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A 5-Day High-Fat, High-Calorie Diet Impairs Insulin Sensitivity in Healthy, Young South Asian Men but Not in Caucasian Men



South Asians (SAs) develop type 2 diabetes at a younger age and lower BMI compared with Caucasians (Cs). 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 (HFHC) diet between both ethnicities. Twelve healthy, young lean male SAs and 12 matched Cs underwent a two-step hyperinsulinemic-euglycemic clamp with skeletal muscle biopsies and indirect calorimetry before and after a 5-day HFHC diet. Hepatic triglyceride content (HTG) and abdominal fat distribution were assessed using magnetic resonance imaging and spectroscopy. At baseline, SAs had higher insulin clamp levels than Cs, indicating reduced insulin clearance rate. Despite the higher insulin levels, endogenous glucose production was comparable between groups, suggesting lower hepatic insulin sensitivity in SAs. Furthermore, a 5-day HFHC diet decreased the insulin-stimulated (nonoxidative) glucose disposal rate only in SA. In skeletal muscle, no significant differences were found between groups in insulin/mammalian target of

rapamycin signaling, 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 SAs. Thus, distinct adaptation to Western food may partly explain their propensity to develop type 2 diabetes.

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The incidence of type 2 diabetes is increasing rapidly worldwide, especially in people of South Asian (SA) descent (1). SAs originate from the Indian subcontinent and represent one fifth of the world's population. Both native and migrant SAs are at high risk of developing type 2 diabetes compared with Caucasians (Cs) (2–4). Not only is the prevalence of type 2 diabetes four to six times higher, but 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 incompletely understood, and only a few in-depth studies have been conducted to

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See accompanying commentary, p. 53.

investigate the pathogenesis of type 2 diabetes in SAs (11,12).

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

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 signaling (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 FA β -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 SAs.

The aim of this study was to investigate whether the metabolic adaptation to a 5-day HF, high-calorie (HFHC) diet is different between young, healthy lean SA males and matched Cs. 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 HFHC diet. Furthermore, hepatic and peripheral insulin sensitivity, substrate oxidation, abdominal fat distribution, skeletal muscle insulin signaling, and mitochondrial respiratory chain content were assessed.

RESEARCH DESIGN AND METHODS

Subjects

Twelve Dutch SAs and 12 Dutch Cs, lean (BMI <25 kg/m²) and healthy males, 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 Center 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 HFHC diet, consisting of the subject's regular diet supplemented with 375 mL of cream per day (1,275 kcal/day, 94% fat). At the end of the first study day, subjects received 15 125-mL cups of cream. 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 HFHC diet to estimate normal dietary intake, maximize compliance with the diet, and check for compliance and compensation behavior. Diaries were entered and analyzed using a specialized Internet application (<http://www.dieetinzicht.nl>, in Dutch). Compliance was measured by asking subjects to bring leftover cups, asking, analyzing the food diaries, and laboratory parameters. Subjects were instructed not to alter lifestyle habits and not to perform physical activity in the last 48 h before the study days. Magnetic resonance (MR) studies were performed shortly before and on the fifth day of the HFHC diet. Metabolic studies were performed 1 day before and 1 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 (Gyrosan ACS-NT15; Philips, Best, the Netherlands) 4 h 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, Leiden, the Netherlands). The number of pixels were converted to centimeters squared and multiplied by the slice thickness (10 mm). Hepatic triglyceride content (HTG) was assessed by proton MR spectroscopy (26). A spectrum without water suppression, four averages, as an internal standard was obtained, and 64 averages were collected with water suppression. The spectra were fitted using Java-base MR user interface software (jMRUI version 2.2) (26). The percentage of hepatic triglyceride signals was calculated as: (signal amplitude hepatic triglycerides/signal amplitude water) \times 100.

Metabolic Studies

Anthropometric measurements, a two-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 (Bodystat 1500; Bodystat Ltd., Douglas, U.K.).

