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Body Fat Partitioning Does Not Explain the Interethnic Variation in Insulin Sensitivity Among Asian Ethnicity: The Singapore Adults Metabolism Study

We previously showed that ethnicity modifies the association between adiposity and insulin resistance. We sought to determine whether differential body fat partitioning or abnormalities in muscle insulin signaling associated with higher levels of adiposity might underlie this observation. We measured the insulin sensitivity index (ISI), percentage of body fat (%body fat), visceral (VAT) and subcutaneous (SAT) adipose tissue, liver fat, and intramyocellular lipids (IMCL) in 101 Chinese, 82 Malays, and 81 South Asians, as well as phosphorylated (p)-Akt levels in cultured myoblasts from Chinese and South Asians. Lean Chinese and Malays had higher ISI than South Asians. Although the ISI was lower in all ethnic groups when %body fat was higher, this association was stronger in Chinese and Malays, such that no ethnic differences were observed in overweight individuals. These ethnic differences were observed even when %body fat was replaced with fat in other depots. Myoblasts obtained from lean South Asians had lower p-Akt levels than those from lean Chinese. Higher adiposity was associated with lower p-Akt levels in Chinese but not in South Asians, and no ethnic differences were observed in overweight individuals. With higher %body fat, Chinese exhibited smaller increases in deep SAT and IMCL compared with Malays and South Asians, which did not explain the ethnic differences observed. Our study suggests that body fat partitioning does not explain interethnic differences in insulin sensitivity among Asian ethnic groups. Although higher adiposity had greater effect on skeletal muscle insulin sensitivity among Chinese, obesity-independent pathways may be more relevant in South Asians.

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South Asians are more insulin-resistant than Caucasians despite similar or even lower BMI (1–3). We have shown that within Asia, South Asians exhibit the greatest insulin resistance, Chinese the least, and Malays are intermediate (4,5). Interestingly, this interethnic difference in insulin resistance was only prominent among lean individuals (2). The association of higher BMI with greater insulin resistance was stronger in Chinese than in other Asian ethnic groups, such that ethnic differences in insulin resistance were no longer evident in overweight or obese individuals (4).

The mechanisms underlying these observations are unclear. One possibility is that individuals from different ethnic groups have a differing propensity to accumulate fat in compartments that are metabolically detrimental, such as within the abdomen, or in the liver or skeletal muscles. Existing evidence suggests that ethnicity does modulate the relationship between total adiposity and the amount of fat in specific depots and lends credence to this hypothesis (1,6,7). However, many of these studies used BMI as a surrogate marker of adiposity and did not accurately measure adiposity. Furthermore, relatively imprecise measures of insulin sensitivity were used.

In this study, we sought to determine whether the propensity to accumulate fat in specific depots with increasing adiposity mediates the interaction between ethnicity, adiposity, and insulin sensitivity. We also measured liver fat content and intramyocellular lipids (IMCL), both being important determinants of whole-body insulin resistance (8,9). Primary cultures of muscle obtained from humans have been shown to retain metabolic characteristics of the donors from which they were taken (10). Thus, we further sought to determine whether abnormalities in skeletal muscle insulin signaling might underlie the interethnic differences in insulin sensitivity.

RESEARCH DESIGN AND METHODS

Subjects

We recruited 264 healthy adult men, comprising 101 Chinese (38.3%), 82 Malays (30.7%), and 81 South Asians (31.1%). The subjects had no prior history of glucose intolerance, hypertension, or dyslipidemia, and all had normal values for fasting blood glucose ($<$7.0 mmol/L). We excluded those with significant changes in diet or weight loss of $>$5 kg, a history of heart disease, epilepsy, insulin allergy, current smoking, a history of ingesting any drug known to alter insulin sensitivity (e.g., corticosteroids), or any hospitalization or surgery during the 6 months before enrollment in our study. Ethics approval was obtained from the National Healthcare Group Domain Specific Review Board (Singapore). All subjects provided informed consent.

Clinical Measurements

Demographic data, medical and drug history, and data on lifestyle factors were collected using interviewer-administered questionnaires. Height was measured using a wall-mounted stadiometer, and weight was measured using a digital scale (SECA, model 803; Vogel & Halke, Hamburg, Germany). BMI was computed using the subject's weight (kg) divided by the square of his height (m). Waist circumference was measured at the midpoint between the lower costal margin and iliac crest during midrespiration. Blood was sampled for glucose, renal function, liver function, lipids, and thyroid-stimulating hormone after a 10-h overnight fast. Body composition was measured using a Hologic Discovery Wi dual-energy X-ray absorptiometry (DEXA) scanner (Hologic, Bedford, MA). Subjects were positioned according to the standard protocol, and all subjects fit within the area of measurement. Manual analyses were performed for regional soft tissue demarcation by three trained DEXA technologists certified by the International Society of Clinical Densitometry.

Insulin sensitivity was assessed after a 10-h overnight fast using the hyperinsulinemic euglycemic clamp technique (11). Insulin was infused at a fixed rate of 40 mU/m^2 body surface area/min for the duration of the clamp (120 min). Blood glucose level was measured every 5 min using the glucose oxidase method (Yellow Spring Glucose Analyzer; YSI Life Sciences, Yellow Spring, OH). The infusion rate of the dextrose 20% solution was adjusted to maintain a constant blood glucose level at ~90 mg/dL (5 mmol/L) throughout the clamp. The insulin sensitivity index (ISI) was calculated using the mean glucose infusion rate and steady-state insulin concentrations (mean 116.8 $\pm$ 35.9 mU/L) during the final 30 min of the clamp.

