Review

Fat Cell Size: Measurement Methods, Pathophysiological Origins, and Relationships With Metabolic Dysregulations

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Abbreviations: AT, adipose tissue; BMI, body mass index; CAD, coronary artery disease; CD, collagenase digestion; CT, computed tomography; FCS, fat cell size; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; HS, histological section; LDL-c, low-density lipoprotein cholesterol; LPL, lipoprotein lipase; NEFA, nonesterified fatty acid; OF, osmium fixation; SCAT, subcutaneous adipose tissue; T2D, type 2 diabetes; TG, triglyceride; VAT, visceral adipose tissue.

Abstract

The obesity pandemic increasingly causes morbidity and mortality from type 2 diabetes, cardiovascular diseases and many other chronic diseases. Fat cell size (FCS) predicts numerous obesity-related complications such as lipid dysmetabolism, ectopic fat accumulation, insulin resistance, and cardiovascular disorders. Nevertheless, the scarcity of systematic literature reviews on this subject is compounded by the use of different methods by which FCS measurements are determined and reported. In this paper, we provide a systematic review of the current literature on the relationship between adipocyte hypertrophy and obesity-related glucose and lipid dysmetabolism, ectopic fat accumulation, and cardiovascular disorders. We also review the numerous mechanistic origins of adipocyte hypertrophy and its relationship with metabolic dysregulation, including changes in adipogenesis, cell senescence, collagen deposition, systemic inflammation, adipokine secretion, and energy balance. To quantify the effect of different FCS measurement methods, we performed statistical analyses across published data while controlling for body mass index, age, and sex.

Key Words: Adipocyte hypertrophy, diabetes, obesity, cardiometabolic disorders, systematic review, meta-analysis

Graphical Abstract

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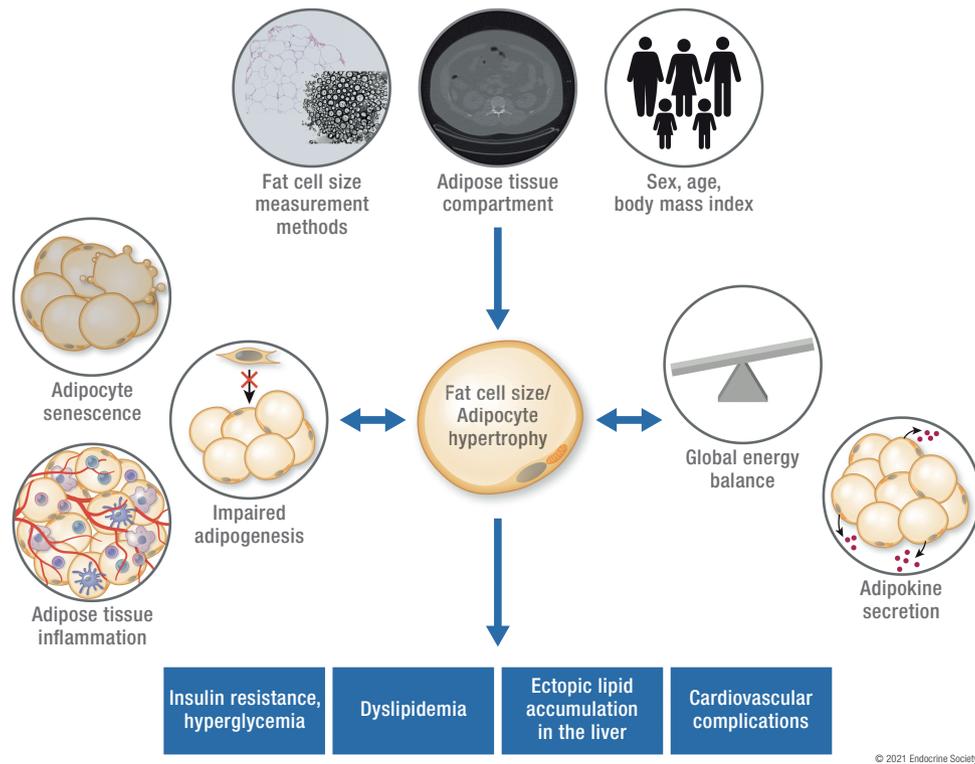
ESSENTIAL POINTS

1. Meta-analyses showed that fat cell size vary according to measurement methods, adipose tissue depots, as well as age and body mass index.
2. Adipocyte hypertrophy is associated with dysregulations in glucose metabolism, lipid metabolism, ectopic fat accumulation, and cardiovascular endpoints independently of body mass index.
3. Adipocyte hypertrophy is related to impaired adipose tissue differentiation potential and rate of adipogenesis, adipose tissue inflammation, and altered adipokine secretion.
4. Metabolic interventions resulting in negative and positive energy balance have led respectively to reduced and increased fat cell size.

The obesity pandemic has gained increasing attention over the last 2 decades. However, the severity of obesity-related metabolic complications does not seem to depend solely on the quantity of excess adipose tissue. In this regard, this differential effect of obesity can be explained by differences in adipose tissue dysmetabolism. An important indicator of such dysmetabolism of adipose tissue is adipocyte hypertrophy. Fat cell size (FCS) predicts numerous obesity-related complications such as lipid dysmetabolism,

ectopic fat accumulation, insulin resistance, and cardiovascular disorders.

Nevertheless, the scarcity of systematic literature reviews on this subject is compounded by diverse methods used to report FCS measurements. Therefore, we performed statistical analyses across published studies to quantify the effects of FCS measurement methods, while also introducing a novel technique to standardize the FCS measurements reported in different dimensions. Furthermore, we present herein an extensive review of the relationships

between adipocyte hypertrophy and systemic metabolic dysregulation. Finally, we also review the mechanistic origins of adipocyte hypertrophy and its relationships with metabolic dysregulation.

Methods for the Systematic Review

Articles identified through primary database screening were assessed for eligibility if they were original, peer-reviewed research conducted on humans and published in English. All articles that were deemed eligible for meta-analysis had to contain measurements of adipocyte size as well as information pertaining to (1) the site(s) at which adipose tissue (AT) samples were collected; (2) the type of method employed for the measurement of cell size; and (3) the type of FCS data (eg, diameter, volume, and cross-sectional area).

The PubMed database was searched up to January 25, 2021, using both Medical Subject Heading terms and keywords. A complete description of the strategy used can be found elsewhere (1).

The study selection process and the assessment for eligibility of articles were done following the Preferred Reporting Items for Systematic reviews and Meta-Analysis guidelines. The evaluation of potential articles, examination of the title, abstract, full text, and supplementary materials were done by the first author. The following data were extracted from the selected articles: general information (ie, title, year published, DOI, study design), characteristics of participants (ie, number of participants in each subgroup, if any, number of women and men, average age, body mass index (BMI), weight, fat mass, percent fat, waist and hip circumference), measures of AT adiposity (ie, adipocyte size, the site of AT biopsy, and the type of methodology employed).

For studies that only analyzed data from an existing cohort that had already been previously evaluated, we made sure that no other study that used the same dataset was included in our quantitative analysis. Furthermore, for studies that investigated clinical samples including pathological conditions, only data from the participants from the control group or those with prediabetes or type 2 diabetes (T2D) were extracted.

Two thousand five hundred and seventy-one articles were identified; after removal of duplicates, 2348 records were obtained. Examination of titles and abstracts allowed the inclusion of 385 articles for eligibility assessment. After full text screening of these articles, 249 articles were retained for qualitative synthesis, and, among these, 154 met the criteria for inclusion in quantitative analysis (Fig. 1). Data from 3681 men and 8434 women, which included 12 705 distinct biopsy samples from different sites, were used for statistical analyses.

Collagenase digestion of adipose tissue followed by microscopic examination (CD) constitutes the most frequently used method and was employed in 89 of the 154 studies; 38 studies used the histological section (HS) technique, while the remaining 26 used the osmium fixation (OF) method (ie, osmium fixation of adipocytes followed by size measurement via a Coulter counter); 1 study used all 3 methods. For adipocyte size information presented in graphical form, an online utility, WebPlotDigitizer (2), was used. This tool is highly reliable for numerical data extraction from published figures (3).

The reporting of adipocyte size across various studies was not standardized; indeed, some expressed average size in terms of diameter (in μm), others reported it as volume (in pL) or mass (in μg of lipid per cell), and still others used cross-sectional area (in $μm^2$). Therefore, for quantitative analysis, all data were converted to measures of diameter. However, an equation exists only to calculate average volume from average diameter (4), which was employed in a previous meta-analysis on adipocyte size by Murphy et al. (5); we have therefore expanded on this idea by developing formulae to solve the reverse problem (1). Similar formulae were also found to convert cell area to cell diameter (1). For adipocyte sizes expressed in terms of micrograms of lipid per cell, the density of triolein (0.915 g/mL) was used to change measurements of mass to volume. Details the statistical approaches used for nonlinear modeling of FCS and tests of group comparisons can be found elsewhere (1).

Adipose Tissue Expansion

Adipose tissues grow through a process known as adipogenesis, which can be defined as the ability of preadipocytes to multiply and to differentiate into mature adipocytes. During the development of obesity as a result of chronic positive energy balance, adipose tissue volume increases via 2 main processes: adipocyte hypertrophy (increase in FCS) and hyperplasia (increase in fat cell number). In contrast to adipocyte hyperplasia, hypertrophy of fat cells portends worse insulin sensitivity, glucose disposal, lipid metabolism, and cardiovascular outcomes independently of the effect of obesity alone.

