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Commonality versus specificity among adiposity traits in normal-weight and moderately overweight adults

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Abstract

BACKGROUND—Many adiposity traits have been related to health complications and premature death. These adiposity traits are intercorrelated but their underlying structure has not been extensively investigated. We report on the degree of commonality and specificity among multiple adiposity traits in normal-weight and moderately overweight adult males and females (mean body mass index (BMI) = 22.9 kg m^{-2}, s.d. = 2.4).

METHODS—A total of 75 healthy participants were assessed for a panel of adiposity traits including leg, arm, trunk, total fat masses and visceral adipose tissue (VAT) derived from dual energy X-ray absorptiometry (DXA), hepatic and muscle lipids from proton magnetic resonance spectroscopy, fat cell volume from an abdominal subcutaneous adipose tissue biopsy (n = 36) and conventional anthropometry (BMI and waist girth). Spearman's correlations were calculated and were subjected to factor analysis.

RESULTS—Arm, leg, trunk and total fat masses correlated positively (r = 0.78–0.95) with each other. VAT correlated weakly with fat mass indicators (r = 0.24–0.31). Intrahepatic lipids (IHL)

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correlated weakly with all fat mass traits (r = 0.09–0.34), whereas correlations between DXA depots and intramyocellular lipids (IMCL) were inconsequential. The four DXA fat mass measures, VAT, IHL and IMCL depots segregated as four independent factors that accounted for 96% of the overall adiposity variance. BMI and waist girth were moderately correlated with the arm, leg, trunk and total fat and weakly with VAT, IHL and IMCL.

CONCLUSION—Adiposity traits share a substantial degree of commonality, but there is considerable specificity across the adiposity variance space. For instance, VAT, IHL and IMCL are typically poorly correlated with each other and are poorly to weakly associated with the other adiposity traits. The same is true for BMI and waist girth, commonly used anthropometric indicators of adiposity. These results do not support the view that it will be possible to identify adequate anthropometric indicators of visceral, hepatic and muscle lipid content in normal-weight and moderately overweight individuals.

Keywords

adiposity; hepatic fat; myocellular fat; visceral adipose tissue; common variants

INTRODUCTION

Scientists and clinicians have been debating for years over the most appropriate adiposity trait to measure in order to predict the risk of morbidities or premature death associated with an excessive amount of stored fat.[1] One school of thought proposed that the accumulation of fat on the trunk and the upper body was closely related to the metabolic complications of obesity.[2–6] A large body of data suggests that abdominal visceral fat augments the risks of cardiovascular disease and type 2 diabetes beyond those conferred by overweight and obesity.[2,7–9] More recently, it has been suggested that preferential storage of excess lipid in the lower body adipose tissue depot may prevent the development of metabolic abnormalities associated with obesity.[10] In addition, adipocyte cell size is a focus of considerable interest. Subcutaneous abdominal adipocyte hypertrophy has been associated with insulin resistance, whereas the hyperplastic adipose tissue phenotype has been suggested to be preventive.[11,12] One current view is that a limited subcutaneous adipose tissue expandability leads to storage of lipids in ectopic sites such as the liver and muscle, which in turn sets the stage for insulin resistance in these organs.[13–15]

A fundamental question that needs to be addressed in the search for the most relevant adiposity traits to predict the risk for the metabolic complications of obesity is the degree of commonality and specificity, as well as the relationship among various adiposity traits. Such data would provide useful information regarding the underlying biological pathways associated with variable amounts of lipid accumulation among depots. It could also be helpful in identifying causal pathways modulating the relationships between adiposity, risk factors levels, morbidities and premature death.

To investigate the quantitative structure underlying these traits, we have taken advantage of a study (InSight) conducted at Pennington Biomedical Research Center on free-living adults who were measured with an extensive panel of adiposity-related traits. The adiposity measures considered in the present analysis include body mass index (BMI), waist girth,

total fat mass, trunk fat, arm fat and leg fat as assessed by dual energy X-ray absorptiometry (DXA), visceral adipose tissue (VAT) measured with a new DXA-based procedure, and intrahepatic (IHL) and intramyocellular (IMCL) lipids measured by proton magnetic resonance spectroscopy (MRS). The relationship between these traits and abdominal fat cell volume is also explored in a subsample.

