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### The epidemiology of abdominal adiposity

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## Validation of ultrasound estimates of visceral fat in black South African adolescents

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#### ABSTRACT

Accurate quantification of visceral adipose tissue (VAT) is needed to understand ethnic variations and their implications for metabolic disease risk. The use of reference methods such as Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) is limited in large epidemiological studies. Surrogate measures such as anthropometry and DEXA do not differentiate between VAT and subcutaneous-abdominal adipose tissue (SCAT). Ultrasound provides a validated estimate of VAT and SCAT in Caucasian populations. This study aimed to validate the use of ultrasound-based assessment of VAT in black South African adolescents.

100 healthy adolescents (boys=48, girls=52) aged 18-19 y participating in the Birth to Twenty cohort study had VAT and SCAT measured by single slice MRI at L4. These MRI 'criterion measures' were related to ultrasound VAT and SCAT thickness, anthropometry (BMI, waist and hip circumferences) and DEXA android region fat.

Ultrasound VAT thickness showed the strongest correlations with MRI VAT (Spearman's correlation coefficients: r=0.72 and r=0.64; in boys and girls, respectively), and substantially improved the estimation of MRI VAT compared to anthropometry and DEXA alone; in regression models the addition of ultrasound VAT thickness to models containing BMI, waist and DEXA android fat improved the explained variance in VAT from 39% to 60% in boys, and from 31% to 52% in girls.

In conclusion, ultrasound substantially increased the precision of estimating VAT beyond anthropometry and DEXA alone. Black South African adolescents have relatively little VAT compared to elderly Caucasians, and we therefore provide new ultrasound-based prediction equations for VAT specific to this group.

#### INTRODUCTION

The prevalence of childhood and adolescence obesity is increasing rapidly worldwide, affecting high and low- and middle-income countries. Obesity and its related co-morbidities, such as diabetes and cardiovascular disease, have become a major public health issues and are contributing substantially to disease burden in countries like South Africa, where obesity co-exists with undernutrition (1); 30% of adolescent girls, and 10% of boys are either overweight or obese (2).

Obesity-related metabolic complications have been attributed to increases in abdominal adiposity, in particular visceral adipose tissue (VAT) (3, 4). However, several studies have shown racial differences in VAT and metabolic risk factors for obesity-related disease (5, 6). At the same level of adiposity, black adults and children have lower VAT than Caucasian adults and children (6-11). However, despite having lower VAT they are more insulin resistant than Caucasians, suggesting that other factors rather than VAT may influence metabolic risk in this ethnic group (5). Subcutaneous-abdominal adipose tissue (SCAT) has been reported to be related to insulin sensitivity and fasting insulin in the same populations (8, 12). This subcutaneous fat compartment appears to be substantially higher in black compared to Caucasian populations for the same level of total body fatness (13, 14). Therefore, investigating variations in VAT and SCAT in certain ethnic groups may help explain the different degrees of health risk associated with obesity and related disease.

Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) are the reference methods used to quantify these abdominal fat compartments. However, their routine use in large-scale-population studies and repeated investigations is limited due to cost, accessibility and, for CT, radiation exposure (15-17). Anthropometry, such as BMI, waist-to-hip ratio and waist circumference, are commonly used as surrogate measures of these fat compartments, but may be inadequate to describe ethnic variations (18, 19). BMI tends to underestimate VAT in non-Caucasian groups (19) and waist circumference and waist-to-hip ratio have been found to be unsuitable for tracking changes in VAT in black African women (9).

DEXA is another technique used to determine whole body composition and regional analysis of the trunk to estimate VAT and SCAT (20). However, a key limitation of the regional analysis is its inability to distinguish between visceral and subcutaneous body fat.

Ultrasound has been shown to be a valid method to estimate both VAT and SCAT in Caucasian populations (16, 21-23). Prediction equations using a combination of ultrasound and anthropometry to

estimate VAT and SCAT in elderly Caucasians have been published (23), but their accuracy has not been tested in other populations. We therefore determined the validity of those equations for estimating VAT and SCAT in black South African adolescents against MRI as the criterion measure.

