Markers of regional subcutaneous adipose tissue (dys)function in childhood-onset versus adult-onset obesity before and after weight loss

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Abstract

Markers of regional subcutaneous adipose tissue (dys)function in childhood-onset versus adult-onset obesity before and after weight loss

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People with childhood-onset obesity have greater risk of type 2 diabetes than people with adult-onset obesity. However, we do not understand the mechanisms contributing to this disease risk discrepancy or whether these mechanisms can be reversed. A decline in subcutaneous adipose tissue (SAT) function (i.e., the ability to safely store lipids and regulate adipokine production) is linked to type 2 diabetes and may therefore vary by age of obesity onset. This dissertation aimed to compare markers of regional SAT (dys)function between people with childhood-onset and adult-onset obesity.

Original article 1 investigated the longstanding yet controversial notion that SAT is hyperplastic (has many small adipocytes) in childhood-onset obesity and hypertrophic (has fewer large adipocytes) in adult-onset obesity. This notion held true only for abdominal SAT in females. The degree of abdominal SAT hypertrophy or hyperplasia was unaffected by age of obesity onset in males. In contrast, femoral SAT was hypertrophic in both males and females with childhood-onset obesity compared to their counterparts with adult-onset obesity.

Original articles 2 & 3 examined preadipocyte DNA damage (γH2AX), senescence markers (p53 and p21), and SAT immune cell profiles in females with childhood-onset and adult-onset obesity, both before and after moderate (~10%) weight loss. Compared to females with adult-onset obesity, those with childhood-onset obesity had a greater proportion of preadipocytes with DNA damage and senescence markers in abdominal and femoral SAT, but a

slightly lower proportion of M1-like ‘pro-inflammatory’ macrophages in abdominal SAT. The proportions of M1-like macrophages in femoral SAT and M2-like ‘anti-inflammatory’ macrophages, $CD3^+CD4^+$ T cells, and $CD3^+CD8^+$ T cells in abdominal and femoral SAT did not differ between groups. After weight loss, preadipocyte DNA damage declined in both females with childhood-onset and adult-onset obesity. Other changes, however, occurred only in females with adult-onset obesity: the intensity of p21 in $p53^+p21^+$ femoral preadipocytes and the proportion of M1-like macrophages in abdominal SAT decreased, while the proportion of $CD3^+CD4^+$ T cells in abdominal and femoral SAT increased.

Recognizing the differences in markers of SAT (dys)function between people with childhood-onset and adult-onset obesity may help guide the development of tailored treatment strategies.

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This PhD journey has been a long one—considering it technically began back in my grade 10 career studies class where I sketched my future self as a 'scientista'. While many are wrong in thinking I never wanted this PhD to end, I *do* prefer beginnings. And from the very beginning of this journey, I was fortunate to have many incredible people in my corner, both in lab and in life.

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Contribution of Authors

PhD Candidate's contribution

When I entered the Metabolism, Obesity, and Nutrition (MON) lab, Sylvia Santosa, the principal investigator, had already secured funding from NSERC to examine baseline differences in adipose tissue characteristics between people with childhood-onset and adult-onset obesity. At this time, preliminary data had been collected and some of the lab protocols were still in the development phase. As such, one of my first roles was to assist with the optimization of our flow cytometry protocol.

Sylvia had planned to turn this obesity-onset project into a weight loss study (with some new assessments), and together, we brought it to fruition. With my input and assistance, Sylvia successfully wrote and secured funding from the Heart and Stroke Foundation of Canada. I amended our consent form, obtained ethical approval, and maintained ethics renewals throughout the project. After initial ethical approval, I launched and managed the study with resources provided by Sylvia and other collaborators. This included creating all study materials and building a database for data entry. Throughout the study, I was actively involved in participant recruitment, screening, and assessments, and coordinating and preparing for all appointments. I delivered the nutrition component of the weight loss intervention and followed up with participants weekly. I also trained and oversaw other students, interns, and volunteers who contributed to the study.

On biopsy days, I fulfilled either the preadipocyte culture/β-gal staining (*original article 2*)/adipocyte sizing (*original article 1 & 3*) role or the flow cytometry (*original article 3*) role—usually the latter. I helped passage/freeze down preadipocyte cultures and conducted all the senescence lab work and analyses (*original article 2*). Using the free code provided by ImageJ-NIH, I developed the Fiji macros for the adipocyte size (*original article 1 & 3*) and senescence immunofluorescence analyses (*original article 2*), seeking expert consultation when needed. Additionally, I analyzed the flow cytometry data (*original article 3*) and conducted all data cleaning and statistical analyses for all articles.

I interpreted all results and wrote the original drafts of all 5 articles included in this dissertation, incorporated co-author feedback, and handled the revisions and responses to the reviewer comments for the published articles.

Co-author contributions

The author contributions for the published articles in this dissertation are listed below using an adapted version of CRediT (contribution roles taxonomy).

Original article 1:

Jessica Murphy: Conceptualization, Project Administration, Investigation, Software, Formal Analysis, Visualization, Writing – Original Draft, Writing – Review & Editing, Article Approval

Abdulrahman Dera: Investigation, Article Approval

José A. Morais: Investigation, Article Approval

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Bjorn T. Tam: Methodology, Article Approval

Sylvia Santosa (corresponding author): Conceptualization, Methodology, Supervision, Funding acquisition, Writing – Review & Editing, Article Approval

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Nino Giorgadze: Methodology, Article Approval

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Original article 3:

Jessica Murphy: Project Administration, Investigation, Formal Analysis, Visualization, Writing – Original Draft, Writing – Review & Editing, Article Approval

José A. Morais: Investigation, Article Approval

Michael A. Tsoukas: Investigation, Article Approval

Alexandra B. Cooke: Investigation, Article Approval

Stella S. Daskalopoulou: Methodology, Article Approval

Sylvia Santosa (corresponding author): Conceptualization, Methodology, Supervision, Funding acquisition, Writing – Review & Editing, Article Approval

The co-authors for the *review article* and the *perspective article* have not yet been finalized. So far, only Sylvia Santosa has reviewed and provided feedback on these articles.

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The age of obesity onset affects changes in subcutaneous adipose tissue macrophages and T cells after weight loss

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INTRODUCTION: Review article.
Childhood-onset versus adult-onset obesity: from adipose tissue biology to cardiometabolic risk and treatment outcomes

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Introduction

Every adult living with obesity has a story, and like every story, theirs has a beginning. For some, their obesity story began in childhood and continued into adulthood. For others, adulthood marked the start of their obesity story. Although researchers have hypothesized that childhood-onset and adult-onset obesity are two types of obesity,[1–3] we do not fully understand how the age of obesity onset shapes disease progression and treatment outcomes.

Excess adipose tissue is a defining feature of obesity. As adipose tissue expands, it undergoes a remodelling process that can turn pathogenic. Dysfunctional adipose tissue loses its ability to safely store lipids and regulate adipokine production. Instead, it directs lipids to ectopic sites like the liver and muscle and shifts toward a pro-inflammatory state, disrupting whole-body metabolism.[4,5]

There is reason to believe that the mechanisms linking adipose tissue remodelling to adipose tissue dysfunction and cardiometabolic disease vary between people with childhood-onset and adult-onset obesity. The biology of obesity development and the ability to adapt to excess adiposity may differ at distinct life stages.[6–8] Furthermore, the length of time a person has lived with obesity likely affects the degree of exposure to metabolic aberrations. Such nuances in cardiometabolic disease mechanisms may indicate a need for treatment strategies tailored to the age of obesity onset.

In this review, we compare the adipose tissue biology, cardiometabolic risk, and treatment outcomes between people with childhood-onset and adult-onset obesity.

Methods

We searched PubMed for human studies published in English or French from inception to March 31, 2024 using variations of the following search strategy: (adult or child or adolescent or teenager or youth or juvenile or pediatric) and (obesity or overweight or adiposity or body mass index or body weight or fat or adipose tissue) and (age of onset or onset or age factors or time factors or time or timing or pattern or trajectory or duration or exposure or change). To identify studies relevant to our objective, we then refined our search by adding terms for adipose tissue characteristics, cardiometabolic risk factors and diseases, or obesity treatments. We also searched the reference lists of included studies.

We discuss all relevant literature on white adipose biology, treatment outcomes, and the following cardiometabolic risk factors and diseases: insulin resistance, systemic inflammation, carotid intima media thickness, arterial stiffness, hypertension, dyslipidemia, non-alcoholic fatty liver disease, type 2 diabetes, and major adverse cardiovascular events (e.g., acute myocardial infarction, stroke).

To facilitate understanding of how obesity progresses from childhood, we described studies that compared the adipose tissue biology of lean children and children with overweight or obesity. We prioritized studies that compared our outcomes of interest between adults with different onsets of common obesity provided at least one group developed overweight or obesity in childhood or adolescence. In addition, we included studies that examined the association between age of obesity onset as a continuous variable (spanning childhood/adolescence to adulthood) and our outcomes of interest.

We also incorporated cohort studies that assessed how adiposity trajectories or changes in adiposity status from childhood to adulthood associate with adult cardiometabolic risk factors or

diseases. These studies included groups with different onsets of obesity, but typically only reported multivariate-adjusted odds ratios (OR), risk ratios (RR), or hazard ratios (HR) with 95% confidence intervals and p-values for each group versus a common reference group (e.g., people with persistent normal weight, *group 0*). We therefore performed a series of computations to statistically compare the odds, risk, or hazard rate of diseases between different obesity-onset groups.

For example, the OR, RR, or HR (relative effect, R) for a group with childhood-onset obesity (*group 1*) versus a group with adult-onset obesity (*group 2*) can be computed as $R_{1vs.2} = R_{1vs.0}/R_{2vs.0}$. The lower ($L$) and upper ($U$) 95% confidence limits for $R_{1vs.2}$ can be computed as $L = R_{1vs.2} \times exp(-1.96s_{1vs.2})$ and $U = R_{1vs.2} \times exp(1.96s_{1vs.2})$, respectively, where s is the standard error. $s_{1vs.2} = (s_{1vs.0}{}^2 + s_{2vs.0}{}^2 - 2C_{1vs.2})$, where $s_{1vs.0} = ln(U_{1vs.0}/ L_{1vs.0})/3.92$, $s_{2vs.0} = ln(U_{2vs.0}/ L_{2vs.0})/3.92$, and C is the covariance.[9] We computed the covariance using the method outlined by Orsini et al.[10] that first requires estimating the number of cases (e.g. number of people with type 2 diabetes) in each group in the analysis given the multivariate-adjusted ln ORs, RRs, or HRs. This first step was possible when the crude number of cases and the total number of participants (for studies reporting OR or RR) or the crude number of cases and the total person-time follow-up (for studies reporting HR) for each group were known. When the total number of participants but not the person-time follow-up for each group was available for studies reporting HR, we used the traditional life-table denominator (number of participants – number of cases/2) as a proportional substitute for person-time.[11] With this information, we estimated the number of cases as per Greenland and Longnecker[12] using the SAS %METADOSE macro developed by Li and Spiegelman.[13]

After computing the covariance then the standard error of *R,* we determined the p-value for the hypothesis $R = 1$ (i.e., no difference between groups) by looking up the absolute Z-statistic ($|\ln(R)/s|$) in a two-tail table for the standard normal distribution using SAS version 9.4 (SAS Institute, Cary, NC, USA). For each study, we performed these computations using the group with the latest obesity onset and, where relevant, the mildest obesity as the reference group (Table S2). We showed sex-stratified analyses when possible. When the above information required to compare odds, risk, or hazard rate of cardiometabolic diseases between obesity-onset groups was not reported or could not be obtained from the study authors, we displayed the original study findings (i.e., with the original reference group) (Table S3) and discussed any reported comparisons between obesity onset groups.

Although we were interested in studying adults with obesity, we included studies that combined adults with overweight and obesity. However, if studies on the same dataset presented results for adults with overweight or obesity or for adults with obesity, we discussed the latter. Furthermore, we presented results for adiposity trajectories from childhood that culminated in adult overweight or adult obesity. For overlapping studies, we selected the one with the largest sample size.

Box 1 highlights key methodological considerations for determining the age of obesity onset.

Box 1. Methodological considerations for determining the age of obesity onset in research studies

Prospective, longitudinal studies (e.g., birth cohort studies) can accurately determine when people develop obesity, per growth charts and BMI criteria[14–17] by regularly collecting

weight and height measurements from childhood through adulthood. However, these studies are resource-intensive and require long-term commitment from both researchers and participants. Although frequent on-site measurements of weight and height would be ideal, they are likely impractical due to personnel constraints and participant burden. Alternatively, if study resources permit, researchers could provide participants with scales and instructions for at-home measurements, observed periodically by trained data assessors through videoconferencing.[18] Electronic scales that transmit data directly to researchers,[19] used successfully by both children[20] and adults,[21] offer another convenient option. Numberless scales with this capability may help avoid potential distress or changes in behaviour associated with frequent weighing. Researchers could routinely compare these home measurements with those taken on calibrated scales during on-site study visits. Despite these methodological advances, to date, there have been no prospective, longitudinal studies specifically designed to examine the association between the age of obesity onset and subsequent health outcomes.

Most studies investigating the age of obesity onset are conducted cross-sectionally in adults with obesity. These studies must rely on retrospective data such as medical records of weight and height, recalled adiposity status, and childhood photographs to determine the age of obesity onset. Given the challenge of determining the exact age of obesity onset, these studies typically strive to broadly classify obesity as childhood-onset or adult-onset. The World Health Organization (WHO) considers adulthood to begin after age 19 years,[22] when most adolescents will have completed puberty (Tanner stage V).[23,24] However, the age cut-off distinguishing adulthood from childhood can vary across health organizations and studies, typically falling between 18 and 21 years.

Medical records of childhood weights and heights provide objective evidence of childhood adiposity status, but they can be difficult to obtain. Participants can sometimes retrieve their weights and heights from immunization records (vaccine booklets), but usually only for infancy and early childhood. This information alone would be insufficient to definitively classify participants as having childhood-onset or adult-onset obesity, as a normal BMI in early childhood does not preclude obesity development later in childhood.

Accordingly, some studies use a participant's height and recalled body weight at the end of adolescence or the start of adulthood (18–21 years) to determine childhood adiposity status. A BMI greater than or equal to 30 kg/m^2 at this age would indicate childhood-onset obesity. While a meta-analysis found that people overestimate their body weight from childhood or young adulthood by an average of only 0.87 kg (95% CI: 0.19, 1.56; $p < 0.001$),[25] factors pertinent to obesity-onset research may influence this accuracy. People with higher BMIs tend to underestimate their past weight, whereas those who gain more weight over the recollection period tend to overestimate their past weight.[26,27] Furthermore, studies have shown that around 20–25% of older adults will not even attempt to recall their past body weight.[27,28] It is unclear how weight recall may be affected if participants know they are in a study focused on the age of obesity onset.

Alternatively, participants can be asked to recall their childhood adiposity status, rather than their childhood body weight, through interviews or questionnaires. People are more likely to remember the age at which they developed obesity if their weight gain occurred suddenly or drastically or coincided with a significant life change (e.g., parents' divorce or starting university). Some may never remember living without obesity, while others may recall being teased for their weight or referred for weight management at a specific age. However, those who

gained weight gradually over several years may struggle to pinpoint when they first developed overweight or obesity. Therefore, most participants would find 'Did you have overweight or obesity as a child or teenager?' easier to answer than 'At what age did you first consider yourself to have overweight or obesity?'. Conducting a thorough weight history interview, as recommended for clinical practice,[29] may help participants recall adiposity changes throughout their lives. Researchers can include prompts about significant life events or periods (e.g., starting school or puberty) to help participants anchor their memories to specific times. Of note, some participants may prefer phrases such as 'excess weight', 'high BMI', or 'larger body size than peers' rather than 'overweight' or 'obesity' to describe their adiposity status.

Importantly, the self-report method used to determine childhood adiposity status may impact whether participants are classified as having childhood-onset or adult-onset obesity. In 1985, Wing et al. found that 64% of applicants to a behavioral weight management program were classified as having childhood-onset obesity based on their response to 'Were you overweight as a child or teenager?'. In contrast, only 37% were classified as having childhood-onset obesity based on self-reported body weight at age 21, which indicated they were at least 20% overweight according to Metropolitan Life Insurance tables.[30] This discrepancy may stem from participants underestimating their body weight at age 21, misinterpreting what constitutes childhood overweight, or misperceiving their childhood adiposity status.

Body rating scales are valuable tools for helping participants recall their body sizes at younger ages. They present a series of silhouettes for each sex, ranging from very thin to very large, allowing participants to select the figure that most closely matches their body size at different ages or life stages. As a visual aid with concrete reference points, these scales can improve the accuracy of recalled adiposity status.[25] The original Stunkard scale features nine

adult figures for each sex,[31] with figures 5 and 6 as the optimal cut-offs for overweight and obesity, respectively.[32–34]

Drawing on Stunkard's scale as a guide, Collins developed a similar scale featuring seven male and female child figures.[35] While optimal cut-offs have not been established for Collins's scale, researchers have used figures 5 and 7 for identifying overweight and obesity, respectively.[36–38] In Truby and Paxton's 7-figure Children's Body Image Scale (CBIS),[39] figures 6 and 7 align, respectively, with the ≥85th (overweight) and ≥95th (obesity) BMI-per-age percentiles on the United States Centres for Disease Control and Prevention growth charts for both boys and girls.[40] Using the International Obesity Taskforce BMI cut-offs, this alignment held for girls, but for boys, the cut-offs corresponded to figures 5 and 6, respectively.[40] Notably, other child and adult body rating scales have been culturally tailored for greater inclusivity.[41–46]

Childhood photographs can help distinguish whether participants have childhood-onset or adult-onset obesity. In today's digital age, accessing old photographs is straightforward, but obtaining printed photographs from older generations may require additional effort. Although photographs provide objective evidence of appearance, interpreting adiposity status still involves subjective judgment by the researcher. To minimize this bias, researchers can compare photographs against standardized body rating scales.

When feasible, researchers should employ multiple methods to determine the age of obesity onset. The primary focus here is on BMI-defined obesity, which indicates a point at which excess adiposity may pose a health risk. Researchers wishing to study the clinical manifestations or stages of childhood-onset versus adult-onset obesity may consider utilizing the Edmonton Obesity Staging Systems (EOSS and EOSS-pediatrics).[47,48] Retrospective application

of these systems could involve detailed interviews or chart reviews to understand the evolution of clinical obesity and its stages over time.

Adipose tissue biology

Adipose tissue expansion

Seminal studies in the 1970s shaped our understanding of human adipose tissue expansion, which would persist well into the 21st century. Researchers established that adipose tissue can expand by increasing adipocyte size (hypertrophy) and/or number (hyperplasia), with a caveat. They inferred that adipocytes can enlarge at all life stages but increase in number only during childhood and adolescence—and never die.[49–51]

Infancy (0–1 year), the age of adiposity rebound (~5–7 years), and adolescence (~10–18 years) are sensitive periods for adipose tissue accrual,[7,52] but whether hyperplasia or hypertrophy dominates at these life stages is debatable.[53] Regardless, both adipose tissue expansion mechanisms are accelerated in children with obesity such that by the time these children reach adulthood, they have both larger and more numerous adipocytes than their lean counterparts (Figure 1).[54,55]

Early studies comparing adipose tissue cellularity (adipocyte size and number) between people with childhood-onset and adult-onset obesity support the idea that fat gain in adulthood can only occur through the enlargement of existing adipocytes. Adults who acquired obesity in adulthood had fewer, larger adipocytes, while those with persistent obesity from childhood had more, smaller adipocytes.[56–60] Furthermore, a small 1970s overfeeding study found that after normal-weight men gained 15–25% of their body weight, the size of their adipocytes from three subcutaneous regions increased, but their adipocyte number remained unchanged.[61]

While the notion that ‘adipocytes are formed in childhood and remain for life’ prevailed, researchers discovered that adults with severe obesity—even when acquired in adulthood—had hyperplastic adipose tissue.[60] This finding provided a glimpse of possibility that adipocytes can form in adulthood. Emerging evidence from *in vitro* experiments further supported this possibility: adipocyte precursor cells from adult humans with and without obesity could differentiate into mature adipocytes.[62–65] Shortly thereafter, *in vitro* experiments also demonstrated that human adipocytes could undergo apoptosis.[66]

In 2008, a landmark carbon dating study officially altered our view of the paradigm that ‘adipocytes are formed in childhood and remain for life’. Spalding et al. discovered that, in adulthood, subcutaneous abdominal adipocytes do indeed turn over—roughly every 10 years.[67] In other words, adipocytes *can* form in adulthood, but they die at the same rate, keeping adipocyte number constant. Accordingly, this study still suggested that adipocyte number is set toward the end of adolescence—at least in their sample of lean adults and adults with childhood-onset obesity. A caveat of this study, however, is that it could not determine how weight gain in adulthood modifies adipocyte turnover. Adipocyte renewal aside, can people with adult-onset obesity experience a net increase in adipocyte number?

Subsequent overfeeding[68] and longitudinal studies[69] confirmed that lean adults can gain fat through both adipocyte hypertrophy *and* hyperplasia. These findings ignited Arner et al.[70] to re-evaluate the earlier work that paralleled childhood-onset obesity with hyperplastic obesity and adult-onset obesity with hypertrophic obesity.[56–60] If adults can increase their number of adipocytes, then why would adipose tissue cellularity differ between people with childhood-onset and adult-onset obesity? A major drawback of earlier studies was their inability to delineate whether cellularity varies by adipose tissue region. After measuring adipocyte size in

one or more adipose regions that often differed among participants, they computed the total number of adipocytes in the body as total fat mass divided by average adipocyte size. This approach assumes little variability in adipose tissue cellularity throughout the body.

Overcoming this limitation, Arner et al. used abdominal subcutaneous adipocyte size and DXA-estimated android SAT mass to specifically examine cellularity in abdominal SAT.[70] Of note, in their study, people with childhood-onset (≤18 years) overweight or obesity were younger yet gained more weight in adulthood than their group with adult-onset (>18 years) overweight or obesity. Accordingly, their childhood-onset group had more android SAT than their adult-onset group, a difference attributed to both greater adipocyte size and number. However, the abdominal SAT morphology index, indicating the degree of hypertrophy or hyperplasia independent of adipose tissue mass,[69] was similar between the two groups.[70]

Correspondingly, our group found a similar mean subcutaneous abdominal adipocyte size among younger (<40 years old) and older (>55 years old) female bariatric surgery patients with a childhood (<18 years old) or adult (>18 years old) onset of obesity matched for BMI and type 2 diabetes status.[71] However, we found that mean visceral adipocyte size was smallest in the younger female group with childhood-onset obesity.[71] Although we were unable to obtain data on regional fat mass and, consequently, adipocyte number, our findings suggest that cellularity may vary with obesity onset across different adipose tissue regions.

Recently, we confirmed this variation in young, healthy adults with mild to moderate obesity. We found that, compared to their counterparts with adult-onset (>18 years old) obesity, only females with childhood-onset (pre-/peri-puberty) obesity had hyperplastic abdominal SAT. In contrast, both sexes with childhood-onset obesity had hypertrophic femoral SAT (Figure 1 and Table S1).[72] These findings challenge the simplistic narrative that childhood-onset obesity is

uniformly hyperplastic and that adult-onset obesity is uniformly hypertrophic. Longitudinal studies are required to determine the evolution of regional adipose tissue cellularity in childhood-onset versus adult-onset obesity.

Preadipocyte (dys)function

Even if people with childhood-onset and adult-onset obesity end up with similar adipose tissue cellularity, their adipose tissue function may differ. Healthy subcutaneous adipose tissue is largely defined by its ability to expand and store lipids—but the mechanism matters. Adipocyte hyperplasia, recognized as a more metabolically favourable expansion mechanism than adipocyte hypertrophy[73], relies on the capacity to generate new adipocytes through adipogenesis. During adipogenesis, adipocyte progenitor cells (APC) commit to the adipocyte lineage as preadipocytes, proliferate, and then differentiate into adipocytes. Abundant and functional preadipocytes are, therefore, essential to healthy adipose tissue expansion.

In children with obesity, APC abundance is not diminished,[55,74] nor is adipogenic potential.[55] In fact, compared to lean children, children with obesity have enhanced proliferation capacity and similar differentiation capacity (Figure 1).[55] But could their increased preadipocyte proliferation in childhood hinder their capacity for healthy adipose tissue expansion in adulthood?

Like all proliferative cells, preadipocytes cannot proliferate forever. Every time a cell divides, its telomeres, the protective caps at the ends of chromosomes, shorten. Critically short or damaged telomeres can trigger the DNA damage response and lead to cellular senescence, a state of permanent cell cycle arrest. Senescent preadipocytes can no longer proliferate or differentiate—nor do they die. They persist in adipose tissue and develop a senescence-associated secretory phenotype (SASP) that can damage neighbouring cells. While cellular

senescence increases with aging and obesity, it is plausible that rapidly proliferating preadipocytes in children with obesity senesce prematurely.

Results from our lab support the idea that, regardless of mechanism, preadipocytes begin the senescence program early in childhood-onset obesity. We found that, compared to females with adult-onset obesity, those with childhood-onset obesity have more preadipocytes with DNA damage and p53/p21 senescence markers in abdominal and femoral subcutaneous adipose tissue (SAT) (Figure 1 and Table S1). This finding occurred despite similar levels of RAD51, a DNA repair marker, in preadipocytes from both obesity-onset groups. In people with childhood-onset obesity, senescent preadipocytes may impair adipogenesis, leaving SAT hypertrophy as the likely expansion mechanism when faced with a positive energy balance.[75] People with adult-onset obesity, on the other hand, may preserve their adipogenic potential, prolonging capacity for hyperplastic SAT expansion. Future studies comparing the adipogenic potential of people with childhood-onset and adult-onset obesity are needed to confirm or refute this hypothesis.

Adipose tissue fibrosis, immune cells, and senescence

During hypertrophic expansion, adipose tissue undergoes structural and functional alterations that drive its pathogenicity over time. As adipocytes enlarge, new blood vessels must form to meet the tissue's oxygen demand. Additionally, the tissue's scaffolding, the extracellular matrix (ECM), must remodel to accommodate larger, lipid-laden adipocytes. In theory, if these adaptive processes were uninhibited, then adipocytes could enlarge indefinitely, and lipids would not 'spill over' and accumulate in ectopic sites. But this is not the case. In time, enlarging adipocytes outstrip their blood supply and uncontrolled hypoxia ensues.[76,77] Hypoxia triggers fibrosis, the excessive deposition of ECM proteins, namely collagens.[78–80] Consequently, the ECM morphs from flexible to rigid and restricts further adipocyte enlargement. Though the

sequence of events is unclear, fibrosis often coincides with immune cell infiltration, adipocyte death, and cellular senescence.[81,82] Together, these features create a proinflammatory milieu and wreak havoc on local and systemic metabolism. What's more, some of these features can appear in adipose tissue as early as childhood, but they may not always assume a villainous role.

Sbarbati et al. were the first to show evidence of fibrosis and immune cell infiltration in abdominal SAT from children with obesity.[83] Using electron microscopy, they observed what they called an 'elementary inflammatory lesion': adipocyte debris with macrophages, a few granulocytes and lymphocytes, and mild fibrosis.[83] Children with severe obesity had the largest SAT inflammatory lesions and children without obesity had none.[83]

Landgraf et al. confirmed an increased macrophage presence in SAT sampled primarily from the lower body of children with obesity. Compared to lean children, those with obesity had double the number of $CD68^+$ macrophages (20 vs. 10) per 100 adipocytes.[55] Additionally, 43% of children with obesity and only 9% of lean children had crown-like structures, where macrophages surround a dead or dying adipocyte.[55] Because CD68 is a general macrophage marker, it does not capture the different macrophage phenotypes, broadly classified as M1-like or proinflammatory and M2-like or anti-inflammatory.

Tam et al. quantified total and M2-like abdominal SAT macrophages in children with and without overweight or obesity. Using HAM56 instead of CD68 as a general macrophage marker, they found a slightly higher number of macrophages per 100 adipocytes in children with overweight or obesity (8 vs. 4) and detected crown-like structures in only one out of the 19 children with overweight or obesity.[84] Unlike Landgraf et al., Tam et al. did not include children with severe obesity, which, combined with the use of a different macrophage marker, may explain the dampened effect of obesity in their study.

As Tam et al. found no difference in the number of M2-like CD206 macrophages between lean children and children with obesity, the increased SAT total macrophage burden in children with obesity likely stems from M1-like macrophages (Figure 1).[84] Mujkić et al., using CD163 as another M2-like macrophage marker, corroborated this finding for abdominal SAT.[85] However, they found more M2-like macrophages in the VAT of children with overweight or obesity.[85] Crown-like structures, though rare, were more common in both the abdominal SAT and VAT of children with overweight or obesity.[85]

It is important to note that the M1/ M2 macrophage classification is oversimplistic. Macrophages can switch between phenotypes and can even exhibit both states simultaneously.[86] Therefore, how macrophages influence adipose tissue inflammation is complex. Furthermore, other immune cells loom in adipose tissue, some of which Tam et al. examined in children. They identified mast cells, very few T cells, and no neutrophils in abdominal SAT, regardless of the children's adiposity status (Figure 1).[84]

Though adipose tissue fibrosis tends to accompany immune cell infiltration as a maladaptive response to obesity in adults,[87] the story may differ in children. Using Masson's trichrome staining, Mujkić et al. found that, compared to lean children, children with overweight or obesity had a similar amount of collagen in VAT and slightly more collagen in abdominal SAT.[85] Using picrosirius staining, the preferred method for collagen quantification, Tam et al. found the opposite for abdominal SAT.[84] Not only did they find less collagen with increasing adiposity (Figure 1), but also with increasing age; the oldest children with the greatest adiposity had the least abdominal SAT collagen.[84] This finding, the authors proposed, may reflect normal remodelling to accommodate increased adipocyte size with growth.[84] Consistent with this idea, they found minimal pericellular fibrosis (Figure 1), the collagen surrounding adipocytes, in both

lean children and children with overweight and obesity.[84] Pericellular fibrosis is more closely linked to adipose tissue dysfunction and metabolic abnormalities than total collagen,[88] especially when rich in collagen IV.[89,90] Irrespective of their adiposity status, the children in Tam et al.'s study had virtually no collagen IV in their abdominal SAT.

Only one study from our lab has compared adipose tissue macrophages and fibrosis between people with childhood-onset and adult-onset obesity. We showed that the number of total, M1-like ($CD11c^+$), and M2-like ($CD163^+$) macrophages per 100 adipocytes in both visceral adipose tissue and abdominal SAT did not differ between younger (< 40 years old) and older (> 55 years old) female bariatric surgery patients with childhood-onset (< 18 years old) or adult-onset (> 18 years old) obesity.[71] We also found no effect of obesity onset on pericellular fibrosis in abdominal SAT and VAT (Figure 1).[71]

Our results, combined with those in children, suggest that while ATM infiltration has a head start in people with childhood-onset obesity, it can catch up in people with adult-onset obesity. Additionally, pericellular fibrosis appears to accumulate predominantly in adulthood, regardless of the age of obesity onset. However, since we studied bariatric surgery patients with severe obesity, we cannot comment on ATM infiltration and fibrosis in the early stages of obesity development in adulthood. To clarify this aspect, studies on people with milder forms of childhood-onset and adult-onset obesity are necessary.

Cellular senescence is another feature of adipose tissue dysfunction contributing to chronic, low-grade inflammation. Besides preadipocytes discussed above, other cells in adipose tissue, including macrophages, endothelial cells, and even adipocytes themselves, can senesce. β-galactosidase (gal) staining in adipose tissue indicates the total number of cells, regardless of type, that have committed to senescence. Interestingly, although females with childhood-onset

obesity had more preadipocytes with p53/p21 senescence markers in abdominal and femoral SAT than those with adult-onset obesity, the groups had a similar percentage of β-gal-positive cells in both SAT regions.[91] Further studies should examine which cells preferentially commit to senescence, and the downstream consequences, in people with different ages of obesity onset.

Adipokine production

Does the ensuing adipose tissue inflammatory environment, shaped by hypertrophic adipocytes, immune cells, and senescent cells, differ between people with childhood-onset and adult-onset obesity? At present, we can only address this question based on adipokine gene expression in adipose tissue. As expected, children with obesity exhibit higher leptin gene expression in SAT and VAT than lean children. In contrast, adiponectin, TNF-α, and resistin gene expression in SAT and VAT, as well as IL-6 gene expression in SAT, are comparable between these two groups of children.[55,92,93] However, our results suggest that differences in adipokine gene expression patterns emerge when these children become adults with obesity. We found that, compared to adult females with childhood-onset obesity, those with adult-onset obesity have increased IL-6 and leptin gene expression but similar TNF-α, resistin, and adiponectin gene expression in both abdominal and femoral SAT (Figure 1 and Table S1).[3] These findings, drawn from select adipokines—out of the hundreds discovered to date—indicate a more proinflammatory SAT environment in those with adult-onset obesity. Future investigations should directly assess how adipokine production in SAT and VAT explants differs between people with childhood-onset and adult-onset obesity.

Lipid handling

Beyond adipokine production, adipose tissue assumes a crucial role in lipid handling and energy homeostasis. Operating as a dynamic hub, adipose tissue responds to energy needs by

releasing (lipolysis) or storing lipids (lipogenesis). Lipolysis is primarily stimulated by catecholamines and suppressed by insulin. An *in vitro* study showed that, compared to lean children, children with obesity have lower basal (unstimulated) lipolysis per adipocyte but similar catecholamine (isoproterenol)-stimulated lipolysis per adipocyte in SAT.[55] An *in vivo* study arrived at a similar conclusion for basal lipolysis normalized for fat mass. However, this study found blunted catecholamine (epinephrine)-stimulated lipolysis in children with obesity (Figure 1).[94] A subsequent *in situ* microdialysis study found that this catecholamine resistance may be due to impaired β2-adrenergic stimulation of lipolysis.[95]

Blunted insulin-mediated suppression of lipolysis largely defines adipose tissue insulin resistance and can be quantified with a multi-step pancreatic clamp or a one-step hyperinsulinemic-euglycemic clamp with a palmitate tracer.[96] However, studies comparing adipose tissue insulin resistance between children with and without obesity have relied on surrogate measures calculated from insulin and free fatty acid concentrations. Using this approach, Reinehr et al. found greater fasting adipose tissue insulin resistance in children with obesity than in those without obesity.[97] Moreover, Hagman et al. found that both fasting and post-prandial adipose tissue insulin resistance indices increased with greater obesity severity in children.[98] Insulin also stimulates lipogenesis, which, to our knowledge, has not been compared between lean children and children with obesity.

Although the measurement technique and data normalization method can influence results,[99,100] contrary to findings in children, obesity in adults generally increases basal lipolysis and decreases catecholamine-stimulated lipolysis in SAT.[101] Obesity in adults also impairs insulin-mediated suppression of lipolysis.[101] While adipose tissue insulin resistance has not been compared between people with childhood-onset and adult-onset obesity, Arner et al. found no

effect of age of obesity onset on *in vitro* basal and catecholamine-stimulated lipolysis in abdominal SAT (Figure 1 and Table S1).[70] Catecholamine-stimulated lipolysis is closely linked to long-term lipid removal in abdominal SAT,[102] which Arner et al. also found was not different between people with an obesity onset before or after age 18 (Table S1).[103]

Our research suggests that lipogenic capacity, on the other hand, may be heightened in people who develop obesity in adulthood. We found that, compared to females with childhood-onset obesity, those with adult-onset obesity have elevated levels of acetyl-CoA in abdominal and femoral SAT.[3] Since the participants were fasting, the acetyl-CoA was likely nucleocytosolic because mitochondrial acetyl-CoA would be consumed in the tricarboxylic acid cycle.[104] Cytosolic acetyl-CoA is critical for de novo lipogenesis (DNL). Correspondingly, we also found that females with adult-onset obesity had increased expression of genes regulating cytosolic acetyl-CoA and DNL in their abdominal and femoral SAT (Figure 1 and Table S1). Adipocyte mitochondria play a central role in energy homeostasis, regulating both lipolysis and lipogenesis.[105] Compared to lean children, children with obesity have compromised mitochondrial function and structure in abdominal SAT (Figure 1).[106] Furthermore, results from our lab suggest that when obesity persists from childhood to adulthood, dysfunctional SAT mitochondria do too. We found that, compared to females with adult-onset obesity, those with childhood-onset obesity have a lower abundance of mitochondrial complex II and IV in abdominal and femoral SAT, despite a higher NAD^+/NADH ratio (Figure 1 and Table S1).[3] Of note, dysfunctional mitochondria contribute to oxidative stress which can drive other aspects of adipose tissue dysfunction, including cellular senescence. It remains to be seen how mitochondrial dysfunction in adipose tissue influences lipid handling and the progression of

metabolic abnormalities like insulin resistance in people with childhood-onset and adult-onset obesity.

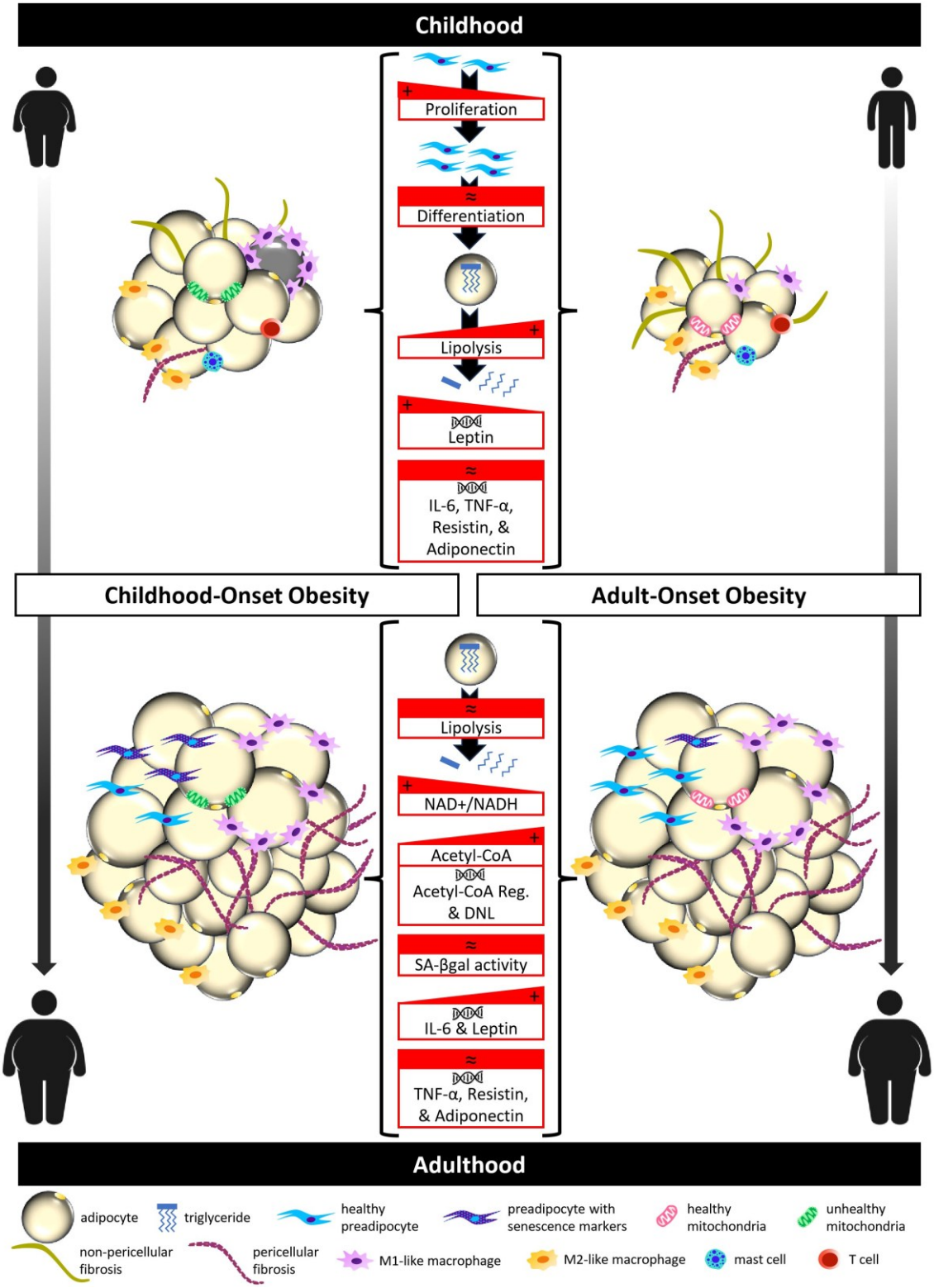

Figure 1. Subcutaneous adipose tissue biology in people with childhood-onset versus adult-onset obesity

The four subcutaneous adipose tissue (SAT) diagrams show changes in structural and cellular characteristics from childhood (*top*) to adulthood (*bottom*) in people with childhood-onset and adult-onset obesity.

Compared to lean children (*top right*), children with obesity (*top left*) have larger, more numerous adipocytes and less non-pericellular fibrosis. Children with obesity also have impaired mitochondrial integrity and more M1-like macrophages and crown-like structures, but a similar number of M2-like macrophages compared to their lean counterparts. Both lean children and children with obesity have minimal mast cells and T cells in their SAT.

As these children transition to adulthood, their adipose tissue can expand by increasing adipocyte size (*top half of adult adipose tissue)* and/or number size (*bottom half of adult adipose tissue*). Both sex and region of the body seem to affect how SAT preferentially expands in adulthood (*Table S1*).

Most studies comparing adipose tissue characteristics between adults with childhood-onset and adult-onset obesity have focused on females with mild to moderate obesity. Compared to females with adult-onset obesity, those with childhood-onset obesity have impaired mitochondrial integrity and more preadipocytes with senescence markers in abdominal and femoral SAT.

Females with severe childhood-onset and adult-onset obesity have a similar M1-like and M2-like macrophage content and degree of pericellular fibrosis in abdominal SAT.

The middle panel illustrates the current evidence on SAT functional characteristics and gene expression patterns. A triangle (+) pointing to the right indicates a higher rate or level in people with childhood-onset obesity than those with adult-onset obesity (and vice versa); a rectangle (≈) indicates no difference in rate or level between obesity onset groups.

Table S1 summarizes the differences in adipose tissue biology between adults with childhood-onset and adult-onset obesity by sex, obesity severity, and adipose tissue region.

Acetyl-CoA reg.= acetyl-coenzyme A regulation; DNL = de novo lipogenesis; IL-6 = interleukin-6 NAD^+/NADH = nicotinamide adenine dinucleotide/the reduced form of NAD^+; TNF-α = tumour-necrosis factor-α

Adipose tissue distribution

Where people store body fat influences their cardiometabolic health. People who accumulate fat centrally (android- or apple-shaped), especially in VAT, have increased cardiometabolic risk compared to people who preferentially store fat in lower body SAT (gynoid- or pear-shaped).[107–111] VAT's pathogenicity stems from both its location and activity. VAT is more lipolytic and proinflammatory than SAT, and because VAT drains into the portal circulation, it exposes the liver to high levels of free fatty acids[112] and proinflammatory cytokines.[113] The latter is most convincingly linked to insulin resistance and metabolic derangements.[113]

In the 1960s, Albrink et al. hypothesized that adipose tissue is peripherally distributed in childhood-onset obesity and centrally distributed in adult-onset obesity.[1] Today, several factors are well-known to affect adipose tissue distribution, including genetics,[114] sex,[115] and age,[116] but does the age of obesity onset belong on this list? Cross-sectional and longitudinal studies of adipose tissue distribution in children and adults with and without obesity suggest that Albrink's hypothesis may, in part, be true. SAT deposition predominates in childhood and adolescence, both with increasing age[117–119] and adiposity.[120–122] Still, compared to lean children, those with obesity have more adipose tissue in all regions of the body.[121] This difference, however, is most striking for abdominal SAT.[121] In other words, in children with obesity, adipose tissue distribution centralizes—subcutaneously.

After puberty, sex differences in adipose tissue distribution become most apparent.[118,123,124] Adult males have more VAT,[118,123] while adult females have more SAT,[118,123] predominately distributed in the lower body.[123] In both sexes, SAT and VAT tend to increase with age but at variable rates.[118,123,125] Throughout adulthood in males and after around age 30 in

females, SAT accumulates less rapidly than VAT, decreasing the proportion of SAT relative to VAT.[118,123] This proportional decline in SAT with age occurs ubiquitously in males, while only in the lower body in females.[123] Females tend to experience the greatest increase in VAT around menopause.[118,123,126] Accordingly, the adipose tissue distribution of post-menopausal females is closer to that of males.

Adipose tissue distribution, nevertheless, can vary widely among people across the lifespan. Even among adolescents with obesity, a subset preferentially stores abdominal adipose tissue viscerally.[127] Compared to adolescents with obesity who preferentially store abdominal adipose tissue subcutaneously, these adolescents have abdominal SAT with larger adipocytes, elevated inflammatory markers, and decreased expression of genes related to adipogenesis, lipogenesis, and insulin sensitivity,[127,128] supporting the view that VAT accumulation signifies SAT dysfunction.[129–132] These adolescents also have worse insulin resistance and more liver fat,[127] which may increase their risk of developing cardiometabolic disease before adulthood.

From the above findings, one would expect that at the same adiposity, healthy people with different ages of obesity onset would, on average, have differently sized adipose tissue depots. Males and females with childhood-onset obesity would have greater abdominal SAT; females with premenopausal-onset obesity, greater lower-body SAT; and males with adult-onset obesity and females with postmenopausal-onset obesity, greater VAT.

The limited studies that have directly compared adipose tissue distribution between people with childhood-onset and adult-onset obesity, however, tell a different story. Using DXA and computed tomography, we found similar quantities of total, gynoid, trunk, and leg fat and android SAT and VAT in a small sample of healthy, premenopausal females with childhood-onset and adult-onset obesity (BMI $\geq$ 30 and $<$ 40 kg/m^2).[91] Arner et al. corroborated these

results for DXA-estimated android visceral fat, but found greater total, gynoid, and android fat in females with childhood-onset overweight than in those with adult-onset overweight (BMI > 25 kg/m^2).[70] Furthermore, Arner et al. found that compared to males with adult-onset overweight, those with childhood-onset overweight had greater quantities of fat in all regions measured.[70] The greater total adiposity and lower age and diabetes prevalence in their childhood-onset groups, nevertheless, muddies interpretation.

Interestingly, studies in the clinical setting with no upper BMI limit routinely find that people with childhood-onset obesity have greater adiposity than those with adult-onset obesity.[133–135] Weight gain does not usually stop once children with obesity become adults; it typically continues throughout adulthood, increasing the risk of severe obesity.[136,137] Therefore, adipose tissue accumulation in adulthood may largely shape adipose tissue distribution in both people with childhood-onset and adult-onset obesity. This idea is supported by a mendelian randomization study that isolated the effects of childhood and adult body size on adult abdominal adipose tissue distribution. Whereas adult body size was independently associated with both abdominal SAT and VAT in adulthood, childhood body size was not associated with adult abdominal SAT and was negatively associated with adult abdominal VAT.[138] Longitudinal studies are needed to understand how adipose tissue distribution changes from the age of obesity onset onward.

Cardiometabolic Risk

How does cardiometabolic disease evolve in people with different ages of obesity onset? Does their unique adipose tissue biology brew unique cardiometabolic risk profiles, and whose risk profile is more likely to lead to overt cardiometabolic disease?

Many children with obesity already bear the burden of cardiometabolic risk factors such as insulin resistance[139] and systemic, low-grade inflammation.[140] The link between childhood obesity and adult cardiometabolic diseases, however, largely stems from the strong tracking of obesity from childhood to adulthood.[141] Mendelian randomization has confirmed that childhood adiposity is not causally related to adult type 2 diabetes and coronary heart disease independently of adult adiposity.[142] What is not clear, though, is whether the pathogenesis of cardiometabolic diseases in people with adult-onset obesity can begin later but progress faster than in people with childhood-onset obesity. For insulin resistance, this may be the case.

Two cross-sectional studies examined the effect of self-reported age of obesity onset on whole-body insulin resistance using the gold standard clamp technique. Muscelli et al. found that after adjusting for age, sex, and BMI, insulin resistance worsened with a later onset of obesity.[143] Similarly, in post-menopausal females, Brochu et al. found that, second to VAT mass, an adult-onset of obesity was the best predictor of insulin resistance.[144]

Are people with childhood-onset obesity, then, less likely to develop cardiometabolic disease? According to three cross-sectional studies of bariatric surgery candidates, the answer is uncertain. In two of these studies, the participants with childhood-onset obesity were over ten years younger than those with adult-onset obesity (age ~ mid-30s vs. ~ late-40s).[133,135] It may seem perplexing, then, why the prevalences of type 2 diabetes, hypertension, and dyslipidemia were lower in participants with childhood-onset obesity in one study[133] and not the other.[135] The only discernible difference between these two studies was the average age of obesity onset in the childhood-onset group: 7 years in the study reporting lower disease prevalence in the childhood-onset group,[133] and 12 years in the study reporting similar disease prevalences between obesity-onset groups.[135] This raises the question of whether a peri-puberty onset of obesity is more

detrimental to metabolic health than a pre-puberty onset of obesity. In the only study to consider childhood-onset (0–11 years), adolescent-onset (12–20 years), and adult-onset (>20 years) obesity, Borgeraas et al. refuted this hypothesis.[145] After adjusting for age and BMI, they found that, compared to adult-onset obesity, adolescent-onset obesity did not increase the odds of type 2 diabetes, coronary heart disease, hypertension, or dyslipidemia;[145] conversely, childhood-onset obesity increased the odds of coronary heart disease by 82% in men and the odds of type 2 diabetes by 25% in women.[145]

Population-based cohort studies tracking adiposity changes and cardiometabolic risk over the life course could provide valuable datasets to clarify the above findings. Unfortunately, many fall short. Using BMI cut points, most of these studies assessed adiposity status once in childhood or adolescence and once in adulthood, time points that spanned up to two decades. Therefore, people with obesity at both time points would have childhood- or adolescent-onset obesity, but their adiposity status could have fluctuated between time points. Furthermore, people with obesity only at the adult time point could, in theory, have childhood-, adolescent-, or adult-onset obesity. At best, these studies allow for comparisons between people with an 'early' versus 'later' onset of obesity without pinpointing the exact age or life stage of onset.

