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Author: Bakker, Leontine E.H.
Title: Pathogenesis of type 2 diabetes and cardiovascular disease in South Asians : effects
of dietary interventions on metabolism and cardiovascular function
Issue Date: 2015-02-18

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

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ABSTRACT

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

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

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

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

INTRODUCTION

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

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

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

METHODS

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

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

Study design

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

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

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

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

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

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

Calculations

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

Laboratory analysis

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

Statistical analysis

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

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

RESULTS

Clinical characteristics

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

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

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

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

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

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

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

Cardiovascular parameters

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

Glucose and lipid levels

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

Cold-induced thermogenesis

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

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

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

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

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

Core and skin temperature

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

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

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

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

Brown adipose tissue volume and activity

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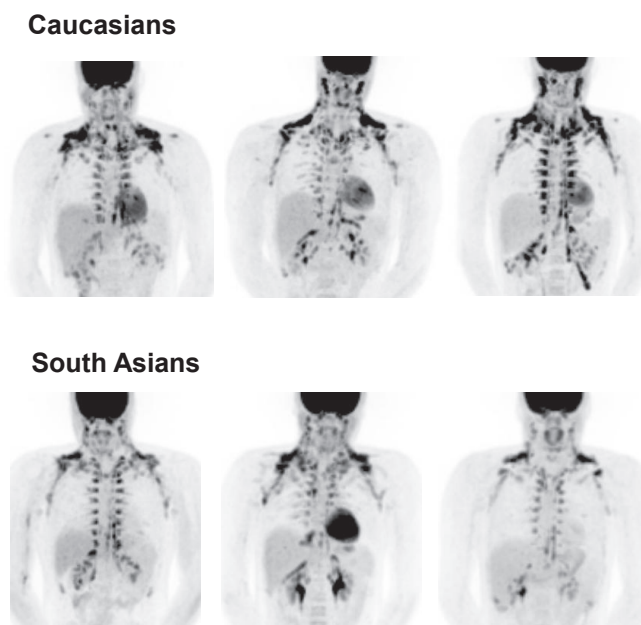
Caucasians

South Asians

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

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

Linear regression analysis showed a clear positive correlation between SUV_{max} and BAT volume (R^2=0.64, β=0.80, p<0.0001) (**Figure 3A**). Furthermore, thermoneutral serum FFA concentration correlated with BAT volume in Caucasian (R^2=0.49, β=0.70, p=0.02), but not in South Asian subjects (R^2=0.0009, β=0.03, p=0.97). Thermoneutral REE tended to correlate positively with BAT volume in both groups, although this correlation did not reach statistical significance per group. After pooling of all subjects, a clear positive correlation was evident between thermoneutral REE and BAT volume (R^2=0.19, β=0.44, p=0.04; **Figure 3B**), also after correction of REE for LBM (R^2=0.27, β=0.52, p=0.01; **Supplementary Figure 2**), strongly suggesting that BAT is involved in total energy metabolism.
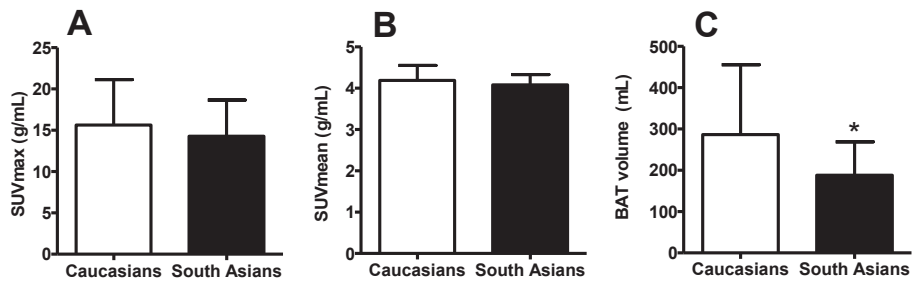
Figure 2. Brown adipose tissue (BAT) activity and volume in healthy young South Asian men and matched white Caucasians as assessed by quantifying ^{18}F-FDG-uptake on PET-CT scans. (A) SUV_{max}, the maximum standardized uptake value (B) SUV_{mean} [g/mL], the average standardized uptake value, and (C) total detectable BAT volume (mL). Results are expressed as mean± SD. * p<0·05 *vs.* Caucasians. BAT, brown adipose tissue. FDG, ^{18}F-fluoro-deoxy-glucose. PET-CT, positron emission tomography and computed tomography. SUV, standard uptake value.
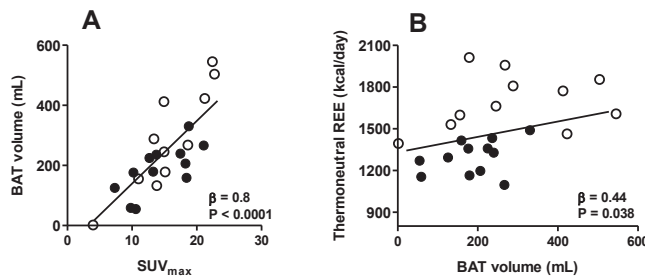

Figure 3. Correlations with brown adipose tissue (BAT). BAT volume in relation to SUV_{max} (A) and thermoneutral REE (B) in healthy young South Asian men (black circles) and matched white Caucasians (white circles). Correlations were determined by linear regression analysis. BAT, brown adipose tissue.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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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SUPPLEMENTARY TECHNICAL APPENDIX

Cooling protocol

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

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

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

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

Temperature registration

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

Calculations

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

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

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

(1)   Respiratory quotient (RQ) = VCO_2 / VO_2

(2)   Lipid oxidation = $1.69*VO_2 - 1.69*VCO_2$

(3)   Glucose oxidation = $4.57*VO_2 - 3.23*VCO_2$

(4)   Resting energy expenditure = $(3.9*VO_2 + 1.1*VCO_2)*1.44$

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

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

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Supplemental figures
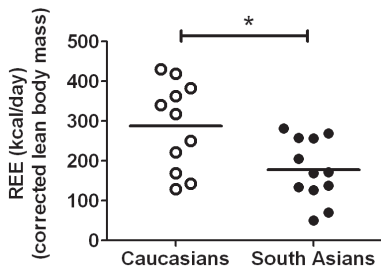
Supplemental figure 1. Resting energy expenditure (REE) after correction for lean body mass. REE in healthy young South Asian men (black circles) and matched white Caucasians (white circles) was corrected for lean body mass by ANCOVA. REE, resting energy expendtiure. *p = 0.03.
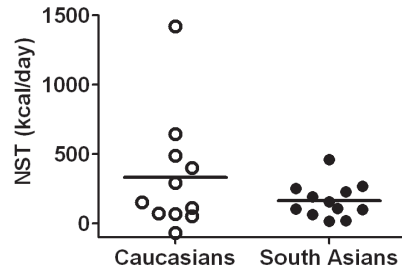
Supplemental figure 2. Individual responses of non-shivering thermogenesis in healthy young South Asian males and matched white Caucasians as assessed by indirect calorimetry. Non-shivering thermogenesis (NST) in healthy young South Asian males (black circles) and matched Caucasians (white circles). p = 0.186.