South Asian males compared to matched white Caucasians. At younger ages, the anti-oxidative function of HDL was comparable between both ethnicities. In contrast, the anti-inflammatory capacity of HDL was markedly lower in South Asian neonates, a difference that was not present at young adult and adult age. However, short-term caloric restriction at adult age significantly impaired anti-inflammatory capacity in South Asians only. Finally, the ability of HDL to induce cholesterol efflux was similar between South Asians and Caucasians, albeit that in both ethnic groups cholesterol efflux was increased after a 5-day HFHCD and reduced after an 8-day VLCD.

HDL functionality between ethnic groups

Several recent studies have reported lower HDL-cholesterol levels in South Asians as compared to Caucasians.[29;31;32;36] Surprisingly, little is known about HDL functionality in South Asians. To date only one cross-sectional, uncontrolled pilot study assessed the anti-oxidative capacity of HDL in 28 South Asian immigrants in the USA.[45] They found dysfunctional HDL, as measured by a cell-free assay, in 50% of the participants, which was significantly correlated with carotid intima media thickness, a surrogate marker of atherosclerosis. However, no Caucasian control group was included, so no statements could be made on the ethnic implication of this percentage. In the present study, HDL-cholesterol levels were comparable between South Asians and Caucasians for all ages. For the neonates this was due to the fact that we matched the groups on HDL-cholesterol levels. For the young adults and adults, this might be due to lack of power to detect differences on HDL levels. However, despite equal HDL-cholesterol levels, we still observed several differences in HDL functionality between both ethnicities, suggesting that the HDL-cholesterol level was not a confounder in the current set-up. Recent evidence indeed suggests that HDL functionality might be affected independent of changes in plasma HDL-cholesterol levels and may be more important than plasma

HDL-cholesterol concentrations with respect to prediction of the cardioprotective effect of HDL.[20;21;24-26]

The oxidative modification of LDL is an important step in the initiation and progression of atherosclerosis as it results in enhanced uptake of LDL by macrophages and subsequent foam cell formation. HDL is able to prevent the oxidation of LDL and hence its subsequent atherogenic actions by various mechanisms.[19;46] In the present study, we observed that the ability of HDL to prevent oxidation of LDL was markedly lower in adult South Asian men compared to matched Caucasian men. Therefore, the reduced anti-oxidative capacity of HDL in South Asians may be involved in the increased risk of cardiovascular disease in this ethnic group. Interestingly, the anti-oxidative capacity of HDL was not impaired at young adult and neonatal age, suggesting that the ability of HDL to protect against LDL oxidation deteriorates with age. Indeed, for South Asians anti-oxidative capacity was significantly lower in adult compared to young adult subjects ($p=0.002$), while for Caucasians this capacity was comparable between both age groups ($p=0.160$). On the underlying mechanism behind this deterioration we can only speculate, but this might be due to exogenous factors such as insulin resistance and type 2 diabetes, which are also known to be considerably more prevalent in people of South Asian origin especially at higher age.[9;11] It has been shown that insulin resistance and type 2 diabetesare associated with a decrease in HDL-cholesterol levels, altered HDL composition and impaired HDL function.[47] In the current study plasma insulin levels were significantly higher in the South Asian adults, pointing to insulin resistance. Interestingly, Mulder *et al*.[39] showed that the anti-oxidative capacity of HDL from type 2 diabetes patients is inversely related to skin autofluorescence, a non-invasive marker of tissue advanced glycation end products, suggesting that impaired anti-oxidative capacity of HDL may contribute to tissue accumulation of advanced glycation end products and thereby to the development of long term diabetic complications. Thus, insulin resistance may affect the ability of HDL to prevent oxidation of LDL or, *vice versa*, HDL dysfunction may also be involved in the increased risk of type 2 diabetes and diabetes-related complications observed in South Asians.

HDL has several anti-inflammatory effects, such as the ability to inhibit cytokine-induced expression of adhesion molecules on endothelial cells, and to control the adaptive immune system.[46;48] Since suppression of immunity in neonates is important during pregnancy to prevent miscarriage, this may explain why we found higher anti-inflammatory capacity of HDL in neonates as compared to adults. Furthermore, the anti-inflammatory capacity of HDL was significantly lower in South Asian neonates (-36%). Interestingly, we previously found higher levels of the adhesion molecule E-selectin in cord blood of the same cohort of South Asian neonates as compared to Caucasians.[30] This is a marker of endothelial activation which may thus be the consequence of the lower anti-inflammatory capacity of HDL. We did not find a difference in anti-inflammatory

capacity of HDL at young adult and adult age. This could suggest that during development the lower anti-inflammatory function in South Asians recovers. However, a basis for atherosclerosis and the concomitant risk of cardiovascular disease is then probably already formed.

Finally, cholesterol efflux capacity did not differ between groups for all ages. The ability of HDL to stimulate cholesterol efflux involves transport of cholesterol from peripheral cells, particularly macrophage foam cells within atherosclerotic plaques, to the liver for final excretion into bile and feces, i.e. the reverse cholesterol transport pathway.[49] This ability of HDL therefore might reverse or prevent the formation of macrophage foam cells and is thus an important atheroprotective property, however, deterioration of this function is likely not involved in the high cardiovascular disease risk of the South Asian population.

Response of HDL functionality to short-term dietary intervention

HDL functionality was also assessed in response to short-term dietary intervention in order to investigate ethnic differences upon dietary stimuli. Previous studies have demonstrated that both HDL-cholesterol levels and HDL functionality can be influenced by dietary intervention.[27;28;50-52]

In the present study, a 5-day HFHCD increased HDL-cholesterol, whereas an 8-day VLCD did not affect HDL-cholesterol levels and decreased HDL-phospholipids. Cholesterol efflux ability was enhanced in response to a 5-day HFHCD, and reduced after an 8-day VLCD, which might be explained by the increase in HDL-cholesterol and phospholipid levels and decrease in phospholipids, respectively. Indeed, in both the young adult group and adult group cholesterol efflux capacity was positively correlated with HDL-cholesterol (**Figure 2**) and phospholipid levels.

Interestingly, anti-inflammatory capacity was significantly impaired after an 8-day VLCD only in South Asians. Hence, instead of being beneficial, caloric restriction appears to be detrimental to South Asians with respect to anti-inflammatory function of HDL. Possibly, this worsening of HDL anti-inflammatory capacity may only be present in the calorie-restricted state, returning to normal or even improving after weight loss and re-introduction of a normal diet. Indeed, our group previously assessed the short- and long-term effects of a 4-month VLCD on low-grade inflammation in obese patients with type 2 diabetes and demonstrated that the beneficial effects on chronic inflammation become apparent only when patients are on a eucaloric diet, suggesting that severe caloric restriction at first increases cytokine production by adipose tissue macrophages and that the beneficial effects of weight loss become apparent only in the eucaloric state.[53] Hence, perhaps the initial response to caloric restriction is worse in South Asians compared to Caucasians, but may normalize later on. This may, at least in part, be due to the fact that South Asians have higher release of pro-inflammatory cytokines, such as

interleukin-6 and tumour necrosis factor-α, by adipocytes, which may aggravate in case of caloric restriction.[54;55] Taken together, further research is needed to elucidate why South Asians respond differently compared to Caucasians to caloric restriction concerning HDL anti-inflammatory functionality.

This study is not without limitations. The sample sizes are relatively small, which might limit generalization potential. On the other hand, subjects were their own controls (with respect to diet intervention), which increases the power to detect relevant differences. Strengths of this study are the assessment of several HDL functions in South Asians, in comparison to Caucasians, at three different age categories, and in response to dietary intervention.

In conclusion, we showed that adult, overweight South Asians have impaired antioxidative capacity of HDL, which is not yet present at a young age and, therefore, possibly at least partly the consequence of environmental factors. Furthermore, anti-inflammatory capacity was reduced in South Asian neonates, and was significantly impaired in response to short-term caloric restriction in South Asian adults. These impairments in HDL functionality may contribute to the excess risk of cardiovascular disease, and possibly of type 2 diabetes in people of South Asian origin. Therefore, future studies should be directed at developing treatment strategies that improve HDL functionality, and at investigating whether these strategies will lower cardiovascular risk in South Asian subjects.

REFERENCES

1. Balarajan R. Ethnic differences in mortality from ischaemic heart disease and cerebrovascular disease in England and Wales. *BMJ* 1991;302(6776):560-4.
2. Chaturvedi N, Fuller JH. Ethnic differences in mortality from cardiovascular disease in the UK: do they persist in people with diabetes? *J Epidemiol Community Health* 1996;50(2):137-9.
3. Forouhi N, Sattar N, Tillin T, McKeigue P, Chaturvedi N. Do known risk factors explain the higher coronary heart disease mortality in South Asians compared with European men? Prospective follow-up of the Southall and Brent studies, UK. *Diabetologia* 2006;49(11):2580-8.
4. Wild SH, Fischbacher C, Brock A, Griffiths C, Bhopal R. Mortality from all causes and circulatory disease by country of birth in England and Wales 2001-2003. *J Public Health (Oxf)* 2007;29(2):191-8.
5. Anand SS, Yusuf S, Vuksan V, Devanesen S, Teo KK, Montague PA *et al*. Differences in risk factors, atherosclerosis and cardiovascular disease between ethnic groups in Canada: the study of health assessment and risk in ethnic groups (SHARE). *Lancet* 2000;356:279-84.
6. Balarajan R. Ethnic differences in mortality from ischaemic heart disease and cerebrovascular disease in England and Wales. *BMJ* 1991;302(6776):560-4.
7. Enas EA, Yusuf S, Mehta JL. Prevalence of coronary artery disease in Asian Indians. *Am J Cardiol* 1992;70(9):945-9.
8. Joshi P, Islam S, Pais P, Reddy S, Dorairaj P, Kazmi K *et al*. Risk factors for early myocardial infarction in South Asians compared with individuals in other countries. *JAMA* 2007;297(3):286-94.
9. Becker E, Boreham R, Chaudhury M, Craig R, Deverill C, Doyle M *et al*. Health Survey for England 2004. Volume 1. The health of minority ethnic groups. In: Sproston K, Mindell J, editors. Cardiovascular disease and diabetes. 1st ed. Leeds: The Information Centre; 2006. p. 63-94.
10. McKeigue PM, Ferrie JE, Pierpoint T, Marmot MG. Association of early-onset coronary heart disease in South Asian men with glucose intolerance and hyperinsulinemia. *Circulation* 1993;87(1):152-61.
11. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res Clin Pract* 2011;94(3):311-21.
12. Assmann G, Schulte H, von Eckardstein A, Huang Y. High-density lipoprotein cholesterol as a predictor of coronary heart disease risk. The PROCAM experience and pathophysiological implications for reverse cholesterol transport. *Atherosclerosis* 1996;124 Suppl:S11-S20.
13. Barter P, Gotto AM, LaRosa JC, Maroni J, Szarek M, Grundy SM *et al*. HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. *N Engl J Med* 2007;357(13):1301-10.
14. Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A *et al*. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA* 2009;302(18):1993-2000.
15. Sharrett AR, Ballantyne CM, Coady SA, Heiss G, Sorlie PD, Catellier D *et al*. Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: The Atherosclerosis Risk in Communities (ARIC) Study. *Circulation* 2001;104(10):1108-13.
16. Wilson PW, Abbott RD, Castelli WP. High density lipoprotein cholesterol and mortality. The Framingham Heart Study. *Arteriosclerosis* 1988;8(6):737-41.
17. Triolo M, Annema W, Dullaart RP, Tietge UJ. Assessing the functional properties of high-density lipoproteins: an emerging concept in cardiovascular research. *Biomark Med* 2013;7(3):457-72.

18. von Eckardstein A, Nofer JR, Assmann G. High density lipoproteins and arteriosclerosis. Role of cholesterol efflux and reverse cholesterol transport. *Arterioscler Thromb Vasc Biol* 2001;21(1):13-27.
19. von Eckardstein A, Hersberger M, Rohrer L. Current understanding of the metabolism and biological actions of HDL. *Curr Opin Clin Nutr Metab Care* 2005;8(2):147-52.
20. Corsetti JP, Gansevoort RT, Sparks CE, Dullaart RP. Inflammation reduces HDL protection against primary cardiac risk. *Eur J Clin Invest* 2010;40(6):483-9.
21. deGoma EM, deGoma RL, Rader DJ. Beyond high-density lipoprotein cholesterol levels evaluating high-density lipoprotein function as influenced by novel therapeutic approaches. *J Am Coll Cardiol* 2008;51(23):2199-211.
22. Sharma M. Combination therapy for dyslipidemia. *Curr Opin Cardiol* 2011;26(5):420-3.
23. Schwartz GG, Olsson AG, Abt M, Ballantyne CM, Barter PJ, Brumm J *et al*. Effects of dalcetrapib in patients with a recent acute coronary syndrome. *N Engl J Med* 2012;367(22):2089-99.
24. Besler C, Heinrich K, Rohrer L, Doerries C, Riwanto M, Shih DM *et al*. Mechanisms underlying adverse effects of HDL on eNOS-activating pathways in patients with coronary artery disease. *J Clin Invest* 2011;121(7):2693-708.
25. Dullaart RP, Annema W, Tio RA, Tietge UJ. The HDL anti-inflammatory function is impaired in myocardial infarction and may predict new cardiac events independent of HDL cholesterol. *Clin Chim Acta* 2014;433:34-8.
26. Riwanto M, Rohrer L, Roschitzki B, Besler C, Mocharla P, Mueller M *et al*. Altered activation of endothelial anti- and proapoptotic pathways by high-density lipoprotein from patients with coronary artery disease: role of high-density lipoprotein-proteome remodeling. *Circulation* 2013;127(8):891-904.
27. Roberts CK, Ng C, Hama S, Eliseo AJ, Barnard RJ. Effect of a short-term diet and exercise intervention on inflammatory/anti-inflammatory properties of HDL in overweight/obese men with cardiovascular risk factors. *J Appl Physiol* 2006;101(6):1727-32.
28. Wang Y, Snel M, Jonker JT, Hammer S, Lamb HJ, de Roos A *et al*. Prolonged caloric restriction in obese patients with type 2 diabetes mellitus decreases plasma CETP and increases apolipoprotein AI levels without improving the cholesterol efflux properties of HDL. *Diabetes Care* 2011;34(12):2576-80.
29. Ajjan R, Carter AM, Somani R, Kain K, Grant PJ. Ethnic differences in cardiovascular risk factors in healthy Caucasian and South Asian individuals with the metabolic syndrome. *J Thromb Haemost* 2007;5(4):754-60.
30. Boon MR, Karamali NS, de Groot CJ, van Steijn L, Kanhai HH, van der Bent C *et al*. E-selectin is elevated in cord blood of South Asian neonates compared with Caucasian neonates. *J Pediatr* 2012;160(5):844-8.
31. Chambers JC, Eda S, Bassett P, Karim Y, Thompson SG, Gallimore JR *et al*. C-reactive protein, insulin resistance, central obesity, and coronary heart disease risk in Indian Asians from the United Kingdom compared with European whites. *Circulation* 2001;104(2):145-50.
32. McKeigue PM, Marmot MG, Syndercombe Court YD, Cottier DE, Rahman S, Riemersma RA. Diabetes, hyperinsulinaemia, and coronary risk factors in Bangladeshis in east London. *Br Heart J* 1988;60(5):390-6.
33. Bhalodkar NC, Blum S, Rana T, Bhalodkar A, Kitchappa R, Kim KS *et al*. Comparison of levels of large and small high-density lipoprotein cholesterol in Asian Indian men compared with Caucasian men in the Framingham Offspring Study. *Am J Cardiol* 2004;94(12):1561-3.

