

The Missing Risk: MRI and MRS Phenotyping of Abdominal Adiposity and Ectopic Fat

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Individual compartments of abdominal adiposity and lipid content within the liver and muscle are differentially associated with metabolic risk factors, obesity and insulin resistance. Subjects with greater intra-abdominal adipose tissue (IAAT) and hepatic fat than predicted by clinical indices of obesity may be at increased risk of metabolic diseases despite their “normal” size. There is a need for accurate quantification of these potentially hazardous depots and identification of novel subphenotypes that recognize individuals at potentially increased metabolic risk. We aimed to calculate a reference range for total and regional adipose tissue (AT) as well as ectopic fat in liver and muscle in healthy subjects. We studied the relationship between age, body-mass, BMI, waist circumference (WC), and the distribution of AT, using whole-body magnetic resonance imaging (MRI), in 477 white volunteers (243 male, 234 female). Furthermore, we used proton magnetic resonance spectroscopy (MRS) to determine intrahepatocellular (IHCL) and intramyocellular (IMCL) lipid content. The anthropometric variable which provided the strongest individual correlation for adiposity and ectopic fat stores was WC in men and BMI in women. In addition, we reveal a large variation in IAAT, abdominal subcutaneous AT (ASAT), and IHCL depots not fully predicted by clinically obtained measurements of obesity and the emergence of a previously unidentified subphenotype. Here, we demonstrate gender- and age-specific patterns of regional adiposity in a large UK-based cohort and identify anthropometric variables that best predict individual adiposity and ectopic fat stores. From these data we propose the thin-on-the-outside fat-on-the-inside (TOFI) as a subphenotype for individuals at increased metabolic risk.

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INTRODUCTION

Obesity is a major risk factor for insulin resistance, type 2 diabetes mellitus, and cardiovascular disease (CVD). However, not every obese patient is insulin resistant or at high risk of diabetes mellitus and CVD (1). Abdominal obesity is associated with an increased predisposition to metabolic and CVD risk with the compartmental distribution of adipose tissue (AT) linked to differences in susceptibility (2,3). Intra-abdominal AT (IAAT) appears to play a major role in the pathogenesis of insulin resistance, diabetes, dyslipidemia, inflammation, hypertension, and CVD, whereas the metabolic consequences of subcutaneous AT (SAT) are less clear (1,4–6).

SAT has been linked to features of the metabolic syndrome independently of intra-abdominal fat, including obesity-related insulin resistance (7,8). However, data suggests that while both IAAT and SAT are correlated with metabolic risk factors, IAAT

remains more strongly associated with an adverse metabolic risk profile even after accounting for anthropometric indices (9,10). Loss of IAAT following diet and exercise is associated with improvements in insulin sensitivity, blood pressure, and circulating lipid levels, whereas comparable loss of SAT by liposuction does not result in amelioration of these metabolic abnormalities (7,11,12). Interestingly, human and animal studies have indicated a possible protective role for SAT; in humans, increased SAT in the leg is associated with decreased risk of perturbed glucose and lipid metabolism (13), whereas in mice, transplantation of SAT into intra-abdominal compartments results in improved glucose metabolism and a reduction in body mass and total fat mass (14). Additional variables such as age, gender, and ethnicity may also be an important confounding factors in the relationship between adiposity stores and metabolic risk.

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BMI is the current benchmark for obesity classification, but like all anthropometric measurements, it only offers a proxy measure of body adiposity (15). Waist circumference (WC) is widely used as a surrogate of central fat distribution, but while easily obtainable, it is unable to distinguish between IAAT and abdominal SAT (ASAT) deposition (16). Magnetic resonance imaging (MRI) is a noninvasive technique that allows accurate measurement of whole-body fat and specific internal stores of AT. MRI studies have demonstrated significant variation among individual AT compartments that is not predicted by total body or trunk fat or standard anthropomorphic characteristics; such as skin-fold measurements, BMI, and waist-to-hip ratio (WHR) (6,17,18). Ectopic fat in organs has also been linked to obesity, insulin resistance, type 2 diabetes mellitus, and in particular, the lipids within the muscle cells (intramyocellular lipids (IMCL)) and the liver (IHCL) (19–22). Recent data have suggested a difference between individual ectopic fat depots with IHCL, but not with IMCL being linked to insulin resistance (23).

In this study, we have employed magnetic resonance techniques to accurately measure the patterns of fat deposition in 477 UK-based white volunteers. In addition to providing gender- and age-specific reference range data, we aimed to describe the relationships between individual anthropometric, adiposity, and ectopic fat measurements. The variation observed in metabolically adverse internal fat deposition led to our proposal of the thin-on-the outside fat-on-the inside (TOFI) subphenotype; a ratio of IAAT and ASAT calculated from the range of abdominal adiposity stores of a defined healthy subset of volunteers, as a simple, quantitative means of identifying those that may be at increased metabolic risk.

METHODS AND PROCEDURES

Subjects

Written, informed consent was acquired from all volunteers. Ethical approval permission for this study was obtained from the research ethics committee of Hammersmith and Queen Charlotte's and Chelsea Research Ethics Committee Hospital, London (Rec: 07Q04011/19). In total, 477 unpaid volunteers (243 male, 234 female) were recruited via advertisements in newspapers, websites, and academic newsletters, inviting male and female volunteers of white ethnicity from the general public. No age constraints were placed on recruitment in order to generate cross-sectional data. Self-reported exclusion criteria included subjects suffering from chronic disease (including diabetes, cardiovascular or liver disease, metabolic conditions, anyone taking prescribed medication and women on the contraceptive pill). Volunteers underwent anthropometric assessment, total body MRI scanning and *in vivo* proton (^1H) magnetic resonance spectroscopy (MRS) of liver and calf muscle.

Anthropometric measurements

Body mass (kg), height (cm), WC (cm), and hip circumference (cm) were measured in each subject by a single experienced observer. WC was measured at the (WHO recommended) midpoint (24) between the distal border of the lowest rib and the superior border of the iliac crest. From these values, BMI (kg/m^2), WHR (waist/hip), and waist-to-height ratio (WHtR, waist/height) were calculated. BMI grouping corresponded to the following ranges: 1: $18.5 < 25 \text{ kg}/\text{m}^2$, 2: $25 < 30 \text{ kg}/\text{m}^2$, 3: $30 < 40 \text{ kg}/\text{m}^2$, 4: $40+ \text{ kg}/\text{m}^2$.