One of the key factors that may pave the way to fat cell hypertrophy is impaired adipogenesis. By inducing differentiation of preadipocytes from abdominal subcutaneous adipose tissue (SCAT), Isakson et al. showed that differentiation potential of these cells was negatively associated with FCS and was reduced in individuals with obesity despite a higher number of CD133-positive preadipocytes (6). Park et al. later corroborated this finding by showing that
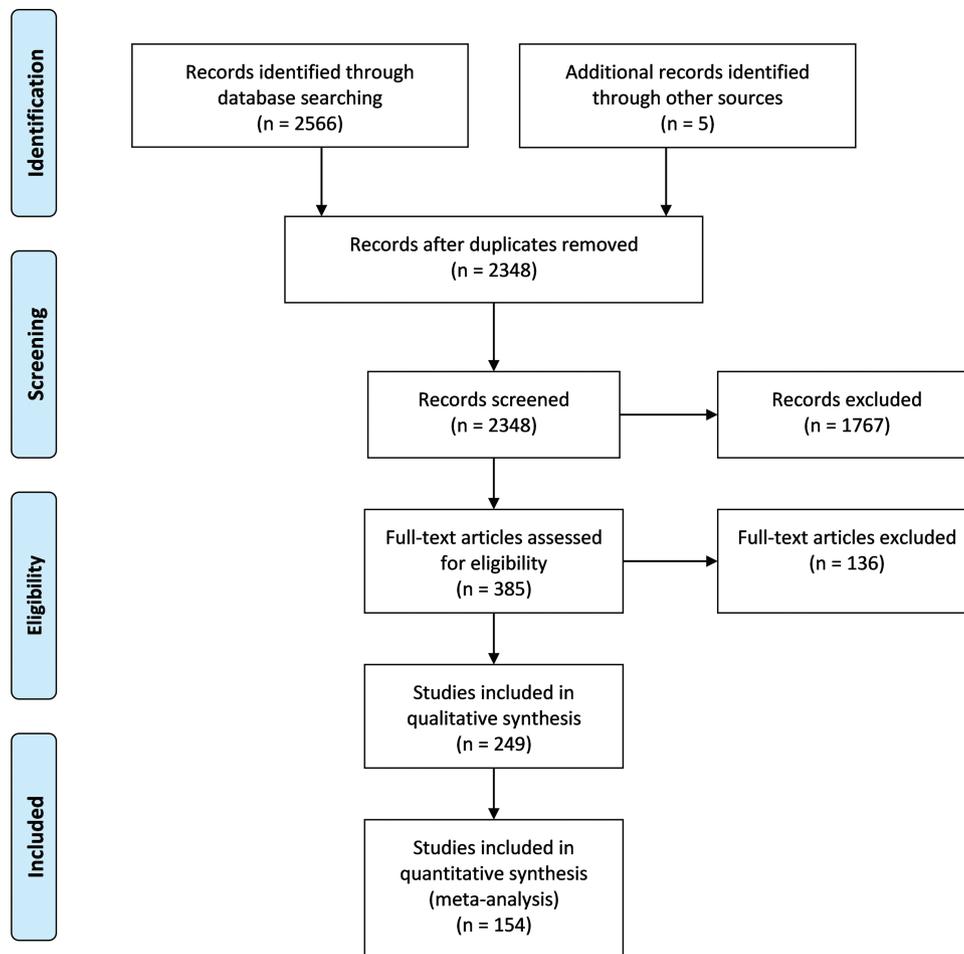
Figure 1. Prisma flowchart showing the number of articles at each step of the literature review and meta-analysis.

SC CD34+/CD31– preadipocyte differentiation was negatively correlated with both SCAT and visceral FCS (7). The rate of production of new adipocytes was also significantly lower in individuals with adipocyte hypertrophy than in those with hyperplasia (8). In a sample of women who are healthy, the negative association between SCAT rate of adipogenesis and visceral FCS was found even after matching for BMI (9).

Furthermore, FCS and the rate of in vitro differentiation of SC adipocytes is influenced by several regulators of adipogenesis. FCS in children was positively correlated with expression of the meteorin-like protein, which decreases PPAR-γ expression and adipocyte differentiation (10). In omental ATs of individuals with severe obesity, FCS is strongly associated with AT content of Pref-1, which inhibits differentiation of preadipocytes (11). In addition, other negative regulators of adipogenesis, such as Wnt (6, 12, 13), Notch (14), and retinoid-related orphan receptor gamma (15), have also been implicated in adipocyte hypertrophy in numerous human studies. Induction of PPAR-γ constitutes an important mediator of adipocyte

differentiation; as such, individuals with obesity have higher expression of mitogen-activated protein kinase 4, an enzyme which is stimulated by TNF-α and blunts the activation of PPAR-γ (6).

In summary, adipocyte hypertrophy is related with impaired AT differentiation potential and rate of adipogenesis. Causality of this association is uncertain. Moreover, there is conflicting data regarding the effects of induction of adipogenesis on FCS in humans.

Relation Between Adipocyte Hypertrophy and Adipose Tissue Pathophysiological Processes

Adipocyte Senescence

Not only has FCS been correlated with reduced adipogenesis, it was also associated with adipocyte death and senescence. In SCAT adipocytes, Gustafson et al. (16) showed that adipocyte hypertrophy was positively correlated with markers of cell senescence, notably plasminogen activator inhibitor-1 (PAI1), *TP53*, and transforming growth factor

beta 1 (TGFB1). In epicardial adipocytes, similar relationships have been found with the expression of p53 (17).

By measuring the number of crown-like structures in AT samples as a surrogate for the frequency of cell death, Cinti et al. (18) found that adipocyte hypertrophy was associated with adipocyte necrosis. Telomere shortening is also known to be a marker of cell senescence. Accordingly, adipocyte telomere length was shorter in individuals with fat cell hypertrophy (19-21). Therefore, the current limited evidence suggests that adipocyte hypertrophy does accompany cell senescence, cell necrosis, and telomere shortening in AT.

Inflammation

A mounting body of evidence has also implicated adipocyte hypertrophy in the development of the inflammatory response. Indeed, there appears to be a stronger correlation between adipocyte size and inflammation than between obesity and inflammation, in terms of macrophage content in ATs (22) and of IL-6 and TNF-α levels (23). In a randomized controlled trial comparing a 26-week treatment with valsartan (an angiotensin receptor antagonist mainly used as an antihypertensive medication) vs placebo, no weight change was observed between the 2 groups; nevertheless, valsartan significantly reduced both adipocyte size and expression of inflammatory genes. Moreover, the change in FCS induced by valsartan was positively correlated with the change in inflammatory gene expression (24).

Higher FCS, determined with histological analysis, was also associated with an increased number of infiltrating macrophages in human AT (22, 25). Using osmium fixation, however, the number of CD68+ and CD163/MAC2+ macrophages was negatively correlated with the size of small and medium-sized fat cells (26). This result is, in fact, consistent with the capacity of osmium fixation to detect small adipocytes. Acosta et al. found that adipocyte hypertrophy was associated with increases in the ratio of M1/M2 macrophages (27). Other studies have also suggested a potential relationship between adipocyte hypertrophy and increased AT M1 macrophages.

Adipocyte hypertrophy is associated with increased gene expression of numerous proinflammatory factors. FCS was correlated with the expression of *CEBPB* (CCAAT Enhancer Binding Protein Beta) mRNA (28), *CD68* (cluster of differentiation 68) (24, 28-31), *TNF-α* (tumor necrosis factor-α) (29), *TNF* (tumor necrosis factor) *receptor* (32), biglycan (33), *MCP-1* (30, 34), *MCP-2* (30), *MIP-1α* (macrophage inflammatory protein-1α) (30), *MIF* (Macrophage migration inhibitory factor) (35), and *RANTES* (Regulated on Activation, Normal T Cell Expressed and Secreted) (36). Using osmium fixation, adipocyte hypertrophy was

characterized by an increase in the proportion of small adipocytes. Accordingly, 2 studies that employed osmium fixation to measure FCS found that the fraction of small cells correlated positively with the expression of inflammatory factors (37), including *MIP-1α* and *MCP-1α* (26). In addition, larger adipocytes expressed more serum amyloid A (*SAA*) gene than smaller adipocytes (38), and serum level of SAA was also correlated with SCAT FCS (39).

The alteration in inflammatory gene expression related to adipocyte hypertrophy is also reflected by changes in downstream secretion of inflammatory factors. Adipocyte enlargement is associated with increased IL-6 (13, 23, 29, 40-42), IL-8 (13, 41), CRP (25, 42-45), TNF-α (23, 27, 41, 42), MCP-1 (13, 41), and MIP-1β (41) secretion. Furthermore, increased FCS is associated with reduction in IL-10 production (41), which may allow M2 polarization of macrophages.

In summary, our review of the literature reveals strong relationships between adipocyte hypertrophy and AT inflammation which could not be merely explained by obesity or increased BMI.

Adipokine Secretion

Adipokines are hormones secreted by adipose tissues that mediate numerous systemic effects. Leptin and adiponectin are the 2 main adipokines that have been extensively investigated in relation to adipocyte hypertrophy.

Leptin is an anorectic hormone that regulates food intake and energy expenditure, but also reduces fat accumulation in the liver and muscles. Many studies have found that individuals with enlarged fat mass had higher plasma concentrations of leptin (29, 43, 46-52), higher AT leptin content (53), increased leptin secretion (41, 51), and higher AT *LEP* mRNA levels (54). Although leptin concentration is positively correlated with BMI (48, 55) and fat mass (46, 48) and decreases proportionally to weight loss (51, 56), leptin level is associated with adipocyte hypertrophy independently of AT mass and BMI. In a large cohort (46), FCS was correlated with leptin levels even with adjustment for AT mass. Along with AT mass, FCS accounted for more than 60% of the variation in leptin levels. AT gene expression of leptin decreased (57) with diet-induced weight reduction and leptin receptor expression increased after Roux-en-Y gastric bypass (58), suggesting an increased leptin sensitivity with caloric restriction.

Adiponectin is associated with increased insulin sensitivity, increased adipogenesis, and decreased inflammation. In various studies, higher FCS was associated with lower serum adiponectin concentrations (19, 28, 33, 42, 49, 52, 59), reduced expression of adiponectin (54, 60), and lower secretion (41) and release (61) of adiponectin in AT. With

weight reduction, improvements in insulin sensitivity (62) and BMI (63) were associated with increased secretion of adiponectin. However, there is insufficient data to conclude whether the association between FCS and adiponectin production is independent of obesity.

The correlation between FCS and adiponectin levels was also weaker and more difficult to detect than that between FCS and serum leptin. This was especially the case when using SCAT and when the histological section or collagenase digestion method was employed. Indeed, a comparative study of the 3 sizing methods revealed that osmium fixation yielded the strongest signals for the correlations between FCS and both plasma adiponectin and leptin (52). At least 3 studies (13, 61, 64) failed to detect any statistically significant correlation between FCS and adiponectin; all of them used the collagenase digestion method and SCAT biopsies.

In conclusion, increased FCS is strongly associated with AT leptin expression, production, secretion, and plasma leptin level. These associations are independent of obesity and cannot be entirely accounted for by genetic and/or epigenetic factors. On the contrary, increased FCS is correlated with reduced adiponectin production and release by AT.

Variation in Fat Cell Size Due to Methodological Factors

The use of different techniques, ie, CD, OF, or HS, to ascertain mean adipocyte size has been shown to yield different results. Independent of the adipose tissue sample and of the BMI of the participant, CD and OF both appear to produce higher cell size estimates than HS; moreover, OF also provides marginally higher measurements than CD, although this difference diminishes with larger BMI. A study directly comparing these 3 methods published by Laforest et al. (52) corroborated the above observations.

Our meta-regression analysis demonstrates that compared to CD, HS yielded significantly lower values of abdominal subcutaneous (SC) adipocyte diameter ($\beta = -20.2385$, $P < .0001$), while OF resulted in higher estimates of cell size ($\beta = 6.3998$, $P = .0166$) (Table 1), after accounting for sex, age, and BMI. For visceral adipocytes, similar results were found, with the HS method revealing smaller average cell diameters than CD ($\beta = -20.3486$, $P < .0001$) (Table 2).