Biochemical analyses were conducted at the National University Hospital Referral Laboratory, which is accredited by the College of American Pathologists. Serum insulin was measured using a chemiluminescence assay (ADVIA Centaur Analyzer, Siemens Healthcare Diagnostics). Serum total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride values were measured using the automated ADVIA 2400 analyzer (Bayer Diagnostics, Tarrytown, NY).

Magnetic Resonance Spectroscopy of Liver and Skeletal Muscle

Fat content in the liver and skeletal muscle was determined using ^{1}H magnetic resonance (MR) spectroscopy using a 3 Tesla MR scanner (Tim Trio, Siemens). The liver spectra were obtained from a 2- $\times$ 2- $\times$ 2-cm^3 voxel from two locations (right and left lobes) using a point-resolved spectroscopy sequence (echo time [TE] = 30 ms, repetition time [TR] = 2,000 ms) and a Siemens body matrix coil. The acquired spectra were fitted using the linear combination of model spectra (LCModel) (12). The liver fat was determined from the concentration of methyl and methylene groups of lipids and the unsuppressed water signal (13). The fat concentration obtained was corrected for T_2 losses and was

also verified by region-of-interest analysis with Dixon imaging. For skeletal muscle MR spectroscopy, the right leg was positioned in a leg holder, and the foot was aligned to eliminate residual dipolar interactions (14,15). The muscle spectrum was obtained from a 2- × 2- × 2-cm^3 voxel within the soleus muscle using a point resolved spectroscopy sequence (TE = 30 ms, TR = 2,000 ms) and a Siemens Tx/Rx 15-Channel Knee Coil. The amount of IMCL was calculated and expressed as the IMCL-to-creatinine ratio.

MR Imaging of the Abdomen

Abdominal fat images were acquired using two 2-point Dixon sequences (TR = 5.28 ms, TE_1 = 2.45 ms, TE_2 = 3.68 ms, flip angle = 9°, slice thickness = 3 mm) during breath-holds of 18–20 s. The fat volume in each abdominal fat compartment was obtained from 80 axial slices covering the L1 to L5 lumbar vertebrae. A fully automatic graph theoretic segmentation algorithm extracted and estimated the subcutaneous (SAT) and visceral (VAT) adipose tissue volumes (16,17). The segmentation algorithm was a two-step process. First, the fat tissues were separated from the nonfat tissues by thresholding. The extracted fat tissues were then classified into SAT and VAT using a graph cut technique that has been validated on the skull-stripping problem (18). A modified version of the distance regularized level set evolution method was used to separate the deep SAT (DSAT) and superficial SAT (SSAT) depots (19). The segmented image volumes of each of the fat depots were quantified by adding all of the voxels of all of the slices and multiplying by the image resolution. This method provides valid estimates of fat volume compared with manual segmentation, with a Dice similarity index ranging from 0.7 to 0.89, where 0 indicates no overlap between the two methods and 1 indicates perfect overlap (unpublished data, manuscript under review).

Primary Human Myoblast Culture

Percutaneous muscle biopsy specimens were obtained from the belly of the vastus lateralis under local anesthesia. One specimen was put immediately into PBS for myoblast cultures. The other samples were snap frozen in liquid nitrogen and stored at −80°C. For myoblast cultures, muscle tissue was minced and digested with 0.2% collagenase type 1A (C5894; Sigma-Aldrich, St. Louis, MO) for 20 min at 37°C. The cultures were then centrifuged, resuspended in PBS, and passed through a 100-μm filter to remove undigested tissue. The cells were resuspended in Dulbecco's modified Eagle's medium, supplemented with 20% FBS (Invitrogen, Carlsbad, CA), 10% horse serum (Invitrogen), 1% penicillin-streptomycin (Invitrogen), and 1% chick embryo extract (C3999; US Biological Life Sciences, Salem, MA) and enriched for myoblasts by preplating on uncoated plates for 3 h. The supernatant containing the myoblasts was transferred onto 10% matrigel-coated plates (Becton, Dickinson and Co., Franklin Lakes, NJ). All cultures were maintained at 37°C and 5% CO_2.

Phosphorylated Akt Immunoblot Analysis

Primary myoblasts were plated at a density of 15,000 cells/cm^2 and incubated with serum-free minimum essential medium-α for 15 min. Then, 2 mL fresh serum-free minimum essential medium-α was added, and the cells were starved for 16 h, followed by stimulation with or without increasing concentrations (0.01 , 0.1, and 1 μmol/L) of insulin for 15 min at 37°C and 5% CO_2. The myoblasts were then washed with PBS and lysed with radioimmunoprecipitation assay buffer (Sigma-Aldrich; Cat#R0278) and complete protease inhibitor cocktail (Roche). Cell lysates were passed through a 26-gauge needle 10 times and centrifuged to pellet cell debris. Total protein (10 μg) was separated by 4–12% SDS-PAGE (Invitrogen) electrophoresis and transferred to nitrocellulose membrane by electroblotting. The membranes were then blocked in 5% milk in Tris-buffered saline with Tween overnight at 4°C, followed by incubation with specific primary antibodies for 3 h at room temperature. The primary antibodies used for immunoblotting were rabbit anti–phosphorylated (p)-Akt antibody (sc-7985-R; Santa Cruz Biotechnology) and mouse α-tubulin antibody (T-9026; Sigma-Aldrich). The membranes were then washed with Tris-buffered saline with Tween and further incubated with anti-rabbit IgG horseradish peroxidase (HRP) conjugate (Bio-Rad) or anti-mouse IgG HRP conjugate (Bio-Rad) secondary antibodies for 1 h at room temperature. The HRP activity was detected using Western Lightning Chemiluminescence Reagent Plus (NEL104; PerkinElmer Life Sciences, Wellesley, MA) and exposure to autoradiography film. Blots were quantified by densitometric analysis using the GS-800 densitometer (Bio-Rad). All p-Akt immunoblots were normalized against α-tubulin levels (Supplementary Fig. 1).