34. Johansson J, Carlson LA, Landou C, Hamsten A. High density lipoproteins and coronary atherosclerosis. A strong inverse relation with the largest particles is confined to normotriglyceridemic patients. *Arterioscler Thromb* 1991;11(1):174-82.
35. Superko HR, Enas EA, Kotha P, Bhat NK, Garrett B. High-density lipoprotein subclass distribution in individuals of Asian Indian descent: the National Asian Indian Heart Disease Project. *Prev Cardiol* 2005;8(2):81-6.
36. Boon MR, Karamali NS, de Groot CJ, van Steijn L, Kanhai HH, van der Bent C *et al*. E-selectin is elevated in cord blood of South Asian neonates compared with Caucasian neonates. *J Pediatr* 2012;160(5):844-8.
37. van der Hoorn JW, de HW, Berbee JF, Havekes LM, Jukema JW, Rensen PC *et al*. Niacin increases HDL by reducing hepatic expression and plasma levels of cholesteryl ester transfer protein in APOE*3Leiden.CETP mice. *Arterioscler Thromb Vasc Biol* 2008;28(11):2016-22.
38. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18(6):499-502.
39. Mulder DJ, de Boer JF, Graaff R, de Vries R, Annema W, Lefrandt JD *et al*. Skin autofluorescence is inversely related to HDL anti-oxidative capacity in type 2 diabetes mellitus. *Atherosclerosis* 2011;218(1):102-6.
40. Annema W, Nijstad N, Tolle M, de Boer JF, Buijs RV, Heeringa P *et al*. Myeloperoxidase and serum amyloid A contribute to impaired in vivo reverse cholesterol transport during the acute phase response but not group IIA secretory phospholipase A(2). *J Lipid Res* 2010;51(4):743-54.
41. Kappelle PJ, de Boer JF, Perton FG, Annema W, de Vries R, Dullaart RP *et al*. Increased LCAT activity and hyperglycaemia decrease the antioxidative functionality of HDL. *Eur J Clin Invest* 2012;42(5):487-95.
42. Tietge UJ, Pratico D, Ding T, Funk CD, Hildebrand RB, Van Berkel T *et al*. Macrophage-specific expression of group IIA sPLA2 results in accelerated atherogenesis by increasing oxidative stress. *J Lipid Res* 2005;46(8):1604-14.
43. Nijstad N, de Boer JF, Lagor WR, Toelle M, Usher D, Annema W *et al*. Overexpression of apolipoprotein O does not impact on plasma HDL levels or functionality in human apolipoprotein A-I transgenic mice. *Biochim Biophys Acta* 2011;1811(4):294-9.
44. Tietge UJ, Nijstad N, Havinga R, Baller JF, van der Sluijs FH, Bloks VW *et al*. Secretory phospholipase A2 increases SR-BI-mediated selective uptake from HDL but not biliary cholesterol secretion. *J Lipid Res* 2008;49(3):563-71.
45. Dodani S, Kaur R, Reddy S, Reed GL, Navab M, George V. Can dysfunctional HDL explain high coronary artery disease risk in South Asians? *Int J Cardiol* 2008;129(1):125-32.
46. HB G, Rao VS, Kakkar VV. Friend Turns Foe: Transformation of Anti-Inflammatory HDL to Proinflammatory HDL during Acute-Phase Response. *Cholesterol* 2011;2011:274629.
47. Rohrer L, Hersberger M, von Eckardstein A. High density lipoproteins in the intersection of diabetes mellitus, inflammation and cardiovascular disease. *Curr Opin Lipidol* 2004;15(3):269-78.
48. Annema W, von Eckardstein A. High-density lipoproteins. Multifunctional but vulnerable protections from atherosclerosis. *Circ J* 2013;77(10):2432-48.
49. Annema W, Tietge UJ. Regulation of reverse cholesterol transport - a comprehensive appraisal of available animal studies. *Nutr Metab (Lond)* 2012;9(1):25.
50. Guay V, Lamarche B, Charest A, Tremblay AJ, Couture P. Effect of short-term low- and high-fat diets on low-density lipoprotein particle size in normolipidemic subjects. *Metabolism* 2012;61(1):76-83.

51. Tremblay AJ, Lamarche B, Guay V, Charest A, Lemelin V, Couture P. Short-term, high-fat diet increases the expression of key intestinal genes involved in lipoprotein metabolism in healthy men. *Am J Clin Nutr* 2013;98(1):32-41.
52. Wadden D, Cahill F, Amini P, Randell E, Vasdev S, Yi Y *et al*. Serum acylated ghrelin concentrations in response to short-term overfeeding in normal weight, overweight, and obese men. *PLoS One* 2012;7(9):e45748.
53. Snel M, van Diepen JA, Stijnen T, Pijl H, Romijn JA, Meinders AE *et al*. Immediate and long-term effects of addition of exercise to a 16-week very low calorie diet on low-grade inflammation in obese, insulin-dependent type 2 diabetic patients. *Food Chem Toxicol* 2011;49(12):3104-11.
54. Peters MJ, Ghouri N, McKeigue P, Forouhi NG, Sattar N. Circulating IL-6 concentrations and associated anthropometric and metabolic parameters in South Asian men and women in comparison to European whites. *Cytokine* 2013;61(1):29-32.
55. Petersen KF, Dufour S, Feng J, Befroy D, Dziura J, Dalla MC *et al*. Increased prevalence of insulin resistance and nonalcoholic fatty liver disease in Asian-Indian men. *Proc Natl Acad Sci U S A* 2006;103(48):18273-7.

12 Summary and conclusions

The risk of developing type 2 diabetes and cardiovascular disease is exceptionally high among both native and migrant South Asians, comprising one fifth of the total world's population and consequently posing a major health and socioeconomic burden worldwide. The underlying cause of this excess risk is still poorly understood. This thesis aimed to gain more insight in the pathogenesis of type 2 diabetes and cardiovascular disease in people of South Asian descent, and to provide new leads for preventive strategies and treatment options.

PART 1: TYPE 2 DIABETES MELLITUS

In **PART 1** of this thesis we focused on the pathogenesis of type 2 diabetes in South Asians.

In **Chapter 2** we reviewed potential pathophysiological mechanisms responsible for the increased risk of type 2 diabetes in South Asians compared to white Caucasians. The predominant mechanism in this ethnic group seems to be insulin resistance rather than impaired insulin secretion, given the consistently found higher insulin levels in South Asians compared to other ethnic groups regardless of age, gender or BMI. We described several possible mechanisms that may underlie or contribute to this increased prevalence of insulin resistance. A gene-environment interaction seems most likely: South Asians seem to have a high genetic susceptibility and enhanced interaction with environmental triggers such as a high fat diet and low levels of physical activity. They have a remarkable disadvantageous metabolic phenotype. South Asians are born relatively small, and already at birth they have high insulin levels and exhibit a thin-fat-phenotype, which remains throughout life. They develop type 2 diabetes at lower ranges of BMI compared to white Caucasians. Furthermore, it has been shown that South Asians have dysfunctional adipose tissue and are in a continuous state of low grade inflammation. Additionally, South Asians seem to have higher hepatic and intramyocellular lipid content, but have less skeletal muscle mass and seem to have lower cardiorespiratory fitness and reduced capacity for fat oxidation during submaximal exercise. Remarkably, as of yet no convincing differences in intracellular signalling cascades and enzymatic process involved in insulin signalling have been found between South Asians and white Caucasians. However, so far only two studies obtained muscle biopsies and investigated mitochondrial function, and only one investigated the insulin signalling pathway. Finally, endothelial and HDL dysfunction have been observed in several studies in South Asians, possibly leading to a decreased NO bioavailability and affecting substrate delivery to skeletal muscle. Hence, so far, an overall biological explanation for their unfavourable phenotype remains to be elucidated. We proposed several other areas of interest that should be explored in South Asians to further investigate this phenotype.

Chapter 3 describes a study investigating the high insulin levels in response to an oral glucose load consistently found in South Asians compared to white Caucasians. These higher insulin levels are considered a compensatory mechanism to overcome insulin resistance and maintain normal glucose tolerance, and might either be caused by a decreased insulin clearance, or an increased β-cell response. Therefore, we investigated if this increased insulin response is due to an increased response of GLP-1, an incretin secreted from the gut in response to eating that stimulates insulin secretion. In addition, we were interested whether this increased insulin response causes reactive hypoglycemia, which is characterized by a drop in glucose level 4-6 hours after a glucose load, and is considered a sign of latent diabetes.[1-3] For this purpose, eight young, healthy South Asian men and ten white Caucasian men were subjected to a prolonged 6-hour 75-g OGTT. This study confirms that young healthy South Asian men are more insulin resistant and have higher insulin levels during an OGTT than white Caucasian men. The high insulin levels were accompanied by increased levels of GLP-1, as reflected by an increased AUC for GLP-1. Since the incretin effect and the direct insulinotropic action of GLP-1 were not assessed in this study, it remains to be elucidated whether this is a compensatory response to facilitate hyperinsulinemia to overcome insulin resistance or reflects a GLP-1 resistant state. The finding that the peak GLP-1 levels preceded the peak insulin response and paralleled the increased β-cell activity suggests, however, a direct relation between the increased GLP-1 response and the insulin secretion by the β-cell. Finally, although insulin levels were higher in South Asians during the whole test, this did not lead to reactive hypoglycemia.

Given the high susceptibility of South Asians to develop type 2 diabetes despite a similar environmental pressure when compared to other ethnicities, a possible explanation for this excess risk might be related to differences in the regulation of energy/nutrient-sensing pathways in metabolic tissues thereby affecting whole-body substrate homeostasis. In **Chapters 4** and **5** we investigated these pathways in young adult and adult subjects, respectively, with a special focus on canonical insulin signalling and mTORC1 pathways. All subjects underwent a 2-step hyperinsulinemic-euglycemic clamp with skeletal muscle biopsies and indirect calorimetry before and after a short-term dietary intervention. In addition, HTG and abdominal fat distribution were assessed using MRI/S.

In **Chapter 4** we compared the metabolic adaptation to a 5-day HFHCD in 12 young healthy South Asian and 12 white Caucasian men. Metabolic clearance rate of insulin and hepatic insulin sensitivity were reduced in South Asians compared to Caucasian subjects both before and after the diet. Strikingly, a 5-day HFHCD was already sufficient to impair insulin-stimulated glucose disposal in South Asians, while such an effect was not observed in Caucasians. The impairment in glucose disposal was primarily due to a decrease in NOGD, suggesting a defect in glycogen storage. However, no obvious

differences were found in expression of proteins and genes involved in glycolysis and glycogen synthesis between groups. At the skeletal muscle level no significant differences were found between groups in mTOR-signalling, nor in insulin signalling, metabolic gene expression and mitochondrial respiratory-chain content, that could explain the diet-induced impairment in insulin-stimulated glucose disposal in South Asians. Furthermore, no differences in HTG and abdominal fat were detected. The fact that we did not find obvious differences between groups might be explained by the relatively good health of our subjects and/or the small sample size. Finally, we cannot exclude the possibility that white adipose tissue might have contributed to the diet-induced impairment in insulin-stimulated glucose disposal in South Asians. About 10-20% of whole-body glucose uptake occurs in white adipose tissue, which corresponds to the observed reduction in glucose disposal in South Asians. In conclusion, HFHC-feeding rapidly induced insulin resistance only in healthy, young, lean South Asian subjects, suggesting that the propensity of South Asians to develop type 2 diabetes may be partly explained by the way they adapt to high fat western food. The mTOR-pathway does not seem to be involved, at least in skeletal muscle.

In **Chapter 5** we assessed the effect of caloric restriction through an 8-day VLCD on skeletal muscle energy/nutrient-sensing pathways in 12 middle-aged overweight South Asian and 12 white Caucasian men. At baseline, South Asians were more insulin resistant compared to Caucasians, as indicated by higher insulin levels (both fasting and during OGTT), and lower hepatic and peripheral insulin sensitivity. In addition, metabolic clearance rate of insulin was lower and hepatic triglyceride content was higher in South Asian subjects. Deposition of fat in the liver is associated with hepatic insulin resistance.[4] The impairment in peripheral insulin sensitivity in South Asians appeared to be due to a reduced rate of NOGD, suggesting a defect in glycogen storage, one of the main defects observed in patients with type 2 diabetes.[5] However, no between-group differences were found in expression of proteins and genes involved in glycolysis and glycogen synthesis, in line with our findings in the young adult group. In addition, no differences were observed before the diet in skeletal muscle insulin and mTOR signalling. Substrate oxidation rates and metabolic flexibility were comparable between groups. Intriguingly, South Asian subjects exhibited a different metabolic adaptation to an 8-day VLCD. In both groups, HTG and abdominal fat distribution were reduced, and hepatic insulin sensitivity was improved in response to the diet, as expected from previous studies.[6-9] However, whereas Caucasian subjects switched from carbohydrate to lipid oxidation in fasted condition and showed an improved insulin effect on substrate oxidation rates, indicating they were metabolically flexible, the shift in whole-body substrate oxidation rates in South Asians was impaired after the diet, both in fasted condition and during hyperinsulinemia, reflecting metabolic inflexibility. Furthermore, in Caucasians peripheral insulin sensitivity was not affected by the diet, in line with other short-term caloric

restriction studies leading to minimal weight loss,[6-8] whereas in South Asians peripheral insulin sensitivity was slightly improved, primarily due to enhanced NOGD, despite lowered insulin levels. Interestingly, skeletal muscle energy/nutrient-sensing pathways were differentially affected, notably with an increase in insulin-induced activation of the ERK-mTOR-S6K1 axis in South Asians. Growing evidence suggests that mTORC1 can suppress fatty acid β-oxidation by inhibiting PPARα and the transcriptional regulation of its target genes.[10-14] Intriguingly, mRNA-expression of PPARA was significantly decreased only in South Asian subjects. Hence, the hyperactive mTOR-pathway in South Asians in response to short-term caloric restriction may have repressed fatty acid β-oxidation by inhibiting PPARα, resulting in impaired metabolic flexibility. mTORC1 is also known to have negative effects on insulin sensitivity. Glucose disposal rate, however, improved in South Asians, apparently primarily accounted for by increased NOGD, although no differences in glycogen metabolism were observed between groups. Interestingly, AMPK expression was significantly increased in South Asians, but not in Caucasians. AMPK activation promotes skeletal muscle glucose uptake and may underlie the improved NOGD in South Asians after caloric restriction, which might explain the improved glucose disposal rate in South Asian subjects. In conclusion, middle-aged overweight South Asian men exhibited a different metabolic adaptation to short-term caloric restriction compared to age- and BMI-matched white Caucasians. Although glucose disposal rate was improved in South Asians in contrast to Caucasians, metabolic flexibility was impaired after an 8-day VLCD, which was accompanied by an increase in insulin-induced activation of the skeletal muscle ERK-mTOR-S6K1 axis.

Recently, brown adipose tissue (BAT) has emerged as a novel player in energy metabolism in humans. In **Chapter 6** we gave an overview of the anatomy, physiology and function of BAT and described how BAT could be manipulated in order to increase energy expenditure and possibly induce weight loss. In contrast to white adipose tissue, BAT takes up glucose and triglyceride-derived fatty acids from the plasma and subsequently burns fatty acids to generate heat through a process called mitochondrial uncoupling.[15] Interestingly, BAT volume and activity, as assessed after exposure to cold by ^{18}F-FDG PET-CT-scans, are inversely related to BMI and percentage of body fat in adult humans, indicating an inverse relationship between BAT and obesity.[16-18] Besides a clear role for BAT in triglyceride metabolism[19] BAT is also thought to contribute to glucose homeostasis, particularly in resting conditions when glucose utilization by skeletal muscle is minimal.[20] Importantly, BAT appears to contribute to NST[18;21] and it has been estimated that fully activated BAT in humans can contribute up to 15-20% of total energy expenditure.[15] Additionally, several pathological conditions that lead to activation of BAT, such as hyperthyroidism and pheochromocytoma, result in increased energy expenditure and in weight loss. Hence, increasing the activity of BAT is considered a promising method

to increase energy expenditure and subsequently induce weight loss. Various ways in which BAT can be manipulated have been identified, e.g. exposure to cold, the use of so-called uncoupling agents or the administration of the hormone irisin.