Analysis of variance also showed statistically significant differences between results obtained with the 3 methods not only in SCAT ($P < .0001$), but also in visceral adipose tissue (VAT) ($P < .0001$). Multiple comparison tests

Table 1. Mixed effects model multiple meta-regression of abdominal subcutaneous adipocyte diameter and cell measurement methods (HS and of compared with CD as the reference method)

Dependent variable: abdominal subcutaneous adipocyte diameter (µm)			
Predictor	Coefficient (β)	Lower bound (95% CI)	Upper bound (95% CI)	*P* value
(Intercept)	65.6814	55.6909	75.6719	<.0001
HS (compared to CD)	−20.2385	−24.1850	-16.2920	<.0001
OF (compared to CD)	6.3998	1.1725	11.6271	.0166
BMI	0.8813	0.6626	1.0999	<.0001
Age	0.2659	0.1094	0.4224	.0009
% Women	−0.0039	−4.5339	4.5261	.9986

Abbreviations: BMI, body mass index; CD, collagenase digestion; CI, confidence interval; HS, histological section; OF, osmium fixation; % Women, percentage of women.

Table 2. Mixed effects model multiple meta-regression of visceral adipocyte diameter and cell measurement methods (HS and OF compared with CD as the reference method)

Dependent variable: visceral adipocyte diameter (µm)			
Predictor	Coefficient (β)	Lower bound (95% CI)	Upper bound (95% CI)	*P*-value
(Intercept)	26.7406	-0.4224	53.9035	.0536
HS (compared to CD)	−20.3486	−26.2228	−14.4744	<.0001
OF (compared to CD)	5.7024	−6.3914	17.7961	.3511
BMI	1.1628	0.8698	1.4558	<.0001
Age	0.7394	0.3419	1.1368	.0004
% Women	−7.2799	−15.5429	0.9830	.0834

Abbreviations: BMI, body mass index; CD, collagenase digestion; CI, confidence interval; HS, histological section; OF, osmium fixation; % Women, percentage of women.

confirmed variations in median values (Fig. 2A and 2B), except for adipocyte measurements between CD and OF, where the differences were nonsignificant.

In addition, nonlinear regression analysis of abdominal subcutaneous adipocyte diameter with respect to BMI using an exponential plateau model, resulted in different maximum plateau diameter values (D_{max}) for the 3 methods: 120.8 vs 112.5 vs 126.7 μm for the CD, HS, and OF methods, respectively (Fig. 3A-3C). In the case of visceral adipocytes, similar results were found, with the CD method yielding higher D_{max} than HS (119.4 vs 85.21 μm) (Fig. 3D and 3E). However, due to the limited number of data points with OF for visceral adipocytes, regression analysis was not performed.

Our meta-analysis corroborated the influence of measurement techniques on average cell diameter. The fact that these differences did not depend on BMI, sex, or age also suggests that they are dependent on the specific methodology. Indeed, many sources of bias in each method may contribute to these observed variations. In both CD and OF, there is the risk of disruption of larger adipocytes due to their increased fragility (65). With OF, there is an added advantage of being able to study cells with very small diameters (<50 μm) (37, 66, 67); nevertheless, because it is the only method in which adipocytes are not directly visualized, there is also the possibility of including small cell fragments. Furthermore, OF is known to cause swelling in
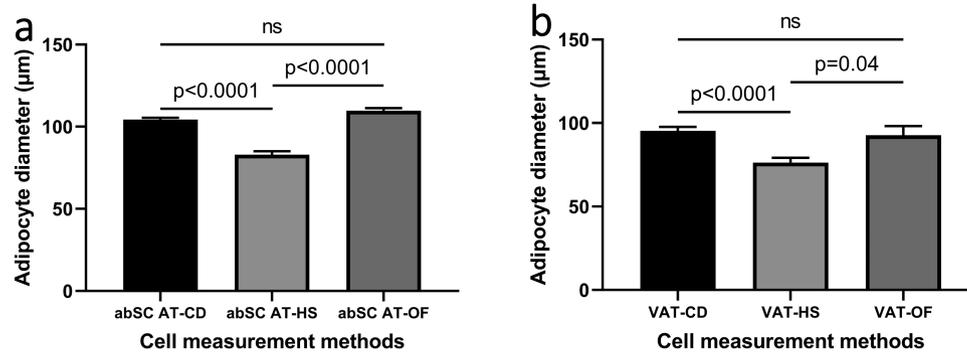
Figure 2. Adipocyte diameter according to different size measurement methods. (A) Abdominal subcutaneous adipocyte diameter. (B) Visceral adipocyte diameter. abSC, abdominal subcutaneous; CD, collagenase digestion; HS, histological section; OF, osmium fixation; VAT, visceral adipose tissue.
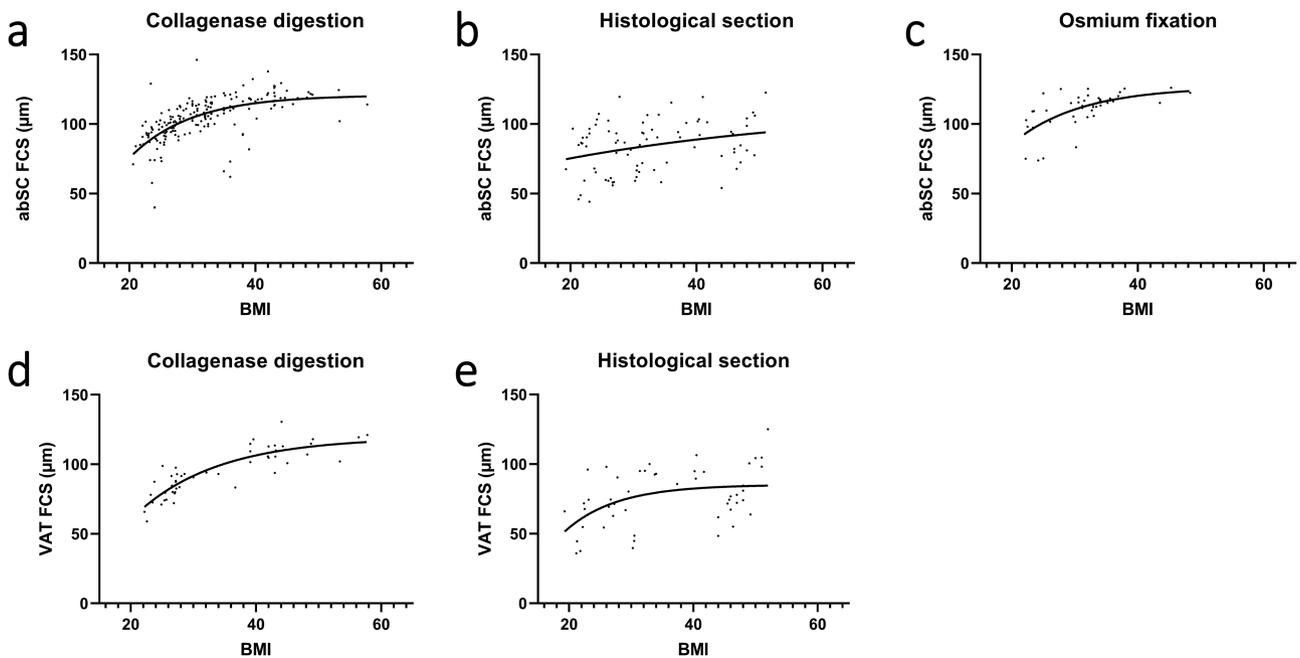
Figure 3. Adipocyte diameter in relation to body mass index (BMI) according to different size measurement methods. (A) Abdominal subcutaneous adipocyte diameter (in μm) assessed using collagenase digestion. (B) Abdominal subcutaneous adipocyte diameter (in μm) assessed using histological section. (C) Abdominal subcutaneous adipocyte diameter (in μm) assessed using osmium fixation. (d) Visceral adipocyte diameter (in μm) assessed using collagenase digestion. (E) Visceral adipocyte diameter (in μm) assessed using histological section. abSC, abdominal subcutaneous; CD, collagenase digestion; FCS, fat cell size; HS, histological section; OF, osmium fixation; VAT, visceral adipose tissue.

a variety of tissues, a phenomenon which has been well documented (68, 69) and reviewed (70, 71). This swelling is thought to be caused by increase in tissue weight due to osmium tetroxide uptake and osmotic pressure gradient and may explain the consistently higher estimates of FCS by OF. The most significant source of bias that accounts for size underestimation with HS is the fact that most cells are likely not cut through their geometric center, hence yielding smaller cross-sectional areas.

In summary, we found that the 3 techniques for FCS measurement generate quantitatively different results, regardless of sex, age, and BMI. HS always produces lower estimates than CD. OF results in slightly higher size measurements than CD in SCAT. These variations are likely due to differences in cell fragility, physical, chemical, statistical, and/or geometric factors.

Regional variations in adipocyte size in relation to cell measurement method

The existing literature suggests that adipocytes are smaller in abdominal SCAT than in femoral (72, 73) and gluteal SCAT (74). However, using the CD method, Berman et al found no differences in cell size between abdominal and gluteal SCAT of women with obesity who were postmenopausal and Caucasian (75). Later, another study using the same method showed smaller abdominal than gluteal SC adipocytes in women with obesity who were African-American but not women with obesity who were non-Hispanic Caucasian (76). Using the HS method, Joffe et al. have provided further evidence supporting this racial difference in regional variations by observing increased cell size in gluteal relative to the abdominal region in women of African descent with and without obesity (77).

Cell size variations between the SC and visceral depots are also very well established. Overall, omental VAT contains adipocytes with smaller diameters than does abdominal SCAT, regardless of the technique used to determine cell size, be it with CD (78-82), HS (83, 84), or OF (85). Nonetheless, van Beek et al. studied abdominal SCAT and VAT in women with obesity and challenged this observation by finding no difference in cell size between these depots (86). Moreover, SC adipocytes are also larger than epicardial AT cells, as demonstrated using both the CD (87) and HS methods (88). Finally, studies using all 3 methods (CD (52), HS (52, 89), and OF (52)) have shown positive correlations between visceral and SC adipocyte diameter.

Because studies that reported cell size other than that of abdominal SC adipocytes usually also measured abdominal SC FSC, we assessed differences in FCS across various AT regions. Results showed that abdominal SCAT FCS was significantly smaller than gluteal SCAT ($P < .0001$) and femoral SCAT FCS ($P < .0001$), and larger than visceral
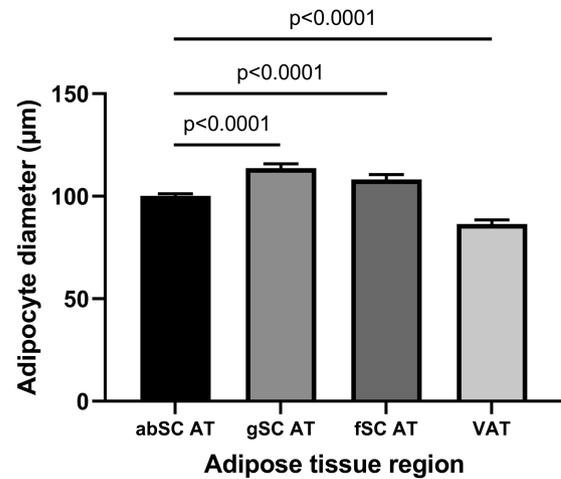

Figure 4. Adipocyte diameter across different adipose tissue regions. abSC, abdominal subcutaneous; AT, adipose tissue; fSC, femoral subcutaneous; gSC, gluteal subcutaneous; VAT, visceral adipose tissue.