MATERIALS AND METHODS

Subjects

Seventy-five healthy and free-living adults (37 women and 38 men; 59 Whites, 10 Blacks and 6 from other ethnicities) participated in the InSight study (ClinicalTrials.gov identifier: NCT00945633), a longitudinal study designed to identify the molecular, physiological and behavioral factors associated with weight gain. The study was approved by Pennington Biomedical Research Center's Institutional Review Board. Written informed consent was obtained from each participant. The subjects were selected using the inclusion criteria of age (20–35 years at the time of screening), BMI (<27.9 kg m^{-2}) and fasting blood glucose (<126 mg dl^{-1} or 6.99 mmol l^{-1}). Recruitment was deliberately focused on normal-weight and moderately overweight subjects in order to identify the most significant predictors of weight gain over time. Inclusion of more severely overweight and obese participants would have made it impossible to identify the predictors that are typically masked by weight gain. Exclusion criteria were the diagnosis of diabetes, select medications (as determined by the study physician from the checklist completed by the participant at screening), injuries or surgeries that could have influenced health status and pregnancy (for women). Women had to be at least 6 months postpartum and have discontinued breastfeeding for at least 3 months before screening. Other exclusion criteria were a history of cancer (including skin cancer) within 5 years, organ transplant, previous diagnosis of human immunodeficiency virus, hepatitis B or C, or tuberculosis, abuse of alcohol or illegal drugs, abnormal electrocardiogram, and presence of pacemaker, defibrillator or an implanted metal prosthesis.

Anthropometry

Weight and height were measured in duplicate to the nearest 0.1 cm and kg, and BMI was calculated (weight in kg divided by height in m^2). Waist girth was measured in duplicate using a nonelastic tape placed midpoint between the inferior border of the rib cage and superior aspect of the iliac crest.[16]

Total and regional adiposity

Total body, arm, leg and trunk fat masses were measured by DXA using the Hologic model QDR 4500 A fan-beam densitometer (Hologic Corp., Bedford, MA, USA)[17–19] and QDR software (version 11.1.2 for Windows). For calibration and quality control assurance, manufacturer-recommended phantom scans were routinely performed.[17]

Visceral adipose tissue

VAT was measured from the DXA scan using APEX software (version 4.0; Hologic Corp.; document number MAN-02354). The software automatically locates the outer and inner

margins of the abdominal wall on both sides of the DXA image based on fat and lean mass profiles in a 5-cm region across the abdomen, with the bottom edge of the region located 1 cm above the iliac crest. The software then measures the total fat mass within the abdominal walls, a region that contains both subcutaneous and visceral fat. The amount of subcutaneous fat between the skin line and outer abdominal wall on both sides of the image is measured, and this estimate is subtracted from the total fat mass measured within the region to yield VAT. Previous studies have reported excellent validity (compared with computed tomography) and reproducibility of the DXA method to estimate VAT.[20] Two validation studies have reported excellent agreement between VAT measured by computerized tomography and DXA.[20,21]

Ectopic lipid depots

Intramyocellular (IMCL) and intrahepatic lipids (IHL) were measured by MRS[22–24] using a 3.0 T whole-body imaging and spectroscopy system (General Electric Medical Systems, Milwaukee, WI, USA) and the Point-resolved spectroscopy technique. Java-based magnetic resonance user interface software[25] with the time domain-fitting model was used to analyze spectra for lipid type.

IMCL—For muscle lipid content, IMCL was measured from the right calf soleus and anterior tibialis muscles as previously described.[24,26] A description of procedures and methods of calculations can be found elsewhere.[23,27]

IHL—Before MRS for IHL, a magnetic resonance imaging scan of the liver was performed to choose a location free from large vessels in the middle of the right lobe. The subject was lightly restrained and advised to maintain shallow breathing to reduce the liver motion induced by respiration.[22,23,27,28] As no respiratory gating was used, spectra for IHL were measured with larger voxels, as compared with calf measures, to represent average liver fat measurement over the mid-right lobe. Details for the spectral collection method and calculations have been published elsewhere.[22]

All lipid signal amplitudes were normalized to the corresponding internal water peaks.[29] Relative to internal water (× 100), peak areas of interest per voxel area[30] were expressed as arbitrary units. For routine quality assurance, an external peanut oil phantom was used.