Another shortcoming is that several cohort studies categorized adiposity status as with or without overweight/obesity at one or both time points, making it impossible to isolate the effect of early- versus later-onset obesity. In these studies, cardiometabolic risk factors seldom differed between people with early-onset and people with later-onset overweight/obesity. Blood CRP concentration did not differ between groups,[146] nor did carotid artery intima media thickness (cIMT)[147,148] or pulse wave velocity (PWV),[149] markers of atherosclerosis and arterial stiffness, respectively. The odds or risk of high-risk cIMT[150,151] or PWV,[151] indicators of subclinical

cardiovascular disease, were also similar between people with early-onset and people with later-onset overweight/obesity. When early- versus later-onset obesity was examined in the Bogalusa Heart Study, however, the cIMT narrative changed. People with obesity at both the childhood/adolescent and adult time points had greater cIMT than those with obesity only at the adult time point.[152]

How studies categorized adiposity status in adulthood also appeared to influence findings for dyslipidemia and hypertension (Tables S2 and S3). In adults with overweight/obesity, childhood overweight/obesity status did not affect the odds of high LDL, HDL, or triglycerides,[153] nor the risk of hypertension.[154] In adults with obesity, this trend held for high HDL and triglycerides,[150] but not always for high LDL and hypertension. In a pooled analysis of two US cohorts, one Australian cohort, and one Finnish cohort, early-onset obesity increased the risk of hypertension in males and high LDL-cholesterol in females compared to later-onset obesity.[150] Moreover, in the National Longitudinal Study of Adolescent to Adult (ADD) Health study, adults with early-onset obesity had greater odds of hypertension than those with later-onset obesity.[155]

In contrast, cohorts from various countries found no difference in the odds of NAFLD[156,157] and MACE[158] and the rate of cardiovascular mortality[159,160] between people with early-onset and later-onset obesity (Tables S2 and S3).

Among the cohort studies assessing adiposity status once in childhood/adolescence and once in adulthood, most found no difference in the odds,[161] risk[150], or hazard rate[162] of type 2 diabetes between people with early- and later-onset overweight or obesity (Table S2). The et al., however, reported a more than 2-fold greater odds of type 2 diabetes in males and females with early-onset obesity compared to those with later-onset obesity.[163] What distinguishes The et al.'s

study from the others is the timing of their childhood/adolescent assessment. The et al. first assessed participants in mid- to late-adolescence (12-17 years), while the other studies included young children. As such, The et al.'s later onset group likely had more participants with adult-onset obesity. Furthermore, compared to childhood obesity, adolescent obesity is more likely to persist into adulthood.[164,165] The et al.'s findings, therefore, may also stem from a greater likelihood of obesity persisting rather than fluctuating between time points in their early-onset group.

Cohort studies that assessed adiposity status twice during childhood/adolescence and once in adulthood confirm that when children with overweight/obesity become normal-weight in adolescence before developing overweight/obesity again by adulthood, their type 2 diabetes odds[166,167] or hazard rate[168] mirror those of people with overweight/obesity only at the adult time point (Tables S2 and S3). Results from 3 pooled British cohorts suggest this pattern holds for coronary heart disease and hypertension[167] (Table S3).

An unresolved question is whether cardiometabolic risk depends on the persistence of overweight/obesity from a specific period in childhood or adolescence. In the BMI Epidemiology Study in Gothenburg, Sweden, there were no differences in the hazard rates of type 2 diabetes,[169] heart failure,[170] stroke,[171] acute coronary events,[172] or cardiovascular mortality[173] between people who developed overweight/obesity before age 8 and people who developed overweight/obesity between age 8 and 20 (Table S2). Findings from Danish, US, and British cohorts support this conclusion for type 2 diabetes. People with persistent obesity, whether from childhood or adolescence, had similar odds,[174] risk,[166] or hazard rates[168] of type 2 diabetes, which were greater than those with adult-onset obesity. An exception was a pooled analysis of three birth cohorts that only detected a difference in type 2 diabetes odds between

those with childhood-onset and adult-onset obesity.[167] In this study, the odds of coronary heart disease also tended to be higher in those with childhood-onset than adult-onset obesity, but the odds of hypertension were unaffected by the age of obesity onset.[167]

The above cohort studies leave us wondering whether the magnitude of changes in adiposity between life stages and the adiposity attained in adulthood plays a role in the link between the age of obesity onset and cardiometabolic risk. When reported, adult BMI tended to increase with an earlier onset of overweight/obesity,[154,167,168,174] yet this was only accounted for in one study.[174]

Attard et al.'s unique approach to analyzing the ADD Health data helped solve these unknowns (Table S3).[175] They derived nine obesity trajectories well-represented in the ADD Health cohort based on BMI at age 15, 20, and 27. Four of these trajectories culminated in mild obesity (BMI ≈ 30 kg/m^2 at age 27), and five culminated in moderate obesity (BMI ≈ 37 kg/m^2 at age 27). Of the mild obesity trajectories, one had an obesity onset before age 15 years (childhood/early adolescent-onset), one between age 15 and 20 years (late adolescent-onset), and two between age 20 and 27 years (adult-onset). The adult-onset trajectories differed in their BMI changes (≈ 1, 4, or 8 kg/m^2) from age 15 to 20 and age 20 to 27. One had a medium BMI increase (≈ 4 kg/m^2) between both age intervals, and the other had a small BMI increase (≈ 1 kg/m^2) between age 15 and 20 and a large BMI increase (≈8 kg/m^2) between age 20 and 27.[175]

All five moderate obesity trajectories had childhood- or adolescent-onset obesity. Four developed obesity by age 15, and one, between ages 15 and 20. One trajectory maintained stable, moderate obesity from age 15 onwards. Three trajectories had mild obesity at age 15 but differed in the magnitude of their BMI changes between age intervals. The trajectory that started with normal weight at age 15 had large increases in BMI between both age intervals.[175]

Attard et al. compared the odds of systemic inflammation (CRP ≥ 3 mg/L), hypertension, and diabetes among the mild obesity trajectories and among the moderate obesity trajectories, by sex.[175] The odds of all outcomes were higher in men and women with severe obesity than those with mild obesity, most strikingly, for systemic inflammation. In women with both mild and moderate obesity, those with the largest BMI increase between age 20 and 27 had the greatest odds of systemic inflammation compared to their respective trajectory groups. For women with mild obesity, those with the highest odds, therefore, had adult-onset obesity. In contrast, for men there was little variability in the odds of systemic inflammation among the mild trajectory groups and among the severe trajectory groups.[175]

The age of obesity onset, rather than the magnitude of BMI change, determined the odds of diabetes for both men and women in the mild obesity trajectories. Compared to people with adult-onset obesity, people with childhood/early adolescent-onset obesity and people with late adolescent-onset obesity had similarly increased odds of diabetes. Likewise, people who acquired moderate obesity before age 15 or between age 15 and 20, tended to have greater odds of diabetes than people who developed moderate obesity after age 20. Conversely, the odds of hypertension varied little among both the mild and moderate obesity trajectories for men and women.[175]

Norris et al. reinforced the importance of obesity severity in cardiometabolic risk.[176] They fitted annual BMI values between ages 10 and 40 for participants from three British birth cohorts, and compared the risk of hypertension, low HDL cholesterol, and diabetes between people with childhood-onset obesity (onset between ages 10 and 20) and four groups of people with adult-onset obesity (onset between ages 20 and 25, 25 and 30, 30 and 35, and 35 and 40) (Table S2). They reported a trend for increased risk of hypertension, low HDL cholesterol, and

diabetes with an earlier onset of obesity. However, when adjusting for average obesity severity, this relationship only persisted for diabetes.[176]

Building on the findings of Attard et al. and Norris et al., some cohort studies used group-based trajectory modelling or growth mixture modelling to identify distinct patterns of overweight/obesity development over the life course (Table S2). Several patterns emerged from these studies. While childhood-onset and adult-onset overweight/obesity usually developed progressively, a few studies identified a trajectory group with stable overweight/obesity from childhood onward. Correa-Burrows et al. found that early adulthood BMI, CRP, and HOMA-IR did not differ between those with progressive adolescent-onset obesity and those with stable, persistent obesity from early childhood.[177] In contrast, other studies suggest that progressive childhood-onset obesity may be more detrimental to cardiometabolic health than stable childhood-onset obesity. Norris et al. found an elevated early adulthood BMI, CRP, and PWV in those with progressive adolescent-onset obesity compared to those with stable childhood-onset obesity; however, they found no difference in cIMT between groups.[178] Furthermore, Fagherazzi et al. found that females with rapid-onset obesity around puberty had a greater hazard rate of type 2 diabetes than those with stable obesity from age 8.[179] These studies only followed participants into their early 20s, so they did not identify a trajectory group with adult-onset obesity.[177–179]

Some cohorts, likely reflecting their pre-obesity epidemic origins, identified a trajectory group with adult-onset overweight that did not escalate to obesity.[178,180–185] When this was the case and adiposity was not adjusted for, the trajectories with childhood-onset obesity had greater odds, risk, or hazard rate of high cIMT,[180] hypertension,[180,181] type 2 diabetes,[180–183] and sometimes dyslipidemia[181] and MACE.[184] In people whose overweight began around the

transition to adulthood (age 18–20) and progressed to obesity, the risk of high cIMT, hypertension, dyslipidemia, and type 2 diabetes did not differ from those whose overweight-to-obesity trajectories began in childhood or adolescence.[180] Though seldom identified, an overweight to obesity trajectory beginning after age 20—true adult-onset overweight/obesity—posed a lower risk of type 2 diabetes than an overweight/obesity onset before age 20.[182,183,186]

Figure 2 summarizes the evidence on cardiometabolic risk in people with childhood-or adolescent-onset obesity versus people with adult-onset obesity, assuming they are of similar age and adiposity. There is compelling evidence that type 2 diabetes risk is greater in people with childhood- or adolescent-onset obesity than in people with adult-onset obesity—even though cross-sectional studies suggest the opposite for insulin resistance. These opposing findings suggest that β-cell dysfunction, rather than insulin resistance, may be the dominant defect leading to type 2 diabetes in people with childhood-onset or adolescent-onset obesity. Aligning with this hypothesis, holding glycemia and insulin resistance constant, adolescence with obesity have hyperresponsive β-cells compared to adults with obesity.[187–189] Hyperresponsive, overworked β-cells could fail prematurely, accelerating the progression to type 2 diabetes.[190] Longitudinal studies are needed determine the impact of the age of obesity onset on the decline in β-cell function and insulin sensitivity in people who develop type 2 diabetes.

The evidence base for the association between the age of obesity onset and other cardiometabolic risk factors and diseases is limited (Figure 2). While the age of obesity onset does not appear to affect the risk of hypertension and low HDL cholesterol, its effect on cIMT, PWV, systemic inflammation, high LDL, high triglycerides, NAFLD, and MACE is inconclusive. Modern cohort studies comparing cardiometabolic risk between people with confirmed childhood-onset and adult-onset obesity are required to clarify these associations.

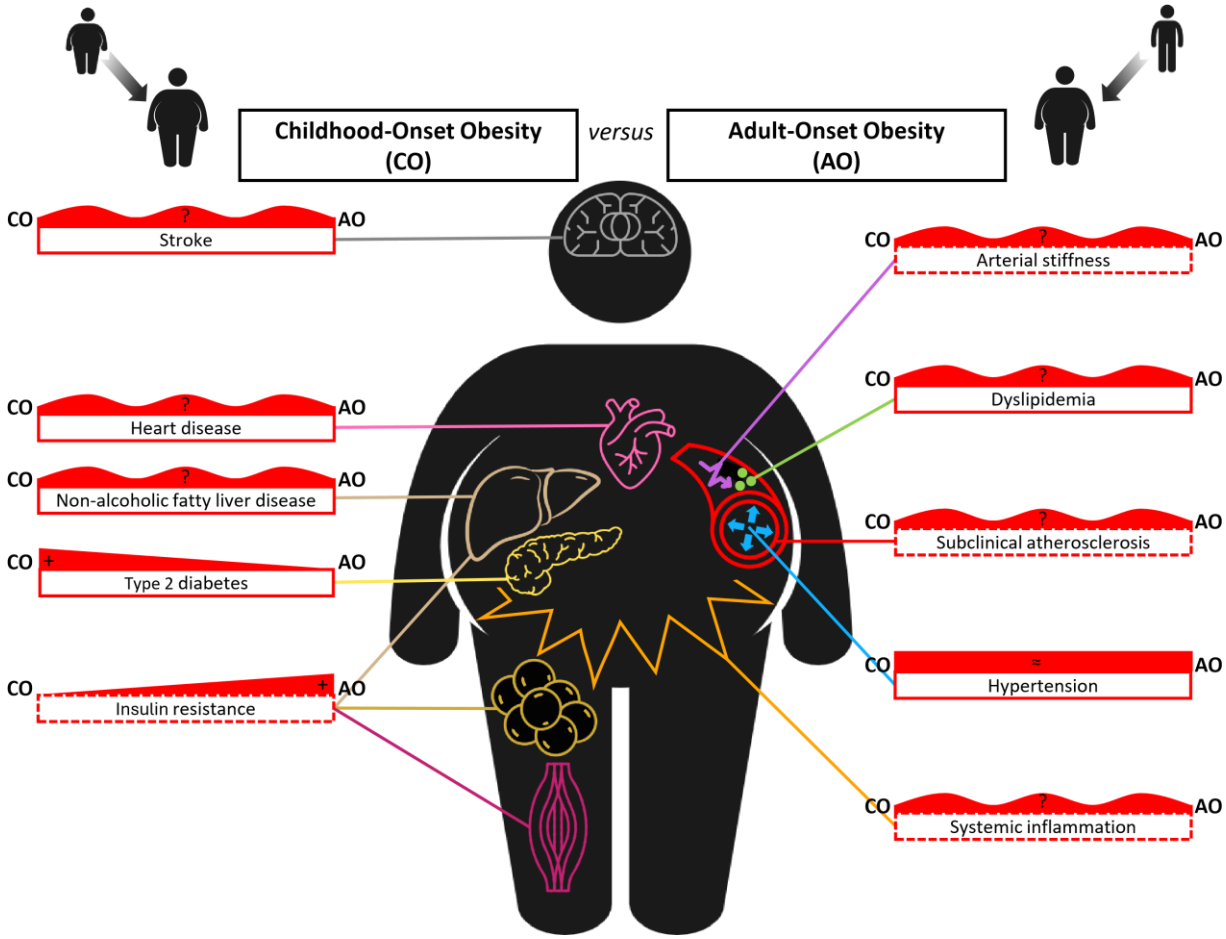

Figure 2. Cardiometabolic risk in people with childhood-onset versus adult-onset obesity

For each cardiometabolic risk factor (dashed outline) and disease (solid outline), a triangle (+) pointing to the right indicates a greater level or risk in people with childhood-onset obesity than in people with adult-onset obesity, and a triangle (+) pointing to the left indicates the opposite; a rectangle (≈) indicates no difference between obesity-onset groups; and a wavy shape (?) indicates that the difference between obesity onset-groups is inconclusive

Treatment outcomes: weight loss and beyond

Weight loss and weight loss maintenance after lifestyle and pharmacological interventions

Childhood-onset obesity has historically been viewed as a barrier to diet-induced weight loss. This notion arose from short-term weight loss studies in the 1950s[191,192] and was perpetuated by the discovery of how adipose tissue cellularity changes with weight loss: adipocyte size decreases while adipocyte number remains stable.[56,61] Since people with

childhood-onset obesity were presumed to have hyperplastic adipose tissue, their adipocytes could theoretically shrink to below-average size with ongoing weight loss[56] and, according to Grinker and Hirsch, acquire a 'starvation-like' state.[193]

The 1950s weight loss studies concluded that compared to adult-onset obesity, childhood-onset obesity was more difficult to treat with a hypocaloric diet (Table S4)[191,192]. But was their conclusion justified? Teasing out sources of weight loss variability (e.g., age of obesity onset) requires careful study design considerations from the energy deficit prescribed to the weight loss outcome examined. Since baseline energy requirements vary among individuals, best practice is to prescribe percent energy deficits and report percent weight loss. Neither Young et al.[191] nor Mullins[192] specified the prescribed energy intake or deficit, and both compared 'weight loss success' between obesity-onset groups. Young et al. deemed 50% of participants with childhood-onset obesity 'complete failures' but did not clearly define weight loss success[191]. Mullins set targets of less than 10% excess weight (defined by Kemsley[194]) for those with mild obesity and less than 20% excess weight for those with moderate or severe obesity.[192] Such weight loss targets would naturally penalize their childhood-onset group that had more people with severe obesity. Therefore, based on these early studies alone, we cannot affirm childhood-onset obesity more resistant to diet-induced weight loss than adult-onset obesity.

Thirteen studies with a similar aim followed (Table S4 and Stein et al.[195] and Bosello et al.[196]), and only three reported worse weight loss outcomes in people with childhood-onset obesity.[195,197] Drenick and Johnson[197] subjected participants to the most extreme intervention, a 2-month hospitalized fast followed by a very low-calorie diet. Their groups had similar excess weight at baseline, yet participants with an obesity onset before age 10 years were least likely to dip below 30% excess weight. However, these participants were also less likely to fast for the

full two months. Stein et al. aimed to identify predictors of weight loss success by conducting a stepwise regression. They included the age of obesity onset as a continuous variable ranging from infancy to 50 years old. Along with intervention length and excess weight at baseline, an older age of obesity onset predicted greater weight loss.[195] Similarly, Bosello et al. reported a moderate, positive correlation between age of obesity onset and percent weight loss after a 6-month, 1000 kcal/day diet.[196]

Three lifestyle intervention studies found slightly greater absolute[198,199] or percent[200] weight loss in people with childhood-onset obesity than people with adult-onset obesity (Table S4). Although the prescribed energy deficits were not specified, higher baseline adiposity in the childhood-onset group may explain this result in two[199,200] of the three studies.

After most lifestyle interventions, however, the age of obesity onset did not dictate weight loss success (Figure 3 and Table S4).[201–206,135,207] Whether the intervention was a very low-calorie formula diet or a multi- component behavioural weight loss program, people with childhood- and adult-onset obesity fared similarly. They both can lose weight, but as two recent studies showed, their success turns challenging over the long term.[135,205] Rupp et al. prescribed the only lifestyle intervention with a formal exercise component. Both their obesity-onset groups lost around 9 kg after 6 months but gradually gained a few kilograms over the next year.[205] Prado et al. tested the only lifestyle intervention combined with pharmacotherapy. They prescribed some medications approved for long-term weight management by health regulatory agencies (liraglutide, a glucagon-like peptide (GLP)-1 receptor agonist (RA); and orlistat, a gastric and pancreatic lipase inhibitor) but did not specify the extent of their use. On average, weight loss stabilized at a modest 2–3 kg after one year, with only 15% of participants losing more than 10% of their initial weight.[135] Semaglutide, a newer GLP-1 RA, and tirzepatide, a dual GLP-

1/glucose-dependent insulinotropic polypeptide (GIP) RA, have shown greater promise for weight loss,[208–211] but no studies have compared their efficacy between people with childhood- and adult-onset obesity. Nevertheless, we await the results of the RESETTLE randomized controlled trial testing the combined effect of lifestyle intervention and semaglutide on the change in BMI in young adults with childhood-onset obesity who had poor success with lifestyle intervention alone.[212]

Between-study differences in obesity-onset categorization and intervention characteristics, often poorly described, preclude us from drawing nuanced conclusions about the suitability of specific lifestyle interventions for people with childhood-onset versus adult-onset obesity. However, we can conclude that childhood-onset obesity is not resistant to diet-induced weight loss, as previously thought.

When studying people in free-living conditions, distinguishing between physiology-driven and adherence-driven sources of weight loss variability can be challenging. Ashwell et al. eliminated this challenge by conducting an inpatient study where they fed participants 800 kcal/day on average for 3 weeks.[213] Their results aligned with those of most outpatient studies. They found no relationship between the age of obesity onset as a continuous variable and short-term weight loss with or without adjusting for baseline adiposity.[213]

Weight loss (typically 5–10%) is only one goal of obesity treatment; maintaining weight loss and preventing weight regain are equally important. With ongoing weight loss, changes in physiology make dietary adherence difficult and favour weight regain.[214] In 1972, Grinker and Hirsch suggested that people with childhood-onset obesity (presumably hyperplastic obesity) fight a harder battle to maintain weight loss and prevent weight regain due their tiny, ‘starved’

post-weight loss adipocytes.[193] The low level of the appetite-supressing hormone leptin that cooccurs with abnormally small adipocytes after weight loss supports this hypothesis.[215]

Björntorp and colleagues, in a way, put Grinker and Hirsch's hypothesis to the test.[216,217] They sought to compare weight loss prognosis between females with hyperplastic and hypertrophic obesity. Participants consumed 1100 kcal/day as outpatients until weight loss plateaued. Both participants with hyperplastic and hypertrophic obesity hit a weight loss plateau when their average adipocyte size from three subcutaneous regions was similar to that of lean controls.[216] After their weight plateaued, the hyperplastic group maintained their weight for 15 weeks, while the hypertrophic group succeeded for 51 weeks.[217] Moreover, weight regain occurred at a rate three times faster in the hyperplastic group than in the hypertrophic group.[217] These findings remained when the groups were matched for total body fat.[217]

Bosello et al. compared both the age of obesity onset and pre-intervention gluteal adipose tissue cellularity among females who either continued to lose weight, maintained their reduced weight, or gained weight in the six months after a 6-month dietary intervention (1000 kcal/day).[196] The age of obesity onset was determined using both recalled body weight and photographic evidence when possible. The age of obesity onset correlated positively with adipocyte size and negatively with adipocyte number. The group that continued to lose weight and the group that maintained their reduced weight had an older average age of obesity onset, a larger adipocyte size, and a lower adipocyte number than the group that regained weight. Notably, the average age of obesity onset still fell in the childhood range for all three groups: 16 years for the weight losers, 13 years for the weight maintainers, and 7 years for the weight regainers.[196]

Taken together, people with childhood-onset obesity may be more susceptible to weight regain after weight loss—*if* childhood-onset obesity is synonymous with hyperplastic obesity.

Weight loss and weight loss maintenance after bariatric surgery

Six studies examined the association between age of obesity onset and weight loss outcomes after bariatric surgery (Table S4). However, most suffered from the same flaws as the lifestyle intervention studies, failing to report baseline demographic characteristics and adiposity in each group. One study reported similar weight loss in people with an obesity onset pre- or post-puberty 5 years after vertical banded gastroplasty,[218] a procedure seldom used today. Erdogdu et al. found that one year after laparoscopic sleeve gastrectomy, those with an obesity onset around puberty lost a greater percent of their excess weight than those with a pre- or post-puberty onset of obesity.[219] Another study reported that one year after Roux-en-Y gastric bypass surgery, people with childhood-onset or adolescent-onset obesity were less likely to lose at least 60% of their excess weight than those adult-onset obesity.[220]

Three bariatric surgery studies used adiposity status at the end of adolescence (18–20 years) to classify obesity-onset groups and reported slightly better weight loss outcomes in people with childhood/adolescent-onset obesity.[134,221,222] Only Kristensson et al.[134] compared percent total weight loss, a recommended weight loss outcome for bariatric surgery,[223–225] between patient groups. Bariatric surgery patients who developed obesity by age 20 experienced a slightly higher percent weight loss than those who developed obesity after age 20, but the difference was not clinically meaningful (Figure 3).[134] On average, participants attained a 25% weight loss one year after bariatric surgery, then gradually gained weight until stabilizing at a 15-20% weight loss 8–10 years post-surgery.[134] Based on this long-term study, the age at which obesity begins does not appear to influence weight-loss success following bariatric surgery.

Cardiometabolic outcomes after obesity treatment

Beyond weight management, obesity treatment aims to improve cardiometabolic health—but does it do so equally for people with childhood-onset and adult-onset obesity? No studies have addressed this question with lifestyle or pharmacological interventions. However, Kristensson et al. compared the cardiometabolic benefit of bariatric surgery between adults who had normal weight, overweight, or obesity at age 20.[134] Compared to controls receiving standard obesity treatment, those who underwent bariatric surgery had increased type 2 diabetes remission and decreased type 2 diabetes incidence after 2 and 10 years of follow-up. The magnitude of these improvements was not influenced by adiposity status at age 20 (Figure 3). The incidence of macrovascular complications up to 26 years after bariatric surgery was also similarly reduced in the three groups compared to controls. Across groups, however, bariatric surgery did not reduce cardiovascular disease incidence compared to standard treatment (Figure 3).[134]

Changes in adipose tissue biology after obesity treatment

While weight gain leads to pathological adipose tissue remodelling that drives cardiometabolic disease, weight loss is presumed to have the opposite effect. Body composition, multi-organ insulin sensitivity, and β-cell function improve with as little as 5% weight loss, but greater weight loss is required to see favourable changes in adipose tissue expression of genes involved in cholesterol flux, lipid synthesis, extracellular matrix remodelling, oxidative stress, and inflammation. These changes occur in a dose-dependant manner between 5 and 16% weight loss.[226]

Few studies have examined the relationship between age of obesity onset and changes in adipose tissue biology after weight loss. Our group found similar changes in adipose tissue distribution after diet- and exercise-induced weight loss.[91] Petrus et al. showed that 5 years after

bariatric surgery, subcutaneous abdominal adipocyte size was similarly reduced in females with childhood-onset and adult-onset obesity.[69] Of note, baseline adipocyte size was also similar between groups. Using the same cohort, Arner et al found that in both groups, the adipocyte size reduction was mainly driven by decreased lipid uptake rather than increased lipid removal (Figure 3).[103]

To prevent metabolic dysfunction, adipose tissue must safely store lipids and secrete anti-inflammatory, insulin-sensitizing adipokines. Cellular senescence can disturb both processes and, therefore, stands as a therapeutic target for cardiometabolic disease. Our group compared changes in abdominal and femoral SAT senescence markers between females with childhood-onset and adult-onset obesity after diet- and exercise-induced weight loss. At baseline, DNA damage, an early event in the senescence process, was more pronounced in the preadipocytes of females with childhood-onset obesity. Interestingly, following 10% weigh loss, DNA damage in femoral preadipocytes decreased to a similar level in both groups. In abdominal preadipocytes, the DNA damage burden declined similarly in both groups, remaining higher in the childhood-onset group after weight loss. Concurrently, the DNA repair protein RAD51 increased comparably across both obesity-onset groups. DNA damage activates the p53/p21 senescence pathway, and β-galactosidase activity indicates senescence commitment. Although the proportion of $p53^+$ and $p21^+$ preadipocytes and β-galactosidase+ cells in SAT remained stable with weight loss, the total p21 intensity in $p53^+/p21^+$ femoral preadipocytes decreased in females with adult-onset obesity (Figure 3). This finding may indicate that moderate weight loss can slow the progression of senescence in people with adult-onset obesity but not eliminate preadipocytes that have already entered the senescence pathway.

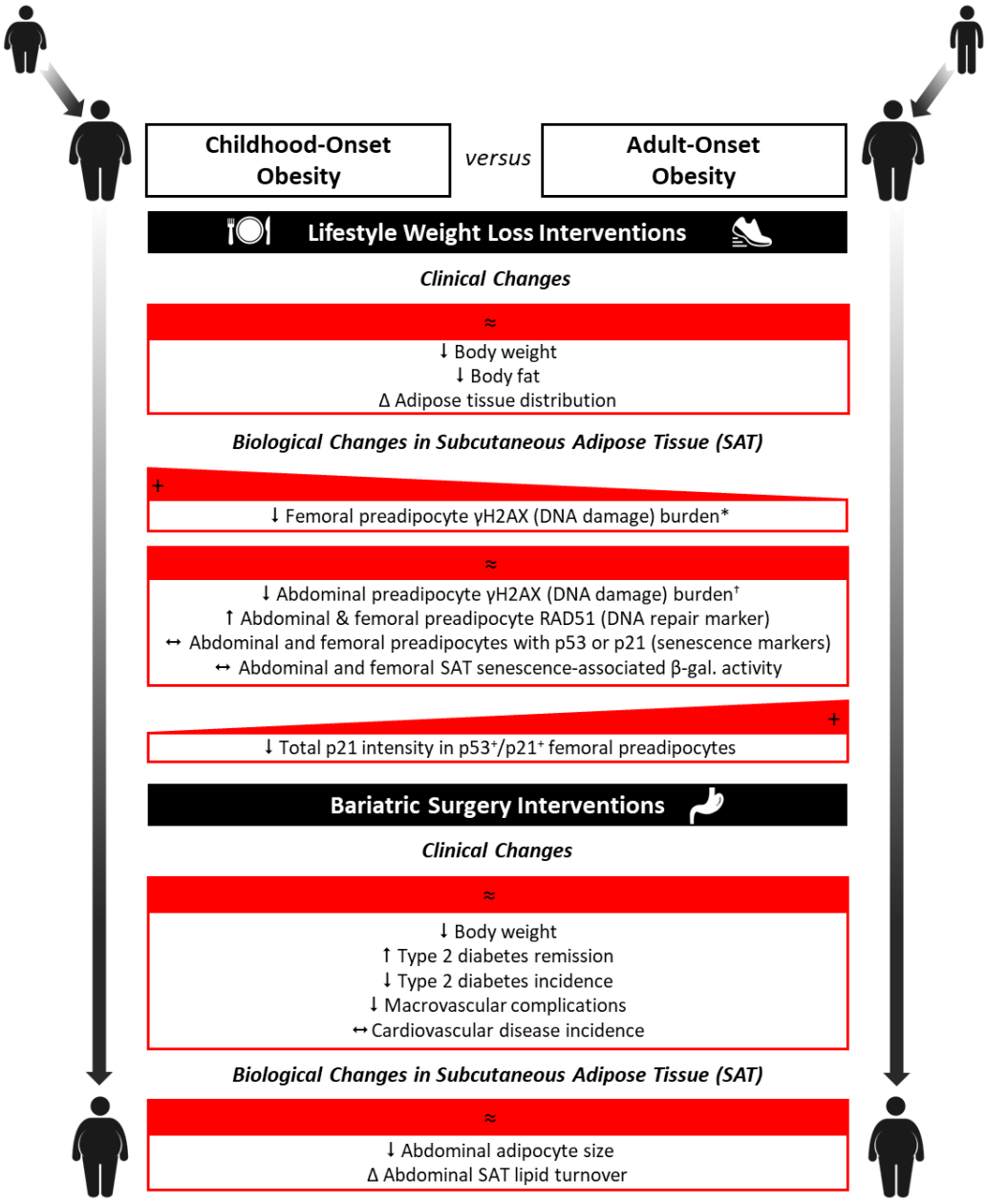

Figure 3. Treatment outcomes in people with childhood-onset versus adult-onset obesity

For each treatment outcome, a triangle (+) pointing to the right indicates a greater change in people with childhood-onset obesity than people with adult-onset obesity, and a triangle (+) pointing to the left indicates the opposite; a rectangle (≈) indicates no difference in the magnitude of the change between obesity-onset groups.

*the childhood-onset group had a greater DNA damage burden than the adult-onset group pre-weight loss, but the groups attained a similar DNA damage burden after weight loss
†the childhood-onset group had a greater DNA damage burden than the adult-onset group both before and after weight-loss

There is still a lot to learn about how adipose tissue biology changes after lifestyle, pharmacological, and surgical obesity treatments in people with childhood-onset and adult-onset obesity. It is crucial to investigate other markers of adipose tissue dysfunction, such as the immune landscape, which modulates local and systemic inflammation. We also need to expand our understanding of how treatment-induced adipose tissue remodelling impacts cardiometabolic risk and weight regain.

Conclusion

Historically, people with childhood-onset obesity and people with adult-onset obesity were considered distinct—in adipose tissue cellularity and weight loss capacity. Today, this simplistic view has evolved. Childhood-onset obesity is not uniformly hyperplastic, nor is adult-onset obesity uniformly hypertrophic. Furthermore, people with childhood-onset obesity do not struggle more to lose weight during lifestyle and surgical interventions.

Recent studies, motivated by growing evidence of increased type 2 diabetes risk in people with childhood-onset obesity, examined features of adipose tissue dysfunction intricately linked to cardiometabolic disease. While the age of obesity onset does not seem to affect pericellular fibrosis and macrophage infiltration in female bariatric surgery patients,[71] it may impact other adipose tissue characteristics in females with milder obesity and no comorbidities. As some markers of adipose tissue dysfunction already present in children with obesity, it is plausible that once severe obesity and cardiometabolic disease manifest in adulthood, biological differences between those with childhood-onset and adult onset become less apparent.

One intriguing finding is that healthy females with childhood-onset obesity have more subcutaneous preadipocytes with senescence markers compared to females with adult-onset obesity.[91] This preliminary finding aligns with the hypothesis that obesity is an accelerated form

of aging. Senescent cells in adipose tissue represent a novel treatment target for age- and obesity-related diseases, but clinical trials of senolytic agents are currently only underway for patients with severe chronic diseases.[227] In females with childhood-onset or adult-onset obesity, moderate weight loss after lifestyle intervention may steer cells away from senescence, but it may not be enough to ameliorate senescent cells already present in adipose tissue. Despite this challenge, bariatric surgery similarly increases type 2 diabetes remission and decreases type 2 diabetes incidence in those with severe childhood-onset and adult-onset obesity.[134]

Beyond preadipocyte aging, we have little biological evidence to explain the increased type 2 diabetes risk in people with childhood-onset obesity compared to those with adult-onset obesity. Furthermore, we have a limited understanding of how the age of obesity onset affects other cardiometabolic diseases, and whether the link between adipose tissue dysfunction and these diseases mirrors that of type 2 diabetes. Future primary studies, using objective measures to determine the age of obesity onset whenever possible, should explore other indicators of adipose tissue (dys)function, including adipogenesis, the immune cell landscape, and secretory profiles, both before and after evidence-based lifestyle, surgical, and pharmacological weight loss interventions. These studies should be conducted in male and female adults with varying stages of obesity and cardiometabolic risk to better understand the effect of age of obesity onset on disease progression. Additionally, studies in which both subcutaneous and visceral adipose tissue samples can be collected from children and adults undergoing elective or bariatric surgery will contribute significantly to this objective.

Cohort studies that rigorously track the age of obesity onset and cardiometabolic risk should also be initiated. The world's adult population with obesity is projected to reach 1·5 billion by the year 2035,[228] due in part to the rising childhood obesity prevalence[228] and strong

tracking of obesity from childhood to adulthood.[229] Most of these adults, however, will develop obesity during adulthood.[165] Fortunately, as techniques to study adipose tissue continue to evolve, so does our potential to identify therapeutic targets that may differ between people with childhood-onset and adult-onset obesity.

Our review underscores the evolving understanding of childhood-onset and adult-onset obesity, which is far from complete. Although we cannot draw firm conclusions about how the age of obesity onset impacts adipose tissue biology, cardiometabolic risk, and treatment outcomes, current evidence affirms that it is a factor worth considering in clinical practice and future research. Importantly, obesity can impair other aspects of health not discussed in this review. The biomechanical and psychosocial consequences of obesity may also differ between people with childhood-onset and adult-onset obesity. Adult obesity is a chronic disease requiring lifelong management. Continuing to uncover the similarities and differences between childhood-onset and adult-onset obesity will help clinicians better manage every chapter of a person's obesity story—regardless of when it began.

Supporting Information

Table S1. Summary of regional adipose tissue biology in adults with childhood-onset versus adult-onset obesity by sex and obesity severity

Characteristic	Subcutaneous adipose tissue						Visceral adipose tissue	
	Femoral		Abdominal					
	Mild to moderate obesity				Severe obesity			
	Males	Females	Males	Females	Males	Females	Males	Females
Structural and cellular characteristics								
Adipocyte hypertrophy	CO > AO	CO > AO	CO ≈ AO	CO < AO	··	··	··	··
Adipocyte hyperplasia	CO < AO	CO < AO	CO ≈ AO	CO > AO	··	··	··	··
Adipocyte size	CO > AO	CO ≈ AO	CO ≈ AO	CO < AO	··	CO ≈ AO	··	CO ≈ AO
Pericellular fibrosis	··	··	··	··	··	CO ≈ AO	··	CO ≈ AO
Preadipocytes with DNA damage (γH2AX)	··	CO > AO	··	CO > AO	··	··	··	··
Preadipocytes with p53 and/or p21	··	CO > AO	··	CO > AO	··	··	··	··
Preadipocyte RAD51 (DNA repair protein)	··	CO ≈ AO	··	CO ≈ AO	··	··	··	··
Mitochondrial integrity	··	CO < AO	··	CO < AO	··	··	··	··
M1-like macrophages	··	··	··	··	··	CO ≈ AO	··	CO ≈ AO
M2-like macrophages	··	··	··	··	··	CO ≈ AO	··	CO ≈ AO
Functional characteristics								
Basal Lipolysis	··	··	CO ≈ AO				··	··
Catecholamine-stimulated lipolysis	··	··	CO ≈ AO				··	··
Lipid turnover	··	··			··	··		··
NAD^+/NADH	··	CO > AO	··	CO > AO	··	··		··
Acetyl-CoA	··	CO < AO	··	CO < AO	··	··		··
Senescence-associated β-galactosidase activity	··	CO ≈ AO	··	CO ≈ AO	··	··		··
Gene expression								
Acetyl-CoA regulation	··	CO < AO	··	CO < AO	··	··	··	··
De novo lipogenesis	··	CO < AO	··	CO < AO	··	··	··	··
Leptin	··	CO < AO	··	CO < AO	··	··	··	··
Adiponectin	··	CO ≈ AO	··	CO ≈ AO	··	··	··	··
Interleukin-6	··	CO < AO	··	CO < AO	··	··	··	··
Tumour necrosis-α	··	CO ≈ AO	··	CO ≈ AO	··	··	··	··
Resistin	··	CO ≈ AO	··	CO ≈ AO	··	··	··	··

Abbreviations:

Acetyl-CoA: acetyl-coenzyme A; **AO:** adult-onset obesity; **CO:** childhood-onset obesity; **NAD^+/NADH:** nicotinamide adenine dinucleotide/the reduced form of NAD+

Table S2. Comparisons of the odds, risk, or hazard rate of cardiometabolic diseases between people with different ages of overweight or obesity onset in cohort studies

Reference	Cohort, country (birth year)	Sample size (% female)	Subgroup	Overweight/ obesity onset group	Ages adiposity assessed	Age outcome assessed	Number with outcome/ Group size	Relative effect			
					Adiposity status[a]	Outcome		Type	Point estimate (95% CI)	*p*-value	Adjustment variables
Hypertension											
Studies assessing adiposity status at two time points											
Merten, 2009[b]	ADD Health, USA (1975–84)	10439 (52% F)		CH/ADL	**12–19 y**[c] → **19–26 y**: OB → OB	**19–26 y** HTN	177/1081	**OR**	1.64 (1.37, 1.96)	<0.001	race/ethnicity, sex, and socioeconomic status in adolescence and adulthood
				ADL/AD	NW/OW → OB		170/1579		*Reference*		
Juonala et al., 2011[d]	BHS, USA (1962–84) MUSC, USA (1952–68) CHAD, Australia (1970–78)	6328 (53% F)	M	CH/ADL	**4–19 y**[e] → **24–42 y**: OW/OB → OB	**24–42 y** HTN	94/254	**RR**	1.39 (1.05, 1.83)	0.020	age, sex, height, length of follow-up, and cohort
				CH/ADL/AD	NW → OB		116/386		*Reference*		
			F	CH/ADL	**4–19 y**[e] → **24–42 y**: OW/OB → OB	**24–42 y** HTN	48/246		1.23 (0.87, 1.75)	0.248	
				CH/ADL/AD	NW → OB		83/426		*Reference*		
Hou et al., 2019[f]	CHNS, China (1974–2006)	2095 (40% F)		CH/ADL_1	**6–17 y**[g] → **18–37 y**[h]: OW/OB → OW/OB	**18–37 y** HTN	11/79		1.01 (0.51, 2.00)	0.976	sex, age, and percentiles of systolic and diastolic blood pressure in childhood, and smoking status and alcohol consumption in early adulthood
				CH/ADL_2	NW → OW/OB		50/301		*Reference*		
Studies assessing adiposity status at three or more time points											
Norris et al., 2020	MRC NSHD, UK (1946) NCDS, UK (1958) BCS70, UK (1970)	20746 (51% F)		CH/ADL	**10–20 y**[e,i] → **20–40 y**[i]: OB → OB	**44–53 y** HTN	92/206	**RR**	1.07 (0.8, 1.42)	0.657	sex, cohort, age at follow-up, ethnicity, birth weight, childhood social class, and obesity severity
				AD_1	NW/OW → OB from 20–25 y		226/442		1.07 (0.88, 1.3)	0.522	
				AD_2	NW/OW → OB from 25–30 y		334/636		1.07 (0.93, 1.22)	0.359	
				AD_3	NW/OW → OB from 30–35 y		460/833		1.13 (1.02, 1.26)	0.024	
				AD_4	NW/OW → OB from 35–40 y		430/739		*Reference*		
Studies assessing adiposity trajectories											
Islam et al., 2019[j]	CHNS, China (1929–83)	5276 (48% F)		CH	**≤ 7 ages: 6–80 y**[h,k]: $^{Stable}OW^{5–20\ y}$ → $^{Prog.}OW^{20–45\ y}$ → $^{Prog.\ Class\ II}OB^{45–80\ y}$	**26–80 y** HTN	181/332	**OR**	2.00 (1.39, 2.88)	<0.001	age, sex, living region, education, smoking, alcohol consumption, physical activity, and unhealthy dietary pattern at the last follow-up
				AD	$^{Stable}NW^{5–18\ y}$ → $^{Prog.}NW/OW^{18–60\ y}$ → $^{Stable}OW^{60–80\ y}$		604/1437		*Reference*		
Buscot et al., 2018[l]	YFS, Finland (1962–74)	2631 (54% F)		CH	**≥ 3 ages: 6–18 y**[e], **19–36 y, 37–49 y**: $^{Stable}OW^{6–15\ y}$ → $^{Prog.\ Class\ III}OB^{15–49\ y}$	**37–49 y** HTN	13/33	**HR**	2.40 (1.31, 4.40)	0.005	sex, year of birth, family history of hypertension, and socioeconomic status and physical activity in adulthood
				ADL	$^{Stable}NW/OW^{6–12\ y}$ → $^{Prog.}OW^{12–25\ y}$ → $^{Stable\ Class\ I}OB^{25–50\ y}$		41/113		1.84 (1.20, 2.82)	0.005	
				AD_1	$^{Stable}NW^{6–20\ y}$ → $^{Prog.}OW^{20–30\ y}$ → $^{Prog.\ Class\ II}OB^{30–50\ y}$		36/110		1.71 (1.07, 2.74)	0.026	
				AD_2	$^{Stable}NW^{6–25\ y}$ → $^{Prog.}OW^{25–40\ y}$ → $^{Stable}OW^{40–49\ y}$		236/879		*Reference*		

Dyslipidemia											
Studies assessing adiposity status at two time points											
Merten, 2009[b]	ADD Health, USA (1975–84)	10439 (52% F)		CH/ADL *ADL/AD*	**12–19 y**[c] → **19–26 y** OB → OB NW/OW → OB	**19–26 y** High cholesterol	111/1081 99/1579	**OR**	1.69 (1.33, 2.15) *Reference*	<0.001	race/ethnicity, sex, and socioeconomic status in adolescence and adulthood
Juonala et al., 2011[d]	Bogalusa, USA (1962–84) MUSC, USA (1952–68) CHAD, Australia (1970–78)	6328 (53% F)	M	CH/ADL *CH/ADL/AD*	**4–19 y**[e] → **24–42 y** OW/OB → OB NW → OB	**24–42 y** High LDL-C	48/254 86/386	**RR**	0.88 (0.62, 1.26) *Reference*	0.492	age, sex, height, length of follow-up, and cohort
			F	CH/ADL *CH/ADL/AD*	**4–19 y**[e] → **24–42 y** OW/OB → OB NW → OB	**24–42 y** High LDL-C	41/246 35/426		2.08 (1.31, 3.3) *Reference*	0.002	
			M	CH/ADL *CH/ADL/AD*	**4–19 y**[e] → **24–42 y** OW/OB → OB NW → OB	**24–42 y** Low HDL-C	120/254 200/386		0.85 (0.48, 1.51) *Reference*	0.580	
			F	CH/ADL *CH/ADL/AD*	**4–19 y**[e] → **24–42 y** OW/OB → OB NW → OB	**24–42 y** Low HDL-C	75/246 109/426		1.10 (0.8, 1.51) *Reference*	0.555	
			M	CH/ADL *CH/ADL/AD*	**4–19 y**[e] → **24–42 y** OW/OB → OB NW → OB	**24–42 y** High TG	86/254 138/386		0.94 (0.73, 1.21) *Reference*	0.616	
			F	CH/ADL *CH/ADL/AD*	**4–19 y**[e] → **24–42 y** OW/OB → OB NW → OB	**24–42 y** High TG	31/246 64/426		0.91 (0.58, 1.42) *Reference*	0.667	
Studies assessing adiposity status at three or more time points											
Norris et al., 2020	MRC NSHD, UK (1946) NCDS, UK (1958) BCS70, UK (1970)	20746 (51% F)		CH/ADL AD_1 AD_2 AD_3 AD_4	**10–20 y**[e,i] → **20–40 y**[i] OB → OB NW/OW → OB from 20–25 y NW/OW → OB from 25–30 y NW/OW → OB from 30–35 y NW/OW → OB from 35–40 y	**44–53 y** Low HDL-C	107/206 251/442 407/636 555/833 521/739	**RR**	1.25 (0.91, 1.72) 1.20 (0.96, 1.51) 1.05 (0.86, 1.28) 1.00 (0.85, 1.18) *Reference*	0.172 0.117 0.626 1.000	sex, cohort, age at follow-up, ethnicity, birth weight, childhood social class, and obesity severity
Studies assessing adiposity trajectories											
Islam et al., 2019[j]	CHNS, China (1929–83)	5276 (48% F)		CH *AD*	**≤ 7 ages: 6–80 y**[h,k] $^{Stable}OW^{5–20\ y}$ → $^{Prog.}OW^{20–45\ y}$ → $^{Prog.\ Class\ II}OB^{45–80\ y}$ $^{Stable}NW^{5–18\ y}$ → $^{Prog.}NW/OW^{18–60\ y}$ → $^{Stable}OW^{60–80\ y}$	**26–80 y** DLP	178/332 661/1437	**OR**	2.48 (1.01, 2.15) *Reference*	0.042	age, sex, living region, education, smoking, alcohol consumption, physical activity, and unhealthy dietary pattern at the last follow-up
Buscot et al., 2018[l]	YFS, Finland (1962–74)	2631 (54% F)		CH ADL AD_1 AD_2	**≥ 3 ages: 6–18 y**[e]**, 19–36 y, 37–49 y** $^{Stable}OW^{6–15\ y}$ → $^{Prog.\ Class\ III}OB^{15–49\ y}$ $^{Stable}NW/OW^{6–12\ y}$ → $^{Prog.}OW^{12–25\ y}$ → $^{Stable\ Class\ I}OB^{25–49\ y}$ $^{Stable}NW^{6–20\ y}$ → $^{Prog.}OW^{20–30\ y}$ → $^{Prog.\ Class\ II}OB^{30–49\ y}$ $^{Stable}NW^{6–25\ y}$ → $^{Prog.}OW^{25–40\ y}$ → $^{Stable}OW^{40–49\ y}$	**37–49 y** High LDL-C	7/33 21/113 20/110 145/879	**HR**	1.35 (0.80, 2.28) 1.07 (0.74, 1.55) 1.16 (0.78, 1.73) *Reference*	0.264 0.716 0.462	sex, year of birth, family history of hypertension, and socioeconomic status and physical activity in adulthood