AT FCS ($P < .0001$) (Fig. 4). Furthermore, gluteal SC and femoral SC FCS also plateaued at higher values of D_{max} (126.3 μm and 111.4 μm, respectively) with increasing level of obesity than did abdominal SC FCS (110.7 μm) and visceral FCS (98.51 μm) (Fig. 5).

Multiple meta-regression was then performed to assess the relationships between abdominal SC FCS and FCS from the other depots. Results (Table 3) showed that adipocyte size from the abdominal SC region, independent of sex, was a strong predictor of cell diameter from the gluteal, femoral, and visceral depots.

To summarize, it is clear from individual studies that femoral and gluteal AT contain larger adipocytes than does abdominal SCAT, whereas VAT contains smaller cells than abdominal SCAT. Furthermore, FCS in abdominal SCAT is positively correlated with FCS in all other ATs. These finding were confirmed by our numerical meta-analyses.

Sex differences in adipocyte size

In contrast to regional differences, sexual dimorphism in FCS was less clearly demonstrated. All 3 cell measurement techniques (CD (90), HS (54), and OF (91)) have shown larger abdominal SC FCS in men. Nevertheless, among studies using CD, Hellström et al. (55) observed no sex variation in abdominal SC FCS, while Votruba et al. (92) found larger adipocytes in femoral SC and gluteal SCAT of women and Couillard et al. (47) have shown increased abdominal SC and femoral SC FCS in women. Using a group of healthy, nondiabetic participants, McLaughlin et al. (93) confirmed this finding by observing higher diameter of large adipocytes in women than in men.

In our meta-regression analysis, there was no statistically significant difference between abdominal SC FCS of
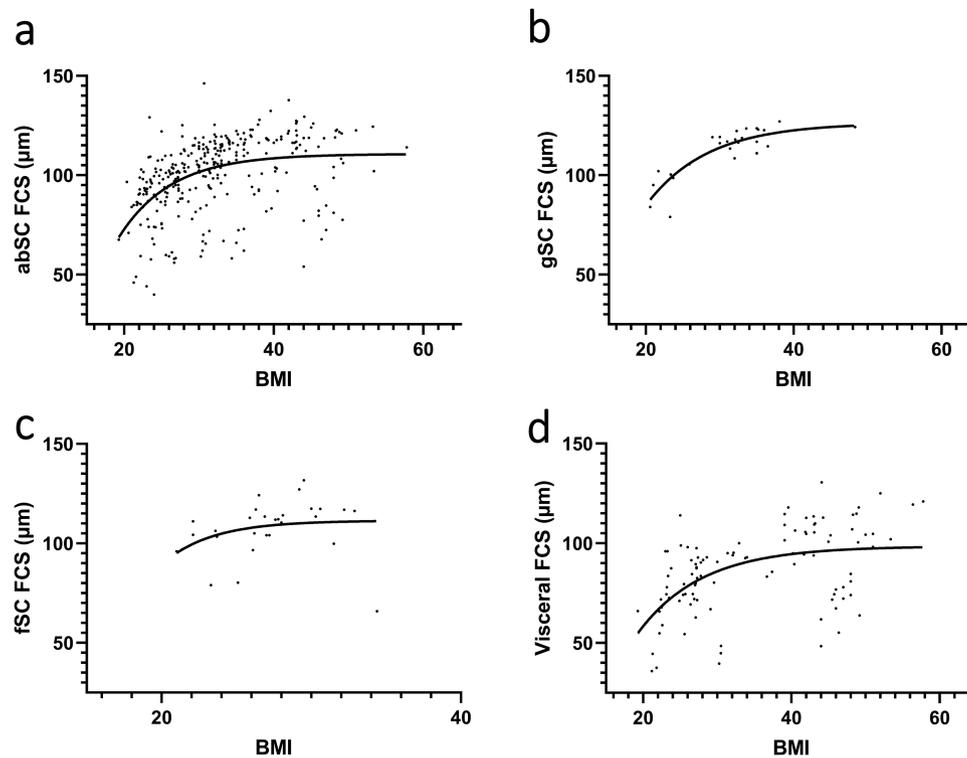
Figure 5. Fat cell size as a function of BMI in different adipose tissue depots. (a) Abdominal subcutaneous fat cell size as a function of BMI. (B) Gluteal subcutaneous fat cell size as a function of BMI. (C)Femoral subcutaneous fat cell size as a function of BMI. (D) Visceral fat cell size as a function of BMI. abSC, abdominal subcutaneous; AT, adipose tissue; BMI, body mass index; FCS, fat cell size; fSC, femoral subcutaneous; gSC, gluteal subcutaneous; VAT, visceral adipose tissue.

Table 3. Mixed effects model multiple meta-regression of gluteal, femoral, and visceral adipocyte diameter as a function of subcutaneous adipocyte diameter and sex

Predictor	Coefficient (β)	Lower bound (95% CI)	Upper bound (95% CI)	*P* value
Dependent variable: gluteal subcutaneous adipocyte diameter (μm)				
(Intercept)	22.5476	14.9479	30.1474	<.0001
abSC FCS	0.8129	0.7424	0.8833	<.0001
% Women	1.7878	−1.9485	5.5242	.3363
Dependent variable: femoral subcutaneous adipocyte diameter (μm)				
(Intercept)	13.6637	−1.7442	29.0715	.0801
abSC FCS	0.9127	0.7639	1.0614	<.0001
% Women	3.4501	−0.7970	7.6973	.1074
Dependent variable: visceral subcutaneous adipocyte diameter (μm)				
(Intercept)	3.8491	−4.7361	12.4344	.3747
abSC FCS	0.9325	0.8535	1.0114	<.0001
% Women	−11.2152	−15.5459	−6.8845	<.0001

Abbreviations: abSC, abdominal subcutaneous; CI, confidence interval; FCS, fat cell size; % Women, percentage of women.

the 2 sexes (Table 1). Studies with a higher proportion of women, however, did tend to reveal reduced FCS in visceral AT (β = −7.2799, *P* = .0834) (Table 2). Additionally, when corrected for abdominal SC FCS, visceral FCS was significantly lower with increasing proportion of women (β = −11.2152, *P* < .0001). The above findings were also confirmed using partial correlation controlling for abdominal SC FCS (Table 4).

Table 4. Partial correlation between FCS and sex

Proportion of Women	Spearman's Rho	*P*-value
Control Variable: abSC FCS		
gSC FCS	0.115	.531
fSC FCS	0.300	.096
Visceral FCS	-0.456	<.0001

Abbreviations: abSC, abdominal subcutaneous; FCS, fat cell size; fSC, femoral subcutaneous; gSC, gluteal subcutaneous.

In summary, data are conflicting regarding possible differences in FCS between men and women; our meta-analyses also failed to demonstrate statistically significant variations. However, we found lower visceral FCS in women, independently of abdominal SC FCS. This finding indicates that for any given degree of SC adipocyte hypertrophy, women are less prone to visceral fat cell hypertrophy.

Adipocyte size and age

We found few results regarding the relationship between adipocyte size and age, although it is known that, during childhood, fat cells are subject to a steady enlargement from ages 1-9 years (94) and that obesity leads to increased FCS throughout adolescence (95). Evidence of an association between age and adipocyte size in adults is scarce (16, 96). Therefore, we used multiple regression to illustrate increased FSC in the abdominal SC (β = 0.2659, *P* = .0009) and visceral (β = 0.7394, *P* = .0004) depots as a function of age, independently of BMI, sex, and cell measurement method (Tables 1 and 2).

Adipocyte size and obesity

The relationship between adipocyte hypertrophy and obesity has already been reviewed elsewhere (97) and a plethora of studies exists that demonstrated the strong positive correlation between FCS and increasing body weight (16, 19, 25, 30, 34, 44, 47, 49-51, 54, 62-64, 66, 67, 76, 77, 81, 83, 89, 93, 96, 98-129). Moreover, the relationship between adipocyte hypertrophy and increased BMI does not appear to be dependent on the presence of dysglycemia, because it was found both in participants with normoglycemia and those with T2D (30, 64). Further supporting evidence of this association is the well-established beneficial effect of weight loss on fat cell shrinkage in individuals with varying demographic profiles. In twin studies, the co-twins with obesity showed greater fat cell hypertrophy than their respective co-twins who were leaner (44), thus underscoring the critical role of acquired environmental and life-style factors in adipocyte enlargement.

Figure 3 shows BMI in relation to abdominal SC and visceral adipocyte size with the 3 different sizing methods (CD, HS, and OF). With multiple meta-regression, BMI is a strong contributor to changes in FCS, regardless of the location of adipose tissue or adjustment for cofounders.

Adipocyte size, insulin resistance, and hyperglycemia

There exists a relatively strong body of evidence supporting an important contribution of adipocyte hypertrophy to the exacerbation of insulin resistance that is independent of the degree of obesity, although a few studies have challenged this assertion.

Among 24 studies that used the CD technique, only 2 found no association between FCS and diabetes or insulin resistance (86, 107). Among 10 studies that have controlled for obesity, either by adjusting for fat area or by matching for BMI (8, 27, 44, 48, 100, 130-134), only 2 found no association between T2D and FCS after adjustment (130, 131). Indeed, when men who were nonobese and had T2D were matched with participants from the control group for BMI, there were significantly higher values of both insulin resistance and abdominal SC FCS in the diabetic group (27). Other studies have reached similar findings but did not control for obesity (102, 103, 135-140).

Since the expandability of adipocytes varies between individuals and is clearly associated with obesity and insulin resistance, it may be posited that genetically inherited factors may be the source of this relationship. In a study of twins who were young, Heinonen et al. (44) showed that, within each pair of twins, intertwin differences in abdominal SC FCS was correlated with differences in Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) independently of body fat variations. While this result indicates that genetic and epigenetic factors are not the sole contributors of adipocyte hypertrophy and insulin resistance, FCS is, in part, dependent on family history of T2D and genetic factors. Indeed, in a large cohort of women after menopause (105), the size of abdominal SC and femoral SC adipocytes was greater in participants with a family history of T2D than those without. Similar results have been found in women after menopause (141) and in men who were middle-aged (60). In addition, Henninger et al. (13) have shown, in a small group of first-degree relatives of patients with T2D who were nonobese and participants from the control group who were nondiabetic and nonobese, that abdominal SC cell size correlated with decreased insulin sensitivity only in first-degree relatives. Furthermore, differences also exist between individuals of African and European descent; in women after menopause with obesity (134), 2-hour postoral glucose tolerance test insulin and

glucose levels were positively correlated with SC and visceral FCS in participants of African but not Caucasian descent.

Among 21 studies that used the HS method to investigate the relationship between FCS and insulin resistance, only 2 found no association. The absence of association in 1 of these 2 (77) may be explained by small sample size, while the other study sampled SCAT from the thoracic instead of the abdominal SC area (88). Furthermore, histomorphological analyses have also revealed that insulin resistance correlates more closely with visceral than SCAT FCS. Indeed, while many studies have shown relationships between increased insulin resistance or the presence of diabetes and increased adipocyte size from both depots (19, 83, 114, 142), a substantial number of investigations have demonstrated an association only with visceral FCS (89, 113, 117, 143-145) or a stronger correlation with visceral than with SCAT FCS (83, 142).