Statistical Analysis

All analyses were performed using SPSS 17.0 software (SPSS, Inc., Chicago, IL). All values are given as means ± SD unless stated otherwise. $P < 0.05$ was considered to be statistically significant. ANOVA was used for comparisons of continuous variables, with Bonferroni corrections applied for group comparisons.

ANCOVA was used to assess the relationship between the ISI and BMI (Fig. 1*A*) and between ISI and percentage of body fat (%body fat) (Fig. 1*B*). The relationship between ISI with the two measures of adiposity was similar, such that among lean individuals, South Asians were less insulin-sensitive than Chinese or Malays. Although the ISI was lower in all ethnic groups when adiposity was higher, this association was stronger in Chinese and Malays, such that in overweight individuals, no ethnic

differences in the ISI were observed. For the subsequent analyses, we used %body fat as the measure of adiposity.

Pearson correlation analysis was used to assess relationships between %body fat or the ISI and the various fat depots. ISI, IMCL, liver fat, SAT, and DSAT were log-transformed to normalize skewed distributions. Next, we used ANCOVA to determine the relationship between ISI and the various fat depots. ISI was entered as the dependent variable, ethnicity (three levels) as a covariate, and the measures of adiposity (VAT, liver fat, IMCL, SAT, SSAT, DSAT) as independent variables in separate analyses. The cross-product interaction term, "fat depot × ethnicity" was included as an independent variable to test for ethnic differences in the relationship between the ISI and each fat depot. We calculated the predicted values for ISI based on the regression model. The predicted value for the ISI was then plotted against each fat depot, stratified by ethnicity. To assess the relationship between %body fat and each fat depot, similar ANCOVA models were constructed with the fat depot (SAT, VAT, liver fat, IMCL, SSAT, DSAT) as the dependent variable, and %body fat, ethnicity, and "%body fat × ethnicity" as independent variables. All analyses were adjusted for age.

For the in vitro myoblasts study, ANOVA was used to test for insulin-stimulated p-Akt levels, and the Student *t* test was used to compare between Chinese and South Asians for each insulin level.

RESULTS

The mean ± SD for age and BMI was 27.3 ± 5.4 years and 24.2 ± 3.2 kg/m^2, respectively. Chinese were older than South Asians (P = 0.006), had a lower BMI than Malays (P = 0.011), and had a smaller waist circumference than South Asians (P = 0.004) (Table 1). There were no significant differences among the ethnic groups for total cholesterol, LDL-cholesterol, triglyceride, and fasting glucose levels. However, Chinese had higher HDL-cholesterol but lower fasting insulin levels than South Asians. South Asians had lower ISI values than the other two ethnic groups. Significant differences in body composition and fat distribution were also noted among ethnic groups. South Asians had the highest %body fat, IMCL, and SAT (both SSAT and DSAT), followed by Malays and then Chinese. These differences persisted even after adjustment for age. Liver fat content did not differ among the ethnic groups. Age-adjusted VAT showed significant interethnic differences. Chinese had a significantly higher VAT-to-SAT ratio than the other ethnic groups, but this difference disappeared after adjustment for age.

Table 2 reports the correlation between %body fat and ISI with fat depots, adjusted for age. %Body fat was positively correlated with fat volume or the percentage in all fat depots across all ethnic groups. ISI was inversely correlated with fat volume or percentage in all fat depots. However, the correlation between IMCL and ISI did not reach statistical significance in Chinese and South Asians.

Table 3 and Fig. 2 show the effect of ethnicity on the relationship between %body fat and each fat depot. %Body fat was positively and significantly correlated with VAT, SAT, SSAT, DSAT, and liver fat content. Ethnicity had no effects on the relationship between %body fat and VAT, SSAT, and liver fat content. However, statistically significant effect modification by ethnicity was noted for the association between %body fat and SAT, in particular, DSAT. Higher %body fat was associated with greater levels of DSAT in Malays and