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 ([6,6-²H₂]-glucose; 0.22 μ mol/kg/min) was used to determine rates

of glucose appearance (R_a) and glucose disposal (R_d). At time (t) = 120 min (step 1) and t = 240 min (step 2), a primed constant infusion of insulin (step 1: 10 mU/m²/min; step 2: 40 mU/m²/min) was started and glucose 20% enriched with 3% [6,6-²H₂]-glucose was infused at a variable rate to maintain the glucose level at 5.0 mmol/L. In basal state (t = 0 min), at the end of the noninsulin-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 FAs (FFAs), and [6,6-²H₂]-glucose-specific activity.

Indirect Calorimetry

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

Skeletal Muscle Biopsies

Muscle biopsies from the medialis vastus lateralis (~75–100 mg) were collected in basal and hyperinsulinemic conditions (at 30 min 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). Nonoxidative glucose disposal (NOGD) was calculated by subtracting the glucose oxidation rate from R_d . The hepatic IR index (HIR) was calculated as the product of noninsulin-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 analyzer (Roche, Mannheim, Germany); serum insulin and C-peptide levels on an Immulite 2500 (Siemens, Breda, the Netherlands); HbA_{1c} on a high-performance liquid chromatography machine, Primus Ultra 2 (Kordia, Leiden, the Netherlands); and plasma FFAs were determined by a colorimetric method (Wako Chemicals, Neuss, Germany). Arterialized whole-blood glucose levels during the clamp were measured by glucose dehydrogenase-NAD technique (Precision Xtra Blood Glucose Monitoring System; Abbott USA, Abbott Park, IL). [6,6-²H₂]-Glucose enrichment was measured in

a single analytical run using gas chromatography-mass spectrometry as described previously (34).

DNA/RNA Isolation and Real-Time PCR

Total RNA was isolated from skeletal muscle biopsies (~25–30 mg) using the phenol-chloroform extraction method (Tripure RNA Isolation reagent; Roche), treated with a DNase kit according to the manufacturer's instructions (TURBO DNase, Life Technologies, Bleiswijk, the Netherlands), and quantified by NanoDrop. First-strand cDNA were synthesized from 1 µg total RNA using a Superscript first strand synthesis kit (Invitrogen, Bleiswijk, the Netherlands). Real-time PCR assays were performed using specific primer sets (sequences provided on request) and SYBR Green on a StepOne Plus Real-Time PCR system (Applied Biosystems). mRNA expression was normalized to ribosomal protein S18 and expressed as arbitrary units. Genomic DNA was extracted using the Qiagen Tissue and Blood Kit (Qiagen, Mainz, Germany), and concentrations were measured spectrophotometrically (GeneQuant; GE Healthcare, Freiburg, Germany). Mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) 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 analyzed genes can be found in Supplementary Table 1.

Western Blot

Skeletal muscle biopsies (~30–45 mg) were homogenized by Ultra-Turrax (22,000 rpm; 2 × 5 s) in a 6:1 (vol/wt) ratio of ice-cold buffer containing: 50 mmol HEPES (pH 7.6), 50 mmol NaF, 50 mmol KCl, 5 mmol NaPPi, 1 mmol EDTA, 1 mmol EGTA, 5 mmol β-GP, 1 mmol Na₃VO₄, 1 mM dithiothreitol, 1% Nonidet P-40, and protease inhibitors cocktail (Complete; Roche). Western blots were performed using phospho-specific (Ser473-protein kinase B [PKB], phospho-Akt substrate, Ser2448-mTOR, and Thr389-ribosomal protein S6 kinase β1 [S6K] from Cell Signaling Technology; Thr246-proline-rich Akt substrate of 40 kDa [PRAS40] from BioSource International) or total primary antibodies (Tubulin, Akt1+2, Akt substrate of 160 kDa; mTOR and S6K from Cell Signaling Technology; PRAS40 from BioSource International; MitoProfile OXPHOS from Abcam; insulin receptor isoform β [IRβ] from Santa Cruz Biotechnology) (36). Blots were quantified by densitometric analysis using ImageJ software (National Institutes of Health).