Since BAT is involved in total energy expenditure and clearance of serum triglycerides and glucose thereby protecting against metabolic disturbances, we hypothesized that a low BAT volume or activity might underlie the disadvantageous metabolic phenotype and susceptibility for type 2 diabetes in South Asians. Therefore, in **Chapter 7**, we investigated REE as well as BAT volume and activity in 12 young healthy lean South Asian men and 12 white Caucasians, using ventilated hoods and cold-induced ^{18}F-FDG-PET-CT-scans. We demonstrated that thermoneutral REE was -32% lower in South Asian subjects compared to Caucasians. In addition, temperature at which shivering started was higher despite a higher total percentage of fat mass, and cold-induced NST was smaller in South Asians. Strikingly, the detectable volume of metabolically active BAT was markedly lower in South Asians (-34%). The fact that this is found already in healthy young adults without differences in the degree of ^{18}F-FDG uptake, as evidenced by equal SUV_{max} and SUV_{mean}, could point to a defect in BAT differentiation. The underlying cause of the lower BAT volume in South Asians may be genetic (i.e. blunted expression of signalling molecules involved in BAT differentiation, e.g. NO, environmental (i.e. clothing behaviour, central heating setting and/or eating pattern), or a combination of the two. These findings suggest that a low BAT volume may underlie the high susceptibility to develop metabolic disturbances, such as obesity and type 2 diabetes, in South Asians. Hence, increasing the volume or activity of BAT might be of great therapeutic potential in this ethnic group, possibly resulting in increased clearance of glucose and fatty acids and increased total energy expenditure.

PART 2: CARDIOVASCULAR DISEASE

In **PART 2** of this thesis we focused on the pathogenesis of cardiovascular disease in South Asians.

In **Chapter 8**, we reviewed potential factors contributing to the increased cardiovascular risk of South Asians and discussed novel therapeutic strategies based on recent insights. The major cause of cardiovascular disease is atherosclerosis, which is present many years before any clinical symptoms of cardiovascular disease become manifest. The development of atherosclerosis may be promoted by metabolic as well as inflammatory risk factors. Metabolic or 'classical' risk factors include dyslipidemia, central obesity and insulin resistance. In addition, although the precise mechanism is still under debate, inflammatory or 'non-classical' risk factors may contribute to development of cardiovascular disease. Among these are systemic inflammation, as well as

HDL dysfunction and endothelial dysfunction which can both give rise to inflammation. In South Asians, classical risk factors associated with cardiovascular disease are highly prevalent. A contributing factor that may underlie the development of this disadvantageous metabolic phenotype is the presence of a lower amount of BAT volume in South Asians, resulting in lower lipid oxidation and glucose uptake. These classical risk factors, however, cannot fully explain the increased South Asian risk for cardiovascular disease. Therefore, other non-classical risk factors must underlie this residual risk. Indeed, the prevalence of inflammatory risk factors including visceral adipose tissue inflammation, endothelial dysfunction, and HDL dysfunction, is higher in South Asians compared to white Caucasians. We concluded that a potential novel therapy to lower cardiovascular disease risk in the South Asian population is to enhance BAT volume or its activity in order to diminish classical risk factors. Furthermore, anti-inflammatory therapy may lower non-classical risk factors in this population and the combination of both strategies may be especially effective.

Chapter 9 aimed to assess whether cardiac dimensions, cardiovascular function and myocardial triglyceride content differ between young, healthy South Asian and white Caucasian men, possibly contributing to the increased cardiovascular disease risk in South Asians. In addition, since insulin resistance and type 2 diabetes are highly prevalent in South Asians[22] and the mortality risk of cardiovascular disease associated with type 2 diabetes is higher in South Asians compared to white Caucasians,[23;24] we hypothesized that the excess cardiac risk in South Asians might be due to a higher cardiac susceptibility to metabolic disorders. Therefore, we assessed cardiac dimensions and cardiovascular function using a 1.5T-MRI/S-scanner in 12 young, healthy male South Asians and 12 white Caucasians, and subjected them to a 5-day HFHCD to study cardiac response to metabolic stress. At baseline, South Asians were more insulin resistant and had higher LDL-cholesterol levels. Cardiac dimensions were smaller in South Asians, as indicated by lower left ventricular mass and end-diastolic volume, indexed for body surface area. Furthermore, differences in diastolic and systolic cardiac function profiles were observed. Parameters of diastolic function, E acceleration and deceleration peak flows, were lower in South Asians, suggesting prolonged cardiac relaxation compared to white Caucasians. In addition, measures of systolic cardiac function, aortic acceleration and deceleration peak flows, were lower in South Asians, indicating prolonged cardiac contraction as well. A 5-day HFHCD did not increase these differences, despite a significant increase in both insulin levels and HOMA-B% only in South Asians, indicating they became even more insulin resistant. Finally, aortic pulse wave velocity, a powerful independent predictor of cardiovascular events,[25] was higher in South Asians at baseline, indicating increased arterial stiffness, which normalized after the diet. Hence, young, healthy South Asians have smaller cardiac dimensions, even when corrected for their

smaller stature, and a different cardiovascular function profile than white Caucasians. Reduced insulin sensitivity and increased LDL-cholesterol might be causally related to the different cardiac function profiles in South Asians.[26;27] Another possibility is that the observed differences in cardiac dimensions and cardiovascular function are innate and are simply representative of differing normal reference values in these two ethnic groups. Whether the observed differences contribute to the higher incidence of cardiovascular disease in South Asians remains to be determined. They cannot be explained by a different metabolic response to short-term dietary fat consumption, as a 5-day HFHCD did not increase the observed differences, despite distinct metabolic effects. It is possible, however, that a longer HF-diet is needed to induce changes.

In **Chapter 10** we assessed whether metabolic and functional cardiovascular flexibility to caloric restriction differs between middle-aged, overweight South Asian and white Caucasian men. Mortality risk of cardiovascular disease associated with type 2 diabetes is higher in South Asians compared to Caucasians,[23] suggesting they have a higher cardiac susceptibility to metabolic disorders. Short-term caloric restriction can be used as a metabolic stress test to study cardiac flexibility. Previous studies in healthy subjects and obese patients with type 2 diabetes with and without cardiovascular disease of white Caucasian descent demonstrated similar metabolic and functional flexibility of the heart in response to both short- and long-term caloric restriction.[9;28-30] It is unknown, however, if caloric restriction has comparable effects in South Asians. Therefore, we assessed cardiovascular function and myocardial triglycerides using a 1.5T-MRI/S-scanner in 12 middle-aged overweight South Asian men and 12 white Caucasians before and after an 8-day VLCD. At baseline, South Asians were more insulin resistant than Caucasians as indicated by higher insulin levels both in fasted condition and during OGTT. Cardiac dimensions were smaller, despite correction for body surface area, and PWV in the distal aorta was higher in South Asians, similar to our findings in the young adult group. The higher PWV in South Asians might be attributed to the higher insulin levels observed in this group. Long-term increased insulin levels, as observed in insulin resistance and type 2 diabetes, are known to compromise aortic elastic function.[31] Systolic and diastolic function, myocardial triglycerides and pericardial fat did not differ significantly between groups. After the VLCD, myocardial triglycerides increased in both ethnicities with 69±18%. Although increased myocardial triglyceride content in insulin resistance is associated with impaired myocardial function,[32-34] the increase in myocardial triglycerides observed after a short-term VLCD is a sign of preserved metabolic flexibility of the heart.[9;28] Given the high risk on cardiovascular disease and type 2 diabetes in South Asians, we hypothesized that the flexibility of the heart to adjust myocardial triglyceride content in response to caloric restriction would be diminished in South Asians. Surprisingly, however, an 8-day VLCD increased myocardial triglycerides similarly in both groups, suggesting a similar physiological flexibility of myocardial lipid

metabolism in both ethnicities. Diastolic cardiac function decreased after the diet in both South Asians and Caucasians, as expected from previous studies,[9;28;30] and can probably be explained by changes in elastic properties of the LV. However, pericardial fat decreased significantly in Caucasians only in response to the dietary intervention, mainly due to a reduction in the paracardial fat layer. Since the paracardial fat layer has been found to be a predictor of cardiovascular disease, the decrease in this specific fat compartment in Caucasians probably conveys less cardiovascular risk.[35] Furthermore, PWV in the proximal and total aorta was reduced after the VLCD in Caucasians only, suggesting that the large arteries are less flexible in South Asians in response to caloric restriction. This might be due to the, probably long-term existing, higher insulin levels observed in South Asians which may have induced irreversible changes in the arterial wall. Hence, myocardial triglyceride stores and diastolic function in middle-aged overweight and insulin resistant South Asians are as flexible and amenable to therapeutic intervention by caloric restriction as age-, sex- and BMI-matched but less insulin resistant white Caucasians This suggests that caloric restriction as a preventive and/or therapeutic strategy against cardiovascular disease is as valuable in South Asians as in white Caucasians. However, paracardial fat volume and PWV showed a differential effect in response to an 8-day VLCD in favour of Caucasians.

Finally, in **Chapter 11** we compared HDL function in neonates, young adults and adults of South Asian and white Caucasian origin. Dysfunction of HDL has been is associated with cardiovascular disease.[36-41] The cardiovascular protective effects of HDL have been attributed to several anti-atherogenic properties, including prevention of LDL oxidation, anti-inflammatory properties, stimulation of cholesterol efflux from foam cells, and inducing vasodilation by induction of NO release.[42-46] Interestingly, multiple studies have repeatedly found lower HDL-cholesterol levels in South Asians compared to Caucasians.[47-54] Hence, a possible contributing factor to the excess high risk of cardiovascular disease in South Asians might be low levels and/or dysfunction of HDL. Therefore, this study determined HDL functionality with respect to cholesterol efflux, anti-oxidation and anti-inflammation *in vitro* using fasting plasma samples from South Asian and white Caucasian neonates (n=14 each), young adult healthy men (n=12 each, 18-25y), and adult overweight men (n=12 each, 40-50y). Furthermore, since HDL function can be influenced by dietary intervention,[55;56] we assessed the effect of short-term dietary intervention on HDL function: young adults were subjected to a 5-day HFHCD and adults to an 8-day VLCD. This study showed that the ability of HDL to prevent oxidation of LDL was impaired in adult overweight South Asian men compared to white Caucasians. At younger ages, the anti-oxidative function of HDL was still comparable between both ethnicities. The underlying mechanism behind this deterioration might be due to exogenous factors such as insulin resistance and type 2 diabetes, highly present in people

of South Asian origin, especially at higher age. Indeed, it has been shown that insulin resistance and type 2 diabetes are associated with a decrease in HDL-cholesterol levels, altered HDL composition and impaired HDL function.[44] In the current study plasma insulin levels were significantly higher in the South Asian adults, pointing to insulin resistance. Interestingly, the anti-oxidative capacity of HDL from diabetic patients is inversely related to skin autofluorescence, a non-invasive marker of tissue AGEs, suggesting that impaired anti-oxidative capacity of HDL may contribute to tissue accumulation of AGEs and thereby to the development of long term diabetic complications.[57] Thus, insulin resistance may affect the ability of HDL to prevent oxidation of LDL or, *vice versa*, HDL dysfunction may also be involved in the increased risk of type 2 diabetes and diabetes-related complication in South Asians. In contrast, the anti-inflammatory capacity of HDL was markedly lower in South Asian neonates, a difference that was not present at young adult and adult age, suggesting that during development the lower anti-inflammatory function in South Asians recovers. However, a basis for atherosclerosis and the concomitant risk of cardiovascular disease is then probably already formed. Furthermore, short-term caloric restriction at adult age significantly impaired anti-inflammatory capacity in South Asians only. Hence, instead of being beneficial, caloric restriction appears to be detrimental to South Asians with respect to anti-inflammatory function of HDL. Possibly, this worsening of HDL anti-inflammatory capacity may only be present in the calorie-restricted state, returning to normal or even improving after weight loss and re-introduction of a normal diet. This may, at least in part, be due to the fact that South Asians have higher release of pro-inflammatory cytokines by adipocytes, which may aggravate in case of caloric restriction.[58;59] Finally, the ability of HDL to induce cholesterol efflux was similar between South Asians and Caucasians. In both ethnic groups cholesterol efflux was increased after a 5-day HFHCD and reduced after an 8-day VLCD. In conclusion, we showed that anti-inflammatory capacity of HDL was reduced in South Asian neonates, and was significantly impaired in response to short-term caloric restriction in South Asian adults. Furthermore, adult overweight South Asians had impaired anti-oxidative capacity of HDL, which was not yet present at a young age and, therefore, likely the consequence of exogenous factors. These impairments in HDL functionality may contribute to the excess risk of cardiovascular disease, and possibly of type 2 diabetes, in people of South Asian origin.

CONCLUDING REMARKS AND FUTURE RESEARCH

The ethnic disparity in diabetic and cardiovascular risk between South Asians and white Caucasians is most likely due to different gene frequencies or expression as well as diverse programming influences (either genetic or programmed by an adverse intra-

uterine environment). This has led to the disadvantageous metabolic South Asian phenotype, which has evolved over generations promoting selective survival in response to certain environmental challenges – such as recurring famine-induced starvation, adverse climate conditions, and varying burdens of infectious diseases – but is now out of step with modern lifestyle and longer life expectancy. In addition, differences in demographic profiles and environmental factors secondary to urbanization will have contributed to the high susceptibility of South Asian people to develop type 2 diabetes and cardiovascular diseases.[60] This thesis aimed to gain more insight in the pathogenesis of these diseases in South Asians. I propose that these various influences have affected not one, but multiple important metabolic mechanisms. Below I will discuss several topics, some of which have been studied in this thesis and some of which still need to be investigated, and suggest ideas for future research.

Insulin signalling, mitochondrial function and GLP-1

Insulin signalling and mitochondrial function are involved in glucose metabolism. Skeletal muscle accounts for the major part of insulin-stimulated glucose disposal.[61] NOGD or glycogen synthesis and oxidative glucose metabolism through glycolysis are the major pathways for glucose disposal.[5;62;63] Furthermore, GLP-1, secreted in the gut in response to eating, is known to have several beneficial effects on glucose regulation.

Compared with control subjects (white Caucasians) a 5-day HFHCD rapidly impaired insulin-stimulated glucose disposal in young, healthy South Asians. Furthermore, in adult overweight South Asians, peripheral insulin sensitivity was lower at baseline. In both age groups, this impairment in glucose disposal was primarily due to reduced NOGD, suggesting a defect in glycogen storage. However, no differences were found in skeletal muscle expression of proteins and genes involved in glycolysis and glycogen synthesis between controls and South Asians. In addition, no differences were observed in skeletal muscle insulin signalling, metabolic gene expression or mitochondrial respiratory-chain content. Another finding is that the higher insulin levels during an OGTT in South Asians were accompanied by increased levels of GLP-1, probably as an adaptive response to facilitate hyperinsulinemia to overcome insulin resistance.

Future research. The role of glycogen metabolism should be further explored by determining skeletal muscle glycogen content and dynamics. The findings on mitochondrial function should be verified via measuring other, more sophisticated mitochondrial markers, such as ex vivo determination of activities of mitochondrial respiratory-chain complexes and citrate synthase activity. Furthermore, we cannot exclude the possibility that differences in white adipose tissue function might have contributed to our findings; hence, the role of white adipose tissue in the pathogenesis of type 2 diabetes in South Asians should be assessed, especially since it has been shown that South Asians have dysfunctional adipose tissue. Regarding GLP-1, it would be interesting to assess the

incretin effect and the direct insulinotropic action of GLP-1 to confirm that the increased GLP-1 response is a compensatory response rather than reflecting a GLP-1 resistant state. This can be studied by an OGTT and an isoglycemic intravenous glucose tolerance test to determine the incretin effect, and by hyperglycemic clamps to determine the direct action of GLP-1.

Energy/nutrient sensing pathways

A possible explanation for the South Asian predisposition for type 2 diabetes might be related to differences in the regulation of energy/nutrient-sensing pathways in skeletal muscle and other metabolic tissues affecting whole-body substrate homeostasis.

Indeed, adult overweight South Asian men exhibited a different metabolic adaptation to short-term caloric restriction. Although glucose disposal rate was improved in contrast to Caucasian subjects, metabolic flexibility was impaired, which was accompanied by an increase in insulin-induced activation of the ERK-mTOR-S6K1 axis. In addition, mRNA expression of PPARA was decreased in South Asians with a concomitant differential effect on several of its target genes, suggesting the hyperactive mTOR-pathway may have repressed fatty acid β-oxidation by inhibiting PPARα resulting in impaired metabolic flexibility. The increase in AMPK expression in South Asians may underlie their improved NOGD after caloric restriction, which might explain the enhanced glucose disposal rate in South Asians only. The fact that we did not observe differences in mTOR, AMPK or other energy/nutrient-sensing pathways in the young adult study might suggest that differences in these pathways develop with age and in a more unfavourable metabolic phenotype.