#### **METHODS**

#### **Study population**

100 healthy black adolescents (boys=48 girls=52) aged 18-19 years old were recruited at random from the Birth to Twenty cohort when the participants presented for annual data collection at the Chris Hani-Baragwanath Hospital in Soweto. The Birth to Twenty (Bt20) cohort started in 1989 with pilot studies to test the feasibility of a long-term follow-up study of children's health and wellbeing (24). Women were enrolled in their second and third trimester of pregnancy through public health facilities and interviewed during regarding their health and social history and current circumstances. Singleton children (n=3,273) born between April and June 1990 and resident for at least 6 months in the municipal area of Soweto-Johannesburg were enrolled into the birth cohort and have been followed up 16 times between birth and 20 years of age (25). During the last 7 years, young people have been seen twice a year, at the Bt20 offices and at home. Attrition over two decades has been comparatively low (30%), mostly occurring during infancy and early childhood, and approximately 2,300 children and their families remain in contact with the study (26). The sample is roughly representative of the demographic parameters of South Africa. Assessments across multiple domains have been made of children, families, households, schools and communities during the course of the study, including growth, development, psychological adjustment, physiological functioning, genetics, school performance, and sexual and reproductive health. Exclusion criteria to the current study included pregnancy. The study was approved by the Ethics Committee on Human Subjects at the University of the Witwatersrand and was performed in accordance with the Declaration of Helsinki. Written consent and was obtained from study participants.

#### Anthropometry

Body Weight (WT) was measured in light clothing without shoes to the nearest 0.1 kg using a digital scale (Tanita model TBF-410; Arlinghton Heights; USA). Height (HT) was measured barefoot to the nearest 0.1 cm using a wall mounted stadiometer (Holtain, Crymych, UK). BMI was calculated as WT

(kg) divided by HT (m) squared. Waist and hip circumferences were measured with a nonstrechable fibreglass insertion tape at the level of the umbilicus and the greater trocanters, respectively. All measurements were performed by trained staff.

#### **Body composition**

#### Dual Energy X ray Absorptiometry (DEXA)

Measurements of body composition using the DEXA fan-beam technology (Hologic Discovery-W,) (Hologic, Bedford, MA, USA) were made in all participants. Total body fat mass and total android fat estimates (g) were derived using the Hologic Discovery Software version 12.1 (Hologic, Bedford, MA, USA). The android region (g) was manually determined for each individual by the same operator using the method described by Ley et al (27). A quadrilateral box is positioned with the lower border above the iliac crest, the upper border at the lower levels of the ribs (excluding any bone) and the lateral boundaries extending to the edge of the abdominal soft tissue. The position of this region of interest was checked by two independent technicians.

#### Ultrasonography

A LOGIQ e ultrasound system (USS) (GE Healthcare, Piscataway, NJ, USA) with a 2-5 MHz 3C-RS curved array transducer was used to determine VAT and SCAT thicknesses. USS VAT thickness was defined as the distance (cm) from the peritoneum to the vertebral bodies, and USS SCAT thickness was defined as the depth (cm) from the skin to the linea alba (16). The scan depth was set at 15 cm for the visceral fat measure and 9 cm for the subcutaneous fat measure to visualise the relevant anatomical structures. Both measurements were obtained where the xyphoid line and the waist circumference met. All USS measurements were taken by one of three trained operators. The relative intra-observer technical error of measurement (TEM) ranged between 1.9 to 2.7% for USS VAT thickness, and 0.9 to 2.8 % for USS SCAT thickness, and the relative inter-observer TEM was 2.2% for USS VAT thickness and 2.5 % for USS SCAT thickness, based on repeated measurements in 15 individuals.

#### MRI

MRI measures of VAT and SCAT were conducted on a whole body 1.5T GE scanner (GE Healthcare, Piscataway, NJ, USA). The L4 vertebral body was placed at the isocentre of the magnet and17 water suppressed  $T_1$ -weighted, turbo spin echo transaxial images were acquired with respiratory gating. The slice thickness was 10 mm (2 mm gap between slices), with an in-plane resolution of 0.94 × 0.94 mm,

and a field of view of 480 × 480 mm. Cross-sectional areas of VAT and SCAT were calculated from one slice that was located at the L4 vertebral body. These areas were generated using a semi-automated method, by using an intensity thresholded map (Analyze 7.0, BIR, Mayo Clinic, Rochester MN), in conjunction with manual input to differentiate VAT and SCAT compartments. In cases where there was an artificial reduction in signal intensity due to artefacts in the MRI image, the thresholded map was corrected by using the auto trace facility within the Analyze software. All the images were reviewed and calculations performed by the same observer (AS).