Statistical Analysis

Data are presented as mean ± SEM when normally distributed or as median (interquartile range [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 modeled as fixed effects, and the subject-specific deviances from the group mean were modeled as random effects. Nonparametric tests (Wilcoxon signed

rank test within group and 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).

RESULTS

Clinical Characteristics

BMI did not differ between groups (SAs: 20.9 ± 0.6 vs. C: 22.2 ± 0.6 kg/m²; $P = 0.11$), but SA subjects were significantly shorter and lighter (Table 1). The percentage of fat mass was significantly higher in SAs 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 SAs after the HFHC diet. Fasting C-peptide levels increased significantly to a similar degree in both groups. HbA_{1c} was higher in SAs, 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 (Supplementary Table 2). The SA diet consisted of fewer calories per day (SA: $2,170 \pm 102$ vs. C: $2,593 \pm 100$ kcal; $P = 0.008$), but corrected for body weight, the amount of calories was similar (SA: 34 ± 2 vs. C: 35 ± 1 kcal/day/kg; $P = 0.91$). Both ethnicities ate the same percentage of fat ($\sim 30\%$), carbohydrates ($\sim 50\%$), and proteins ($\sim 16\%$). Both groups complied

well with the diet. Mean daily calorie intake was $\sim 55\%$ higher compared with their normal diet, and $\sim 54\%$ of energy was derived from fat (Supplementary Table 2).

Fat Distribution

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

Endogenous Glucose Production and R_d

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 SAs compared with Cs before and after the HFHC diet; no diet effect was observed. The MCR_i was significantly lower in SAs 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 SAs. Furthermore, no diet effect was observed. However, the calculated HIR index was higher in SAs compared with Cs ($P = 0.065$ before diet, $P = 0.002$ after diet) and showed a significant increase after the diet only in SAs (P diet effect = 0.008). Suppression of EGP by insulin was comparable between groups and was $\sim 24\%$ 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 SAs

Table 1—Clinical characteristics, body composition, and fasting plasma and serum levels before and after a 5-day HFHC diet in healthy, young South Asian males and matched Caucasians

	Caucasians		South Asians	
	Before	After	Before	After
Clinical characteristics				
Age (years)	22.1 ± 0.6		22.2 ± 0.7	
Height (m)	1.84 ± 0.01		1.74 ± 0.02**	
Weight (kg)	75.1 ± 1.8	75.6 ± 1.8	63.2 ± 2.3**	63.7 ± 2.3†,**
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
HTG 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††,*
Insulin (pmol/L)	34 (32)	49 (46)	49 (29)	73 (34)†††,**,‡‡
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
Triglyceride (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). * $P < 0.05$; ** $P < 0.005$ vs. Caucasians; † $P < 0.05$; †† $P < 0.005$ within group vs. before diet; ††† $P < 0.005$ diet effect vs. Caucasians.

Table 2—Metabolic parameters of a two-step hyperinsulinemic-euglycemic clamp with stable isotopes before and after a 5-day HFHC diet in healthy, young South Asian males and matched Caucasians

	Caucasians		South Asians	
	Before	After	Before	After
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)†,*
EGP = R _d (μmol/kg _{LBM} /min)	16.3 ± 0.4	17.0 ± 0.3	17.5 ± 0.5	17.5 ± 0.4
HIR (μmol*pmol/kg _{LBM} /min/L)	562 (600)	760 (778)	763 (512)	1,269 (520)††,**,‡
MCR _g (mL/kg _{LBM} /min)	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} /min)	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} /min)	15.3 ± 0.8	17.4 ± 1.2	16.2 ± 0.9	15.3 ± 1.1
MCR _g (mL/kg _{LBM} /min)	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} /min)	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} /min)	41.7 ± 2.9	41.0 ± 2.8	48.7 ± 2.9**	39.0 ± 2.1†††,‡‡
R _d /insulin (μmol*L/kg _{LBM} /min/mU)	1.14 ± 0.13	1.07 ± 0.12	0.87 ± 0.07	0.72 ± 0.05†,*
MCR _i (mL/m ² /min)	1,076 (397)	1,054 (270)	735 (70)**	771 (164)†,**,‡
MCR _g (mL/kg _{LBM} /min)	8.8 ± 0.7	8.7 ± 0.6	10.7 ± 0.8**	8.6 ± 0.5†††,‡‡