Future research. To explore the underlying mechanisms in more detail, in depth studies should be performed on the role of mTOR and AMPK in glucose and FFA metabolism and metabolic flexibility using clamping techniques and skeletal muscle and white adipose tissue biopsies before and after a single exercise test and short-term exercise and dietary interventions. As chronic mTORC1 activation is believed to contribute to the development of insulin resistance and type 2 diabetes,[11] the response to long-term caloric restriction on mTORC1 signalling and insulin sensitivity merits investigation. Interestingly, not only mTORC1 but also mTORC2 appears to be a central regulator of lipid metabolism, regulating for example lipolysis in white adipose tissue.[64] mTORC2 is therefore another interesting research topic. In light of the impaired metabolic flexibility, other possible explanations apart from suppressed skeletal muscle lipid oxidation via mTORC1/PPARα, such as a lower portion of slow-twitch type 1 oxidative muscle fibres, or preferential storage instead of oxidation of FFAs into complex lipids (IMCL) must be studied.

Brown adipose tissue

Since BAT is involved in total energy expenditure and clearance of serum triglycerides and glucose,[15] a low BAT volume or activity, leading to a disturbed energy homeostasis, might underlie the disadvantageous metabolic phenotype and susceptibility for type 2 diabetes and cardiovascular disease in South Asians.

Indeed, the detectable volume of metabolically active BAT was lower in young, healthy South Asians compared to controls. The underlying cause of the lower BAT volume may be genetic (i.e. blunted expression of signalling molecules involved in BAT differentiation) and/or- environmental, i.e. clothing behaviour, central heating setting or eating pattern, or a combination of the two.

*Future research***.** Future studies should investigate the underlying cause of lower BAT volume in South Asians. Several key molecules have been shown to be involved in BAT differentiation in rodents, including NO.[65] Interestingly, South Asians appear to have reduced bioavailability of NO compared to white Caucasians.[66] Thus, an inborn reduction in NO bioavailability might play a role in the diminished BAT volume in South Asians. Furthermore, studies should be directed towards the development of novel strategies (e.g. cold exposure or medication) to increase BAT volume and activity. For example, regarding the role of NO in BAT differentiation and the observed lower NO bioavailability in South Asians, it would be interesting to perform a randomized placebo controlled trial to determine the effect of oral supplementation of L-arginine, a semi-essential amino acid and the precursor of NO, on energy expenditure and insulin sensitivity, using cold-induced ^{18}F-FDG PET-CT-scans, indirect calorimetry and clamping techniques. Also interesting is to study the effect of cold exposure on BAT recruitment in South Asians.

HDL function

HDL has several anti-atherogenic properties[46;67-71] and dysfunction of HDL may not only directly aggravate atherosclerosis development as a consequence of lower cholesterol uptake from the vascular wall, but also indirectly through induction of inflammation as well as through reduced vasodilatation, due to less stimulation of NO release. The latter leads to increased endothelial shear stress and, hence, to endothelial activation. Reduced vasodilatation also leads to decreased delivery of insulin to tissues that take up glucose.

Anti-inflammatory capacity of HDL was reduced in South Asian neonates, and was significantly impaired in response to short-term caloric restriction in South Asian adults. This impairment is possibly only present in the calorie-restricted state and may be due to higher release of pro-inflammatory cytokines. Furthermore, adult overweight South Asians had impaired anti-oxidative capacity of HDL, which was not yet present at a young age and, therefore, likely the consequence of exogenous factors. Cholesterol efflux capacity of HDL was similar between groups.

Future research. Studies should be directed at developing treatment strategies that improve HDL functionality. The next step would be to investigate whether these strategies will indeed lower cardiovascular and diabetic risk in South Asians. In this context it is worthwhile to investigate whether HDL-cholesterol of South Asians is functionally capable of stimulating NO release by the endothelium. This can be studied by isolating HDL from blood samples and incubate HDL with endothelial cells; the amount of NO release by the endothelium is a measure for the functionality of HDL.

Endothelial function

Endothelial activation is involved in the development of atherosclerosis.[72] A hallmark of endothelial activation is a reduction in the bioavailability of endothelium-derived NO. NO not only has vasodilating properties, but also anti-platelet, anti-proliferative, and anti-inflammatory properties.[73;74] The vasodilating effect of NO is related to insulin resistance and type 2 diabetes: vasodilatation increases delivery of insulin to tissues that take up glucose, and, vice versa, insulin stimulates the release of NO from the endothelium thereby inducing capillary recruitment.[75;76]

Signs of endothelial dysfunction have been demonstrated in South Asians.[48;77;78] Furthermore, South Asians appear to have lower circulating EPCs compared to white Caucasians.[66;78] This may lead to a reduced capacity for endothelial repair, and exercise-induced EPC mobilization. In addition, EPC mobilization was reduced in South Asian men,[66] presumably as a consequence of their reduced NO bioavailability.

Future research. It would be interesting to assess the role of endothelial function in insulin sensitivity, atherosclerosis and BAT metabolism in South Asians compared to white Caucasians. Endothelial function can be determined by isolating EPCs from blood samples and performing in vitro function tests e.g. NO production. The effect of hyperinsulinemia on insulin-induced capillary recruitment and the response to infusion of NO precursor L-arginine on capillary density, EPC mobilization, degree of atherosclerosis and insulin-stimulated glucose disposal should be further explored. Furthermore, the effect of submaximal exercise on capillary recruitment is worth investigating in light of a possible defect in substrate delivery to muscle.[79]

Sympathetic nervous system activity

The sympathetic nervous system is involved in many homeostatic mechanisms by innervating tissues in virtually every organ system, and is recognized to play a role in energy expenditure. Interestingly, reduced muscle sympathetic nervous system activity has been found in Pima Indians, a population with a similar high prevalence of obesity, insulin resistance and type 2 diabetes as South Asians. Furthermore, sympathetic nervous system activity was not related to body fat mass and energy expenditure in Pima Indians, whereas in Caucasians a significant correlation was observed.[80]

Plasma FFAs increased less in response to short-term caloric restriction in adult overweight South Asians and in response to cold in young healthy South Asians. This was accompanied with a lower increase in whole-body lipid oxidation in adult South Asians, and a lower cold-induced increase in lipid oxidation and systolic blood pressure in adolescent South Asians. This may suggest that South Asians rather store than burn fat (or energy), and may point to differences in sympathetic activation and/or peripheral resistance to sympathetic outflow in white adipose tissue and the vasculature.

Future research. Future studies should investigate a potentially different, organ-specific sympathetic response in South Asians compared to white Caucasians and the potential link with energy expenditure, in fasted resting condition and in response to glucose, insulin and exercise.

Finally, there are other areas that merit further exploration as well, including: 1) *Adipocyte function*: South Asians appear to have dysfunctional adipocytes, leading to impaired release of FFA's, adipokines and pro-inflammatory cytokines, which may contribute to their increased diabetic and cardiovascular risk. 2) *Cortisol metabolism*: the typical South Asian thin-fat phenotype might suggest differences in the hypothalamic-pituitary-adrenal-target organ-axis with (tissue-specific) impaired cortisol metabolism. 3) *Irisin*: a recently discovered myokine that increases with exercise and is involved in browning of white adipose tissue.[81] Considering the fact that South Asians have lower muscle mass and lower physical activity levels, irisin might have a role in insulin resistance and the amount of BAT. Given the findings of a recent study, though, it is not certain that irisin will have a similar beneficial effect in humans as in mice.[82] 4) *Gut microbiota*: the gut microbiota of obese subjects appears to be different from that of lean subjects and is thought to be associated with insulin resistance.[83] Possibly, the gut microbiota also differ between various ethnicities. 5) *Resveratrol*: a natural polyphenol produced by plants and present in low concentrations in plant-based foods, which mediates some of its effects via activation of AMPK. Recent studies in rats showed that intrauterine growth restriction increased the susceptibility to HF-diet induced alterations of fat distribution, adipocyte size, lipid metabolism, and insulin signalling pathways,[84] and that resveratrol reduced this susceptibility.[85] It would be interesting to investigate the effect of resveratrol in South Asians compared to white Caucasians.

To conclude, there are still many promising areas to explore in order to find new strategies for the prevention and treatment of type 2 diabetes and cardiovascular disease in people of South Asian origin thereby hopefully reducing the major health and socioeconomic burden that we are currently facing worldwide.

REFERENCES

1. Anderson JW, Herman RH. Classification of reactive hypoglycemia. *Am J Clin Nutr* 1969;22(5):646-50.
2. Conn JW, Fajans SS, Seltzer HS. Spontaneous hypoglycemia as an early manifestation of diabetes mellitus. *Diabetes* 1956;5(6):437-42.
3. Faludi G, Bendersky G, Gerber P. Functional hypoglycemia in early latent diabetes. *Ann N Y Acad Sci* 1968;148(3):868-74.
4. Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, Goto T, Westerbacka J, Sovijarvi A *et al*. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J Clin Endocrinol Metab* 2002;87(7):3023-8.
5. Shulman GI, Rothman DL, Jue T, Stein P, DeFronzo RA, Shulman RG. Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. *N Engl J Med* 1990;322(4):223-8.
6. Christiansen MP, Linfoot PA, Neese RA, Hellerstein MK. Effect of dietary energy restriction on glucose production and substrate utilization in type 2 diabetes. *Diabetes* 2000;49(10):1691-9.
7. Jazet IM, Pijl H, Frolich M, Romijn JA, Meinders AE. Two days of a very low calorie diet reduces endogenous glucose production in obese type 2 diabetic patients despite the withdrawal of blood glucose-lowering therapies including insulin. *Metabolism* 2005;54(6):705-12.
8. Markovic TP, Jenkins AB, Campbell LV, Furler SM, Kraegen EW, Chisholm DJ. The determinants of glycemic responses to diet restriction and weight loss in obesity and NIDDM. *Diabetes Care* 1998;21(5):687-94.
9. van der Meer RW, Hammer S, Smit JW, Frolich M, Bax JJ, Diamant M *et al*. Short-term caloric restriction induces accumulation of myocardial triglycerides and decreases left ventricular diastolic function in healthy subjects. *Diabetes* 2007;56(12):2849-53.
10. Peng T, Golub TR, Sabatini DM. The immunosuppressant rapamycin mimics a starvation-like signal distinct from amino acid and glucose deprivation. *Mol Cell Biol* 2002;22(15):5575-84.
11. Ricoult SJ, Manning BD. The multifaceted role of mTORC1 in the control of lipid metabolism. *EMBO Rep* 2013;14(3):242-51.
12. Sengupta S, Peterson TR, Laplante M, Oh S, Sabatini DM. mTORC1 controls fasting-induced ketogenesis and its modulation by ageing. *Nature* 2010;468(7327):1100-4.
13. Sipula IJ, Brown NF, Perdomo G. Rapamycin-mediated inhibition of mammalian target of rapamycin in skeletal muscle cells reduces glucose utilization and increases fatty acid oxidation. *Metabolism* 2006;55(12):1637-44.
14. Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M *et al*. Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature* 2004;431(7005):200-5.
15. van Marken Lichtenbelt WD, Schrauwen P. Implications of nonshivering thermogenesis for energy balance regulation in humans. *Am J Physiol Regul Integr Comp Physiol* 2011;301(2):R285-R296.
16. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB *et al*. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* 2009;360(15):1509-17.
17. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND *et al*. Cold-activated brown adipose tissue in healthy men. *N Engl J Med* 2009;360(15):1500-8.
18. Vijgen GH, Bouvy ND, Teule GJ, Brans B, Schrauwen P, van Marken Lichtenbelt WD. Brown adipose tissue in morbidly obese subjects. *PLoS One* 2011;6(2):e17247.

19. Bartelt A, Bruns OT, Reimer R, Hohenberg H, Ittrich H, Peldschus K *et al*. Brown adipose tissue activity controls triglyceride clearance. *Nat Med* 2011;17(2):200-5.
20. Stanford KI, Middelbeek RJ, Townsend KL, An D, Nygaard EB, Hitchcox KM *et al*. Brown adipose tissue regulates glucose homeostasis and insulin sensitivity. *J Clin Invest* 2013;123(1):215-23.
21. Yoneshiro T, Aita S, Matsushita M, Kameya T, Nakada K, Kawai Y *et al*. Brown adipose tissue, whole-body energy expenditure, and thermogenesis in healthy adult men. *Obesity (Silver Spring)* 2011;19(1):13-6.
22. Chiu M, Austin PC, Manuel DG, Shah BR, Tu JV. Deriving ethnic-specific BMI cutoff points for assessing diabetes risk. *Diabetes Care* 2011;34(8):1741-8.
23. Forouhi NG, Sattar N, Tillin T, McKeigue PM, Chaturvedi N. Do known risk factors explain the higher coronary heart disease mortality in South Asian compared with European men? Prospective follow-up of the Southall and Brent studies, UK. *Diabetologia* 2006;49(11):2580-8.
24. Wilkinson P, Sayer J, Laji K, Grundy C, Marchant B, Kopelman P *et al*. Comparison of case fatality in south Asian and white patients after acute myocardial infarction: observational study. *BMJ* 1996;312(7042):1330-3.
25. Mitchell GF, Hwang SJ, Vasan RS, Larson MG, Pencina MJ, Hamburg NM *et al*. Arterial stiffness and cardiovascular events: the Framingham Heart Study. *Circulation* 2010;121(4):505-11.
26. Celentano A, Vaccaro O, Tammaro P, Galderisi M, Crivaro M, Oliviero M *et al*. Early abnormalities of cardiac function in non-insulin-dependent diabetes mellitus and impaired glucose tolerance. *Am J Cardiol* 1995;76(16):1173-6.
27. Rietzschel ER, Langlois M, De Buyzere ML, Segers P, de Bacquer D, Bekaert S *et al*. Oxidized low-density lipoprotein cholesterol is associated with decreases in cardiac function independent of vascular alterations. *Hypertension* 2008;52(3):535-41.
28. Hammer S, van der Meer RW, Lamb HJ, de Boer HH, Bax JJ, de Roos A *et al*. Short-term flexibility of myocardial triglycerides and diastolic function in patients with type 2 diabetes mellitus. *Am J Physiol Endocrinol Metab* 2008;295(3):E714-E718.
29. Hammer S, Snel M, Lamb HJ, Jazet IM, van der Meer RW, Pijl H *et al*. Prolonged caloric restriction in obese patients with type 2 diabetes mellitus decreases myocardial triglyceride content and improves myocardial function. *J Am Coll Cardiol* 2008;52(12):1006-12.
30. Hammer S, van der Meer RW, Lamb HJ, Schar M, de Roos A, Smit JW *et al*. Progressive caloric restriction induces dose-dependent changes in myocardial triglyceride content and diastolic function in healthy men. *J Clin Endocrinol Metab* 2008;93(2):497-503.
31. Stapleton PA, James ME, Goodwill AG, Frisbee JC. Obesity and vascular dysfunction. *Pathophysiology* 2008;15(2):79-89.
32. Christoffersen C, Bollano E, Lindegaard ML, Bartels ED, Goetze JP, Andersen CB *et al*. Cardiac lipid accumulation associated with diastolic dysfunction in obese mice. *Endocrinology* 2003;144(8):3483-90.
33. McGavock JM, Lingvay I, Zib I, Tillery T, Salas N, Unger R *et al*. Cardiac steatosis in diabetes mellitus: a 1H-magnetic resonance spectroscopy study. *Circulation* 2007;116(10):1170-5.
34. Szczepaniak LS, Dobbins RL, Metzger GJ, Sartoni-D'Ambrosia G, Arbique D, Vongpatanasin W *et al*. Myocardial triglycerides and systolic function in humans: in vivo evaluation by localized proton spectroscopy and cardiac imaging. *Magn Reson Med* 2003;49(3):417-23.
35. Sicari R, Sironi AM, Petz R, Frassi F, Chubuchny V, de Marchi D *et al*. Pericardial rather than epicardial fat is a cardiometabolic risk marker: an MRI *vs.* echo study. *J Am Soc Echocardiogr* 2011;24(10):1156-62.