#### Calculations

Estimated VAT and SCAT based on anthropometry and USS parameters were calculated using published predication equations derived in older Caucasian adults (23). The equations used were for boys:  $[VAT \text{ cm}^2 = (3.2*BMI \text{ kg/m}^2) + (1.7*waist \text{ cm}) + (14.6*USS VAT thickness \text{ cm}) -184.9] \& [SCAT \text{ cm}^2 = [(3.6*BMI \text{ kg/m}^2) + (4.5*waist \text{ cm}) + (29.4*USS SCAT thickness \text{ cm}) - 381.7]$  for girls:  $[VAT \text{ cm}^2 = [(-1.8*BMI \text{ kg/m}^2) + (1.8*waist \text{ cm}) + (15.9*VAT thickness \text{ cm}) -96.1] \& [SCAT \text{ cm}^2 = [(17.5*BMI \text{ kg/m}^2) + (-1.3*waist \text{ cm}) + (30.2*USS SCAT thickness \text{ cm}) -165]$ 

#### **Statistical Analysis**

Statistical analyses were performed using STATA (version 11.0 StataCorp, College Station, Texas, USA). Statistical significance was set at P <0.05. Sex differences were tested using unpaired t-tests and all subsequent analyses were performed separately in boys and girls.

Spearman rank coefficients were used to investigate associations between the different measures and estimates of abdominal fat. Bland-Altman analysis (28) was used to investigate the agreement between MRI criterion measures and estimated VAT and SCAT calculated from the USS and anthropometry-based prediction equations. Mean bias was calculated and significance was assessed by paired t-tests.

Univariable linear regression was performed for each USS measure, to quantify the proportion of variance of VAT and SCAT explained by these variables. Stepwise multivariable regression models were subsequently built by adding anthropometry, DEXA and USS measures to derive the optimal prediction of MRI VAT and SCAT. The models were constructed using a hierarchical and pragmatic approach. The likelihood ratio test was used to assess the incremental value of adding USS measures to anthropometry and DEXA variables. Co-linearity between parameters was indicated by a variance inflation factor (VIF) > 5, and such models were considered invalid.

### RESULTS

Characteristics of the study population are shown in Table 1. Boys were taller, but had smaller waist and hip circumferences, and less DEXA % total body fat and android fat mass than girls, but no differences in body weight or waist-to-hip ratio. Furthermore, boys had less MRI VAT and SCAT than girls, but had higher VAT to SCAT ratios.

Table 1. Characteristics of the study participants by sex (Mean  $\pm$  SD)

	Boys	Girls		
	( <i>n</i> = 48)	( <i>n</i> = 52)	P-value <sup>a</sup>	
Age (yr)	18.9±0.1	18.8±0.1	0.1	
Anthropometry				
Weight (kg)	61.1±10.2	60.1±13.0	0.7	
Height (cm)	171.0±7.5	160.0±5.1	< 0.0001	
BMI (kg/m <sup>2</sup> )	21.0±3.5	23.6±5.2	0.005	
Waist circumference (cm)	73.9±8.8	79.2±11.4	0.01	
Hip circumference (cm)	91.2±10.6	99.4±14.0	0.001	
Waist:Hip ratio	0.8±0.1	0.8±0.1	0.5	
DEXA <sup>f</sup>				
Total fat (%)	13.8±5.4	32.7±7.0	< 0.0001	
Total fat mass (kg)	8.3±5.2	20.1±8.3	< 0.0001	
Android fat (kg)	0.6±0.5	$1.8 \pm 1.4$	< 0.0001	
MRI <sup>b</sup>				
VAT <sup>c</sup> (cm <sup>2</sup> )	12.3±8.1	17.3±8.7	0.004	
$SCAT^{d}$ (cm <sup>2</sup> )	79.1±89.7	212.8±150	< 0.0001	
VAT:SCAT ratio	0.2±0.1	0.1±0.05	< 0.0001	
USS <sup>e</sup>				
Visceral thickness (cm)	2.2±1.0	2.3±0.8	0.6	
Subcutaneous fat thickness (cm)	1.1±0.4	1.7±0.7	< 0.0001	
Visceral: subcutaneous thickness ratio	2.1±0.9	1.5±0.8	0.0005	
<sup>a</sup> Sex differences by T-test				