In line with the findings by Svensson et al. (133), Rojas-Rodriguez et al. observed higher SCAT adipocyte size in women who were pregnant with gestational diabetes compared to those with normal glucose tolerance (143). Consistent with studies that have employed CD, results using the HS technique also support the notion that the influence of adipocyte hypertrophy on insulin resistance and glucose tolerance is independent of obesity and fat mass (19, 23, 59, 83, 146, 147). Moreover, FCS measured using HS is not only associated with levels of fasting insulin (96, 110, 148), but also with fasting glucose (148), HOMA-IR (15, 149), glucose disposal rate (59, 146), and 2-hour postoral glucose tolerance test glucose (149).

All except 1 of 17 studies that used OF have concluded that AT hypertrophy contributes to impaired insulin sensitivity and T2D. As discussed previously, 1 of the key distinguishing features of the OF method is that it allows for the identification and quantification of very small adipocytes. The inclusion of this population of cells, which cannot be detected by the HS and CD techniques (52), results in a bimodal distribution of cell size. Indeed, Azuma et al. have shown that the average cell diameter did not vary between individuals with and without diabetes (125). This result was in agreement with an investigation by McLaughlin et al. (150), who also observed that average cell size did not differ between the insulin sensitive and the insulin resistant groups matched for BMI, but that the insulin-resistant group had a higher proportion of very small cells. Many studies have therefore also analyzed the mode of and the size distribution of the large and small adipocyte populations. These have concluded that insulin resistance and T2D are associated both with an increased large adipocyte size (50, 52, 62, 67, 151, 152) and a higher relative number of small adipocytes (127, 150, 153-155). Nonetheless, some studies that have used the OF method also found association between the presence of diabetes (42, 156, 157) or impaired insulin response (85, 157, 158) and increased average cell size.

Meta-regression analysis corroborated the positive relationship between FCS and impaired insulin sensitivity and glucose intolerance (Fig. 6). Abdominal SCAT FCS was predicted by fasting insulin ($\beta = 0.1089$, $P < .0001$), HOMA-IR ($\beta = 3.9202$, $P < .0001$), and M-value ($\beta = -1.8483$, $P < .0001$) independently of the percentage of women, age, and FCS methodology. Abdominal SC FCS was, however, not predicted by fasting glucose ($P > .1$). Similar results have been reached between visceral FCS
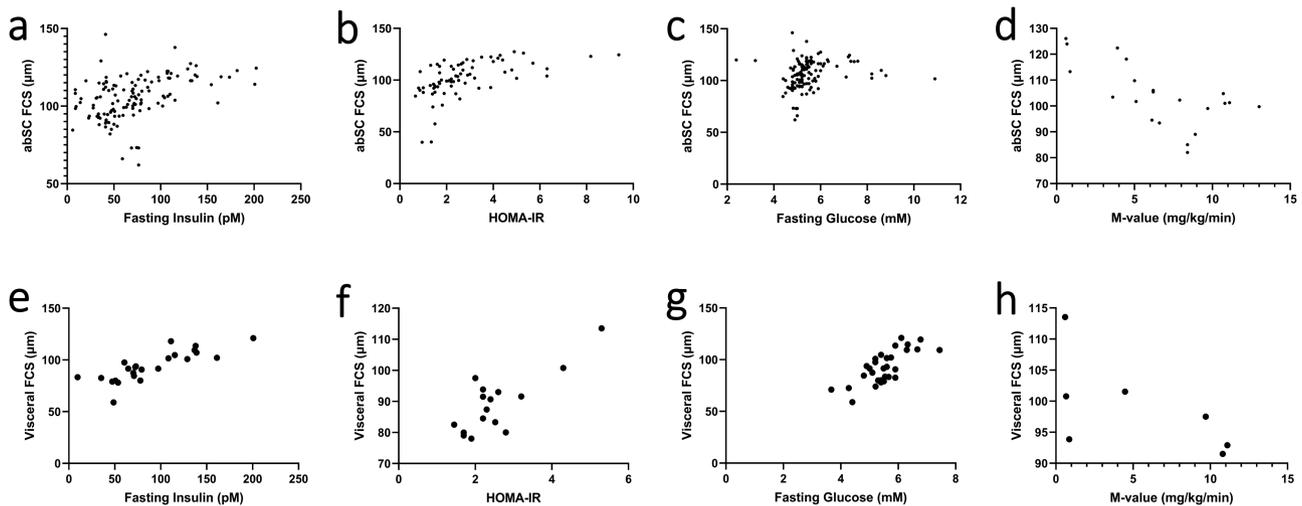

Figure 6. Relationships between abdominal subcutaneous fat cell size and (A) fasting insulin; (B) HOMA-IR; (C) fasting glucose; (D) M-value. Relationship between visceral fat cell size and (E) fasting insulin; (F) HOMA-IR; (G) fasting glucose; and (H) M-value. abSC, abdominal subcutaneous; FCS, fat cell size; HOMA-IR, homeostatic model assessment of insulin resistance.

and fasting insulin ($\beta = 0.2712$, $P < .0001$), fasting glucose ($\beta = 4.9258$, $P = .0006$), and HOMA-IR ($\beta = 5.0827$, $P = .001$). When BMI was included in the model, no significant relationship could be found between abdominal SC FCS and any of the above indexes. For visceral FCS, however, fasting glucose ($\beta = 2.5795$, $P = .03$) and HOMA-IR ($\beta = 3.4731$, $P = .0054$) remained significant predictors, while fasting insulin ($\beta = 0.1132$, $P = .0787$) was of borderline statistical significance.

In summary, FCS increases with diabetes status, impaired glucose tolerance, and insulin resistance. These associations are stronger in VAT than SCAT but are not independent of obesity or BMI in all studies. Genetic, epigenetic, and family history of diabetes all seem to contribute to this relationship. We confirmed these correlations in meta-regression analyses and showed that visceral FCS is associated with hyperglycemia and insulin resistance independently of BMI.

Adipocyte size and lipid metabolism

Adipocyte hypertrophy may exert its influence on systemic lipid metabolism through changes in adipose tissue lipid uptake, storage and lipolytic rates (Fig. 7). Under physiological conditions, triglyceride (TG) transported by blood lipoprotein particles bind to lipoprotein lipase (LPL) and are then hydrolyzed as nonesterified fatty acids (NEFAs), incorporated into adipocytes to be either oxidized or re-esterified and stored as TG, or released into the circulation (ie, NEFA spillover) (159). Change in LPL activity with fat cell hypertrophy has thus been the topic of numerous investigations. Indeed, individuals who are obese have higher AT LPL activity than those who are lean due to
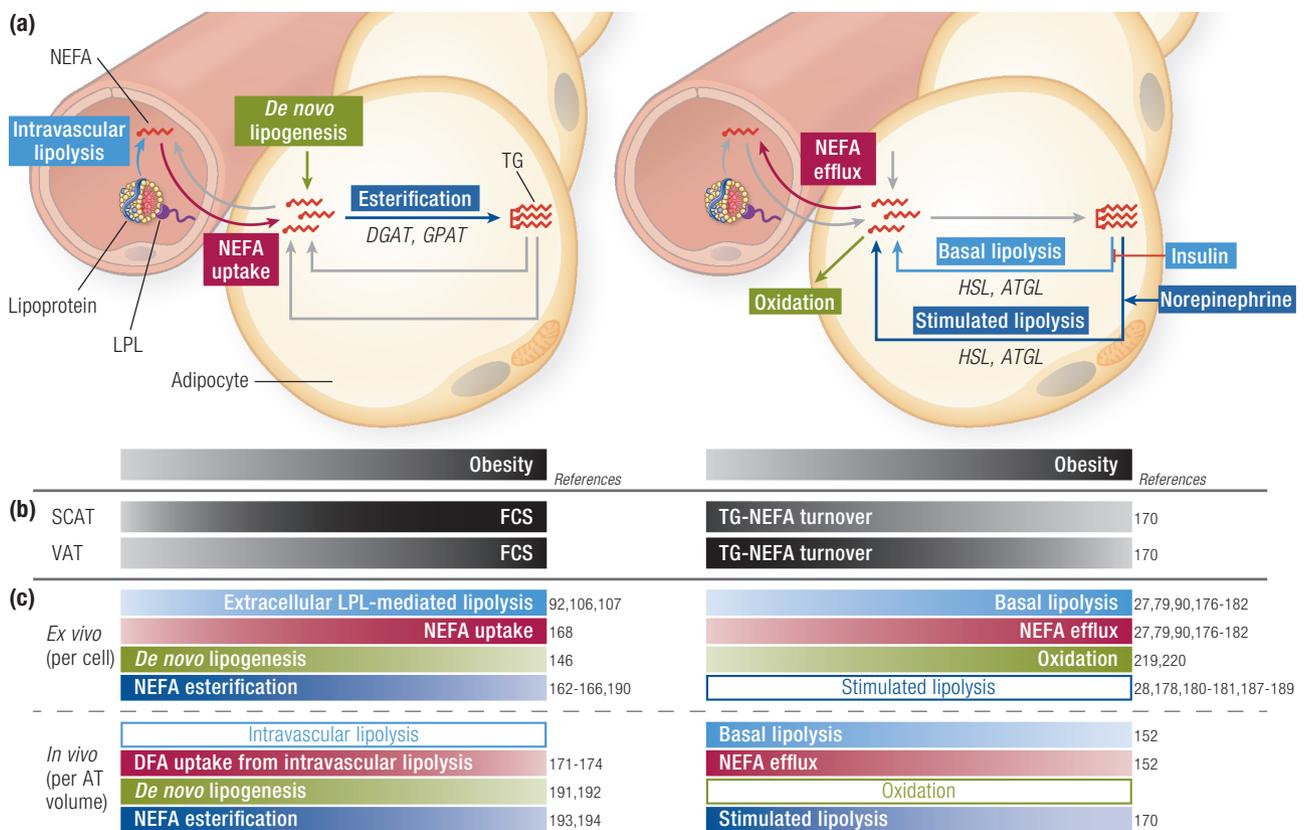