36. Assmann G, Schulte H, von Eckardstein A, Huang Y. High-density lipoprotein cholesterol as a predictor of coronary heart disease risk. The PROCAM experience and pathophysiological implications for reverse cholesterol transport. *Atherosclerosis* 1996;124 Suppl:S11-S20.
37. Barter P, Gotto AM, LaRosa JC, Maroni J, Szarek M, Grundy SM *et al*. HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. *N Engl J Med* 2007;357(13):1301-10.
38. Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A *et al*. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA* 2009;302(18):1993-2000.
39. Gordon DJ, Rifkind BM. High-density lipoprotein—the clinical implications of recent studies. *N Engl J Med* 1989;321(19):1311-6.
40. Sharrett AR, Ballantyne CM, Coady SA, Heiss G, Sorlie PD, Catellier D *et al*. Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: The Atherosclerosis Risk in Communities (ARIC) Study. *Circulation* 2001;104(10):1108-13.
41. Wilson PW, Abbott RD, Castelli WP. High density lipoprotein cholesterol and mortality. The Framingham Heart Study. *Arteriosclerosis* 1988;8(6):737-41.
42. Movva R, Rader DJ. Laboratory assessment of HDL heterogeneity and function. *Clin Chem* 2008;54(5):788-800.
43. Nofer JR, Kehrel B, Fobker M, Levkau B, Assmann G, von Eckardstein A. HDL and arteriosclerosis: beyond reverse cholesterol transport. *Atherosclerosis* 2002;161(1):1-16.
44. Rohrer L, Hersberger M, von Eckardstein A. High density lipoproteins in the intersection of diabetes mellitus, inflammation and cardiovascular disease. *Curr Opin Lipidol* 2004;15(3):269-78.
45. von Eckardstein A, Nofer JR, Assmann G. High density lipoproteins and arteriosclerosis. Role of cholesterol efflux and reverse cholesterol transport. *Arterioscler Thromb Vasc Biol* 2001;21(1):13-27.
46. von Eckardstein A, Hersberger M, Rohrer L. Current understanding of the metabolism and biological actions of HDL. *Curr Opin Clin Nutr Metab Care* 2005;8(2):147-52.
47. Ajjan R, Carter AM, Somani R, Kain K, Grant PJ. Ethnic differences in cardiovascular risk factors in healthy Caucasian and South Asian individuals with the metabolic syndrome. *J Thromb Haemost* 2007;5(4):754-60.
48. Boon MR, Karamali NS, de Groot CJ, van Steijn L, Kanhai HH, van der Bent C *et al*. E-Selectin is Elevated in Cord Blood of South Asian Neonates Compared with Caucasian Neonates. *J Pediatr* 2011.
49. Chambers JC, Eda S, Bassett P, Karim Y, Thompson SG, Gallimore JR *et al*. C-reactive protein, insulin resistance, central obesity, and coronary heart disease risk in Indian Asians from the United Kingdom compared with European whites. *Circulation* 2001;104(2):145-50.
50. Chandalia M, Abate N, Garg A, Stray-Gundersen J, Grundy SM. Relationship between generalized and upper body obesity to insulin resistance in Asian Indian men. *J Clin Endocrinol Metab* 1999;84(7):2329-35.
51. Ehtisham S, Crabtree N, Clark P, Shaw N, Barrett T. Ethnic differences in insulin resistance and body composition in United Kingdom adolescents. *J Clin Endocrinol Metab* 2005;90(7):3963-9.
52. McKeigue PM, Marmot MG, Syndercombe Court YD, Cottier DE, Rahman S, Riemersma RA. Diabetes, hyperinsulinaemia, and coronary risk factors in Bangladeshis in east London. *Br Heart J* 1988;60(5):390-6.
53. McKeigue PM, Shah B, Marmot MG. Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. *Lancet* 1991;337(8738):382-6.

54. Raji A, Gerhard-Herman MD, Warren M, Silverman SG, Raptopoulos V, Mantzoros CS *et al*. Insulin resistance and vascular dysfunction in nondiabetic Asian Indians. *J Clin Endocrinol Metab* 2004;89(8):3965-72.
55. Roberts CK, Ng C, Hama S, Eliseo AJ, Barnard RJ. Effect of a short-term diet and exercise intervention on inflammatory/anti-inflammatory properties of HDL in overweight/obese men with cardiovascular risk factors. *J Appl Physiol* 2006;101(6):1727-32.
56. Wang Y, Snel M, Jonker JT, Hammer S, Lamb HJ, de Roos A *et al*. Prolonged caloric restriction in obese patients with type 2 diabetes mellitus decreases plasma CETP and increases apolipoprotein AI levels without improving the cholesterol efflux properties of HDL. *Diabetes Care* 2011;34(12):2576-80.
57. Mulder DJ, de Boer JF, Graaff R, de Vries R, Annema W, Lefrandt JD *et al*. Skin autofluorescence is inversely related to HDL anti-oxidative capacity in type 2 diabetes mellitus. *Atherosclerosis* 2011;218(1):102-6.
58. Peters MJ, Ghouri N, McKeigue P, Forouhi NG, Sattar N. Circulating IL-6 concentrations and associated anthropometric and metabolic parameters in South Asian men and women in comparison to European whites. *Cytokine* 2013;61(1):29-32.
59. Petersen KF, Dufour S, Feng J, Befroy D, Dziura J, Dalla MC *et al*. Increased prevalence of insulin resistance and nonalcoholic fatty liver disease in Asian-Indian men. *Proc Natl Acad Sci U S A* 2006;103(48):18273-7.
60. Yusuf S, Reddy S, Ounpuu S, Anand S. Global burden of cardiovascular diseases: part I: general considerations, the epidemiologic transition, risk factors, and impact of urbanization. *Circulation* 2001;104(22):2746-53.
61. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes* 1981;30(12):1000-7.
62. Cline GW, Petersen KF, Krssak M, Shen J, Hundal RS, Trajanoski Z *et al*. Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes. *N Engl J Med* 1999;341(4):240-6.
63. Rothman DL, Shulman RG, Shulman GI. 31P nuclear magnetic resonance measurements of muscle glucose-6-phosphate. Evidence for reduced insulin-dependent muscle glucose transport or phosphorylation activity in non-insulin-dependent diabetes mellitus. *J Clin Invest* 1992;89(4):1069-75.
64. Lamming DW, Sabatini DM. A Central role for mTOR in lipid homeostasis. *Cell Metab* 2013;18(4):465-9.
65. Nisoli E, Clementi E, Paolucci C, Cozzi V, Tonello C, Sciorati C *et al*. Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. *Science* 2003;299(5608):896-9.
66. Cubbon RM, Murgatroyd SR, Ferguson C, Bowen TS, Rakobowchuk M, Baliga V *et al*. Human exercise-induced circulating progenitor cell mobilization is nitric oxide-dependent and is blunted in South Asian men. *Arterioscler Thromb Vasc Biol* 2010;30(4):878-84.
67. Annema W, von Eckardstein A. High-density lipoproteins. Multifunctional but vulnerable protections from atherosclerosis. *Circ J* 2013;77(10):2432-48.
68. Barter PJ, Baker PW, Rye KA. Effect of high-density lipoproteins on the expression of adhesion molecules in endothelial cells. *Curr Opin Lipidol* 2002;13(3):285-8.
69. Navab M, Hama SY, Cooke CJ, Anantharamaiah GM, Chaddha M, Jin L *et al*. Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: step 1. *J Lipid Res* 2000;41(9):1481-94.

70. Navab M, Ananthramaiah GM, Reddy ST, Van Lenten BJ, Ansell BJ, Hama S *et al*. The double jeopardy of HDL. *Ann Med* 2005;37(3):173-8.
71. Sugatani J, Miwa M, Komiyama Y, Ito S. High-density lipoprotein inhibits the synthesis of platelet-activating factor in human vascular endothelial cells. *J Lipid Mediat Cell Signal* 1996;13(1):73-88.
72. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atherosclerotic risk. *Arterioscler Thromb Vasc Biol* 2003;23(2):168-75.
73. Jin RC, Loscalzo J. Vascular Nitric Oxide: Formation and Function. *J Blood Med* 2010;2010(1):147-62.
74. Kawashima S. The two faces of endothelial nitric oxide synthase in the pathophysiology of atherosclerosis. *Endothelium* 2004;11(2):99-107.
75. Cersosimo E, DeFronzo RA. Insulin resistance and endothelial dysfunction: the road map to cardiovascular diseases. *Diabetes Metab Res Rev* 2006;22(6):423-36.
76. Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. *Circulation* 2006;113(15):1888-904.
77. Chambers JC, McGregor A, Jean-Marie J, Kooner JS. Abnormalities of vascular endothelial function may contribute to increased coronary heart disease risk in UK Indian Asians. *Heart* 1999;81(5):501-4.
78. Murphy C, Kanaganayagam GS, Jiang B, Chowienczyk PJ, Zbinden R, Saha M *et al*. Vascular dysfunction and reduced circulating endothelial progenitor cells in young healthy UK South Asian men. *Arteriosclerosis, Thrombosis, and Vascular Biology* 2007;27(4):936-42.
79. Hall LM, Moran CN, Milne GR, Wilson J, MacFarlane NG, Forouhi NG *et al*. Fat oxidation, fitness and skeletal muscle expression of oxidative/lipid metabolism genes in South Asians: implications for insulin resistance? *PLoS One* 2010;5(12):e14197.
80. Spraul M, Ravussin E, Fontvieille AM, Rising R, Larson DE, Anderson EA. Reduced sympathetic nervous activity. A potential mechanism predisposing to body weight gain. *J Clin Invest* 1993;92(4):1730-5.
81. Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC *et al*. A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 2012;481(7382):463-8.
82. Raschke S, Elsen M, Gassenhuber H, Sommerfeld M, Schwahn U, Brockmann B *et al*. Evidence against a beneficial effect of irisin in humans. *PLoS One* 2013;8(9):e73680.
83. Cani PD, Osto M, Geurts L, Everard A. Involvement of gut microbiota in the development of low-grade inflammation and type 2 diabetes associated with obesity. *Gut Microbes* 2012;3(4):279-88.
84. Rueda-Clausen CF, Dolinsky VW, Morton JS, Proctor SD, Dyck JR, Davidge ST. Hypoxia-induced intrauterine growth restriction increases the susceptibility of rats to high-fat diet-induced metabolic syndrome. *Diabetes* 2011;60(2):507-16.
85. Dolinsky VW, Rueda-Clausen CF, Morton JS, Davidge ST, Dyck JR. Continued postnatal administration of resveratrol prevents diet-induced metabolic syndrome in rat offspring born growth restricted. *Diabetes* 2011;60(9):2274-84.

13 Nederlandse samenvatting

DEEL 1: TYPE 2 DIABETES MELLITUS IN ZUID AZIATEN

Bij diabetes (suikerziekte) kan het lichaam de bloedsuiker niet meer regelen. Normaal regelt het lichaam de bloedsuikerspiegel heel precies met het hormoon insuline. Insuline wordt gemaakt en uitgescheiden door de alvleesklier. Het zorgt ervoor dat glucose uit het bloed wordt opgenomen door onder meer de skeletspieren en het vetweefsel, en het remt de glucoseproductie door de lever. Tegelijkertijd remt insuline ook de afbraak van vet. Mensen met diabetes maken zelf geen insuline meer, of hun lichaam reageert niet meer op de insuline, afhankelijk van het type diabetes (type 1 of 2). Bij type 2 diabetes is het lichaam minder gevoelig voor insuline (insulineresistentie). Eerst maakt de alvleesklier nog extra insuline aan om te compenseren voor de verminderde insulinegevoeligheid, maar na verloop van tijd wordt dit steeds minder en stijgt de bloedsuikerspiegel; men spreekt dan van diabetes. Diabetes kan nadelige gevolgen hebben in het hele lichaam. Veel voorkomende complicaties van diabetes zijn hart- en vaatziekten, achteruitgang van de nierfunctie en ogen, en zenuwschade.

Diabetes is één van de meest voorkomende chronische aandoeningen wereldwijd en neemt nog steeds toe in aantal. Opvallend is dat er sterke geografische variatie is in het voorkomen van type 2 diabetes, waarschijnlijk ten gevolge van genetische, gedrags, en omgevingsfactoren. Dit geldt onder meer voor mensen van Zuid Aziatische afkomst, bij wie sprake is van een snelle toename in het voorkomen van type 2 diabetes. Zuid Aziaten komen oorspronkelijk van het Indiase subcontinent – India, Pakistan, Bangladesh, Nepal en Sri Lanka – en vertegenwoordigen één vijfde van de wereldbevolking. Het risico is verhoogd voor zowel Zuid Aziaten die zijn blijven wonen op het Indiase subcontinent als voor Zuid Aziaten die geëmigreerd zijn, zoals de Hindostaanse bevolkingsgroep in Nederland. In 2008 had 26.7% (age-standardized prevalence) van de Hindostanen diabetes in vergelijking tot 5.5% van de blanke autochtone Nederlanders en tot 10% wereldwijd. Ni*et al*leen komt type 2 diabetes 4 tot 6 keer vaker voor in Zuid Aziaten, het treedt ook op jongere leeftijd (ongeveer 10 jaar eerder) op en bij een lagere body mass index (BMI) in vergelijking tot blanke mensen van Kaukasische afkomst. Bovendien ontwikkelen ze eerder en ernstiger complicaties.

De snelle toename van type 2 diabetes wereldwijd is geassocieerd met een Westerse levensstijl (calorierijk eten, weinig lichaamsbeweging). Zuid Aziaten lijken echter een uitzonderlijk hoog risico te hebben voor het ontwikkelen van diabetes bij eenzelfde levensstijl in vergelijking tot andere bevolkingsgroepen. Hoewel bepaalde risicofactoren zoals een zittende levensstijl en ongezond dieet zeker van toepassing zijn voor Zuid Aziaten, mede het gevolg van de snelle ontwikkelingen in Zuid Aziatische landen in de afgelopen decennia, kunnen zij niet volledig het hoge risico verklaren en hebben genetische invloeden waarschijnlijk ook een belangrijke rol.

De hoge prevalentie van diabetes in de Zuid Aziatische populatie vormt wereldwijd een belangrijk gezondheids- en sociaaleconomisch probleem. Een verklaring voor het hoge risico is echter nog onduidelijk. In dit proefschrift worden studies beschreven gericht op het verkrijgen van meer inzicht in de pathogenese van type 2 diabetes in Zuid Aziaten en om zo nieuwe aanknopingspunten te vinden voor het ontwikkelen van preventieve maatregelen en behandelopties van diabetes in deze populatie. Hiertoe werden jonge (18 tot 25 jaar) gezonde slanke mannen en oudere (40 tot 50 jaar) mannen met overgewicht (BMI 25 tot 30 kg/m^2) onderzocht. De Zuid Aziatische proefpersonen die deel hebben genomen aan deze studies zijn Nederlandse Hindostanen. Deze groep komt oorspronkelijk uit Noord-India. Na het afschaffen van de slavernij in 1862 in de Nederlandse koloniën, zoals Suriname, mochten de Nederlanders van de Britten in 1873 werknemers rekruteren in Noord-India voor Suriname. Vlak voor en vlak na de onafhankelijkheid van Suriname in 1975 zijn veel Hindostanen gemigreerd naar Nederland. In 2010 woonden er circa 123.000 – 147.000 Hindostanen in Nederland; de grootste groep woont in Den Haag (circa 45.000). Blanke autochtone Nederlanders vormen de controlegroep van de studies beschreven in dit proefschrift.