<sup>b</sup>MRI magnetic resonance imaging

<sup>c</sup>VAT visceral adipose tissue

<sup>d</sup>SCAT subcutaneous adipose tissue

<sup>e</sup>USS ultrasound system

<sup>f</sup> Dual Energy x-ray absorptiometry

#### Correlations with MRI measures of abdominal fat

Spearman's correlation coefficients between the MRI criterion measures and all other measures are shown in Table 2. USS VAT thickness was the measure that was most strongly correlated with MRI VAT in both boys and girls; anthropometry and DEXA measures showed only moderate or weak correlations with MRI VAT. DEXA total fat mass was the measure that was most strongly correlated with MRI SCAT in both boys and girls. BMI was also strongly correlated with MRI SCAT in girls. USS VAT:SCAT thickness ratio was the only parameter that showed positive correlations with MRI VAT.

 Table 2. Spearman correlation coefficients between MRI adiposity areas and anthropometry, DEXA and ultrasound parameters, in boys and girls

		Boys			Girls			
		VAT <sup>a</sup>	SCAT <sup>b</sup>	VAT:SCA	VAT <sup>a</sup>	SCAT <sup>b</sup>	VAT:SCAT	
		$(cm^2)$	$(cm^2)$	T ratio	$(cm^2)$	$(cm^2)$	ratio	
Anthropometry	Weight (kg)	0.3	0.53	-0.3	0.62	0.79	-0.24	
	BMI (kg/m2)	0.37	0.71	-0.39	0.59	0.88	-0.33	
	Waist circumference (cm)	0.39	0.58	-	0.41	0.61	-	
	Hip circumference (cm)	0.44	0.64	-	0.57	0.81	-	
	Waist:Hip ratio	-	-	-0.09	-	-	-0.15	
DEXA	Total fat mass (kg)	0.46	0.85	-	0.58	0.93	-	
	Android fat (kg)	0.44	0.75	-	0.34	0.73	-	
USS	visceral thickness (cm)	0.72	0.34	-	0.64	0.46	-	
	subcutaneous fat thickness (cm)	0.39	0.82	-	0.38	0.61	-	
	visceral:subcutaneous thickness ratio	-	-	0.64	-	-	0.4	

<sup>a</sup>VAT visceral adipose tissue area

<sup>b</sup>SCAT subcutaneous adipose tissue area

#### Validation of existing prediction equations to estimate abdominal fat tissues

Bland Altman analyses showed that estimates calculated from the existing predication equations, derived from elderly Caucasians (23), substantially over-estimated VAT in black South African boys (mean difference = 28.0; 95% CI: 19.7-36.41 cm<sup>2</sup>) and girls (23.6; 18.4-28.9 cm<sup>2</sup>). Conversely, these predication equations (23) under-estimated SCAT in boys (-20.1; -32.4 to -7.9 cm<sup>2</sup>) and girls (-16.2; -36.9 to -4.5 cm<sup>2</sup>).

		Model	Constant	$B^a \pm SE$				$R^2 \%$	RMSE <sup>c</sup>
			-	BMI	WC <sup>b</sup>	DXA android	US visceral		
						fat			
				$(kg/m^2)$	(cm)	(kg)	(cm)		
Boys	VAT (cm <sup>2</sup> )	1	-9.6	1.0 ±0.3	-	-	-	20	7.3
		2	-26.0	$0.1 \pm 0.5$	$0.5 \pm 0.2$	-	-	32	6.8
		3	-17.8	-0.1±0.4	$0.4 \pm 0.2$	3.9±3.1	-	39	6.4
		4	-17.8	-0.2±0.4	$0.3 \pm 0.2$	0.4±2.7	4.6±1.1	60	5.4
Girls	VAT $(cm^2)$	1	-3.0	$0.9 \pm 0.2$	-	-	-	27	7.6
		2	2.7	1.1 ±0.4	-0.2±0.2	-	-	28	7.6
		3	0.8	1.4 ±0.4	-0.2±0.2	-1.1±0.9	-	31	7.5
		4	-1.2	$0.9 \pm 0.4$	-0.2±0.1	-0.8±0.7	5.5±1.3	52	6.4

Table 3. Prediction models for VAT using anthropometry, DEXA variables and ultrasound visceral thickness

<sup>a</sup> B represents expected change in VAT per

unit increase in the covariate.