MRI scanning: Total body and regional AT content

Rapid T_1 -weighted MR images were acquired using a 1.5T Phillips Achiva scanner (Phillips, Best, the Netherlands), as previously described (17). Subjects lay in a prone position with arms straight above the head, and were scanned from fingertips to toes, acquiring 10-mm thick contiguous transverse images throughout the body. Images were analyzed using SliceOmatic (Tomovision, Montreal, Quebec, Canada). Regional volumes were recorded in liters (l); comprising: ASAT, non-ASAT (NASAT), IAAT, and non-abdominal internal-AT (NAIAT) which includes internal AT in the head, neck, chest, pelvis, arms, and legs as previously described (17). The abdominal region was defined as the image slices from the slice containing the femoral heads, to the slice containing the top of the liver/bottom of the lungs; therefore the measurement of IAAT contains a mixture of visceral, perirenal, and retroperitoneal AT (17). Total AT was calculated from the sum of SAT and internal adipose stores: $\text{TAT} = \text{SAT} + \text{Internal}$. SAT was subdivided into ASAT and non-NASAT: $\text{SAT} = \text{ASAT} + \text{NASAT}$. Total internal AT (Internal) was subdivided into IAAT and non-abdominal internal AT (NAIAT): $\text{Internal} = \text{IAAT} + \text{NAIAT}$. In order to gauge abdominal adiposity as a whole, "trunk" fat was derived from the sum of IAAT and ASAT: $\text{Trunk} = \text{IAAT} + \text{ASAT}$.

MRS of liver and muscle fat

During the same scanning session, ^1H MR spectra were also acquired at 1.5T, using a surface coil. Transverse images of the liver were used to ensure accurate positioning of the ($20 \times 20 \times 20 \text{ mm}$) voxel in the liver, avoiding blood vessels, the gall bladder, and fatty tissue. Spectra were obtained from the right lobe of the liver using a PRESS sequence (repetition time 1,500 ms, echo time 135 ms) without water saturation and with 128 signal averages. Intrahepatocellular lipids (IHCL) were measured relative to liver water content, as previously described (21). IMCL were measured in the soleus (S-IMCL) and tibialis (T-IMCL) muscles by ^1H MRS. Proton MR spectra were acquired from $20 \times 20 \times 20 \text{ mm}$ voxels localized to the soleus and tibialis muscles of the left calf using a PRESS sequence (repetition time 1,500 ms, echo time 135 ms). IMCL were subsequently measured, relative to total muscle creatine signal, as previously described (25). Spectra from both S-IMCL and T-IMCL muscles were obtained because their differences in fiber composition and fuel requirements (tibialis primarily utilizes carbohydrate; soleus predominantly utilizes lipid) result in different lipid levels and metabolism. MRS data (IHCL, S-IMCL, and T-IMCL) are presented as the geometric mean, whereas statistical analysis was performed on \log_e transformed variables, due to the positively skewed distribution of these datasets (21). Of the male volunteers (total = 243) IHCL data was available for 234 individuals, S-IMCL for 239, T-IMCL for 239 subjects; whereas for females (total = 234): IHCL was available for 169, S-IMCL for 179, and T-IMCL for 178. We have previously published data concerning the reproducibility of the MRI (26) and MRS protocols for IHCL (21) and IMCL (25) measurements implemented here.

Definition of healthy controls

To identify subjects who had a fat distribution which deviated from "normal" it was necessary to define a healthy control group from our larger population. The following criteria were used to define white individuals within our test population as healthy controls: (i) Absence of disease/metabolic condition; (ii) BMI: $18.5 < 25 \text{ kg}/\text{m}^2$ (WHO guidelines (27)); (iii) WC: male $\leq 94 \text{ cm}$, female $\leq 80 \text{ cm}$ (WHO guidelines (28)); (iv) WHR: male ≤ 0.90 , female ≤ 0.80 (29); (v) age: male (18–50 years), female (18–39 years): the younger age group in female subjects was chosen in order to eliminate the effects of menopause from our control group, as there is a significant increase in obesity related-metabolic disorders after menopause, which has been linked to alterations in body adiposity, notably an increase in IAAT. (vi) Activity level: sedentary subjects were identified using Baecke (30) and/or I-PAQ questionnaires (31). Both tests calculate weekly physical activity, classifying individuals into low, moderate, or high categories based on specific criteria; I-PAQ

Table 1 Gender-specific variable data

	Male (n = 243)		Female (n = 234)		P
	Mean ± s.d.	Range	Mean ± s.d.	Range	
Age (years)	40.3 ± 13	17–70	34.5 ± 12.4	17–71	<0.001
Weight (kg)	87.6 ± 16.2	59.0–146.6	71.4 ± 17.6	40.7–146.8	<0.001
BMI (kg/m ²)	27.3 ± 4.8	18.6–44.5	26.2 ± 6.6	15.5–57.3	<0.05
WC (cm)	95.4 ± 13.3	70.0–131	81.4 ± 13.8	56.5–131	<0.001
Hip (cm)	103.5 ± 8.4	85.4–136	102.3 ± 10.7	76–134	0.23
Height (cm)	179 ± 7.3	143–199	165.2 ± 6.6	145.5–182	<0.001
WHR	0.92 ± 0.07	0.75–1.11	0.8 ± 0.07	0.58–1.04	<0.001
WHtR	0.53 ± 0.08	0.37–0.85	0.49 ± 0.09	0–0.78	<0.001
IHCL ^a	6.8 ± 14.0	0–89.6	2.8 ± 8.5	0–65.0	<0.001
S-IMCL ^a	15.5 ± 9.7	2.9–100.1	11.5 ± 6.9	2.28–51.0	<0.001
T-IMCL ^a	6.3 ± 3.8	0.25–30.5	6.7 ± 4.1	0.96–35.4	0.25
TAT (l)	24.9 ± 10.9	6–67.7	31.2 ± 15.9	8.3–106.2	<0.001
SAT (l)	18.6 ± 8.4	4.2–58.2	26.6 ± 13.5	7.3–90.5	<0.001
ASAT (l)	5.3 ± 3.0	0.7–20.2	7.6 ± 4.9	1.5–29.7	<0.001
NASAT (l)	13.3 ± 5.6	1.3–38	19.0 ± 8.8	5.8–60.9	<0.001
Internal (l)	6.3 ± 3.3	0.7–15.8	4.6 ± 2.8	1–15.7	<0.001
IAAT (l)	3.5 ± 2.1	0.2–9.4	2.3 ± 1.8	0.4–9.6	<0.001
NAIAT (l)	2.8 ± 1.4	0.5–7.9	2.4 ± 1.1	0.6–6.2	<0.001
Trunk (l)	8.8 ± 4.7	1.0–25.5	9.9 ± 6.4	1.9–39.3	<0.05
IAAT/ASAT	0.7 ± 0.3	0.18–1.64	0.3 ± 0.1	0.09–0.97	<0.001

Mean and range variable data. Adipose tissue deposits are in liters (l).