Fat cell volume

Abdominal fat cell volume was determined in a subsample of 36 subjects (18 from each sex). Adipose tissue biopsies were obtained from the subcutaneous abdominal region two-thirds of the way from the iliac spine to the umbilicus using a miniliposuction procedure, and the tissue was fixed with osmium tetroxide.[31] The diameter of cells (based on >2000 cells) was measured using the Coulter principle method,[32] and the volume was calculated using the formula:[33]

$$\text{Cell volume}\,(nL) = \frac{4}{3}\pi \times (\text{diameter in}\,\mu\text{m}/2)^3 \times 10^6$$

The instrument was calibrated by running fixed-size cell standards (20, 43 and 90 μm) along with each measurement. The arithmetic mean of the volume was calculated for each subject as the sum of the volume of all adipocytes measured divided by their number.

Statistical analysis

The data were analyzed using SAS v9.3 (SAS Institute Inc., Cary, NC, USA). Spearman's correlations were computed to quantify the associations among adiposity-related variables. Data were adjusted for age, sex and height squared (m^2) through regression procedures.

Factor analysis was undertaken to define the underlying structure among the following adiposity traits: arm fat, leg fat, trunk fat, total body fat, VAT, IHL and IMCL. Factor analysis was performed on rank-order correlations in order to minimize the potential role of strong deviations from distribution symmetry in some of the variables. Varimax rotation was then applied to maximize the independence of the factors extracted from the data. The first four factors accounted for 96% of the variance. These four factors were retained for further analysis. Spearman's correlations were used to investigate whether and to what extent BMI, waist girth and fat cell volume were related to the primary adiposity traits.

RESULTS

Descriptive statistics

Mean, s.d., median, minimum and maximum values for all variables are provided in Table 1. The mean age of subjects was 26.8 ± 4.5 years, with a range from 20 to 35 years. The sample as a whole tended to be on the lean side, with a mean BMI of 22.9 ± 2.4 kg m^{-2} and a range from 18.5 to 27.7 kg m^{-2}. Table 1 also includes the data for men and women of the study, with the *P*-value column pertaining to the differences between the means of each sex. There were no differences in the mean age, VAT, IHL or fat cell size between men and women and only marginal differences for BMI and trunk fat.

Correlation analysis

Correlation matrices calculated with age-adjusted scores (above diagonal) are presented for reference, but the present study focuses on data adjusted for age, sex and height squared (below the diagonal), as depicted in Table 2. Highly significant ($P \leq 0.0001$), moderate-to-strong positive correlations ($r = 0.78–0.95$) were found among the arm fat, leg fat, trunk fat and total fat masses. Although statistically significant, VAT was weakly correlated with these adiposity traits ($r = 0.24–0.31$). Correlations between IMCL and the other adiposity traits were trivial. In contrast, IHL was significantly ($P \leq 0.05$) but weakly correlated with all DXA adiposity traits. IHL was not correlated with VAT ($r = 0.09$).

Factor analysis

A factor analysis based on rank-order correlations ($N = 75$) was conducted on seven variables: arm fat, leg fat, trunk fat, total fat, VAT, IHL and IMCL. The first four factors explained 96% of the variance space (Table 3). After varimax rotation, these factors resulted in well-defined and clearly interpretable orthogonal traits as shown in Table 4. With high positive loadings, total fat (0.98), arm fat (0.95), leg fat (0.91) and trunk fat (0.94) all

defined a global adiposity trait (factor 1 accounting for 57% of the variance for adiposity traits; Table 4). Factor 2 (16% of the overall variance) was strongly defined by VAT with a factor loading of 0.98. Factor 3 (13% of the variance) loaded very highly on IHL (0.98), whereas factor 4 (11% of the variance) was defined by IMCL (0.99).