<sup>b</sup> WC waist circumference

<sup>c</sup>RMSE root mean squared error.

Table 4. Prediction models for SCAT using anthropometry, DEXA variables and ultrasound visceral thickness

		Model	Constant	$B^a \pm SE$					RMSE <sup>c</sup>
				BMI	$WC^b$	DXA android	US visceral		
						fat			
				$(kg/m^2)$	(cm)	(kg)	(cm)		
Boys	SCAT (cm <sup>2</sup> )	1	-331.7	19.6±2.4	-	-	-	59	58.2
		2	-587.7	4.7±2.5	$7.7 \pm 1.0$	-	-	81	39
		3	-411.7	$3.9 \pm 2.2$	5.0±1.1	67.6 ±16.1	-	87	33.7
		4	-353.0	1.2 ±2.3	4.4±1.1	63.3 ±16.7	43.6 ±18.8	90	31.2
Girls	SCAT (cm <sup>2</sup> )	1	-424.1	$27.0 \pm 1.4$	-	-	-	88	51.1
		2	-497.9	23.4 ±2.3	$2.0 \pm 1.1$	-	-	89	50.6
		3	-490.0	22.3 ±2.7	2.1±1.1	$5.1 \pm 5.7$	-	89	50.5
		4	-490.3	22.5 ±2.8	2.1±1.1	5.6±6.1	-4.1±12.6	90	50.1

<sup>a</sup> B represents expected change in SCAT per unit increase in the covariate.

<sup>b</sup> WC waist circumference

<sup>c</sup>RMSE root mean squared error.

#### Estimating VAT and SCAT in black South African adolescents

In univariable models, USS VAT thickness explained 55% and 46% of the variance in VAT in boys and girls, respectively. USS SCAT thickness explained 69% and 33% of the variance in SCAT in boys and girls, respectively.

Multivariable regression models are shown in Tables 3 and 4. Height and hip circumference did not contribute to the models ( $p \ge 0.1$ ) and DEXA total fat mass was excluded due to co-linearity with DEXA android fat mass. The addition of USS VAT thickness to models with only BMI, waist circumference and DEXA android fat improved the explained variance in VAT from 39% to 60% in boys (LR test p<0.0001), and from 31% to 52% in girls (p<0.0001) (Table 3). The precision (reduction in the root mean squared error) increased by 26% in boys and 16% in girls.

In contrast to the results for VAT, BMI and waist circumference alone explained a high proportion of the variance in SCAT (model R-square: 81% and 89% in boys and girls, respectively) (Table 4). The addition of DEXA android fat mass improved the estimation of USS SCAT thickness in boys (to 87%) but not in girls. Further addition of USS SCAT thickness made a statistically significant (p=0.02), but minor improvement in the estimation of SCAT in boys (to 90%), but not in girls (Table 4), and improved the precision by 46% in boys and only 2% in girls.

#### DISCUSSION

This study shows the utility of USS to estimate VAT and SCAT in black South African adolescents, against gold-standard MRI criterion measures. The reproducibility of our USS measurements was high in this setting, indicated by the low TEM's. However, the published equations derived in older Caucasian individuals based on anthropometry and USS to estimate VAT and SCAT cannot be extrapolated to black South African adolescents as their use introduced considerable bias. We therefore, developed new prediction equations for the estimation of abdominal fat compartments for this population. USS VAT thickness substantially improved the estimation of VAT over and above the contributions of anthropometry and DEXA in both boys and girls. Conversely, USS made only limited improvement in the estimation of SCAT in boys and no improvement in girls.

Our findings concur with other studies in adult women, obese and diabetic adults and older individuals, which reported strong correlations ranging from 0.75 to 0.82 between USS VAT thickness and VAT measured by MRI or CT (16, 21-23, 29, 30). The utility of USS to estimate SCAT was similar in boys to a previous study in Japanese adults (29), but in girls our correlations were far weaker than the results in that report. Again, compared to the results in older Caucasians (23, 31), our correlations were substantially higher in boys, but slightly lower in girls. A potential explanation is that because USS only measures the anterior SCAT thickness, possible variation in the anterior versus posterior distribution of SCAT in black South African girls could lead to lower correlations with MRI which measures total SCAT area at L4.