Hoofdstuk 2 is een review over studies die onderzocht hebben waarom mensen van Zuid Aziatische afkomst een verhoogd risico hebben op het ontwikkelen van type 2 diabetes in vergelijking tot mensen van blanke Kaukasische afkomst. Het voornaamste mechanisme in Zuid Aziaten lijkt insulineresistentie te zijn. Deze insulineresistentie is meest waarschijnlijk het gevolg van een interactie tussen genen en omgeving. Zuid Aziaten hebben een opmerkelijk ongunstig metabool fenotype: ze worden relatief klein geboren en hebben al bij de geboorte hoge insulinewaarden en een ongunstige lichaamsbouw (het zogenaamde 'thin-fat-phenotype': dunne armen en benen (dat wil zeggen weinig spiermassa), en relatief veel vet aan de romp, zowel rondom de organen, zogenaamd visceraal vet, als onderhuids). Dit uit zich ook in een hoger vetpercentage bij een vergelijkbare BMI. Bovendien is hun vetweefsel dysfunctioneel: er is minder opslagvermogen voor vetten en een gestoorde uitscheiding van vrije vetzuren, adipokinen en pro-inflammatoire ('pro ontsteking') cytokinen waardoor er continu sprake is van een laaggradige ontsteking. Dit leidt tot opslag van vet elders (in de lever, spier; ook wel ectopisch vet genoemd), verstoring van de insuline signaalroute en toename van insulineresistentie. Enkele studies tonen een gestoorde functie van het endotheel (de binnenbekleding van bloedvaten) leidend tot onvoldoende vaatverwijding en verminderde afgifte van insuline aan de weefsels. Dysfunctie van het HDL-cholesterol zou mogelijk ook een rol kunnen spelen. Verschillen in dieetgewoonten lijken geen belangrijke rol te spelen in het verhoogde risico. Wel lijken het dagelijks minder bewegen en hun lagere cardiorespiratoir uithoudingsvermogen bij te dragen aan het frequente voorkomen van insulineresistentie.

Dit literatuuroverzicht maakt echter ook duidelijk dat er maar weinig studies gedaan zijn bij Zuid Aziaten op weefselniveau, en dat een verklaring voor hun ongunstige metabole fenotype nog niet opgehelderd is.

In **Hoofdstuk 3** wordt een onderzoek beschreven dat ingaat op de bij herhaling gevonden hoge insulinewaarden na een orale glucosebelasting in Zuid Aziaten vergeleken met blanke Kaukasiërs. Deze hogere insulinewaarden worden gezien als een compensatoir mechanisme voor insulineresistentie om normale glucosewaarden te behouden. De hoge waarden kunnen het gevolg zijn van een verminderde klaring van insuline uit het bloed, of door een verhoogde uitscheiding van insuline door de alvleesklier. In deze studie is onderzocht of de verhoogde insulinewaarden het gevolg zijn van een verhoogde respons van GLP-1. GLP-1 is een hormoon dat door de darm uitgescheiden wordt in reactie op eten, en dat de uitscheiding van insuline stimuleert. Verder waren we geïnteresseerd of de hoge insulinewaarden een reactieve hypoglykemie (lage bloedsuikerspiegel) veroorzaken, gekenmerkt door een daling in de bloedsuikerspiegel(<= 3 mmol/L) 4 tot 6 uur na de glucosebelasting; dit wordt gezien als een teken van beginnende diabetes. Hiertoe ondergingen 8 jonge, gezonde Zuid Aziatische mannen en 10 blanke Kaukasische mannen een 6-uur durende orale glucose belastingstest (OGTT). De Zuid Aziatische proefpersonen waren meer insulineresistent en hadden hogere insulinewaarden conform andere studies. De hoge insulinewaarden gingen vergezeld met verhoogde waarden van GLP-1. De piek GLP-1 waarden gingen vooraf aan de piek insulinewaarden, dit suggereert een direct verband tussen de verhoogde GLP-1 respons en insuline afscheiding door de alvleesklier. Aanvullend onderzoek is echter nodig om te bepalen of de verhoogde GLP-1 concentraties inderdaad de oorzaak zijn van de verhoogde insulinewaarden, of dat deze hoge GLP-1 waarden een uiting zijn van ongevoeligheid voor GLP-1. Er was verder geen sprake van reactieve hypoglykemie.

Het belangrijkste orgaan dat glucose onder invloed van insuline opneemt uit het bloed is de skeletspier (75-80%). In de spier wordt de glucose vervolgens ofwel verbrand tot energie (oxidatieve glucose stofwisseling (Rd)) ofwel opgeslagen als glycogeen (niet-oxidatieve glucose verwijdering (NOGD)). Deze processen lopen via de zogenaamde 'insuline signaalroute' (insulin signaling pathway). Bij mensen met diabetes lijkt het belangrijkste probleem in de skeletspier de NOGD te zijn. Opvallend genoeg zijn bij Zuid Aziaten slechts twee onderzoeken gedaan op weefselniveau, waarvan in één de insuline signaalroute onderzocht is.

Gezien het hoge risico van Zuid Aziaten om diabetes te ontwikkelen bij eenzelfde levensstijl in vergelijking tot andere bevolkingsgroepen, zou een verklaring voor hun predispositie ook gelegen kunnen zijn in een verschillende regulatie van 'energie/ nutriënt-gevoelige routes' in metabole weefsels wat effect kan hebben op de gehele

stofwisseling. Een belangrijke signaalroute hierbij is de mTOR route. mTOR is een eiwit dat aan de hand van beschikbaarheid van voedingsstoffen en energieniveau van de cel de celgroei reguleert. mTOR reageert op insuline en andere groeifactoren, stress, zuurstof en niveau van voedingsstoffen. Het bevordert de eiwitaanmaak, celgroei en -differentiatie, en kan de insuline signaalroute remmen. Verder lijkt het ook een cruciale rol te spelen in de mitochrondriële biogenese en oxidatieve stofwisseling.

In de **Hoofdstukken 4 en 5** zijn deze signaalroutes onderzocht in jong volwassen en volwassen proefpersonen voor en na een dieet.

INTERMEZZO: UITLEG ONDERZOEKSMETHODEN

In dit proefschrift is gebruik gemaakt van diverse onderzoekstechnieken.

2-staps hyperinsulinemische-euglycemische clamp. Met deze test kan de gevoeligheid van weefsels voor insuline worden bepaald. De proefpersoon krijgt zowel insuline als glucose toegediend. Onder invloed van insuline wordt glucose uit het bloed opgenomen door met name de spieren; hierdoor gaat de bloedglucosewaarde omLaag. Hoe meer glucose men iemand moet toedienen om de bloedglucosespiegel stabiel te houden hoe gevoeliger de spier is voor insuline (perifere insulinegevoeligheid, R_d). De lever produceert zelf ook glucose (endogene of hepatische glucoseproductie, EGP). Om onderscheid te kunnen maken tussen het door lever geproduceerde glucose en het toegediende glucose wordt gelabelde glucose toegediend (glucose gelabeld met 2 waterstofatomen, de stabiele isotoop deuterium). Hiermee kun je de gevoeligheid van de lever voor insuline bepalen (hoe gevoeliger hoe minder glucoseproductie). De test bestaat uit 2 stappen, een lage insuline stap waarin met name de lever insulinegevoeligheid goed te meten is, en een hoge insuline stap waarin juist de perifere insulinegevoeligheid (met name spier; R_d) goed te meten is.

Spierbiopsie. Met een holle naald wordt onder lokale verdoving een biopt afgenomen uit skeletspierweefsel (uit het bovenbeen), zowel voor als na het dieet, en voor en tijdens de clamp; tijdens de clamp kan dan het effect van insuline op de insulinegevoelige signaalroutes in spier worden bestudeerd. In het spierweefsel wordt gekeken naar eiwitexpressie (door middel van Western Blot) en genexpressie (door middel van qPCR) van eiwitten betrokken in diverse signaalroutes.

Indirecte calorimetrie. Vlak voor en tijdens de clamp ondergingen de proefpersonen een geventileerde kapmeting (indirecte calorimetrie). Tijdens deze meting krijgt de proefpersoon een geventileerde doorzichtige kap over zijn hoofd waarin het zuurstofgehalte (O2) en koolzuurgasgehalte (CO2) in de in- en uitademingslucht wordt gemeten en met elkaar wordt vergeleken. Hieruit kan men bepalen wat iemands energiegebruik is in rust (resting energy expenditure, REE), en hoeveel koolhydraten, vetten en eiwitten iemand verbrandt. Insuline remt de vetverbranding en stimuleert de glucoseverbranding. Tijdens de clamp waarbij insuline wordt toegediend verwacht men dus een afname van de vetverbranding en een toename van de glucoseverbranding. Hoe resistenter iemand is voor insuline, hoe minder sterk dit effect zal zijn. Bij caloriebeperking gaat het lichaam over op vetverbranding om glucose te sparen voor de glucoseafhankelijke weefsels (de hersenen en

rode bloedcellen). Na het dieet verwacht men daarom in de niet-insuline-gestimuleerde toestand een toename van de vetverbranding en een afname van de glucoseverbranding. Het effect van insuline zal in principe sterker zijn door toegenomen insulinegevoeligheid. Het vermogen om de vetverbranding te verhogen na caloriebeperking en om te switchen van vet- naar glucoseverbranding tijdens insulinetoediening wordt metabole flexibiliteit genoemd.

MRI/MRS. Voorafgaand aan en 1 dag na het dieet werd een MRI (magnetic resonance imaging) en spectroscopie (MRS) verricht. Hiermee is gekeken naar de hartfunctie, het hartvet (myocardial triglyceride content), levervet (hepatic triglyceride content, HTG), buikvetverdeling en de stijfheid van de aorta (pulse wave velocity, PWV).

^{18}F-FDG-PET-CT scan. Voor de bruin vet studie (Hoofdstuk 7) is gebruik gemaakt van een ^{18}F-FDG-PET-CT scan. Een ^{18}F-FDG PET-onderzoek (positron emissie tomografie) geeft informatie over de glucosestofwisseling in weefsels die glucose opnemen, zoals bruin vet. Een PET-camera kan glucose in beeld brengen wanneer het radioactief is gemerkt (^{18}F-FDG). De PET-scan wordt gecombineerd met een CT-scan om de glucoseopname nauwkeurig te kunnen lokaliseren. Om de detectiekans van bruin vet te vergroten op de PET-CT scan wordt een koelprocedure toegepast; bruin vet wordt immers geactiveerd door kou. Tijdens het koelen met speciale koelmatten wordt de proefpersoon gedurende circa 3 uur blootgesteld aan milde kou (± 16 °C). Het doel is om het bruine vet maximaal te activeren zonder dat de proefpersoon gaat rillen.

Hoofdstuk 4 vergelijkt de metabole aanpassing aan een 5-dagen hoog-vet-hoog-calorisch dieet (HFHCD, eigen dieet plus 375 mL slagroom per dag (= 1275 kcal per dag extra bestaande uit 94% vet)) in de jonge groep. Het bleek dat een 5-dagen HFHCD al voldoende is om de perifere insulinegevoeligheid te verminderen in Zuid Aziaten, terwijl er geen effect te zien was in de blanke controles. Deze afname in insulinegevoeligheid was voornamelijk het gevolg van een afname in NOGD wat een defect in de glycogeenopslag suggereert. Er werden echter geen duidelijke verschillen tussen de groepen gevonden in de expressie van eiwitten en genen betrokken in glycogeenafbraak en -opslag. Op het niveau van de skeletspier werden verder ook geen significante verschillen gezien tussen de groepen in mTOR en insuline signalering, en ook niet in de expressie van metabole genen en mitochondriële functie (mitochrondria zijn de energiecentrales van de cel). Verder waren levervet en buikvetverdeling niet verschillend tussen Zuid Aziaten en blanke controles. Wel was sprake van een lagere insuline klaringssnelheid en lever insulinegevoeligheid in Zuid Aziaten, zowel voor als na het dieet.

Redenen waarom geen duidelijke verschillen werden gevonden op weefselniveau kunnen de relatief goede gezondheid van de proefpersonen en/of de kleine groepsgrootte zijn. Verder kan niet worden uitgesloten dat het witte vetweefsel bijgedragen heeft aan de afname in perifere insulinegevoeligheid in Zuid Aziaten. Ongeveer 10-20% van de insulinegestimuleerde glucoseopname treedt namelijk op in wit vetweefsel.

Concluderend gaf een HFHCD snel insulineresistentie in jonge gezonde slanke Zuid Aziatische proefpersonen. Dit suggereert dat de vatbaarheid van Zuid Aziaten voor het ontwikkelen van type 2 diabetes deels verklaard kan worden door de manier waarop ze zich aanpassen aan een Westers vetrijk dieet. De mTOR route in skeletspierweefsel lijkt daarbij nog geen rol te hebben.

Hoofdstuk 5 bestudeert het effect van caloriebeperking door middel van een 8-dagen zeer-laag-calorisch-dieet (VLCD, ~450 kcal per dag) op energie/nutriënt-gevoelige signaalroutes in skeletspierweefsel in de oudere groep. Voorafgaand aan het dieet waren de Zuid Aziaten meer insulineresistent dan de blanke controles: ze hadden hogere insulinewaarden zowel in nuchtere toestand als tijdens orale glucosebelasting, en een lagere lever en perifere insulinegevoeligheid. Verder was de insuline klaringssnelheid lager en het levervet hoger in de Zuid Aziatische proefpersonen. Opslag van vet in de lever is geassocieerd met insulineresistentie van de lever. De lagere perifere insulinegevoeligheid in Zuid Aziaten leek het gevolg te zijn van een verminderde NOGD. Dit suggereert een defect in glycogeenopslag, één van de belangrijkste problemen in patiënten met type 2 diabetes. Er werden echter geen verschillen tussen de groepen gevonden in de expressie van eiwitten en genen betrokken bij glycogeenafbraak en -opslag, zoals eerder ook in de jonge groep was gezien (**Hoofdstuk 4**). Verder werden voorafgaand aan het dieet geen verschillen gevonden in insuline en mTOR signalering, en was de verbranding van koolhydraten en vetten en de metabole flexibiliteit vergelijkbaar.

De Zuid Aziatische proefpersonen reageerden echter metabool anders op het VLCD dan de blanke controles. In beide groepen namen lever- en buikvet af en verbeterde de insulinegevoeligheid van de lever. De blanke controles waren metabool flexibel: ze switchten van glucose naar vetverbranding in de nuchtere toestand en lieten een versterkt insuline effect zien op de glucose- en vetverbranding. In de Zuid Aziatische groep was deze switch echter gestoord na het dieet, zowel in de nuchtere toestand als tijdens de clamp, en was derhalve sprake van metabole inflexibiliteit. Verder werd in de blanke groep geen dieeteffect gezien op de perifere insulinegevoeligheid, overeenkomend met andere kortdurende studies met caloriebeperking en minimaal gewichtsverlies. In de Zuid Aziaten werd juist een lichte verbetering gezien in de perifere insulinegevoeligheid ondanks een afname in insulinewaarden, voornamelijk door een toename in NOGD. Op spierniveau bleken energie/nutriënt-gevoelige signaalroutes anders aangedaan, met een toename in insuline geïnduceerde activatie van de mTOR (ERK-mTOR-S6K1) signaalroute in Zuid Aziaten.

Er is steeds meer bewijs dat mTOR de vetzuurverbranding kan onderdrukken door het remmen van PPARα, een transcriptiefactor. PPARα regelt de transcriptie van genen betrokken in onder meer de opname van vetzuren in de cel en mitochondria en vetzuurverbranding. Inderdaad bleek mRNA expressie van PPARA en enkele doelgenen van

PPARA alleen in Zuid Aziaten significant afgenomen te zijn. Het is dus mogelijk dat de activatie van de mTOR signaalroute in Zuid Aziaten in reactie op caloriebeperking de vetzuurverbranding heeft onderdrukt door het remmende effect van mTOR op PPARα, leidend tot metabole inflexibiliteit.

Een opvallende bevinding was de toegenomen perifere insulinegevoeligheid in Zuid Aziaten, terwijl mTOR hierop juist een remmend effect zou hebben. De toename in perifere insulinegevoeligheid lijkt met name verklaard te worden door een toegenomen NOGD, hoewel er wederom geen verschillen in het glycogeenmetabolisme gevonden werden. Interessant in dit opzicht is dat alleen in de Zuid Aziatische proefpersonen de expressie van AMPK significant toenam na het dieet. AMPK activatie bevordert de glucoseopname in de spier en zou daarom onderliggend kunnen zijn aan de verbeterde NOGD in Zuid Aziaten.