ASAT, abdominal subcutaneous adipose tissue; IAAT, intra-abdominal adipose tissue; IHCL, intrahepatocellular lipid; IMCL, intramyocellular lipid (S, soleus, T, tibialis); internal, total internal; NAIAT, non-abdominal internal adipose tissue; NASAT, non-abdominal subcutaneous adipose tissue; SAT, subcutaneous adipose tissue; TAT, total adipose tissue; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio.

^aMRS data (IHCL (M: 234, F: 169), S-IMCL (M: 239, F: 179), and T-IMCL (M: 239, F: 178)) is presented as the geometric mean, while statistical analysis was performed on log₁₀ transformed variables. All data are presented as mean ± s.d. Male vs. female data analyzed by Student's *t*-test.

assesses the duration and frequency of walking, moderate intensity, and vigorous intensity activity. We included subjects with a high or moderate score in our definition of “healthy” as this level of activity was considered sufficient to maintain cardiovascular health while individuals reported in the “low” physical activity group from either test were excluded. Subjects with a fat distribution that deviated by 2 s.d. from that found in healthy controls were identified by comparing the ratio of intra-abdominal to ASAT (IAAT/ASAT) and against BMI.

Age- and BMI-related reference ranges

Age group (16–25 years, 26–35 years, 36–45 years, and 56+ years) and BMI group reference ranges were calculated for each anthropometric and adipose variable for male and female subjects. For each dependent variable a fractional polynomial model was fitted to predict the mean curve estimated using generalized least squares (32). The s.d. was assumed to be constant and estimated using the residual mean square from the model. The reference ranges were calculated on the logarithmic scale and back transformed to the original scale of measurement for presentation. A logarithmic scale was used for the y axis in each graph. For IHCL the y axis contains (IHCL + 0.03) as it is not possible to plot IHCL values of zero on a logarithmic scale as the log of zero is not defined. Residuals from each fitted model were assessed for normality using normal plots and Shapiro and Francia's *W*²-test of normality (33). Log_e transformations were used for all six dependent variables to improve the assumption of normality of residuals of the models. As there were some values of zero for IHCL, the statistical analysis used a log (IHCL + 0.03) transformation. For T-IMCL a log (T-IMCL + 0.8) transformation was used to improve the normality of the residuals.

Multiple regression analysis

Regression models predicting AT were fitted to 11 variables: TAT, SAT, ASAT, NASAT, total internal, IAAT, NAIAT, trunk AT, S-IMCL, T-IMCL, and IHCL. Separate regression models were fitted to males and females. Log_e transformations were used for all dependent variables to improve the assumption of normality of residuals of the models. As there were some values of zero for IHCL, the statistical analysis used a log (IHCL + 0.03) transformation. For T-IMCL a log (T-IMCL + 0.8) transformation was used to improve the normality of the residuals. For each dependent variable six regression models were fitted. Independent variables considered were WC, BMI, hip, age, WHtR, and weight. The six regression models were: 1a: WC BMI; 1b: WC BMI hip; 1c: WC BMI hip age; 2a: WHtR weight; 2b: WHtR weight hip; 2c: WHtR weight hip age. The Bayesian Information Criterion was used to compare the goodness-of-fit of these six models (34).

Statistical analysis

Gender differences were analyzed using the Student's *t*-test. Significance is taken as *P* < 0.05. All data are presented as mean ± s.d. The statistical analysis was performed using Stata Release 11. Age- and BMI-related reference ranges were fitted to the data using established Stata routines (32).

RESULTS

Descriptive statistics

The mean age of all subjects was 37 years (range 18–71 years) with 25.0% of all subjects classified as overweight (32.6% of