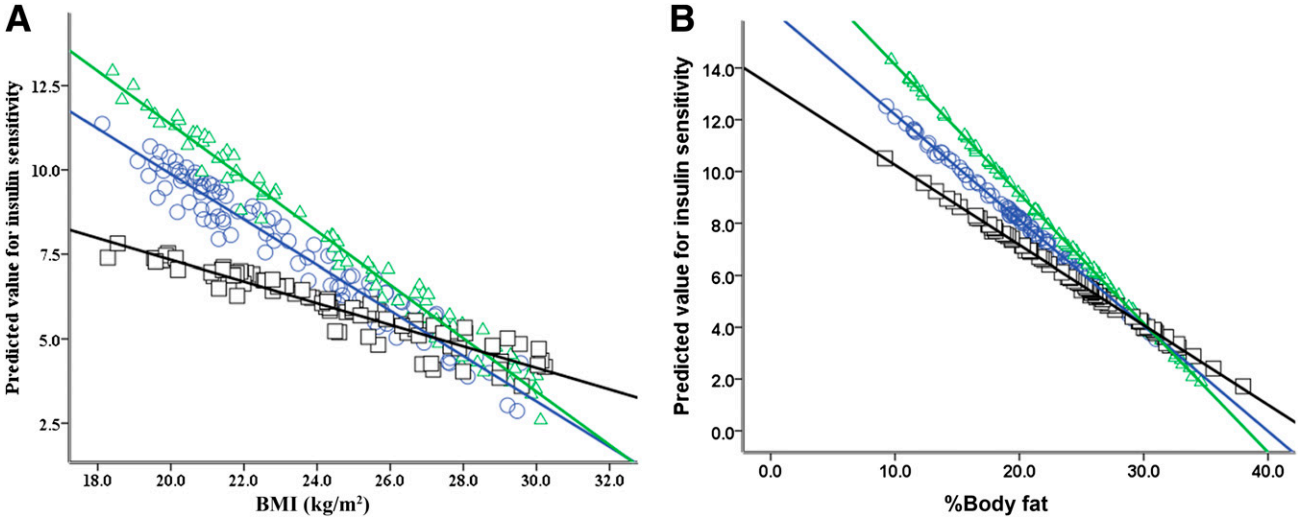

Figure 1—Age-adjusted fitted regression plots of ISI (mg/kg/min per μU/mL) against BMI (kg/m^2) (*A*) and ISI (mg/kg/min per μU/mL) against %body fat (*B*) for Chinese (○), Malay (△), and South Asian (□) subjects. P = 0.010 for ethnicity × BMI interaction variable; P = 0.047 for ethnicity × %body fat interaction variable.

Table 1—Anthropometric characteristics and fat depots of study participants by ethnicity

	Chinese (n = 101)	Malays (n = 82)	South Asians (n = 81)	P ANOVA*	P Adjusted**
Age, years	28.4 ± 6.0	27.6 ± 5.1	25.9 ± 4.6	**0.007**	—
Height, cm	172.2 ± 5.8	171.0 ± 6.4	173.7 ± 6.0	**0.019**	**0.022**
Weight, kg	69.4 ± 10.1	72.7 ± 11.3	74.1 ± 10.8	**0.009**	**0.001**
BMI, kg/m^2	23.5 ± 2.9	24.9 ± 3.5	24.5 ± 3.2	**0.009**	**0.001**
Waist circumference, cm	81.5 ± 8.4	84.4 ± 10.4	86.4 ± 9.4	**0.005**	**<0.001**
Fasting glucose, mmol/L	4.53 ± 0.41	4.50 ± 0.35	4.46 ± 0.32	0.502	0.974
Fasting insulin, pmol/L	10.90 ± 9.62	11.00 ± 7.00	15.51 ± 20.76	**0.044**	**0.030**
Cholesterol, mmol/L					
Total	4.92 ± 0.96	4.93 ± 0.89	4.86 ± 1.00	0.880	0.849
HDL	1.29 ± 0.27	1.21 ± 0.23	1.17 ± 0.23	**0.003**	**<0.001**
LDL	3.13 ± 0.88	3.18 ± 0.81	3.16 ± 0.84	0.914	0.478
Triglycerides, mmol/L	1.10 ± 0.63	1.19 ± 0.72	1.16 ± 0.62	0.581	0.208
Total lean mass, kg	50.69 ± 6.21	51.42 ± 5.74	52.04 ± 5.88	0.312	0.200
%Body fat, %	21.25 ± 5.55	23.34 ± 7.00	24.07 ± 5.95	**0.006**	**<0.001**
ISI, mg/kg/min per μU/mL	7.58 ± 3.62	7.53 ± 4.93	5.87 ± 3.09	**0.007**	**0.001**
Liver fat content, %	9.09 ± 8.14	10.25 ± 10.23	10.35 ± 10.38	0.657	0.119
IMCL	9.12 ± 3.93	10.42 ± 5.08	14.01 ± 7.87	**<0.001**	**<0.001**
SAT, ×10^3 cm^3	2.06 ± 0.97	2.62 ± 1.44	2.85 ± 1.38	**<0.001**	**<0.001**
VAT, ×10^3 cm^3	1.03 ± 0.65	1.12 ± 0.71	1.15 ± 0.69	0.461	**0.003**
VAT-to-SAT ratio	0.51 ± 0.22	0.45 ± 0.19	0.41 ± 0.18	**0.006**	0.101
SSAT, ×10^3 cm^3	1.17 ± 0.40	1.31 ± 0.50	1.38 ± 0.44	**0.011**	**<0.001**
DSAT, ×10^3 cm^3	0.74 ± 0.52	1.15 ± 0.87	1.31 ± 0.89	**<0.001**	**<0.001**

Significant P values are indicated as bold. *P ANOVA value for comparison among the ethnic groups. **P value for comparison among the ethnic groups adjusted for age.

South Asians than in Chinese. A statistically significant ethnic interaction was also noted between %body fat and IMCL, especially between Chinese and South Asians (P = 0.006).

Figure 3 shows the relationships between ISI and various fat depots and the influence of ethnicity on these relationships. ISI was significantly lower, with greater levels of fat volume or percentage in all depots ($P < 0.001$ for all). In general, the patterns of association mirrored that for %body fat and ISI (i.e., South Asians were less insulin-sensitive when fat volume or percentage was low). This association was stronger in Chinese and Malays, such that at higher levels of fat volume or percentage, no ethnic differences in ISI were observed. This was observed in all fat depots, and the interaction term (fat depot × ethnicity) was statistically significant for the

Table 2—Correlation between %body fat and ISI with fat depots by ethnic group, adjusted by age

	Chinese				Malays				South Asians			
	%Body fat		ISI		%Body fat		ISI		%Body fat		ISI	
	r	P	r	P	r	P	r	P	r	P	r	P
Liver fat content, %	0.621	**<0.001**	−0.434	**<0.001**	0.665	**<0.001**	−0.445	**<0.001**	0.526	**0.004**	−0.384	**0.001**
IMCL	0.233	**0.044**	−0.176	0.128	0.510	**<0.001**	−0.391	**0.001**	0.399	**0.010**	−0.203	0.083
VAT, × 10^3 cm^3	0.759	**<0.001**	−0.628	**<0.001**	0.771	**<0.001**	−0.623	**<0.001**	0.738	**<0.001**	−0.575	**<0.001**
SAT, ×10^3 cm^3	0.861	**<0.001**	−0.590	**<0.001**	0.919	**<0.001**	−0.576	**<0.001**	0.883	**<0.001**	−0.471	**<0.001**
SSAT, × 10^3 cm^3	0.838	**<0.001**	−0.566	**<0.001**	0.892	**<0.001**	−0.628	**<0.001**	0.824	**<0.001**	−0.450	**<0.001**
DSAT, × 10^3 cm^3	0.850	**<0.001**	−0.513	**<0.001**	0.899	**<0.001**	−0.574	**<0.001**	0.880	**<0.001**	−0.454	**<0.001**

r, correlation coefficient. Significant correlation is indicated as bold.