Concluderend reageerden volwassen Zuid Aziatische mannen met overgewicht metabool anders op kortdurende caloriebeperking in vergelijking tot blanke mannen van Kaukasische afkomst. Hoewel de perifere insulinegevoeligheid toenam, was de metabole flexibiliteit gestoord. Dit ging gepaard met een toename in insuline geïnduceerde activatie van de ERK-mTOR-S6K1 signaalroute op spierniveau.

Bruin vet is een recent ontdekte speler in de energie stofwisseling van mensen. **Hoofdstuk 6** geeft een overzicht over de anatomie, fysiologie en functie van bruin vet en beschrijft hoe bruin vet gemanipuleerd zou kunnen worden om het energiegebruik te verhogen en daarmee mogelijk gewichtsverlies te induceren. In tegenstelling tot het witte vet neemt bruin vet glucose en vetten op uit het bloed en verbrandt deze vervolgens tot warmte. Dit mechanisme draagt in baby's bij aan het handhaven van hun lichaamstemperatuur. De hoeveelheid bruin vet neemt in mensen echter snel af na de peutertijd. In 2009 is met een speciale scantechniek (^{18}F-FDG PET-CT scan) echter ontdekt dat volwassen mensen nog functioneel bruin vet hebben. Kou activeert bruin vet; de beste detectiekans heb je daarom door voorafgaand aan het toedienen van het FDG de proefpersoon te koelen. Bruin vet kan in belangrijke mate bijdragen aan het energiegebruik (tot 15-20% van het totale dagelijkse energiegebruik). Een interessante bevinding is dat mensen met overgewicht minder bruin vet lijken te hebben. Verder resulteren bepaalde aandoeningen die leiden tot activatie van bruin vet, zoals een snelle schildklierfunctie en een feochromocytoom (een neuroendocriene (bijnier)tumor dat teveel stresshormonen aanmaakt), tot een verhoogd energiegebruik en gewichtsverlies. Het stimuleren van bruin vet wordt daarom gezien als een mogelijk preventief en therapeutisch doel in onze strijd tegen overgewicht en aan overgewicht gerelateerde ziekten, zoals type 2 diabetes. Verschillende methoden waarop bruin vet gemanipuleerd kan worden zijn geïdentificeerd met wisselend succes, zoals het stimuleren van aanwezig bruin vet door stimulatie van het sympathisch zenuwstelsel, via blootstelling aan kou

en bepaalde medicamenten, en het rekruteren van bruin vet door het stimuleren van de differentiatie van vetcellen naar bruine vetcellen.

Aangezien bruin vet betrokken is in het totale energiegebruik en de klaring van vetten en glucose en daarmee beschermt tegen metabole verstoringen, was onze hypothese dat een laag bruin vet volume of activiteit onderliggend zou kunnen zijn aan het ongunstige metabole fenotype van Zuid Aziaten (overgewicht, gestoorde vetbalans, insulineresistentie) en aan hun vatbaarheid voor diabetes. In **Hoofdstuk 7** is daarom zowel het rust energiegebruik (REE) als het volume en de activiteit van bruin vet onderzocht in de jonge groep met behulp van de geventileerde kapmeting en koude-geïnduceerde ^{18}F-FDG-PET-CT-scans. REE was 32% lager in de Zuid Aziatische proefpersonen. Bovendien lag de temperatuur waarop het rillen begon hoger in deze groep ondanks een hoger totaal vetpercentage, en de koude geïnduceerde 'non-shivering' thermogenese (warmteproductie niet door spieractiviteit (rillen)) was kleiner. Het bruin vet volume was aanzienlijk lager in de Zuid Aziatische groep (34%), terwijl er geen verschil was in de activiteit. Een verklaring voor het lagere bruin vet volume in Zuid Aziaten zou genetisch kunnen zijn (verminderde expressie van signaalmoleculen betrokken bij de differentiatie van bruin vet), en/of omgeving gerelateerd (kleedgedrag, instelling centrale verwarming, en/of eetpatroon). Concluderend zou een laag bruin vet volume onderliggend kunnen zijn aan het hoge risico van Zuid Aziaten tot het ontwikkelen van metabole aandoeningen als obesitas en type 2 diabetes. Het vergroten van het volume of activiteit van bruin vet zou daarom een belangrijk therapeutisch aangrijpingspunt kunnen zijn, mogelijk leidend tot een verhoogde klaring van glucose en vetten en een verhoogd totaal energiegebruik.

DEEL 2: HART- EN VAATZIEKTEN IN ZUID AZIATEN

Hart- en vaatziekten komen veel voor in de algemene bevolking en zijn wereldwijd een belangrijke doodsoorzaak. Onder hart- en vaatziekten vallen hartinfarct, herseninfarct of beroerte, en perifeer vaatlijden (bijv. 'etalagebenen'). De belangrijkste oorzaak is atherosclerose (aderverkalking), wat vaak al vele jaren aanwezig is voordat symptomen optreden. Belangrijke oorzaken van atherosclerose zijn leefstijlfactoren zoals roken, ongezonde voeding en te weinig beweging, stress, overgewicht, ongunstig vetprofiel (hoog LDL, laag HDL, hoog triglyceridengehalte), verhoogde bloeddruk (hypertensie) en diabetes. Daarnaast spelen ook erfelijke factoren een rol bij het ontstaan van hart- en vaatziekten.

Net als voor diabetes hebben mensen van Zuid Aziatische afkomst een verhoogd risico op het ontwikkelen van hart- en vaatziekten in vergelijking tot blanke Kaukasische mensen. Zuid Aziatische mensen krijgen eerder een hartinfarct (circa 5 jaar), kennen

een agressiever beloop en hoger overlijdensrisico op jongere leeftijd. Het hoge risico op hart- en vaatziekten in Zuid Aziaten komt waarschijnlijk door een wisselwerking tussen genetische gevoeligheid en omgevingsfactoren. In dit proefschrift worden studies beschreven gericht op het verkrijgen van meer inzicht in de pathogenese van hart- en vaatziekten in Zuid Aziaten en om zo nieuwe aanknopingspunten te vinden voor het ontwikkelen van preventieve maatregelen en behandelopties in deze populatie. Hiertoe werden dezelfde proefpersonen onderzocht als eerder beschreven onder Type 2 diabetes.

In **Hoofdstuk 8** worden mogelijke factoren besproken die bij kunnen dragen aan het verhoogde cardiovasculaire risico van Zuid Aziaten evenals nieuwe therapeutische strategieën. De belangrijkste oorzaak van hart- en vaatziekten is atherosclerose (aderverkalking), wat al vele jaren aanwezig kan zijn voordat symptomen optreden. De ontwikkeling van atherosclerose wordt beïnvloed door zowel metabole als inflammatoire risicofactoren. Metabole of 'klassieke' risicofactoren zijn dyslipidemie (gestoord vetprofiel), abdominaal overgewicht (vet met name in de buik) en insulineresistentie. Inflammatoire of 'niet klassieke' risicofactoren zijn systemische ontsteking, HDL dysfunctie en endotheel dysfunctie. In Zuid Aziaten komen de klassieke risicofactoren veel voor. Een mogelijke bijdragende factor aan de ontwikkeling hiervan is een lager volume van bruin vet, resulterend in een afgenomen vetverbranding en glucoseopname. De klassieke factoren kunnen echter niet volledig het verhoogde risico verklaren. Andere, niet-klassieke risicofactoren moeten daarom aanwezig zijn. Inderdaad is de prevalentie van inflammatoire risicofactoren, waaronder ontsteking van het viscerale (orgaan) vetweefsel, endotheel dysfunctie en HDL dysfunctie hoger in de Zuid Aziatische populatie vergeleken met mensen van blanke Kaukasische afkomst. Concluderend zou het hoge risico op hart- en vaatziekten in Zuid Aziaten mogelijk verminderd kunnen worden door behandelmethoden gericht op het vergroten van het bruin vet volume of activiteit, daarmee de klassieke risicofactoren verminderend. Verder zou anti-inflammatoire therapie de niet-klassieke risicofactoren in deze bevolkingsgroep kunnen verlagen. Een combinatie van beide strategieën zal met name effectief zijn.

In de **hoofdstukken 9 en 10** zijn de cardiale dimensies, cardiovasculaire functie en het vetgehalte van de hartspier onderzocht middels MRI en spectroscopie. De mortaliteit van hart- en vaatziekten bij diabetes is hoger in Zuid Aziaten in vergelijking tot blanke Kaukasiërs. Dit suggereert een hogere cardiale vatbaarheid voor metabole verstoringen. Kortdurende dieetinterventies, zoals hoog vet belasting en caloriebeperking, kunnen gebruikt worden als een metabole stresstest voor het onderzoeken van de cardiovasculaire flexibiliteit.

In **Hoofdstuk 9** is onderzocht of de cardiale dimensies, cardiovasculaire functie en het vetgehalte van de hartspier op jonge leeftijd verschillen tussen Zuid Aziaten en blanke Nederlanders, met het idee dat dit bij zou kunnen dragen aan het verhoogde risico op hart- en vaatziekten in Zuid Aziaten. Verder is de respons op een 5-dagen HFHCD bekeken. Voorafgaand aan het dieet bleken de cardiale dimensies kleiner in Zuid Aziaten, ook na correctie voor hun kleinere lichaamspostuur. Verder werden er verschillen in diastolische en systolische cardiale functie gezien passend bij een langere cardiale relaxatie en contractie. Een 5-dagen HFHCD vergrootte deze verschillen niet, ondanks een significante toename in insulinewaarden in Zuid Aziaten aangevend dat ze meer insulineresistent waren geworden. Tot slot was de pulse wave velocity (PWV) van de aorta hoger (dat wil zeggen een stijvere aorta) in Zuid Aziaten voorafgaand aan het dieet; dit normaliseerde na het dieet. De PWV van de aorta is een krachtige onafhankelijke voorspeller van cardiovasculaire complicaties. Verminderde insulinegevoeligheid en verhoogd LDL-cholesterol zouden causaal gerelateerd kunnen zijn aan de verschillende cardiale functieprofielen. Een andere mogelijkheid is dat de geobserveerde verschillen in cardiale dimensies en cardiovasculaire functie aangeboren zijn en simpelweg representatief zijn voor verschillende normale referentiewaarden in deze twee etnische groepen. Of de waargenomen verschillen bijdragen aan de hogere incidentie van hart- en vaatziekten in Zuid Aziaten moet nog worden vastgesteld. Ze kunnen in ieder geval niet verklaard worden door een verschillende metabole respons op een kortdurend hoog vet dieet, aangezien een 5-dagen HFHCD de waargenomen verschillen niet vergrootte ondanks duidelijke metabole effecten. Het is echter mogelijk dat een langer durend HFHCD nodig is om veranderingen teweeg te brengen.

In **Hoofdstuk 10** is onderzocht of de metabole en functionele cardiovasculaire flexibiliteit op caloriebeperking verschillend is tussen volwassen Zuid Aziaten en blanke Kaukasiërs. Eerdere studies in gezonde proefpersonen en mensen met overgewicht en diabetes met en zonder hart- en vaatziekten van blanke Kaukasische afkomst toonden een vergelijkbare metabole en functionele flexibiliteit van het hart aan in reactie op zowel kort- als langdurende caloriebeperking. Het is echter onbekend of caloriebeperking vergelijkbare effecten heeft in Zuid Aziaten. In deze studie werd daarom de volwassen groep onderzocht voor en na een 8-dagen VLCD. Voorafgaand aan het dieet bleken de cardiale dimensies kleiner ondanks correctie voor hun kleinere postuur, en was de PWV in de aorta hoger in Zuid Aziaten, conform onze bevindingen in de jonge groep. De hogere PWV zou toegeschreven kunnen worden aan de hogere insulinewaarden. Langdurig verhoogde insulinewaarden, zoals bij insulineresistentie en diabetes, kunnen de elastische functie van de aorta verminderen. Systolische en diastolische functie, vetgehalte in de hartspier en rondom het hart waren niet significant verschillend. Na het VLCD, steeg het vetgehalte in de hartspier in beide groepen met 69%. Hoewel een verhoogd myocardiaal vetgehalte bij insulineresistentie geassocieerd is met een ges-

toorde hartfunctie, is de toename van myocardiale vetopslag na een kortdurend VLCD een teken van behouden metabole flexibiliteit van het hart. Gezien het hoge risico op hart- en vaatziekten en diabetes in Zuid Aziaten was de hypothese dat de flexibiliteit van het hart afgenomen zou zijn in deze populatie. Het vetgehalte van het hart steeg echter in gelijke mate in beide groepen. Diastolische cardiale functie nam af na het dieet in beide groepen, conform eerdere studies, en kan waarschijnlijk verklaard worden door veranderingen in elastische eigenschappen van de linkerkamer. Daarentegen nam het pericardiale vet (vet rondom het hart) alleen in de Kaukasische groep significant af na het dieet, voornamelijk door een afname in de paracardiale vetlaag. Aangezien de paracardiale vetlaag een voorspeller is voor hart- en vaatziekten, geeft de afname in Kaukasiërs waarschijnlijk minder cardiovasculair risico. Verder nam alleen in de Kaukasische groep de PWV in een deel van de aorta af na het dieet. Dit suggereert dat de grote slagaderen minder flexibel zijn in Zuid Aziaten in respons op caloriebeperking. Dit kan het gevolg zijn van de, waarschijnlijk langdurig bestaande, hogere insulinewaarden met irreversibele veranderingen in de arteriewand tot gevolg. Concluderend zijn de myocardiale vetopslag en diastolische hartfunctie van volwassen insulineresistente Zuid Aziaten net zo flexibel en ontvankelijk voor caloriebeperking als van gematchte, maar minder insulineresistente blanke Kaukasiërs. Daarentegen was sprake van een verschillend dieeteffect op het paracardiale vetvolume en de PWV ten gunste van Kaukasiërs.

Tot slot werd in **Hoofdstuk 11** de HDL functie vergeleken tussen Zuid Aziaten en blanke Kaukasiërs in pasgeborenen, de jonge groep en de volwassen groep.

De belangrijkste oorzaak van hart- en vaatziekten is atherosclerose (aderverkalking). Atherosclerose ontstaat door een continue afzetting van vetten en cholesterol in de binnenwand van slagaders (juist onder het endotheel). De ontwikkeling ervan start al vele jaren voordat er symptomen ontstaan en begint met schade en dysfunctie van de binnenwand van slagaders (het endotheel), bijvoorbeeld door een te hoge bloeddruk, waardoor er ontstekingscellen worden aangetrokken naar de plekken met endotheelschade. Een te hoog LDL-cholesterol (het 'slechte' cholesterol) is een belangrijke factor die het risico op aderverkalking verhoogt. LDL kan geoxideerd raken en op den duur door de vaatwand heen dringen en vervolgens ter plekke neerslaan. Deze geoxideerde LDL-deeltjes worden opgeruimd door macrofagen, een bepaald type ontstekingscel, met gevolg dat deze cellen gaan schuimen. De schuimcellen hopen zich op in de vaatwand en vormen uiteindelijk een vetophoping (een plaque). Dit trekt weer ontstekingscellen aan wat de ontwikkeling van atherosclerose verder in stand houdt. De plaque kan verder groeien en zo vernauwing van het bloedvat veroorzaken met belemmering van de bloedstroom tot gevolg. Ook kan er een hard kapje (fibreuze kap) over de plaque gevormd worden als een soort bescherming. Echter, als de plaque blijft groeien kan het kapje scheuren, waardoor er een bloedstolsel gevormd wordt met mogelijk complete

blokkade van het bloedvat tot gevolg. Hierdoor krijgt het weefsel achter de blokkade geen zuurstof meer met tot gevolg dat dit weefsel afsterft (infarct).

Dysfunctie van HDL (het 'goede' cholesterol) is geassocieerd met hart- en vaatziekten. De cardiovasculaire beschermende effecten van HDL worden toegeschreven aan enkele anti-atherogene eigenschappen, waaronder 1) het voorkomen van LDL oxidatie, een belangrijke stap in het begin en de progressie van atherosclerose, 2) anti-inflammatoire eigenschappen, 3) stimulatie van cholesterol efflux uit macrofaag schuimcellen in atherosclerotische plaques naar de lever met uiteindelijk uitscheiding van cholesterol in gal, en 4) het induceren van vaatverwijding door stimulatie van NO (stikstof oxide) afgifte door endotheelcellen, waardoor er minder spanning staat op de vaatwand en daardoor minder ontwikkeling van atherosclerose.