				≥ 3 ages: 6–18 y[e], 19–36 y, 37–49 y	**37–49 y**						
			CH	$^{Stable}OW^{6–15\ y} \rightarrow {}^{Prog.\ Class\ III}OB^{15–49\ y}$		13/33		1.10 (0.74, 1.67)	0.635		
			ADL	$^{Stable}NW/OW^{6–12\ y} \rightarrow {}^{Prog.}OW^{12–25\ y} \rightarrow {}^{Stable\ Class\ I}OB^{25–49\ y}$	Low HDL-C	45/113		1.14 (0.75, 1.72)	0.540		
			AD_1	$^{Stable}NW^{6–20\ y} \rightarrow {}^{Prog.}OW^{20–30\ y} \rightarrow {}^{Prog.\ Class\ II}OB^{30–49\ y}$		46/110		1.09 (0.32, 3.67)	0.891		
			AD_2	$^{Stable}NW^{6–25\ y} \rightarrow {}^{Prog.}OW^{20–40\ y} \rightarrow {}^{Stable}OW^{40–49\ y}$		231/879		*Reference*			
				≥ 3 ages: 6–18 y[e], 19–36 y, 37–49 y	**37–49 y**						
			CH	$^{Stable}OW^{6–15\ y} \rightarrow {}^{Prog.\ Class\ III}OB^{15–49\ y}$		6/33		1.11 (0.40, 3.05)	0.839		
			ADL	$^{Stable}NW/OW^{6–12\ y} \rightarrow {}^{Prog.}OW^{12–25\ y} \rightarrow {}^{Stable\ Class\ I}OB^{25–49\ y}$	High TG	29/113		1.47 (0.96, 2.25)	0.080		
			AD_1	$^{Stable}NW^{6–20\ y} \rightarrow {}^{Prog.}OW^{20–30\ y} \rightarrow {}^{Prog.\ Class\ II}OB^{30–49\ y}$		30/110		1.77 (1.07, 2.92)	0.026		
			AD_2	$^{Stable}NW^{6–25\ y} \rightarrow {}^{Prog.}OW^{20–40\ y} \rightarrow {}^{Stable}OW^{40–49\ y}$		156/879		*Reference*			
Non-Alcoholic Fatty Liver Disease											
Studies assessing adiposity status at two time points											
Yan et al., 2017[d]	Beijing BP, China (1969–1982)	1350 (46% F)	M	**6–18 y[e]**	**28–45 y**	**28–45 y**					
			CH/ADL	OW/OB	→ OB	NAFLD	27/40		0.89 (0.35, 2.29)	0.812	childhood age, length of follow-up, and total cholesterol, triglycerides, HDL-C, LDL-C, smoking, alcohol consumption, and physical activity in adulthood
			CH/ADL/AD	NW	→ OB		50/66		*Reference*		
			F	**6–18 y[e]**	**28–45 y**	**28–45 y**		**OR**			
			CH/ADL	OW/OB	→ OB	NAFLD	10/14		2.56 (0.49, 13.26)	0.263	
			CH/ADL/AD	NW	→ OB		18/29		*Reference*		
Cuthbertson et al., 2023[d]	YFS, Finland (1962–1977)	2020 (54% F)		**3–18 y[e]**	**34–49 y**	**34–49 y**					
			CH/ADL	OW/OB	→ OB	NAFLD	49/95		1.11 (0.64, 1.93)	0.707	age, sex, adulthood alcohol consumption, physical exercise, and smoking
			CH/ADL/AD	NW	→ OB		148/317		*Reference*		
Type 2 diabetes											
Studies assessing adiposity status at two time points											
The et al., 2013[b]	ADD Health, USA (1975–84)	10481 (53% F)	M	**12–17 y[c]**	**18–34 y**	**24–34 y**					
			CH/ADL	OB	→ OB	Diabetes	173/1324[m]		2.27 (1.41, 3.64)[n]	0.001	age, race/ethnicity, education, and parental history of diabetes
			ADL/AD	NW/OW	→ OB		139/2244[m]		*Reference*		
			F	**12–17 y[c]**	**18–34 y**	**24–34 y**		**OR**			
			CH/ADL	OB	→ OB	Diabetes	173/1324[m]		2.08 (1.34, 3.24)[n]	0.001	
			ADL/AD	NW	→ OB		139/2244[m]		*Reference*		
Liang et al., 2015[b]	Beijing BP, China (1969–89)	1209 (55% F)		**6–18 y[o]**	**30–42 y[h]**	**30–42 y**					
			CH/ADL	OB	→ OB	Diabetes	18/95		1.95 (0.88, 4.35)	0.100	age, sex, family history of diabetes, smoking, alcohol consumption, and physical inactivity in adulthood
			CH/ADL/AD	NW/OW	→ OB		14/140		*Reference*		
Juonala et al., 2011[d]	Bogalusa, USA (1962–84) MUSC, USA (1952–68) CHAD, Australia (1970–78) YFS, Finland (1962–77)	6328 (53% F)	M	**4–19 y[e]**	**24–42 y**	**24–42 y**					
			CH/ADL	OW/OB	→ OB	T2D	18/254		1.37 (0.67, 2.83)	0.389	age, height, length of follow-up, and cohort
			CH/ADL/AD	NW	→ OB		18/386	**RR**	*Reference*		
			F	**4–19 y[e]**	**24–42 y**	**24–42 y**					
			CH/ADL	OW/OB	→ OB	T2D	19/246		1.09 (0.57, 2.07)	0.802	
			CH/ADL/AD	NW	→ OB		29/426		*Reference*		

Ohlsson et al., 2019[f]	BEST Gottenburg, Sweden (1945–61)	36176 (0% F)		**8 y**[c,p]		**20 y**[q]	**20–55 y**		**HR**	1.03 (0.79, 1.35)	0.816	birth year and country of birth
			CH	OW/OB	→	OW/OB	T2D	85/947		*Reference*		
			CH/ADL	NW	→	OW/OB		152/1719				
				8 y[c,p]		**20 y**[q]	**> 55 y**			1.07 (0.75, 1.53)	0.724	
			CH	OW/OB	→	OW/OB	T2D	64/608		*Reference*		
			CH/ADL	NW	→	OW/OB		74/1044				
Choudhary et al., 2022[f]	NFBC, Finland (1966)	6372 (54% F)		**7 y**[e]		**31 y**	**32–50 y**			0.83 (0.36, 1.92)	0.669	sex, education, smoking, physical activity, and diet score at 31 y
			M CH	OW/OB	→	OW/OB	T2D	10/98		*Reference*		
			M *CH/ADL/AD*	NW	→	OW/OB		64/823				
				7 y[e]		**31 y**	**32–50 y**			1.12 (0.51, 2.45)	0.784	
			F CH	OW/OB	→	OW/OB	T2D	11/123		*Reference*		
			F *CH/ADL/AD*	NW	→	OW/OB		48/600				
Studies assessing adiposity status at three or more time points												
Norris et al., 2020	MRC NSHD, UK (1946) NCDS, UK (1958) BCS70, UK (1970)	20746 (51% F)		**10–20 y**[e,i]		**20–40 y**[i]	**44–53 y**			1.50 (1.09, 2.07)	0.014	sex, cohort, age at follow-up, ethnicity, birth weight, childhood social class, and obesity severity
			CH/ADL	OB	→	OB		106/206		1.55 (1.22, 1.98)	<0.001	
			AD_1	NW/OW	→	OB from 20–25 y		190/442	**RR**	1.20 (0.98, 1.47)	0.083	
			AD_2	NW/OW	→	OB from 25–30 y	Diabetes	212/636		1.15 (0.94, 1.40)	0.164	
			AD_3	NW/OW	→	OB from 30–35 y		246/833		*Reference*		
			AD_4	NW/OW	→	OB from 35–40 y		185/739				
Bjerregaard et al., 2018[r]	CSHRR, Denmark (1930–89)	62565 (0% F)		**7 y**[c]		**13 y**[c]	**17–26 y**	**30–60 y**		1.23 (1.04, 1.46)	0.015	Intelligence-test score, education, and age at conscription
			CH	OW/OB	→	OW/OB	→ OW/OB			1.19 (1.01, 1.42)	0.043	
			CH/ADL	NW	→	OW/OB	→ OW/OB	T2D		0.79 (0.58, 1.06)	0.114	
			CH	OW/OB	→	NW	→ OW/OB			*Reference*		
			ADL/AD	NW	→	NW	→ OW/OB	451/2807	**HR**			
				7 y[c]		**13 y**[c]	**17–26 y**	**60–76 y**		0.93 (0.69, 1.25)	0.632	
			CH	OW/OB	→	OW/OB	→ OW/OB			1.31 (1.01, 1.71)	0.045	
			CH/ADL	NW	→	OW/OB	→ OW/OB	T2D		0.75 (0.47, 1.17)	0.205	
			CH	OW/OB	→	NW	→ OW/OB			*Reference*		
			ADL/AD	NW	→	NW	→ OW/OB	184/2807				
Studies assessing adiposity trajectories												
Islam et al., 2019[j]	CHNS, China (1929–83)	5276 (48% F)		**≤ 7 age: 6–80 y**[h,k]			**26–80 y**			2.84 (1.82, 4.44)	<0.001	age, sex, living region, education, smoking, alcohol consumption, physical activity, and unhealthy dietary pattern at the last follow-up
			CH	$^{Stable}OW^{5–20\ y}$ → $^{Prog.}OW^{20–45\ y}$ → $^{Prog.\ Class\ II}OB^{45–80\ y}$			T2D	70/332	**OR**	*Reference*		
			AD	$^{Stable}NW^{5–18\ y}$ → $^{Prog.}NW/OW^{18–60\ y}$ → $^{Stable}OW^{60–80\ y}$				158/1437				
Buscot et al., 2018[l]	YFS, Finland (1962–74)	2631 (54% F)		**≥ 3 ages: 6–18 y**[e]**, 19–36 y, 37–49 y**			**37–49 y**			7.89 (3.69, 16.91)	<0.001	sex, year of birth, family history of hypertension, and socioeconomic status and physical activity in adulthood
			CH	$^{Stable}OW^{6–15\ y}$ → $^{Prog.\ Class\ III}OB^{15–49\ y}$				7/33				
			ADL	$^{Stable}NW/OW^{6–12\ y}$ → $^{Prog.}OW^{12–25\ y}$ → $^{Stable\ Class\ I}OB^{25–49\ y}$			T2D	14/113	**RR**	4.46 (2.16, 9.24)	<0.001	
			AD_1	$^{Stable}NW^{6–20\ y}$ → $^{Prog.}OW^{20–30\ y}$ → $^{Prog.\ Class\ II}OB^{30–49\ y}$				19/110		4.83 (2.08, 11.24)	<0.001	
			AD_2	$^{Stable}NW^{6–25\ y}$ → $^{Prog.}OW^{25–40\ y}$ → $^{Stable}OW^{40–49\ y}$				31/879		*Reference*		

Yacamán-Méndez et al., 2021[s]	SDPP, Sweden (1936–63)	7203 (67% F)		**5 ages: 7 y[t], 18 y[t], 25–46 y, 30–51 y, 35–56 y**	**35–76 y**			1.55 (1.09, 2.21)	0.015	age, family history of type 2 diabetes, general health, comorbidities, self-reported physical activity, smoking, and alcohol consumption for both sexes, and history of gestational diabetes for females
			M	CH: $^{Stable}OW/OB^{7–55\ y}$			42/141			
			M	AD_1: $^{Stable}NW^{7–18\ y} \rightarrow {}^{Prog.}OW/OB^{18–38\ y} \rightarrow {}^{Stable}OW/OB^{38–55\ y}$	T2D		199/654	1.60 (1.23, 2.08)	<0.001	
			M	AD_2: $^{Stable}NW^{7–38\ y} \rightarrow {}^{Prog.}OW/OB^{38–55\ y}$			60/334	*Reference*		
				5 ages: 7 y[t], 18 y[t], 25–46 y, 30–51 y, 35–56 y	**35–76 y**					
			F	CH: $^{Stable}OW/OB^{7–55\ y}$			62/313	1.22 (0.89, 1.67)	0.215	
			F	AD_1: $^{Stable}NW^{7–18\ y} \rightarrow {}^{Prog.}OW/OB^{18–38\ y} \rightarrow {}^{Stable}OW/OB^{38–55\ y}$	T2D		184/822	1.51 (1.18, 1.93)	<0.001	
			F	AD_2: $^{Stable}NW^{7–38\ y} \rightarrow {}^{Prog.}OW/OB^{38–55\ y}$			95/652	*Reference*		
Fagherazzi et al., 2015[u]	E3N, France (1925–51)	81110 (100% F)		**4 ages: 8 y[v], age of menarche[v], 20–25 y[v], 35–40 y[v]**	**42–82 y**					education, smoking, physical activity, hypertension, hypercholesterolemia, family history of diabetes, use of hormone replacement therapy, age at menarche, number of children, use of oral contraceptives, menopausal status, birth length, and birth weight
				CH: $^{Stable}OB^{8–40\ y}$	T2D		135/4056	0.75 (0.58, 0.96)	0.023	
				CH/ADL: $NW^{8\ y} \rightarrow {}^{Rapid}OB^{age\ of\ menarche} \rightarrow {}^{Stable}OB^{age\ of\ menarche–40\ y}$			125/2433	*Reference*		
Zheng et al., 2017[w]	NHS, USA (1921–46) HPFS, USA (1911–46)	122498 (69% F)		**7 ages: 5 y[v], 10 y[v], 20 y[v], 30 y[v], 40 y[v], 50 y[x], 55 y[x]**	**> 55 y**	**HR**				height, race, smoking, regular aspirin use, menopausal hormone therapy, physical activity, alcohol consumption, Alternate Healthy Eating Index score, and family history of diabetes
			M	CH: $^{Stable}OW^{5–20\ y} \rightarrow {}^{Prog.}OW^{20–48\ y} \rightarrow {}^{Prog.}OB^{48–55\ y}$	Diabetes		396/3708	1.19 (1.05, 1.34)	0.005	
			M	*AD*: $^{Prog.}NW^{5–45\ y} \rightarrow {}^{Prog.}OW^{40–55\ y}$			979/10703	*Reference*		
				7 ages: 5 y[v], 10 y[v], 20 y[v], 30 y[v], 40 y[v], 50 y[x], 55 y[x]	**> 55 y**					
			F	AD_1: $^{Stable}NW^{5–10\ y} \rightarrow {}^{Prog.}NW^{10–33\ y} \rightarrow {}^{Prog.}OW^{35–45\ y} \rightarrow {}^{Prog.}OB^{45–55\ y}$	Diabetes		1372/8562	0.91 (0.84, 0.97)	0.008	
			F	AD_2: $^{Prog.}NW^{5–48\ y} \rightarrow {}^{Prog.}OW^{48–53\ y} \rightarrow {}^{Prog.}OB^{53–55\ y}$			2138/11983	*Reference*		
Luo et al., 2020[y]	ALSWH. Australia (1973–78)	11192 (100% F)		**2–7 ages: 18–42 y**	**21–42 y**					age, physical activity, smoking, alcohol consumption, education, income adequacy, stress score, and history of gestational diabetes
				CH/ADL_1: $^{Prog.}OB^{20–33\ y} \rightarrow {}^{Prog.}OB^{33–39\ y}$			32/3118	6.75 (3.78, 12.05)	<0.001	
				CH/ADL_2: $^{Prog.}OW^{20–28\ y} \rightarrow {}^{Prog.}OB^{28–39\ y}$	T2D		67/2335	3.19 (2.08, 4.89)	<0.001	
				AD: $^{Prog.}NW^{20–28\ y} \rightarrow {}^{Prog.}OW^{28–39\ y}$			21/289	*Reference*		
Lv et al., 2020[z]	CHNS, China (1943–1991)	7289 (52 % F)		**3–9 ages: 20–50 y**	**20–50 y**					age, sex, BMI, smoking, and alcohol consumption
				CH/ADL: $^{Prog.}OW^{20–35\ y} \rightarrow {}^{Prog.}OB^{35–40\ y} \rightarrow {}^{Stable}OB^{40–50\ y}$	Diabetes		27/239	1.50 (0.58, 3.91)	0.407	
				AD: $^{Prog.}NW^{20–35\ y} \rightarrow {}^{Prog.}OW^{35–50\ y}$			82/1914	*Reference*		
Major adverse cardiovascular events										
Studies assessing adiposity status at two time points										
Morrison et al., 2012[f]	NHLBI LRC & PFS, USA (1953–71)	770 (54% F)		**5–20 y[c]** → **29–48 y**	**29–48 y**	**OR**				NA
				CH: OW/OB → OB	MACE		7/113	1.57 (0.51, 4.81)	0.428	
				CH/ADL/AD: NW → OB			6/149	*Reference*		
Ohlsson et al., 2016[f]	BEST Gottenburg, Sweden (1945–61)	37672 (0% F)		**8 y[c,p]** → **20 y[q]**	**>20 y**	**HR**				birth year and country of birth
				CH: OW/OB → OW/OB	CVD Mortality		30/990	0.77 (0.5, 1.19)	0.244	
				CH/ADL: NW → OW/OB			66/1800	*Reference*		
				8 y[c,p] → **20 y[q]**	**>30 y**					
				CH: OW/OB → OW/OB	CVD Mortality		29/969	0.75 (0.49, 1.16)	0.198	
				CH/ADL: NW → OW/OB			66/1757	*Reference*		
				8 y[c,p] → **20 y[q]**	**>40 y**					
				CH: OW/OB → OW/OB	CVD Mortality		28/939	0.74 (0.47, 1.15)	0.176	
				CH/ADL: NW → OW/OB			65/1721	*Reference*		

			CH *CH/ADL*	**8 y**[c,p] OW/OB → OW/OB NW → OW/OB	**20 y**[q]	**>50 y** CVD Mortality	20/897 41/1643		0.83 (0.49, 1.42) *Reference*	0.498	
Ohlsson et al., 2017[f]	BEST Gottenburg, Sweden (1945–61)	37669 (0% F)	CH *CH/ADL*	**8 y**[c,p] OW/OB → OW/OB NW → OW/OB	**20 y**[q]	**>20 y** Stroke	36/990 67/1800		0.94 (0.63, 1.42) *Reference*	0.783	birth year and country of birth
Kindblom et al., 2018[f]	BEST Gottenburg, Sweden (1945–61)	37670 (0% F)	CH *CH/ADL*	**8 y**[c,p] OW/OB → OW/OB NW → OW/OB	**20 y**[q]	**>20 y** Heart Failure	21/990 40/1800		0.91 (0.54, 1.52) *Reference*	0.711	birth year and country of birth
Kindblom et al., 2021[f]	BEST Gottenburg, Sweden (1945–61)	37672 (0% F)	CH *CH/ADL*	**8 y**[c,p] OW/OB → OW/OB NW → OW/OB	**20 y**[q]	**>20 y** ACE	74/990 156/1800		0.82 (0.63, 1.08) *Reference*	0.168	birth year and country of birth
			CH *CH/ADL*	**8 y**[c,p] OW/OB → OW/OB NW → OW/OB	**20 y**[q]	**>20 y** ACE Mortality	13/990 41/1800		0.54 (0.29, 1.01) *Reference*	0.052	
Studies assessing adiposity trajectories											
Yang et al., 2019[aa]	MCCS, Australia (1920–54)	29881 (59% F)	CH/ADL CH/ADL AD_1 *AD_2*	**4 ages: 18–21 y, 40–69 y, 43–77 y, and 48–85 y** $^{Prog.}OW^{18–28\ y}$ → $^{Prog.}OB^{28–60\ y}$ → $^{Stable.\ Class\ II}OB^{60–90\ y}$ $OW^{18\ y}$ → $^{Stable\ Class\ I}OB$ $^{Prog.}NW^{18–28\ y}$ → $^{Prog.}OW/OB^{18–60\ y}$ → $^{Stable\ Class\ I}OB^{60–90\ y}$ $^{Prog.}NW^{18–35\ y}$ → $^{Prog.}OW/OB^{35–60\ y}$ → $^{Stable}OW/OB^{60–90\ y}$		**>48–85 y** CVD mortality	43/948 68/933 138/2893 349/8130		2.67 (1.63, 4.37) 1.51 (0.95, 2.39) 1.28 (0.92, 1.79) *Reference*	<0.001 0.083 0.145	age, birth cohort, height, sex, country of birth, Socioeconomic Indexes for Areas, education, smoking status at latest BMI assessment, and Mediterranean Diet Score, alcohol consumption, and physical activity at baseline (age 40–69 y)
Zheng et al., 2017[w]	NHS, USA (1921–46) HPFS, USA (1911–46)	122498 (69% F)	M CH M *AD*	**7 ages: 5 y, 10 y, 20 y, 30 y, 40 y, 50 y, 55 y** $^{Stable}OW^{5–20\ y}$ → $^{Prog.}OW^{20–48\ y}$ → $^{Prog.}OB^{48–55\ y}$ $^{Prog.}NW^{5–45\ y}$ → $^{Prog.}OW^{40–55\ y}$		**>55 y** MACE	463/3938 1470/11162	**HR**	1.05 (0.95, 1.17) *Reference*	0.312	height, race, smoking, regular aspirin use, menopausal hormone therapy, physical activity, alcohol consumption, Alternate Healthy Eating Index score, and family history of diabetes
			F AD_1 F *AD_2*	**7 ages: 5 y, 10 y, 20 y, 30 y, 40 y, 50 y, 55 y** $^{Stable}NW^{5–10\ y}$ → $^{Prog.}NW^{10–33\ y}$ → $^{Prog.}OW^{35–45\ y}$ → $^{Prog.}OB^{45–55\ y}$ $^{Prog.}NW^{5–48\ y}$ → $^{Prog.}OW^{48–53\ y}$ → $^{Prog.}OB^{53–55\ y}$		**>55 y** MACE	788/9690 979/13201		1.12 (1.02, 1.23) *Reference*	0.013	
			M CH M *AD*	**7 ages: 5 y^{v}, 10 y^{v}, 20 y^{v}, 30 y^{v}, 40 y^{v}, 50 y^{x}, 55 y^{x}** $^{Stable}OW^{5–20\ y}$ → $^{Prog.}OW^{20–48\ y}$ → $^{Prog.}OB^{48–55\ y}$ $^{Prog.}NW^{5–45\ y}$ → $^{Prog.}OW^{40–55\ y}$		**>55 y** CHD	354/3942 1116/11170		1.06 (0.95, 1.2) *Reference*	0.304	
			F AD_1 F *AD_2*	**7 ages: 5 y^{v}, 10 y^{v}, 20 y^{v}, 30 y^{v}, 40 y^{v}, 50 y^{x}, 55 y^{x}** $^{Stable}NW^{5–10\ y}$ → $^{Prog.}NW^{10–33\ y}$ → $^{Prog.}OW^{35–45\ y}$ → $^{Prog.}OB^{45–55\ y}$ $^{Prog.}NW^{5–48\ y}$ → $^{Prog.}OW^{48–53\ y}$ → $^{Prog.}OB^{53–55\ y}$		**>55 y** CHD	448/9717 528/13245		1.17 (1.04, 1.33) *Reference*	0.012	
			M CH M *AD*	**7 ages: 5 y^{v}, 10 y^{v}, 20 y^{v}, 30 y^{v}, 40 y^{v}, 50 y^{x}, 55 y^{x}** $^{Stable}OW^{5–20\ y}$ → $^{Prog.}OW^{20–48\ y}$ → $^{Prog.}OB^{48–55\ y}$ $^{Prog.}NW^{5–45\ y}$ → $^{Prog.}OW^{40–55\ y}$		**>55 y** Stroke	419/11183 121/3951		0.99 (0.81, 1.21) *Reference*	0.922	
			F AD_1 F *AD_2*	**7 ages: 5 y^{v}, 10 y^{v}, 20 y^{v}, 30 y^{v}, 40 y^{v}, 50 y^{x}, 55 y^{x}** $^{Stable}NW^{5–10\ y}$ → $^{Prog.}NW^{10–33\ y}$ → $^{Prog.}OW^{35–45\ y}$ → $^{Prog.}OB^{45–55\ y}$ $^{Prog.}NW^{5–48\ y}$ → $^{Prog.}OW^{48–53\ y}$ → $^{Prog.}OB^{53–55\ y}$		**>55 y** Stroke	371/9714 504/13240		1.03 (0.9, 1.18) *Reference*	0.647	

The table displays the results of cohort studies in which the odds, risk, or hazard rate of cardiometabolic diseases could be statistically compared between different obesity-onset groups as detailed in the Methods. These studies assessed adiposity status at two or three time points between childhood and adulthood or assessed adiposity trajectories between childhood and adulthood. Obesity-onset was classified based on these adiposity status changes or trajectories. We used the group with the latest overweight/obesity onset (and where relevant, the least severe overweight/obesity) as the reference group. We discuss other relevant comparisons in the text.

[a]Adiposity status in adulthood was defined according to standard BMI categories unless otherwise indicated (normal weight: 18.5–<25 kg/m^2; overweight: 25–<30 kg/m^2; obesity: ≥ 30 kg/m^2): Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser 894, i–xii, 1–253 (2000).

[b]Model also included the following adiposity status changes: NW/OW -> NW/OW and OB -> NW/OW

[c]Adiposity status in childhood/adolescence was defined according to the United States Centers for Disease Control and Prevention sex- and age-specific percentiles (normal weight: 5–<85th; overweight: 85–<95th; obesity: ≥ 95th): Kuczmarski, R. J. et al. 2000 CDC Growth Charts for the United States: methods and development. Vital Health Stat. 11. 1–190 (2002).

[d]Model also included the following adiposity status changes: NW -> NW/OW and OW/OB -> NW/OW

[e]Adiposity status in childhood/adolescence was defined according to the international sex- and age-specific percentiles that extrapolate to the standard adult BMI categories: Cole, T. J., Bellizzi, M. C., Flegal, K. M. & Dietz, W. H. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ 320, 1240 (2000).

[f]Model also included the following adiposity status changes: NW → NW and OW/OB → NW

[g]Adiposity status in childhood/adolescence was defined according to the Working Group on Obesity in China sex and age-specific BMI percentiles that extrapolate to BMI cutoffs of overweight (24 kg/m^2) and obesity (28 kg/m^2) at 18 years in Chinese adults: Li H, et al. Body mass index cut-offs for overweight and obesity in Chinese children and adolescents aged 2-18 years. Zhonghua Liu Xing Bing Xue Za Zhi. 31, 616–620 (2010).

[h]Adiposity status in adulthood was defined according to the Working Group on Obesity in China (overweight: 24–<28 kg/m^2; obesity: ≥ 28 kg/m^2): Bei-Fan, Z. & the Cooperative Meta-analysis Group of Working Group on Obesity in China. Predictive values of body mass index and waist circumference for risk factors of certain related diseases in Chinese adults: study on optimal cut-off points of body mass index and waist circumference in Chinese adults. Asia Pac. J. Clin. Nutr. 11, (2002).

[i]Models were used to fit annual BMI values between 10 and 40 years for each participant.

[j]Model also included the following adiposity trajectories: stable NW and low-NW progressive to NW that stabilizes in mid-adulthood

[k]Adiposity status in childhood/adolescence was defined according to the Working Group on Obesity in China sex and age-specific BMI percentiles (normal weight: 5–<85th; overweight: 85–<95th; obesity: ≥ 95th): Ji, C.-Y. & Working Group on Obesity in China. Report on childhood obesity in China (1)--body mass index reference for screening overweight and obesity in Chinese school-age children. Biomed. Environ. Sci. BES 18, 390–400 (2005).

[l]Model also included the following adiposity trajectories: stable NW and OW/OB resolving to NW in adulthood

[m]For males and females combined

[n]Reported by study authors

[o]Adiposity status at age 6 was defined as in [c] and adiposity status at age 7-18 y was defines as in [k].

[p]BMI was calculated using all paired height and weight measurements from 6.5 to 9.5 y and age-adjusted to 8 y using linear regression models

[q]BMI was calculated using all paired height and weight measurements from 17.5 to 22 y and age-adjusted to 20 y using linear regression models

[r]Model also included the following adiposity status changes: NW → NW → NW, OW/OB → NW → NW, OB/OW → OW/OB → NW, and NW → OW/OB → NW

[s]Model also include the following adiposity trajectories: Stable NW and low-NW progressing to NW in early-adulthood and stabilizing in mid-adulthood

[t]Adiposity status was determined based on the question, 'Compared to others of your same age, what was your weight status at age 7 and 18' (1) very lean, (2) somewhat lean, (3) normal weight, (4) somewhat overweight, (5) very overweight.

[u]Model also included the following adiposity status trajectories: stable NW, stable high-NW, low-NW progressing to NW and low-NW increasing to NW at puberty and remained stable

[v]Participants were asked to recall their adiposity status using the Stunkard body rating scale: Stunkard, A. J., Sørensen, T. & Schulsinger, F. Use of the Danish Adoption Register for the study of obesity and thinness. Res. Publ. - Assoc. Res. Nerv. Ment. Dis. 60, 115–120 (1983).

[w]Model also included the following adiposity status trajectories: stable low-NW, stable NW, low-NW that progresses to NW by mid-adulthood

[x]Measured BMI was converted to the same scale as the somatotypes in the Stunkard body rating scale: Stunkard, A. J., Sørensen, T. & Schulsinger, F. Use of the Danish Adoption Register for the study of obesity and thinness. Res. Publ. - Assoc. Res. Nerv. Ment. Dis. 60, 115–120 (1983).

[y]Model also included the following adiposity trajectories: stable low-NW, stable mid-NW, and low-NW that progressively increases to mid-NW

[z]Model also included the following adiposity trajectories: low NW increasing to mid-NW

[aa]Model also included the following adiposity trajectories: stable low-NW and stable high-NW

Abbreviations:

ACE: acute coronary events; **AD:** adult-onset; **ADL:** adolescent-onset; **ADD Health:** the National Longitudinal Study of Adolescent to Adult Health; **ALSWH:** Australian Longitudinal Study on Women's Health; **BCS70:** The British Cohort Study 1970; **Beijing BP:** the Beijing Blood Pressure Cohort Study; **BEST Gottenburg:** the BMI Epidemiology Study Gothenburg; **BMI:** body mass index; **BHS:** the Bogalusa Heart Study; **CDAH:** the Childhood Determinants of Adult Health study; **CH:** childhood-onset; **CHD:** coronary heart disease; **CHNS:** China Health Nutrition Survey; **CI:** confidence interval; **CSHRR:** the Copenhagen School Health Record Register; **CVD:** cardiovascular disease; **DLP:** dyslipidemia; **E3N:** the Etude Epidémiologique auprès de femmes de la Mutuelle Générale de l'Education Nationale cohort; **F:** females; **GCS:** the Golestan Cohort Study; **HDL-C:** high-density lipoprotein cholesterol; **HR:** hazard ratio; **HTN:** hypertension; **LDL-C:** low-density lipoprotein cholesterol; **M:** male; **MACE:** major adverse cardiovascular event; **MCCS:** The Melbourne Collaborative Cohort Study; **MRC NSHD:** Medical Research Council National Survey of Health and Development; **MUSC:** the Muscatine Study; **NA:** not applicable **NAFLD:** non-alcoholic fatty liver disease; **NCDS:** The National Childhood Development Survey; **NFBC 1966:** Northern Finland Birth Cohort 1966; **NHLBI LRC & PFS:** National Heart, Lung, and Blood Institute Lipid Research Clinics study and Princeton follow-up study; **NHS:** the Nurses' Health Study; **HPFS:** the Health Professionals follow-up Study; **NHSII:** The Nurses' Health Study II; **NW:** normal weight; **OW:** overweight; **OB:** obesity; **OR:** odds ratio; **PHBPC:** Prevention of High Blood Pressure in Children study; **Prog.:** progressive; **RR:** risk ratio; **SDPP:** the Stockholm Diabetes Prevention Program cohort; **T2D:** type 2 diabetes; **TG:** triglycerides; **UK:** United Kingdom; **USA:** United States of America; **YFS:** the Cardiovascular Risk in Young Finns Study

Table S3. Cohort studies assessing the odds, risk or hazard rate of cardiometabolic diseases in people with different ages of overweight or obesity onset versus people who have never lived with overweight or obesity

Reference	Cohort, country (birth year)	Sample size (% female)	Subgroup	Overweight/ obesity onset group	Ages adiposity assessed					Age outcome assessed	Relative effect			Comparisons between obesity onset groups
					Adiposity status[a]					Outcome	Type	Point estimate (95% CI)	Adjustment variables	
Hypertension														
Studies assessing adiposity status at three or more time points														
Park et al, 2013[b]	MRC NSHD, UK (1946) NCDS, UK (1958) BCS70, UK (1970)	11476 (51% F)			**7-10 y[c]**		**15–16 y[c]**		**34–53 y**	**34–53 y**	OR		sex, year of birth, exact age and height at childhood BMI measurement, birth weight, socioeconomic status at birth and in adulthood, and smoking status in adulthood	
				CH_1	OW/OB	→	OW/OB	→	OB			2.56 (1.40, 4.68)		$CH_1 \approx ADL/AD$[d]
				CH/ADL	NW	→	OW/OB	→	OB			3.01 (2.11, 4.29)		
				CH_2	OW/OB	→	NW	→	OB	HTN		2.91 (1.54, 5.49)		
				ADL/AD	NW	→	NW	→	OB			2.28 (1.76, 2.95)		
				Never	*NW*	→	*NW*	→	*NW*			*Reference*		
Attard et al, 2013	ADD Health, USA (1975–83)	13984 (48% F)	M; moderate obesity		**15 y**		**20 y**		**27 y**	**24–32 y**			age in 1996, smoking status in 1996, 2001-2002, and 2008-2009, race, region, cluster at the school level, parental history of diabetes, anti-inflammatory medication use, presence of subclinical infection markers, and current infections/inflammatory disease	
				CH/ADL_1	$OB^{BMI\sim36}$	→	$OB^{\sim36\ kg/m^2}$	→	$OB^{\sim36\ kg/m^2}$			3.56 (2.74, 4.61)		$CH/ADL_2 > CH/ADL_3$[d]
				CH/ADL_2	$OB^{\sim30\ kg/m^2}$	→	$OB^{\sim36\ kg/m^2}$	→	$OB^{\sim36\ kg/m^2}$			3.99 (3.16, 5.03)		
				CH/ADL_3	$OB^{\sim30\ kg/m^2}$	→	$OB^{\sim33\ kg/m^2}$	→	$OB^{\sim36\ kg/m^2}$			3.35 (2.77, 4.06)		
				CH/ADL_4	$OB^{\sim30\ kg/m^2}$	→	$OB^{\sim30\ kg/m^2}$	→	$OB^{\sim36\ kg/m^2}$	HTN		3.30 (2.64, 4.12)		
				ADL	$NW^{\sim23\ kg/m^2}$	→	$OB^{\sim30\ kg/m^2}$	→	$OB^{\sim36\ kg/m^2}$			3.38 (2.48, 4.61)		
				Never	*$NW^{\sim23\ kg/m^2}$*	→	*$NW^{\sim23\ kg/m^2}$*	→	*$NW^{\sim23\ kg/m^2}$*			*Reference*		
			M; mild obesity		**15 y**		**20 y**		**27 y**	**24–32 y**				
				CH/ADL_1	$OB^{\sim30\ kg/m^2}$	→	$OB^{\sim30\ kg/m^2}$	→	$OB^{\sim30\ kg/m^2}$			2.08 (1.81, 2.39)		$AD_1 > AD_2$[d]
				ADL_2	$NW^{\sim23\ kg/m^2}$	→	$OB^{\sim30\ kg/m^2}$	→	$OB^{\sim30\ kg/m^2}$			2.13 (1.73, 2.61)		
				AD_1	$NW^{\sim23\ kg/m^2}$	→	$OW^{\sim26\ kg/m^2}$	→	$OB^{\sim30\ kg/m^2}$	HTN		1.99 (1.66, 2.38)		
				AD_2	$NW^{\sim23\ kg/m^2}$	→	$NW^{\sim23\ kg/m^2}$	→	$OB^{\sim30\ kg/m^2}$			1.76 (1.44, 2.14)		
				Never	*$NW^{\sim23\ kg/m^2}$*	→	*$NW^{23\ kg/m^2}$*	→	*$NW^{23\ kg/m^2}$*			*Reference*		
			F; moderate obesity		**15 y**		**20 y**		**27 y**	**24–32 y**				
				CH/ADL_1	$OB^{\sim36\ kg/m^2}$	→	$OB^{\sim36\ kg/m^2}$	→	$OB^{\sim36\ kg/m^2}$			2.84 (2.27, 3.55)		$CH/ADL_2 > CH/ADL_1$ & CH/ADL_3[d]
				CH/ADL_2	$OB^{\sim30\ kg/m^2}$	→	$OB^{\sim36\ kg/m^2}$	→	$OB^{\sim36\ kg/m^2}$			3.43 (2.84, 4.13)		
				CH/ADL_3	$OB^{\sim30\ kg/m^2}$	→	$OB^{\sim33\ kg/m^2}$	→	$OB^{\sim36\ kg/m^2}$			2.90 (2.46, 3.42)		
				CH/ADL_4	$OB^{\sim30\ kg/m^2}$	→	$OB^{\sim30\ kg/m^2}$	→	$OB^{\sim36\ kg/m^2}$	HTN		2.85 (2.34, 3.47)		
				ADL	$NW^{\sim23\ kg/m^2}$	→	$OB^{\sim30\ kg/m^2}$	→	$OB^{\sim36\ kg/m^2}$			3.20 (2.54, 4.02)		
				Never	*$NW^{\sim23\ kg/m^2}$*	→	*$NW^{\sim23\ kg/m^2}$*	→	*$NW^{\sim23\ kg/m^2}$*			*Reference*		

			F; mild obesity	**15 y**		**20 y**		**27 y**	**24–32 y**				
				CH/ADL$_1$	OB$^{\sim 30\ kg/m^2}$	→	OB$^{\sim 30\ kg/m^2}$	→	OB$^{\sim 30\ kg/m^2}$			1.83 (1.63, 2.07)		AD$_1$ > AD$_2$[d]
				ADL$_2$	NW$^{\sim 23\ kg/m^2}$	→	OB$^{\sim 30\ kg/m^2}$	→	OB$^{\sim 30\ kg/m^2}$			2.06 (1.76, 2.41)		
				AD$_1$	NW$^{\sim 23\ kg/m^2}$	→	OW$^{\sim 26\ kg/m^2}$	→	OB$^{\sim 30\ kg/m^2}$	HTN		1.93 (1.69, 2.20)		
				AD$_2$	NW$^{\sim 23\ kg/m^2}$	→	NW$^{\sim 23\ kg/m^2}$	→	OB$^{\sim 30\ kg/m^2}$			1.71 (1.47, 2.00)		
				Never	*NW$^{\sim 23\ kg/m^2}$*	→	*NW$^{23\ kg/m^2}$*	→	*NW$^{23\ kg/m^2}$*			*Reference*		
Dyslipidemia													
Studies assessing adiposity status at two time points													
Yan et al., 2019[e]	BHS, USA (1955–92) MUSC, USA (1952–73) NGHS, USA (1977–78) PHBPC, USA (1969–72) YFS, Finland (1962–77)	5195 (60% F)		**3–19 y**[f]		**20–52 y**			**25–52 y**	**OR**		2.02 (1.74, 2.78)	race, sex, age, and cohort	No group differences[g]
				CH/ADL	OW/OB	→	OW/OB							
				CH/ADL/AD	NW	→	OW/OB		High LDL-C			2.37 (1.84, 3.03)		
				Never	*NW*	→	*NW*					*Reference*		
				3–19 y[f]		**20–52 y**			**25–52 y**			3.88 (3.18, 4.72)		No group differences[g]
				CH/ADL	OW/OB	→	OW/OB							
				CH/ADL/AD	NW	→	OW/OB		Low HDL-C			3.38 (2.74, 4.19)		
				Never	*NW*	→	*NW*					*Reference*		
				3–19 y[f]		**20–52 y**			**25–52 y**			3.05 (2.35, 3.97)		No group differences[g]
				CH/ADL	OW/OB	→	OW/OB							
				CH/ADL/AD	NW	→	OW/OB		High TG			3.20 (2.43, 4.22)		
				Never	*NW*	→	*NW*					*Reference*		
Type 2 diabetes													
Studies assessing adiposity status at three or more time points													
Power and Thomas, 2011	NCDS, UK (1958)	7855 (50% F)		**7,11, or 16 y**[c]		**23 or 33 y**		**45 y**	**45 y**	**OR**		4.38 (1.86, 10.31)	BMI, waist circumference, sex, total cholesterol, HDL-C, family history of diabetes, menopausal status, smoking and alcohol consumption at 42 years, social class in childhood and at 45 years, and qualifications by 42 years	Linear trend from Never to CH/ADL[d]
				CH/ADL	OB	→	OB	→	OB					
				CH/ADL/AD	NW/OW	→	OB	→	OB	T2D		3.96 (2.10, 7.43)		
				AD	NW/OW	→	NW/OW	→	OB			1.13 (0.61, 2.08)		
				Never	*NW/OW*	→	*NW/OW*	→	*NW/OW*			*Reference*		
Park et al, 2013[b]	MRC NSHD, UK (1946) NCDS, UK (1958) BCS70, UK (1970)	11476 (51% F)		**7–10 y**[c]		**15–16 y**[c]		**34–53 y**	**34–53 y**			12.60 (6.61, 23.98)	sex, year of birth, exact age and height at childhood BMI measurement, birth weight, socioeconomic status at birth and in adulthood, and smoking status in adulthood	CH$_1$ > ADL/AD[d]
				CH$_1$	OW/OB	→	OW/OB	→	OB					
				CH/ADL	NW	→	OW/OB	→	OB			6.61 (3.61, 12.09)		
				CH$_2$	OW/OB	→	NW	→	OB	T2D		4.70 (1.89, 11.67)		
				ADL/AD	NW	→	NW	→	OB			5.47 (3.39, 8.82)		
				Never	*NW*	→	*NW*	→	*NW*			*Reference*		

Attard et al, 2013	ADD Health, USA (1975–83)	13984 (48% F)	M; moderate obesity		**15 y**		**20 y**		**27 y**	**24–32 y**			
				CH/ADL$_1$	OB$^{BMI\sim36}$	→	OB$^{\sim36\ kg/m^2}$	→	OB$^{\sim36\ kg/m^2}$			4.37 (3.07, 6.22)	CH/ADL$_2$ > CH/ADL$_3$ & CH/ADL$_4$ & ADL[d]
				CH/ADL$_2$	OB$^{\sim30\ kg/m^2}$	→	OB$^{\sim36\ kg/m^2}$	→	OB$^{\sim36\ kg/m^2}$			4.90 (3.10, 7.74)	
				CH/ADL$_3$	OB$^{\sim30\ kg/m^2}$	→	OB$^{\sim33\ kg/m^2}$	→	OB$^{\sim36\ kg/m^2}$	Diabetes		3.39 (2.53, 4.55)	CH/ADL$_4$ > ADL[d]
				CH/ADL$_4$	OB$^{\sim30\ kg/m^2}$	→	OB$^{\sim30\ kg/m^2}$	→	OB$^{\sim36\ kg/m^2}$			2.99 (2.20, 4.08)	
				ADL	NW$^{\sim23\ kg/m^2}$	→	OB$^{\sim30\ kg/m^2}$	→	OB$^{\sim36\ kg/m^2}$			3.02 (1.77, 5.17)	
				Never	*NW$^{\sim23\ kg/m^2}$*	→	*NW$^{\sim23\ kg/m^2}$*	→	*NW$^{\sim23\ kg/m^2}$*			*Reference*	
			M; mild obesity		**15 y**		**20 y**		**27 y**	**24–32 y**			
				CH/ADL	OB$^{\sim30\ kg/m^2}$	→	OB$^{\sim30\ kg/m^2}$	→	OB$^{\sim30\ kg/m^2}$			2.33 (1.92, 2.83)	CH/ADL$_1$ & ADL$_2$ & AD$_1$ > AD$_2$[d]
				ADL	NW$^{\sim23\ kg/m^2}$	→	OB$^{\sim30\ kg/m^2}$	→	OB$^{\sim30\ kg/m^2}$			2.35 (1.51, 3.66)	
				AD$_1$	NW$^{\sim23\ kg/m^2}$	→	OW$^{\sim26\ kg/m^2}$	→	OB$^{\sim30\ kg/m^2}$	Diabetes		1.84 (1.37, 2.47)	ADL$_2$ > AD$_1$[d]
				AD$_2$	NW$^{\sim23\ kg/m^2}$	→	NW$^{\sim23\ kg/m^2}$	→	OB$^{\sim30\ kg/m^2}$			1.44 (1.10, 1.87)	
				Never	*NW$^{\sim23\ kg/m^2}$*	→	*NW$^{23\ kg/m^2}$*	→	*NW$^{23\ kg/m^2}$*			*Reference*	
			F; moderate obesity		**15 y**		**20 y**		**27 y**	**24–32 y**			
				CH/ADL$_1$	OB$^{\sim36\ kg/m^2}$	→	OB$^{\sim36\ kg/m^2}$	→	OB$^{\sim36\ kg/m^2}$			2.99 (2.16, 4.14)	CH/ADL$_2$ > CH/ADL$_3$ & CH/ADL$_4$ & ADL[d]
				CH/ADL$_2$	OB$^{\sim30\ kg/m^2}$	→	OB$^{\sim36\ kg/m^2}$	→	OB$^{\sim36\ kg/m^2}$			3.41 (2.41, 4.81)	
				CH/ADL$_3$	OB$^{\sim30\ kg/m^2}$	→	OB$^{\sim33\ kg/m^2}$	→	OB$^{\sim36\ kg/m^2}$	Diabetes		2.46 (1.93, 3.13)	CH/ADL$_4$ > ADL[d]
				CH/ADL$_4$	OB$^{\sim30\ kg/m^2}$	→	OB$^{\sim30\ kg/m^2}$	→	OB$^{\sim36\ kg/m^2}$			2.18 (1.62, 2.94)	
				ADL	NW$^{\sim23\ kg/m^2}$	→	OB$^{\sim30\ kg/m^2}$	→	OB$^{\sim36\ kg/m^2}$			2.30 (1.52, 3.49)	
				Never	*NW$^{\sim23\ kg/m^2}$*	→	*NW$^{\sim23\ kg/m^2}$*	→	*NW$^{\sim23\ kg/m^2}$*			*Reference*	
			F; mild obesity		**15 y**		**20 y**		**27 y**	**24–32 y**			
				CH/ADL	OB$^{\sim30\ kg/m^2}$	→	OB$^{\sim30\ kg/m^2}$	→	OB$^{\sim30\ kg/m^2}$			1.88 (1.58, 2.23)	CH/ADL$_1$ & ADL$_2$ & AD$_1$ > AD$_2$[d]
				ADL	NW$^{\sim23\ kg/m^2}$	→	OB$^{\sim30\ kg/m^2}$	→	OB$^{\sim30\ kg/m^2}$			1.98 (1.39, 2.82)	
				AD$_1$	NW$^{\sim23\ kg/m^2}$	→	OW$^{\sim26\ kg/m^2}$	→	OB$^{\sim30\ kg/m^2}$	Diabetes		1.58 (1.25,1.99)	ADL$_2$ > AD$_1$[d]
				AD$_2$	NW$^{\sim23\ kg/m^2}$	→	NW$^{\sim23\ kg/m^2}$	→	OB$^{\sim30\ kg/m^2}$			1.27 (0.99, 1.62)	
				Never	*NW$^{\sim23\ kg/m^2}$*	→	*NW$^{23\ kg/m^2}$*	→	*NW$^{23\ kg/m^2}$*			*Reference*	
Yeung et al, 2010[b]	NHSII, USA (1947–64)	109172 (100% F)			**10 y**[h]		**18 y**		**25–42 y**	**41–58 y**	**RR**		age, race, smoking status, parental history of diabetes, parity, age at first birth, and physical activity
				CH$_1$	OW/OB	→	OW/OB	→	OW/OB			15.10 (13.21, 17.26)	CH$_1$ & CH/ADL > CH$_2$ & AD[g]
				CH/ADL	NW	→	OW/OB	→	OW/OB			14.37 (12.73, 16.22)	
				CH$_2$	OW/OB	→	NW	→	OW/OB	T2D		8.72 (7.47, 10.18)	
				AD	NW	→	NW	→	OW/OB			8.23 (7.41, 9.15)	
				Never	*NW*	→	*NW*	→	*NW*			*Reference*	

Major adverse cardiovascular events												
Studies assessing adiposity status at two time points												
Shimazu et al., 2009[i]	Ohsaki NHI, Japan (1915–54)	30080 (51% F)		**20 y**		**40–79 y**		**40–85 y**			age, sex, smoking status, alcohol consumption, daily, walking duration, and education	No group differences[g]
			CH/ADL_1	OB	→	OB		CVD mortality		2.74 (1.36, 5.54)		
			CH/AD_2	OW	→	OB				1.80 (0.99, 3.28)		
			AD	NW	→	OB				1.58 (0.97, 2.57)		
			Never	*NW*	→	*NW/OW*				*Reference*		
Etemadi et al., 2014[j]	GCS, Iran (1929–64)	50006 (58% F)		**15 y**[k]		**30 y**[k]		**40–75 y**	**HR**		age, smoking, socioeconomic status, ethnicity, residence, education, opium use, and physical activity	No group differences[g]
			CH/ADL	OB	→	OB				1.32 (0.93, 1.88)		
		M	ADL/AD	UW/NW/OW	→	OB				1.57 (1.17, 2.10)		
			NA	*NW*	→	*NW*				*Reference*		
			CH/ADL	OB	→	OB		CVD mortality		1.41 (1.12, 1.78)		No group differences[g]
		F	ADL/AD	NW/OW	→	OB				1.71 (1.30, 2.25)		
			Never	*NW*	→	*NW*				*Reference*		
Studies that assessed adiposity status at three or more time points												
Park et al, 2013[b]	MRC NSHD, UK (1946) NCDS, UK (1958) BCS70, UK (1970)	11476 (51% F)		**7–10 y**[c]		**15–16 y**[c]	**34–53 y**	**34–53 y**	**OR**		sex, year of birth, exact age and height at childhood BMI measurement, birth weight, socioeconomic status at birth and in adulthood, and smoking status in adulthood	CH > ADL/AD (trend)[d]
			CH_1	OW/OB	→	OW/OB	→ OB			6.62 (1.94, 22.67)		
			CH/ADL	NW	→	OW/OB	→ OB			3.74 (1.35, 10.35)		
			CH_2	OW/OB	→	NW	→ OB	T2D		1.10 (0.14, 8.48)		
			ADL/AD	NW	→	NW	→ OB			3.83 (1.98, 7.42)		
			Never	*NW*	→	*NW*	→ *NW*			*Reference*		

The table displays the results of cohort studies in which the odds, risk, or hazard rate of cardiometabolic diseases could not be statistically compared between different obesity-onset groups. The results are therefore displayed with the original reference group (those with persistent normal weight). These studies assessed adiposity status at two or three time points between childhood and adulthood. Obesity-onset was classified based on these adiposity status changes. We present the results of comparisons between obesity-onset groups, as stated by the study authors or derived from our interpretation.