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. †*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.

compared with Cs before the diet (SA: 48.7 ± 2.9 vs. C: 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 SAs despite similar insulin levels, whereas no diet effect was found in Cs (SA: 39.0 ± 2.1 μmol/kg_{LBM}/min [*P* < 0.001] vs. C: 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 conditions and step 1 of the clamp were comparable for both groups before and after the HFHC diet (Table 3). In step 2, however, glucose oxidation increased significantly in SAs, whereas no diet effect was observed in Cs. Interestingly, NOGD in step 2 was significantly higher in SAs compared with Cs at baseline (*P* < 0.001), but decreased significantly after the HFHC diet only in SAs (SA: 34.4 ± 4.0 vs. 19.3 ± 2.0 μmol/kg_{LBM}/min [*P* < 0.001]; C: 24.1 ± 2.1 vs. 23.8 ± 1.6 μmol/kg_{LBM}/min [*P* = 0.87]; *P* diet effect < 0.001).

Skeletal Muscle Signaling

The protein expression and phosphorylation state of key molecules involved in the insulin and mTOR signaling

pathways were determined in basal condition and during the hyperinsulinemic-euglycemic clamp in skeletal muscle (Fig. 1). A trend for a reduced IRβ expression was observed in SAs. During hyperinsulinemia, the phosphorylation state of key proteins involved in the insulin/mTOR pathway (PKB, Akt substrate of 160 kDa [AS160], PRAS40, mTOR, and S6K1) was significantly increased when compared with basal, as expected (Fig. 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 FA metabolism was determined (Supplementary Table 1).

At baseline, no significant differences between groups were observed in the transcript levels of all analyzed genes. The HFHC diet induced significant downregulation of solute carrier family 2, member 4, glycogen synthase kinase 3A, glycogen synthase 1, glycogen debranching enzyme, protein phosphatase 1 regulatory subunit 3A, pyruvate dehydrogenase kinase 2 (PDK2), acetyl-CoA carboxylase α, and peroxisome proliferator-activated receptors A and D mRNA expression in C subjects, with a comparable response in SAs. Only pyruvate kinase

Table 3—Parameters for indirect calorimetry before and after a 5-day HFHC diet in healthy, young South Asian males and matched Caucasians

	Caucasians		South Asians	
	Before	After	Before	After
Basal				
REE (kcal/day)	1,469 ± 50	1,523 ± 38	1,220 ± 31**	1,224 ± 22**
REE (kcal/day/kg _{LBM})	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	14.3 ± 1.0	13.6 ± 1.1	13.9 ± 1.3	14.7 ± 1.5
Lipid oxidation	2.4 ± 0.3	2.7 ± 0.3	2.7 ± 0.4	2.4 ± 0.5
NOGD	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	16.2 ± 1.6	16.4 ± 1.6	14.3 ± 1.7	14.8 ± 1.5
Lipid oxidation	2.2 ± 0.5	1.9 ± 0.4	2.6 ± 0.5	2.3 ± 0.5
NOGD	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	17.7 ± 1.5	18.2 ± 1.8	14.4 ± 1.2	19.2 ± 1.5†
Lipid oxidation	1.8 ± 0.4	1.6 ± 0.4	2.5 ± 0.4	1.4 ± 0.4
NOGD	24.1 ± 2.1	23.8 ± 1.6	34.4 ± 4.0**	19.3 ± 2.0††,‡‡

Data are presented as mean ± SEM ($\mu\text{mol}/\text{kg}_{\text{LBM}}/\text{min}$). ** $P < 0.005$ vs. Caucasians; † $P < 0.05$; †† $P < 0.005$ within group vs. before diet; ‡‡ $P < 0.005$ diet effect vs. Caucasians.

muscle isozyme 2 was differentially affected in SAs in response to the HFHC diet.