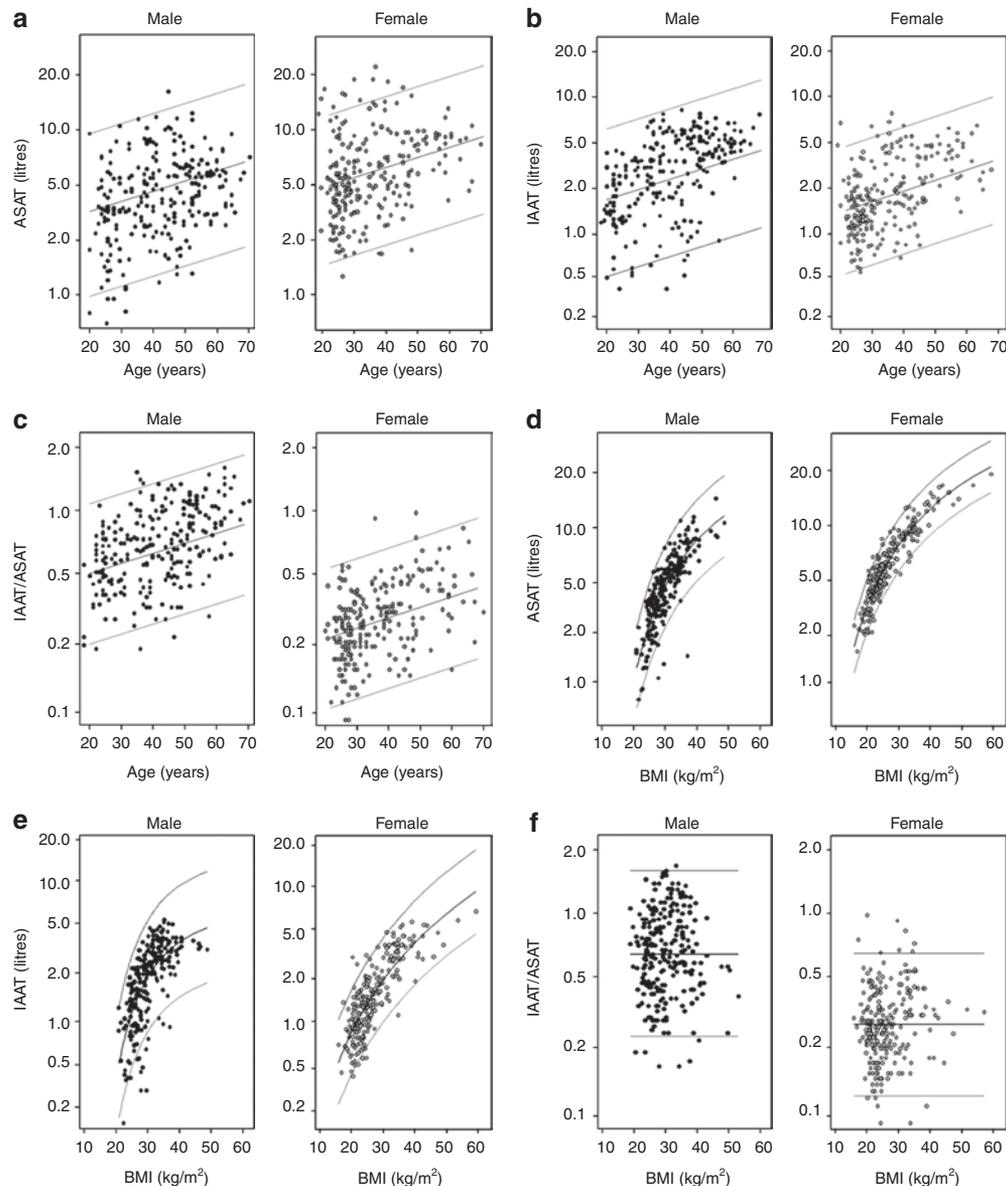


Figure 1 Abdominal subcutaneous (ASAT), intra-abdominal adipose tissue (IAAT), and IAAT/ASAT ratio by age and BMI group in male and female volunteers. (a–c) Age group and (d–f) BMI group-specific variation in (a, d) abdominal subcutaneous (ASAT) and (b, e) intra-abdominal adipose tissue (IAAT) in male and female volunteers. Adiposity stores are presented in litres. The ratio of IAAT/ASAT is also presented by (c) age and (f) BMI groups. The graphs present the fitted mean curve, and the 2.5th and 97.5th centiles, calculated assuming normal errors.

men, 17.0% women, BMI: 25 < 30), 24.7% qualifying as obese (26.8% of men, 22.6% of women, BMI: 30 < 40) and 2.9% morbidly obese (2.1% of men, 3.8% of women, BMI: 40+). Gender-specific characteristics are shown in [Table 1](#). Overall, female subjects were characterized by greater TAT, SAT, ASAT, NASAT, and lower internal, IAAT and lower IAAT/ASAT ratio than males ($P < 0.001$ for all, [Table 1](#)). The gender-specific range observed in IAAT and ASAT stores is shown in [Figure 1a–f](#). Given that males were slightly older than females (male: 40 ± 13 years vs. female: 36 ± 12 years, $P < 0.01$, [Table 1](#)) correction for age by multiple linear regression analysis was performed in order to examine gender differences ([Supplementary Table S1](#)). Males had a significantly greater weight, WC, height and WHR, but lower NASAT and NAIAT than females, when

correcting for age (all $P < 0.05$, [Supplementary Table S1](#)). Male volunteers also demonstrated a trend toward increased IHCL ($P = 0.08$) compared to females (adjusting for age) ([Supplementary Table S1](#)). The range of IHCL, S-IMCL, and T-IMCL values, as categorized by age and BMI group, are illustrated in [Figure 2a–f](#). All variables categorized by BMI and age group can be found in [Supplementary Tables S2–S4](#).

Age was positively correlated with all anthropometric variables (apart from a negative influence on height), ectopic fat stores (IHCL, S-IMCL, and T-IMCL) and adiposity depots collapsed across gender ([Supplementary Table S1](#)). NAIAT and IAAT/ASAT ratio showed a significantly positive interaction between age and gender (gender \times age P : NAIAT: 0.01, IAAT/ASAT: <0.001) ([Supplementary Table S1](#)). Age was associated