[a]Adiposity status in adulthood was defined according to standard BMI categories unless otherwise indicated (normal weight: 18.5–<25 kg/m^2; overweight: 25–<30 kg/m^2; obesity: ≥ 30 kg/m^2): Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser 894, i–xii, 1–253 (2000).

[b]Model included adiposity statuses OW/OB → NW → NW, OB/OW -> OW/OB → NW, and NW → OW/OB -> NW

[c]Adiposity status in childhood/adolescence was defined according to the international sex- and age-specific percentiles that extrapolate to the standard adult BMI categories: de Onis, M. et al. Development of a WHO growth reference for school-aged children and adolescents. Bull. World Health Organ. 85, 660–667 (2007).

[d]Reported by the study authors

[e]Model included adiposity status OW/OB → NW

[f]NW: BMI < race-sex specific median; OW/OB: BMI > race-sex specific median

[g]Our interpretation

[h]Participants were asked to recall their adiposity status using the Stunkard body rating scale (OW/OB: somatotype ≥ 5): Stunkard, A. J., Sørensen, T. & Schulsinger, F. Use of the Danish Adoption Register for the study of obesity and thinness. Res. Publ. - Assoc. Res. Nerv. Ment. Dis. 60, 115–120 (1983).

[i]Model also included the following adiposity status changes: NW → OW and OW → OW

[j]Model included adiposity statuses UW → UW, UW → NW/OW/OB, and OB → NW/OW

[k]Participants were asked to recall their adiposity status using the Stunkard body rating scale (UW: somatotype 1; NW: somatotype 2 & 3; OW: somatotype 4 & 5; OB: somatotype ≥ 6): Stunkard, A. J., Sørensen, T. & Schulsinger, F. Use of the Danish Adoption Register for the study of obesity and thinness. Res. Publ. - Assoc. Res. Nerv. Ment. Dis. 60, 115–120 (1983).

Abbreviations:

AD: adult-onset; **ADL:** adolescent-onset; **ADD Health:** the National Longitudinal Study of Adolescent to Adult Health; **BCS70:** The British Cohort Study 1970; **BMI:** body mass index; **BHS:** the Bogalusa Heart Study; **CH:** childhood-onset; **CI:** confidence interval; **CVD:** cardiovascular disease; **F:** females; **GCS:** Golestan Cohort Study; **HDL-C:** high-density lipoprotein cholesterol; **HR:** hazard ratio; **HTN:** hypertension; **LDL-C:** low-density lipoprotein cholesterol; **M:** male; **MRC NSHD:** Medical Research Council National Survey of Health and Development; **NCDS:** The National Childhood Development Survey; **HPFS:** the Health Professionals follow-up Study; **NHSII:** The Nurses' Health Study II; **NW:** normal weight; **Ohsaki NHI:** National Health Insurance Cohort Study **OW:** overweight; **OB:** obesity; **OR:** odds ratio; **RR:** risk ratio; **T2D:** type 2 diabetes; **TG:** triglycerides; **UK:** United Kingdom; **UW:** underweight; **USA:** United States of America; **YFS:** the Cardiovasc

Table S4. Weight loss outcomes in people with childhood-onset and adult-onset obesity

Reference	Intervention		Obesity onset		Baseline participant characteristics			Weight loss	
	Description	Length[a] or follow-up period[b]	Method of determination	Group	*N* (% F)	Mean (SD or range) or n (%)[c]			Group difference summary[d]
						Age (y)	Weight/ adiposity measures	Outcome	
Lifestyle and/or pharmacological weight loss interventions									
Young et al., 1955	▪ Experimental community nutrition clinic ▪ Weekly visits with physician and nutritionist ▪ A variety of WL diets	≥3 wk	*Self-report* ▪ Weight history interview	CO$^{<18\ y}$	143 (85% F[e])	(6-70)	NR	'complete failures': 50% 'fairly successful': 30%	CO < AO
				AO$^{\geq 18\ y}$				'complete failures': 15% 'fairly successful': 57%	
Mullins et al., 1958	▪ Hypocaloric diet (kcal/d NR) ▪ Drugs, mainly dextroamphetamine sulphate, when not possible to treat by dietary means alone	4 mo	*Self-report* ▪ Classified according to whether they had been known as 'fatty' at school ('the most practical single criterion')	CO	32 (84%)	(18-69)	EW 20-25%: 4 (12%) EW 25-50%: 11 (35%) EW >50%: 17 (35%)	Poor[f]: 20 (62%) Moderate[g]: 8 (25%) Good[h]: 0 (0%) Not yet known: 4 (13%)	CO < AO
				AO	69 (71%)	(20-79)	EW 20-25%: 28 (41%) EW 25-50%: 21 (30%) EW >50%: 20 (29%)	Poor[f]: 27 (39%) Moderate[g]: 17 (25%) Good[h]: 10 (14%) Not yet known: 15 (22%)	
Johnson et al, 1976	▪ Hypocaloric diet (800 to 1200 kcal/d) ▪ Encouraged to establish a regular exercise program	4-28 mo	NR	CO$^{1-11\ y}$	32 (59%)	33	EW: 93 %	WL ≥ 10% or EW ≤ 20% at 4-6 mo: 19 (59%) at 7-10 mo: 10 (67%) at 11-15 mo: 11 (65%) at 16-28 mo: 7 (64%)	Ø
				CO/AO$^{\geq 12\ y}$	22 (82%)	44	EW: 74 %	WL ≥ 10% or EW ≤ 20% at 4-6 mo: 10 (45%) at 7-10 mo: 4 (50%) at 11-15 mo: 9 (56%) at 16-28 mo: 6 (60%)	
Rodin et al, 1977	▪ Hospital outpatient weight reduction clinic	10 wk	NR	CO$^{<10\ y}$	18 (100%)	32.5	EW: 85.5 %	WL: 8.7 kg	NR
				AO$^{>19\ y}$	15 (100%)	40.5	EW: 69.7 %	WL: 6.7 kg	
Drenick et al., 1978	▪ Hospitalized fast (planned for 2 mo duration but could be longer if desired by the patient and considered medically safe by the physician). ▪ Patients with OW after fasting were encouraged to adhere to a VLCD (500 kcal/d; 50 g of protein) and to continue to reduce weight as outpatients (not followed regularly)	~2 mo	*Self-report* ▪ Weight history (NR if interview or questionnaire)	CO$^{0-5\ y}$	27 (7%)	42 (25-76)	EW: 95 % W: 147 kg	EW <30%: 7 (25.9%)	CO$^{\leq 10\ y}$ < CO/AO$^{>10\ y}$
				CO$^{6-10\ y}$	15	42	EW: 88 % W: 139 kg	EW <30%: 4 (26.7%)	
				CO^{11-15y}	19	37	EW: 85 % W: 139 kg	EW <30%: 4 (47.4%)	
				CO$^{16-21\ y}$	32	40	EW: 93 % W: 152 kg	EW <30%: 9 (40.6%)	
				AO$^{\geq 22\ y}$	68	44	EW: 90 % W: 146 kg	EW <30%: 27 (39.7%)	

Jeffery et al., 1978	▪ Behavioural weight loss Intervention in clinical setting ▪ Weekly individual meetings with therapist to review eating and exercise records ▪ Weekly group sessions with lessons on the following topics: self-monitoring, stimulus control techniques, slowing the rate of eating, generating social support, exercise, dietary planning, preplanning, and individual problem solving	20 wk	*Self-report* ▪ Weight history questionnaire	CO$^{\leq 20\ y}$	47 (82%)	41 (16-63)	EW: 65 %	WL: 7.0 kg	CO > AO
				AO$^{> 20\ y}$	24		EW: 72 %	WL : 5.4 kg	
Genender et al., 1982	▪ Hypocaloric diet (Optifast; 300 kcal/d) ▪ Offered extended care resources: psychological support groups, individual psychotherapy (when indicated or requested), behavior modification classes, and nutrition and exercise counseling	22 wk (mean)	*Self-reported* ▪ NR if interview or questionnaire	CO$^{1\text{-}12\ y}$ *F; EW 40-150%*	34 (100%)	39 (18-64)	EW: 79 (40-150) % W: 109 kg	EWL: 58 % WL: 26 kg	Ø
				CO$^{13\text{-}19\ y}$ *F; EW 40-150%*	62 (100%)	33 (16-61)	EW: 81 (40-150) % W: 108 kg	EWL: 58 % WL: 27 kg	
				AO$^{\geq 20\ y}$ *F; EW 40-150%*	302 (100%)	44 (21-67)	EW: 75 (40-150) % W: 102 kg	EWL: 61 % WL: 27 kg	
				CO$^{1\text{-}12\ y}$ *M; EW 40-150%*	42 (0%)	39 (18-64)	EW:78 (40-150) % W:126 kg	EWL : 70 % WL: 38 kg	Ø
				CO$^{13\text{-}19\ y}$ *M; EW 40-150%*	86 (0%)	33 (16-61)	EW: 82 (40-150) % W: 133 kg	EWL : 58 % WL : 33 kg	
				AO$^{\geq 20\ y}$ *M; EW 40-150%*	445 (0%)	44 (21-67)	EW: 74 (40-150) % W:121 kg	EWL : 66 % WL : 33 kg	
				CO$^{13\text{-}19\ y}$ *F; EW 150-250%*	2 (100%)	43 (16-61)	EW: 178 (150-250) % W: 151 kg	EWL: 17 % WL: 17 kg	Ø
				AO$^{\geq 20\ y}$ *F; EW 150-250%*	4 (100%)	47 (21-67)	EW: 170 (150-250) % W: 153 kg	EWL: 34 % WL: 33 kg	
				CO$^{13\text{-}19\ y}$ *M; EW 150-250%*	4 (0%)	32 (16-61)	EW: 188 (150-250) % W: 200 kg	EWL: 48 % WL: 64 kg	Ø
				AO$^{\geq 20\ y}$ *M; EW 150-250%*	5 (0%)	47 (21-67)	EW: 170 (150-250) % W: 177 kg	EWL: 57 % WL: 62 kg	
Nasr et al., 1982	▪ Behavioural weight management intervention ▪ Dietary regimen (protein sparing modified fast; 1.5-2.0 g protein/kg IW), fitness counseling, behavior modification, and supportive psychotherapy	3 mo	*Self-report* ▪ Weight history questionnaire and interview	CO$^{\leq 11\ y}$	8 (75%)	33 (26-39)	BMI: 46 (29-73) kg/m^2	WL: 19.5 (3.6) %	CO > AO
				AO$^{\geq 18\ y}$	8 (75%)	39 (26-63)	BMI: 39 (26-63) kg/m^2	WL: 16.2 (2.8) %	
Sorbara et al., 2002	▪ Medically supervised liquid formula diet (900 kcal/d): 5 packets of Procal (R-Kane) and	4 wk	*Self-report*	CO$^{< 18\ y}$	36 (70%)	42 (11)	W: 102 (19) kg BMI: 37 (6) kg/m^2 FM: 44 (7) %	BMIL: NR kg/m^2	Ø

	▪ 3.5 cups of 1% milk, plus 2 packets of fiber supplements weekly one-on-one counseling sessions		▪ Questionnaire: Eating and Weight Patterns-Revised (QEWP-R)	AO$^{\geq 18\ y}$	20 (70%)	44 (9)	W: 98 (17) kg BMI: 37 (4) kg/m^2 FM: 42 (5) %	BMIL: NR kg/m^2	
Guerdjikova et al., 2007	▪ Fee-for-service weight management program ▪ Psychopharmacological treatment of any identified psychiatric illness that might contribute to overeating and/or physical inactivity ▪ Behavioral dietary counseling (focusing on healthy eating, exercise, and other lifestyle changes) strongly encouraged but not mandatory	6 mo	*Self-report* ▪ Written report of weight history and obesity onset and 2 interviews ▪ Patients recollected and recorded the weight, height, and age when they first considered themselves to have OW/OB and felt distressed because of their weight ▪ Calculated BMI, Z-score for weight-for-age, and percentile for BMI-for-age ▪ Childhood-onset (2-17 y): Z-score > 2 and percentile for BMI-for-age > 95 ▪ Adult-onset (≥ 18 y): BMI ≥ 30 kg/m^2	CO$^{2\text{-}17\ y}$	44 (66%)	42 (9)	W: 136 (32) kg BMI: 45 (9) kg/m^2	WL: 6.3 %	Ø
				AO$^{\geq 18\ y}$	69 (65%)	44 (13)	W: 119 (27) kg BMI: 43 (9) kg/m^2	WL: 5.3 %	
Rupp et al., 2016	▪ Participants were randomized into one of three intervention groups: standard behavioral weight loss; standard behavioral weight loss with additional strategies at the initiation of the intervention; or standard behavioral weight loss with additional strategies at predetermined times over the intervention period. ▪ All three intervention groups received group intervention sessions; a dietary prescription for reduced caloric intake (1200 kcal/d for participants weighing ≤ 90 kg) and 1500 kcal/d for participants weighting > 90 kg; 20-30% fat) and a prescription for physical activity (progressive from 100 min/wk to 200 min/wk at moderate-to-vigorous intensity) ▪ Additional strategies in 2 of the intervention groups included supervised exercise at group sessions, additional campaign to promote physical activity, and additional telephone contacts	1.5 y	*Self-report* ▪ Weight history questionnaire asked 'Select whether you were extremely underweight, underweight, normal weight, overweight or extremely overweight at each of the following ages': pre-school; elementary school; junior high (12–14 y); high school (15–18 y); 19–25 y; and 26–35 y ▪ 'juvenile onset': overweight or extremely overweight at age 15–17 y ▪ 'adult onset': overweight or extremely overweight at age ≥ 19 y ▪ Excluded if reported being overweight or extremely overweight at age < 15 y but not at age 15–17 y ▪	CO$^{\leq 18\ y}$	61 (82%)	40 (32-48)[j]	W[k]: 102 (99, 105) kg BMI: 34 (3) kg/m^2	WL[k] at 6 mo: 8.7 (6.7, 10.8) kg at 1 y: 7.8 (5.2, 10.4) kg at 1.5 y: 5.2 (2.5, 8.0) kg	Ø
				AO$^{> 18\ y}$	116 (77%)	47 (40-50)[j]	W[k]: 99 (97, 102) kg BMI: 33 (3) kg/m^2	WL[k] at 6 mo: 9.4 (7.9, 11.0) kg at 1 y: 8.8 (6.9, 10.6) kg at 1.5 y: 7.1 (5.2, 9.1) kg	

Akter et al., 2020	▪ Hypocaloric diet (1120 kcal/d): randomized to total meal replacement (Optifast 800) or typical diet (portion control to reduce calories but no significant changes to typical foods eaten)	3 wk	*Self-report* ▪ Weight history questionnaire asked, 'At what age did you first consider yourself overweight?' ▪ Response options: never; childhood (0-12); adolescence (13-17); young adult (18-25); adult (26-49); late adult (50+)	CO[0-12 y]	8 (63%)	32 (13)	BMI: 35 (3) kg/m^2	WL[i]: 3.05 kg FL[i]: 2.11 kg	Ø
				CO[13-17 y]	4 (50%)			WL[i]: 3.8 kg FL[i]: 0.06 kg	
				AO[18-25 y]	8 (50%)			WL[i]: 4.57 kg FL[i]: 2.82 kg	
				AO[26-49 y]	7 (57%)			WL[i]: 2.97 kg FL[i]: 1.64 kg	
				AO[≥ 50 y]	1 (100%)			WL[i]: 1.5 kg FL[i]: 0.9 kg	
Prado et al., 2023	▪ Obesity outpatient clinic ▪ Medical, psychological, and nutritional care appointments ▪ Prescription of medications for weight loss: sibutramine, topiramate, liraglutide, fluoxetine, orlistat, bupropion, or a combination of two or more drugs	2 y (mean)	*Self-report* ▪ Questionnaire	CO[< 18 y]	40 (78%)	36 (10)	W: 124 (28) kg BMI: 46 (9) kg/m^2	at 1 y WL[j]: 2.6 (-0.4, 6.1) kg WL[j]: 2.1 (-0.3, 6.4) % WL ≥ 10%: 6 (15.0%) at end of treatment WL[j]: 2.4 (-2.4, 6.6) kg	Ø
				AO[≥ 18 y]	84 (87%)	48 (11)	W: 109 (19) kg BMI: 41 (6) kg/m^2	at 1 y WL[j]: 3.0 (-0.8, 7.1) kg WL[j]: 2.8 (-0.9, 7.5) % WL ≥ 10%: 12 (14.5%) at end of treatment WL[j]: 2.4 (-1.2, 7.9) kg	
Almeida et al., 2024	▪ Hypocaloric diet (20% energy deficit): 50–60% carbohydrate, 20–30% fat, 20% protein. ▪ 10% increase in energy expenditure through aerobic exercise: three 45-minute treadmill or elliptical sessions per week with one session supervised ▪ Weekly follow-ups	12 wk	*Self-report* ▪ Weight history interview ▪ Aked to recall body size at age 10, puberty, age 20, and current age using the Collins[l] and Stunkard[m] body rating scales as appropriate. *Objective* ▪ Participants provided photographic proof of body size around puberty (10–14 years old for females and 12–15 years for males). ▪ The photograph was compared against the Collins[l] body rating scale	CO[pre/peri-puberty]	13 (68%)	30 (1)[n]	W: 95 (3)[n] kg FM: 40 (1)[n] kg FM: 43 (1)[n] %	WL: 3.6 kg FM: 2.5 kg FM: 1.1 %	Ø
				AO[> 18 y]	12 (67%)	31 (1)[n]	W: 91 (3)[n] kg FM: 38 (1)[n] kg FM: 42 (1)[n] %	WL: 3.6 kg FM: 3.5 kg FM: 2.1 %	

Bariatric surgery interventions									
Pekkarinen et al., 1994	▪ Vertical banded gastroplasty	5.4 y (mean)	NR	$Co^{pre\text{-}puberty}$ $CO/AO^{post\text{-}puberty}$	27 (70%)	36 (22-48)	W: 141 (108-206) kg BMI: 50 (39-65) kg/m^2 EW: 116 (NR) %	WL: NR kg WL: NR kg	Ø
Rowe et al., 2000	▪ Silastic ring vertical stapled gastroplasty	6 mo	*Self-report* ▪ Weight history interview	$CO^{< 18\ y}$ $AO^{\geq 18\ y}$	35 (91%)	41 (21-54)	W: 124 (94-167) kg BMI: 46 (36-60) kg/m^2	BMIL: NR % BMIL: NR %	$CO^{\leq 18\ y} > AO^{> 18\ y}$
Sillén et al.,2017	▪ Roux-en-Y gastric bypass surgery	1 y	*Self-report* ▪ Questionnaire	$CO^{Childhood}$ $CO^{Adolescence}$ AO	281 (70%)	41 (16-67)	W: 136 (85-254) kg BMI: 46 (32-81) kg/m^2	EWL ≥ 60%: 63 (63.6%) EWL ≥60%: 57 (71.3%) EWL ≥ 60%: 75 (81.5%)	$CO^{Childhood} <$ $CO^{Adolescence} <$ AO
Erdogdu et al., 2018	▪ Laparoscopic sleeve gastrectomy s	1 y	*Self-report* ▪ NR if questionnaire or interview ▪ Puberty period defined by pubertal development scale ▪ Childhood obesity: BMI percentile > 95th ▪ Obesity onset was classified as pre-puberty, puberty, and post-puberty	$CO^{pre\text{-}puberty}$ $CO^{puberty}$ $CO/AO^{post\text{-}puberty}$	81 (88%) 22 (95%) 62 (79%)	37 (16-59) 32 (22-45) 41 (23-61)	W: 130 (101-183) kg BMI: 47 (40-67) kg/m^2 W: 126 (101-175) kg BMI: 46 (35-56) W: 123 (92-165) kg BMI: 45 (35-67) kg/m^2	EWL: 62.9 (15.9) % EBMIL: 76.1 (19.8) % EWL: 72.7 (12.8) % EBMIL: 85.6 (13.8) % EWL: 62.2 (17.0) % EBMIL: 75.2 (21.8) %	EWL: $CO^{puberty} < CO^{pre\text{-}puberty}$ & $CO/AO^{post\text{-}puberty}$ EBMIL: Ø
Kristensson et al., 2020	▪ Gastric bypass, banding, or vertical-banded gastroplasty	10 y	*Self-report* ▪ Questionnaire: weight at age 20 y ▪ BMI at age 20 y: <25 kg/m^2 (normal weight), 25–29.9 kg/m^2 (overweight), or ≥30 kg/m^2 (obesity)	$CO^{OB \leq 20\ y}$ $CO^{OW \leq 20\ y,\ OB > 20\ y}$ $AO^{NW \leq 20\ y,\ OB > 20\ y}$	528 (69%) 744 (69%) 725 (74%)	45 (6) 47 (6) 49 (6)	W: 127 (18) kg BMI: 44 (5) kg/m^2 W: 120 (16) kg BMI: 42 (4) kg/m^2 W: 118 (15) kg BMI:42 (4) kg/m^2	WL[o] at 6 mo: 22.9 (22.3, 23.5) % at 1 y: 26.7 (25.9, 27.5) % at 2 y: 25.1 (24.2, 26.1) % at 3 y: 23.2 (22.1, 24,2) % at 4 y: 21.2 (20.2, 22.3) % at 6 y: 19.9 (18.8, 21.0) % at 8 y: 18.5 (17.3, 19.6) % at 10 y: 18.3 (17.1, 19.5) % WL[o] at 6 mo: 21.7 (21.1, 22.2) % at 1 y: 24.3 (23.6, 25.0) % at 2 y: 22.8 (22.0, 23.5) % at 3 y: 20.2 (19.4, 21.0) % at 4 y: 19.1 (18.2, 20.0) % at 6 y: 17.3 (16.4, 18.3) % at 8 y: 17.0 (15.9, 18.0) % at 10 y: 17.0 (16.0, 18.0) % WL[l] at 6 mo: 21.6 (21.1, 22.2) % at 1 y: 24.5 (23.8, 25.3) % at 2 y: 23.2 (22.4, 24.0) % at 3 y: 20.6 (19.8, 21.5) % at 4 y: 19.8 (18.9, 20.7) % at 6 y: 17.7 (16.8, 18.7) % at 8 y: 17.1 (16.0, 18.1) % at 10 y: 17.1 (16.1, 18.2) %	$CO^{OB \leq 20\ y} >$ $CO^{OW \leq 20\ y,\ OB > 20\ y}$ & $AO^{NW \leq 20\ y,\ OB > 20\ y}$

Fink et al., 2023	▪ Roux-en-y gastric bypass, sleeve gastrectomy, or one-anastomosis gastric bypass	1 y	NR	CO$^{<18\ y}$ AO$^{\geq 18\ y}$	14404 (74%)	44 (12)	W: 142 (27) kg BMI: 49 (8) kg/m^2	OR of WL < 20% at 1-year post-surgery (AO vs. CO): 0.84	CO$^{<18\ y}$ > AO$^{\geq 18\ y}$

[a]For lifestyle and/or pharmacological interventions

[b]For bariatric surgery interventions

[c]Unless otherwise stated or not reported

[d]Based on $p<0.05$

[e]Percent female is from original cohort (N = 152)

[f]EWL < 30%

[g]EWL > 30% but EW > 10% in cases of mild (EW 20-25%) obesity and EW >20% in cases of moderate (EW 25-50%) or severe (EW >50%) obesity

[h]EW < 10% in cases of mild (EW 20-25%) obesity and EW <20% in cases of moderate (EW 25-50%) or severe (EW >50%) obesity

[i]Adjusting for age, sex, diet group, and preintervention body weight, fat mass, and BMI

[j]Median (interquartile range)

[k]Adjusted for age, gender, race, treatment, group, and treatment group-by-time interaction

[l]Collins ME. Body figure perceptions and preferences among preadolescent children. Int J Eat Disord. 1991;10(2):199-208

[m]Stunkard AJ, Sorensen T, Schulsinger F. Use of the Danish Adoption Register for the study of obesity and thinness. Res Publ Assoc Res Nerv Ment Dis. 1983;60:115-120.

[n]Standard error of the mean

[o]Mean (95% confidence interval)

Abbreviations:
AO: group with adult-onset obesity; **BMI:** body mass index; **BMIL:** body mass index loss (decrease in BMI); **CO:** group with childhood-onset obesity; **EBMIL:** excess body mass index loss; **EW:** excess weight; **EWL:** excess weight loss; **F:** female; **FL:** fat loss (by bioelectrical impedance analysis); **FM:** fat lass; **OR:** odds ratio; **OW:** overweight; **OB:** obesity; **SD:** standard deviation; **NR:** not reported; **W:** weight; **WL:** weight loss

A note on animal models of childhood-onset versus adult-onset obesity

Although animal models have been indispensable for understanding obesity and driving treatment development, they have contributed little to distinguishing childhood-onset obesity from adult-onset obesity. Nonetheless, evidence from these models aligns with human studies, which show worse insulin resistance in adult-onset obesity despite a greater risk of type 2 diabetes in childhood-onset obesity.

Two studies have investigated how diet-induced obesity initiated at different ages in C57BL/6J mice affects glucose homeostasis. Cordoba-Chacon et al. fed male C57Bl/6J mice a high-fat diet from 4 weeks old (peripubertal-onset) to 20 weeks old or from 12 weeks old (young-adult-onset) to 25 weeks old.[230] Nishikawa et al. fed both male and female C57Bl/6J mice a high-fat diet for 9 weeks starting at 4 weeks old (peripubertal-onset) or 52 weeks old (mid-adult-onset).[231]

In both studies, adult-onset obesity resulted in greater weight gain compared to peripubertal-onset obesity,[230,231] although this difference was less pronounced in female mice.[231] Nishikawa et al. also reported a larger increase in the ratio of fat to body weight in mice with mid-adult-onset obesity compared to peripubertal-onset obesity, consistent with findings that C57BL/6J mice are relatively resistant to diet-induced obesity until 8 weeks of age.[232,233]

Despite greater weight gain in mice with adult-onset obesity, their glucose tolerance was either better[231] or not different[230] from those with peripubertal-onset obesity. Insulin tolerance measured over 120 minutes was also not different between mice with peripubertal- and mid-adult-onset obesity.[230] However, insulin tolerance during the first 60 minutes, a more accurate reflection of whole-body insulin sensitivity,[234] was impaired in the mice with mid-adult-onset obesity.[230] While advancing age could theoretically also reduce insulin sensitivity in the mid-adult-onset group, this is rarely observed in C57BL/6J mice under these experimental conditions.[235]

Worse insulin sensitivity but similar glucose tolerance in mice with adult-onset obesity compared to those with peri-pubertal-onset obesity may suggest that type 2 diabetes pathogenesis varies by age of obesity onset. Since glucose tolerance reflects both insulin sensitivity and insulin secretion, the absence of

a clear difference between onset groups may suggest that β-cell dysfunction is accelerated in mice with peri-pubertal onset obesity. However, C57BL/6J mice rarely develop β-cell dysfunction or diabetes on a high-fat diet, making them suboptimal for studying diabetes risk.[233]

Using the Swiss Webster mouse strain, susceptible to high-fat diet-induced β-cell dysfunction and diabetes, Glavas et al. confirmed that despite greater insulin resistance in male mice with adult-onset obesity, those with peri-pubertal onset obesity have a greater incidence and earlier onset of diabetes.[236] Female Swiss Webster mice did not develop diabetes even into old age, regardless of the age of obesity onset. However, adult-onset obesity in these female mice was associated with hepatic steatosis and gonadal white adipose tissue fibrosis.[236]

Mechanistic studies and detailed examinations of adipose tissue biology in animal models should aim to uncover the underlying biological processes, such as inflammatory pathways, lipid handling, and cellular aging, that contribute to the distinct metabolic risk in childhood-onset and adult-onset obesity.

Large animal models, such as sheep and pigs, which more closely approximate human physiology, have been developed for studying childhood-onset obesity.[237–239] However, comparative studies including large animals with adult-onset obesity are lacking. These models hold significant potential for bridging the gap between rodent studies and clinical applications.[233]

Moving forward, refining animal models to better capture the complexities of childhood-onset and adult-onset obesity will be essential. This includes carefully aligning the timing of high-fat or cafeteria-style feeding with developmental stages that parallel human life[240] and considering genetic diversity, sex, and strain in experimental designs.[233] These refinements will enhance the translational value of animal studies, addressing key gaps in our understanding of how childhood-onset and adult-onset obesity shape long-term metabolic health and facilitating the development of tailored treatments.

Rationale, objectives, and hypotheses

People with childhood-onset obesity have a greater risk of type 2 diabetes than people with adult-onset obesity. However, we do not understand the mechanisms contributing to this disease risk discrepancy or whether these mechanisms can be reversed. A decline in subcutaneous adipose tissue (SAT) function (i.e., the ability to safely store lipids and regulate adipokine production) is linked to type 2 diabetes and may therefore occur differently in people with childhood-onset and adult-onset obesity. Comparing markers of SAT dysfunction between people with childhood-onset and adult-onset obesity, before the onset of comorbidities, could help identify potential targets for tailored treatment strategies. Additionally, examining how these markers change after a conventional diet and exercise intervention will provide preliminary evidence on whether weight loss alone can modify SAT dysfunction in each group or if alternative strategies should be considered.

The overarching objective of this study was to compare markers of regional SAT (dys)function and cardiometabolic characteristics between people with childhood-onset and adult-onset obesity before and after moderate (~10% weight loss).

The primary objectives of the present dissertation were as follows:

Original article 1

- To examine the effect of age of obesity onset (childhood-onset vs. adult-onset), sex, and their interaction on abdominal and femoral SAT morphology (degree of adipocyte hyperplasia or hypertrophy).

Original article 2

- To examine the effect of age of obesity onset (childhood-onset vs. adult-onset) on senescence markers in abdominal and femoral SAT before and after moderate (~10%) weight loss.

Original article 3

- To examine the effect of age of obesity onset (childhood-onset vs. adult-onset) on abdominal and femoral SAT immune cells (macrophage and T-cell populations) before and after moderate (~10%) weight loss.

We hypothesized the following:

Original article 1

- Compared to males and females with adult-onset obesity, those with childhood-onset obesity will have hyperplastic abdominal and femoral SAT.

Original article 2

- Compared to people with adult-onset obesity, those with childhood-onset obesity will have more cells with senescence markers in abdominal and femoral SAT at baseline.
- Compared to people with adult-onset obesity, those with childhood-onset obesity will have a blunted decrease in these senescence markers after moderate (~10%) weight loss.

Original article 3

- Compared to people with adult-onset obesity, those with childhood-onset obesity will have a greater proportion of M1-like, proinflammatory macrophages and cytotoxic T cells in abdominal and femoral SAT at baseline.
- Compared to people with adult-onset obesity, those with childhood-onset obesity will have a blunted decrease in these immune cells after moderate (~10%) weight loss.

Bridge 1

In *original article 1*, we open this dissertation by investigating the longstanding notion that subcutaneous adipose tissue is hyperplastic in childhood-onset obesity and hypertrophic in adult-onset obesity. Given that hyperplastic SAT is considered metabolically protective, this notion does not align with the increased risk of type 2 diabetes associated with childhood-onset obesity.

Original article 1.
Age of obesity onset affects subcutaneous adipose tissue cellularity differently in the abdominal and femoral region

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- Presented as published (Editors' choice article):

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Abstract

Objective: We aimed to examine the effect of age of obesity onset, sex, and their interaction on abdominal and femoral subcutaneous adipose tissue (SAT) morphology (degree of adipocyte hyperplasia or hypertrophy).

Methods: In this cross-sectional study, we isolated adipocytes via collagenase digestion from abdominal and femoral SAT biopsies taken from male and female adults with childhood-onset obesity (CO; n = 8 males, n = 16 females) or adult-onset obesity (AO; n = 8 males, n = 13 females). Regional body composition was measured with dual-energy x-ray absorptiometry and a single-slice abdominal computed tomography scan. Mean adipocyte size was measured in abdominal and femoral SAT and was used to quantify morphology in android and gynoid subcutaneous fat, respectively.

Results: Abdominal SAT morphology was more hyperplastic in females with CO than females with AO (p = 0.004) but did not differ between males with CO and males with AO (p = 0.996). Conversely, femoral SAT morphology was more hypertrophic in males and females with CO than those with AO.

Conclusions: Age of obesity onset appears to affect SAT morphology differently in the abdominal and femoral regions of male and female adults. Our findings challenge the notion that SAT is uniformly hyperplastic in CO and hypertrophic in AO.

Introduction

In the 1970s, a paradigm arose that equated childhood-onset obesity (CO) with hyperplastic obesity (many small adipocytes) and adult-onset obesity (AO) with hypertrophic obesity (fewer large adipocytes). However, the studies that shaped this paradigm had limitations.[56–60] They measured adipocyte size in one or multiple adipose tissue regions, which often varied among participants, and then divided total fat mass by average adipocyte size to estimate total adipocyte number. Because adipocyte size can vary largely among regions,[59] and adipose tissue is not evenly distributed throughout the body, this approach can over- or underestimate total adipocyte number. Moreover, it cannot determine whether the degree of hypertrophy or hyperplasia varies across adipose tissue regions.

Since the 1970s, additional evidence has accumulated questioning the rigidity of the cellularity-based classification of CO and AO. Hyperplastic adipose tissue is considered metabolically protective, and hypertrophic adipose tissue is considered metabolically harmful[73]; however, cardiometabolic risk is greater in CO than AO.[167] Could this paradox mean that hyperplasia is not always benign or that AO is not always hypertrophic? Weight gain studies in lean adults have supported both possibilities. Contrary to the longstanding belief that adipocyte number is established in childhood, adults can accumulate adipose tissue through hyperplasia,[69,241,242] which, in turn, can associate with poor metabolic health.[243]

Nevertheless, the labeling of CO as hyperplastic and AO as hypertrophic has only recently been challenged. Overcoming the limitations of earlier studies, Arner et al. measured

adipocyte size in abdominal subcutaneous adipose tissue (SAT) and computed adipocyte number in dual-energy x-ray absorptiometry (DXA)-estimated abdominal subcutaneous fat, not total body fat.[70] They concluded that the degree of abdominal SAT hypertrophy or hyperplasia does not differ between people with CO and AO.[70] This novel contribution to the literature stemmed from a secondary analysis that classified CO and AO based on recalled body weight at age 18 years. The groups differed in age, adiposity, and health status, and the group with CO gained more weight per year during adulthood than the group with AO. Also, the generalizability of the findings to other adipose tissue regions remains unknown.

In the present study, we aimed to assess the effect of age of obesity onset (CO vs. AO), sex, and their interaction on abdominal and femoral SAT cellularity. We also explored how the degree of hypertrophy or hyperplasia in abdominal and femoral SAT relates to cardiometabolic risk factors, stratified by age of obesity onset and sex.

Methods

Participants and procedures

Between April 1, 2016, and November 30, 2019, we recruited healthy adults from Montréal, Québec, Canada, to participate in a study investigating adipose tissue characteristics in CO and AO. Eligibility criteria were as follows: obesity onset either pre- or peri-puberty (CO) or after the age of 18 years (AO); age between 25 and 40 years; body mass index (BMI) ≥ 30 kg/m2 and < 40 kg/m2; sedentary or lightly active lifestyle; weight stability (±2 kg) for at least 2 months; no use of nicotine-containing products; and no medication use (e.g., antidepressants, antihypertensives), surgeries (e.g., gastric bypass), or past or current medical conditions that could affect metabolism or the ability to complete the study. Females who were menopausal, pregnant, or breastfeeding were also ineligible. Participants provided photographic proof of body

size around puberty (age ~10–14 years for females, age ~12–15 years for males) and verified their body size at different ages using the Collins Childhood Body Rating Scales[35] and the Stunkard Body Rating Scale.[31] Ethical approval was obtained from the Concordia University Human Research Ethics Committee. All participants provided written informed consent.

Study procedures occurred after a 12-h fast and included measurements of body composition and arterial stiffness, a blood draw, and an abdominal (lateral periumbilical region) and femoral (lateral upper thigh) SAT biopsy. The biopsy procedure has been previously described.[244]

Body composition

Total, android, and gynoid fat mass (detailed elsewhere[245]) were assessed using DXA (Lunar Prodigy Advance, GE HealthCare, Madison, Wisconsin) with Encore Software (version 14.10, GE HealthCare). The android region (waist) extends upward from the pelvis cut by 20% of the distance between the pelvis and neck cuts.[245] The upper boundary of the gynoid region (hips/upper thighs) is below the pelvis cut by 1.5 times the height of the android region. The height of the gynoid region is two times the height of the android region.

SAT and visceral adipose tissue (VAT) were quantified from an L2–L3 single-slice (10 mm) computed tomography (Revolution Evo, GE Medical Systems, Inc., Milwaukee, Wisconsin) image using sliceOmatic Software (version 5.0, TomoVision, Montréal, Québec). The ratios of SAT to total adipose tissue and VAT to total adipose tissue were multiplied by the DXA android fat region to compute android SAT and VAT quantities, respectively.[91]

Cardiometabolic risk factors

Serum concentrations of glucose, triglycerides, total cholesterol, and high-density lipoprotein (HDL) cholesterol were measured at the McGill University Health Centre central

laboratory in Montréal using standard procedures.[91] Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation.[246] Plasma insulin concentration was measured by enzyme-linked immunosorbent assay (ELISA; R&D Systems, Toronto, Ontario), and the homeostatic model assessment for insulin resistance (HOMA-IR) was computed as follows: (glucose in millimoles per liter insulin in picomoles per liter)/135.[247] To assess arterial stiffness, we measured carotid-femoral pulse wave velocity using applanation tonometry (SphygmoCor, ATCOR Medical, Sydney, Australia) as previously described.[248]

Adipokines

Plasma concentrations of leptin, adiponectin, interleukin (IL)-8 (R&D Systems), and IL-6 (Abcam, Cambridge, UK) were measured by ELISA. Plasminogen activator inhibitor-1 (PAI-1), monocyte chemoattractant protein-1 (MCP-1), and resistin were measured using a Human ProcartaPlex Simplex Kit (Invitrogen, Thermo Fisher Scientific Inc., Waltham, Massachusetts). Adiponectin is an anti-inflammatory, insulin-sensitizing adipokine, whereas the other selected adipokines are proinflammatory and implicated in cardiometabolic risk.[249–251]

Regional SAT cellularity

Adipocytes were isolated from approximately 1 g of abdominal and femoral SAT by collagenase digestion and then imaged using phase contrast microscopy (Motic AE2000 TRI, Motic [Xiamen] Electric Group Co., Ltd., Xiamen, China), as previously described.[252] Consistent with the literature, the areas of 100 random adipocytes were measured (FIJI software[253]) and converted to volumes assuming a spherical shape. Mean adipocyte mass was calculated from mean adipocyte volume using a density of 0.915 g/mL (triolein).[254] Adipocyte number in each region was determined by dividing subcutaneous android and gynoid fat mass by mean abdominal and femoral adipocyte mass, respectively. We computed the abdominal and femoral

SAT morphology index developed by Arner et al.[255] based on the relationship between mean adipocyte volume and regional fat mass derived by Spalding et al.[67]: mean adipocyte volume (in picoliters [pL]) = a regional fat mass (in kilograms)/(1 + [b regional fat mass (in kilograms)]). To do so, we fit our data to the equation using PROC NLIN in SAS version 9.4 (SAS Institute, Inc., Cary, North Carolina). We then computed the morphology index for each participant as the difference between actual and predicted mean adipocyte volume. Therefore, a positive morphology value indicates hypertrophic SAT, and a negative morphology value indicates hyperplastic SAT, relative to the predicted morphology for a given fat mass.

Statistical analyses

Two-way ANOVA was used to test the effects of obesity onset, sex, and their interaction on participant characteristics, adipokine concentrations, and regional adipose tissue cellularity (adipocyte size, adipocyte number, and morphology). When the interaction was statistically significant, group differences were tested with Tukey post-hoc tests. The normality of residuals for each model was assessed using the Shapiro–Wilk test and visual inspection. Dependent variables were natural log-transformed when necessary. When the homogeneity of variances assumption was violated, as per Levene's test and visual inspection, robust standard errors were used. Results are presented as least-squares means and 95% confidence intervals (CI).

Because adiposity can influence adipocyte size and number,[254,256] we considered regional fat mass (android and gynoid subcutaneous fat as appropriate) as a potential covariate in our ANOVA for adipocyte size and number. Of note, the morphology index is independent of regional fat mass. We therefore examined the relationships (Pearson correlation) between regional fat mass and adipocyte size and number. We considered it necessary to adjust for regional fat mass when it differed among our groups and was linearly related to our outcome

(i.e., it met the criteria for a confounder) or when it improved the model R^2. Before including the covariate in the model, we verified that regression slopes were similar across groups by testing the interaction between group and the covariate.

Spearman rho (ρ) correlation was used to assess the relationships between the regional SAT morphology indexes and cardiometabolic variables by sex and obesity onset. Missing data were dealt with by pairwise deletion. All analyses were conducted using SAS version 9.4. Statistical significance was set at $p < 0.05$.

Results

Participant characteristics

Figure S1 shows the participant flow diagram. Our final sample consisted of 8 males and 16 females with CO and 8 males and 13 females with AO. Body fat percentage was highest in females with CO and AO, intermediate in males with CO, and lowest in males with AO (Table 1). Regardless of obesity onset, males had more visceral fat than females. Compared with the other three groups, males with AO had less total and android subcutaneous fat. Although the four groups were similar in most cardiometabolic risk factors, HOMA-IR was higher in participants with CO than those with AO across sexes.

Group differences in leptin concentrations paralleled those for body fat percentage (Table 2). Regardless of obesity onset, IL-8 was higher and resistin lower in males than females. All other adipokine concentrations were similar among groups.

Table 1. Demographic and clinical characteristics by sex and obesity onset

Characteristic	Females		Males		Effect, *p* value		
	Childhood-onset Obesity (*n* = 16)	Adult-onset Obesity (*n* = 13)	Childhood-onset Obesity (*n* = 8)	Adult-onset Obesity (*n* = 8)	Obesity Onset	Sex	Obesity Onset x Sex
Age (years)	30.5 (28.6, 32.4)	30.9 (28.8, 33.1)	30.5 (27.8, 33.2)	30.3 (27.5, 33.0)	0.943	0.780	0.780
Body Mass Index (kg/m^2)	33.6 (32.2, 35.1)	34.2 (32.6, 37.8)	35.9 (33.9, 37.9)	30.8 (28.8, 32.8)[d,e]	0.015	0.509	0.003
Lean Body Mass (kg)[a]	46.6 (44.1, 49.2)	48.2 (45.5, 50.9)	62.7 (54.3, 71.0)	63.4 (57.8, 69.0)	0.669	<0.001	0.869
Total Body Fat (kg)	40.4 (37.1, 43.8)	42.7 (39.0, 46.3)	43.1 (38.4, 47.8)	32.9 (28.2, 37.6)[d,e]	0.062	0.093	0.005
Total Body Fat (%)	45.0 (43.0, 47.0)	45.4 (43.3, 47.6)	39.6 (36.9, 42.4)[c,d]	33.1 (30.3, 35.9)[c-e]	0.016	<0.001	0.006
Android Visceral Fat (kg)	0.75 (0.53, 0.98)	0.83 (0.57, 1.07)	1.51 (1.19, 1.82)	1.66 (1.34, 1.98)	0.432	<.0.001	0.744
Android Subcutaneous Fat	2.9 (2.6, 3.2)	2.8 (2.5, 3.2)	3.1 (2.7, 3.5)	1.8 (1.4, 2.2)[c-e]	<0.001	0.038	0.001
Gynoid Fat (kg)	7.3 (6.5, 8.0)	8.0 (7.2, 8.8)	6.5 (5.5, 7.5)	5.0 (4.0, 6.1)[c-e]	0.392	<0.001	0.019
Log HOMA-IR[a,b]	2.1 (1.5, 2.8)	1.9 (1.7, 2.1)	2.2 (1.7, 2.8)	1.4 (1.1, 1.7)	0.027	0.415	0.214
Log Triglycerides(mmol/L)[b]	0.05 (-0.19, 0.28)	0.10 (-0.16, 0.36)	0.38 (0.05, 0.71)	0.43 (0.10, 0.76)	0.733	0.027	0.981
HDL Cholesterol (mmol/L)	1.3 (1.2, 1.5)	1.2 (1.1, 1.4)	1.2 (1.0, 1.4)	1.1 (0.9, 1.3)	0.268	0.059	0.893
LDL Cholesterol (mmol/L)	2.7 (2.3, 3.1)	2.6 (2.2, 3.1)	2.8 (2.3, 3.4)	2.7 (2.1, 3.2)	0.684	0.750	0.848
Total/HDL Cholesterol	3.5 (3.0, 4.0)	3.7 (3.2, 4.3)	4.3 (3.6, 5.0)	4.2 (3.5, 4.9)	0.926	0.052	0.628
cfPWV (m/s)	7.2 (6.7, 7.6)	7.2 (6.7, 7.8)	7.5 (6.8, 8.3)	7.6 (6.7, 7.8)	0.862	0.269	0.896

Results are least-squares means (95% CI). For females with CO, *n* = 14 for HOMA-IR. For males with CO, *n* = 7 for HOMA-IR and cfPWV.

When the obesity onset x sex interaction was statistically significant ($p < 0.05$), groups were compared with Tukey post-hoc tests (all $P < 0.05$):

[a]Robust standard errors used due to unequal variances.

[b]Natural log-transformed.

[c]Different from females with CO, $p < 0.05$.

[d]Different from females with AO, $p < 0.05$.

[e]Different from males with CO, $p < 0.05$

Abbreviations:
cfPWV = carotid-femoral pulse wave velocity; **HDL** = high-density lipoprotein; **HOMA-IR** = homeostatic model assessment of insulin resistance; **LDL** = low-density lipoprotein

Table 2. Adipokine concentrations by sex and obesity onset

Characteristic	Females		Males		Effect, *P*		
	Childhood-onset Obesity (*n* = 16)	Adult-onset Obesity (*n* = 13)	Childhood-onset Obesity (*n* = 8)	Adult-Onset Obesity (*n* = 8)	Obesity Onset	Sex	Obesity Onset x Sex
Log Leptin(pg/mL)[a,b]	10.9 (10.8, 11.1)	11.0 (10.8, 11.1)	10.3 (10.1, 10.5)[c,d]	9.6 (9.2, 10.0)[c-e]	0.009	<0.001	0.004
Adiponectin (µg/mL)	4.9 (4.0, 5.7)	4.3 (3.4, 5.3)	4.2 (2.9, 5.5)	3.3 (1.9, 4.7)	0.208	0.126	0.746
Log IL-6 (pg/mL)[b]	1.5 (1.2, 1.8)	1.3 (1.0, 1.6)	1.0 (0.6, 1.5)	1.3 (0.8, 1.7)	0.815	0.160	0.279
Log IL-8 (pg/mL)[b]	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	1.5 (1.2, 1.8)	1.6 (1.3, 1.8)	0.682	0.007	0.672
Log PAI-1 (pg/mL)[b]	8.9 (8.6, 9.3)	8.9 (8.5, 9.3)	9.2 (8.7, 9.7)	8.9 (8.4, 9.5)	0.427	0.412	0.606
Log MCP-1 (pg/mL)[b]	4.6 (4.4, 4.9)	4.8 (4.6, 5.1)	4.7 (4.4, 5.0)	5.0 (4.7, 5.3)	0.062	0.476	0.696
Resistin (ng/mL)	5.7 (3.8, 7.7)	7.1 (4.9, 9.2)	4.1 (1.4, 6.8)	3.4 (0.6, 6.1)	0.796	0.031	0.393

Results are least-squares means (95% CI). For males with CO, $n = 7$ for leptin, adiponectin, IL-6, and IL-8. For males with AO, $n = 6$ for adiponectin.
When the obesity onset x sex interaction was statistically significant ($p < 0.05$), groups were compared with Tukey post-hoc tests.

[a]Robust standard errors used due to unequal variances.

[b]Natural log-transformed.

[c]Different from females with CO, $p < 0.05$.

[d]Different from females with AO, $p < 0.05$.

[e]Different from males with CO, $p < 0.05$

Abbreviations:
IL = interleukin; **MCP-1** = monocyte chemoattractant protein-1; **PAI-1** = plasminogen activator inhibitor-1

Regional SAT cellularity

Figure 1 shows the relationships between regional fat mass and mean adipocyte size used to compute the morphology indexes. Of note, because the best fit line was flat in the abdominal region, the morphology index was equivalent to adipocyte volume but on a different scale reflecting relative hypertrophy (positive value) or hyperplasia (negative value).

Because our groups differed in adiposity, we needed to determine whether regional fat mass should be included as a covariate in our models assessing the effects of obesity onset, sex, and their interaction on adipocyte volume and number. To do so, we assessed the linear relationships between regional fat mass and adipocyte volume and number. In both regions, fat mass was not associated with adipocyte volume (Figure S2). Moreover, adding regional fat mass to the models predicting adipocyte volume did not improve the model R^2. Android subcutaneous fat mass associated with abdominal adipocyte number ($r = 0.53$; $p < 0.001$), but gynoid fat mass did not associate with femoral adipocyte number. For models predicting both abdominal and femoral adipocyte number, the R^2 improved when regional fat mass was added as a covariate. For consistency, we displayed the unadjusted and adjusted analyses for abdominal and femoral adipocyte size and number. However, we based our interpretation on the unadjusted models for adipocyte size and adjusted models for adipocyte number. For illustrative purposes, we also correlated all SAT cellularity outcomes with BMI and percent body fat (Figures S3 and S4). Only abdominal adipocyte number correlated with percent body fat ($r = 0.39$; $p = 0.008$).