Interessant is dat meerdere studies bij herhaling lagere HDL-cholesterol waarden hebben gevonden in Zuid Aziaten in vergelijking tot mensen van blanke Kaukasische afkomst. Een bijdragende factor aan het hoge risico op hart- en vaatziekten in Zuid Aziaten zou daarom een lage concentratie en/of dysfunctie van HDL kunnen zijn. Deze studie onderzocht de HDL functionaliteit met betrekking tot cholesterol efflux, anti-oxidatie en anti-inflammatie in bloedmonsters van de eerder genoemde groepen. Aangezien de HDL functie beïnvloed kan worden door dieetinterventie werd ook het effect van kortdurende dieetinterventie op de HDL functie onderzocht (5-dagen HFHCD in de jonge groep, 8-dagen VLCD in de volwassen groep).

Deze studie toont aan dat het vermogen van HDL om oxidatie van LDL te voorkomen gestoord was in volwassen Zuid Aziaten. Op jongere leeftijd is deze functie nog vergelijkbaar. Een verklaring voor deze verslechtering zouden exogene factoren kunnen zijn, zoals insulineresistentie en diabetes. Beide zijn geassocieerd met een afname in HDL-cholesterol waarden, veranderde HDL samenstelling en gestoorde HDL functie. Interessant is dat een gestoord anti-oxidatief vermogen van HDL andersom ook lijkt bij te dragen aan het verhoogde risico op diabetes en diabetes gerelateerde complicaties in Zuid Aziaten.

Het anti-inflammatoire vermogen van HDL was aanzienlijk lager in pasgeboren Zuid Aziaten. Dit verschil was niet aanwezig in de jonge en volwassen groep, suggererend dat tijdens de ontwikkeling de verminderde anti-inflammatoire functie zich herstelt. Echter, er is dan waarschijnlijk al wel een basis voor atherosclerose en het daarmee gepaard gaande risico op hart- en vaatziekten gelegd. Kortdurende caloriebeperking op volwassen leeftijd verstoorde alleen in de Zuid-Aziatische proefpersonen de anti-inflammatoire capaciteit. Dus in plaats van gunstig te zijn, lijkt caloriebeperking eerder schadelijk voor Zuid Aziaten wat betreft de anti-inflammatoire functie. Mogelijk is deze verslechtering alleen aanwezig in de caloriebeperkte toestand, en normaliseert deze zich weer na gewichtsverlies en herintroducering van een normaal dieet. Dit zou deels

het gevolg kunnen zijn van de hogere afgifte van pro-inflammatoire cytokinen door vetcellen in Zuid Aziaten, die verergerd kan worden in geval van caloriebeperking.

Tot slot was het vermogen van HDL om cholesterol efflux te induceren vergelijkbaar tussen de bevolkingsgroepen. In beide groepen nam de cholesterol efflux toe na een 5-dagen HFHCD en af na een 8-dagen VLCD.

Concluderend hebben we aangetoond dat de anti-inflammatoire functie van HDL verminderd was in Zuid Aziatische pasgeborenen, en significant gestoord was na kortdurende caloriebeperking in volwassen Zuid Aziaten. Verder was de anti-oxidatieve capaciteit van HDL gestoord in volwassen Zuid Aziaten, wat nog niet het geval was op jonge leeftijd, en daarom mogelijk het gevolg is van exogene factoren. Deze verstoringen in HDL functionaliteit zouden bij kunnen dragen aan het overmatige risico op hart- en vaatziekten, en mogelijk ook diabetes, in mensen van Zuid Aziatische afkomst.

CONCLUSIE EN TOEKOMSTIG ONDERZOEK

De etnische ongelijkheid tussen Zuid Aziaten en blanke Kaukasiërs in het risico op diabetes en hart- en vaatziekten is waarschijnlijk te wijten aan verschillen in genen, maar ook aan andere programmeringsinvloeden (gedurende de afgelopen eeuwen maar bijvoorbeeld ook door ongunstige omstandigheden in de baarmoeder zoals bij maternale ondervoeding of hyperglykemie), leidend tot het huidige ongunstige metabole Zuid Aziatische fenotype. Dit fenotype was oorspronkelijk gunstig voor de overleving onder bepaalde nadelige omstandigheden, zoals terugkerende hongersnood, ongunstige klimatologische omstandigheden en bepaalde infectieziekten, maar past nu niet meer bij de moderne Westerse levensstijl en langere levensverwachting. Daarnaast zullen ook demografische en omgevingsfactoren secundair aan verstedelijking hebben bijgedragen aan de gevoeligheid van Zuid Aziatische mensen voor het ontwikkelen van diabetes en hart- en vaatziekten. Dit proefschrift had als doel meer inzicht te krijgen in de pathogenese van deze aandoeningen in Zuid Aziaten. Het lijkt erop dat bovenstaande factoren niet één, maar meerdere belangrijke metabole mechanismen beïnvloed hebben. Een aantal van deze mechanismen zijn in dit proefschrift onderzocht.

Ten eerste de *insuline signaalroute*. Een kortdurende HFHCD verminderde al de perifere insulinegevoeligheid in de jonge Zuid Aziatische groep, en in de oudere groep was deze al in de uitgangssituatie lager. In beide leeftijdsgroepen leek dit het gevolg te zijn van een afgenomen NOGD wat een probleem in de glycogeenopslag suggereert. Op spierniveau werden echter geen verschillen gevonden in expressie van eiwitten en genen betrokken bij de afbraak en opslag van glycogeen. Daarnaast waren er geen verschillen in de insuline signaalroute, expressie van metabole genen of mitochondriele elektronentransportketen (een indirecte maat voor de mitochondriële functie). Een

andere bevinding was dat hogere insulinewaarden tijdens een OGTT in Zuid Aziaten gepaard gingen met hogere waardes van GLP-1, waarschijnlijk als aanpassing aan de insulineresistentie. Verder onderzoek naar de rol van het glycogeenmetabolisme, mitochondriële functie en GLP-1 is nodig. Ook zou het interessant zijn om de rol van wit vetweefsel te onderzoeken, aangezien ongeveer 10-20% van de insulinegestimuleerde glucoseopname optreedt in wit vetweefsel.

Ten tweede de *energie/nutriënt-gevoelige signaalroutes*. De oudere Zuid Aziatische groep liet een andere metabole aanpassing zien aan kortdurende caloriebeperking in vergelijking tot blanke mannen van Kaukasische afkomst met een toename in insuline geïnduceerde activatie van de ERK-mTOR-S6K1 signaalroute op spierniveau. Dit ging onder meer gepaard met een gestoorde metabole flexibiliteit. Verder onderzoek naar de rol van energie/nutriënt-gevoelige signaalroutes in de glucose- en vetstofwisseling en metabole flexibiliteit in spier en wit vet voor en na inspanning en langer durende dieetinterventies zou interessant zijn.

Ten derde *bruin vet*. Bruin vet is betrokken in het totale energiegebruik. Het volume van bruin vet bleek lager te zijn in de jonge groep Zuid Aziaten. Toekomstig onderzoek moet bekijken wat de onderliggende oorzaak hiervan is, zoals een verminderde expressie van signaalmoleculen betrokken in de differentiatie van bruin vet. Ook is het interessant om onderzoek te doen gericht op het ontwikkelen van nieuwe strategieën om bruin volume en/of activiteit te vergroten.

Ten vierde *HDL functie*. Er was sprake van een leeftijdsafhankelijke dysfunctie van HDL in de Zuid Aziatische proefpersonen wat mogelijk bij zou kunnen dragen aan hun hoge risico op hart- en vaatziekten. Toekomstige studies moeten bijvoorbeeld gericht zijn op het ontwikkelen van behandelstrategieën die de HDL functionaliteit kunnen verbeteren en of dit daadwerkelijk het cardiovasculaire risico vermindert in Zuid Aziaten.

Ook zijn er mechanismen die niet in dit proefschrift onderzocht zijn maar wel aandacht verdienen. Zoals de *endotheel functie*. Endotheel dysfunctie is betrokken in de ontwikkeling van atherosclerose. Enkele studies hebben al tekenen van endotheel dysfunctie aangetoond in Zuid Aziaten. Het *sympathische zenuwstelsel*. Het sympathisch zenuwstelsel speelt onder meer een rol in het energiegebruik. De mindere toename in vrije vetzuren na caloriebeperking in volwassen Zuid Aziaten en na koude expositie in jonge Zuid Aziaten, samen met een mindere toename in vetverbranding in de volwassen groep en een lagere koude geïnduceerde toename in vetverbranding en bloeddruk in de jonge groep kan wijzen op verschillen in sympathische activatie en/of resistentie voor sympathische activatie. Andere interessante gebieden om nog te onderzoeken zijn bijvoorbeeld de *functie van witte vetcellen*, het *cortisol metabolisme* en het *darm microbioom* (bacteriën in het maag-darmkanaal).

Concluderend zijn er nog tal van veelbelovende onderzoeksgebieden om nieuwe strategieën voor de preventie en behandeling van type 2 diabetes en hart- en vaatziekten in mensen van Zuid Aziatische afkomst te vinden en daarmee uiteindelijk het grote gezondheids- en sociaaleconomische probleem wereldwijd aan te pakken.

List of publications

Meijer JA, Bakker LEH, Valk GD, de Herder WW, de Wilt JH, Netea-Maier RT, Schaper N, Fliers E, Lips P, Plukker JT, Links TP, Smit JA. Radioactive iodine in the treatment of medullary thyroid carcinoma: a controlled multicenter study. *Eur J Endocrinol* 2013;168(5):779-86.

Bakker LEH*, Boon MR*, Meinders AE, van Marken Lichtenbelt WD, Rensen PC, Jazet IM. Brown adipose tissue: the body's own weapon against obesity? *Ned Tijdschr Geneeskd* 2013;157(20):958-64. (Bruin vet: een lichaamseigen middel in de strijd tegen obesitas?)

Bakker LEH*, van Schinkel LD*, Jonker JT, de Roos A, Pijl H, Meinders AE, Jazet IM, Smit JWA, Lamb HJ. Functional and metabolic imaging of the cardiovascular system in young healthy South Asians and Caucasians unveils early differences. *Diabetes Care* 2013;36(10):e178-9.

Bakker LEH*, Sleddering MA*, Schoones JW, Meinders AE, Jazet IM. Pathogenesis of type 2 diabetes in South Asians. *Eur J Endocrinol* 2013;169(5):R99-R114.

Bakker LEH, van Schinkel LD, Guigas B, Streefland TCM, Jonker JT, van Klinken JB, van der Zon GCM, Lamb HJ, Smit JWA, Pijl H, Meinders AE, Jazet IM. A 5-day high fat, high calorie diet impairs insulin sensitivity in healthy, young South Asian men but not in Caucasian men. *Diabetes* 2014;63(1):248-58.

Sleddering MA, Bakker LEH, Janssen LGM, Meinders AE, Jazet IM. Higher insulin and glucagon-like peptide-1 (GLP-1) levels in healthy, young South Asians as compared to Caucasians during an oral glucose tolerance test. *Metabolism* 2014;63(2):226-32.

Bakker LEH*, Boon MR*, van der Linden RAD, Pereira Arias-Bouda L, Smit F, Jukema JW, Tamsma JT, Havekes LM, van Marken Lichtenbelt WD, Jazet IM, Rensen PCN. Brown adipose tissue volume in healthy lean South Asian adults compared with white Caucasians: a prospective, case-controlled observational study. *Lancet Diabetes Endocrinol* 2014;2(3):210-7.

Bakker LEH*, Boon MR*, van der Linden RAD, Pereira Arias-Bouda L, van Marken Lichtenbelt WD, Jazet IM, Rensen PCN. Supraclavicular skin temperature as a measure of 18F-FDG uptake by BAT in human subjects. *Plos One* 2014;9(6):e98822.

Bakker LEH, Guigas B, van Schinkel LD, van der Zon GCM, Streefland TCM, Lamb HJ, Smit JWA, Pijl H, Meinders AE, Jazet IM. Middle-aged overweight male South Asians exhibit a different metabolic adaptation to short-term energy restriction compared with Europeans. *Diabetologia* 2015;58(1):165-77.

Bakker LEH*, Wijngaarden MA*, van der Zon GCM, 't Hoen PAC, Willems van Dijk K, Jazet IM, Pijl H, Guigas B. Regulation of skeletal muscle energy/nutrient-sensing pathways during metabolic adaptation to fasting in healthy humans. *Am J Physiol Endocrinol Metab* 2014;307(10):E885-95.

Bakker LEH*, Boon MR*, Haks MC, Quinten E, van Schinkel LD, van Beek L, Wang Y, van Harmelen V, Meinders AE, Ottenhoff THM, Willems van Dijk K, Guigas B, Jazet IM, Rensen PCN. Short-term high-fat diet increases macrophage markers in skeletal muscle accompanied by impaired insulin signaling in healthy male subjects. *Clin Sci* 2015;128(2):143-51.

Bakker LEH*, Boon MR*, van der Linden RAD, van Ouwekerk A, de Goeje PL, Counotte J, Jazet IM, Rensen PCN. High prevalence of cardiovascular disease in South Asians: novel insights in classical and non-classical risk factors including BAT. *Accepted for publication in Crit Rev Clin Lab Sci.*

Bakker LEH*, van Schinkel LD*, Jonker JT, de Roos A, Pijl H, Meinders AE, Jazet IM, Lamb HJ, Smit JWA. Cardiovascular flexibility in middle-aged overweight South Asians *vs.* white Caucasians: response to short-term caloric restriction. *Accepted for publication in Nutr Metab Cardiovasc Dis.*

Bakker LEH*, Boon MR*, Annema W, Dikkers A, Jukema JW, Havekes LM, Meinders AE, Tietge UJF, Jazet IM, Rensen PCN. South Asians exhibit disturbed HDL functionality as compared to white Caucasians. *Submitted.*

Curriculum Vitae

Leontine Erica Henriëtte Bakker werd geboren op 11 februari 1981 te Leiden. Na het behalen van haar gymnasiumdiploma aan het Stedelijk Gymnasium te Leiden in 1999 startte zij met de studie Informatica aan de Universiteit van Leiden, waarvoor zij in 2003 haar Bachelor diploma behaalde. Haar bachelorproject verrichtte zij op de afdeling Klinische Informatiekunde in het LUMC onder begeleiding van Prof.dr. J.H.M. Zwetsloot-Schonk en ging over de herinrichting van de informatieuitwisseling voor het zorgtraject Dementieel Syndroom. In 2003 startte zij met de studie Geneeskunde aan de Universiteit van Leiden. In het derde jaar van deze studie deed zij een public health stage van de IFMSA in Malawi. Tijdens de doctoraalfase was ze betrokken bij wetenschappelijk onderzoek op de afdeling Endocrinologie van het LUMC gericht op het medullair schildkliercarcinoom onder begeleiding van J.A.A. Meijer en Prof.dr. J.W.A. Smit. Aansluitend volgde zij hier haar wetenschapsstage. In 2009 behaalde zij zowel het doctoraal- als het artsexamen *cum laude*. Van oktober 2009 tot en met januari 2010 was zij kortdurend werkzaam als arts-assistent niet in opleiding op de afdeling interne geneeskunde van het Bronovo Ziekenhuis te Den Haag. In februari 2010 startte ze vervolgens met haar promotieonderzoek op de afdeling Endocrinologie in het LUMC onder begeleiding van Dr. I.M. Jazet, Prof.dr. A.E. Meinders en Prof.dr. H. Pijl. De resultaten van dit onderzoek staan beschreven in dit proefschrift. In juli 2013 is zij begonnen met de opleiding Interne Geneeskunde in het Rijnland Ziekenhuis te Leiderdorp (opleiders Dr. M.F.J.M. Janssen en S. Anten, en Prof.dr. J.W. de Fijter)