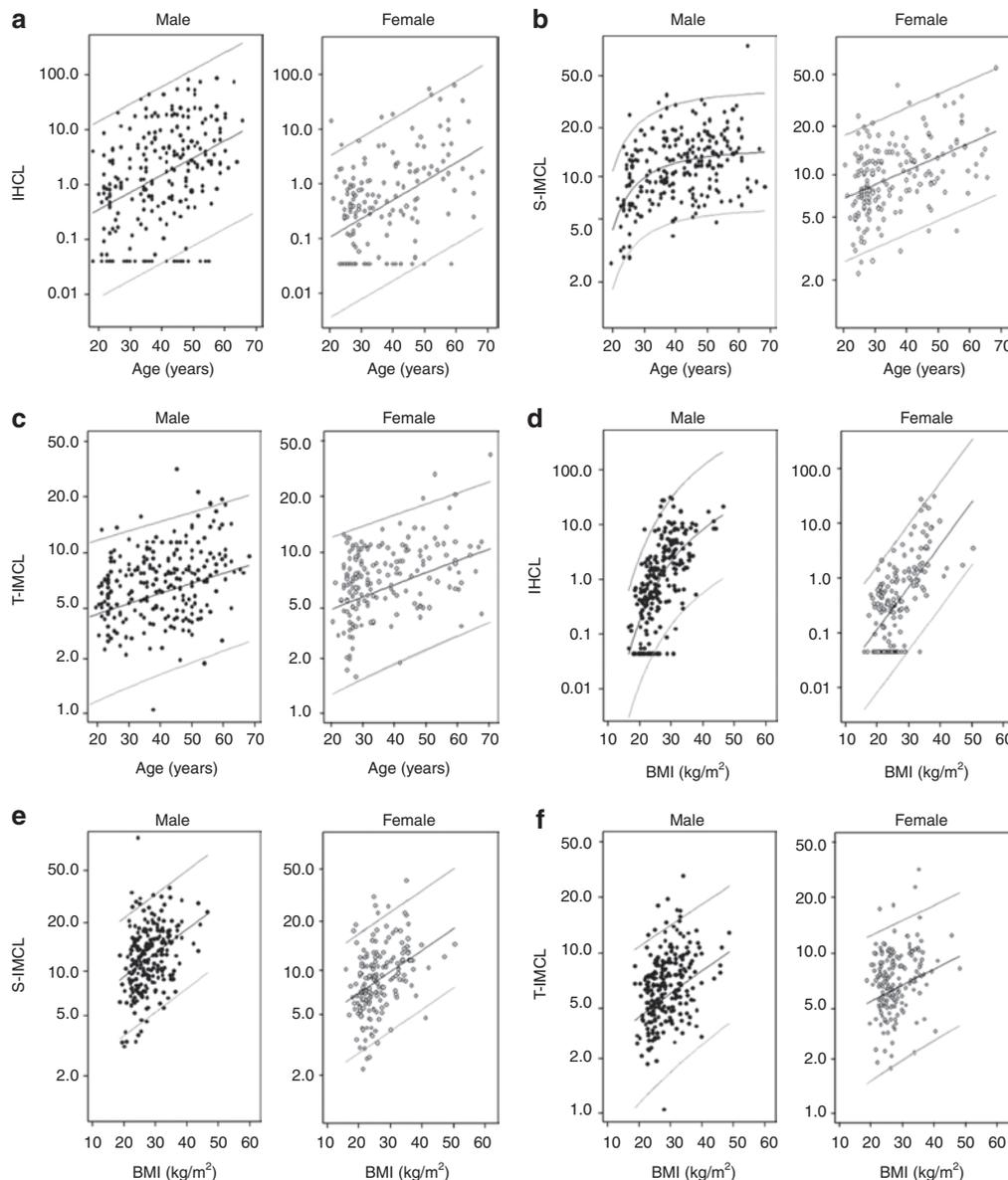


Figure 2 Ectopic lipid stores by BMI and age group in male and female volunteers. (a–c) Age group and (d–f) BMI group-specific variation in (a, d) intrahepatocellular lipid (IHCL), (b, e) intramyocellular muscle in the soleus (S-IMCL), and (c, f) tibialis (T-IMCL) in male and female volunteers. The graphs present the fitted mean curve, and the 2.5th and 97.5th centiles, calculated assuming normal errors.

with increased IAAT/ASAT in both genders, but had a greater effect in males ($\beta: 0.005 \pm 0.001$, **Supplementary Table S1**).

Correlation analysis

Gender-specific correlation analysis for all anthropometric variables, AT (in l) and ectopic fat stores are shown in **Table 2** (men) and **Table 3** (women). Apart from a few exceptions, all parameters correlated with each other to a significant degree ($P < 0.01$). In male subjects, WC was the variable which correlated to the greatest degree with individual adiposity stores (TAT: $r = 0.915$, SAT: $r = 0.878$, ASAT: $r = 0.850$, NASAT: $r = 0.868$, internal: $r = 0.804$, IAAT: $r = 0.815$, NAIAT: $r = 0.7147$, trunk: $r = 0.919$, $P < 0.01$, **Table 2**), whereas in female subjects BMI had the strongest correlation with individual adiposity stores (TAT: $r = 0.9514$, SAT: $r = 0.944$, ASAT: $r = 0.937$, NASAT:

$r = 0.927$, internal: $r = 0.850$, IAAT: $r = 0.839$, NAIAT: $r = 0.777$, trunk: $r = 0.949$, $P < 0.01$, **Table 3**).

IHCL correlated most strongly with WC in male subjects ($r = 0.712$, $P < 0.01$, **Table 2**) and WHtR in female subjects ($r = 0.644$, $P < 0.01$, **Table 3**). In male subjects, WC was also the strongest correlate of S-IMCL ($r = 0.504$, $P < 0.01$) and T-IMCL ($r = 0.389$, $P < 0.01$) (**Table 2**), whereas in females S-IMCL and T-IMCL correlated most strongly with BMI (S-IMCL: $r = 0.450$, T-IMCL: $r = 0.265$, $P < 0.01$ for both, **Table 3**). IAAT was the depot which correlated most strongly with ectopic fat in both genders (male IAAT: IHCL $r = 0.716$, S-IMCL; $r = 0.473$, T-IMCL; $r = 0.417$, $P < 0.01$ for all, **Table 2**; female IAAT: IHCL; $r = 0.720$, S-IMCL; $r = 0.506$, T-IMCL; $r = 0.319$, $P < 0.01$ for all, **Table 3**). In both male and female subjects, mass, BMI, WC, and WHtR correlated more strongly

Table 2 Linear correlation analysis between anthropometric measurements, lipid stores, and body fat stores in male subjects

Male n = 243	Age	Anthropometric variable					Ectopic fat store ^a					Adiposity depot (liters)								
		Weight	BMI	WC	Hip	Height	WHR	WHR	WHtR	IHCL	S-IMCL	T-IMCL	TAT	SAT	ASAT	NASAT	Internal	IAAT	NAIAT	Trunk
Weight	0.288**																			
BMI	0.378**	0.883**																		
WC	0.478**	0.881**	0.901**																	
Hip	0.311**	0.914**	0.841**	0.861**																
Height	-0.124	0.352**	-0.120	0.090	0.355**															
WHR	0.510**	0.603**	0.706**	0.856**	0.479**	-0.108														
WHtR	0.493**	0.745**	0.912**	0.950**	0.766**	-0.216*	0.885**													
IHCL	0.405**	0.551**	0.625**	0.712**	0.559**	-0.070	0.663**	0.707**												
S-IMCL	0.389**	0.377**	0.378**	0.504**	0.437**	0.054	0.427**	0.463**	0.440**											
T-IMCL	0.314**	0.293**	0.343**	0.389**	0.300**	-0.051	0.375**	0.384**	0.417**	0.590**										
TAT	0.376**	0.836**	0.832**	0.915**	0.863**	0.098	0.712**	0.855**	0.663**	0.427**	0.386**									
SAT	0.271**	0.836**	0.823**	0.878**	0.876**	0.116	0.632**	0.816**	0.590**	0.368**	0.338**	0.975**								
ASAT	0.231**	0.829**	0.841**	0.850**	0.858**	0.074	0.599**	0.806**	0.598**	0.358**	0.359**	0.922**	0.944**							
NASAT	0.280**	0.801**	0.775**	0.868**	0.862**	0.133*	0.632**	0.799**	0.567**	0.357**	0.311**	0.958**	0.984**	0.870**						
Internal	0.550**	0.635**	0.659**	0.804**	0.642**	0.028	0.750**	0.761**	0.692**	0.451**	0.414**	0.825**	0.678**	0.647**	0.663**					
IAAT	0.548**	0.629**	0.669**	0.815**	0.632**	-0.004	0.779**	0.778**	0.716**	0.473**	0.417**	0.804**	0.659**	0.639**	0.976**	0.639**				
NAIAT	0.501**	0.585**	0.580**	0.714**	0.598**	0.075	0.638**	0.664**	0.589**	0.462**	0.370**	0.781**	0.642**	0.598**	0.637**	0.943**	0.847**			
Trunk	0.396**	0.822**	0.848**	0.919**	0.840**	0.047	0.740**	0.874**	0.711**	0.435**	0.421**	0.962**	0.911**	0.939**	0.854**	0.859**	0.865**	0.769**		
IAAT/ ASAT	0.460**	-0.026	0.012	0.187**	-0.013	-0.071	0.369**	0.182**	0.330**	0.195**	0.169**	0.125	-0.064	-0.132*	-0.024	0.571**	0.603**	0.467**	0.185**	

All data are presented as Pearson partial correlation r values. Bold typeface indicates a significant correlation; shaded boxes indicate the anthropometric variable with the strongest correlation.