Skeletal Muscle Mitochondrial Respiratory Chain Content

The protein expression of several mitochondrial respiratory chain complex subunits was determined (Fig. 2A). Although at baseline no differences were observed between groups, the expression of respiratory chain complex 1 and 2 was significantly increased after the HFHC diet only in Cs (Fig. 2B). However, the complex 2-on-complex 1 ratio, as a measure of change in fat versus glucose oxidation, was not significantly different between both ethnicities (Fig. 2C). The mtDNA-on-nDNA ratio was significantly lower in SAs compared with Cs, but was not affected in response to the diet (Fig. 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 (Supplementary Table 1).

DISCUSSION

This is the first study in SAs in which a two-step hyperinsulinemic-euglycemic clamp with stable isotopes was performed to measure peripheral and hepatic insulin sensitivity and the first one in this ethnicity that assessed the effect of HF feeding on both insulin sensitivity and skeletal muscle insulin and mTOR signaling. Strikingly, a 5-day HFHC diet was already sufficient to impair insulin-stimulated (nonoxidative) glucose disposal in SAs, while such an effect was not observed in Cs.

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 SAs compared with Cs at baseline in both basal and insulin-stimulated conditions (12,38,40–42). Instead, SAs seemed to have even higher insulin-stimulated peripheral insulin sensitivity. However, insulin levels during the clamp were higher in SAs 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 SAs, which has been shown before (40). The lower MCR_i together with the higher HIR index in SAs indicates lower hepatic insulin sensitivity both at baseline and after the diet.

The difference in above-mentioned findings compared with the 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 HFHC Diet

The mean daily calorie intake during the HFHC diet was ~55% higher compared with their normal diet, and both groups reached ~54% of energy derived from fat compared with ~30% of their normal daily energy intake. HTG increased significantly after the diet in both groups,