Table 3—Effects of ethnicity on the relationship between %body fat and abdominal fat depots (cm^3), hepatic fat content (%), and IMCL

	SAT		VAT		SSAT		DSAT		Liver fat content		IMCL	
	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*
Constant	−930.8	0.002	−1,889.1	<0.001	−242.9	0.025	−547.8	0.007	−16.4	<0.001	0.3	0.911
Chinese	—	—	—	—	—	—	—	—	—	—	—	—
Malay	−573.8	0.122	−24.9	0.909	−102.6	0.427	−473.0	0.048	−2.6	0.609	−4.3	0.159
South Asian	−1,058.8	0.010	−94.3	0.695	7.0	0.017	−1,014.9	<0.001	1.2	0.816	−4.2	0.211
%Body fat	148.4	<0.001	73.3	<0.001	58.3	<0.001	78.5	<0.001	0.6	<0.001	0.1	0.605
Age	−4.9	0.541	48.7	<0.001	12.8	<0.001	−12.7	0.020	0.4	<0.001	0.3	<0.001
Chinese × %body fat	—	—	—	—	—	—	—	—	—	—	—	—
Malay × %body fat	33.2	0.041	−2.2	0.816	4.4	0.440	29.0	0.006	0.1	0.620	0.2	0.074
South Asian × %body fat	57.3	0.001	4.6	0.652	3.9	0.527	53.9	<0.001	0.0	0.897	0.4	0.006
P (ethnicity × %body fat)	**0.005**		0.779		0.717		**<0.001**		0.788		**0.022**	
Model R^2, %	75.9		70.8		77.0		74.4		29.3		30.1	

β, regression coefficient. The analyses were adjusted for age. R^2 indicates the proportion of variance of the dependant variables attributable to ethnicity and %body fat. $P < 0.05$ is considered statistically significant. Significant values are indicated as bold.

relationship between ISI and each of the following: IMCL, SAT, SSAT, and DSAT.

For the in vitro primary myoblast study, we selected myoblasts from 20 subjects (10 Chinese and 10 South Asians) with low %body fat (14.5 ± 2.7%) and 20 subjects (10 Chinese and 10 South Asians) with high %body fat (29.9 ± 3.4%). Figure 4 shows the relative p-Akt levels for unstimulated and insulin-stimulated myoblasts