ASAT, abdominal subcutaneous adipose tissue; IAAT, intra-abdominal adipose tissue; IHCL, intrahepato cellular lipid; IMCL, intramyocellular lipid; S, soleus; T, tibialis; internal, total internal; NAIAT, non-abdominal internal adipose tissue; NASAT, non-abdominal subcutaneous adipose tissue; SAT, subcutaneous adipose tissue; TAT, total adipose tissue; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio.

^aStatistical analysis of IHCL (M: 234), S-IMCL (M: 239), and T-IMCL (M: 239) performed on \log_{10} transformed variables.

* $P < 0.05$, ** $P < 0.01$.

Table 3 Linear correlation analysis between anthropometric measurements, lipid stores, and body fat stores in female subjects

Female n = 234	Anthropometric variable				Ectopic fat store ^a				Adiposity depot (liters)										
	Age	Weight	BMI	WC	Hip	Height	WHR	WHR	IHCL	S-IMCL	T-IMCL	TAT	SAT	ASAT	NASAT	Internal	IAAT	NAIAT	Trunk
Weight	0.276**																		
BMI	0.357**	0.945**																	
WC	0.477**	0.862**	0.892**																
Hip	0.350**	0.882**	0.874**	0.848**															
Height	-0.261*	0.114	-0.208*	-0.049	0.052														
WHR	0.456**	0.488**	0.530**	0.825**	0.405**	-0.158*													
WHR	0.462**	0.734**	0.832**	0.973**	0.801**	-0.227*	0.832**												
IHCL	0.477**	0.501**	0.622**	0.623**	0.510**	-0.309*	0.539**	0.644**											
S-IMCL	0.501**	0.397**	0.450**	0.436**	0.402**	-0.133	0.333**	0.402**	0.404**										
T-IMCL	0.371**	0.196*	0.265**	0.278**	0.191*	-0.162*	0.295**	0.239**	0.399**	0.448**									
TAT	0.292**	0.959**	0.951**	0.854**	0.879**	-0.032	0.461**	0.762**	0.584**	0.426**	0.174*								
SAT	0.256**	0.959**	0.944**	0.834**	0.878**	-0.013	0.431**	0.738**	0.552**	0.387**	0.138	0.995**							
ASAT	0.227**	0.945**	0.937**	0.845**	0.874**	-0.038	0.455**	0.753**	0.545**	0.384**	0.151	0.970**	0.974**						
NASAT	0.266**	0.946**	0.927**	0.803**	0.852**	-0.001	0.408**	0.706**	0.509**	0.378**	0.127	0.987**	0.992**	0.939**					
Internal	0.424**	0.821**	0.850**	0.772**	0.704**	-0.117	0.532**	0.721**	0.686**	0.505**	0.299**	0.879**	0.828**	0.807**	0.822**				
IAAT	0.461**	0.796**	0.839**	0.787**	0.690**	-0.165*	0.577**	0.745**	0.720**	0.506**	0.319**	0.852**	0.802**	0.799**	0.786**	0.974**			
NAIAT	0.322**	0.773**	0.777**	0.657**	0.643**	-0.031	0.408**	0.596**	0.552**	0.445**	0.232**	0.828**	0.781**	0.734**	0.790**	0.935**	0.829**		
Trunk	0.302**	0.943**	0.949**	0.879**	0.874**	-0.075	0.511**	0.797**	0.637**	0.448**	0.212**	0.978**	0.967**	0.986**	0.936**	0.887**	0.888**	0.792**	
IAAT/ ASAT	0.446**	0.053	0.122	0.232**	0.075	-0.184*	0.335**	0.267**	0.373**	0.303**	0.304**	0.122	0.038	0.009	0.0535	0.509**	0.539**	0.409**	0.158*

All data are presented as Pearson partial correlation r values. Bold typeface indicates a significant correlation; shaded boxes indicate the anthropometric variable with the strongest correlation.

ASAT, abdominal subcutaneous adipose tissue; IAAT, intra-abdominal adipose tissue; IHCL, intrahepatic adipose tissue; IMCL, intramyocellular lipid; IMCL, total internal; NAIAT, non-abdominal internal adipose tissue; NASAT, non-abdominal subcutaneous adipose tissue; SAT, subcutaneous adipose tissue; TAT, total adipose tissue; WHR, waist-to-hip ratio; WHR, waist-to-hip ratio.

^aStatistical analysis of IHCL (F: 169), S-IMCL (F: 179), and T-IMCL (F: 178) performed on \log_{10} transformed variables.

* $P < 0.05$, ** $P < 0.01$.

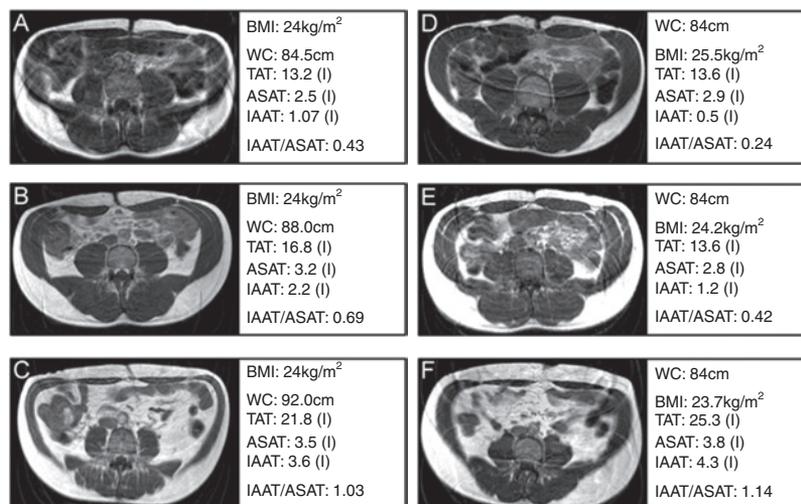


Figure 3 Anthropometric variation in abdominal adiposity. Umbilical MRI images obtained from six different male subjects with a (a–c) BMI of 24.0, or a (d–f) WC of 84.0 cm. ASAT, subcutaneous abdominal adipose tissue (l); IAAT, intra-abdominal adipose tissue (l); TAT, total adipose tissue (liters); WC, waist circumference. In male subjects, the TOFI phenotype is attributed to individuals with BMI of 18.5–24.99 kg/m² and a IAAT/ASAT ratio greater or equal to 1.0.