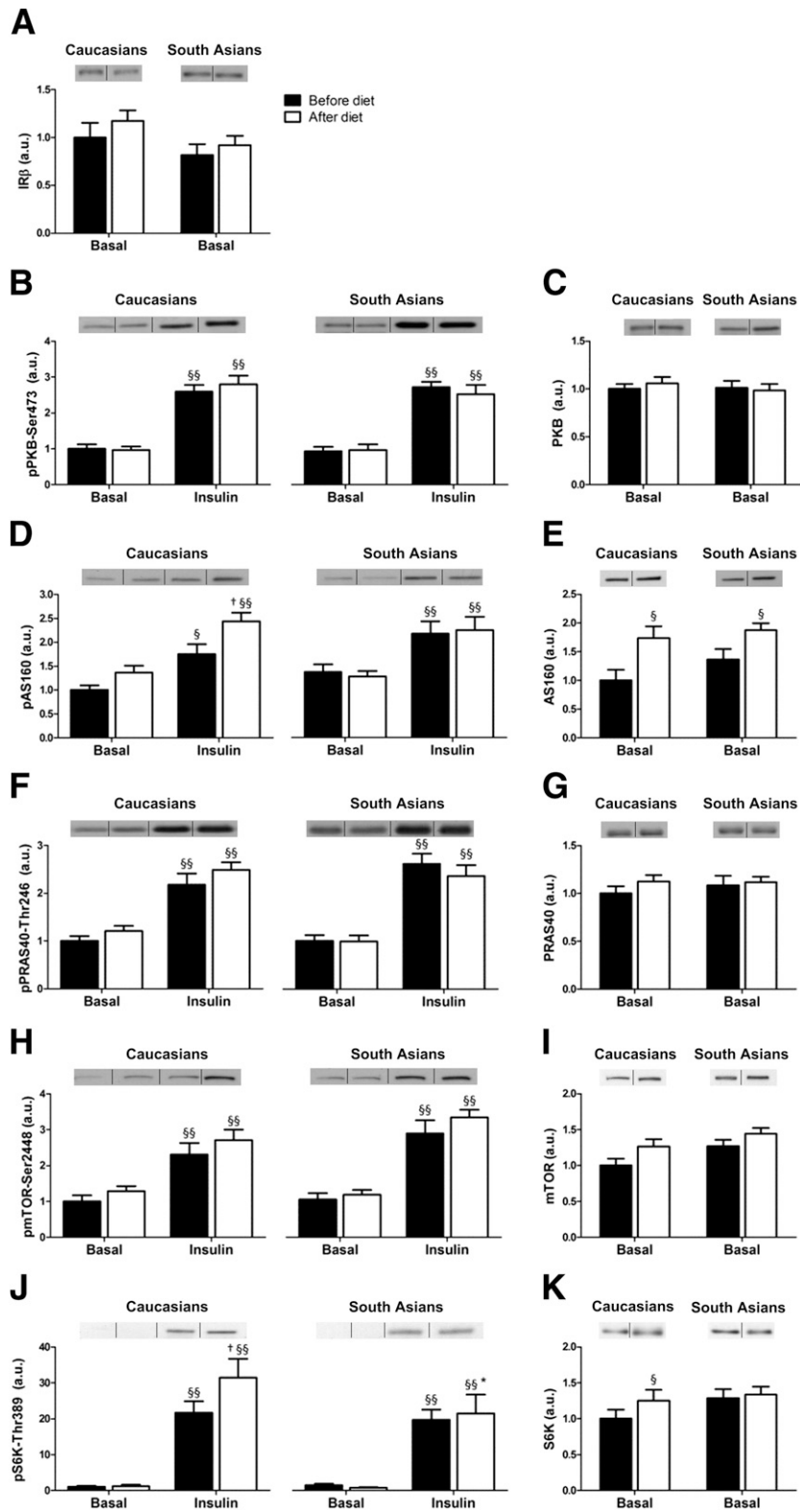


Figure 1—Insulin and mTOR signaling in skeletal muscle from healthy, young SA males and matched Cs before (black bars) and after (white bars) a 5-day HFHC diet. The protein expression of IRβ (A), phospho-(p)PKB–Ser473 (B), PKB (C), pAS160 (D), AS160 (E), pPRAS40–Thr246 (F), PRAS40 (G), pmTOR–Ser2448 (H), mTOR (I), pS6K–Thr389 (J), and S6K (K) were assessed by Western blot. The phosphorylation state in basal and hyperinsulinemic (step 2) conditions (B, D, F, H, and J) or the protein expression in basal conditions (A, C, E, G, I, and K) are shown. Representative blots for one subject per group are shown. Results are normalized to C subjects (before diet, basal condition) and expressed as mean ± SEM. Due to a small amount of tissue, two C subjects were excluded for Western blot analysis. §P < 0.05; §§P < 0.005 within groups vs. basal condition; †P < 0.05 within group vs. before diet; *P < 0.05 vs. Cs. a.u., arbitrary units.