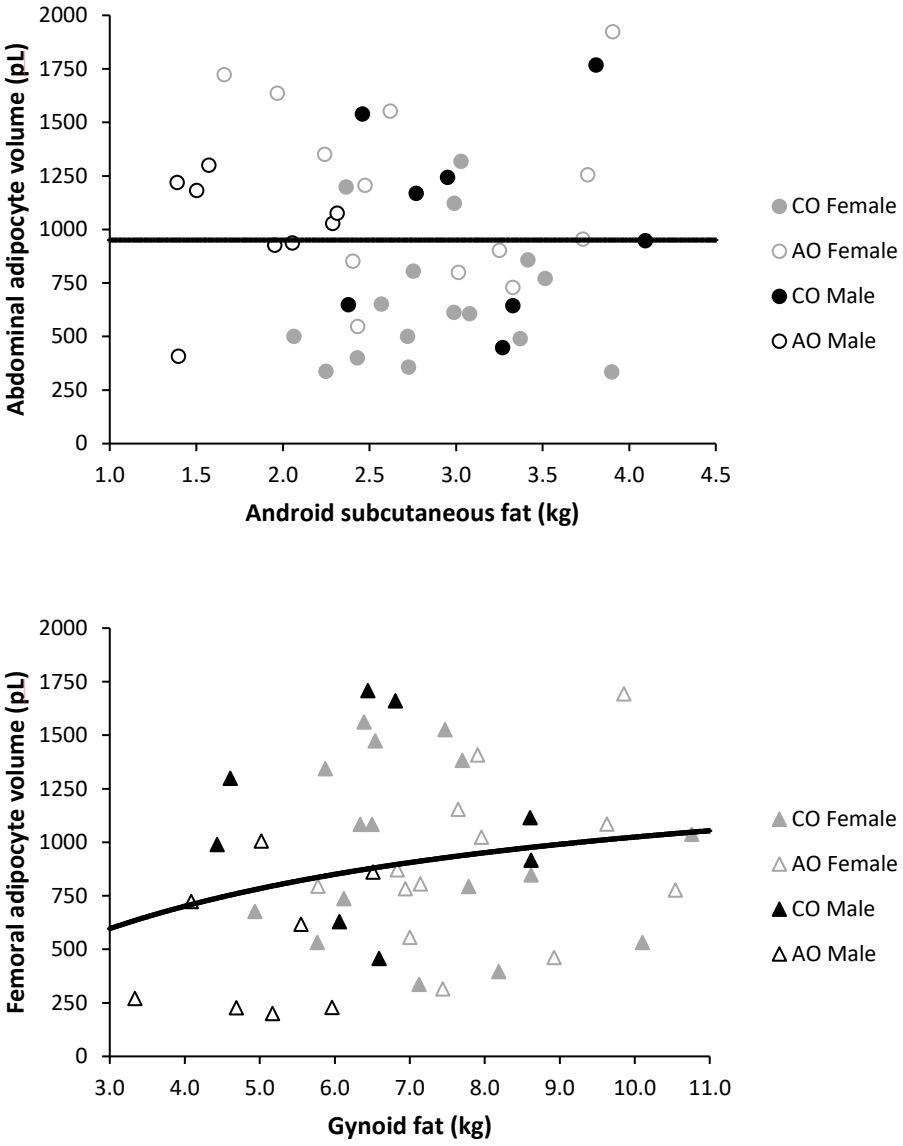

Figure 1. The relationships between regional fat mass and mean adipocyte size
AO = adult-onset obesity; CO = childhood-onset obesity

Table 3 shows the abdominal and femoral SAT cellularity results by sex and obesity onset. Mean abdominal adipocyte volume was lower in females with CO than females with AO ($p = 0.004$) but did not differ between males with CO and males with AO ($p = 0.996$). Abdominal adipocyte number was greater in participants with CO than those with AO across

sexes with ($p < 0.001$) and without ($p = 0.034$) adjusting for android subcutaneous fat mass. Abdominal SAT morphology was more hyperplastic (less hypertrophic) in females with CO than females with AO ($p = 0.004$) but did not differ between males with CO and males with AO ($p = 0.996$). Both males with CO and AO had an abdominal SAT morphology that was relatively hypertrophic (morphology index > 0) on average. Femoral adipocyte volume was lower in males with AO than males with CO ($p = 0.027$) but did not differ between females with CO and females with AO ($p = 0.981$). Femoral adipocyte number was greater in participants with AO than those with CO across sexes ($p = 0.030$), but this difference only persisted in males ($p = 0.027$) when adjusting for gynoid fat mass. Femoral SAT morphology was more hyperplastic (less hypertrophic) in participants with AO than those with CO across sexes ($p = 0.024$).

Table 3. Abdominal and femoral subcutaneous adipose tissue cellularity by sex and obesity onset

	Female		Male		Effect, *p* value		
	Childhood-onset Obesity (*n* = 16)	Adult-onset Obesity (*n* = 13)	Childhood-onset Obesity (*n* = 8)	Adult-onset Obesity (*n* = 8)	Obesity Onset	Sex	Obesity Onset x Sex
Abdominal subcutaneous adipose tissue cellularity							
Mean adipocyte volume (pL)	679 (491, 868)	1187 (978, 1396)[d]	1051 (784, 1317)	1010 (743, 1276)	0.052	0.409	0.023
Mean adipocyte volume (pL)[a]	682 (488, 876)	1189 (976, 1402)[d]	1059 (776, 1342)	992 (663, 1321)	0.117	0.476	0.039
Adipocyte number ($x10^9$)[b]	5.0 (4.0, 6.4)	2.7 (2.1, 3.5)	3.5 (2.5, 4.9)	2.0 (1.4, 2.8)	<0.001	0.034	0.832
Adipocyte number ($x10^9$)[a,b]	4.7 (3.8, 5.9)	2.6 (2.0, 3.3)	3.0 (2.2, 4.2)	2.8 (1.9, 4.1)	0.034	0.200	0.094
Morphology index (pL)	-272 (-460, -83)	236 (27, 445)[d]	100 (-167, 367)	59 (-208, 325)	0.052	0.409	0.023
Femoral subcutaneous adipose tissue cellularity							
Mean adipocyte volume (pL)	959 (759, 1158)	902 (681, 1123)	1097 (815, 1379)	517 (235, 799)[e]	0.014	0.321	0.040
Mean adipocyte volume (pL)[c]	955 (751, 1159)	890 (648, 1131)	1101 (814, 1389)	539 (209, 870)	0.017	0.495	0.070
Adipocyte number ($x10^9$)[b]	9.0 (6.8, 11.8)	10.4 (7.7, 14.1)	6.9 (4.7, 10.1)	12.7 (8.6, 18.6)	0.030	0.843	0.177
Adipocyte number ($x10^9$)[b,c]	8.6 (6.6, 11.2)	9.1 (6.7, 12.5)	7.3 (5.0, 10.5)	16.1 (10.5, 24.7)[e]	0.012	0.310	0.038
Morphology index (pL)	50 (-151, 250)	-42 (-264, 181)	227 (-56, 13)	-262 (-546, 21)	0.024	0.864	0.116

Results are least-squares means (95% CI). When the obesity onset x sex interaction was statistically significant ($p < 0.05$), groups were compared with Tukey post-hoc tests

[a]Adjusted for android subcutaneous fat mass.

[b]Natural log-transformed prior to analysis but results are displayed as back-transformed values.

[c]Adjusted for gynoid fat mass.

[d]Different from females with CO, $p < 0.05$.

[e]Different form males with CO, $p < 0.05$.

Relationships between regional SAT cellularity and cardiometabolic variables

Table S1 shows the correlations between regional SAT morphology and cardiometabolic variables by group. In females with CO, abdominal SAT hypertrophy correlated with MCP-1 ($\rho = 0.54$; $p = 0.033$), while femoral SAT morphology did not correlate with any cardiometabolic variables. In females with AO, both abdominal and femoral SAT hyperplasia correlated with resistin ($\rho = 0.62$; $p = 0.025$ and $\rho = 0.78$; $p = 0.002$, respectively). Femoral SAT hyperplasia also correlated with PAI-1 ($\rho = 0.59$; $p = 0.033$) and arterial stiffness ($\rho = 0.59$; $p = 0.033$). Femoral SAT hypertrophy, on the other hand, correlated with triglycerides ($\rho = 0.61$; $p = 0.028$). In males with CO, abdominal SAT morphology did not correlate with any cardiometabolic variables. Femoral SAT hyperplasia correlated with android VAT ($\rho = 0.74$; $p = 0.037$) and the total-to-HDL-cholesterol ratio ($\rho = 0.76$; $p = 0.028$). In males with AO, abdominal SAT morphology did not correlate with any cardiometabolic variables, while femoral SAT hyperplasia correlated negatively with HDL cholesterol ($\rho = 0.81$; $p = 0.015$).

Discussion

We sought to investigate the longstanding notion that hyperplastic SAT is a feature of CO and that hypertrophic SAT is a feature of AO. Our findings were distinct in abdominal and femoral SAT. Abdominal SAT morphology was hyperplastic in females with CO compared with females with AO but did not significantly differ between males with CO and males with AO. Conversely, femoral SAT morphology was hyperplastic in both males and females with AO compared with those with CO.

Our study was, in part, motivated by the methodological limitations of the 1970s studies that shaped the paradigm equating CO with hyperplastic obesity and AO with hypertrophic obesity.[56–60] These studies could not delineate which adipose tissue regions drove their findings

because they lacked the technology to measure regional fat mass. The recent study by Arner et al. overcame this limitation using DXA-estimated android SAT and concluded that abdominal SAT morphology does not vary by age of obesity onset.[70] We enhanced their approach by directly measuring abdominal SAT with the gold standard, computed tomography, but concluded the opposite in females. Our studies differed in other important ways, which may explain the conflicting results. The study by Arner et al. classified overweight/obesity onset based on recalled body weight at age 18 years.[70] In contrast, we defined CO as obesity acquired pre- or peri-puberty, a sensitive period for body fat accrual, and we verified the age of obesity onset with photographic evidence and body rating scales. Moreover, we employed narrow inclusion criteria that resulted in groups well-matched for potential confounders, including age and health status.

Collectively, findings from prospective studies align with the greater abdominal SAT hypertrophy in our females with AO and the greater femoral SAT hyperplasia in our males and females with AO compared with their counterparts with CO. A longitudinal study found that children with obesity enter adulthood with greater gluteal adipocyte size and number than lean children.[54] Based on cross-sectional studies comparing lean adults with those with obesity,[56,60,257] we anticipate this finding to be consistent across SAT regions. To our knowledge, no studies have examined how continued weight gain in adults with CO affects SAT cellularity. However, an overfeeding study showed that lean adults gained SAT primarily through hypertrophy in the abdominal region and hyperplasia in the femoral region.[241] This study also showed that baseline adipocyte size determined how adipose tissue expanded. Femoral SAT hyperplasia occurred at lower baseline femoral adipocyte sizes in males than females, and abdominal SAT hyperplasia occurred only in females with large baseline abdominal adipocytes.[241] A longitudinal study

confirmed that, in lean female adults, abdominal adipocytes increase in both size and number after an 18% body weight gain over 10 years.[69]

Our study was also spurred by the conflicting concept that hyperplastic SAT is considered both a feature of CO[56–60] and metabolic health[73]; however, CO increases type 2 diabetes risk compared with AO.[167] By design, we studied young adults without any comorbidities, but those with CO still had higher HOMA-IR than those with AO. Given that excess adipose tissue is the first prerequisite for obesity, studying SAT expansion mechanisms via cellularity is a way to capture initial abnormalities that may, in turn, contribute to adipose tissue dysfunction and eventual disease risk. In our study, circulating adipokines and cardiometabolic risk factors did not consistently correlate with SAT hypertrophy or hyperplasia in any group. Because this analysis was exploratory, the correlations should be interpreted with caution. It is possible that studying participants with more variability in cardiometabolic risk factors would have captured more consistent correlations with SAT morphology. Nevertheless, we can draw from the current literature to understand how the distinct SAT cellularity profiles in males and females with CO and AO might impact cardiometabolic health over the long-term.

We found that increased femoral SAT hypertrophy distinguished both males and females with CO from those with AO. Although abdominal SAT hypertrophy has been extensively linked to insulin resistance, high-risk lipid profiles, and systemic inflammation and can even predict type 2 diabetes,[73,258] lower body SAT hypertrophy has been less well-studied. In males with obesity and hypertension, Achimastos et al. showed that gluteal adipocyte size associated with mean arterial pressure.[259] Across a wide adiposity range, Imbeault et al. reported that femoral adipocyte size correlated with fasting plasma insulin in premenopausal females and with high-risk lipid profiles in both males and premenopausal females.[260] Moreover, Espinosa De Ycaza et

al. found that femoral adipocyte size correlated as strongly as abdominal subcutaneous adipocyte size with adipose tissue insulin resistance.[261]

In our study, abdominal SAT hypertrophy was similar in males with CO and AO but was greater in females with AO than those with CO. This finding ignites the question of why hypertrophic femoral SAT in females with CO may be more harmful than hypertrophic abdominal SAT in females with AO. We hypothesize that the pathological potential of abdominal SAT hypertrophy in females with AO is offset by their hyperplastic femoral SAT, which can still safely accommodate lipids. Conversely, in females with CO, limited lipid storage capacity in metabolically protective femoral SAT could promote lipid storage in more harmful abdominal SAT and VAT depots, as observed after lower body SAT lipectomy.[262] Our previous findings in female bariatric surgery patients (mean BMI = 48 kg/m^2) with CO and AO matched for BMI and diabetes prevalence supports this hypothesis.[71] Although we did not examine lower body SAT, we found that abdominal subcutaneous adipocyte size did not differ between obesity-onset groups.[71] Because males have lower gluteofemoral fat storage capacity than females,[263] it is possible that abdominal SAT turns hypertrophic at a lower obesity severity in males with CO.

It is also plausible that abdominal adipocytes in females with CO become dysfunctional at a lower size threshold. According to the expandability hypothesis, enlarged adipocytes indicate impaired adipogenic potential.[264] Both enlarged adipocytes and reduced adipogenesis have been linked to senescent preadipocytes in abdominal SAT.[75] Correspondingly, we have previously shown that females with CO have more senescent preadipocytes in abdominal and femoral SAT than those with AO.[91] Even though females with CO have hyperplastic abdominal and hypertrophic femoral SAT, their abdominal SAT has more senescent preadipocytes than their femoral SAT.[91] In females with CO, we found that abdominal SAT hypertrophy correlated

with MCP-1, a chemokine that attracts monocytes and is linked to macrophage infiltration.[265] A study in children with obesity showed that subcutaneous adipocyte size associated with macrophage infiltration, systemic inflammation, and insulin resistance.[55] Therefore, the pathological nature of enlarged adipocytes, even in predominantly hyperplastic SAT, may begin in childhood.

An alternative view is that hyperplasia is not a benign expansion mechanism. White et al. showed that, in lean males, an increase in proportion of small adipocytes during overfeeding correlated with visceral and ectopic fat accumulation.[242] They also showed that, in females with obesity, in vivo adipogenesis in both abdominal and femoral SAT related to VAT and insulin resistance.[243] In our study, femoral SAT hyperplasia correlated with inflammatory markers and cardiometabolic risk factors in females with AO and males with CO and AO. Another interesting observation was that males with AO had a VAT mass similar to those with CO despite having lower total adiposity. We suspect that the femoral SAT hyperplasia in AO may not be as protective in males as it is in females. We hypothesize that, in males, hyperplasia may reflect failed adipocyte hypertrophy. The hypertrophic femoral SAT in males with CO may be due to the greater capacity for peripheral SAT expansion in childhood. After puberty, central adipose tissue expansion predominates in males. Others have shown that both a high proportion of small adipocytes and an increased peak diameter of large adipocytes associate with cardiometabolic risk.[266] This characteristic adipocyte size distribution has even been seen in insulin-resistant children with a high ratio of visceral to subcutaneous abdominal fat.[128] Therefore, both SAT hypertrophy and hyperplasia may contribute to cardiometabolic risk. Further studies are needed to elucidate the mechanisms.

We used the collagenase digestion method to measure adipocyte size. Although this is a common and well-accepted methodology, it yields one adipocyte population and is limited to a measure of mean adipocyte size. We therefore could not examine the small and large adipocyte populations that are identified with the osmium tetroxide technique. Our study is also limited by its small sample size. Larger studies, especially in males, are required to replicate our findings. We acknowledge that we cannot generalize our findings to adults with severe obesity or comorbidities.

The present study challenges the view that SAT is uniformly hyperplastic in CO and hypertrophic in AO. For the first time, we found that this cellularity-based classification of CO and AO depends on sex and does not apply to all SAT regions. We found that hyperplastic abdominal SAT was characteristic of CO in females only, whereas hyperplastic femoral SAT was characteristic of AO in both males and females. Longitudinal studies are necessary to clarify when regional SAT hypertrophy and hyperplasia diverge in males and females with different ages of obesity onset. Future studies are also required to better understand the cardiometabolic consequences of regional SAT hyperplasia versus hypertrophy in adults with newly acquired versus lifelong obesity.

Supporting Information

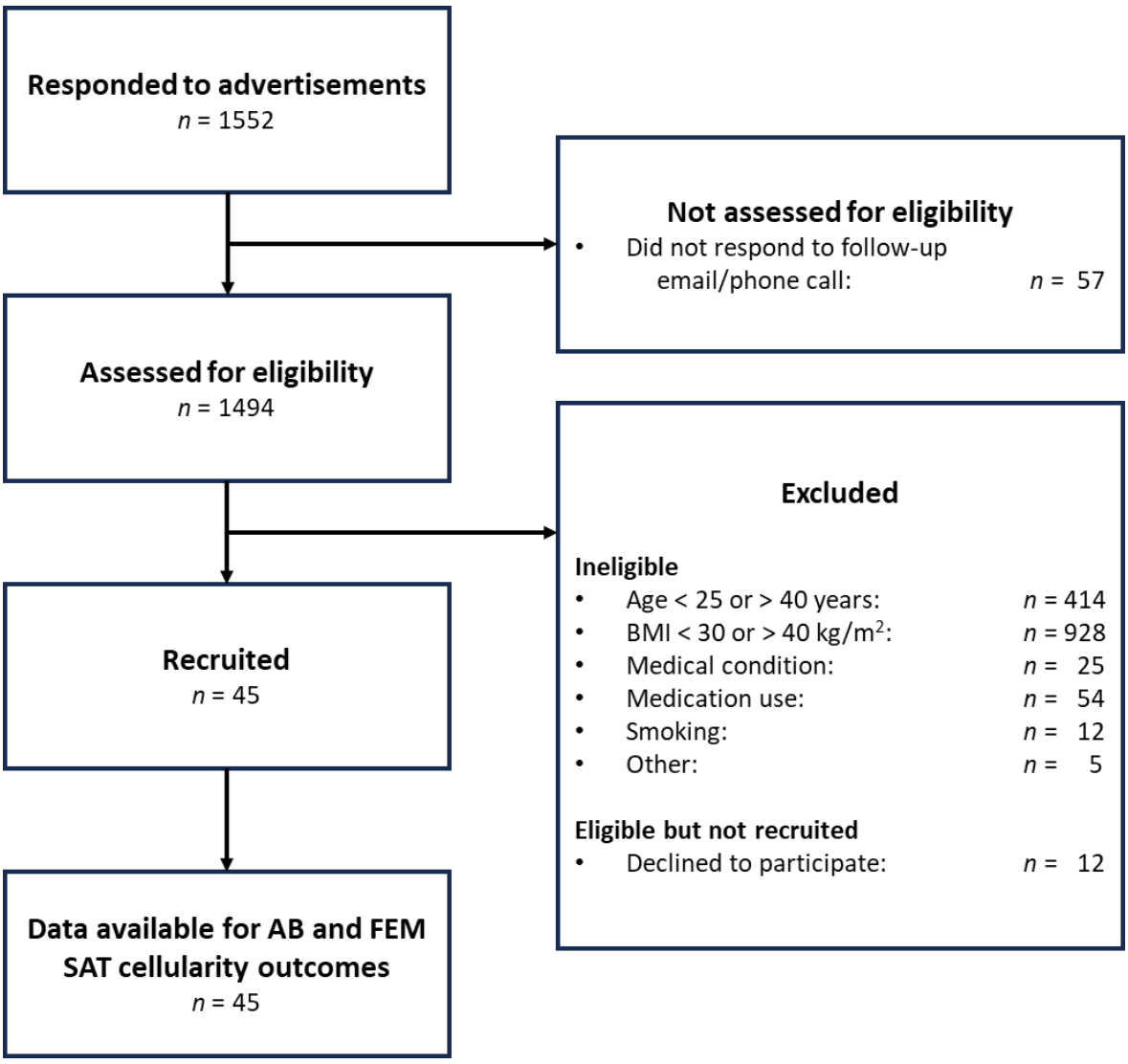

Figure S1. Participant flow diagram

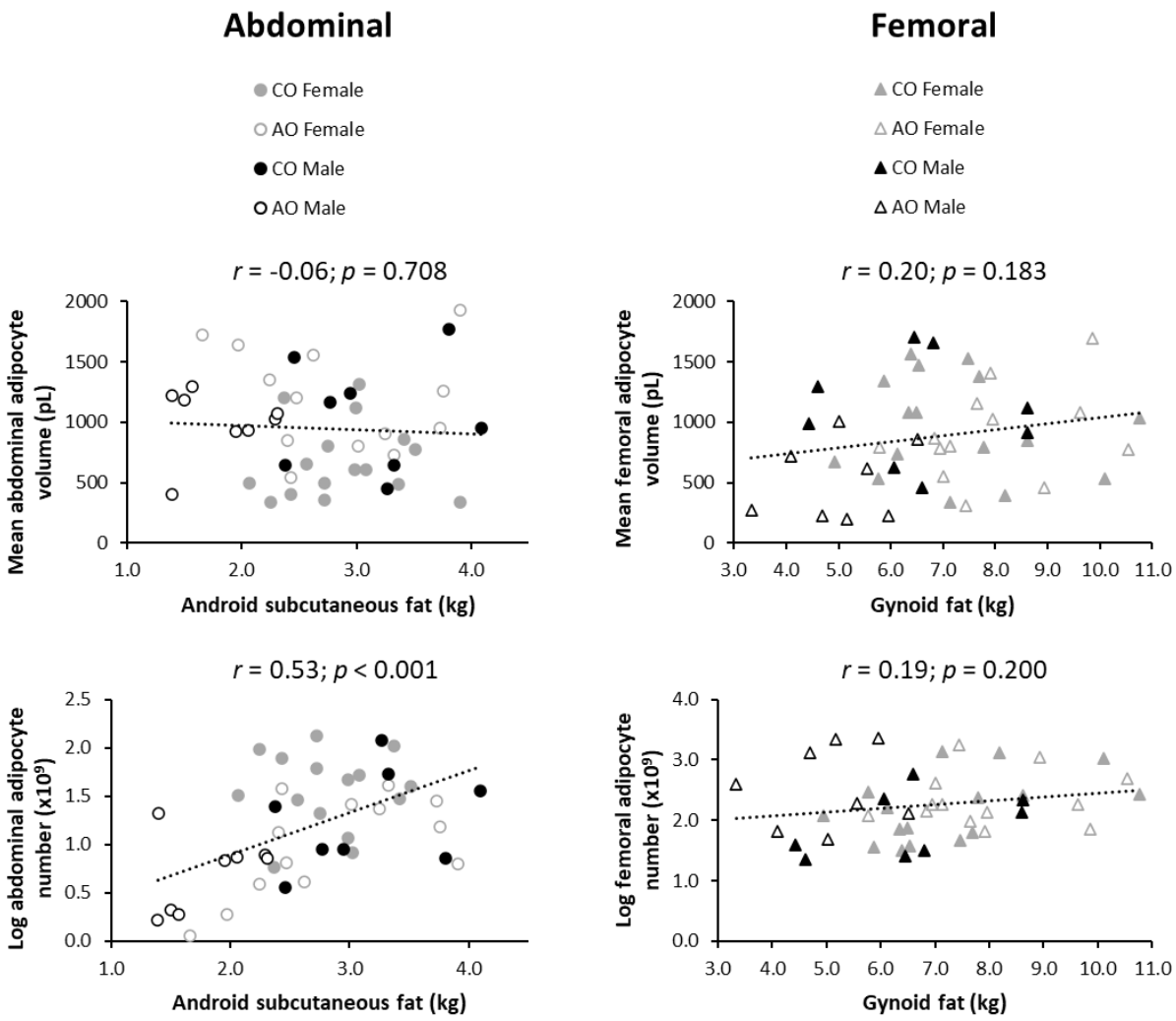

Figure S2. The linear relationships between regional subcutaneous fat mass and adipose tissue cellularity

r = Pearson's correlation coefficient

AO = adult-onset obesity; CO = childhood-onset obesity

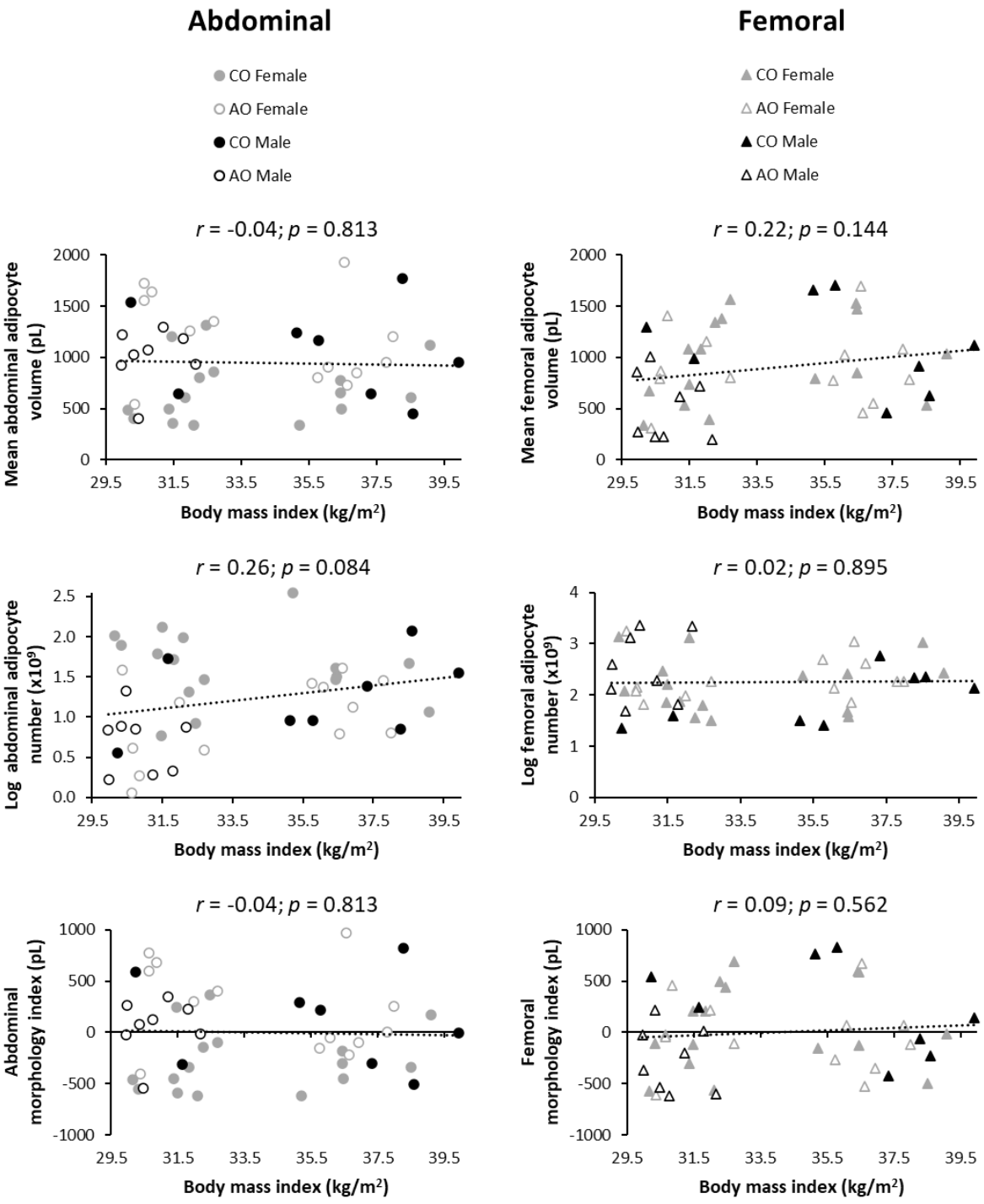

Figure S3. The linear relationships between body mass index and adipose tissue cellularity

r = Pearson's correlation coefficient

AO = adult-onset obesity; CO = childhood-onset obesity

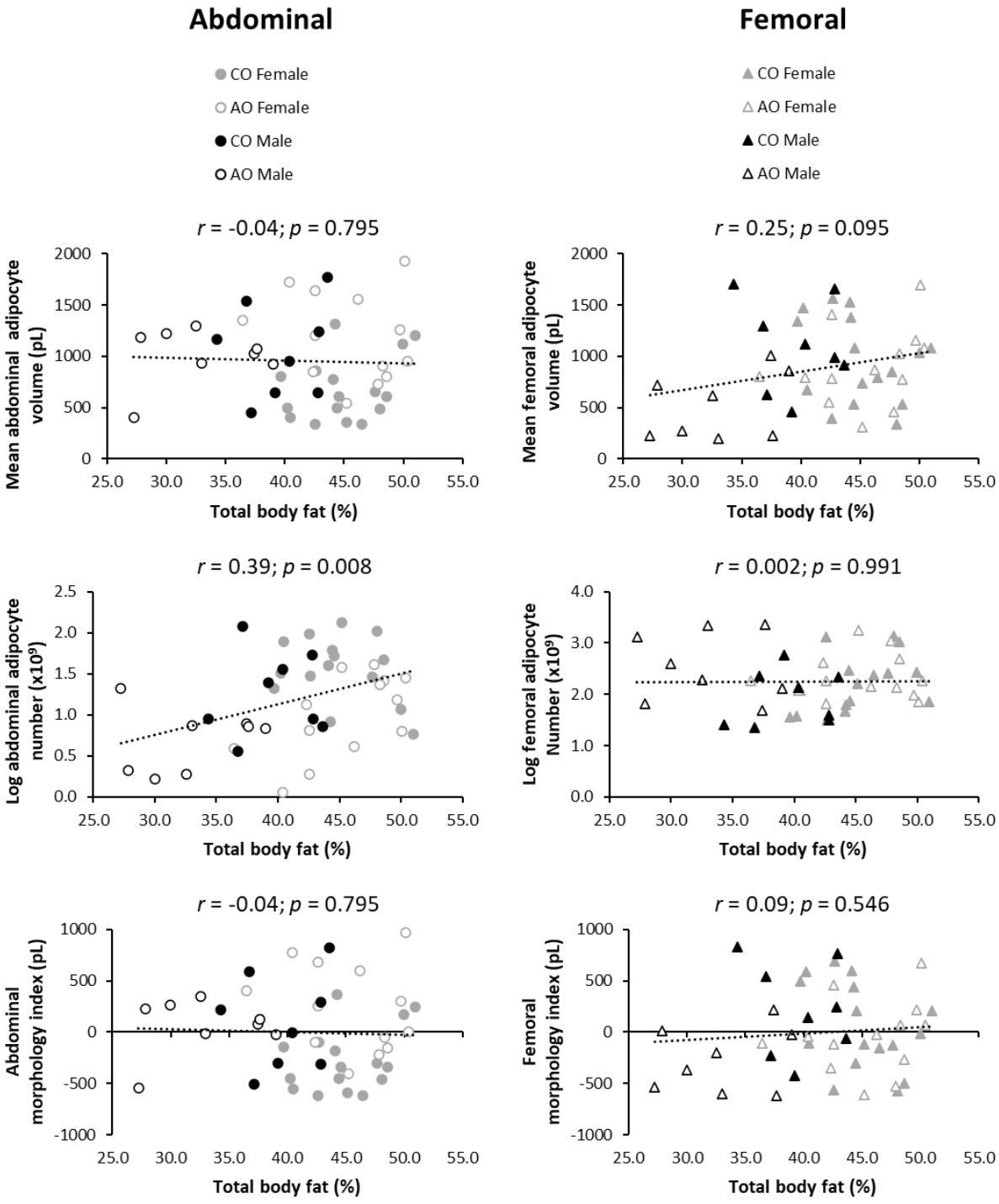

Figure S4. The linear relationships between percent body fat and adipose tissue cellularity

r = Pearson's correlation coefficient

AO = adult-onset obesity; CO = childhood-onset obesity

Table S1. Spearman correlations between regional subcutaneous adipose tissue morphology and cardiometabolic outcomes by sex and obesity onset

Cardiometabolic outcome	Females								Males							
	Childhood-onset Obesity (n = 16)[a]				**Adult-onset Obesity** (n = 13)[b]				**Childhood-onset Obesity** (n = 8)[c]				**Adult-onset Obesity** (n = 8)[d]			
	Abdominal		Femoral		Abdominal		Femoral		Abdominal		Femoral		Abdominal		Femoral	
	ρ	p	ρ	p	ρ	p	ρ	p	ρ	p	ρ	p	ρ	p	ρ	p
Leptin	0.01	0.983	-0.44	0.085	-0.02	0.958	0.01	0.979	0.18	0.702	0.61	0.148	0.43	0.289	-0.24	0.570
Adiponectin	-0.03	0.923	0.14	0.610	-0.10	0.748	-0.07	0.817	0.04	0.939	0.32	0.482	0.20	0.704	0.54	0.266
IL-6	0.29	0.279	0.24	0.380	-0.31	0.297	0.21	0.482	0.29	0.535	0.07	0.879	-0.33	0.420	-0.24	0.570
IL-8	0.33	0.217	0.16	0.557	-0.25	0.415	-0.12	0.707	0.04	0.939	0.61	0.148	0.55	0.160	0.48	0.233
PAI-1	-0.20	0.465	-0.21	0.444	-0.35	0.247	-0.59	0.033	-0.24	0.570	0.29	0.493	0.14	0.736	-0.10	0.823
MCP-1	0.54	0.033	0.33	0.209	-0.25	0.405	-0.23	0.448	-0.38	0.352	0.40	0.320	-0.52	0.183	0.43	0.289
Resistin	-0.19	0.492	-0.28	0.300	-0.62	0.025	-0.78	0.002	0.33	0.420	-0.07	0.867	0.31	0.456	-0.43	0.289
Android VAT mass	0.43	0.094	0.34	0.196	0.25	0.415	0.49	0.090	-0.48	0.233	-0.74	0.037	-0.55	0.160	0.02	0.955
HOMA-IR	-0.31	0.288	-0.28	0.326	-0.04	0.901	0.41	0.162	-0.25	0.589	0.29	0.535	0.12	0.779	0.14	0.736
Triglycerides	-0.20	0.465	0.06	0.820	0.49	0.089	0.61	0.028	0.10	0.823	-0.10	0.823	0.07	0.867	-0.19	0.651
LDL cholesterol	-0.27	0.316	-0.18	0.509	0.20	0.505	0.14	0.655	0.45	0.260	0.52	0.183	0.07	0.867	0.81	0.015
HDL cholesterol	-0.09	0.745	0.31	0.247	0.29	0.334	0.33	0.271	0.02	0.955	-0.38	0.352	0.33	0.420	0.24	0.570
Total-to-HDL Cholesterol	0.03	0.914	0.32	0.226	0.06	0.845	0.16	0.591	-0.43	0.289	-0.76	0.028	0.38	0.352	0.05	0.911
Arterial Stiffness	-0.05	0.854	0.13	0.644	-0.43	0.144	-0.59	0.033	-0.25	0.589	-0.36	0.432	0.29	0.493	-0.52	0.183

ρ: Spearman's rho; [a]n = 14 for HOMA-IR;[b]n = 11 for leptin; [c]n = 7 for Leptin, Adiponectin, IL-6, and IL-8; [d]n = 6 for Adiponectin

p values have not been adjusted for multiplicity

Bridge 2

In *original article 1*, we challenged the longstanding notion that SAT is uniformly hyperplastic in childhood-onset obesity and hypertrophic in adult-onset obesity. Our findings indicated that SAT expansion patterns in childhood-onset and adult-onset obesity vary by sex and body region.

In *original article 2*, we shift perspectives to investigate whether childhood-onset obesity accelerates adipose tissue aging and whether lifestyle intervention can reverse this process. To do so, we compare senescence-related markers in abdominal and femoral SAT—and their changes after moderate (~10%) weight loss—between females with childhood-onset and adult-onset obesity.

Original article 2.
Senescence markers in subcutaneous preadipocytes differ in childhood- versus adult-onset obesity before and after weight loss

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- Presented as published:

  Murphy J, Tam BT, Kirkland JL, Tchkonia T, Giorgadze N, Pirtskhalava T, Tsoukas MA, Morais JA, Santosa S. Senescence markers in subcutaneous preadipocytes differ in childhood- versus adult-onset obesity before and after weight loss. *Obesity.* 2023 Jun;31(6):1610–9. DOI: 10.1002/oby.23745

Abstract

Objective: The aim of this study was to determine the effect of age of obesity onset on senescence-related markers in abdominal (AB) and femoral (FEM) subcutaneous adipose tissue (SAT) before and after moderate (~10%) weight loss.

Methods: AB and FEM SAT were collected from human females with childhood-onset obesity (CO) or adult-onset obesity (AO) before and after diet- and exercise-induced weight loss. Immunofluorescence analysis of γH2AX/RAD51 (DNA damage/ repair markers) and p53/p21 (senescence markers) was conducted in cultured preadipocytes, and senescence-associated β-galactosidase (SA-β-gal) activity was measured in SAT.

Results: CO had proportionately more AB and FEM preadipocytes with DNA damage ($\gamma H2AX^+$) and senescence markers ($p53^+$ and/or $p21^+$) than AO at baseline. The proportion of $\gamma H2AX^+$ FEM preadipocytes declined with weight loss in CO and was similar between groups after weight loss. The number of γH2AX foci in $\gamma H2AX^+$ preadipocytes decreased similarly between groups and regions with weight loss in parallel with an increase in RAD51. The proportion of $p53^+$ and $p21^+$ preadipocytes and SA-β-gal^+ cells in SAT did not change with weight loss, but the total p21 intensity in $p53^+/p21^+$ FEM preadipocytes declined in AO.

Conclusions: These results provide preliminary evidence that females with CO have an accelerated preadipocyte aging state that improves with weight loss in terms of DNA damage but not senescence.

Introduction

The path to adult obesity and the disease risk that follows are not uniform. A pooled analysis of three birth cohorts showed that adults with persistent obesity from childhood have twice the odds of developing type 2 diabetes than those who acquired obesity in adulthood.[167] As childhood obesity commonly tracks into adulthood,[229] understanding the biological mechanisms driving this disease risk discrepancy is imperative to inform treatment strategies.

We hypothesize that adults with childhood-onset obesity (CO) are at greater risk for metabolic disease because of accelerated aging.[267] Adipose tissue senescence is one fundamental aging process that is strongly implicated in metabolic dysfunction.[268] Senescent cells accumulate in human subcutaneous adipose tissue (SAT) with aging and obesity,[75,269] and they correlate with reduced replicative potential, impaired adipogenesis,[75,270,271] and insulin resistance.[272] Increased type 2 diabetes risk in lifelong obesity may, therefore, stem from an exacerbated senescent cell burden in SAT.

Characterized by a state of stable cell growth arrest, senescent cells prevent the proliferation of aged or damaged cells but concurrently develop a proinflammatory secretory phenotype. Cellular senescence can be initiated by multiple stimuli including telomere damage and oxidative stress that trigger the DNA damage response (DDR), classically marked by γH2AX. The DDR drives DNA repair and activates the tumor suppressor protein p53. When DNA repair is delayed or not possible, persistent p53 signaling upregulates the cyclin-dependent

kinase inhibitor p21, leading to cell cycle arrest. Senescence-associated β-galactosidase (SA-β-gal) activity is associated with a committed senescent state.

Weight loss through lifestyle modification remains a key objective of initial obesity treatment. In mice with diet-induced obesity, markers of AT senescence decrease after weight loss through calorie restriction[273] or exercise.[274] Whether weight loss can alleviate AT senescence in humans, independent of obesity onset, remains unknown. The present study aimed to determine the effect of CO versus adult-onset obesity (AO) on the multistep process of senescence in regional SAT before and after moderate (~10%) weight loss.

Methods

Participants and study design

We recruited healthy, nonsmoking, premenopausal females (age = 25–40 years; BMI = 30–39 kg/m^2) from Montréal, Québec, Canada, who acquired obesity either in pre /peri-puberty (CO; n = 8) or after the age of 18 (AO; n = 9). To determine obesity onset, participants provided photographic proof of body size around puberty (~10–14 years old). Participants were also interviewed about their weight history and they verified their body size at different ages using the Collins' Childhood Body Rating Scales[35] and the Stunkard Body Rating Scale.[31] To be included, participants had to be sedentary or lightly active and weight stable (±2 kg) for at least 2 months. We excluded individuals who used medications (e.g., antidepressants, antihypertensives) or had any surgeries (e.g., gastric bypass) or past or current medical conditions that could affect research outcomes or the ability to complete the study. Females who were pregnant or breastfeeding were also excluded. Ethical approval was obtained from the Concordia University Human Research Ethics Committee, and all participants provided written informed consent.

All study visits were conducted at Concordia University's PERFORM Centre. The study consisted of a baseline 2-week weight stabilization period, a weight loss period that ended when participants lost approximately 10% of their initial body weight, and a final 2-week weight stabilization period. Assessments were completed toward the end of each weight stabilization period. Participants were instructed to maintain their usual eating and physical activity habits during the weight stabilization periods and to refrain from exercise for at least 48 hours before assessments. The visits occurred after a 12-hour fast and included anthropometric and body composition measurements, indirect calorimetry, a blood draw, and abdominal (AB) (lateral periumbilical region) and femoral (FEM) (lateral upper thigh) SAT biopsies. Indirect calorimetry and clinical blood measurements are described in the online Supporting Information Methods. The biopsy procedure has been previously described.[244] Five participants with CO and six participants with AO completed both baseline and final assessments.

Anthropometric and body composition measurements

Participants wore light clothing and no shoes during the anthropometric and body composition measurements. Height was recorded to the nearest 0.1 cm at the first study visit using a fixed-wall stadiometer (Seca 216, Seca Corp., Chino, California). Body weight was measured to the nearest 0.1 kg using a calibrated scale (DIN 2, AmCells Corp., Vista, California).

Total and regional body composition was assessed by one of two highly trained operators using dual-energy x-ray absorptiometry (DXA; Lunar Prodigy Advance, GE Healthcare, Madison, Wisconsin) with Encore Software (version 14.10; GE Healthcare). When required, regions of interest were manually adjusted and verified by both operators. The DXA scanner has

shown high intraobserver agreement for measurement of regional percent fat (Supporting Information Table S1).

SAT and visceral AT (VAT) were quantified from an L2–L3 single-slice (10 mm) computed tomography (CT; Revolution Evo, GE Healthcare) image using SliceOMatic Software (version 5.0; Tomovision, Montréal, Québec). The ratios of SAT and VAT to total AT (TAT) were multiplied by the DXA android fat region to compute android SAT and VAT quantities (e.g., CT VAT [cm^2]/CT TAT [cm^2] DXA android total fat [kg] = android VAT [kg]).[275]

Preadipocyte culture and immunofluorescence staining of senescence-related markers

Preadipocytes from approximately 1 g of SAT were isolated and cultured as previously described.[276] Cells were grown to subconfluence on gelatin-coated cover slips in 24-well plates and fixed in 4% paraformaldehyde for 15 minutes. Cells were then washed three times with phosphate-buffered saline (PBS), permeabilized with 0.3% Triton X-100 (in PBS) for 12 minutes, washed three times with PBS, and blocked with 1.5% bovine serum albumin (BSA) (in PBS) for 1 hour. After another three washes with PBS, cells were double-stained and incubated with the following two primary antibody cocktails (diluted in 1.5% BSA) overnight at 4 °C: (1) anti-γH2AX (3:200; Santa Cruz Biotechnology, Dallas, Texas) and anti-RAD51 (1:300; Abcam, Toronto, Ontario); and (2) anti-phospho-p53 (Ser15) (3:500; Thermo Fisher Scientific [Invitrogen], Waltham, Massachusetts) and anti-p21 (3:200; Santa Cruz Biotechnology). γH2AX and RAD51 are markers of DNA damage and repair, respectively; and p53 and p21 are proteins in the p53/p21 pathway of cell cycle arrest. After three 5-minute washes, cells were incubated with Alexa 488 (1:300) and Alexa 568 secondary (1:200) antibodies (Thermo Fisher Scientific [Thermo Scientific]) in blocking solution for 1 hour at room temperature then counterstained with DAPI (1:1500) to visualize nuclei.

Images were acquired using fluorescence microscopy (Leica DMI6000, Concord, Ontario), and data from five random fields (500–600 cells) were generated using macros in FIJI.[253] The FIJI Analyze Particle tool was used to create a region of interest (ROI) around each nucleus so that results could be expressed per nucleus. We quantified the number of γH2AX and phospho-p53 nuclear foci using the FIJI FindFoci plugin.[277] We also computed the percentage of cells positive (≥1 foci) for these markers. Because p21 was present in only a select number of nuclei per image and stained throughout the nucleus, we quantified the percentage of cells positive for p21 and the total p21 intensity within these cells. RAD51 stained diffusely throughout most nuclei, so we quantified the total RAD51 intensity per cell.

Assessment of SA-β-gal activity in AT

SA-β-gal activity was assessed in approximately 100 mg of SAT as previously described.[278] SA-β-gal positive cells, identified as blue dots, were manually counted from five random fields using phase contrast microscopy (Leica DMI6000). Nuclei were imaged in the same field using fluorescence microscopy and counted using FIJI software. The percentage of cells positive for SA-β-gal was calculated as the number of blue dots divided by the number of nuclei.

Ex vivo analysis of AT inflammation

To test the association between senescence markers and AT inflammation, AB and FEM SAT explants were cultured in Medium 199 (M199; Thermo Fisher Scientific [Gibco]) supplemented with insulin, dexamethasone, antibiotics, and NaHCO3 (3 mL medium per 100 mg SAT). After 24 hours, the medium was replaced with fresh medium that did not contain insulin and dexamethasone. The conditioned medium was collected 24 hours later, and the concentration

of the anti-inflammatory, insulin-sensitizing adipokine adiponectin was measured by enzyme-linked immunosorbent assay (ELISA; R&D Systems, Oakville, Ontario).

Lifestyle weight loss protocol

Baseline daily energy needs were calculated as resting energy expenditure multiplied by an activity factor of 1.2 to 1.3 (representing a sedentary to lightly active lifestyle). The goal of the weight loss protocol was to achieve a 30% energy deficit through a 20% reduction in energy intake and a 10% increase in energy expenditure based on a previous protocol.[279] Follow-ups and weigh-ins were scheduled weekly throughout the protocol. Participants were instructed how to achieve their daily energy intake goal using exchange lists for meal planning that aimed to provide 50% to 60% carbohydrate, 20% protein, and 20% to 30% fat.

To achieve the energy expenditure target, participants engaged in three 45-minute moderate-to-vigorous intensity aerobic exercise sessions per week while wearing a heart rate monitor. The exercise program comprised treadmill and/or elliptical sessions at the PERFORM Centre and it was preprogrammed on a Technogym key (Technogym USA Corp., Fairfield, New Jersey). Target heart rates were calculated from the Karvonen equation (heart rate reserve [HRR] intensity + resting heart rate), where HRR is age-predicted maximum heart rate (220—age) minus resting heart rate.[280] Exercise intensity was prescribed at 40% to 50% HRR for weeks 1 to 2 and 50% to 60% HRR for weeks 3 to 4. Thereafter, intervals alternating between 60% and 80% HRR were incorporated into the sessions. Participants were taught how to use the Borg Rating of Perceived Exertion scale to gauge exercise intensity for sessions that needed to occur outside of the PERFORM Centre without a heart rate monitor. Participants' exercise activity was monitored weekly throughout the study.

Statistical analyses

Data were analyzed using SAS version 9.4 (SAS Institute Inc.). Baseline participant characteristics were expressed as means (SEM) and analyzed with independent t tests.

For our primary analyses, we used marginal models (SAS PROC MIXED with REPEATED statement) with restricted maximum likelihood estimation to accommodate unbalanced data and flexibly alter the covariance structure of the residuals. The models included the senescence-related markers as outcomes and group (obesity onset), SAT region, time (weight loss), and all two-way and three-way interactions as fixed factors. The models for body composition and metabolic variables (secondary outcomes) included group, time, and the group-by-time interaction as fixed factors. We selected a compound symmetry, compound symmetry heterogeneous, or unstructured covariance structure for each model based on the results of likelihood ratio χ^2 tests. The normality of residuals for each model was assessed by the Shapiro-Wilk test and visual inspection. When necessary, dependent variables with values greater than zero were natural log- or square-root-transformed, and dependent variables with zero values were square-root- or cube-root-transformed. The degrees of freedom were estimated using the Kenward–Roger method.[281] Significant interactions were decomposed with relevant within- and between-group contrasts. When the three-way interaction was significant, the group-by-time and group-by-region interactions were tested at each level of the third variable (region and time, respectively) and then decomposed with simple contrasts if significant.

The model results were expressed as least-squares means (lsmeans) (95% CI) or differences in lsmeans (95% CI). The lsmeans from transformed data were back-transformed to the original scale to facilitate interpretation. When reported, the differences in lsmeans were only back-transformed for the logged outcomes since the result gives the ratio of the geometric

lsmeans,[282] and it can be interpreted as a fold difference. Effect sizes were computed as Cohen's d ($d = 2t(\text{sqrt}(df))$) using the model t values and degrees of freedom (df),[283] then converted to Hedge's g ($g = d(1 - 3/(4df - 1))$ to adjust for small sample size bias.[284] Statistical significance was set at $p < 0.1$ for interactions and $p < 0.05$ for main effects and contrasts as in previous studies.[285,286]

We used Spearman correlation coefficient (ρ) to assess the relationship between senescence markers and the following: obesity duration, homeostatic model assessment of insulin resistance, and android VAT at baseline; and adiponectin concentrations in AT-conditioned media before and after weight loss.