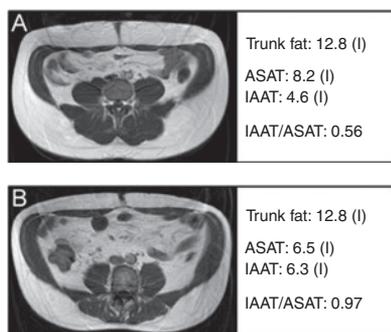


Figure 4 Truncal variation in abdominal adiposity. Umbilical magnetic resonance imaging (MRI) images obtained from two different male subjects with an equal amount truncal fat (liters), but markedly different IAAT and ASAT deposits. ASAT, subcutaneous abdominal adipose tissue (l); IAAT, intra-abdominal adipose tissue (l).

with each other than individual percentage adiposity stores (Tables 2 and 3).

Multiple regression analysis—Bayesian Information Criterion

The goodness-of-fit of six regression models using Bayesian Information Criterion is shown in Supplementary Table S5. WC provides the best model for the majority of adipose and ectopic variables in male subjects. In female subjects, BMI contributes the most for each dependent variable.

The TOFI phenotype

The gender-specific variable data from healthy, active individuals used to define the TOFI phenotype can be found in Supplementary Table S6. The mean IAAT/ASAT ratio for healthy white individuals was 0.59 (male) and 0.25 (female). Two standard deviations above the mean IAAT/ASAT of healthy individuals (+2 s.d. male: 1.04, female: 0.45) was used

to define the cutoff for TOFI classification. Thus, individuals with a BMI 18.5 < 25 kg/m² with an IAAT/ASAT ratio above 1.0 (males) and 0.45 (females) were classified as TOFI; this corresponds to 14% of men (15/106) and 12% of women (17/132) in our cohort. Gender-specific variable data for TOFI and non-TOFI individuals can be found in Supplementary Table S7. Significantly greater IHCL, S-IMCL, and T-IMCL were observed in both male and female TOFI female volunteers (Supplementary Table S7). Subjects classified with the TOFI phenotype based on their IAAT, could not be predicted from BMI, WHR or WHtR or trunk fat because there was no significant difference in these variables between TOFI and non-TOFI healthy subjects (Supplementary Table S7).

WHR was the anthropometric variable which correlated most strongly with the IAAT/ASAT ratio in both men ($r = 0.369$, $P < 0.01$) (Table 2) and women ($r = 0.335$, $P < 0.01$) (Table 3). The variation in IAAT/ASAT in male subjects with either an identical BMI (24.0 kg/m²) or WC (84.0 cm) is illustrated in Figure 3. Axial MRI scan data in Figure 4 demonstrates the variation in ASAT and IAAT from two individuals with identical trunk fat (IAAT + ASAT).

DISCUSSION

There is a growing recognition that the increased health risks of obesity and metabolic syndrome are more strongly associated with central rather than total adiposity, with an excess in IAAT and liver fat being the key determinants (1). In this study, we have employed MRI-based techniques in order to determine patterns of fat distribution in a large heterogeneous cohort and used the ratio of intra-abdominal (IAAT) and subcutaneous (ASAT) AT in healthy subjects as a means of identifying white individuals at potentially increased metabolic risk. In addition, we demonstrate a large variation in IAAT, ASAT, and ectopic fat deposition in the liver and skeletal muscle that is not fully predicted by conventionally used anthropometric measurements.

Age and gender effects on fat depots

Gender differences in energy regulation and body fat distribution are well established; with women demonstrating proportionally higher total AT and greater fat deposition in the lower body, whereas men are predisposed to increased upper body fat accumulation. Our results reflect these findings, with women demonstrating significantly higher percentage total and subcutaneous fat stores than the males. There is conflicting data in the literature regarding gender differences in intra-abdominal deposition, with some studies suggesting that men have greater IAAT than women (18), while others reveal no difference (35). When correcting for age in our cohort we found significantly greater IAAT in males compared to females. In agreement with previous studies, we reveal significantly greater subcutaneous fat depots in male subjects as age increases but a variable picture in female subjects (18,36). Our MRI data corroborate previous observations indicating an abrupt reduction in subcutaneous fat stores with the menopause as recorded in white females in the 46–55 years compared to 36–45 years groups (37) (**Supplementary Table S4**).

Ectopic fat stores

In addition to the major subcutaneous and abdominal fat compartments, ectopic fat depots in the liver and skeletal muscle are implicated in the pathogenesis of insulin resistance, a key facet of the metabolic syndrome (1). The ectopic fat hypothesis suggests that a lack of sufficient adipocytes and/or limited capacity results in excess adipose storage around tissues and organs such as the liver, heart, and kidneys (38). The exact mechanism by which ectopic fat accumulation affects tissue and organ function is unknown, but may include physical compression, altering local secretory profiles, and lipotoxicity (1). More tangible is the strong association between increased ectopic fat storage and obesity, type 2 diabetes mellitus, and insulin resistance (39).

Here, we report male subjects demonstrate significantly higher levels of IHCL, similar levels of S-IMCL and lower levels of T-IMCL compared to women, correcting for age. There are contradictory reports concerning the effects of age on ectopic fat deposition, with one study reporting an age-related increase in IHCL and IMCL (40) whereas others have found no association (18,41). In our cohort, correlation analysis indicates a significant positive relationship between age and IHCL and both IMCL ectopic fat depots in both male and female subjects. In agreement with previous data showing a significant relationship between ectopic fat deposition and internal adiposity (18,21) we demonstrate IHCL, S-IMCL, and T-IMCL all correlate strongest to abdominal adiposity stores, in particular IAAT. As such, separate analysis with multiple methods of data presentation would seem prudent when analyzing cohort body composition data.

Anthropometric variables as markers of fat deposition

Anthropometric measurements are easily obtainable, inexpensive, and commonly used determinants of both obesity and the metabolic syndrome (27,42). We found anthropometric

variables generally correlated with total and subcutaneous stores better than with internal depots or ectopic fat stores. Previous studies have demonstrated that both WC and WHtR correlate well with abdominal fat mass (both subcutaneous and intra-abdominal) and cardiometabolic disease (43). There is a growing body of evidence endorsing WHtR as the best measure of obesity and metabolic risk, regardless of ethnicity (44,45) with the incorporation of adjustment for stature the rationale behind its improvement in correlation over WC (46). In our analysis, we found that both WC and WHtR correlated strongly with ectopic fat depots and percentage adiposity stores. However, as illustrated in **Figure 3** there is a large amount of variation in abdominal fat at a given WC measurement. Both correlation and multiple regression analysis reveal a clearly defined segregation between the strongest anthropometric determinants for either subcutaneous or internal adipose stores, specific to gender. In agreement with a recent study by Flegal *et al.* we found BMI to be the best anthropometric predictor for individual adiposity stores in women (47). By contrast, WC was the best predictor of individual adiposity depot volumes in males, while WHtR was the best predictor of IHCL in both genders. Overall, we found anthropometric variables to be more closely related to each other than individual adiposity or ectopic fat stores but generally correlated well within gender.