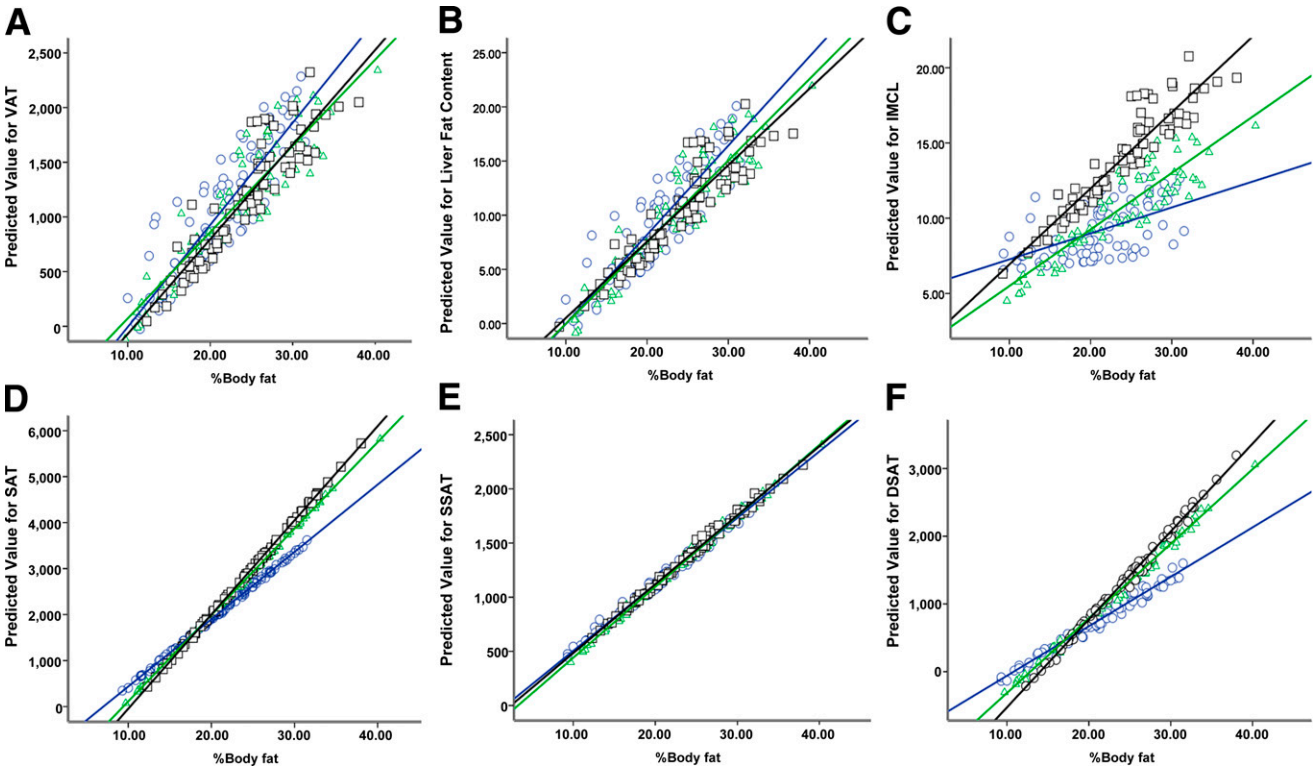

Figure 2—Age-adjusted fitted regression plots of VAT (cm^3), liver fat (%), IMCL, SAT (cm^3), SSAT (cm^3), and DSAT (cm^3) against %body fat for Chinese (O), Malay (Δ), and South Asian (□) subjects. Relationship between %body fat and VAT ($P = 0.779$ for interaction) (*A*), liver fat content ($P = 0.788$ for interaction) (*B*), IMCL ($P = 0.022$ for interaction) (*C*), SAT ($P = 0.005$ for interaction) (*D*), SSAT ($P = 0.717$ for interaction) (*E*), and DSAT ($P < 0.001$ for interaction) (*F*).

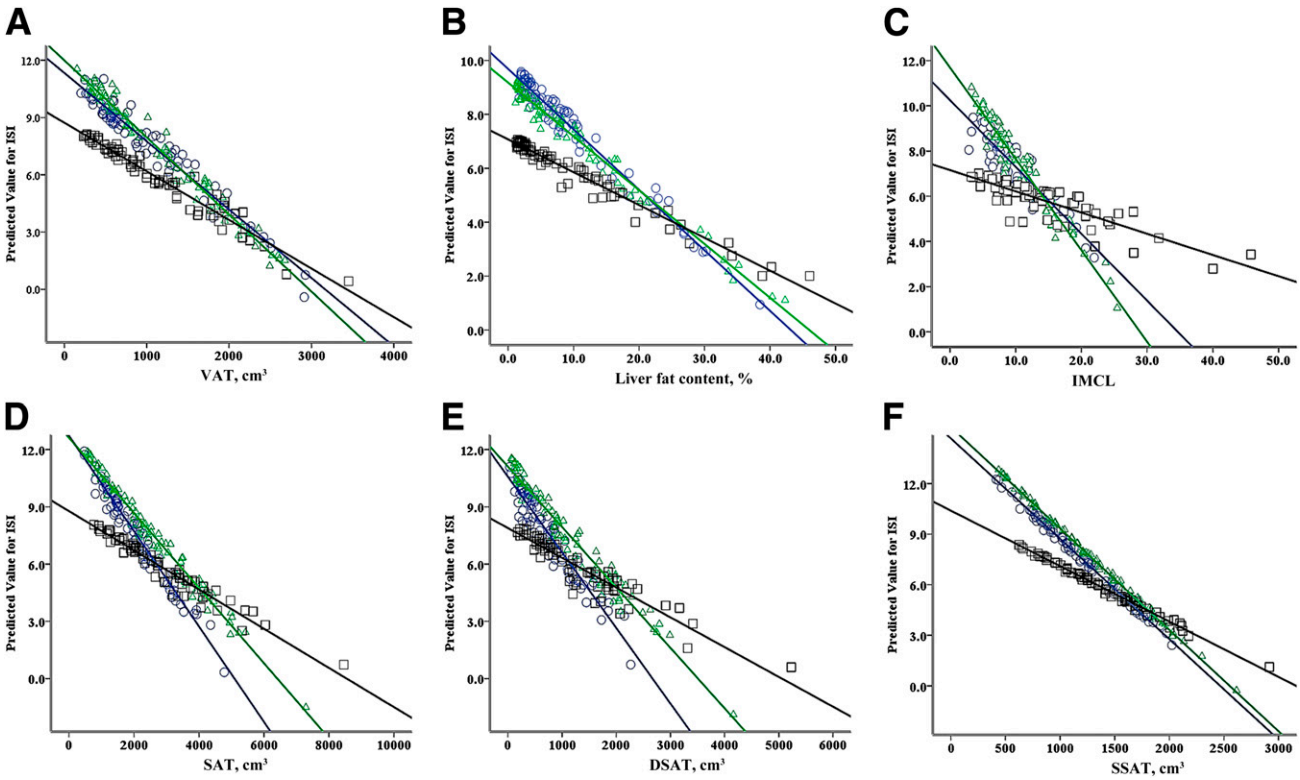

Figure 3—Age-adjusted fitted regression plots of ISI (mg/kg/min per μU/mL) against VAT (cm^3), liver fat (%), IMCL, SAT (cm^3), SSAT (cm^3), and DSAT (cm^3) for Chinese (O), Malay (Δ), and South Asian (□) subjects. Relationship of ISI and VAT ($P = 0.065$ for interaction) (*A*), liver fat content ($P = 0.268$ for interaction) (*B*), IMCL ($P = 0.019$ interaction) (*C*), SAT ($P = 0.016$ for interaction) (*D*), DSAT ($P = 0.011$ for interaction) (*E*), and SSAT ($P = 0.043$ for interaction (*F*).

normalized to α-tubulin levels. As expected, p-Akt levels increased with exposure to increasing insulin concentrations, irrespective of %body fat and ethnic groups. Among those from individuals with a lower %body fat, myoblasts from Chinese had higher basal p-Akt levels compared with myoblasts from South Asians. Compared with lean Chinese, basal and insulin-stimulated p-Akt levels were lower in myoblasts from overweight Chinese. In contrast, little difference was observed between myoblasts from lean and overweight South Asians.