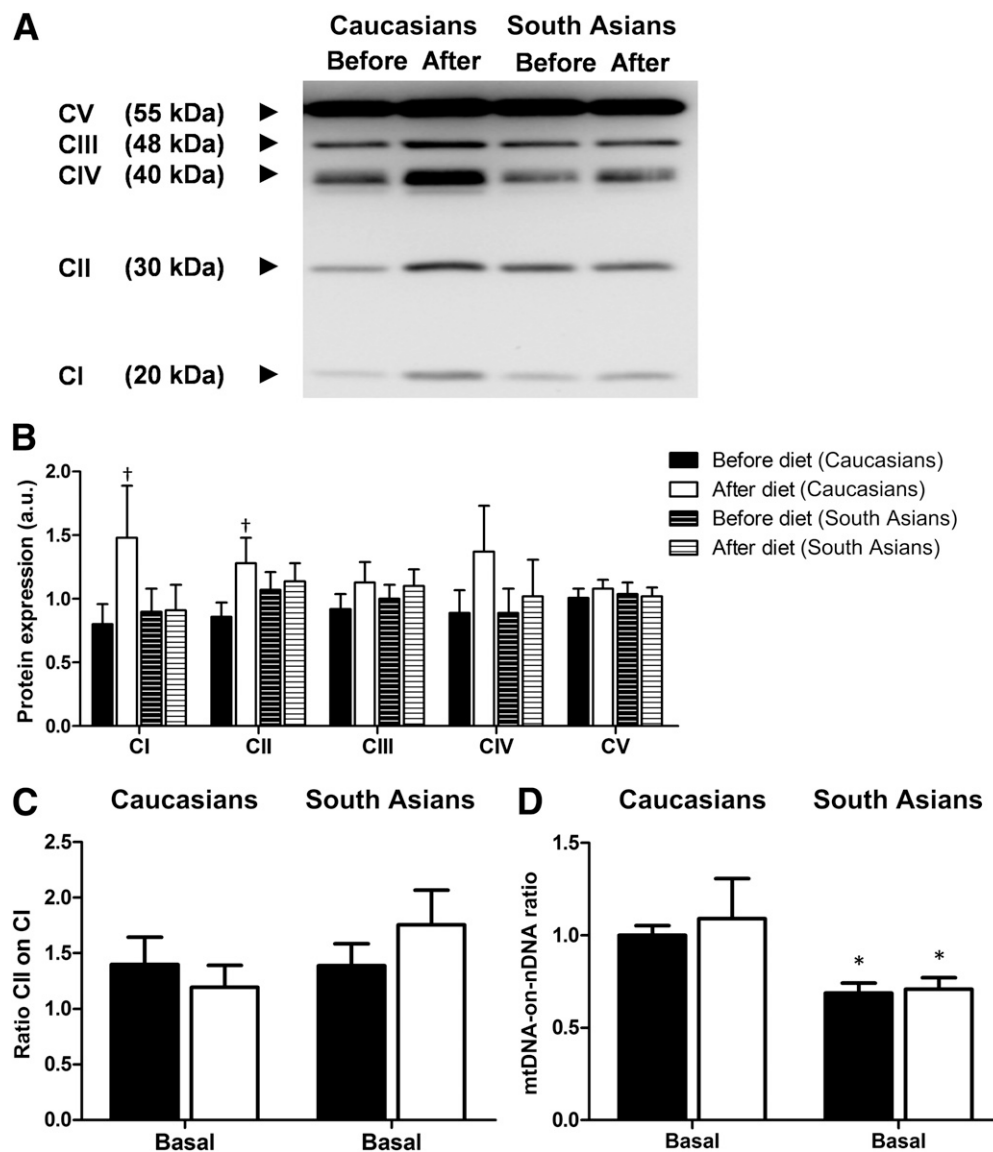


Figure 2—Protein expression of mitochondrial respiratory chain subunits in skeletal muscle from healthy, young SA males and matched Cs before and after a 5-day HFHC diet. *A*: Representative blots for one subject per group. *B*: The expression of various mitochondrial respiratory chain subunits (CI: NDUF8; CII: SDHB; CIII: UQCRC2; CIV: MTCO1; and CV: ATP5A) were assessed by Western blot in basal conditions. *C*: The respiratory chain complex 2-on-complex 1 ratios were calculated. *D*: The mtDNA-on-nDNA ratio as assessed by quantitative PCR in basal conditions ($n = 7/12$ [C/SA]). Results are normalized to C subjects (before diet) and expressed as mean \pm SEM. Due to a small amount of tissue, two C subjects were excluded for Western blot analysis. $\dagger P < 0.05$ within group vs. before diet; $*P < 0.05$ vs. Cs. CI–V, mitochondrial respiratory chain subunits I–V.