Results

Participant characteristics

Table 1 shows the baseline demographic and clinical characteristics of the participants with CO and AO. The groups were similar in mean age, body mass index (BMI), and insulin sensitivity. Mean serum glucose and lipid concentrations were comparable between groups and they fell within the normal ranges. Baseline characteristics did not differ between participants who completed and did not complete the weight loss protocol (data not shown). Participants who completed the weight loss protocol lost an average of 8 kg (CO: 8.4 ± 0.6 kg; AO: 8.2 ± 1.9 kg) or 9% of their initial weight (CO: 9.3% ± 0.7%; AO: 8.5% ± 1.8%). On average, more than 75% of the lost weight was fat (CO: 75.4% ± 5.1%; AO: 79.3% ± 5.9%).

Table 1. Demographic and clinical characteristics of study participants

Characteristic	Childhood-onset Obesity (n = 8)	Adult-onset Obesity (n = 9)	p value
Age (years)	31.0 (1.3)	31.2 (0.8)	0.884
Body Mass Index (kg/m^2)	33.2 (1.1)	33.3 (1.0)	0.940
Duration of Obesity (years)	22.6 (2.3)	7.8 (1.7)	<0.001
Glucose (mmol/L)	4.5 (0.2)[a]	4.6 (0.1)	0.690
Log Insulin (U/mL)	3.2 (0.4)[a]	3.5 (0.1)	0.379
Log HOMA-IR	1.6 (0.4)[a]	1.9 (0.1)	0.354
Triglycerides (mmol/L)	1.0 (0.2)	1.2 (0.1)	0.520
Total Cholesterol (mmol/L)	4.4 (0.3)	4.4 (0.3)	0.846
HDL-Cholesterol (mmol/L)	1.3 (0.1)	1.3 (0.1)	0.742
LDL-Cholesterol (mmol/L)	2.6 (0.2)	2.6 (0.3)	0.859

Results are means (SEM)

[a] n = 7

Abbreviations:

HDL = high-density lipoprotein; **HOMA-IR** = homeostatic model assessment of insulin resistance; **LDL** = low-density lipoprotein

Body composition and resting metabolism

There were no group or group-by-time interaction effects on regional body composition (Table 2). Across groups, fat mass decreased in the trunk and legs. Android SAT and VAT mass also declined. Although the android to gynoid fat ratio did not change with weight loss, the trunk to total fat ratio decreased and the leg to total fat ratio increased, suggesting a preferential loss of trunk fat and preservation of leg fat. The changes in percent fat in all regions of the body exceeded the least significant changes previously reported (Table S1). Lean tissue declined in the total body but not the legs.

There were no group or group-by-time interaction effects on metabolic outcomes (Table 2). Resting energy expenditure decreased with weight loss across groups, but the respiratory exchange ratio did not change.

Table 2. Weight, body composition, and resting metabolism before and after weight loss

Characteristic	Baseline		Final		Effect, *p* value		
	Childhood-onset Obesity (*n* = 8)	Adult-onset Obesity (*n* = 9)	Childhood-onset Obesity (*n* = 5)	Adult-onset Obesity (*n* = 6)	Group	Time	Group x Time
Weight (kg)	91.1 (84.6, 97.6)	93.9 (87.7, 100.0)	82.7 (75.9, 89.4)	85.8 (79.4, 92.1)	0.494	<0.001	0.874
Total Body Fat (kg)	40.4 (36.0, 44.8)	42.5 (38.3, 46.6)	33.9 (29.3, 38.6)	36.3 (32.0, 40.7)	0.442	<0.001	0.838
Total Body Fat (%)	44.7 (41.5, 47.9)	45.5 (42.5, 48.5)	41.0 (37.7, 44.3)	42.5 (39.4, 45.6)	0.569	<0.001	0.530
Total Lean Mass (kg)	47.1 (43.1, 51.0)	48.1 (44.4, 51.8)	45.3 (41.3, 49.3)	46.6 (42.9, 50.4)	0.641	0.002	0.739
Leg Fat (kg)[a]	13.8 (11.7, 16.2)	15.1 (12.9, 17.6)	11.8 (10.0, 13.9)	13.3 (11.3, 15.6)	0.326	<0.001	0.450
Leg Lean Mass (kg)	17.8 (14.7, 20.8)	17.9 (15.0, 20.8)	17.0 (15.1, 18.8)	17.4 (15.7, 19.0)	0.767	0.433	0.839
Trunk Fat (kg)	21.5 (19.1, 24.0)	22.0 (19.7, 24.3)	17.6 (14.9, 20.2)	18.3 (15.9, 20.8)	0.699	<0.0001	0.791
Android VAT (kg)[b]	0.7 (0.4, 0.9)	0.8 (0.6, 1.1)	0.5 (0.2, 0.7)	0.6 (0.4, 0.9)	0.324	0.008	0.754
Android SAT (kg)[c]	3.1 (2.7, 3.6)	2.9 (2.4, 3.3)	2.5 (2.1, 3.0)	2.5 (2.0, 2.9)	0.551	<0.001	0.111
Android Fat (kg)/Gynoid Fat (kg)	0.55 (0.47, 0.62)	0.48 (0.41, 0.55)	0.51 (0.44, 0.59)	0.48 (0.41, 0.55)	0.300	0.289	0.083
Trunk Fat (kg)/Total Fat (kg)	0.54 (0.50, 0.57)	0.52 (0.49, 0.55)	0.52 (0.49, 0.56)	0.50 (0.47, 0.53)	0.333	0.036	0.802
Leg Fat (kg)/Total Fat (kg)	0.35 (0.31, 0.38)	0.36 (0.33, 0.39)	0.36 (0.32, 0.40)	0.37 (0.34, 0.41)	0.587	0.046	0.858
Resting Energy Expenditure (kcal/day)	1681 (1547, 1814)	1776 (1650, 1902)	1531 (1369, 1693)	1685 (1537, 1834)	0.156	0.049	0.587
Respiratory Exchange Ratio	0.86 (0.83, 0.89)	0.86 (0.84, 0.89)	0.84 (0.81, 0.88)	0.86 (0.82, 0.89)	0.637	0.453	0.722

Results are means (95 % CI)

[a]natural log transformed prior to analyses with results presented as back-transformed means (95% CI)

[b]calculated as computed tomography VAT (cm^2)/computed tomography total adipose tissue (cm^2) × dual-energy absorptiometry android total fat (kg)

[c]calculated as computed tomography SAT (cm^2)/ computed tomography total adipose tissue (cm^2) × dual-energy absorptiometry android total fat (kg)

Abbreviations: SAT = subcutaneous adipose tissue; **VAT** = visceral adipose tissue

Senescence-related markers in preadipocytes and adipose tissue

Figures S1–S3 show representative microscope images for the senescence-related markers by group, region, and time. There was a group-by-region-by-time interaction on the percentage of γH2AX$^+$ cells (Figure 1A). The group-by-time interaction was statistically significant in FEM but not AB. The percentage of γH2AX$^+$ FEM preadipocytes was greater in CO than AO at baseline (15.9 percentage points [95% confidence interval (CI): 1.0 to 30.9]; p = 0.039; g = 1.14), and it decreased with weight loss in CO only (23.6 percentage points [95% CI: 45.9 to 1.2]; p = 0.041; g = 1.62). As a result, the percentage of γH2AX$^+$ FEM preadipocytes was similar between groups after weight loss. In contrast, the percentage of γH2AX$^+$ AB preadipocytes was greater in CO than AO across time (10.8 percentage points [95% CI: 1.4 to 20.2]; p = 0.028; g = 1.36), and it did not significantly change with weight loss across groups. The group-by-region interaction was not statistically significant at baseline; AB had a greater percentage of γH2AX$^+$ cells than FEM across groups (9.3 percentage points [95% CI: 2.3 to 16.4]; p = 0.013; g = 1.43), and this regional difference persisted in CO (23.7 percentage points [95% CI: 12.5 to 34.8]; p = 0.002; g = 3.77) but not AO after weight loss (group-by-region interaction after weight loss).

There was a main effect of time on the mean number of γH2AX foci (Figure 1B) and total RAD51 intensity (Figure 1C) in γH2AX$^+$ cells. On average, these cells had 0.50 (95% CI: 0.07-0.92; p = 0.024; g = 0.79) less γH2AX foci and a 1.17-fold (12,450 arbitrary units [au] (95% CI: 163-24,736; p = 0.047; g = 0.68) greater total RAD51 intensity after weight loss. There was a significant group-by-region interaction on the total RAD51 intensity in both γH2AX$^+$ (Figure 1C) and γH2AX$^-$ cells (Figure 1D). Across time, the total RAD51 intensity did not differ between regions in CO; however, in AO, FEM had a 1.23-fold (17,058 au [95% CI: 1361-

32,754]; $p = 0.0341$; $g = 0.78$) and 1.26-fold (95% CI: 1.08-1.49; $p = 0.0089$; $g = 1.93$) greater total RAD51 intensity than AB in $\gamma H2AX^+$ and $\gamma H2AX^-$ preadipocytes, respectively.

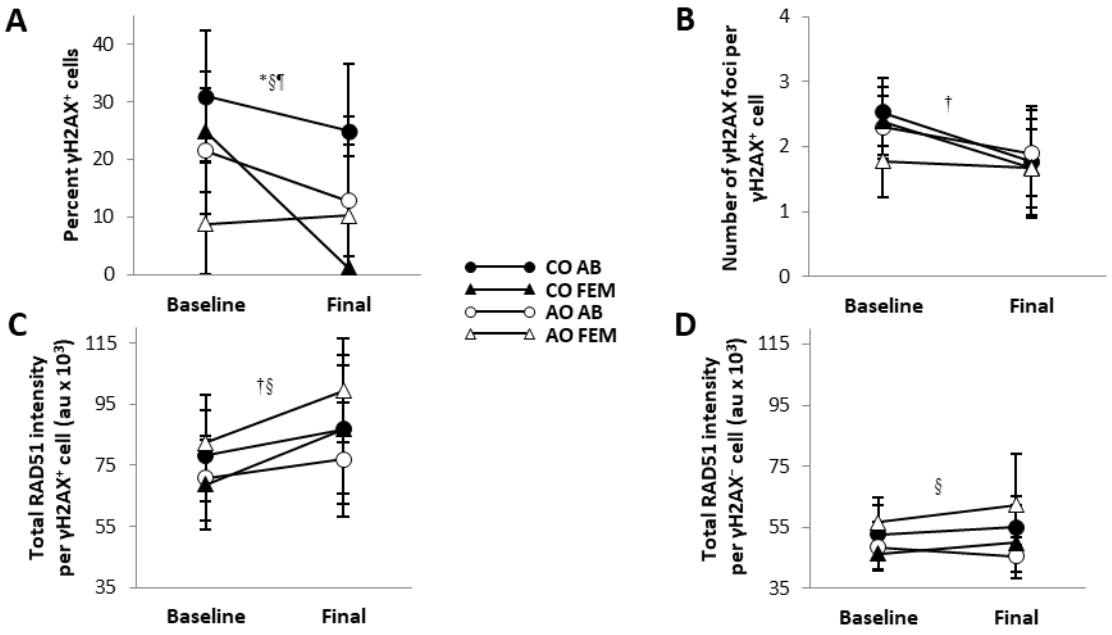

Figure 1. Regional preadipocyte γH2AX and RAD51 content in females with childhood-onset and adult-onset obesity before and after moderate weight loss

A-D Outcomes by group and subcutaneous adipose tissue region over time.

In **A**, negative confidence limits were replaced by 0. The outcome was natural log-transformed in **D** prior to analysis but is displayed as back-transformed values. Results are presented as least-squares means (95% CI).

In **A**, *region, $p < 0.001$; §group-by-region, $p = 0.064$; ¶group-by-region-by-time, $p = 0.013$: group-by-time in AB, $p = 0.815$ (across time: CO > AO, $p = 0.028$; across groups: baseline = final, $p = 0.217$); group-by-time in FEM, $p = 0.097$ (baseline: CO > AO, $p = 0.039$; CO: baseline > final, $p = 0.041$; AO: baseline = final, $p = 0.881$; final: CO = AO, $p = 0.363$); group-by-region at baseline, $p = 0.341$ (across groups: AB > FEM, $p = 0.013$); group-by-region at final, $p = 0.016$ (CO: AB > FEM, $P = 0.002$; AO: AB = FEM, $p = 0.422$).

In **B**, †time, $p = 0.024$: baseline > final.

In **C**, †time, $p = 0.047$: baseline < final; §group-by-region, $p = 0.076$ (across time in CO: AB = FEM, $p = 0.604$; across time in AO: AB < FEM, $p = 0.034$).

In **D**, §group-by-region, $p = 0.001$ (across time in CO: AB = FEM, $p = 0.120$; across time in AO: AB < FEM, $p = 0.009$)

AB = abdominal region; AO = adult-onset obesity; CO = childhood-onset obesity; FEM = femoral region

Most preadipocytes were negative for both p53 and p21 (Figure 2A). The AB region had a lower proportion of $p53^{-}$ /$p21^{-}$ cells (region effect, $p < 0.001$; $g = 3.34$) and a higher proportion of $p53^{-}$ /$p21^{+}$ cells (region effect, $p = 0.002$; $g = 2.63$) than FEM. CO had a lower proportion of $p53^{-}$ /$p21^{-}$ cells (group effect, $p = 0.034$; $g = 1.16$) and hence a higher proportion of $p53^{+}$ and/or $p21^{+}$ cells. There was a three-way interaction on the percentage of $p53^{+}$/$p21^{-}$ cells, but the group-by-time and group-by-region interactions were not significant at each level of region and time, respectively. There was no time effect across groups in either region and no region effect across groups at each time point.

There were no obesity onset, region, time, or interaction effects on the number of p53 foci in $p53^{+}$/$p21^{-}$ cells (Figure 2B) or the total p21 intensity in $p53^{-}$/$p21^{+}$ cells (Figure 2C).

There was a group-by-region-by-time interaction on the number of p53 foci in $p53^{+}$/$p21^{+}$ cells (Figure 2D). The group-by-region interaction was not significant at baseline; the number of p53 foci was similar between regions across groups. The group-by-time interaction was significant in AB whereby the p53 foci count increased nonsignificantly in AO and decreased nonsignificantly in CO. In FEM, the group-by-time interaction was not statistically significant; the number of p53 foci did not change across groups. Still, there was a group-by-region interaction after weight loss; the number of p53 foci was 2.42-fold (95% CI: 1.11-5.24; $p = 0.027$; $g = 0.90$;) lower in FEM than AB in AO but it did not differ between regions in CO.

There was also a three-way interaction on the total p21 intensity in $p21^{+}$/$p53^{+}$ cells (Figure 2E). The group-by-time interaction was significant in FEM but not AB. In FEM, the total p21 intensity was not different between groups at baseline and it declined with weight loss in AO (1.57-fold [95% CI: 1.05-2.34]; $p = 0.029$; $g = 0.85$) but not CO. Hence, the FEM p21 intensity was 1.61-fold (95% CI: 1.01-2.54; $p = 0.044$; $g = 0.68$) lower in AO than CO after weight loss.

In AB, the total p21 intensity was similar between groups across time and it did not change with weight loss. The region-by-group interaction was not significant at baseline or after weight loss; the regions did not differ across groups at both time points.

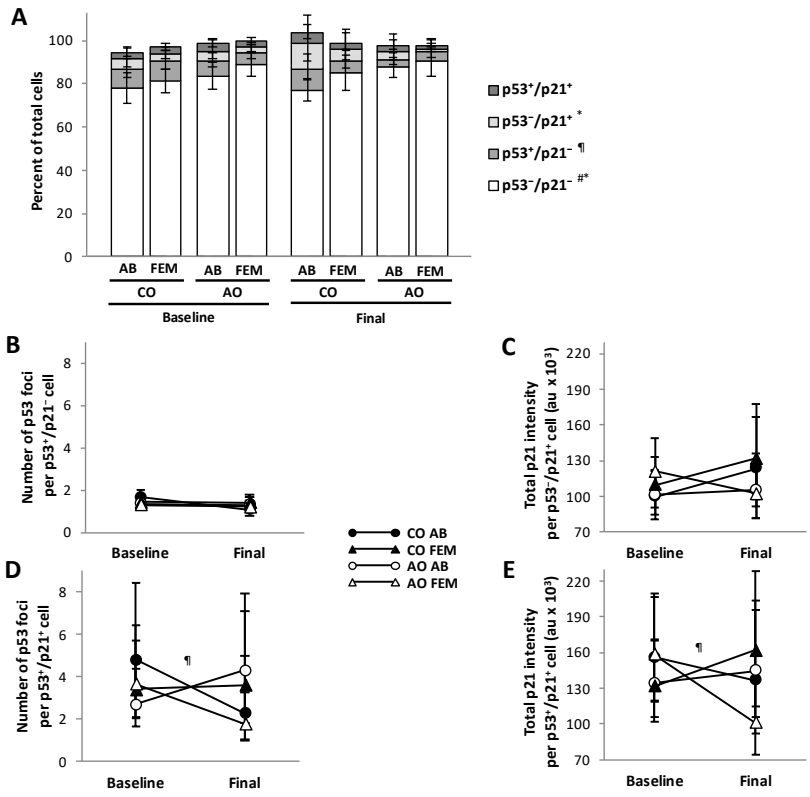

Figure 2. Regional preadipocyte p53 and p21 content in females with childhood-onset and adult-onset obesity before and after moderate weight loss

A-**E** Outcomes by group and subcutaneous adipose tissue region over time. Outcomes were square root-transformed in **A** (except for $p53^-p21^-$) and natural log-transformed in **B**-**E** prior to analysis but are displayed as the back-transformed values. Results are presented as least-squares means (95% CI).

In **A** $p53^-p21^-$, #group, $p = 0.034$: CO < AO; *region, $p < 0.001$: AB < FEM**.** In **A** $p53^+p21^-$, ¶group-by-region-by-time, $p = 0.097$: group-by-time in AB, $p = 0.133$ (across time: CO = AO, $P = 0.090$; across groups: baseline = final, $p = 0.345$); group-by-time in FEM, $p = 0.430$ (across time: CO = AO, $p = 0.279$; across groups: baseline = final, $p = 0.138$); group-by-region at baseline, $p = 0.428$ (across groups: AB = FEM, $p = 0.790$); group-by-region at final, $p = 0.133$ (across groups: AB = FEM, $p = 0.511$). In **A** $p53^-p21^+$, *region, $p = 0.002$: AB > FEM.

In **D**, ¶group-by-region-by-time, $p = 0.014$: group-by-time in AB, $p = 0.037$ (baseline: CO = AO, $p = 0.122$; CO: baseline = final, $p = 0.093$; AO: baseline = final, $p = 0.191$; final: CO = AO, $p = 0.198$); group-by-time in FEM, $p = 0.158$ (across time: CO = AO, $p = 0.332$; across groups: baseline = final, $p = 0.225$); group-by-region at baseline, $p = 0.168$ (across groups: AB = FEM, $p = 0.398$); group-by-region at final, $p = 0.034$ (CO: AB = FEM, $p = 0.337$; AO: AB > FEM, $p = 0.027$).

In **E**, ¶group-by-region-by-time, $p = 0.014$: group-by-time in AB, $p = 0.487$ (across time: CO = AO, $p = 0.768$; across groups: baseline = final, $p = 0.834$); group-by-time in FEM, $p = 0.027$ (baseline: CO = AO, $p = 0.329$; CO: baseline = final, $p = 0.315$; AO: baseline > final, $p = 0.029$; final: CO > AO, $p = 0.044$); group-by-region at baseline, $p = 0.182$ (across groups: AB = FEM, $p = 0.974$); group-by-region at final, $p = 0.110$ (across groups: AB = FEM, $p = 0.569$).

AB = abdominal region; AO = adult-onset obesity; CO = childhood-onset obesity; FEM = femoral region

There was a region effect (FEM > AB; $p = 0.008$; $g = 1.97$) but no group, time, or interaction effects on the percentage of SA-β-gal$^+$ cells in SAT (Figure 3).

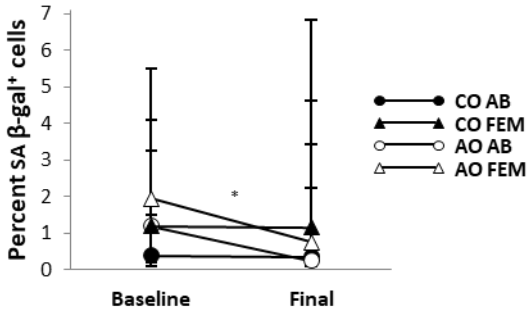

Figure 3. Regional subcutaneous adipose tissue senescence-associated β-galactosidase activity in females with childhood-onset and adult-onset obesity before and after moderate weight loss

Percent SA-β-gal$^+$ cells by group and subcutaneous adipose tissue region over time. The outcome was cube root-transformed prior to analysis but is displayed as the back-transformed values. Results are presented as least-squares means (95% CI).

*region, $p < 0.001$: AB < FEM.

AB = abdominal region; AO = adult-onset obesity; CO = childhood-onset obesity; FEM = femoral region

Obesity duration tended to correlate with the percentage of FEM γH2AX$^+$ cells (ρ = 0.49; $p = 0.066$) and the number of p53 foci in AB p53$^+$p21$^+$ cells (ρ = 0.53; $p = 0.051$) and negatively with the RAD51 intensity in FEM γH2AX$^+$ cells (ρ = 0.48; $p = 0.069$). Homeostatic model assessment of insulin resistance (ρ = 0.67; $p = 0.008$) and VAT (ρ = 0.47; $p = 0.088$) were positively associated with the p21 intensity in AB p53$^-$p21$^+$ cells. At baseline, adiponectin concentration in SAT-conditioned medium was negatively associated with the percentage of p53$^+$p21$^-$ cells in AB (ρ = 0.66; $p = 0.014$) and the number of p53 foci in p53$^+$p21$^-$ cells in FEM (ρ = 0.53; $p = 0.051$). After weight loss, adiponectin was not associated with the senescence markers in AB but it tended to correlate with the percentage of p53$^-$p21$^-$ cells in FEM (ρ = 0.61; $p = 0.060$).

Discussion

This study is the first to examine the effect of obesity onset age on regional SAT senescence and the senolytic potential of moderate weight loss in females. We found that compared with females with AO, those with CO had proportionately more AB and FEM preadipocytes with DNA damage (γH2AX) and senescence markers (p53 and/or p21). Preadipocyte DNA damage decreased in both SAT regions with weight loss regardless of obesity onset and it became similar between groups in the FEM region. Though most senescence markers did not change with weight loss, the total p21 intensity in $p53^{+}/p21^{+}$ FEM preadipocytes declined in females with AO.

Different mechanisms may contribute to the increased DNA damage and senescent cell burden in CO. SAT telomere length is inversely associated with adiposity and age.[287] Moreover, children with obesity have higher rates of preadipocyte proliferation than their lean counterparts.[288] It is therefore conceivable that preadipocytes from adults with lifelong obesity would reach replicative senescence prematurely. We have previously shown that females with CO have impaired SAT mitochondrial integrity compared with females with AO.[3] Hence, an earlier and prolonged exposure to excess adiposity may exacerbate some of the triggers that accelerate preadipocyte aging.

We identified distinct baseline regional differences in senescence-related markers at the preadipocyte and AT level. Both the percentages of $\gamma H2AX^{+}$ and $p53^{-}/p21^{+}$ preadipocytes were greater in AB than FEM SAT, but p53 content was not different between regions. In line with our results, mitochondrial dysfunction is exacerbated in AB compared with gluteal SAT in females with obesity.[3] Furthermore, DNA damage can activate p53-independent upregulation of p21 through transforming growth factor-β (TGFβ) signaling[289]; and obesity elevates TGFβ

release from AB SAT.[290] Our findings also correspond with the observation that AB SAT has proportionately more M1 macrophages than FEM SAT.[291] Senescent preadipocytes can recruit proinflammatory macrophages via their senescence-associated secretory phenotype.[269]

In contrast to our preadipocyte results, we found a greater proportion of SA-β-gal$^+$ cells in FEM than AB SAT, which is consistent with a recent study.[278] There are potential explanations for why our findings at the preadipocyte and adipose tissue level do not align. Positive β-gal staining has been reported in adipose tissue endothelial cells,[292] macrophages,[293] and adipocytes,[294] so it is possible that the SA-β-gal$^+$ cells in SAT represent other cell types besides preadipocytes. Moreover, the p53/p21 senescence pathway is preferentially activated in response to telomere attrition,[295] whereas SA-β-gal activity is a marker for lysosomal number that is associated with cellular senescence.[296]

Preadipocyte DNA damage declined with weight loss across groups and SAT regions, most drastically in the FEM region of the CO group. In the Comprehensive Assessment of the Long-Term Effects of Reducing Intake of Energy (CALERIE) randomized controlled trial, DNA damage in whole blood significantly decreased after moderate weight loss by calorie restriction with or without exercise.[297] The effect of weight loss on DNA damage may therefore act locally in adipose tissue and systemically. A decline in DNA damage can result from a reduction in DNA damaging stimuli or an improvement in DNA repair. RAD51 is vital for homologous recombination, one of the mechanisms that repairs DNA double-stranded breaks, especially in telomeres.[298] RAD51 also plays a role in the general maintenance of telomere integrity.[299] As RAD51 foci form rapidly in response to DNA damage,[300] the diffuse RAD51 staining pattern observed in our study indicates a lack of active homologous recombination.[301] Nuclei with γH2AX foci but no RAD51 foci are characteristic of persistent, irreparable DNA damage[300] that

can occur in uncapped telomeres.[302] It is plausible that increased RAD51 protein content in $\gamma H2AX^+$ cells with weight loss represents an enhanced capacity for RAD51 foci formation and hence DNA repair. The same concept may apply to the greater RAD51 protein content in FEM than AB preadipocytes in the females with AO.

Despite the decline in DNA damage with weight loss, the downstream target of the DDR, p53, did not change. Still, the total p21 intensity in $p53^+p21^+$ FEM preadipocytes decreased in the AO group. The same degree of p53 activation can generate variable p21 protein levels, which, on the lower end, may reflect upregulated p21 degradation to maintain genomic stability.[303] Low levels of p21 are less likely to inhibit cell cycle progression.[304] Therefore, although there was no reduction in the percentage of $p53^+/p21^+$ preadipocytes, a lower p21 protein level may prevent senescence commitment.

The unique group-specific responses to weight loss—reduced $\gamma H2AX^+$ cells in the CO group and lower p21 burden in the AO group—occurred in the FEM region. This finding is interesting because the AB preadipocytes had a greater senescence burden at baseline, and females in both groups lost most of their fat from the upper body. It is possible that senescence markers in FEM preadipocytes decline at a lower weight loss threshold. Magkos et al. showed that a weight loss of 11% to 16% was required to downregulate the expression of inflammatory genes in AB SAT.[226] How greater weight loss affects senescence markers in AB preadipocytes requires further research.

From a primary prevention standpoint, we studied young adults without obesity-related comorbidities. Metabolically healthy obesity is often transient,[305] and early adulthood can be an opportune time for weight loss interventions.[306] In mice, adipose tissue senescence precedes the development of insulin resistance, a major risk factor for type 2 diabetes.[307] The aged

preadipocytes in our participants, especially the CO group, may indicate subclinical metabolic dysfunction that can in part improve with weight loss. Although our results are preliminary and they do not have direct clinical implications, they support the recommendation to consider the age of obesity onset when evaluating disease risk and developing care plans for people living with obesity.[29]

Our study has several strengths. The translational, multidisciplinary nature of this study is unique and advances our understanding of human health. We took extra care to verify age of obesity onset by both photographic proof and body rating scales. We included participants within a narrow age range to avoid potential overlap of obesity duration in our two groups and minimize the confounding effect of age. Our groups were similar in important covariates, namely age, body composition, and health status, both before and after weight loss. These similarities allowed us to better understand the effects of age of obesity onset on our outcomes. Furthermore, we studied multiple markers implicated in the multistep process of senescence in both AB and FEM SAT. By using immunofluorescence analysis, we were able to assess colocalization of related markers.

We acknowledge that our research has limitations. We studied a small sample of young, healthy females, which limits the generalizability of our findings. Subsequent studies should replicate our research and examine the added complexities of severe obesity, comorbidities, and advanced age. Research that includes males and investigates sex differences is also warranted. We recognize that our assessment of regional SAT senescence was not exhaustive. However, our results provide a foundation for further investigation as they show for the first time that, compared with females with AO, females with CO have signs of exacerbated preadipocyte aging that are less improved with weight loss. How the age of obesity onset affects other senescence

markers and components of the SAT inflammatory environment warrants further investigation. Future studies should also assess the effects of continued weight loss, weight maintenance, and weight regain.

Our study provides initial evidence that adult females with CO have accelerated preadipocyte aging. After moderate weight loss, preadipocyte DNA damage declined regardless of obesity onset. In terms of senescence markers, only the p21 intensity in FEM $p53^{+}$/ $p21^{+}$ preadipocytes decreased in females with AO. Hence, moderate weight loss does not seem to eliminate senescent preadipocytes, but it may reduce senescence initiation and, in AO, alter the senescence fate. Whether our findings impact the type 2 diabetes risk discrepancy between individuals with CO and AO remains to be determined. Further characterization of the effects of age of obesity onset on the evolution of the disease is necessary. Investigation of complementary treatments that target senescent cells in obesity, such as senolytic agents, is an important avenue for future research.

Supporting Information

Supplementary Methods: Indirect Calorimetry

Participants rested supine for 1 hour in a darkened, quiet, thermoneutral room prior to indirect calorimetry. Oxygen consumption and carbon dioxide production were quantified continuously for 30 minutes using a calibrated, flow-through open-circuit indirect calorimeter (Field Metabolic System and Flow Kit 500, Sable Systems, Las Vegas, NV, USA). The first 10 minutes of the measurement were excluded to account for participant acclimatization.

Supplementary Methods: Clinical Blood Measurements

At baseline, serum concentrations of glucose, total cholesterol, HDL cholesterol, and triglyceride~~s~~ were measured on the Beckman Coulter AU5800 system (Brea, CA, USA) at the

McGill University Health Centre Central Laboratory. LDL cholesterol was calculated as total cholesterol – (HDL cholesterol + (triglycerides/2.2)). Plasma insulin was measured by ELISA (R&D Systems, ON, Canada). We computed the homeostatic assessment of insulin resistance (HOMA-IR) index to assess insulin sensitivity.[1]

Supplementary References

1. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.

Table S1. Changes in regional fat percentages with weight loss and reported precision of the DXA Lunar Prodigy with enCORE software

Characteristic	Decrease		Effect, *p* value			Precision[a]			
	Childhood-onset Obesity	Adult-onset Obesity	Group	Time	Group x Time	ICC	RMS-SD	Coefficient of Variation (%)	Least Significant Change
Total Body Fat (%)	3.7 (1.9, 5.6)	3.0 (1.3, 4.7)	0.569	<0.001	0.530	0.994	0.26	2.0	1.7
Trunk Fat (%)	4.6 (1.9, 7.3)	3.4 (0.9, 5.8)	0.866	<0.001	0.459	0.990	0.43	3.1	2.8
Leg Fat (%)	2.9 (1.6, 4.2)	2.6 (1.4, 3.8)	0.380	<0.001	0.725	0.997	0.37	1.8	1.6
Android Fat (%)	5.5 (2.2, 8.8)	3.2 (0.2, 6.3)	0.928	0.002	0.278	0.992	0.54	1.6	1.6
Gynoid Fat (%)	3.6 (2.1, 5.2)	4.2 (2.8, 5.7)	0.274	<0.001	0.535	0.995	0.51	1.2	1.2

Decreases are differences in least-squares means (95 % CI) from before to after weight loss

[a]reported in a heterogeneous population with a wide age and BMI range (n = 253) who had 3 repeat scans performed by the same technician: Kaminsky LA, Ozemek C, Williams KL, Byun W. Precision of total and regional body fat estimates from dual-energy X-ray absorptiometer measurements. J Nutr Health Aging. 2014;18(6):591-4.

Abbreviations:

ICC = intra-class correlation coefficient; **RMS-SD** = root mean square standard deviation

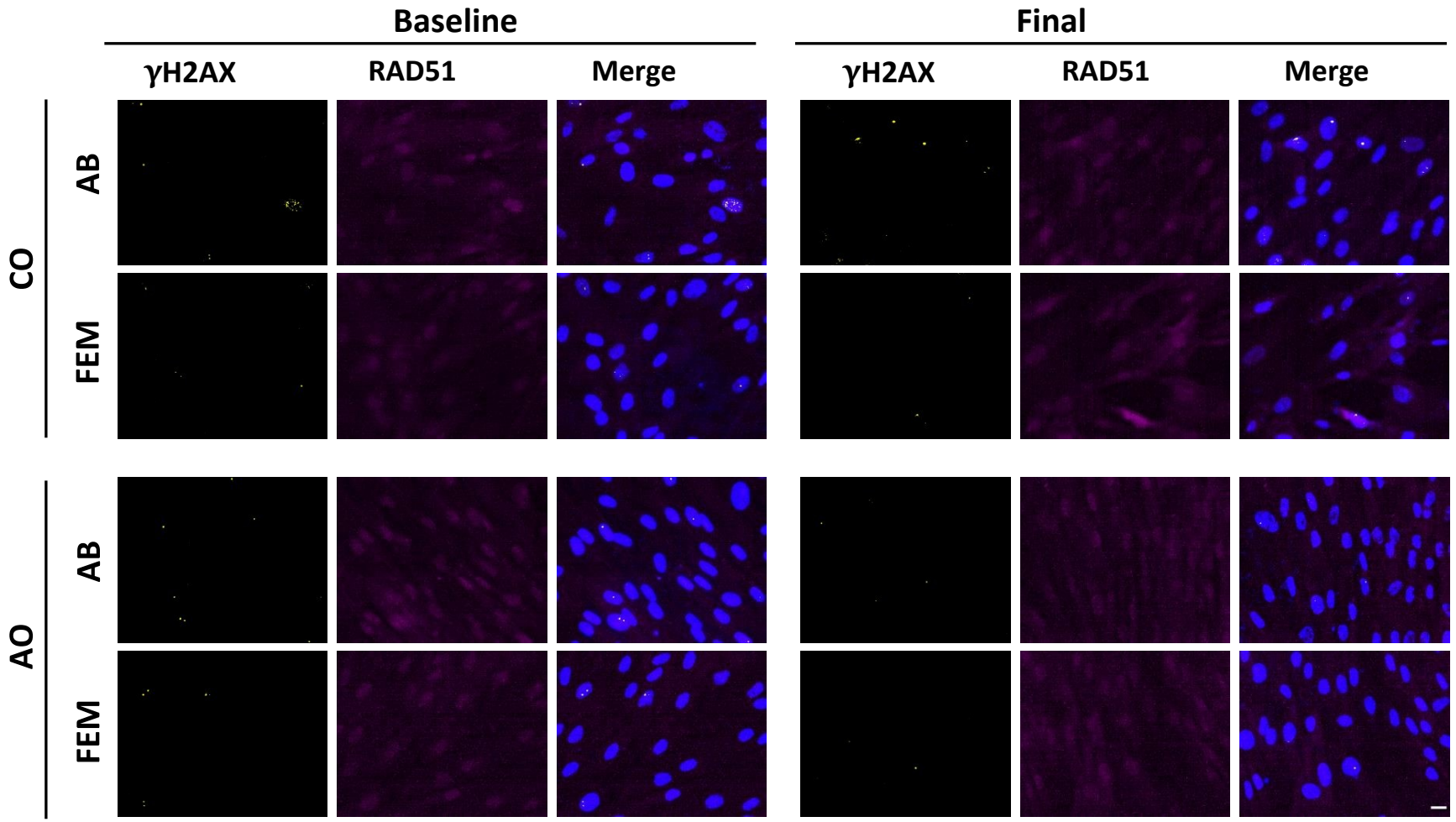

Figure S1. Representative immunofluorescence images of regional preadipocyte γH2AX and RAD51 content in females with childhood-onset and adult-onset obesity before and after moderate weight loss

Preadipocytes were stained with anti-γH2AX (left panel, yellow) and anti-RAD51 (middle panel, magenta). The right panel shows the images merged with DAPI (blue) that stains nuclei. The brightness and contrast of γH2AX and RAD51 were adjusted equally across images. Scale bar is 10 µm.

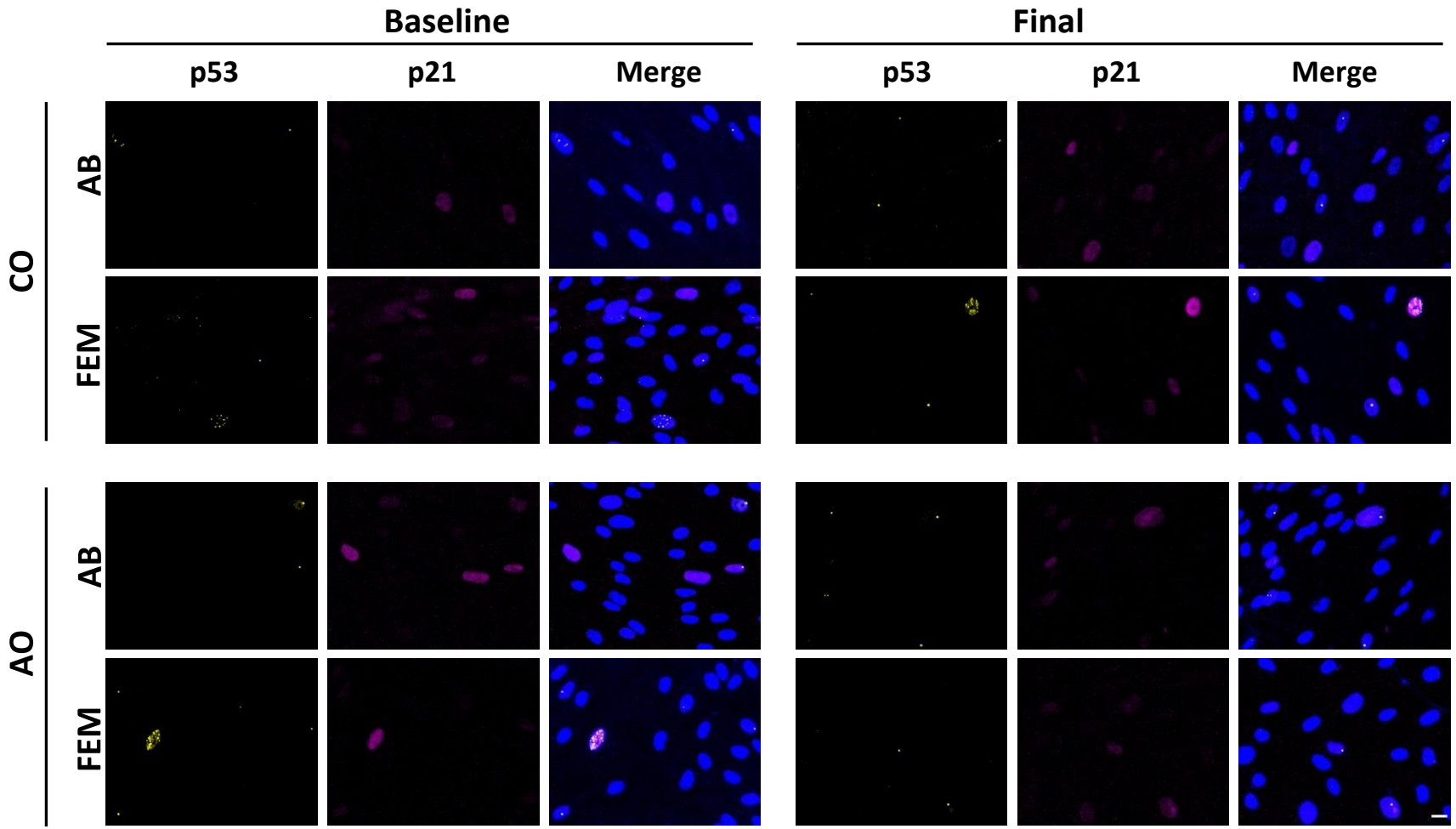

Figure S2. Representative immunofluorescence images of regional preadipocyte p53 and p21 content in females with childhood-onset and adult-onset obesity before and after moderate weight loss

Preadipocytes were stained with anti-phospho-p53 (Ser15) (left panel, yellow) and anti-p21 (middle panel, magenta). The right panel shows the images merged with DAPI (blue) that stains nuclei. The brightness and contrast of phospho-p53 (Ser15) and p21 were adjusted equally across images. Scale bar is 10 µm.

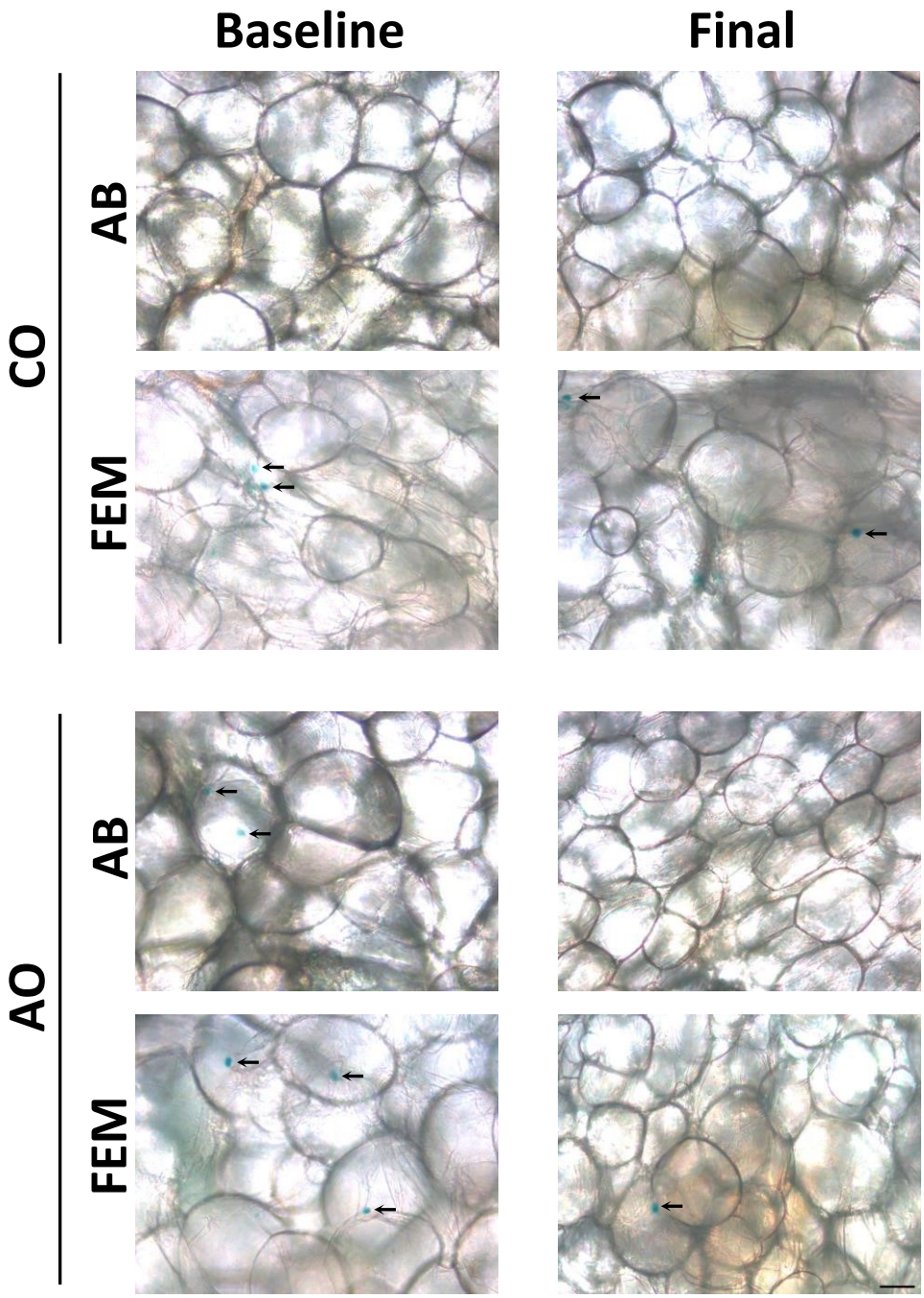

Figure S3. Representative images of regional subcutaneous adipose tissue senescence-associated β-galactosidase activity in females with childhood-onset and adult-onset obesity before and after moderate weight loss

Adipose tissue was stained with senescence-associated-β-galactosidase (SA-β-gal) activity solution. Arrows point to blue dots that represent SA-β-gal^{+} cells. Scale bar is 25 µm.

Bridge 3

In *original article 2*, we found that compared to females with adult-onset obesity, those with childhood-onset obesity have an accelerated preadipocyte aging state that improves with weight loss in terms of DNA damage but not senescence markers.

In *original article 3*, we shift our focus to another component of SAT (dys)function by comparing SAT macrophage and T cell profiles—and their changes after moderate (~10%) weight loss—between females with childhood-onset and adult-onset obesity.

Original article 3.
The age of obesity onset affects changes in subcutaneous adipose tissue macrophages and T cells after weight loss

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Murphy J, Morais JA, Tsoukas MA, Cooke AB, Daskalopoulou SS, Santosa S

Abstract

Context: Adipose tissue inflammation may explain why adults with childhood-onset obesity (CO) have a higher risk of type 2 diabetes than those with adult-onset obesity (AO).

Objective: We aimed to determine whether the age of obesity onset (CO vs. AO) affects abdominal and femoral subcutaneous adipose tissue (SAT) immune cell proportions before and after moderate (~10%) weight loss.

Methods: We collected abdominal and femoral SAT from females with CO or AO before (CO: $n = 14$; AO: $n = 13$) and after (CO: $n = 8$; AO: $n = 6$) diet- and exercise-induced weight loss. We used flow cytometry to quantify the proportions of macrophages and T cells in the stromovascular fraction of both SAT regions.

Results: Abdominal $CD68^+CD206^-$ macrophages were slightly higher in AO than CO at baseline but declined in AO, equalizing between groups after weight loss. Femoral $CD68^+CD206^-$ macrophages, as well as abdominal and femoral $CD68^+CD206^+$ macrophages and $CD3^+CD8^+$ T cells, did not differ between groups at baseline or change after weight loss. Abdominal and femoral $CD3^+CD4^+$ T cells increased after weight loss in AO but remained unchanged in CO.

Conclusion: Unexpectedly, females with AO have slightly higher abdominal M1-like 'pro-inflammatory' macrophages compared to those with CO, but this difference diminishes after weight loss. In contrast, baseline T cells are unaffected by age of obesity onset, but the $CD3^+CD4^+$ population—potentially pro- or anti-inflammatory—increases after weight loss only in those with AO. SAT immune cell profiles may not fully explain the increased type 2 diabetes

risk associated with CO or the broader effects of moderate weight loss on cardiometabolic health.

Introduction

Childhood obesity often persists into adulthood,[229] bringing with it a host of metabolic abnormalities.[308] Compared to people who develop obesity as adults, those with childhood-onset obesity (CO) face a heightened risk of type 2 diabetes.[163,166,168,174–176,182,183,186] While the mechanisms behind this increased risk remain unclear, chronic, low-grade inflammation—implicated in both insulin resistance and β-cell dysfunction[309]—may play a key role. Inflammation also contributes to arterial stiffness, a marker of subclinical cardiovascular disease.[310] Increased arterial stiffness often precedes the development of insulin resistance and type 2 diabetes[311,312] and is linked to the microvascular and macrovascular complications of type 2 diabetes.[313]

Adipose tissue serves as a central hub linking inflammation to cardiometabolic disease. As both an immunological and endocrine organ, it harbors diverse immune cells and secretes inflammatory factors that can act locally or systemically. During obesity development in mice, adipose tissue immune cell and secretory profiles shift from anti-inflammatory to pro-inflammatory.[314] Children with obesity already exhibit macrophage infiltration in adipose tissue and elevated circulating inflammatory markers, such as C-reactive protein.[55] Whether their adipose tissue and systemic inflammation worsen as they enter adulthood remains unknown.

Our group has compared adipose tissue macrophage (ATM) populations between female bariatric surgery patients with CO and adult-onset obesity (AO), matched for type 2 diabetes status. We found that the age of obesity onset did not affect M1-like 'pro-inflammatory' and M2-like 'anti-inflammatory' macrophage content in abdominal subcutaneous adipose tissue (SAT)

and visceral adipose tissue (VAT).[71] However, we do not know if such similarities manifest in adults with milder obesity before the onset of comorbidities or if they extend to other immune cells and circulating adipokines.

Furthermore, we do not fully understand whether conventional weight loss treatments can effectively target inflammation in people with CO and AO. While some pro-inflammatory adipose tissue immune cells decrease after bariatric surgery,[315,316] this is not always the case after lifestyle interventions—despite cardiometabolic improvements.[261,317] We suspect that the age of obesity onset may contribute to this variability.

The present study aimed to examine the effect of age of obesity onset (CO vs. AO) on abdominal and femoral SAT macrophage and T-cell populations (primary outcomes), as well as circulating adipokines and subclinical cardiometabolic risk factors (hyperinsulinemia and arterial stiffness) before and after moderate (~10%) weight loss.