Figure 7. Adipocyte size and lipid metabolism. (A) Left panel: mechanisms leading to adipocyte triglyceride (TG) accumulation include nonesterified fatty acids (NEFAs) uptake from the circulatory pool or from lipoprotein lipase (LPL)-mediated hydrolysis of TG-rich lipoproteins, and de novo synthesis of fatty acids from carbohydrates (de novo lipogenesis). Right panel: mechanisms leading to adipocyte TG mobilization include basal and norepinephrine-stimulated intracellular TG lipolysis leading to NEFA efflux into the circulation or NEFA oxidation. (B) Change in fat cell size in relation to obesity is depicted for abdominal subcutaneous adipose tissue (SCAT) vs visceral adipose tissue (VAT) along with change in triglyceride-nonesterified fatty acid (TG-NEFA) turnover based on in vivo studies. (C) Change in the mechanisms leading to adipocyte TG accumulation (left panels) or mobilization (right panels) according to the degree of obesity and fat cell size (FCS) measured ex vivo (upper panels) or in vivo (lower panels). Changes are expressed by the color tone, light tones for low metabolic rates or smaller levels and dark tones for high metabolic rates or higher levels. Empty bands indicate conflicting results or insufficient data to indicate increase or decrease with obesity or fat cell size change. ACS, acetyl-CoA synthetase; ATGL, adipose triglyceride lipase; DGAT, diglyceride acyltransferase; DFA, dietary fatty acid; GPAT, glycerol-3-phosphate acyltransferase; HDL-c, high-density lipoprotein cholesterol; HSL, hormone sensitive lipase; LDL-c, low-density lipoprotein-cholesterol.

an increased amount of fat mass and adipocyte size (106). In women and men who are nonobese or overweight, there exists a significant, positive relationship between fasting LPL activity and SCAT FCS (92). Furthermore, there are also variations in LPL activity with respect to the location of AT. In women who are nonobese, abdominal SCAT has lower LPL activity than gluteal SCAT (160) but *LPL* mRNA expression is higher in abdominal SCAT than visceral AT (109). This result agrees with the notion that during nutritional excess, the surplus of TG is preferentially stored in subcutaneous rather than visceral depots. This has been shown directly in vivo after only a 7-day overfeeding in healthy subjects using molecular imaging of dietary fat uptake in all organs of the body (161). In an elegant study in women with obesity, Serra et al. showed that abdominal SCAT of those who had low proportions of visceral fat relative to total fat demonstrated a significant and positive correlation between adipocyte volume and LPL activity. The strength of this association was substantially attenuated in abdominal SCAT of those with higher proportions of visceral fat relative to total fat and was absent altogether in gluteal SCAT (107). Therefore, the increase in LPL activity in AT as a function of FCS was more manifest in abdominal SCAT and in individuals who were leaner and metabolically healthier.

Furthermore, FCS is also associated with decreased intracellular enzymes involved in TG synthesis, namely acetyl-CoA synthetase (162), diglyceride acyltransferase (162-165), and glycerol-3-phosphate acyltransferase (166), as well as reduced expression of genes involved in de novo lipogenesis (146), suggesting a potential role of adipocyte hypertrophy in impaired TG storage. In a study of men and women with overweight who were middle-aged, however, Rajjo et al. found that NEFA storage rate was higher in large adipocytes in proportion to their larger volume (167). It has also been shown that obesity is associated with increased adipose tissue fatty acid uptake (115, 168) and that SCAT is responsible for the majority of total AT very-low-density lipoprotein-TG storage (169). The increase in LPL activity in the presence of less efficient NEFA esterification would theoretically increase NEFA spillover. However, this phenomenon may be attenuated through the concomitant reduction in the expression of factors involved in de novo lipogenesis seen with adipocyte hypertrophy. Furthermore, since the visceral adipose tissue maintains its metabolic flexibility prior to the establishment of severe obesity, the increase in subcutaneous adipose tissue NEFA spillover may also be curbed by increased NEFA uptake in visceral adipose tissue due to the higher flux of circulating NEFA (159).

By measuring visceral and SCAT TG age, Spalding et al. estimated TG storage capacity of ATs of a large cohort consisting of individuals who were lean, overweight, or morbidly obese (170). Among those with smaller average abdominal FCS, individuals who were metabolically unhealthy had slower lipid turnover rates than the metabolically healthy. Nonetheless, among individuals with more pronounced SCAT adipocyte hypertrophy, no difference of lipid turnover was observed between the metabolically healthy and unhealthy groups. Across all participants, participants who were metabolically healthy had smaller cells than those who were metabolically unhealthy. Furthermore, although individuals who were lean had significantly higher subcutaneous lipid turnover rates, there was no difference in the turnover rate among those who were overweight, obese, or morbidly obese. However, in visceral AT, lipid turnover rate decreased only in participants who had reached the morbidly obese status. Because slower lipid turnover reflects increased storage capacity relative to lipid removal capacity, this suggests that, early on during the development of obesity, SC adipocytes adapt by slowing down their lipid turnover to accommodate excess lipids; this adaptive response may become saturated during the late stages of obesity, during which visceral adipocytes play a more important role. In morbidly obese individuals, this metabolic flexibility is overwhelmed in both SC and visceral adipose tissue depots, leading to reduced uptake of dietary fatty acid and increased dietary fatty acid spillover that are reversible only upon caloric restriction (171). This is consistent with the finding of impaired dietary fatty acid uptake and storage capacity with impaired glucose tolerance (172-174). In addition, in a large cross-sectional study, Sato et al. showed that the cross-sectional area of SCAT measured by abdominal computed tomography (CT) increased as a function of VAT cross-sectional area. However, after VAT cross-sectional area reaches a critical point (at 100 cm^2), increases in SCAT area became less pronounced despite continued expansion of VAT (175). Together, these results corroborate the assumption that excess lipids are stored primarily in subcutaneous tissues during the early stages of obesity, as we found with short-term overfeeding in individuals who were healthy (161); the visceral depots appear to retain their expansion capacity after SC adipocytes slow their expansion and have developed some degree of hypertrophy and increased adipogenesis.

Obesity is generally associated with higher rates of adipose tissue lipolysis, and numerous studies have documented increased ex vivo basal lipolytic rate with larger adipocytes (27, 79, 90, 91, 176-182). Accordingly, increased basal lipolysis has been associated with overexpression of hormone sensitive lipase (45, 176, 183, 184) and adipose triglyceride lipase (45), as well as decreased perilipin levels (81, 176) in larger adipocytes. The increased basal lipolytic activity in hypertrophic fat cells is also associated with a blunted ex vivo antilipolytic effect of insulin (90, 177,

185). In support of these observations, we have also recently shown that the increase in postprandial NEFA flux in individuals with impaired glucose tolerance was entirely due to increase in NEFA flux originating from AT intracellular lipolysis, not dietary fatty acid spillover (186). In the latter study, insulin resistance was however not associated with increased postprandial NEFA flux and we found inverse relationship between insulin resistance and glycerol flux, a marker of adipose tissue lipolysis.

Whether FCS affects catecholamine-stimulated lipolysis is more controversial, with some studies observing increased (28, 178, 180, 181, 187, 188) and others showing decreased (179, 189) lipolysis rates after stimulation with adrenergic agonists. Whether these observations made from ex vivo experiments can predict adipose tissue lipolysis in vivo, however, is unclear. For example, we found an inverse association between change in SCAT FCS and change in plasma glycerol appearance rate or change in NEFA spillover induced by bariatric surgery in individuals with morbid obesity without or with T2D (152). This demonstrates that those patients with the greatest reduction in SCAT FCS display the lowest reduction in in vivo adipose tissue lipolytic rate. In addition, the increase in NEFA flux associated with insulin resistance may also be a result of impaired re-esterification of NEFAs originating from intracellular lipolysis (159). Accordingly, it has been shown that NEFA re-esterification was reduced in participants who had diabetes (165, 190) and that TG storage was impaired in those with insulin resistance (191-193) or obesity (194).

Increased abdominal adipocyte size, especially in visceral AT, has also been associated with decreased HDL-c (19, 132, 134) and increased circulating TG (19, 45, 89, 131, 132, 134, 139, 148, 151, 195), NEFAs (165), total cholesterol (19, 195), and LDL-c (44, 139, 195). Results from meta-regression analyses are in line with the association between adipocyte hypertrophy and poor circulating lipid profile (Fig. 8). For abdominal SCAT, FCS was predicted by TG (β = 5.9983, P = .0098), NEFAs (β = 0.0371, P = .0077), total cholesterol (β = 6.2174, P = .0044), and by low HDL-c (β = -24.3195, P = .0029), independently of age, sex, and cell measurement methodology. However, when BMI was also included in the model, only total cholesterol remained a significant predictor (β = 6.1120, P = .0025). For visceral AT, FSC was predicted by TG (β = 19.4356, P = .0004) and low HDL-c (β = −52.6697, P = .0026); both remained significant after controlling for BMI.

To summarize, as adipocytes expand, basal lipolysis measured by ex vivo methods also increases, possibly due to greater amounts of intracellular TG substrates. However, increased insulin levels and impaired catecholamine action lead to reduced lipolysis rate per adipose tissue TG mass in vivo (159). The rapid adipocyte expansion followed by a decrease and stabilization in turnover suggests an early recruitment and loss of adipocyte expandability in the subcutaneous adipose tissue. In visceral adipose tissue, lipid turnover rate decreases only in individuals who have reached the morbidly obese status. The relationships between FCS and TG, NEFA, total cholesterol, and reduced HDL-c were confirmed using meta-regression and are not solely dependent on BMI. Furthermore, adipocyte hypertrophy is associated with AT LPL activity and impairment of lipid storage, all of which may eventually contribute to ectopic lipid accumulation.
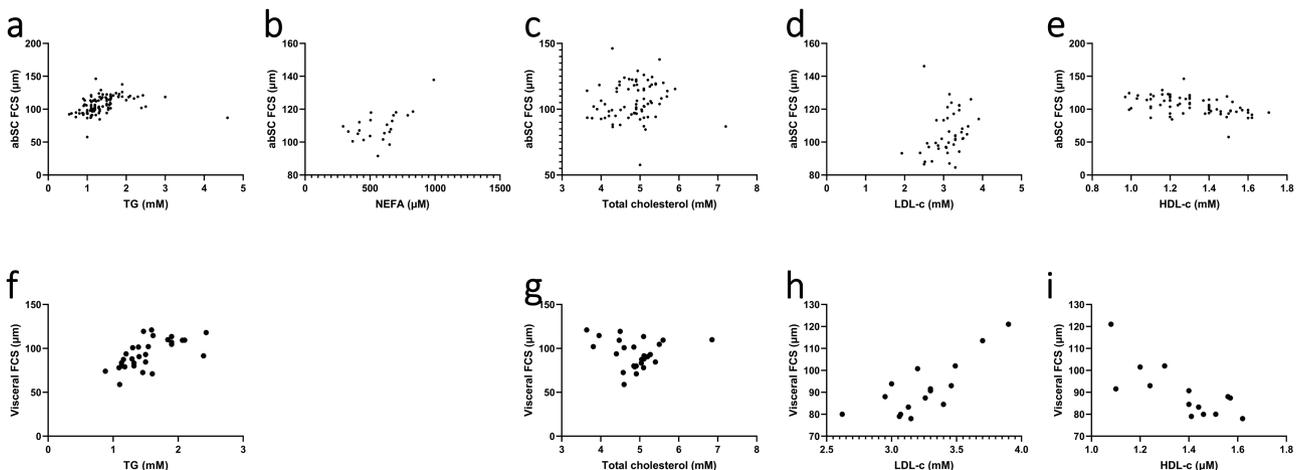
Figure 8. Relationships between abdominal subcutaneous fat cell size and (A) plasma triglycerides; (B) nonesterified fatty acid; (C) total cholesterol; (D) low-density lipoprotein-cholesterol; (E) and high-density lipoprotein-cholesterol. Relationships between visceral fat cell size and (F) triglycerides; (G) total cholesterol; (H) low-density lipoprotein-cholesterol; and (I), high-density lipoprotein-cholesterol. abSC, abdominal subcutaneous; FCS, fat cell size; NEFA, free fatty acid; HDL-c, high-density lipoprotein-cholesterol; LDL-c, low-density lipoprotein-cholesterol; TG, triglyceride.