Our study sample had substantially lower VAT values compared to elderly Caucasians and this difference likely explains the over-estimation of VAT in this sample by those earlier prediction equations. Mean VAT in these black South African adolescents (12.3 cm<sup>2</sup> in boys, 17.3 cm<sup>2</sup> in girls) was an order of magnitude lower than that in elderly Caucasians (mean±SD: 155.1±73.6 cm<sup>2</sup> in men, 105.3±59.8 cm<sup>2</sup> in women) (23). In contrast, values for SCAT were more comparable (mean±SD in elderly Caucasians was:  $235.6\pm75.1 \text{ cm}^2$  in men and  $278.2\pm95.9 \text{ cm}^2$  in women). Abdominal adiposity increases progressively with advancing age (32, 33). Furthermore, other studies have reported that Caucasians have more VAT than blacks at the same level of adiposity (6-11). However, despite their lower VAT, blacks are more hyperinsulimic (34, 35) and may have higher risk of diabetes (11). Several investigators have suggested that SCAT might be a determinant of insulin resistance in black populations as it is related to basal and 2-h insulin (8, 12). SCAT occurs into deep and superficial subcutaneous adipose layers separated by a fascial plane (36). Deep SCAT is metabolically active and in black it may confer greater metabolic health risk than in Caucasians despite having lower VAT (11). Future research examining the metabolic sequelae of these abdominal fat compartments in different ethnic populations could valuably inform their biological relevance. However, ethnic differences in these fat distributions mean that indirect estimates by anthropometry and DEXA are likely to be inappropriate. Our results suggest that USS is a valid method when the gold standard methods are not feasible.

As body composition and fat distribution change with age, it may have been unsurprising that the prediction equations for abdominal fat derived in older people do not fit in younger populations. However, to our knowledge, these are the only two reported prediction equations for VAT and SCAT using a combination of anthropometry and USS parameters, and with the same USS protocol as in our

current study (De Lucia et al, Gradmark et al). Other studies in adults (37), in women only (38) and in children (39) used different anatomical landmarks to determine VAT and SCAT (peritoneum-aorta anterior wall, ratios between subcutaneous and peritoneal areas) or only presented associations with the gold standard measurements rather than predictions and therefore they were not considered for analysis. Interestingly, anthropometry and DEXA parameters showed poor correlations with VAT, suggesting that these measures are sub-optimal indicators of this fat region in this population. Possibly, these measures are not sensitive enough to pick up very low levels of VAT. In this group, BMI and waist performed surprisingly well when predicting SCAT.

A limitation of our study is that without cross-validating the new prediction equations, the applicability of our results to other black populations remains in question. The validity of these new predication equations will need to be evaluated in future studies. A further limitation is that we used single thickness measures of VAT. Other investigators have used different abdominal ultrasound protocols that directly visualise specific visceral compartments, in particular pre-peritoneal (PP) fat pad (39, 40). However, we are not aware of any studies that have validated those measures against VAT assessed by MRI or CT. Mook-Kanamori et al.(39) compared ultrasound against CT measures of PP, rather than VAT, while Semiz et al (40) used the ultrasound PP measure as a gold standard to assess the utility of anthropometry. In contrast, there are now a growing number of studies (16, 21-23, 30, 31) that have reported the more indirect ultrasound measure of total visceral thickness which appears to correlate strongly with VAT assessed by CT and/or MRI, and therefore chose this latter approach in our study. Those former direct measures require different ultrasound probes and settings, which would have added considerably to the duration of the assessments, and therefore not included in our measurement protocol. In conclusion, we demonstrated that USS is reliable and substantially improved the prediction of VAT in black South African adolescents, and we developed new prediction equations for VAT and SCAT specific to this population. The use of USS in large epidemiological studies could potentially help exploring the relevance of marked ethnic differences in abdominal fat distribution to metabolic disease risk. This application is critical for understanding and developing future preventative and intervention programs specific to a population group.

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