DISCUSSION

In this study, we confirmed that ethnicity modulates the association between adiposity and insulin sensitivity by using a more precise assessment of body fat and insulin sensitivity (hyperinsulinemic euglycemic clamp). This

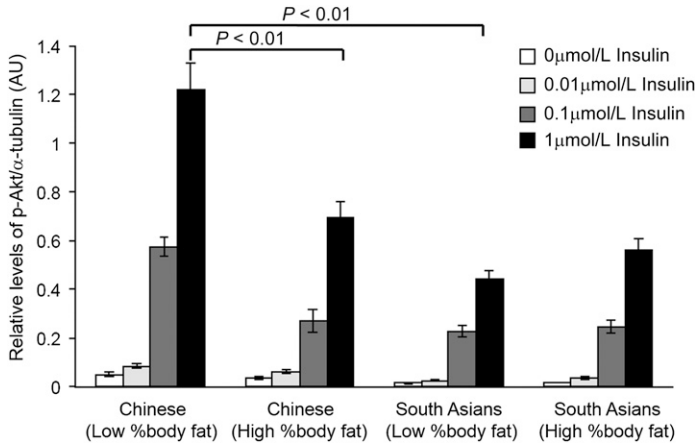

Figure 4—Graph shows densitometric analysis of p-Akt protein levels in human primary myoblasts isolated from low and high %body fat Chinese and South Asian individuals at basal level (0 μmol/L) or after treatment with increasing concentrations of insulin (0.01, 0.1, and 1 μmol/L). Graph displays mean p-Akt levels ± SEM in arbitrary units (AU) normalized against α-tubulin protein levels.

agrees with our previous findings, which used the homeostasis model assessment of insulin resistance as a measure of insulin resistance and BMI as the measure of adiposity (4). We showed that with higher %body fat, all three ethnic groups accumulate fat in the liver and in VAT and SSAT compartments to a similar extent. However, Chinese with higher levels of %body fat seemed to accumulate DSAT and IMCL to a lesser extent compared with other ethnic groups. Fat in both of these compartments has been thought to be detrimental, and therefore, this reduced accumulation of fat in these depots does not explain the stronger association between high adiposity and low ISI observed in Chinese than in other ethnic groups. Although insulin sensitivity was generally lower with greater fat volume or percentage in each adipose tissue depot, the effect was greater among Chinese and Malays than in South Asians. As such, our data do not support the hypothesis that a difference in fat accumulation in various fat depots is responsible for these ethnic differences. Studies performed in primary myoblasts suggest that these differences relate to differences in the expression of p-Akt in the muscle of these ethnic groups. This is the first study to explore these relationships in relation to insulin sensitivity and also the first study to compare Chinese, Malays, and South Asians. These ethnic groups (or related ethnic groups) represent almost two-thirds of the world's populations living in areas where the prevalence of type 2 diabetes is projected to escalate tremendously.

Several studies have demonstrated ethnic differences in the distribution of fat (6,7). Lear et al. (6) showed that the higher levels of VAT with greater adiposity were more prominent in Chinese, followed by Europeans and then South Asians, resulting in higher VAT in South Asians at low levels of adiposity and lower VAT in South Asians at higher levels of adiposity. However, the authors did not measure insulin sensitivity, and whether the differences in VAT accumulation translate into differences in insulin sensitivity is unclear. Nazare et al. (7) had recently showed that the relationship between BMI and fat distribution differed between ethnic groups. In particular, East Asians (from Japan, China, and Korea) had a tendency to accumulate more VAT with greater levels of adiposity (both BMI and SAT) compared with Hispanics, African Caribbean blacks, and Southeast Asians. At moderate levels of adiposity, East Asians showed lower amounts of VAT; however, with higher BMI, East Asians had greater levels of VAT. In relation to SAT, East Asians accumulated less SSAT and DSAT relative to VAT with higher BMI, which explains their higher levels of VAT despite generally lower overall adiposity in this ethnic group. This study had several limitations. First, Southeast Asians originated mainly from Malaysia and Thailand and comprise a mixture of Chinese, Malay, and South Asian ethnicities. Second, the study did not include South Asians, an ethnic group that is thought to suffer most from the effects of insulin resistance and visceral adiposity. Finally, few of the ethnic differences in the relationship between fat volume and cardiometabolic risk factors reached statistical significance. This may relate to the fact that insulin resistance was assessed using a variety of cardiometabolic variables that do not precisely represent insulin stimulated glucose uptake.

Of specific relevance to our present study, the Multicultural Community Health Assessment Trial showed that Chinese exhibited more VAT per kilogram of total body fat than Europeans, whereas South Asians exhibited less VAT per kilogram of total body fat (6); however, there was no direct comparison between Chinese and South Asians. Our study agrees with these studies (6,7) and confirms that East Asians tend to accumulate VAT with higher levels of adiposity. In addition, our study provides a direct comparison between Chinese and South Asians in relation to fat partitioning with adiposity and is the first to include liver fat content and IMCL. We showed that differential fat partitioning with higher levels of adiposity between Chinese and South Asians is observed, particularly for DSAT and IMCL.

Excessive accumulation of DSAT is associated with adverse lipid profiles and with insulin resistance (20). The DSAT depot has higher saturated lipids content compared with SSAT (21). The expression profiles of 11β-hydroxysteroid dehydrogenase-1, leptin, and resistin in DSAT are closer to VAT (22). Taken together, DSAT represents a distinct abdominal fat depot that may have pathogenic potential in the development of obesity-associated disorders. However, our data do not suggest that the differences in DSAT are likely to explain the ethnic differences in insulin sensitivity. This greater propensity to deposit DSAT with higher %body fat was observed in Malays and South Asians, whereas Chinese showed the least tendency to accumulate DSAT. However, the negative relationship of higher %body fat and lower insulin sensitivity was greater in Chinese than in the other ethnic groups.