Adipocyte size and ectopic lipid accumulation

It is hypothesized that adipocyte hypertrophy resulting from chronic energy imbalance may lead to insulin resistance via accumulation of ectopic fat in the liver and skeletal muscles. However, existing literature has delineated a more complex interplay between FCS and increased lipid content in these ectopic sites. In a large cohort of individuals who were obese, abdominal SCAT FCS explained 21% of the variance of liver fat content measured by proton magnetic resonance spectroscopy, independently of age, sex, BMI, and the ratio of visceral vs SC abdominal fat mass (196). Similar positive correlations between intrahepatic lipid levels and abdominal SCAT FCS have been observed in groups of participants with overweight (126), obesity (127), severe obesity, and morbid obesity (64, 89, 124). Furthermore, Anand et al. found that individuals of South Asian descent had more body fat, visceral adiposity, and liver fat than people of Caucasian descent and that abdominal SCAT FCS was associated with the observed difference in liver fat between these 2 ethnic groups (197). By studying a group of participants who were monozygotic twins, Pietiläinen et al. have also shown that variations in intrahepatic lipids within each pair of twins were associated with variations in leptin levels, which were, in turn, predicted by SCAT FCS (29). Despite these findings, other investigators have reported no significant correlation between SCAT adipocyte size and liver fat in smaller cohorts of individuals who were lean (123), overweight (198), or obese (199). As shown in Table 5, these have generally included participants with lower BMIs; it can be speculated that the relationship between SCAT FCS and ectopic lipid accumulation in the liver is, in part, dependent on BMI and is observed mostly in those with more severe obesity. In particular, 1 of these studies (198) investigated the effect of a hypercaloric diet on liver fat in participants with a BMI below 32 kg/m^2 and found that despite increases in both abdominal SCAT FCS and intrahepatic lipid with weight gain, no correlation was found between these variables. The investigators have further shown that regardless of fat mass, having larger adipocytes at baseline curbed hepatic fat accumulation induced by overfeeding. It may be argued, therefore, that in individuals with relatively low BMI, those who have larger SCAT adipocytes may display efficient adipose tissue fat storage that protects against liver fat accretion. A more recent twin study (44) showed that although twins with obesity and more pronounced adipocyte hypertrophy had increased liver fat, differences in SCAT FCS were not correlated with differences in hepatic lipid content.

Visceral adiposity is a better predictor of metabolic endpoints. Furthermore, it has also been hypothesized that due to the venous drainage of omental and mesenteric AT

to the liver, hypertrophy of the visceral AT may be better associated with liver fat content than would SCAT fat cell enlargement. This is in agreement with findings in individuals with morbid obesity, in which both visceral and SCAT FCS correlated with hepatic steatosis severity assessed by liver biopsy, while only visceral AT FCS was associated with hepatic fibrosis (89). These observations were later corroborated by Wree et al. (43) in a larger cohort of participants with more severe obesity.

In contrast to the liver, the relationship between lipid accumulation in muscles and SCAT FCS has not been well demonstrated. Four studies (64, 123, 126, 198) found no significant correlation; 1 of them (126) revealed a positive association between SCAT FCS and liver fat but not intramuscular lipid content. Nevertheless, CT densitometry showed increased muscle fat accumulation in women with higher percentage of visceral fat mass relative to total fat mass than those with less visceral adiposity (107). As discussed earlier, the former had larger SCAT FCS and lower rate of increase in AT LPL activity with respect to SCAT adipocyte size.

We found no study investigating the possible link between muscle fat content and visceral FCS. However, compared with SCAT FCS, visceral AT FCS is more strongly correlated with decreased adiponectin, an adipokine that is associated with increased insulin sensitivity, lower risk of T2D, and increased lipid oxidation in muscles and liver (203, 204). In line with these findings, plasma adiponectin is negatively correlated with fat levels in skeletal myocytes (64). Furthermore, although the NEFA fractional extraction rate was similar in the skeletal muscles of individuals with vs without obesity, it was correlated with serum TG and was increased in individuals with obesity and T2D (115).

In summary, abdominal SCAT adipocyte size is positively associated with liver fat in large cross-sectional studies. The mechanism explaining the association between SCAT adipocyte hypertrophy and liver fat content is currently unclear. In contrast, visceral AT FCS appears to be more closely associated with the severity of hepatic steatosis. Nevertheless, FCS in the SCAT does not seem to be associated with muscle lipid accumulation.

Adipocyte size and cardiovascular complications

In general, increased FCS accompanies poor cardiovascular endpoints. A positive association between blood pressure and adipocyte hypertrophy was observed in numerous studies (48, 103, 107, 117, 137, 205). This relationship seems to be stronger for visceral FCS, consistent with the fact that cardiometabolic dysfunctions are better correlated with visceral than SC fat mass. Furthermore, a recent study showed a strong, positive correlation between SCAT

Table 5. Relationships between liver and muscle fat content and FCS, insulin resistance, and adipogenesis

Findings	FCS-liver fat corr.	FCS-muscle fat corr.	AT	N	Age	Sex	BMI	T2D	Ref.
Histology assessment of ectopic lipid content									
• Visceral FCS correlated with: liver injury (ALT, AST) and NAS severity (histology)	↑	NA	Vis. HS	93	43	72 W	52	Unk.	(43)
• Visceral and SC FCS correlated with degree of hepatic steatosis • Visceral FCS: predictor of liver fibrosis	↑	NA	Abd. HS and CD	35	41.8	25 W	49.5	6	(89)
• Visceral FCS correlated with Pref-1 expression, which correlated with degree of hepatic steatosis	↑ (?)	NA	Abd. HS and CD	29	42.3	20 W	48.6	Unk.	(11)
Proton magnetic resonance spectroscopy assessment of ectopic lipid content									
• Compared to individuals with IS, individuals with IR have ↑ VAT and intrahepatic lipid • Δ peak diameter predicted Δ intrahepatic lipid with overfeeding	↑	NA	Abd. OF	31	30-60 Mean ≈ 56	15 W	25-35 Mean ≈ 30	0	(127)
• FCS correlated with serum leptin, which correlated with liver fat • The co-twins who was heavier had ↑ SAT, ↑ FCS, and ↑ liver fat • Liver fat correlated with serum insulin	↑ (?)	NA	Abd. CD	34	24-27	16 W	Mean ≈ 26.8	0	(29)
• Co-twins with hypoplastic obesity: ↑ liver fat • Co-twins with hypertrophic obesity: ↓ fat cell number, ↑ liver fat • Δ FCS: not correlated with Δ liver fat	↑	NA	Abd. CD	40	28.4	30 W	28	0	(44)
• FCS explained 21% of liver fat variation	↑	NA	Abd. CD	119	40	83 W	30	0	(196)
• FCS: correlated with VAT and liver fat, but not muscle fat	↑	—	Abd. OF	46	37.5	Unk. Mixed.	25-30 Mean ≈ 27.7	0	(126)
• Participants of South Asian descent have more liver fat than those of Caucasian descent. Adjustment for FCS and fat distribution decreased the difference in liver fat	↑ (?)	NA	Abd. HS	108	35.5	Unk. Mixed.	Mean ≈ 27.5	0	(197)
• No correlation between FCS and liver fat • AT adiponectin correlated negatively with liver fat • Liver fat: correlated with crown-like structures and macrophages in SCAT, correlated with PAI-1	—	NA	Abd. HS	27	61	9 W	31.4	27	(199)
• In high visceral fat group: ↑ hepatic fat	NA	NA	Abd. OF	38	15.1	14 W	36.8	Unk.	(124)
• FCS is not correlated with hepatic, muscle, or visceral fat	—	—	Abd. OF	75	26.8	37 W	22.9	0	(123)
• No association between FCS and liver or muscle fat	—	—	Abd. OF	29	26.8	0 W	25.5	0	(198)
• No difference in liver fat between T2D and control	NA	NA	Abd. CD	30	58	10 W	30.8	15	(165)
• FCS: positively correlated with liver fat, but not muscle fat	↑	—	Abd. CD	53	25.8	21 W	35.4	0	(64)
• FCS: positively correlated with liver lipids	↑	—	Abd. CD	53	42	0 W	Unk.	0	(225)
CT assessment of ectopic lipid content									
• In high liver fat group: ↑ CD68, chemokines monocyte chemoattractant protein-1, and PAI-1, ↓ PPAR-γ and adiponectin	NA	NA	SC CD	20	Mean ≈ 40.5	20 W	Mean ≈ 36	0	(226)
• In individuals with T2D: ↑ liver fat, ↑ muscle fat	↑ (?)	↑ (?)	Abd. OF	102	58	59 W	Mean ≈ 33.6	67	(125)
• Women with higher proportions of visceral fat have higher fat accumulation in the muscle	NA	↑ (?)	Abd., g. CD	48	Mean ≈ 60	48 W	Mean ≈ 31.5	0	(107)
• Liver fat: associated with IL-6 and number of macrophages in SCAT	NA	NA	Abd. HS	36	37	17 W	26	0	(227)

Abbreviations: ↑ (in first column), increase in; ↓ (in first column): decrease in; ↑ (in second and third columns, positive correlation; Δ, change in; abd., abdominal subcutaneous; AT, adipose tissue assessment; BMI, body-mass index; CD, collagenase digestion; corr., correlation; CT, computed-tomography; FCS, fat cell size; HS, histological section; IR, insulin resistant; IS, insulin sensitive; N, number of participants; NA, not applicable or not reported; NAS, nonalcoholic steatosis; OF, osmium fixation; Ref., reference number; SAT, subcutaneous adipose tissue; Sex, number of women; T2D, type 2 diabetes; Unk., unknown; VAT, visceral adipose tissue; W, women.

FCS and the Framingham risk score for cardiovascular disease; this increased risk may be caused by higher NEFA levels due to increased lipolysis and decreased lipogenesis (177). The accumulation of lipids in cardiomyocytes may then lead to altered cardiac function. Another causal mechanism of hypertension implicating adipocyte hypertrophy is stiffening of arteries. Using pulse wave velocity as a proxy for arterial stiffness, Arner et al. showed that pulse wave velocity was positively correlated with SCAT and visceral FCS (206).