Methods

Participants and study design

We recruited healthy, non-smoking adult females (age = 25-40 years; BMI = 30-39 kg/m^2) who acquired obesity either pre-/peri-puberty (CO; females: n = 14); or after the age of 18 (AO; females: n = 13). A subset of these participants were included in a previously published study.[91] Participants provided photographic proof of body size around puberty (~10-14 years old) and verified their body size at different ages using the Collins' Childhood Body Rating Scales[35] and the Stunkard Body Rating Scale.[31] Detailed eligibility criteria have been previously described.[91] Ethical approval was obtained from the Concordia University Human Research Ethics Committee. All participants provided written informed consent.

The study design has been previously detailed.[91] In brief, the study consisted of a baseline 2-week weight stabilization period, a weight loss period, and a final 2-week weight stabilization period. The weight loss period ended when participants lost approximately 10% of their initial body weight. The following assessments were completed toward the end of each weight stabilization period after a 12-hour fast: anthropometric and body composition measurements (dual-energy x-ray absorptiometry and L2-L3 single-slice computed tomography), indirect calorimetry, a blood draw, an arterial stiffness measurement, and an abdominal (lateral periumbilical region) and femoral (lateral upper thigh) SAT biopsy. Standard clinical blood measurements (serum glucose, total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides) were taken only at baseline. The clinical assessments[91] and biopsy procedure[318] have been previously described. Eight participants with CO and six participants with AO completed assessments both before and after weight loss.

Lifestyle weight loss protocol

Details of the weight loss protocol have been previously outlined.[91] Participants were instructed how to decrease their energy intake by 20% using exchange lists for meal planning and to increase their energy expenditure by 10% through moderate-to-vigorous intensity aerobic exercise (treadmill and/or elliptical), targeting a total energy deficit of 30%. The protocol was adapted from a previously successful weight loss study.[279]

Subclinical cardiometabolic risk assessments

We assessed plasma insulin concentrations and arterial stiffness as markers of subclinical cardiometabolic risk. Plasma insulin concentration was measured by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, ON, Canada). Arterial stiffness was assessed as

carotid-femoral pulse wave velocity (cfPWV) using applanation tonometry (SphygmoCor, AtCor Medical, Sydney, Australia) as previously described.[248]

Circulating Adipokines

Plasma concentrations of plasminogen activator inhibitor-1 (PAI-1), monocyte chemoattractant protein-1 (MCP-1), and resistin were measured using a Human ProcartaPlex™ Simplex Kit (Thermo Fisher Scientific [Invitrogen], Waltham, MA, USA). Plasma concentrations of leptin, adiponectin, interleukin (IL)-8 (R&D Systems), and IL-6 (Abcam, ON, Canada) were measured by ELISA. Adiponectin is an anti-inflammatory, insulin-sensitizing adipokine, while the others are pro-inflammatory.

SAT immune cell analysis

SAT immune cells were isolated and analysed as detailed by our lab.[318] Briefly, the stromovascular cells from approximately 1 g of SAT were isolated by collagenase digestion, purified, stained with CD68, CD206, CD3, CD4, and CD8 antibodies (Table S1), and analyzed using an 8-color BD FACSVerse (BD Biosciences, San Jose, California) and FlowJo software version 9.3.2 (Treestar Inc., Ashland, Oregon). Table S2 shows our single-stain and fluorescence-minus-one controls. We quantified the number of $CD68^{+}CD206^{-}$ (M1-like) macrophages, $CD68^{+}CD206^{+}$ (M2-like) macrophages, $CD3^{+}CD4^{+}$ (T helper or T regulatory) cells, and $CD3^{+}CD8^{+}$ (cytotoxic) T cells. Our gating strategy for immune cell identification is displayed in Figure S1. We expressed immune cell quantities as a percentage of live stromovascular cells.

Complementary adipose tissue analyses

We measured mean adipocyte volume as previously described.[319] Additionally, we conducted an *ex vivo* analysis of SAT secretions (conditioned media) to measure adiponectin using ELISA, as detailed elsewhere.[320]

Statistical Analyses

Data analyses were conducted using SAS version 9.4 (SAS Institute Inc.). Baseline participant characteristics were reported as means (SEM) and compared between groups with independent t tests.

For our main analyses, we used marginal models (SAS PROC MIXED with the REPEATED statement) and applied restricted maximum likelihood estimation to handle unbalanced data and adjust the residuals' covariance structure flexibly. The models included the adipose tissue immune cell proportions, adipocyte size, or SAT-secreted adiponectin as outcomes and group (obesity onset), SAT region, time (weight loss), and all two-way and three-way interactions as fixed factors. The models for circulating adipokines included group, time, and the group-by-time interaction as fixed factors. We used likelihood ratio χ^2 tests to select the appropriate covariance structure (compound symmetry, compound symmetry heterogeneous, or unstructured) for each model. We assessed the normality of residuals for each model using the Shapiro-Wilk test and by visual inspection, and log-transformed dependent variables when necessary. The Kenward–Roger method was used to estimate the degrees of freedom.[281] We decomposed significant interactions graphically and with relevant within- and between-group contrasts. When the three-way interaction was significant, we tested the group-by-time and group-by-region interactions at each level of the third variable (region and time, respectively) and examined simple contrasts as needed.

We expressed model results as least-squares means (lsmeans) (95% CI) or differences in lsmeans (95% CI). The lsmeans from transformed data were back-transformed to the original scale for easier interpretation. The differences in lsmeans were back-transformed for logged outcomes, giving the ratio of the geometric lsmeans[282] or fold difference. We computed Cohen's d ($d = 2t(\text{sqrt}(df))$) using the model t values and degrees of freedom (df),[283] then converted it to Hedge's g ($g = d(1 - 3/(4df - 1))$) to adjust for small sample size bias.[284] Statistical significance was set at $p < 0.1$ for interactions and $p < 0.05$ for main effects and contrasts, consistent with prior research.[285,286]

Results

Participant characteristics

At baseline, participants with CO and AO did not differ in mean age, BMI, or clinical blood measurements (Table 1). There were no differences in baseline characteristics between participants who completed the weight loss protocol and those who did not (data not shown).

Table 1. Demographic and clinical characteristics of study participants

Characteristic	Childhood-onset Obesity (n = 14)	Adult-onset Obesity (n = 13)	*p* value
Age (years)	30.2 (1.0)	30.9 (0.8)	0.583
Body Mass Index (kg/m^2)	33.5 (0.8)	33.9 (0.8)	0.732
Glucose (mmol/L)	4.6 (0.1)[a]	4.7 (0.1)	0.663
Triglycerides (mmol/L)	1.0 (0.1)	1.2 (0.1)	0.405
Total Cholesterol (mmol/L)	4.5 (0.2)	4.4 (0.2)	0.961
HDL-Cholesterol (mmol/L)	1.3 (0.1)	1.2 (0.1)	0.616
LDL-Cholesterol (mmol/L)	2.7 (0.2)	2.6 (0.2)	0.879

Results are means (SEM)

[a] n = 13

Abbreviations: HDL = high-density lipoprotein; **LDL** = low-density lipoprotein

Participants lost an average of 8 kg, or 9 % of their initial weight. Total and percent body fat, as well as visceral and subcutaneous android fat, did not differ between obesity-onset groups at baseline and declined similarly across groups with weight loss. The android-to-gynoid fat ratio remained unchanged with weight loss in both groups (Table 2).

Table 2. Body weight and composition before and after weight loss

Characteristic	Baseline		Final		Effect, *p* value		
	Childhood-onset Obesity (*n* = 14)	Adult-onset Obesity (*n* = 13)	Childhood-onset Obesity (*n* = 8)	Adult-onset Obesity (*n* = 6)	Group	Time	Group x Time
Weight (kg)	91.3 (85.9, 96.6)	94.0 (88.5, 99.5)	82.6 (77.1, 88.2)	85.9 (80.0, 91.8)	0.428	<0.001	0.806
Total Body Fat (kg)	41.1 (37.3, 44.8)	42.7 (38.7, 46.6)	33.4 (29.8, 37.0)	37.5 (33.4, 41.7)	0.176	0.004	0.402
Total Body Fat (%)	45.2 (42.9, 47.5)	45.4 (43.1, 47.8)	41.7 (39.4, 44.1)	42.4 (39.9, 45.0)	0.768	<0.001	0.619
CT Android VAT (kg)	0.79 (0.56, 1.0)	0.82 (0.56, 1.1)	0.67 (0.43, 0.91)	0.61 (0.36, 0.86)	0.921	0.003	0.332
CT Android SAT (kg)	2.9 (2.6, 3.3)	2.8 (2.5, 3.2)	2.4 (2.0, 2.7)	2.4 (2.1, 2.8)	0.903	<0.001	0.173
Android Fat (kg)/Gynoid Fat	0.54 (0.48, 0.60)	0.47 (0.41, 0.53)	0.53 (0.45, 0.60)	0.48 (0.39, 0.56)	0.196	0.780	0.552

Results are least squares means (95 % CI)

Abbreviations: CT = computed tomography; **SAT** = subcutaneous adipose tissue; **VAT** = visceral adipose tissue

Subclinical cardiometabolic risk factors

The obesity-onset groups did not differ in plasma insulin concentration or arterial stiffness at baseline. Across groups, plasma insulin concentration decreased 1.33-fold (95% CI: 1.07, 1.66; $p = 0.016$; $g = 1.50$) and cfPWV decreased by 0.64 m/s (95% CI: 0.30, 0.99; $p = 0.001$; $g = 1.98$) (Figure 1A and D).

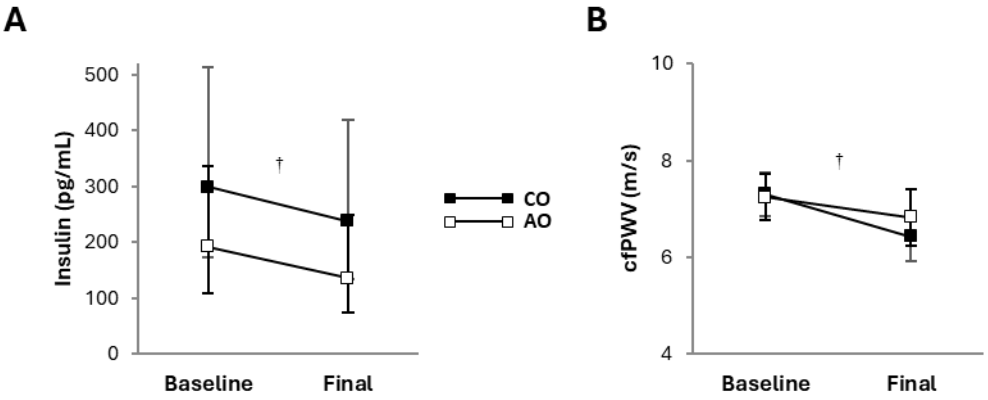

Figure 1. Subclinical cardiometabolic risk factors in females with childhood-onset and adult-onset obesity before and after moderate weight loss

Subclinical cardiometabolic risk factors by group over time. Results are presented as least-squares means (95% CI).

In **A**, †time, $p = 0.016$: baseline > final.
In **B**, †time, $p = 0.001$: baseline > final.

cfPWV = carotid-femoral pulse wave velocity (arterial stiffness); AO = group with adult-onset obesity; CO = group with childhood-onset obesity

Adipokines

After weight loss, plasma leptin concentration decreased 1.86-fold (95% CI: 1.50, 2.30; $p < 0.001$; $g = 2.77$) and plasma PAI-1 concentration decreased 1.24-fold (95% CI: 1.05, 1.46; $p = 0.016$; $g = 1.46$) across groups (Figure 2A and D). There were no group, time, or group-by-time interaction effects on the plasma concentrations of other proinflammatory adipokines (Figure 2B, C, E, and F) or adiponectin (Figure 3A). The adiponectin concentration in SAT-conditioned

media, however, increased after weight loss (1.31-fold (95% CI: 1.02, 1.68); $p = 0.032$; $g = 0.56$) across groups and SAT regions (Figure 3B)

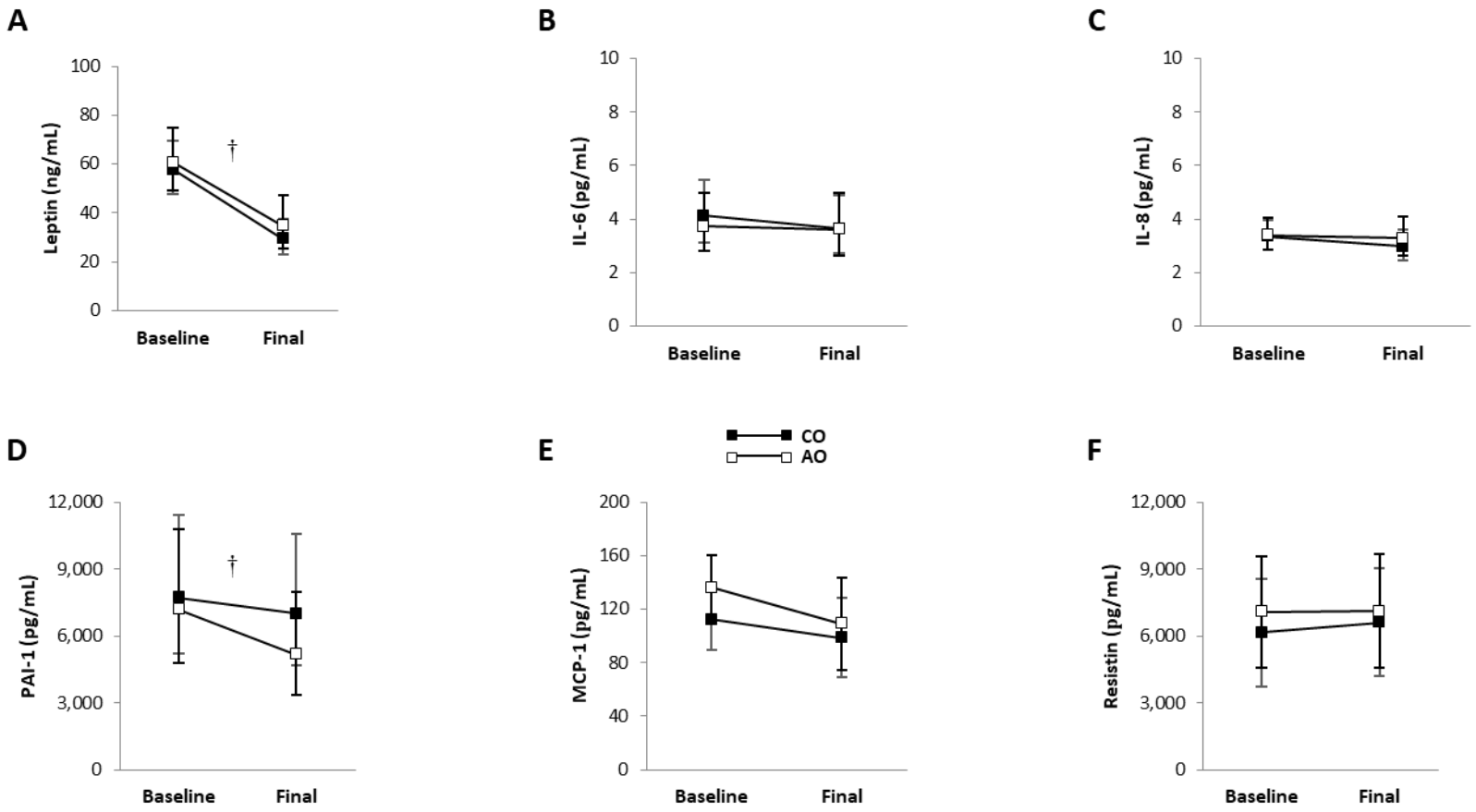

Figure 2. Plasma proinflammatory adipokine concentrations in females with childhood-onset and adult-onset obesity before and after moderate weight loss

Plasma proinflammatory adipokine concentrations by group over time. Results are presented as least-squares means (95% CI).

In **A**, †time, $p < 0.001$: baseline > final.
In **D**, †time, $p = 0.016$: baseline > final.

AO = group with adult-onset obesity; CO = group with childhood-onset obesity; IL = interleukin; MCP-1 = monocyte chemoattractant protein-1; PAI-1 = plasminogen activator inhibitor-1

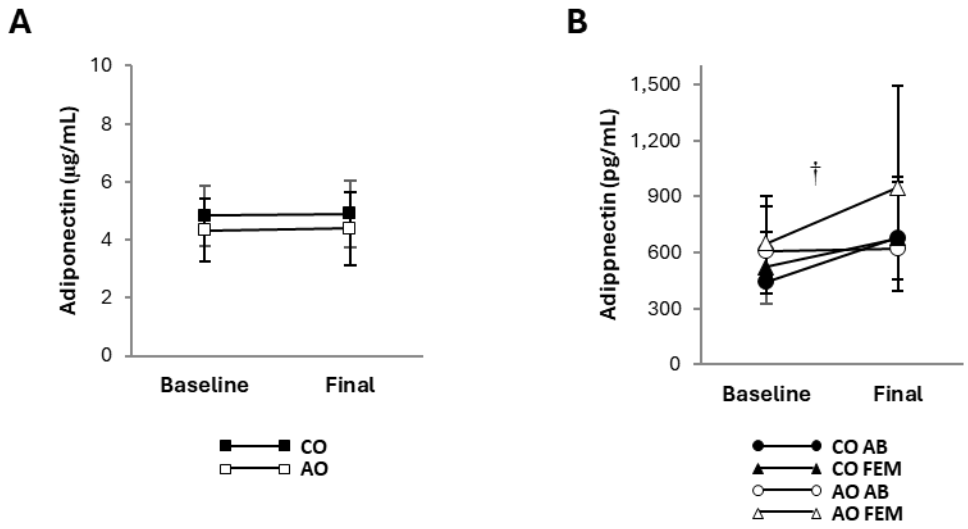

Figure 3. Plasma and subcutaneous adipose tissue-conditioned media adiponectin concentrations in females with childhood-onset and adult-onset obesity before and after moderate weight loss

Plasma and subcutaneous adipose tissue-conditioned media adiponectin concentration by group over time. Results are presented as least-squares means (95% CI).

In **B**, †time, $p = 0.032$: baseline < final.

AB = abdominal region; AO = group with adult-onset obesity; CO = group with childhood-onset obesity; FEM = femoral region

Adipocyte Size

There was a group-by-region-by-time interaction on mean adipocyte size (Figure 4). The group-by-region interaction was significant at baseline; mean adipocyte size was greater in the femoral region than in the abdominal region in the CO group (313 pL [95% CI: 129, 497]; $p = 0.002$; $g = 1.36$) but greater in the abdominal region than in the femoral region in the AO group (285 pL [95% CI: 95, 476]; $p = 0.005$; $g = 1.19$). The group-by-time interaction was not significant in the abdominal or femoral region. Across time, mean adipocyte size was 394 pL (95% CI: 180, 609) smaller in the CO group than in the AO group in the abdominal region ($p = 0.001$; $g = -1.68$) and not different between groups in the femoral region. Across groups, adipocyte size decreased with weight loss by 329 pL (95% CI: 120, 539) in the abdominal region ($p = 0.004$; $g = -1.43$) and by 280 pL (95% CI: 48, 512) in the femoral region ($p = 0.021$; $g = -$

1.16). A slight difference in the change over time between regions in the AO group (larger decrease in the abdominal region) led to a group-by-region interaction after weight loss that manifested differently than at baseline. While adipocyte size remained greater in the femoral region than in the abdominal region in the CO group (176 pL [95% CI: 31, 321]; $p = 0.021$; $g = 1.43$), there was no regional difference in the AO group after weight loss.

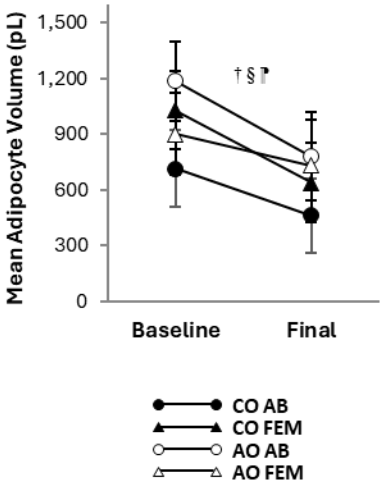

Figure 4. Regional subcutaneous adipocyte size in females with childhood-onset and adult-onset obesity before and after moderate weight loss

Adipocyte size by group and subcutaneous adipose tissue region over time. Results are presented as least-squares means ± 95% CIs.

†time, p =0.005; §region-by-onset, $p < 0.001$; ¶group-by-region-by-time, $p = 0.004$: group-by-time in AB, $p = 0.454$ (across time: CO < AO, $p = 0.001$; across groups: baseline > final, $p = 0.004$); group-by-time in FEM, $p = 0.331$ (across time: CO = AO, $p = 0.867$); group-by-region at baseline, $p < 0.001$ (CO: AB < FEM, $p = 0.002$; AO: AB > FEM, $p = 0.005$); group-by-region at final, $p = 0.049$ (CO: AB < FEM, $p = 0.021$; AO: AB = FEM, $p = 0.547$)

AB = abdominal region; AO = group with adult-onset obesity; CO = group with childhood-onset obesity; FEM = femoral region

SAT immune cells

There was a group-by-region-by-time interaction on the proportion of M1-like $CD68^{+}CD206^{-}$ macrophages (Figure 5A) indicating that the group differences in the change over time were not consistent across regions or that group differences in regional variations were not

consistent before and after weight loss. However, the group-by-region interaction before and after weight loss and the group-by-time interaction in the abdominal and femoral region were not significantly different from zero, suggesting that these two-way interactions did not fully explain the three-way interaction. Across time, the proportion of $CD68^{+}CD206^{-}$ macrophages was not significantly different between the CO and AO groups before and after weight loss. Across groups the proportion of $CD68^{+}CD206^{-}$ macrophages was 1.40-fold (95% CI: 1.08, 1.83; p = 0.013; g = 1.20) greater in the abdominal region than in the femoral region at baseline and declined in the abdominal region (2.40-fold [95% CI: 1.02, 5.62]; p = 0.045; g = 1.00) but not in the femoral region with weight loss. As a result, there was no regional difference across groups post-weight loss. Simple contrasts, however, indicated that the regional difference at baseline and the decline in the abdominal region were specific to the AO group. These contrasts help explain the three-way interaction that can be depicted on the graph: the change over time was less parallel for the CO and AO groups in the abdominal region (higher at baseline and larger decrease in AO compared to CO, though non-significant) than the femoral region; and the regional difference was more pronounced in AO than CO before weight loss compared to after weight loss. There were no group, region, time, or interaction effects on the proportion of M2-like $CD68^{+}CD206^{+}$ macrophages (Figure 5B).

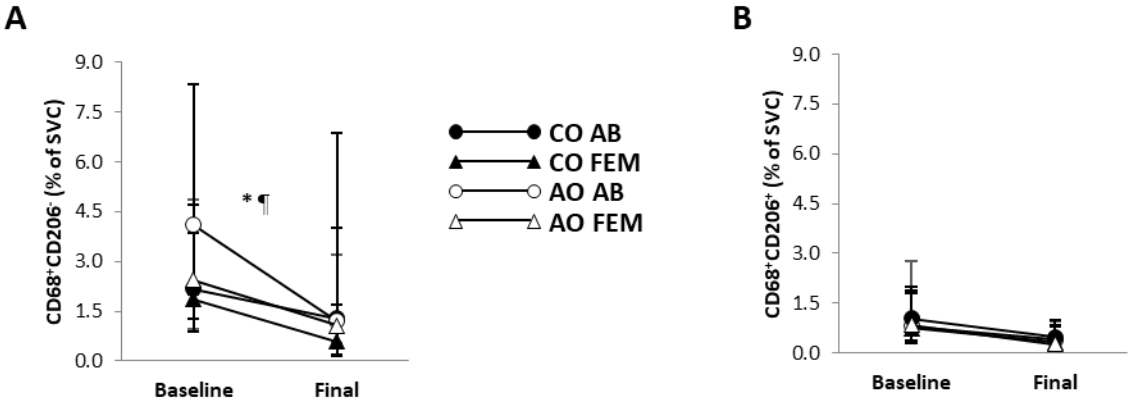

Figure 5. Regional subcutaneous adipose tissue macrophage populations in females with childhood-onset and adult-onset obesity before and after moderate weight loss

A-B Macrophage populations by group and subcutaneous adipose tissue region over time. Outcomes were natural log-transformed prior to analysis but are displayed as back-transformed values. Results are presented as least-squares means ± 95% CIs.

In **A**, *region, $p = 0.024$; ¶group-by-region-by-time, $p = 0.076$: group-by-time in AB, $p = 0.402$ (across time: CO = AO, $p = 0.537$; across groups: baseline > final, $p = 0.045$); group-by-time in FEM, $p = 0.748$ (across time: CO = AO, $p = 0.428$; across groups: baseline = final, $p = 0.076$); group-by-region at baseline, $p = 0.166$ (across groups: AB > FEM, $p = 0.013$); group-by-region at final, $p = 0.215$ (across groups: AB = FEM, $p = 0.106$). Although these two-way interactions cannot fully explain the three-way interaction, simple contrasts indicated that the regional difference (AB > FEM) at baseline and the change in AB (baseline > final) occurred in AO and not CO. These contrasts correspond with the graphical depiction showing that the change over time was less parallel for CO and AO in AB compared to FEM.

AB = abdominal region; AO = group with adult-onset obesity; CO = group with childhood-onset obesity; FEM = femoral region; SVC = stromovascular cells

There was a region effect but no group, time, or interaction effects on the proportion of $CD3^+CD8^+$ T cells (Figure 6B). The percentage of $CD3^+CD8^+$ T cells was greater in the femoral than abdominal region across groups and time (1.17 percentage points [95% CI: 0.48, 1.86,]; $p = 0.001$; $g = 1.07$). Similarly, the percentage of $CD3^+CD4^+$ T cells was greater in the femoral than abdominal region across groups and time (1.65 percentage points [95% CI: 0.87, 2.43]; region effect, $p < 0.001$; $g = 1.32$). In addition, there was a group-by-time interaction on the percentage of $CD3^+CD4^+$ T cells (Figure 6A). Across regions, the percentage of $CD3^+CD4^+$ T cells did not differ between groups pre-weight loss and increased after weight loss in the AO group only (1.54 percentage points [95% CI: 0.20, 2.87]; $p = 0.025$; $g = 0.64$). Therefore, the percentage of $CD3^+CD4^+$ T cells was higher in the AO group than in the CO group across regions post-weight loss (2.86 percentage points [95% CI: 0.99, 4.73]; $p = 0.004$; $g = 0.85$).

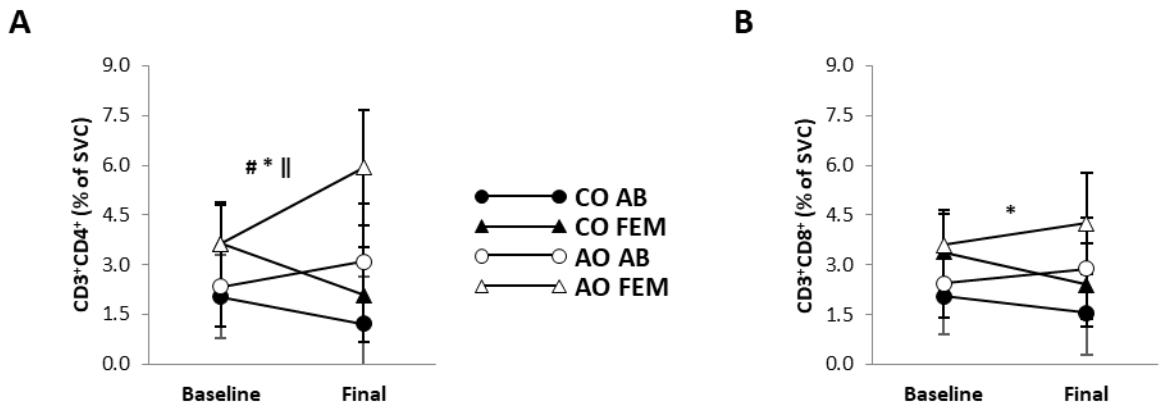

Figure 6. Regional subcutaneous adipose tissue T-cell populations in females with childhood-onset and adult-onset obesity before and after moderate weight loss

A-B T-cell populations by group and subcutaneous adipose tissue region over time. Results are presented as least-squares means ± 95% CIs.

In **A**, #group, $p = 0.043$; *region, $p < 0.001$: AB < FEM; ‖group-by-time, $p = 0.004$ (across regions at baseline CO = AO, $p = 0.843$; across regions in CO: baseline = final, $p = 0.063$; across regions in AO: baseline < final, $p = 0.025$; across regions at final: CO < AO, $p = 0.004$)

In **B**, *region, $p < 0.001$: AB < FEM

AB = abdominal region; AO = group with adult-onset obesity; CO = group with childhood-onset obesity; FEM = femoral region; SVC = stromovascular cells

Discussion

This study was motivated by the increased risk of type 2 diabetes associated with CO and is the first of its kind to examine the effects of age of obesity onset on regional SAT immune cells and systemic inflammation before and after weight loss. Contrary to our expectations, we did not find a more pro-inflammatory SAT immune cell profile in females with CO than in those with AO. Instead, we found a slightly higher proportion of M1-like macrophages in the abdominal SAT of females with AO, and no other differences in macrophage or T-cell proportions between groups. Fasting plasma adipokine and insulin concentrations, as well as arterial stiffness, were also not significantly different between groups. Moderate (~10%) weight loss similarly reduced arterial stiffness and plasma concentrations of insulin, leptin, and PAI-1 in

females with CO and AO. However, SAT immune cell proportions only shifted in females with AO. After weight loss, their M1-like macrophages in abdominal SAT decreased, while their $CD3^+CD4^+$ T cells in abdominal and femoral SAT increased.

Consistent with our baseline findings on M1-like macrophages, females with AO exhibit greater IL-6 gene expression in abdominal SAT than those with CO.[3] Furthermore, Attard et al. found that 27-year-old females with obesity who experienced the greatest weight gain over the past 7 years had increased odds of systemic inflammation (CRP >3 mg/L) compared to those who gained most of their weight earlier in life.[175] Circulating concentrations of CRP, but not IL-6, correlate with pro-inflammatory macrophages and IL-6 gene expression in SAT.[321] Therefore, it is not surprising that we did not find a difference in plasma IL-6 concentration between groups.

A mouse study by Wernstedt Asterholm et al.[322] prompts us to question whether increased abdominal SAT pro-inflammatory macrophages in AO represent a temporary adaptive response to recent weight gain rather than a driver of chronic inflammation. Wernstedt Asterholm et al. found that, during the early stages of obesity development in mice, adipose tissue inflammation is crucial for proper extracellular matrix remodelling and angiogenesis, processes that promote adipogenesis.[322] They postulated that by promoting healthy tissue expansion, acute adipose tissue inflammation can resolve before becoming chronic.[322] While no studies have compared adipogenic capacity between people with CO and AO, our lab has provided evidence suggesting increased lipogenic capacity in females with AO.[3]

Although it is appealing to view the increased abdominal SAT proinflammatory macrophages in AO as an adaptive response to recent weight gain, we must consider that adult obesity typically develops over several months or even years in humans. This raises questions about what constitutes an ‘acute’ inflammatory response to weight gain in humans and how long healthy adipose tissue remodelling can persist before chronic, low-grade inflammation takes

over. Short-term overfeeding studies in healthy adults show that after a ~3-kg weight gain, the total number of macrophages in abdominal SAT remains stable. However, the M1/M2 macrophage ratio increases along with markers of extracellular matrix remodelling and angiogenesis. Additionally, the circulating concentration of CRP increases, while that of IL-6 remains unchanged.[323,324] What remains unclear is whether these changes after weight gain are tempered once weight stabilizes over the long term. In our study, participants were weight-stable for at least three months, yet we still observed slight differences in abdominal SAT M1-like macrophages between those with recent weight gain (AO) and those with CO. Studies comparing bariatric surgery patients with CO and AO indicate that by the time they have severe obesity with comorbidities, their ATM profiles[71] and circulating CRP concentrations[133] converge. The adiposity trajectories leading to this convergence remain to be studied.

Even though our findings suggest that SAT immune cells and circulating adipokines may not explain the increased type 2 diabetes risk associated with childhood-onset obesity, they do not discount the role of inflammation entirely. While the degree of SAT and systemic inflammation is one consideration for cardiometabolic risk, the duration of exposure to inflammation is another. Still, this reasoning does not align with our subclinical findings. If those with CO experience chronic, low-grade inflammation for longer, why don't they exhibit worse arterial stiffness than those with AO? Differences in other triggers of arterial stiffness, such as oxidative stress, endothelial dysfunction, or structural changes in the artery, could provide an explanation.

However, both obesity-onset groups had average cfPWV values (~7 m/s) in the upper-normal range for young healthy adults,[325] well below the clinical concern threshold of 10 m/s.[326] While the duration of obesity accelerates arterial stiffness in middle-aged and older adults,[327] this may not be the case for younger adults. Evidence shows that adiposity does not correlate with

arterial stiffness until middle age.[328–331] At younger ages, researchers proposed, the vasculature may temporarily adapt to the increased cardiac output associated with obesity by decreasing arterial stiffness.[330,331]

The average plasma insulin concentration was ~100 pmol/L greater in females with CO than in those with AO, and although this difference was not statistically significant, it may still be clinically relevant. Hyperinsulinemia, a well-established risk factor for type 2 diabetes, remains difficult to define due to variability in insulin assays. However, the mean plasma insulin concentrations of ~300 pmol/L in our CO group and ~200 pmol/L in our AO group are notably higher than the ~60 pmol/L we measured in a small sample of lean females using the same methodology (unpublished). Plasma insulin concentrations reflect the balance between insulin secretion and clearance. An increase in the former and a decrease in the latter can lead to fasting and postprandial hyperinsulinemia in people with obesity.[332]

Drawing from current evidence, we suspect that compensatory hyperinsulinemia may be more common in AO, whereas hypersecretion-induced hyperinsulinemia may be more common in CO. Studies using the hyperinsulinemic-euglycemic clamp technique have found worse insulin resistance in people with AO compared to those with CO.[143,144] Moreover, at the same level of glycemia and insulin sensitivity, children with obesity have hyperresponsive β-cells compared to adults with obesity.[187–189] This hyperresponsiveness could lead to accelerated β-cell exhaustion and an earlier onset of type 2 diabetes in adults with CO.[190] Studies comparing insulin kinetics and multi-organic insulin sensitivity between adults with CO and AO over time are required to test this hypothesis.

Many cardiometabolic risk factors improve following lifestyle weight loss interventions,[226,333,334] but whether changes in adipose tissue immune cell profiles drive these improvements remains unclear. Collectively, human studies suggest that total abdominal SAT

macrophage content increases or remains stable in the early phases of rapid or gradual diet and/or exercise-induced weight loss (< ~10%), despite improvements in cardiometabolic risk factors.[226,261,317,335–338] However, with continued weight loss (> ~15%), total abdominal SAT macrophage content decreases.[226,335,339] There is also evidence that exercise, with or without weight loss, shifts abdominal SAT macrophages toward an anti-inflammatory M2-like phenotype.[336,340] In our study, only females with AO experienced a decrease in the proportion of abdominal SAT M1-like macrophages after weight loss, eliminating the baseline difference between those with AO and CO. Therefore, in some people, a 10% weight loss achieved through diet and exercise may reduce pro-inflammatory macrophages, with baseline levels possibly playing a role.

In our study, the proportions of abdominal and femoral SAT $CD3^+CD8^+$ T cells (pro-inflammatory) did not change after weight loss, regardless of obesity onset. In contrast, the proportion of $CD3^+CD4^+$ T cells increased in both SAT regions in females with AO, but not in those with CO. Our findings in females with AO align with those of Kratz et al., who examined changes in abdominal SAT T-cell populations after a 1-year lifestyle intervention (~7% weight loss) in people with obesity and type 2 diabetes.[317] Given their participants were older (~50 years), it is plausible that a higher proportion had adult-onset obesity.

SAT Treg cells, an anti-inflammatory subset of $CD3^+CD4^+$ T cells, are both reduced and less functional in adults with obesity.[341] Due to chronic antigen stimulation, they adopt an exhausted phenotype marked by impaired activity and a diminished ability to proliferate.[341] Interestingly, Cottom et al. found that adipose tissue Treg cells remain low and maintain and an exhausted phenotype after weight loss in obese mice.[342] If the increase in $CD3^+CD4^+$ T cells in people with AO is due to a rise in Treg cells, it might suggest that their baseline Treg cells—even if similar in quantity to the CO group—are less exhausted. Conversely, in those with CO,

10% weight loss may not be enough to recover Treg cell exhaustion. Further phenotyping of $CD3^+CD4^+$ T cells in people with CO and AO, both before and after weight loss, is needed to explore this possibility.

For people with obesity engaged in lifestyle interventions, an initial weight loss target of 5-10% is considered clinically significant—but is it biologically significant? We found that after 10% weight loss, females with CO and AO improved their plasma insulin concentration and arterial stiffness. However, our findings, along with those of others,[226,261] suggest that 10% weight loss may not be sufficient to induce positive changes in certain aspects of SAT biology—at least in some people. Consistent with other studies,[226,343] we found that circulating concentrations of only select adipokines, leptin and PAI-1, declined in both groups after moderate weight loss. We also found that adipocyte size decreased and adiponectin secreted from SAT increased in both groups, whereas SAT immune cell profiles only changed in those with AO.

While these results may suggest that changes in SAT immune cells profiles are not required for short-term improvements in cardiometabolic risk factors, we wonder whether they are necessary to sustain improvements over the long-term. Since immune cell infiltration is one of the first changes with weight gain, could it be the last to resolve with weight loss before lasting metabolic health is restored? In mice with diet-induced obesity, a proinflammatory immune cell profile in adipose tissue persisted after weigh loss and, along with glucose tolerance, worsened after weight regain. The authors speculated that a 'memory-like immunological imprinting' may contribute to the exacerbated metabolic dysfunction associated with weight regain. Interestingly, after a 10% diet-induced weight loss in humans, the lower the reduction in gene expression of immune cell integrins in abdominal SAT, the greater the weight regain over the following nine months. These findings may be particularly relevant to those with

CO, whose SAT immune cell profile did not change after 10% weight loss and whose hyperplastic abdominal SAT may predispose them to weight regain.[217] Longitudinal studies are required to determine whether those with CO need greater weight loss or alternative interventions compared to those with AO to prevent weight regain and sustain metabolic improvements.

We acknowledge that the M1/M2 classification may oversimplify the inflammatory and functional diversity of macrophages. We were also unable to assess $CD3^+CD4^+$ T-cell subtypes, which limits our understanding of how they contribute to the SAT inflammatory milieux. Moreover, our analysis was limited to macrophages and T cells in SAT, preventing a comprehensive understanding of the adipose tissue immune landscape. Our findings are derived from a small sample of healthy females with obesity, which limits generalizability to other populations, including males and people with varying degrees of cardiometabolic risk.

A major strength of our study is the use of the gold standard technique, flow cytometry, to quantify immune cell proportions in two SAT regions. SAT immune cell proportions quantified by flow cytometry demonstrate good test-retest reliability,[344] which is crucial for our pre-post design. Another strength is that we verified the age of obesity onset through photographic evidence and body rating scales, ensuring accurate classification of participants.

Our findings provide new evidence that the age of obesity onset influences changes in SAT macrophages and T cells following moderate weight loss. Unexpectedly, females with AO had slightly more proinflammatory macrophages at baseline in abdominal SAT compared to those with CO, though this difference diminished after weight loss. In contrast, T-cell populations in both abdominal and femoral SAT were unaffected by the age of obesity onset; however, $CD3^+CD4^+$ T cells increased after weight loss only in those with AO.

Our results suggest that SAT immune cell profiles may not fully account for the elevated type 2 diabetes risk in people with CO or the short-term cardiometabolic benefits of weight loss. However, the long-term implications of SAT immune changes—or lack thereof—after weight loss remain unclear. Future research should determine if sustained metabolic improvements in CO and AO require specific changes in SAT immune cell populations and whether targeted interventions are needed to support these changes.

Supporting Information

Table S1. Antibody and fluorochrome pairings with supplier and clone information

	PE	PE-Cy7	APC	APC-Cy7	BV510	Compensation Beads
SS PE	CD4					Positive/ negative
SS PE-Cy7		CD68				Positive/ negative
SS APC			CD206			Positive/ negative
SS APC-Cy7				CD8		Positive/ negative
SS BV510					CD3	Positive/ negative
FMO PE		CD68	CD206	CD8	CD3	
FMO PE-Cy7	CD4		CD206	CD8	CD3	
FMO APC	CD4	CD68		CD8	CD3	
FMO APC-Cy7	CD4	CD68	CD206		CD3	
FMO BV510	CD4	CD68	CD206	CD8		

Table S2. Single stain (SS) cocktails and fluorescence minus one (FMO) controls for flow cytometry experiment

Antibody*	**Fluorochrome**	**Supplier**	**Clone**	**Titrated concentration** (µl/10^6 cells)
CD4	PE	BioLegend	SK3	1.5
CD68	PE-Cy7	eBioscience	815CU17	1.5
CD206	APC	eBioscience	19.2	1.5
CD8	APC-Cy7	BioLegend	SK1	1.5
CD3	BV510	BioLegend	SK7	1.5

*Antibodies were titrated on human adipose tissue to determine the optimal staining volume per 10^6 cells.

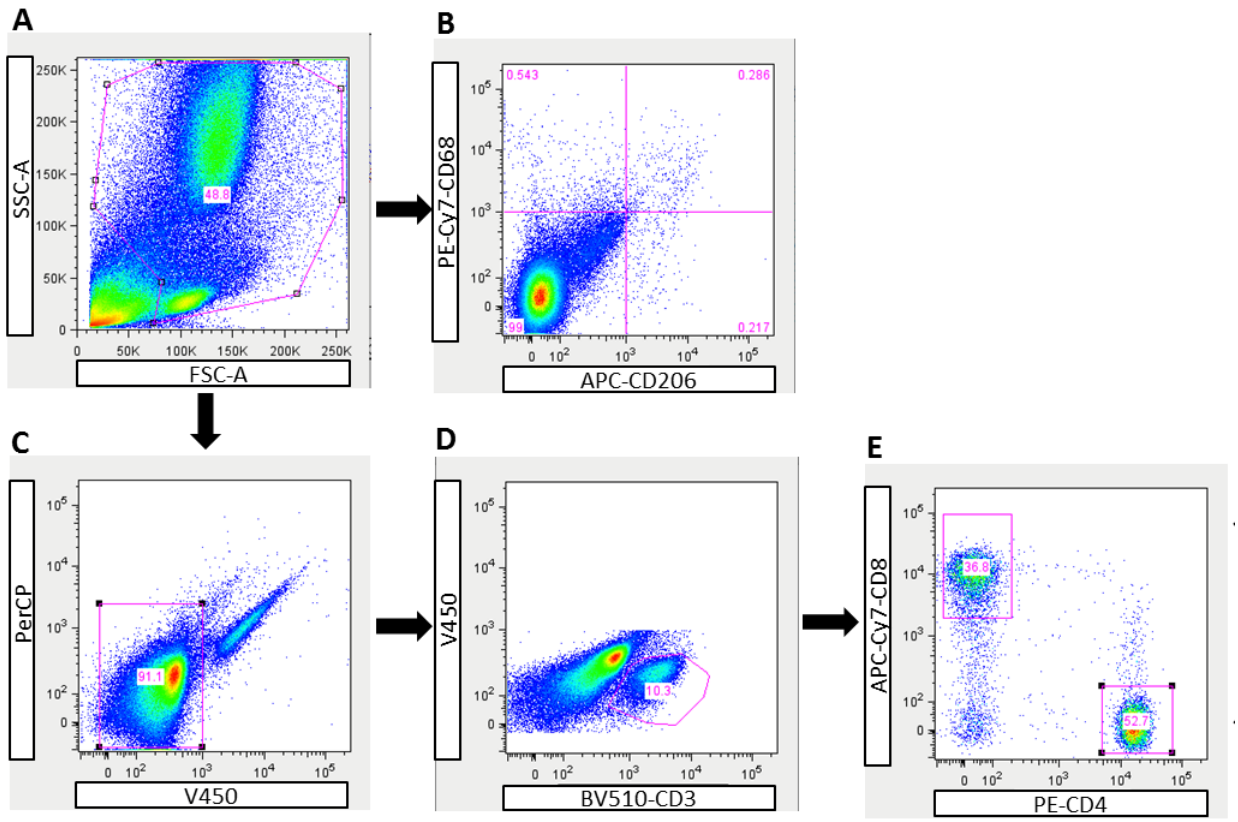

Figure S1. Representative gating strategy for the identification of immune cell populations
(**A**) Live cells were gated, and (**B**) macrophage (CD68+) populations were identified as CD206+ or CD206-. (**C**) Unwanted events were eliminated by gating out the negative cells on the two dump channels, and (**D**) T cells were identified as CD3+. (**E**) From the CD3+ gate, T-cell subpopulations were identified as CD8+ or CD4+.

Bridge 4

We conclude this dissertation by synthesizing the findings of *original articles 1-3* and other relevant literature in a *perspective article* that explores whether childhood-onset and adult-onset obesity are two types of obesity.

DISCUSSION: Perspective article.
Are childhood-onset and adult-onset obesity two types of obesity?

Classifying diseases by age of onset—such as early- versus late-onset type 2 diabetes (< 40 vs. ≥ 40 years), colorectal cancer (<50 vs. ≥ 50 years), and Alzheimer's disease (<65 vs. ≥ 65 years)—has long proven clinically valuable. The age of onset often reveals crucial details about a disease's pathophysiology and clinical course, which can guide treatment strategies. Recognizing this importance, the European Association for the Study of Obesity even included *onset* in its recent obesity taxonomy, designed to bridge policy and practice.[345]

Obesity is unique because it often begins in childhood and tracks into adulthood[229]—though most cases develop during adulthood.[165] Adult obesity can therefore be broadly classified as childhood-onset (≤ 18 years) or adult-onset (>18 years). While obesity history certainly has its place in clinical decision-making,[29] the value of applying a *childhood-onset versus adult-onset* classification to adults with obesity remains unclear. In this Perspective, we explore whether childhood-onset and adult-onset obesity represent two distinct types of the disease and consider the potential implications for treatment strategies.

Obesity development starts with excess adipose tissue expansion. For decades, the mechanisms of expansion were believed to biologically distinguish these two 'types' of obesity. The prevailing notion held that adipose tissue expanded mainly through hyperplasia (an increase in adipocyte number) in childhood-onset obesity and hypertrophy (an increase in adipocyte size) in adult-onset obesity.[56–60] Hyperplasia in adulthood was considered rare and limited to severe obesity[60]—a concept that has since been refuted.[67]

Our recent findings further challenge the idea that the age of obesity onset reliably dictates whether adipose tissue becomes hyperplastic or hypertrophic.[319] In young, healthy adults with mild to moderate obesity, the 'hyperplastic-childhood-onset/hypertrophic-adult-onset'

classification only applied to abdominal subcutaneous adipose tissue (SAT) in females. The degree of abdominal SAT hypertrophy or hyperplasia did not differ between males with childhood-onset and adult-onset obesity. Furthermore, femoral SAT was hypertrophic in both males and females with childhood-onset obesity compared to those with adult-onset obesity (*original article 1*).[319]

Nevertheless, excess adipose tissue—whether hyperplastic or hypertrophic—is just one defining feature of obesity. Dysfunctional adipose tissue with the potential to impair health is another. Unfortunately, we do not yet fully understand how the age of obesity onset influences most disease processes and health outcomes. Type 2 diabetes risk is an exception: population-based cohort studies have exposed a heightened risk in people with childhood-onset obesity compared to those with adult-onset obesity, even when accounting for age and adiposity.[163,166,168,174–176,182,183,186] Still, insulin resistance tends to be more pronounced in those with adult-onset obesity.[143,144] These findings suggest that the clinical course of type 2 diabetes may differ between childhood-onset and adult-onset obesity. It is plausible that β-cells may fail at a lower degree of insulin resistance in people with childhood-onset obesity, perhaps due to prolonged hyperresponsiveness.

Dysfunctional adipose tissue serves as a critical link to insulin resistance and β-cell exhaustion through its pro-inflammatory state and reduced capacity to store lipids.[4,346,347] Children with obesity exhibit SAT characteristics that tend to favour—rather than impair—lipid storage. Compared to lean children, children with obesity have decreased lipolysis, enhanced preadipocyte proliferation and similar differentiation capacity.[55,94] They also have less total fibrosis, which may permit greater adipocyte enlargement.[84] However, these potential adaptations may come at a cost, both in the short and long term.

Children with obesity already have reduced mitochondrial function in their SAT,[106] which, along with increased preadipocyte proliferation, may accelerate preadipocyte senescence in adults with childhood-onset obesity compared to those with adult-onset obesity. In support of this hypothesis, we found that preadipocytes in the abdominal and femoral SAT of females with childhood-onset obesity had a greater burden of DNA damage and p53/p21 senescence markers compared to those in females with adult-onset obesity (*original article 2*).[320] Senescent cells not only promote inflammation through their senescence-associated secretory phenotype (SASP) but also impair adipogenesis, compromising lipid storage capacity.[75] Our research also supports that lipogenesis may be impaired in childhood-onset obesity. We found that compared to females with adult-onset obesity, those with childhood-onset obesity had lower fasting abdominal and femoral SAT acetyl-CoA, a central metabolite in *de novo* lipogenesis.[3]

Senescent preadipocytes do not act alone in promoting inflammation and impairing adipogenesis; adipose tissue macrophages also play a role.[348] Although children with obesity have more pro-inflammatory macrophages in their SAT compared to lean children,[84] this pattern does not appear to worsen when both groups of children become adults with obesity. Unexpectedly, we found that females with childhood-onset obesity had a slightly lower proportion of M1-like 'pro-inflammatory' macrophages (*original article 3*) and reduced IL-6 gene expression in their abdominal SAT compared to those with adult-onset obesity. In contrast, we found no differences in the proportions of M1-like macrophages in femoral SAT and M2-like 'anti-inflammatory' macrophages, $CD3^+CD4^+$ T cells, and $CD3^+CD8^+$ T cells in abdominal and femoral SAT between these females with childhood-onset and adult-onset obesity (*original article 3*). We suspect that increased SAT inflammation in those with adult-onset obesity may represent a temporary adaptation to recent weight gain, facilitating SAT remodelling[322]; however, this hypothesis remains untested in humans.