indicating good compliance to the diet, and was consistent with a previous study in which young, healthy C males were subjected to a 3-day HF diet (26). In contrast, fasting glucose and insulin levels increased significantly only in SAs. 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 SAs only. Strikingly, insulin-stimulated R_d was significantly impaired after the diet in SAs, whereas no diet effect was observed in Cs.

The response to an HF diet on (skeletal muscle) insulin sensitivity in people of C 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 body weight, and method used to assess insulin sensitivity. In general, HF diets of several hours up to 3 days induce whole-body IR (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 SAs 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 SAs compared with Cs, but this was possibly due to the higher insulin levels in SAs. Because of the impairment in NOGD in SAs after the diet, we also analyzed proteins (Supplementary Fig. 1) and genes involved in glycolysis and glycogen synthesis. However, no obvious differences were found between groups. The mRNA expression of glycogen synthase 1 was significantly reduced in both groups after the diet (Supplementary Table 1). Of note, in contrast to what was observed in SAs in the current study, in several short-term HF-diet studies in Cs, 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 PDK4 and a corresponding decrease in pyruvate dehydrogenase enzyme complex in basal and insulin-stimulated conditions (44,47,48). In the current study, PDK4 was not affected by the diet, and pyruvate dehydrogenase enzyme complex was reduced only in SAs (Supplementary 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 SAs.

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 IR together with increased ectopic fat deposition in SAs versus Cs. Interestingly, mTORC1 appears to have negative effects on insulin signaling (17). There are various mechanisms through which this negative-feedback loop of mTORC1 on insulin signaling is initiated. When activated by mTORC1, downstream target S6K1 can suppress IRS1 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 β -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 IR and type 2 diabetes in SAs. However, we did not find obvious differences in the mTOR pathway between or within groups, neither at baseline nor after a 5-day HFHC diet. 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 signaling, 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 SAs, which is in line with a previous study in which young, healthy C males were subjected to a 5-day HFHC diet (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 with 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 SAs in whom skeletal muscle biopsies were obtained to assess insulin signaling and/or mitochondrial function, and none assessed the mTOR pathway. Nair et al. (12) found no impairment in mitochondrial function in healthy, middle-aged SAs, even despite the finding that they were more insulin resistant than matched Cs. Correspondingly, Hall et al. (11) reported that healthy, young, lean SA males did not exhibit lower expression of skeletal muscle oxidative and lipid metabolism genes compared with matched white Cs 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 SAs was observed. Thus, both studies concluded that mitochondrial dysfunction did not account for the observed IR in SAs, which is in line with our present findings concerning the effect of a HFHC diet. Additionally, the study by Hall et al. (11) showed that SAs had reduced skeletal muscle protein expression of key insulin-signaling proteins in the fasted state. In that study, insulin sensitivity, as measured by the Matsuda insulin sensitivity index, was, however, significantly lower in SAs. Thus, these subjects might have been more insulin-resistant, explaining the reduced expression of insulin signaling proteins as compared with our study. Other possibilities for the different findings on insulin signaling are the larger group size in the study by Hall et al. (11) 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 SAs. Indeed, ~10–20% of whole-body glucose uptake occurs in white adipose tissue, which corresponds to the observed reduction in R_d in SAs (mean percentage decrease: $20 \pm 5\%$).

In conclusion, we showed that a 5-day HFHC diet is already sufficient to affect insulin-stimulated (non-oxidative) glucose disposal in healthy, young, lean SA males, whereas no diet effect was found in age- and BMI-matched Cs, suggesting that the propensity of SAs 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 IR and type 2 diabetes in SAs.

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