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

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ABSTRACT

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

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

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

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

INTRODUCTION

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

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

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

METHODS

Participants

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

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

Study design

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

MR studies

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

Metabolic Studies

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

DNA/RNA isolation and real-time RT-PCR

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

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

Western Blot

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

Statistical analysis

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

RESULTS

Clinical characteristics

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

Fat distribution

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

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

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

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

EGP and glucose disposal

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

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

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

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

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

REE, substrate oxidation and NOGD

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

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

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

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

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

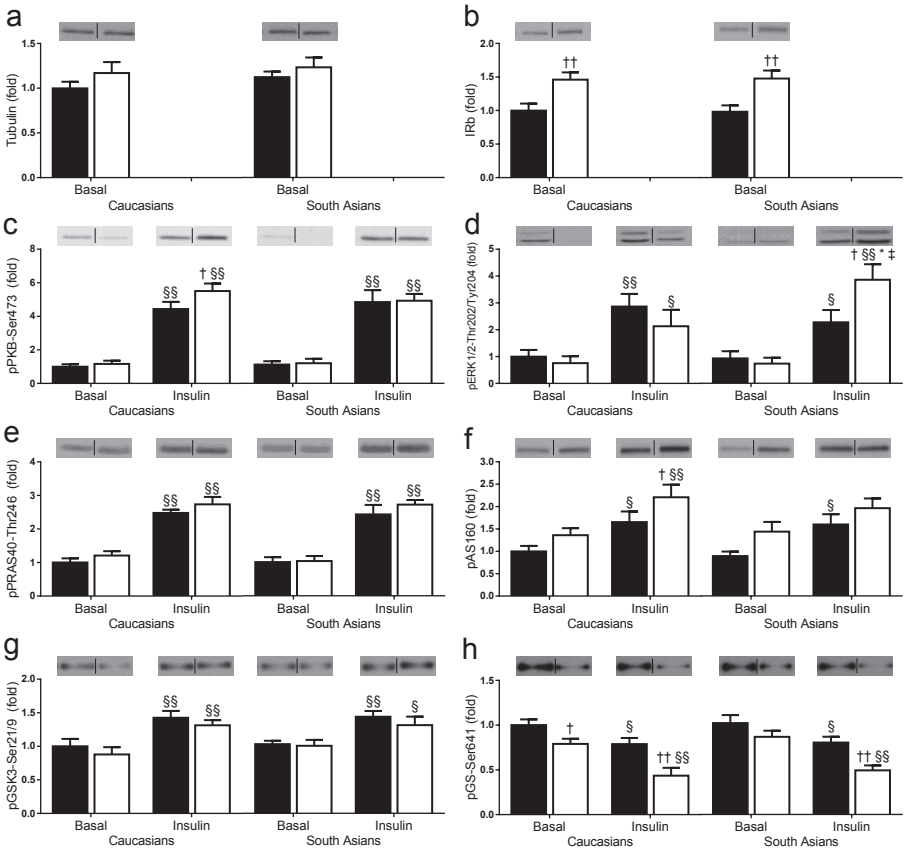

Figure 1. Effect of VLCD on insulin signalling pathways in skeletal muscle from South Asian and white Caucasian men in basal state and during a hyperinsulinemic-euglycemic clamp. The protein expression of tubulin (a), insulin receptor β (b) and the phosphorylation states of Ser473-PKB (c), Thr202/Tyr204-ERK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

Energy/nutrient-sensing signalling pathways in skeletal muscle

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

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

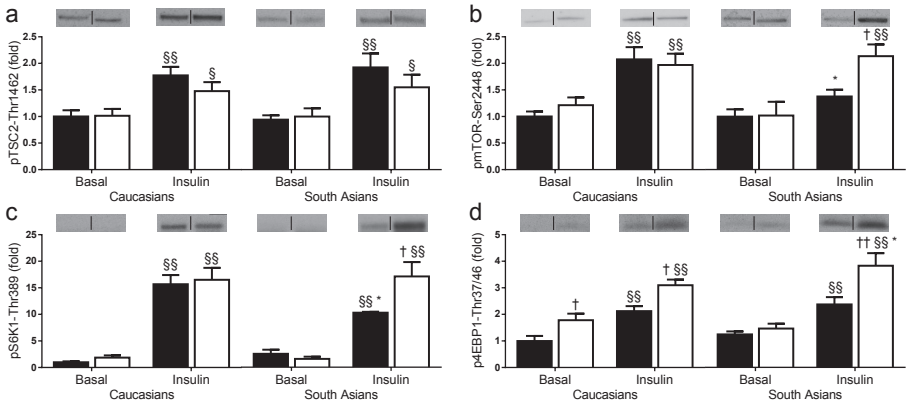

Figure 2. Effect of VLCD on mTOR signalling in skeletal muscle from South Asian and white Caucasian men in basal state and during a hyperinsulinemic-euglycemic clamp. The phosphorylation states of Thr1462-TSC2 (a), Ser2448-mTOR (b), Thr389-S6K1 (c) and Thr37/46-4EBP1 (d) were assessed by Western Blot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