Analyzing adipose distribution as a percentage of body mass (%kg) or as a percentage of total AT (%TAT) is often implemented as it provides an insight into both fat-free mass and the distribution of specific adipose stores, respectively. Correlation and multiple regression analysis revealed similar gender patterns of significance between adiposity data transformed via these two approaches and data analyzed as an absolute volume in liters (data not shown).

Individuals matched for ASAT, with high levels of IAAT demonstrate significantly increased insulin resistance and decreased glucose tolerance compared to those with less IAAT (48–50). Several more recent studies have also shown that IAAT is a stronger correlate of metabolic syndrome associated risks than either ASAT or anthropometric variables (4,6,10). Indeed, there is conflicting data in the literature concerning the use of multiple anthropometric variables to predict cardiovascular risk. It has been suggested that combining BMI with WC increases the cardiovascular risk prediction than either measure (51) whereas other data suggests BMI reduces the discriminatory power for CVD risk factors, supporting the use of WHtR as the sole measure of obesity (52).

Multiple regression analysis performed in our cohort suggests that specific combinations of anthropometric variables can be used to predict 60–70% of ASAT and IAAT values. However, despite this significant degree of correlation we reveal a wide range of IAAT and ASAT values by BMI and age group. In addition, we illustrate a significant variation in IAAT and ASAT in subjects with identical BMI's or WC measurements (**Figure 3**). Anthropometric variables such as WC and WHtR can give no indication of the proportion of IAAT or ASAT in seemingly “lean” subjects and are therefore inappropriate for

classifying individuals that may be at increased metabolic risk within a “normal” BMI range.

The “thin outside fat inside (TOFI)” subphenotype

Methods of measuring abdominal obesity such as Viscan and dual-energy X-ray absorptiometry offer a faster, less expensive alternative to the MRI protocol implemented here (53,54). However, while there is no doubt that a strong correlation exists between abdominal fat and metabolic risk, these methods are unable to differentiate between individual abdominal adipose stores. Here, we propose using a ratio of IAAT and ASAT (IAAT/ASAT) to identify individuals at potentially increased metabolic risk. We have determined the range of IAAT and ASAT in healthy individuals in order to define the limits by which individuals with a proportionally elevated IAAT, or TOFI (thin outside fat inside) can be classified. When considering our entire cohort, this healthy control group represents 10–20% of individuals; the same proportion of healthy individuals observed in the normal population (55). For ease of reference, we propose using an IAAT/ASAT ratio cutoffs of >1.0 in white male subjects and 0.45 in white female subjects to define this phenotype.

The components of the IAAT/ASAT ratio were selected in order to identify patterns of abnormal body fat distribution. We employed ASAT due to its abdominal location and potentially protective role against cardiovascular and metabolic risk compared to IAAT (9), as opposed to whole-body SAT or NASAT. Anthropometric measurements, such as WHR and WC, identify individuals with larger waists and hence more total abdominal fat. As a predictor for abnormal body fat distribution these measurements rely on a strong relationship between total abdominal fat and visceral fat across a wide range of abdominal fat. However, we found a notable variation in visceral adiposity observed in our healthy population, who represent individuals with “normal” WCs, highlighting the fact that on an individual basis visceral adiposity may vary despite similar waist or abdominal fat. Indeed, as illustrated in **Figure 4**, individuals can show identical amounts of trunk fat and yet have entirely different amounts of IAAT and ASAT. Other measurements that could potentially reflect the TOFI phenotype, such as fat-free mass or percentage body fat, would only reflect excess total adiposity either relative to body size (fat-free mass index, fat mass index), lean tissue (FM:FFM ratio) or as a percentage of weight (% body fat) and therefore not accurately represent fat distribution. If used in combination with waist measurements these ratios may imply excess adiposity around the abdomen, however this does not necessarily reflect visceral fat.

Correlation analysis revealed relatively weak associations between the IAAT/ASAT ratio that we have used to define TOFI individuals and other physiological characteristics. Indeed, it was age, a nonanthropometric variable, which provided the strongest correlation to IAAT/ASAT. There was a notable lack of statistical difference in anthropometric variables between TOFI and non-TOFI individuals save for changes in internal fat depots. These data suggest that MRI analysis is currently

the only means of successfully identifying individuals with a disproportionately high amount of intra-abdominal fat.

Our data and that of other studies have previously reported a significant positive relationship between liver fat and IAAT content (18,21). Furthermore, we and others have also found that individuals with a phenotype opposite to that of the TOFI (the so called “fat-fit”), have reduced intra-abdominal and IHCL compared to weight matched individuals (56,57). In addition to a strong correlation between the IAAT/ASAT ratio and individual ectopic fat depots, we also observed a significant increase in IHCL, S-IMCL, and T-IMCL in TOFI compared to non-TOFI individuals, for both sexes. When our proposed IAAT/ASAT cutoff values were applied to individuals with an increased BMI (greater than the 18.5 <25 kg/m² range) we found that 16% of females and 23% of males were registered as TOFI. This increase in male classification is likely a reflection of the increased proportion and deposition of IAAT observed at a higher BMI in males.

Further work will be required to characterize healthy control individuals within increased BMI ranges to accurately define those with excessive IAAT. Here, we attribute the TOFI phenotype to 12–13% of European white volunteers that fall within a normal BMI range (28). Additional studies may also reveal the applicability of the TOFI index to additional ethnic populations given the established differences in body fat distribution between racial groups (58,59). Currently, the TOFI index provides a quantitative means of comparing intra-abdominal fat deposition. Clearly, the utility of the TOFI phenotype to classify this “at risk” group of individuals will only be fully realized once it has been correlated with markers of the metabolic syndrome. Further work will also be required to determine the physiological basis for the wide variation in abdominal fat partitioning we have recorded here. The mechanism is likely to be complex, with a multitude of genetic-, environmental- and age-related determinants.

In summary, we reveal the pattern of regional adiposity in a large cohort of UK-based volunteers, providing gender- and age-specific reference range data and elucidate the relationships between individual fat depots. We found anthropometric variables to be more closely related to each other than adiposity or ectopic fat stores but overall correlated well within gender. Furthermore, our data demonstrate that specific anthropometric variables should be used to best predict individual adiposity stores and ectopic fat stores for each gender; WC in men, and BMI in women. Finally, we have used the ratio of IAAT and ASAT in a defined “healthy” subset of our cohort to define the TOFI subphenotype, a potential means of evaluating abdominal obesity and identifying individuals at potentially increased metabolic risk.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/oby>

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DISCLOSURE

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