Correlations of adiposity traits with BMI, waist girth and fat cell volume

BMI and waist girth significantly correlated ($r = 0.75$) with each other. Correlations between BMI, waist girth, fat cell size and the DXA adiposity traits adjusted for age, sex and height squared are shown in Table 5. Arm fat, leg fat, trunk fat and total fat masses were moderately to strongly correlated ($r \geq 0.74$) with BMI. VAT was weakly correlated with BMI ($r = 0.32$), whereas IHL and IMCL were not significantly correlated with BMI. The waist girth was strongly correlated with the trunk fat ($r = 0.80$), whereas less strongly associated with the arm fat, leg fat and total fat masses (ranging from 0.56–0.73). VAT, IHL and IMCL were all weakly but significantly correlated with waist girth ($r = 0.29$–0.31).

Arm fat, leg fat, trunk fat and total fat were all moderately but significantly correlated with subcutaneous abdominal adipocyte volume ($r = 0.52$–0.64). In contrast, fat cell volume was not significantly correlated with VAT, IHL or IMCL.

DISCUSSION

The present study deals with the relationships among body composition, fat distribution, ectopic fat depots and adipocyte volume in normal-weight and moderately overweight young adults of both sexes. Our main finding is that DXA total and regional fat depots, VAT, IHL and IMCL stores segregated into four independent factors. Moreover, strong evidence of commonality was observed between DXA total and regional fat depots with BMI and waist girth. One goal was to understand whether common anthropometric indices or global indicators of adiposity could be used as reliable surrogate measures of visceral fat mass or hepatic and skeletal muscle lipid accretion in normal-weight and moderately overweight individuals. The findings of the present study are quite clear in this regard: none of these measures are valid surrogates for VAT, IHL and IMCL in normal-weight and moderately overweight young adults. The lower than expected correlations between waist girth and the other adiposity traits, particularly VAT, may indicate that waist girth is not a strong predictor of DXA adiposity or that our predominantly lean study sample is inadequate for investigating this relationship compared with a population with a wide range of adiposity.

When correlations among DXA adiposity variables and MRS-measured intracellular hepatic and skeletal muscle lipid stores were compared, the results were mixed. Notably, DXA fat mass, arm fat, leg fat and trunk fat correlated significantly but weakly with IHL. This is supportive of the low-to-moderate associations between whole-body and regional adiposity with elevated hepatic lipid content reported by others.[13,34] We found trivial associations between IMCL and the DXA variables in accordance with previous reports,[35] a pattern of relationship that is compatible with the potentially critical role of intramyocellular stores in conferring increased metabolic risk independent of the other lipid depots.[36] We found only small, insignificant relationships among VAT, IHL and IMCL, which is partly discordant

from the findings of some studies[37] but in general agreement with the observations of others.[13,35,38] The levels of associations that we observed between waist girth and VAT, IHL and IMCL translate in shared variance on the order of 9% ($r^2 \times 100$). In contrast, some studies have reported stronger associations between DXA adipose tissue depots and VAT, IHL and IMCL, but they included obese individuals or subjects covering a wide range of adiposity levels.[39] Our sample included young, healthy, free-living, normal-weight and moderately overweight adults, which may explain these discrepancies.

To put these low-level relationships into perspective, ectopic fat accumulation in the liver or skeletal muscle amounts to rather small quantities of lipid accretion compared with the substantial storage capacity of the adipose organ. This suggests that it is unlikely that IHL or IMCL results primarily from a reduced lipid storage ability of hypertrophic abdominal adipocytes as has been suggested.[35,40] In the present study, we found no significant relationship between mean abdominal fat cell volume and VAT, IHL or IMCL. The physiological basis for the different regulation of IHL and total and regional adipose tissue stores is unknown. The reported lower level of CD36 expression in adipose versus liver tissue suggests one potential mechanism by which fat storage may be favored in the liver compared with the adipose tissue.[34] Multiple mechanisms are undoubtedly involved as suggested by the remarkable degree of specificity among total or regional adiposity, VAT, IHL and IMCL levels.