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

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

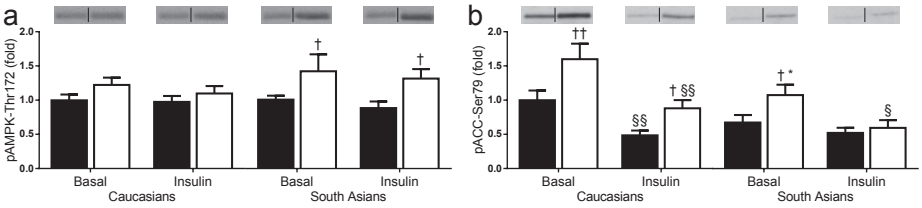

Figure 3. Effect of VLCD on AMPK signalling in skeletal muscle from South Asian and white Caucasian men in basal state and during a hyperinsulinemic-euglycemic clamp. The phosphorylation states of Thr172-AMPK (a) and Ser79-ACC (b) were assessed by Western Blot in the same conditions as described in legend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

Skeletal muscle mitochondrial respiratory-chain content

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

Skeletal muscle metabolic gene expression

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

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

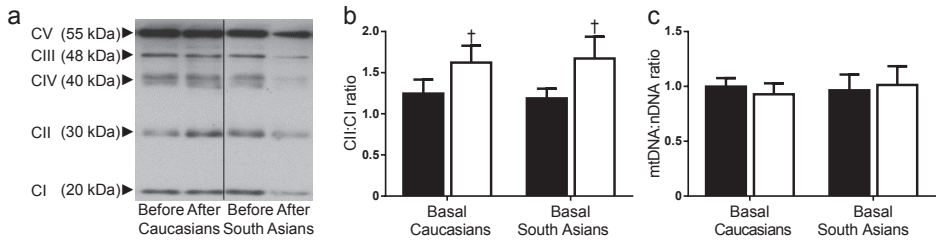

Figure 4. Effect of VLCD on protein expression of mitochondrial respiratory-chain subunits and mtDNA-to-nDNA ratio in skeletal muscle from South Asian and white Caucasian men in basal state and during a hyperinsulinemic-euglycemic clamp. The protein expression of various mitochondrial respiratory-chain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

DISCUSSION

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

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

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

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

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

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

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

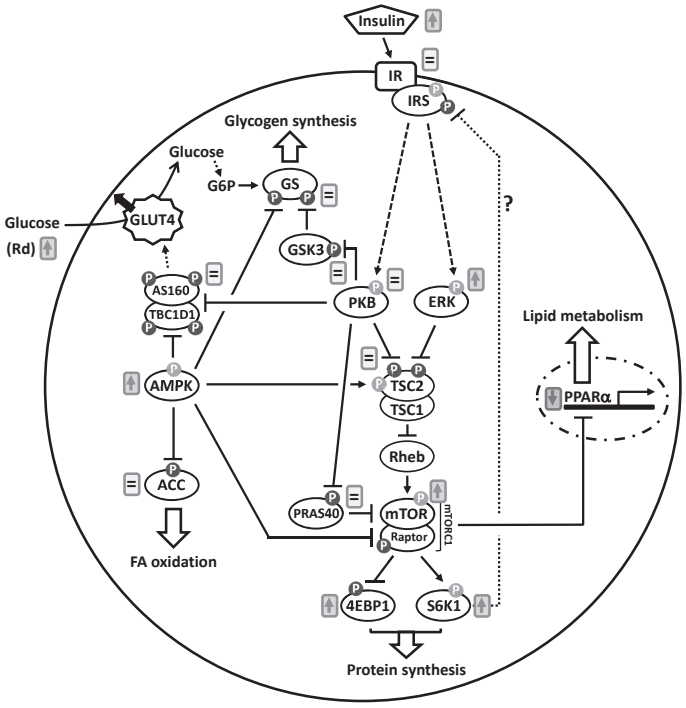

Figure 5. Proposed mechanism(s) underlying the different metabolic adaptations to short-term caloric restriction in South Asian compared to white Caucasian men. The differences in skeletal muscle response to caloric restriction between South Asians and white Caucasians during step 2 of the hyperinsulinemic-euglycemic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

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

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

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

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

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

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

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

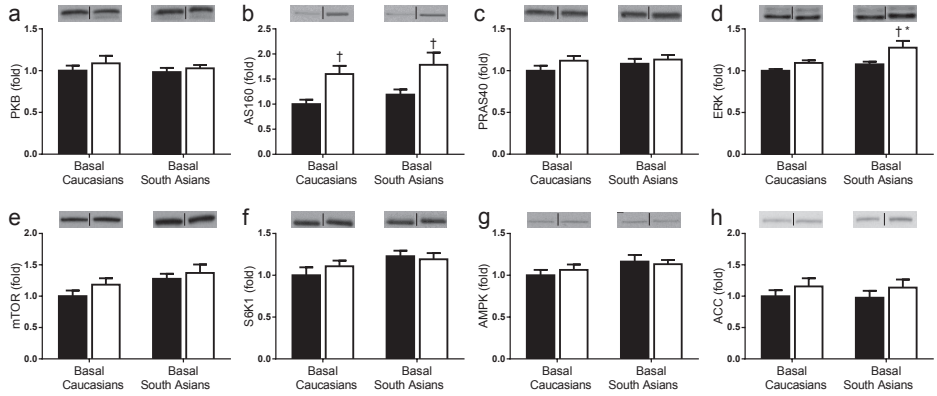
Supplemental Table 1. Overview of metabolic gene expression analysis in skeletal muscle from middle-aged overweight South Asian men and matched white Caucasians before and after an 8-day VLCD. (continued)

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

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

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

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



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