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

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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.

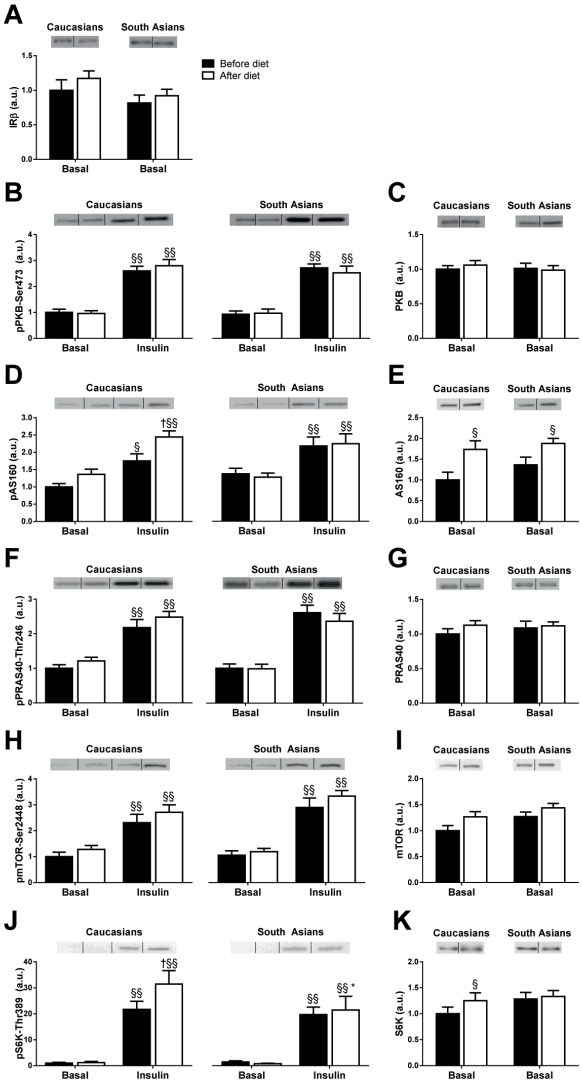

Figure 1. Insulin and mTOR signalling in skeletal muscle from healthy, young South Asian men and matched white Caucasians before (black bars) and after (white bars) a 5-day HFHCD. The protein expression of A. IRβ, B. Ser473-PKB, C. PKB, D. phospho-AS160, E. AS160, F. Thr246-PRAS40, G. PRAS40, H. Ser2448-mTOR, 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.

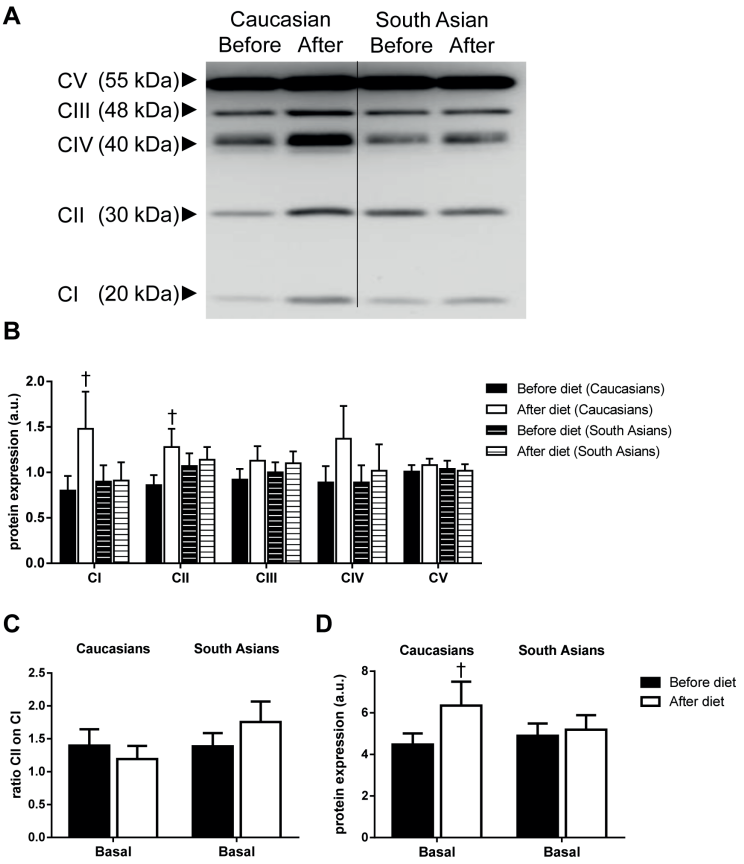

Figure 2. Protein expression of mitochondrial respiratory-chain subunits in skeletal muscle from healthy, young South Asian (striped bars) men and matched white Caucasians (closed bars) before (black bars) and after (white bars) a 5-day HFHCD. A. Representative blots for one subject per group. B. The 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.

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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)

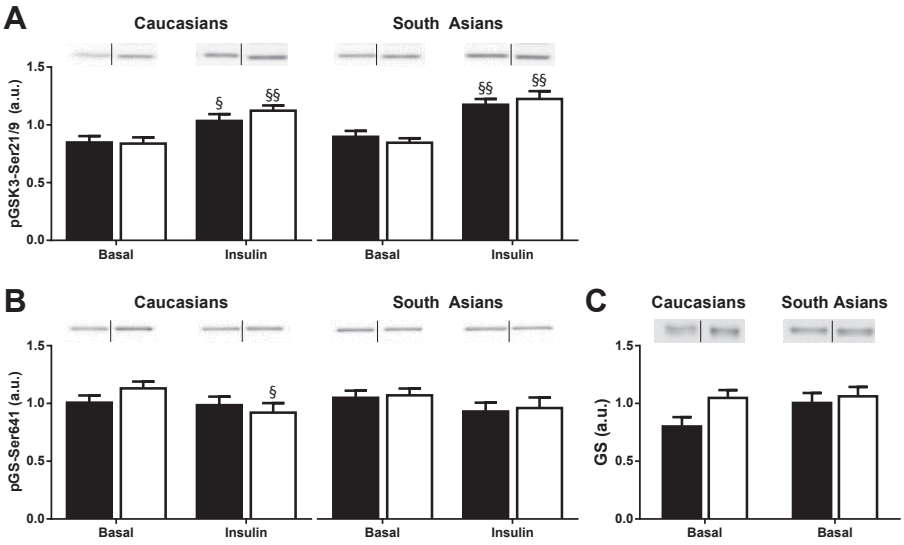
Gene name	Gene symbol	Entrez gene	white Caucasians		South Asians		Interaction p-value
			before	after	before	after	
Mitochondrial metabolism							
Mitochondrial biogenesis							
PPARα, coactivator 1α (PGC-1α)	PPARGC1A	10891	1.00 ± 0.16	**0.52 ± 0.09†**	0.73 ± 0.11	0.44 ± 0.09	0.35
PPARβ, coactivator 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.