In relation to IMCL, South Asians tend to accumulate more IMCL with higher levels of adiposity than Malays or Chinese. However, this did not explain the ethnic differences in insulin sensitivity observed. Indeed, the associations between IMCL and insulin sensitivity were weak in all three ethnic groups. The reason for the weak correlation between IMCL and insulin sensitivity is unclear and may be context-dependent. That higher levels of IMCL are associated with enhanced insulin sensitivity in endurance-trained athletes is well known (23). Although our participants were not endurance-trained athletes, they were young. A study in mice showed that young mice exhibited high IMCL but were insulin-sensitive, in contrast to old mice, which had high IMCL but were insulin-resistant (24). Thus, one possibility is that age modulates the association between IMCL and insulin sensitivity and that in young subjects (such as ours), the association between IMCL and insulin sensitivity is less strong.

We have also shown that these in vivo findings are recapitulated in vitro. Akt is a downstream effector of phosphatidyl inositol 3-kinase in the insulin-signaling pathway and plays a key role in regulating GLUT4 trafficking for intracellular glucose uptake. Consistent with the in vivo ISI, we observed that the basal and insulin-stimulated p-Akt levels were lower in myoblasts from lean South Asians compared with those from lean Chinese. Higher %body fat was associated with lower p-Akt in myoblasts from Chinese subjects but not in those from South Asians. In lean South Asians, basal and insulin-stimulated p-Akt levels were similar to Chinese with high %body fat. The mechanisms underlying this observation are not clear at this time. However, we suggest that a primary defect in the muscles that is unaffected by increasing adiposity may underlie the insulin resistance observed in South Asians. The hypothesis that non–obesity-mediated pathways may be important in the pathogenesis of insulin resistance is consistent with the findings of Abbasi et al. (25), who reported that obesity explains only 22% of the variance in insulin sensitivity.

In contrast to our study, Chandalia et al. (1) found that for any given BMI level, greater insulin resistance in South Asians was accompanied by greater truncal adiposity compared with Caucasians. These authors also showed that South Asians had higher levels of adiposity in the truncal subcutaneous compartment than in the visceral compartment. Nonetheless, these differences in fat mass in different compartments failed to explain the ethnic differences in insulin resistance. In fact, the authors showed that insulin resistance in young South Asian men can be observed even without an increase in the intraperitoneal fat mass and is related to large subcutaneous adipose adipocytes size.

The molecular basis underpinning the greater effect of adiposity on insulin resistance in Chinese remains unsolved. One prevailing hypothesis is that limited fat expandability in these susceptible individuals gives rise to dysfunctional adipose function, greater inflammation, and altered adipokines production. This hypothesis is further suggested by Chandalia et al. (1), who showed that South Asians had high levels of nonesterified fatty acid and low levels of adiponectin compared with Caucasians (1). Ethnic differences in the relationship between fat volume or percentage and adipokines, myokines, or inflammatory markers that mediate the biological cross talk between adipose tissue and skeletal muscle deserve further study.

There are limitations in our study. This study included only men. For future studies, it will be important to extend the findings in this study to women, taking into account the variation in insulin resistance that occurs in different phases of the menstrual cycle. In addition, our cohort consisted of young, healthy men who have low risk of diabetes or cardiometabolic diseases in the short-term. However, we do believe that the propensity for diabetes in these individuals is high (in Singapore, more than 30% of the population aged older than 50 years has diabetes), and studying them when they are young and healthy allows us to avoid any confounding that occurs due to reverse causation that may occur after chronic diseases develop. Furthermore, our findings are similar to our previous findings (which included a much older population), and as such, we do not feel that this is a major limitation of this study. We also did not systematically measure hepatic insulin sensitivity. The insulin sensitivity measured using the euglycemic clamp reflects primarily skeletal muscle insulin sensitivity. Future studies on differences in insulin sensitivity among the ethnic groups will need to take into consideration the use of radiolabeled glucose tracers to quantify hepatic insulin resistance.

In summary, irrespective of ethnic groups, higher %body fat is associated with higher fat volume or percentage in various depots or organs. Higher %body fat is also associated with lower insulin sensitivity. Ethnicity modulates the relationship between adiposity and insulin sensitivity, although this phenomenon appears complex and cannot be purely explained by differences in body fat partitioning alone. Our study suggests that in South Asians, non–obesity-related insulin resistance may predominate in skeletal muscles. In contrast, Chinese and Malays appear to be more affected by higher levels of adiposity. Further studies of adipose tissue and skeletal muscle from these ethnic groups will be required to dissect the pathways to insulin resistance. These findings may have relevance to other populations because obesity is known to explain only a small proportion of the variance in insulin sensitivity.

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Author Contributions. C.M.K. and M.K.-S.L. were responsible for the study execution and for manuscript preparation. C.M.K. and K.V. were responsible for data management and data analyses. C.M.K., M.K.-S.L., K.V., and E.Y.H.K. performed study experiments. S.A.S. and S.S.V. were responsible for the MR imaging and MR spectroscopy. R.L. and Y.T.O. performed the myoblasts experiment. R.K. and C.M. were responsible for the design and interpretation of the myoblasts study. P.D.G., Y.S.L., and Y.S.C. provided critical review of the manuscript. E.S.T. wrote the grant proposal, designed the experiments, and reviewed and edited the final manuscript. C.M.K. and E.S.T. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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