In this regard, factor analysis identified four clear, independent clusters among all DXA, VAT, IHL and IMCL depots. Factor 1 had strong positive loadings on all four DXA total and regional adiposity variables, whereas VAT, IHL and IMCL clustered independently onto factors 2, 3 and 4, respectively. These four factors accounted for 96% of the total variance among the seven adiposity-related measures. These observations are concordant with the notion that whole-body DXA, VAT, IHL and IMCL represent distinct adiposity traits with markedly different causal pathways and functional roles and are suggestive of diverse etiologies.

In a subsample of 36 participants, we explored the relationships between abdominal fat cell volume and VAT, DXA-derived adiposity traits, IHL and IMCL. It has been shown before that large fat cell size in the subcutaneous abdominal adipose tissue is reflective of overall adiposity and of upper body adipose tissue mass.[41] As shown previously,[41] moderate and highly significant positive correlations were observed between abdominal fat cell volume with all adiposity traits, but not with VAT, IHL or IMCL. The lack of a relationship between subcutaneous abdominal fat cell size and VAT reported in the current study is in contrast to what has been previously reported in a large group of men and women with a wide range of age and BMI.[41] This discrepancy is likely to be explained by the restricted range of age and adiposity level in the present study. Moreover, our data do not support the finding of a negative association between subcutaneous abdominal adipocyte size and lower body fat mass reported by others.[41] Finally, we found no significant correlations between fat cell volume and IHL or IMCL.

In conclusion, the present study reveals that there is a high degree of commonality among the various DXA indicators of adiposity in normal-weight and moderately overweight

adults. However, DXA-VAT, intrahepatic fat content and IMCL levels exhibit almost complete independence from global indicators of adiposity including BMI and waist girth. Factorial analysis indicated that there were in fact four orthogonal components that were easily recognized as representing DXA adiposity measures (total fat mass and upper and lower adiposity levels), VAT, hepatic fat content and IMCL depot, respectively. However, from a practical point of view, BMI, waist girth, trunk fat and total fat mass were highly intercorrelated, providing further justification for their use as common indicators of weight and adiposity status in free-living young adults. In contrast, BMI, waist girth, total adiposity, trunk fatness and lower body fat share little common variance with the VAT or hepatic and skeletal muscle lipid content. As a result of this degree of specificity, we conclude that it will likely be an insurmountable challenge to identify valid anthropometric indicators of the visceral fat or hepatic and skeletal muscle lipid accretion in normal-weight and moderately overweight individuals.

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Table 1

Descriptive characteristics of the sample and included adiposity traits

Variables	*Total sample*				*Males*				*Females*				*M vs W*
	N	*Mean*	*s.d.*	*Median*	*N*	*Mean*	*s.d.*	*Median*	*N*	*Mean*	*s.d.*	*Median*	P-value[a]
Age (years)	75	26.8	4.5	26.0	38	26.3	3.9	26.5	37	27.3	5.0	26.0	0.36
Height (cm)	75	172.8	9.6	171.5	38	179.7	6.9	179.9	37	165.8	6.4	166.5	<0.0001
Weight (kg)	75	68.7	10.9	68.9	38	75.8	8.2	76.7	37	61.4	8.0	60.1	<0.0001
BMI (kg m^{-2})	75	22.9	2.4	23.1	38	23.5	2.6	23.6	37	22.3	2.2	22.2	0.029
Waist girth (cm)	75	77.5	7.5	77.9	38	81.3	6.4	82.0	37	73.5	6.5	72.1	<0.0001
Arm fat (kg)	75	1.7	0.6	1.8	38	1.4	0.5	1.3	37	2.0	0.7	2.0	<0.0001
Leg fat (kg)	75	6.2	2.4	6.3	38	4.7	1.6	4.3	37	7.8	2.1	7.7	<0.0001
Trunk fat (kg)	75	6.6	2.5	6.6	38	6.0	2.2	6.0	37	7.2	2.7	6.8	0.034
Total Fat (kg)	75	15.4	5.1	15.0	38	13.1	4.1	12.4	37	17.7	5.0	17.1	<0.0001
VAT (cm^2)	75	53.2	22.2	49.2	38	55.1	20.1	48.8	37	51.2	24.4	49.5	0.457
IHL (AU)	75	0.007	0.01	0.003	38	0.010	0.017	0.003	37	0.004	0.007	0.002	0.077
IMCL (AU)	75	0.006	0.002	0.005	38	0.006	0.003	0.006	37	0.005	0.002	0.004	0.008
Fat cell size (nl)	36	0.66	0.28	0.63	18	0.738	0.348	0.697	18	0.588	0.180	0.600	0.114

