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.