Due to their proximity to the myocardial tissue and vessels, epicardial and perivascular ATs are of particular interest when studying the effects of AT dysregulation of lipid metabolism and adipokine secretion on cardiac dysfunction. In a given individual, the average size of epicardial adipocyte is smaller than that of visceral and SCAT adipocytes (34, 87, 88, 207). In addition, the epicardial AT fat cells were hypertrophied in patients with coronary artery disease (CAD) relative to those without CAD (87, 208, 209). Epicardial AT FCS was also higher in those with T2D compared to individuals who were nondiabetic (88). In line with these findings, patients with CAD had reduced expression of adiponectin in their epicardial AT (87, 88), which may limit adipocyte hyperplasia and promote inflammation. Moreover, blood pressure is also associated with increased Pref-1 expression in omental AT (11) and decreased adiponectin in epicardial AT (210), indicating reduced adipogenesis. The proinflammatory profile in epicardial AT of patients with CAD or epicardial fat cell hypertrophy was evidenced by higher expression of MCP-1 (88), CD68 (88), IL-6 (211), TLR-2 (208), and TLR-4 (208), more CD11c-positive macrophages (208), and lower expression of adiponectin (17). Nevertheless, in a cohort of 22 patients with or without CAD, Eiras et al. found reduced epicardial MCP-1 expressions in those with larger epicardial FCS, despite higher MCP-1 plasma concentrations (34). Furthermore, elevated PAI-1 level increases the risk for CAD (212-214) and is positively correlated with FCS of both the SC and visceral depots (16, 54, 215).

Hence, there is solid evidence that SCAT adipocyte hypertrophy accompanies a wide range of dysregulations in cardiometabolic health, with high blood pressure, increased Framingham risk score, and arterial stiffening. In line with these findings, epicardial FCS is also associated with CAD and diabetes status. These associations may be mediated through a proinflammatory profile of hypertrophied epicardial fat cells.

Effect of energy balance and therapeutic interventions on adipocyte hypertrophy

Energy balance and therapeutic interventions. Long-term energy imbalance is among the most important

determinants of excessive weight and, perhaps, adipocyte hypertrophy. Changes in energy intake have been shown to affect FCS in numerous studies. In women with obesity, dietary caloric restriction resulted in decreased SCAT FCS to the level of control participants (74). Dietary weight loss also reduced the size of both gluteal and abdominal SCAT adipocyte size in men with obesity (216). Other subsequent studies have reached similar conclusions with different diets and in different test populations (57, 99, 178, 217, 218). One study in adults who were healthy, but insulin-resistant and overweight showed that changes in FCS were significantly associated with changes in weight (weight loss of 4.3 kg) and in insulin resistance (151). In the latter study, changes in FCS, but not in BMI, predicted improvement in insulin sensitivity.

The effects of weight fluctuation on FCS and adipocyte number are specific to the type of adipose depots. Increase in weight (by 3.1 kg (99) or by 4.6 kg (219)) was associated with increase in the quantity of both upper-body and lower-body SCAT. However, whereas the increase in upper-body SCAT mass (by 1.9 kg) was associated with adipocyte hypertrophy, the increase in lower-body SCAT mass (by 1.6 kg) did not appear to be associated with cell hypertrophy, but with cell hyperplasia (219). With subsequent weight reduction (of 2.4 kg) in the same participants, FCS in both regions decreased, but adipocyte numbers did not change (99). With a 15% weight loss, FCS decrease was more important in the abdominal SCAT than in the gluteal AT (218). In addition, another study showed that dietary intervention in men with obesity (resulting in an average weight loss of 13.1 kg) reduced abdominal FCS, with modest decrease in gluteal FCS that was not statistically significant (216). Hence, it seems that upper-body FCS is more reactive to weight fluctuations, while lower-body AT responds to weight gain through adipogenesis, which confers more durable increase in lipid storage capacity. Other investigators have found that AT cell numbers in the femoral (74) and abdominal (115, 137) depots were not affected by weight loss interventions (with losses of 25 kg (115) and 33% (137)).

Significant decrease in abdominal SCAT FCS was observed after bariatric surgery with Roux-en-Y gastric bypass (58, 115, 137), duodenal switch (152), and sleeve gastrectomy (115). Initial FCS was also an important negative predictor of the beneficial effects of bariatric surgery on the diabetes status and metabolic dysfunctions (hyperinsulinemia, hyperglycemia, hypertension, dyslipidemia, and central obesity). Indeed, patients with larger FCS displayed decreased effectiveness of gastric bypass on remission of diabetes status and improvement of diabetes risk factors (141). The rates of basal and stimulated ex vivo AT lipolysis were reduced with gastric

banding and lifestyle interventions (178), which may thus explain the decrease in lean tissue steatosis with weight reduction. However, in vivo NEFA appearance rate did not change despite very important weight loss and reduction in SCAT FCS after duodenal switch in patients without or with T2D (152).

The level of physical activity constitutes a determinant of overall energy expenditure that is associated with adipocyte size. Individuals with a sedentary lifestyle have larger adipocytes than individuals who were physically active (148); this difference, however, may be due to higher BMI in the former group. Nonetheless, while dietary restriction did reduce body weight (by 10.4 kg) in a study conducted by You et al., it did not change SCAT FCS, which was decreased only with the addition of exercise (220). A 3-month aerobic exercise program reduced gluteal and abdominal SC FCS in men (216). In individuals with overweight, FCS was correlated with intrahepatic lipid content; both were significantly reduced following dietary restriction with physical exercise (126). Finally, in 7 pairs of volunteers who were monozygotic twins, Ravussin et al observed a decrease in FCS with a negative energy balance imposed by exercise concomitantly with a slight increase in plasma ghrelin concentration (221).

In addition, thiazolidinediones are antidiabetic medications known to mediate their effects through activation of PPAR-γ and induction of adipogenesis. Accordingly, 2 studies have found significant increases in the proportion and absolute number of small cells in individuals treated with thiazolidinediones (222, 223). Other studies have concluded that the induction of PPAR-γ using pioglitazone (14) and troglitazone (224) was associated with increased FCS by measuring the average size of adipocytes.

In summary, metabolic interventions involving dietary restriction result in reduced FCS with weight reduction and suggest an effect of FCS correction on insulin resistance that is independent of change in BMI. On the contrary, caloric excess with overfeeding leads to increased FCS. Furthermore, dietary interventions appear to have different effects depending on the AT depot: abdominal SCAT reacted to weight change through change in FCS whereas lower-body SCAT responded to weight gain preferentially through change in the number of adipocytes. Other interventions aimed at restricting excess energy balance, including bariatric surgery and physical activity, have also consistently led to reduced FCS. Induction of adipogenesis via treatment with thiazolidinediones may also increase AT storage capacity by increasing the number of small cells and by promoting subsequent enlargement of these adipocytes.

AT oxidative metabolism. Adipose tissue of individuals with obesity generally consumes less oxygen than those of individuals who are lean; this reduced oxygen consumption seems to be related to altered mitochondrial function in fat cells (225, 226). However, it is not entirely clear whether this discrepancy is merely due to diminished mitochondrial function in both small and large fat cells in obesity or if it is caused by a relative decrease in oxygen consumption per gram of lipid as adipocytes enlarge. Fischer et al. measured the mitochondrial oxidative phosphorylation capacity in adipocytes, which was negatively correlated with BMI. They also determined that respiratory capacity was not significantly different between small and large fat cells (227). Yet in another study, oxygen consumption rate was higher in AT of participants who were obese compared to those who were lean, when it was expressed per number of adipocytes. Nevertheless, it was lower in obesity when expressed as a function of quantity of adipose tissue (226). Furthermore, a more recent study found that the oxygen consumption rate and citrate synthase activity were reduced in AT of individuals who were obese vs whose who were lean. When expressed per number of cells, oxygen consumption rate and citrate synthase activity were higher in large adipocytes than in small adipocytes of the same individuals, although these differences did not reach statistical significance (225). However, while not explicitly presented in the article, the oxygen consumption rate and citrate synthase activity were lower in large cells when expressed per gram of lipids. It thus appears that, at the cellular level, adipocyte hypertrophy is associated with an increase in oxygen consumption, although such an increase is not commensurate with the growth in cell volume.

In conclusion, the few studies on AT oxidative metabolism and FCS indicate that the reduced oxidative metabolism in hypertrophied AT of individuals with obesity may be a result of increases of adipocyte volume that surpass the increase in oxygen consumption displayed individually by larger adipocytes. The relationship between impaired oxidative metabolism and adipocyte hypertrophy may be caused by acquired variations in expression of genes implicated in mitochondrial function.

Conclusion

To summarize, we first assessed variations in FCS due to measurement methods and demographic factors. We found that, regardless of sex, age, and BMI, HS produces lower FCS estimates than CD, while OF results in slightly higher size measurements than CD in SCAT. These variations are probably the result of differences in cell fragility, physical, chemical, statistical, and/or geometrical factors. Numerical analyses confirmed that FCS in SCAT is positively correlated with cell size in all other AT compartments and that femoral and gluteal AT contain the largest adipocytes, whereas visceral AT contains smaller cells compared to abdominal

SCAT. Nevertheless, data are conflicting regarding possible differences in FCS between men and women in SCAT; our meta-analyses also failed to demonstrate statistically significant variations between sexes. Despite the lack of studies directly measuring the relationship between age and adipocyte size, we were able to show, using meta-regression, that FCS increases with age in both SCAT and visceral AT independently of measurement method, sex, and BMI. This finding is in line with the observation that increases in FCS is associated with adipocyte senescence.

Our review of the physiopathological processes associated with adipocyte hypertrophy revealed that adipocyte hypertrophy is related to impaired AT differentiation potential and rate of adipogenesis. Furthermore, hypertrophy of fat cells also accompanies cell senescence, with increased cell necrosis and reduced telomere length. We also found an extensive body of evidence for the strong relationships between adipocyte hypertrophy and AT inflammation. Moreover, we have also consistently found studies indicating that the associations between FCS and these physiopathological processes are not solely the result of obesity or increased BMI.

Next, we reviewed the relationships between adipocyte hypertrophy and systemic metabolic endpoints. FCS increases with diabetes status, impaired glucose tolerance, and insulin resistance. Our meta-regression analyses confirmed these correlations and showed that visceral AT (but not SCAT) FCS is associated with glucose intolerance and insulin resistance independently of BMI. In terms of lipid metabolism, adipocyte hypertrophy is associated with AT LPL activity, impaired lipid storage, and increased intracellular lipolysis, all of which may contribute to ectopic lipid accumulation. Consistent with this observation, we found that abdominal SCAT adipocyte size is positively associated with liver fat content in large cross-sectional studies. In contrast, visceral FCS appears to be more closely associated with the severity of hepatic steatosis, possibly due to the direct venous drainage from visceral AT. Finally, SCAT adipocyte hypertrophy is related to numerous indicators of cardiometabolic dysregulations, including high blood pressure, increased Framingham risk score, and arterial stiffening.

Metabolic interventions involving dietary restriction and overfeeding resulted respectively in reduced and increased FCS. Other interventions aimed at inducing negative energy imbalance, including bariatric surgery and physical activity, have also consistently led to reduced FCS. In line with these observations with change in energy expenditure, studies also suggest that the reduced oxidative metabolism in hypertrophied AT of individuals with obesity may be the result of the increase of adipocyte volume that is proportionally greater than the increase in oxygen metabolism at the individual cell level. This may lead to decreased overall AT oxidative metabolism per mass of AT.

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