Abbreviations: AU, arbitrary unit; IHL, intrahepatic lipids; IMCL, intramyocellular lipids; M, men; VAT, visceral adipose tissue; W, women.

[a] *P*-value based on two-sample *t*-test.

Table 2

Spearman's correlation coefficients among primary adiposity variables

	Age-adjusted correlations						
	Arm fat	**Leg fat**	**Trunk fat**	**Total fat**	**VAT**	**IHL**	**IMCL**
Age-, sex- and height (m^2)-adjusted correlations							
Arm fat		0.86*	0.86*	0.95*	0.16	0.20	−0.08
Leg fat	0.87*		0.68*	0.90*	0.04	0.01	−0.20
Trunk fat	0.88*	0.78*		0.92*	0.21	0.40**	0.09
Total fat	0.94*	0.92*	0.95*		0.15	0.23****	−0.06
VAT	0.29***	0.24****	0.31***	0.28****		0.28****	0.12
IHL	0.30***	0.24****	0.34***	0.30***	0.09		0.27****
IMCL	0.11	0.10	0.18	0.15	0.05	0.21	

Abbreviations: IHL, intrahepatic lipids; IMCL, intramyocellular lipids; VAT, visceral adipose tissue.

* $P \leq 0.0001$,

** $P \leq 0.001$,

*** $P \leq 0.01$,

**** $P \leq 0.05$.

Table 3

Results of factor analysis of adiposity variables previously adjusted for age, sex, and height^2 (N = 75)

Principal components	Eigenvalue	Proportion	Cumulative
1	3.964	0.566	0.566
2	1.099	0.157	0.723
3	0.888	0.127	0.850
4	0.755	0.108	0.958
5	0.202	0.029	0.987
6	0.087	0.013	0.999
7	0.005	0.001	1.000

Table 4

Factor loadings for the first four factors after varimax rotation

	Factor 1	Factor 2	Factor 3	Factor 4
Arm fat	0.95	0.13	0.13	0.02
Leg fat	0.91	0.08	0.07	0.03
Trunk fat	0.94	0.14	0.14	0.09
Total fat	0.98	0.11	0.12	0.07
VAT	0.18	0.98	0.03	0.02
IHL	0.18	0.03	0.98	0.11
IMCL	0.08	0.02	0.10	0.99

Abbreviations: IHL, intrahepatic lipids; IMCL, intramyocellular lipids; VAT, visceral adipose tissue.

Table 5

Spearman's correlation coefficients among adiposity variables

	Age-, sex- and height (m^2)-adjusted correlations		
	BMI	**Waist girth**	**Fat cell volume**
Arm fat	0.79*	0.71*	0.63*
Leg fat	0.74*	0.56*	0.52**
Trunk fat	0.78*	0.80*	0.63*
Total fat	0.81*	0.73*	0.64*
VAT	0.32***	0.29****	0.21
IHL	0.21	0.29****	0.15
IMCL	0.16	0.31***	0.08

Abbreviations: BMI, body mass index; IHL, intrahepatic lipids; IMCL, intramyocellular lipids; VAT, visceral adipose tissue. Correlations for fat cell volume were based on 76 subjects. All others were based on 75 subjects.

* $P \leq 0.0001$,

** $P \leq 0.001$,

*** $P \leq 0.01$,

**** $P \leq 0.05$.