The differences in SAT biology between people with childhood-onset and adult-onset obesity may carry important treatment implications. For people with mild to moderate obesity and no comorbidities, lifestyle interventions targeting a 5–10% reduction in body weight are the first-line treatment. Our lab has found that while some SAT characteristics improve after a 10% weight loss induced by diet and exercise, notable differences exist between females with childhood-onset and adult-onset obesity.

Preadipocyte DNA damage decreased in both abdominal and femoral SAT after 10% weight loss, regardless of the age of obesity onset. In femoral SAT, the decrease was more pronounced in those with childhood-onset obesity, resulting in a similar DNA damage burden between groups post-weight loss. Conversely, in abdominal SAT, the decrease was comparable between groups, so the DNA damage burden remained higher in those with childhood-onset obesity post-weight loss.[91] The percentage of $p53^+$ or $p21^+$ preadipocytes, which did not change after weight loss in either group, also remained elevated in females with childhood-onset obesity. Interestingly, the total p21 intensity in $p53^+/p21^+$ femoral preadipocytes decreased only in females with adult-onset obesity (*original article 2*). This change may indicate a possible shift in senescence commitment.[91] Therefore, moderate weight loss may help reduce senescence initiation—and possibly senescence progression in those with adult-onset obesity—but may not eliminate preadipocytes that have already begun the senescence program.

Senolytic therapies may represent a complementary strategy to mitigate the effects of cellular senescence, especially in those with childhood-onset obesity. Although clinical trials of senolytic drugs have only been initiated in patients with severe chronic diseases,[349] Chaib et al. proposed how these therapies could be safely used in people with obesity.[350] By targeting and clearing senescent cells, these therapies hold promise for improving metabolic health and addressing the underlying mechanisms of obesity-related complications.

SAT macrophage and T-cell populations also appear to be more responsive to diet- and exercise-induced weight loss in females with adult-onset obesity. After 10% weight loss, the proportion of M1-like macrophages in their abdominal SAT decreased, while the proportion of $CD3^+CD4^+$ T cells in their abdominal and femoral SAT increased—with no such changes observed in females with childhood-onset obesity (*original article 3*).

Omega-3 fatty acids and drugs with anti-inflammatory properties have potential to target adipose tissue immune cells and inflammation.[351,352] However, before evaluating whether these interventions might be beneficial for individuals with childhood-onset or adult-onset obesity, several questions regarding immune cell changes after weight loss need to be clarified. Do M1-like macrophages decline in females with adult-onset obesity only because they are slightly higher at baseline, leaving more room for improvement? Is the increase in $CD3^+CD4^+$ T cells in females with adult-onset obesity driven by anti-inflammatory T regulatory or T helper (Th) 2 cells or proinflammatory Th1 or Th17 cells? Does the lack of change in SAT immune cell profiles pose any consequences for females with childhood-onset obesity?

Since cardiometabolic risk factors improve after weight loss without changes in SAT immune cell profiles, the answer to the last question may appear to be 'no'. However, studies in mice suggest that adipose tissue can retain an 'immune memory' after weight loss, leading to exacerbated inflammation and metabolic dysfunction after weight regain.[342,353] Further functional and inflammatory phenotyping of SAT immune cells in people with childhood-onset and adult-onset obesity, both before and after weight loss, is required to better understand immune memory and its potential role in long-term metabolic health. Given that the inflammatory process begins earlier in childhood-onset obesity, it is plausible that their SAT immune memory is more solidified and resistant to change.

This concept of immune memory is concerning because females with childhood-onset obesity may be more susceptible to weight regain[217] due to their smaller abdominal subcutaneous adipocytes (*original articles 1 & 3*).[319] When adipocytes shrink to a size smaller than that of lean controls, plasma concentrations of the appetite-supressing hormone leptin also becomes abnormally low.[215] Notably, even before weight loss, leptin gene expression in SAT is lower in females with childhood-onset obesity than in those with adult-onset obesity.[3] While we did not find an abnormal decline in plasma leptin in females with childhood-onset obesity after a 10% weight loss (*original article 3*), hypoleptinemia—and hyperphagia—may arise with more substantial weight loss.

GLP-1-based therapies, which decrease appetite and food intake, could address this issue. These therapies work through various mechanisms including central nervous system effects and delayed gastric emptying,[354] and also seem to impact leptin secretion from adipose tissue. A randomized controlled trial found that after a 12% diet-induced weight loss, administration of liraglutide (a GLP-1 receptor agonist) during a 1-year weight maintenance period diminished the weight loss-induced decrease in plasma leptin.[355] However, given the tendency for weight regain upon discontinuation of GLP-1-based therapies[356,357] and the uncertainties surrounding their prolonged use,[358] these treatments may not provide a long-term solution for weight management.

In contrast, sustained high levels of physical activity are often credited for the success of long-term weight-loss maintainers.[359] However, no study has assessed whether physical activity plays a more significant role in preventing weight regain among those with childhood-onset obesity relative to those with adult-onset obesity.

Long-term post-treatment weight trajectories have only been compared between people with childhood-onset and adult-onset obesity following bariatric surgery. Kristensson et al. found that in both obesity-onset groups, weight declined by 25% one year after surgery, gradually

increased, and then stabilized at 15–20% below pre-surgery weight 8 years later.[134] This similar weight-loss trajectory between the onset groups may not be surprising, since, unlike their counterparts with mild to moderate obesity and no comorbidities, female bariatric surgery candidates with childhood-onset obesity do not exhibit smaller subcutaneous abdominal adipocyte sizes than those with adult-onset obesity, both before[69,71] and after weight loss.[69] Interestingly, female bariatric surgery candidates with childhood-onset and adult-onset obesity, matched for age and type 2 diabetes status, share other similar SAT and VAT characteristics, including M1-like and M2-like macrophage content and degree of pericellular fibrosis.[71] Therefore, by the time comorbidities manifest in people with childhood-onset and adult-onset obesity, previous differences in some adipose tissue characteristics may diminish. While the specific changes in these characteristics after bariatric surgery remain unknown, the comparable rates of type 2 diabetes remission between the two onset groups are encouraging.[134]

Although distinctions in adipose tissue characteristics between childhood-onset and adult-onset obesity may converge by the time metabolic diseases emerge, proactively classifying these types of obesity could prove valuable, especially given the increased type 2 diabetes risk in people with childhood-onset obesity. Since fasting hyperinsulinemia is not affected by the age of obesity (*original article 3*) onset and insulin resistance is more pronounced in adult-onset obesity, β-cell insulin hypersecretion is likely a key abnormality in childhood-onset obesity. In theory, any intervention that steers lipids away from the liver and muscle and reduces nutrient overload to the β-cells could improve these type 2 diabetes risk factors.

In this Perspective, we saw that markers of SAT dysfunction—which can signify metabolic dysfunction—respond better to diet- and exercise-induced weight loss in females with adult-onset obesity than in those with childhood-onset obesity. Females with childhood-onset obesity exhibit preadipocytes with a more senescent or ‘aged’ phenotype, which, along with their

adipose tissue immune cell composition, appear unresponsive to such interventions. This observation raises the question of whether females with childhood-onset obesity could benefit from complementary treatments, such as senolytics, to delay their progression to type 2 diabetes. Because of their smaller abdominal subcutaneous adipocytes, females with childhood-onset obesity may also require tailored strategies to prevent weight regain and associated metabolic complications.

The findings in this Perspective are primarily based on small studies of young (age: 25–40 years) females with mild to moderate obesity (BMI: 30.0–39.9 kg/m²) and no comorbidities. They should, therefore, be interpreted with caution and not generalized to broader populations until replicated in larger studies. The robustness of the weight loss results could have been enhanced by incorporating control groups assessed before and after a weight maintenance period. Nevertheless, the assessment of age of obesity onset using childhood photographs and body rating scales enhances the rigor of these studies compared to secondary analyses that might rely on recalled body weight at age 18. Furthermore, the groups were similar in age and BMI, which strengthens the validity of the comparisons.

By studying young adults prior to the onset of obesity-related comorbidities, these studies captured abnormalities in abdominal and femoral SAT that may emerge early in the pathogenesis of type 2 diabetes, even when people are still clinically considered ‘metabolically healthy’. These findings illustrate the subtle progression of disease beneath the surface, underscoring the importance of early interventions to prevent the clinical manifestation of ‘metabolically unhealthy’ obesity. Young adulthood is recognized as a crucial period for initiating targeted weight loss interventions.[306]

Future studies should leverage advanced techniques to study adipose tissue dysfunction across subcutaneous and visceral depots in males and females with varying severities of

childhood-onset and adult-onset obesity. Samples of VAT obtained during bariatric or elective surgery will be necessary to fulfill this objective. Broad outcomes of interest include adipogenesis, extracellular matrix remodelling, immune cell infiltration and function, cellular aging, lipid metabolism, secretory function, and inflammation. Pairing biological outcomes with assessments of multi-organ insulin sensitivity and β-cell function in obesity-onset groups with different levels of glucose tolerance will enhance our understanding of type 2 diabetes progression in childhood-onset versus adult-onset obesity. Additionally, mechanistic studies in animal models of childhood-onset and adult-onset obesity[230,237] will help delineate how features of adipose tissue dysfunction interact and relate to metabolic disease.

We need to clarify the relationship between age of obesity onset and health outcomes beyond the risk of type 2 diabetes. The risk of diseases such as cardiovascular disease, non-alcoholic fatty liver disease, and cancer, as well as biomechanical and psychosocial health, may also differ between people with childhood-onset and adult-onset obesity. Longitudinal studies that carefully track weight trajectories and health outcomes from childhood through adulthood will facilitate this pursuit.

A growing understanding of the biological and clinical differences between childhood-onset and adult-onset obesity will help inform the design of interventions tailored to each group for testing in clinical trials. Until this time comes, collecting age of obesity onset data at enrollment in randomized controlled trials of new anti-obesity medications and other interventions will enable subgroup analyses comparing treatment efficacy between people with childhood-onset and adult-onset obesity.

Although we do not have a complete picture of how the age of obesity onset impacts the course of adult obesity, current findings illustrate that children with obesity who become adults with obesity are not merely *adults with obesity*; they are adults with childhood-onset obesity—

distinct from those with adult-onset obesity. Incorporating this perspective into clinical practice guidelines has the potential to improve obesity management for people with childhood-onset and adult-onset obesity alike.

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Appendices

Appendix 1. Participant consent form

INFORMATION AND CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Study Title:
Acute and Chronic Effects of Obesity

Researcher:
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Source of funding for the study:
Canada Research Chair, Tier 2 – Clinical Nutrition, Natural Science and Engineering Research Council of Canada, and Heart and Stroke Foundation of Canada

You are being invited to participate in the research study mentioned above. This form provides information about what participating would mean. Please read it carefully before deciding if you want to participate or not. If there is anything you do not understand, or if you want more information, please ask the researcher.

A. PURPOSE

You have been invited to take part in a study on aging, fat tissue risk factors for disease, and weight loss. By participating, you will help us to better understand whether weight loss changes disease risk differently depending on the age when a person develops overweight.

B. PROCEDURES

If you participate, your involvement in the study will last about 6 months or shortly after you lose around 10 % of your starting body weight. The study includes a screening period, a pre-weight loss stabilization period ending with assessments, a weight loss period, and a post-weight loss stabilization period ending with assessments.

The description below provides a general outline of the study protocol. It is possible to schedule several assessments at a given visit. Please note that there may be times where assessments and sample collection (e.g. a blood draw) occur outside of the usual timeline for reasons such as scheduling conflicts.

Information Session, Screening, and Health Assessment

◆***Maximum of 2 visits of up to 1.5 hours each***

The screening process will determine whether you are eligible for the study, and the health assessment will provide information for designing your weight loss protocol.

- ✓ ***Information Session and Screening.*** You will meet with members of the research staff to discuss the study and have your questions answered. If you agree to enter the study you will sign the consent form and provide medical records of height and weight and/or pictures from childhood (around the age of puberty). You will also be asked to review and sign a behavioural contract agreeing to do your best to engage fully in the research project. A member of the research team will confirm your eligibility for the study by reviewing the information you provided via email or telephone, and by measuring your height and weight.

- ✓ ***Health Assessment.*** You will be interviewed and complete questionnaires about your demographic characteristics, medical history, weight history, dietary habits, and physical activity level.

Pre- and Post-Weight Loss Stabilization Periods

You will be instructed to follow your usual diet to maintain a stable weight for 2 weeks both before and after the weight loss protocol. To ensure that you are weight stable, you will be

weighed at every study visit during this time period. You will also record your food intake for 3 days (2 weekdays and 1 weekend day).

Weight Loss Protocol

2-4 visits a week for approximately 5 months

During this period you will follow a protocol to help you lose weight by diet and exercise. You will be instructed on how to decrease the amount of calories you eat in your diet by 20 % and increase the number of calories you burn by 10 % through moderate intensity exercise. You will be required to keep a record of your daily diet periodically throughout the study. All exercise will be performed on cardio equipment at the study site at a self-selected frequency (2-4 visits) that will meet your weekly target. In addition, your weight will be measured and your weight loss progress will be monitored on a weekly basis. Optional educational and support group sessions will be offered occasionally. *You will follow the weight loss protocol until you lose around 10 % of your starting body weight, which is estimated to take approximately 5 months.*

Assessments

Approximately 2-3 fasted visits and 2 non-fasted visits per time point

You will have assessments at the following 3 time points:
(1) Towards the end/after pre-weight loss stabilization period
(2) 12-weeks after starting weight loss protocol
(3) Towards the end/after post-weight loss stabilization period

The following assessments will be conducted at time points (1), (2) and (3):

Non-fasted Assessments. These assessments can occur at any time of day.

- ✓ ***Fitness Assessment.*** The fitness assessment will involve 3 tests.
    - You will undergo a submaximal fitness test on a bike. You will pedal on a stationary bike for approximately 15 minutes. Throughout this time, the resistance will be gradually increased, and your heart rate and blood pressure will be periodically assessed.
    - You will perform a shuttle run/walk test. You will run/walk back and forth between two lines in time with an audio 'beep'. The period of time required to reach the line will get progressively shorter. The test will end when you can no longer continue or when you do not reach the line before the 'beep' on 2 consecutive occasions. The test takes approximately 10 minutes.

- You will perform a handgrip strength test. You will squeeze your fist for around 5 seconds around a device that measures your strength. The test will be conducted 2 times per hand with at least 30 seconds rest between each test. The total time for the test is around 10 minutes.

Fasted Assessments. These assessments must occur in the morning after an overnight fast of at least 8 hours. Water is allowed.

- ✓ ***Body Composition Assessment by Dual Energy X-ray Absorptiometry (DEXA).*** For the DEXA scan you will be positioned on the table and be asked to lie still as the DEXA arm passes over you. Total scan time is usually about 15 minutes.

- ✓ ***Circumference Measurements.*** The circumferences of different parts of your body (e.g. waist, hip, chest, arm, thigh) will be measured with a measuring tape. This process takes approximately 15 minutes.

- ✓ ***Energy Expenditure Assessment by Indirect Calorimetry.*** You will rest comfortably for 90 minutes before the test. You will breathe normally under a clear, plastic canopy for around 30 minutes while lying down. This will allow us to measure the rate at which your body burns calories (your energy expenditure). Information from this assessment will help us determine how many calories you need to maintain weight or to lose weight at a certain rate. Your blood pressure and heart rate will be measured after the test.

- ✓ ***Blood Draw.*** *A sample of* your blood will be drawn. This procedure takes approximately 15 minutes.

The following assessments will be conducted at time points (1) and (3) only:

Non-fasted Assessments. These assessments can occur at any time of day.

- ✓ ***Questionnaires.*** You will be asked to complete questionnaires about your health, eating behaviour, and quality of life. The questionnaires take approximately 30 minutes to complete.

- ✓ ***Arterial Measurement.*** You will be asked to not consume caffeine or alcohol for at least 12 hours prior to this visit. On the day of this visit, exercise or strenuous physical activity should be avoided before the assessment. The hardening of your arteries will be measured while you are resting using a pen-like pressure sensor that will be placed on

your skin on top of your pulse at three sites (wrist, neck, and crease of the leg). The procedure takes approximately 30 minutes.

Fasted Assessments. These assessments must occur in the morning after an overnight fast of at least 8 hours. Water is allowed.

- ✓ ***Body Composition Assessment by Computed Tomography (CT).*** If you are female, a urine pregnancy test will be conducted prior to this test to ensure you are not pregnant. During this test, you will lie on a table that will be passed through a large, open circular tube. The CT machine will take pictures of your abdomen. The scan takes approximately 5-10 minutes to complete.

- ✓ ***Biopsies.*** This procedure takes approximately 90 minutes. You will also be asked to not consume caffeine or alcohol for at least 24 hours prior to this visit. Exercise or strenuous physical activity should also be avoided for at least 24 hours before the procedure. You will provide a small urine sample prior to the procedure.

    - ***Fat.*** A sample will be taken from the fat in your stomach and thigh region. The procedure will be performed by a physician. The physician will first clean your skin to remove any germs, numb your skin by injecting a local anesthesia (to freeze the area) with a thin needle, and injecting fluid (water and salt solution) similar to the composition of your body just below your skin. The physician then makes a small nick incision and suctions out the fluid that was injected using a small hollow tube attached to a syringe. As the fluid is removed, a small amount of fat tissue just below the skin will be removed as well. The procedure will not require stitches, as the incisions are small; the physician will simply place sterile tape to close the incision. After the biopsies are done, post-biopsy care will be explained and you will be provided with written instructions.

    - ***Muscle.*** A sample will be taken from the muscle on the outside side of your thigh. The procedure will be performed by a physician. The physician will first clean your skin to remove any germs, numb your skin by injecting a local anesthesia (to freeze the area) with a thin needle, and make a small incision. A needle (hollow tube) will be inserted to remove a small piece of muscle. The procedure will not require stitches, as the incisions are small; the physician will simply place sterile tape to close the incision. Firm pressure will be applied to the area for 10 minutes to prevent bruising. After the biopsy is done, post-biopsy care will be explained and you will be provided with written instructions.

A typical timeline of study visits and assessments is shown in the table below:

Study Visit Timeline														
Time Point	**(1)** Screening/Pre-Weight Loss Stabilization Period						**(2)** 12-weeks into Weight Loss Protocol			**(3)** Post-Weight Loss Stabilization Period				
Visit #	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***14***
Procedures														
Screening	✓													
Health Interview		✓												
Questionnaires	✓										✓			
Bike Fitness Test		✓					✓			✓				
Shuttle Test						✓		✓			✓			
Handgrip Strength Test		✓					✓			✓				
Energy Expenditure			✓						✓			✓		
DEXA Scan			✓						✓			✓		
Circumferences			✓						✓			✓		
CT Scan				✓									✓	
Arterial Measurement				✓									✓	
Blood Draw					✓				✓					✓
Biopsies					✓									✓
Exercise/Diet Overview						✓								

*Your weight will be measured at every visit and weekly throughout the weight loss protocol
*You will complete cardio exercise sessions 2-3 times per week throughout the weight loss protocol

C. RISKS AND BENEFITS

You might face certain risks by participating in this research. These risks include:

◆***Blood Draws.*** There is a risk of discomfort, pain, fainting, bruising or infection (rare) from the blood draw. The amount of blood drawn at each time point will vary. Total blood drawn throughout the study will not exceed 2 cups (~500 mL). It is recommended that you avoid taking aspirin 3 days before and after the blood draws, and that you don't donate blood for up to 8 weeks following your participation in the study.

◆***Fat and Muscle Biopsies.*** The most common risks of fat and muscle biopsies include pain, a small dent or bump and bruising at the site where the sample was taken. The bruising may last one to two weeks. Less common risks of biopsies include bleeding, infection, a small scar, and

numbness of the skin around the biopsy site. The chance of these risks is less than 1% (1 in 100). There is also a chance of an allergic reaction to the lidocaine used for local anaesthesia. Care will be taken to reduce the chances of these risks. Aspirin should be avoided 3 days before and after the biopsies. It is not advised to participate in any vigorous activities for 3 days before and after the biopsies. Exposure to water for prolonged periods should be avoided (e.g bathtubs, hot tubs or swimming) for 5 days after the biopsies. Showering is permitted; however, band-aids must be changed afterwards. Normal daily activities will not be affected.

◆***Indirect Calorimetry***. There is a slight risk of discomfort and hyperventilation from claustrophobia when under the clear, plastic hood. Staff will be present and the hood is easily removable.

◆***DEXA and CT Scans.*** You will be exposed to some radiation with the DEXA and CT scan. The amount of radiation used is considered too low to cause any harmful side effects. Radiation exposure from the DEXA scan is similar to the amount you would receive from sun exposure on a sunny day ($1/10^{th}$ that of a chest x-ray). The amount of radiation you are exposed to from the CT scan is less than your exposure from one return transatlantic plane flight (about 2-3 chest x-rays). The radiation does not remain in the body after the scan. Should you have any concerns, the research team will be happy to address them with you.

◆***Fitness Tests and Exercise.*** You may experience some discomfort from physical exertion during the fitness test and the exercise component of the weight loss protocol.

Your assessments will be supervised by experienced research staff who will make every effort to keep you comfortable during the study.

Although the assessments conducted as part of this protocol are not expected to provide you with any direct benefits, the results will tell you more about your health and metabolism and you may see positive changes in your health during weight loss.

D. CONFIDENTIALITY

We will gather the following information as part of this research: demographic information, contact information, and the results of all study procedures described above.

We will not allow anyone to access the information, except people directly involved in conducting the research, and except as described in this form. We will only use the information for the purposes of the research described in this form.

The information gathered will be coded. That means that the information will be identified by a code. The researcher will have a list that links the code to your name.

All of your paper-based information will be kept in a filing cabinet in a secure and private research office. All of your electronic information will be stored on a password-protected research computer. The urine, blood, fat and muscle samples will be coded and safely stored at Concordia University. Any samples or data the are sent to external collaborators is coded.

We intend to publish the results of the research. However, it will not be possible to identify you in the published results.

E. BIOLOGICAL SAMPLES

You will be asked to provide the following biological samples as part of the research: urine, blood, fat and muscle.

Taking these specimens involves urinating into a plastic container, blood draws, and fat and muscle biopsies as described in the procedures section above.

We will use your urine sample to assess your overall health with a standard urinalysis, and to do a pregnancy test for female participants. We will use your blood samples to measure things like sugar, cholesterol and inflammatory markers. We will use the biopsy samples to assess the health of your fat and muscle. This includes things like the size of your fat cells, the amount and type of inflammatory markers in your fat, and how well your muscle uses energy.

We will keep the specimens for up to 25 years after the end of the study. After that, they will be destroyed.

If we find anything that might be relevant to your health, we will contact you and direct you to the appropriate service.

F. CONDITIONS OF PARTICIPATION

You do not have to participate in this research. It is purely your decision. If you do participate, you can stop at any time. You can also ask that the information you provided not be used, and your choice will be respected. If you decide that you don't want us to use your information, you must tell the researcher before withdrawing from the study. In addition, the research team may withdraw you from the study if you are not compliant.

As a compensatory indemnity for participating in this research, you will receive $500. If you withdraw before the end of the research, you will receive an amount proportional to your progress in the study and the assessments you completed, as assessed by the research team. To

make sure that research money is being spent properly, auditors from Concordia or outside will have access to a coded list of participants. It will not be possible to identify you from this list.

We will tell you if we learn of anything that could affect your decision to stay in the research.

There are no negative consequences for not participating, stopping in the middle, or asking us not to use your information.

We will not be able to offer you compensation if you are injured in this research. However, you are not waiving any legal right to compensation by signing this form.

G. PARTICIPANT'S DECLARATION

I have read and understood this form. I have had the chance to ask questions and any questions have been answered. I agree to participate in this research under the conditions described.

NAME (please print)

_____

SIGNATURE

_____

DATE _____

If you have questions about the scientific or scholarly aspects of this research, please contact the researcher. Their contact information is on page 1.

If you have concerns about ethical issues in this research, please contact the Manager, Research Ethics, Concordia University, 514.848.2424 ex. 7481 or oor.ethics@concordia.ca.

H. FUTURE RESEARCH PROJECTS?

Do you agree that your research data may be used to carry out other research projects?

These research projects will be evaluated and approved by the Research Ethics Board at Concordia University prior to their realization. Please note that your research data will be kept securely by the researcher responsible for this research project. In order to preserve your identity and the confidentiality of your research data, you will only be identified by a unique numerical code. The code key will be kept by the researcher responsible for this research project.

Your research data may be published or be part of scientific discussions, but it will not be possible to identify you.

Your research data will be retained for as long as it can be useful for the advancement of scientific knowledge. When it is no longer needed, your research data will be destroyed. Please note that at any time you may request that your research data not be used by contacting the researcher responsible for this research project or the ombudsman office at Concordia University.

The Research Ethics Board of Concordia University will monitor and control the data that is collected. In addition, for monitoring, control, protection, and security purposes, your research data may be accessed by a person appointed by regulatory bodies, as well as representatives of the granting agency, Concordia University or the Research Ethics Board of Concordia University. These individuals and organizations adhere to a privacy policy.

Do you agree that your research data will be used under these conditions?
□ Yes □ No

Do you agree that the principal investigator of this research project or a member of his research staff may contact you again to suggest that you participate in other research projects? Of course, during this call, you will be free to agree or refuse to participate in the research projects suggested.
□ Yes □ No

Appendix 2. Participant screening questionnaire

Telephone Screening Questionnaire

Part 1

<table>
<tr><td>Name:</td><td colspan="2">Sex: M / F
[If F] Premenopausal / Postmenopausal
(natural or surgical)
(circle one)</td></tr>
<tr><td>Age: yrs</td><td colspan="2">DOB: / /
(mm/ dd/ yyyy)</td></tr>
<tr><td>Height: _____ feet _____ inches
or
_____ cm</td><td>Weight: _____ lbs
or
_____ kg</td><td>BMI : _____ kg/m2</td></tr>
<tr><td colspan="3">Did you have overweight or obesity as a child/teenager? Y / N
[For participants with BMI ≥ 30 kg/m2] When did you start gaining extra weight (age)? ____
a. Do you have a recorded weight from when you were a child? Y / N (ask to bring in record)
b. If not, do you have a picture from childhood around the age of puberty? (girls ~10-14 y; boys ~12-15)
Y / N</td></tr>
</table>

Part 2

1) Weight History:

Have you been weight stable for at least 2 months? Y / N

Do you plan on losing weight? Y / N

What was your highest weight in your adult life? _____ kg/lb at age _____

What was your lowest weight in your adult life? _____ kg/lb at age _____

2) Have you ever had any surgery or medical procedures for weight loss? Y / N

3) Please describe your current physical activity level

_____
_____
_____
_____

4) Do you exercise? Y / N

*[*If Y*]* Please describe (frequency, intensity, time, type)

_____
_____
_____
_____

5) Do you currently smoke or use any other nicotine containing products? Y / N

*[*If Y*]* Please specify how often (i.e. cigarettes per day).

_____

6) Have you smoked or used any other nicotine containing products in the past? Y / N

*[*If Y*]* Please specify
- time period _____
- how often (i.e. cigarettes per day)_____
- quit date _____

7) Do you currently use electronic cigarettes? Y / N

*[*If Y*]* Please specify how often.

_____

8) Have you used electronic cigarettes in the past? Y / N

*[*If Y*]* Please specify
- time period _____
- how often (i.e. cigarettes per day)_____
- quit date _____

9) Do you currently use cannabis? Y / N

*[*If Y*]* Please specify how often.

_____

10) Do you currently consume alcohol? Y / N

*[*If Y*]* Please specify how often.

_____

11) *[*If Female*]* Are you pregnant or planning on becoming pregnant in the near future? Y / N

12) Medical history / Surgeries / Diseases / Conditions:

_____
_____

_____
_____

_____

13) Are you currently taking any medications, drugs, or supplements? (prescription, over-the-counter, herbal/natural, or recreational) Y / N

*]*If Y*]* Please specify the following:

Name	Frequency	Dose	Other Details

14) Allergies or hypersensitivities to food, medications or other products? Y / N

If Y, please specify.

_____
_____
_____

15) Where did you find out about the study? _____

Appendix 3. Body rating scales

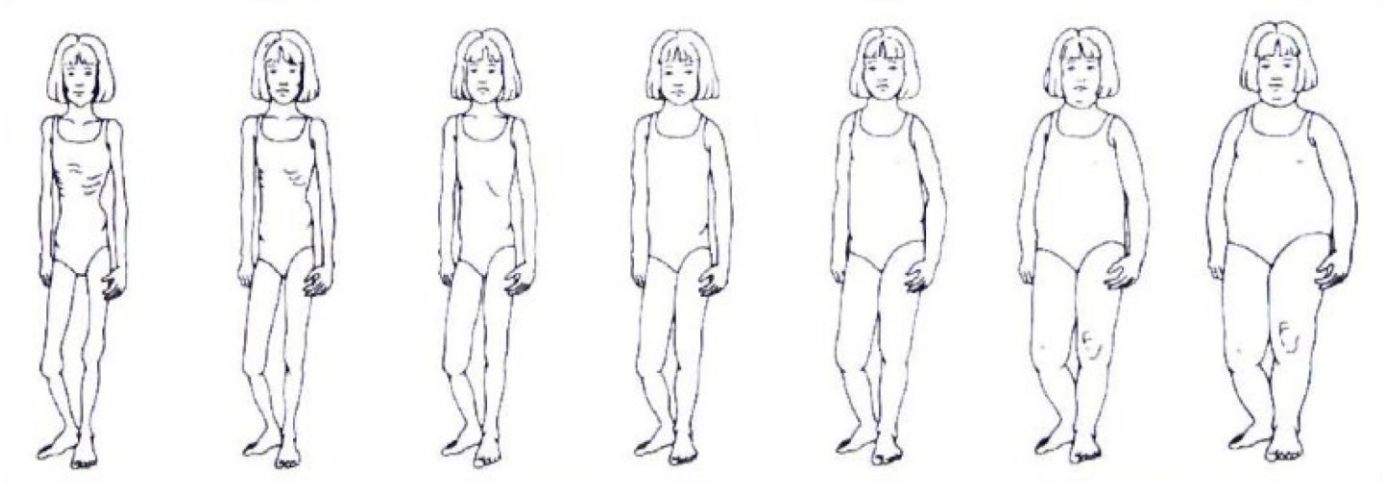
Body Rating Chart

	Figure number								
	1	2	3	4	5	6	7	8	9
Age 5	☐	☐	☐	☐	☐	☐	☐		
Age 10	☐	☐	☐	☐	☐	☐	☐		
Puberty (Age _____)	☐	☐	☐	☐	☐	☐	☐		
Age 20	☐	☐	☐	☐	☐	☐	☐	☐	☐
Currently (Age _____)	☐	☐	☐	☐	☐	☐	☐	☐	☐

Childhood Body Rating Scale: Collins' Figure Drawings

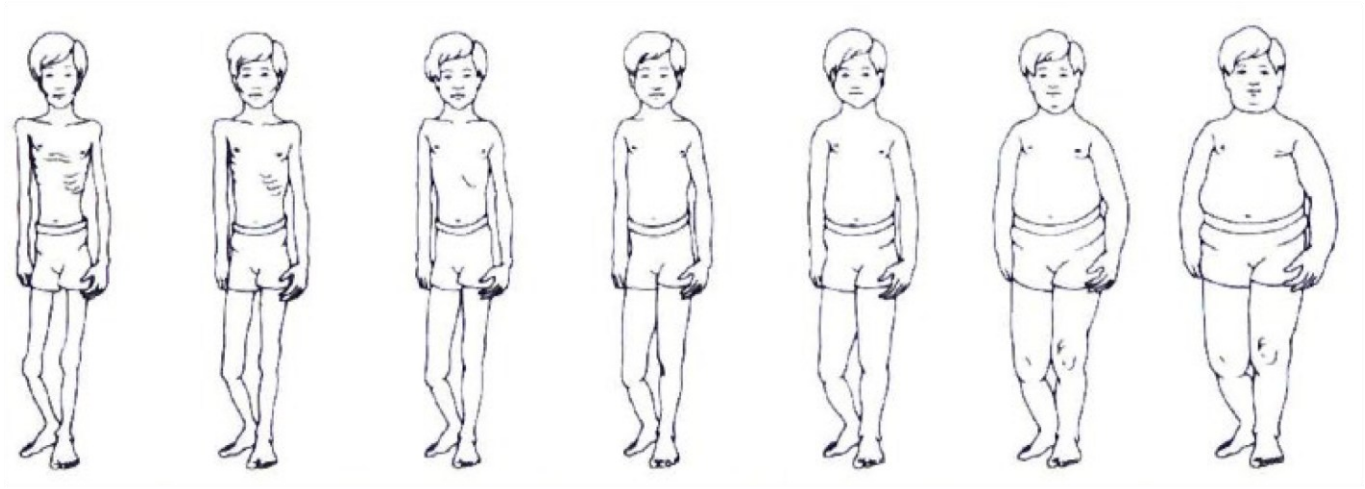
Reference: Collins ME. Body figure perceptions and preferences among preadolescent children. Int J Eat Disord 1991; 10: 199–208. Reprinted with permission from John Wiley and Sons

Girls



1 2 3 4 5 6 7

Boys

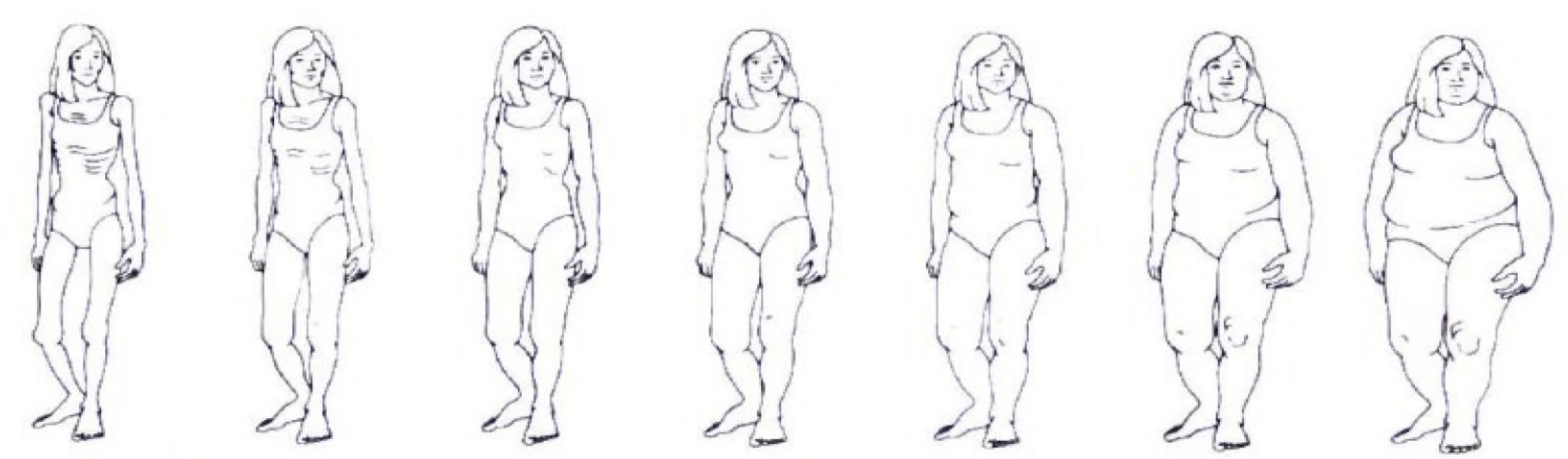


1 2 3 4 5 6 7

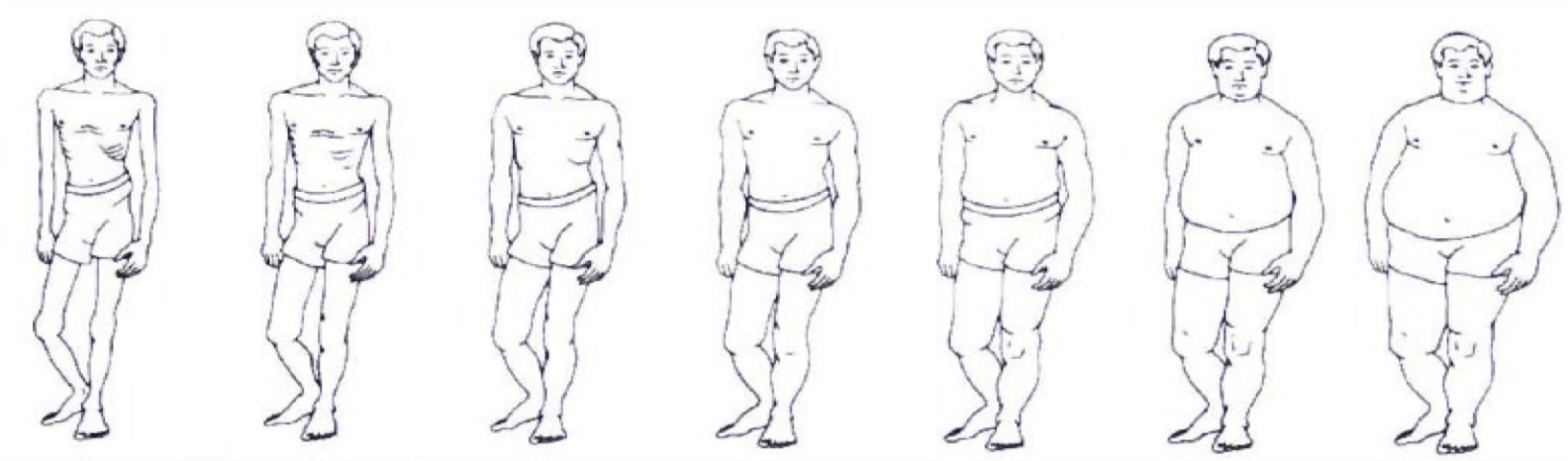
Adolescent Body Rating Scale: Collins' Figure Drawings

Reference: Collins ME. Body figure perceptions and preferences among preadolescent children. Int J Eat Disord 1991; 10: 199–208. Reprinted with permission from John Wiley and Sons

Girls



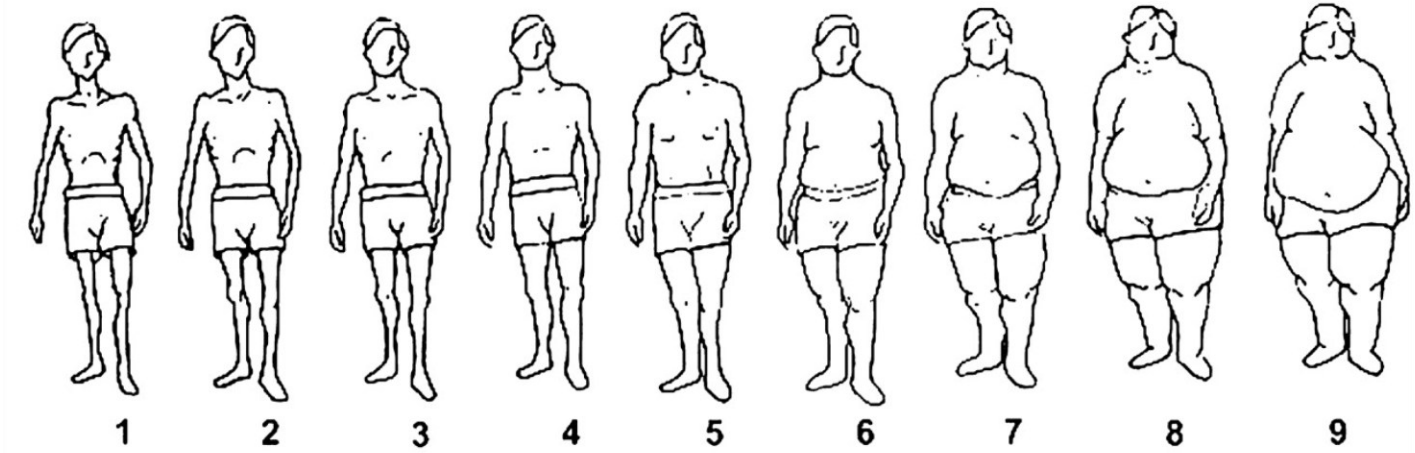
Boys



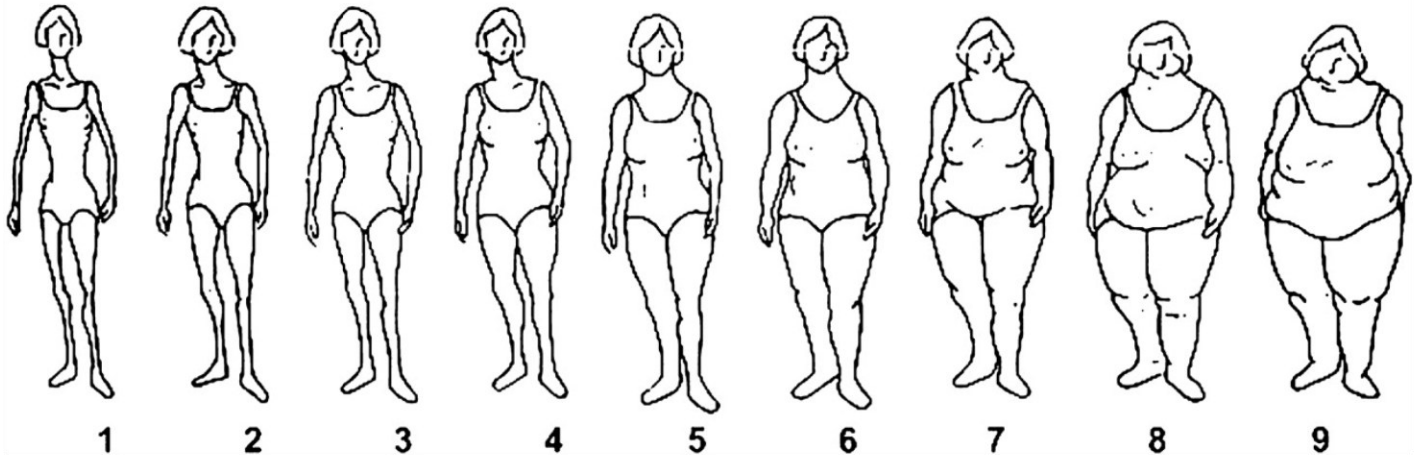
Adulthood Body Rating Scale: Stunkard Body Rating Scale

Reference: Sørensen TI, Stunkard AJ. Does obesity run in families because of genes? An adoption study using silhouettes as a measure of obesity. Acta Psychiatr Scand Suppl. 1993;370:67–72.
Reprinted with permission from John Wiley and Sons

Men



Women



Appendix 4. Weight loss protocol information

Monitoring adherence

Adherence to the prescribed calorie deficit was assessed by plotting participants' weekly weights on a weight graph predicted by the NIH Body Weight Planner according to the energy balance model of Hall et al.: Hall, K. D. *et al.* Quantification of the effect of energy imbalance on bodyweight. *Lancet* **378**, 826–37 (2011).

Behavioural change techniques

To promote weight loss, we used several behaviour change techniques (BCT) in the 'goals and planning', 'feedback and monitoring', 'shaping knowledge', 'comparison of behaviour', and 'reward and threat' clusters of the behaviour change taxonomy derived by Michie et al.: Michie, S. et al. The Behavior Change Technique Taxonomy (v1) of 93 Hierarchically Clustered Techniques: Building an International Consensus for the Reporting of Behavior Change Interventions. *Ann. Behav. Med*. **46**, 81–95 (2013).

Specific BCT in each cluster with examples are as follows:

Goals and planning

- Goal setting (behaviour and outcome)
    - setting daily calorie goal using exchange lists for meal planning
    - setting weekly exercise goal (3x cardio sessions per week)
    - setting weekly weight goal based on predicted weight graph
- Action planning
    - planning days/times of weekly exercise sessions
- Review behaviour and outcome goals
    - reviewing food diaries

    - reviewing weekly weight loss progress by plotting weights on predicted weight graph
- Behavioural contract
    - a written agreement to adhere to the weight loss protocol and attend study appointments (signed by participant; signed by research personnel as witness)

Feedback and monitoring

- Feedback on behavior and outcome
    - feedback on food diaries and exercise reporting
    - feedback on weekly weight loss progress
- Self-monitoring of behavior
    - keeping food diaries (and tracking allotted dietary exchanges) first week of study and periodically throughout

Shaping knowledge

- Instruction on how to perform a behavior
    - instruction on how to use treadmill/elliptical with heart rate monitor

Comparison of behaviour

- Social comparison
    - showing progress of all participants (anonymously) using a sticker chart

Reward and threat

- Social reward
    - congratulating participants for meeting weekly weight loss goals and letting them choose and place a sticker on the participant progress chart
- Incentive (outcome)

    - inform participants they will be financially compensated if they reach their weight loss goal and complete the study

- Reward (outcome)
    - providing rewards (e.g., gift cards, mugs, water bottles) to participants periodically throughout the study when on track with weight loss goals and providing financial compensation once final weight loss goal is met and final assessments are completed

Samples of select participant nutrition material

Food Journal

Notes:

Date:

Time	Quantity	Food/Beverage

Duration	Intensity	Physical Activity/Exercise

1400 calories

Fruit
Vegetable
Starch
Milk
Fat
Protein
Water

ID:

Food Groups

PROTEINS

¼ — Tuna canned in water

1oz (30g) — Chicken or turkey (without skin), veal (except cutlets), lean beef, lean pork (fresh ham, fillet), fish, light cheese

½ — 1 % cottage cheese

1 — Whole egg or 3 egg whites

1 table — Peanut butter

FATS

1 tea — Margarine, mayonnaise, vegetable oil (olive and canola are best)

1 table — Salad dressing, light cream cheese

MILK PRODUCTS

½ — Evaporated skim milk

1 — Skim or 1 % milk, plain low-fat yogurt

STARCHES

¾ — Whole-grain unsweetened breakfast cereals, oatmeal or hot cereals

1/3 — Concentrated bran cereals, cooked legumes (chickpeas, kidney beans, navy beans, lima beans)

3 — Popcorn without added fat

1 — Slice of whole-wheat bread, tortilla (6 inch diameter), small baked potato

5 — Melba toast

½ — Cooked pasta, rice or bulgur, corn, green peas, mashed potatoes, low-fat bran flakes

1 — Low-fat croutons

½ — Bagel, English muffin, hamburger or hot dog bun, pita (6 inch diameter)

3 — Plain rice cakes

VEGETABLES AND FRUITS

1 — Raw vegetables (cabbage, shallots, celery, mushrooms, radishes, cucumber, parsley, cabbage, zucchini, bean sprouts, hot peppers)

1 — Tomato

½ — Cooked vegetables, vegetable juice (asparagus, Brussels sprouts, broccoli, cabbage, carrots, cauliflower, eggplant, green peppers, beets, leeks, turnips, spinach zucchini)

2 — Plums

½ — Banana, grapefruit, mango

1 — Medium-sized fresh fruit (apple, nectarine, orange, pear, peach), large kiwi

1 — cantaloupe, papaya, raspberries

1/3 — Canned pineapple; cranberry, grape or prune juice

¾ — Fresh pineapple, fresh blueberries

½ — Unsweetened apple sauce, canned apricots, peaches, pears or fruit mixtures, juice (apple, grapefruit, orange or pineapple)

1 ¼ — Watermelon

FREE FOODS

1 table — Ketchup, mustard, vinegar, barbecue sauce, taco sauce

FREE — Herbs, spices, lemon, lime, garlic, lettuce, spinach, etc.

Cup Piece

Spoon Oz / gr

Smart choices in restaurants

It's not always easy to keep on track of your diet when going out. Don't worry; we've got you covered! Here are some easy ways to keep it all under control

Veggie Kingdom

Make sure your meal has plenty of vegetables and protein

Take it to go

If the meal is too big, you can always save it for later, you don't have to finish it all

"Big size"

Try to avoid temptations. If you're still hungry, look for some side veggies or salad.

Read Avoid the words "creamy, breaded, crisp, sauced, stuffed, buttery, gravy, sautéed, fried, or au gratin", they have a lot of calories!

GO FOR: Baked, broiled, fresh, grilled, steamed, roasted, poached, blacken or lightly sautéed.

Different dressing Use vinegar, lemon, mustard or a little bit of olive oil to avoid creamy dressings.

Not every salad is your Be careful with the dressings options and the toppings you choose, try not to include bacon bits, croutons, cheese or mayonnaise.

The drinks Drink water or unsweetened tea or coffee. Try not to drink exotic or mixed drinks. A light beer, wine, vodka tonic or a simple martini are better choices.

Share the Get one dessert for 2, so you only get half the calories. You could also try fresh fruit.

Don't be afraid to Request different options for side dishes, like salad, vegetables, or fruit. Change white bread or rice for whole grain, this will fill you up faster. Ask for the ingredients and the preparation of your meal and ask politely for any changes. Solicit not to add extra butter or sauces to your food.

Enjoy your Stop eating when you feel full, also take your time to eat.

Remember: Small steps first

Exercise program

- Three 45 minutes moderate-to-vigorous intensity aerobic exercise sessions (treadmill and/or elliptical) per week

    - 5–10-minute warm-up and 5-minute cool-down
    - Intensity of main workout (expressed as a percent of heart rate reserve):
        - Week 1-2: **40-50%**
        - Week 3-4: **50-60%**
        - Week 5-24: intervals alternating between 80% and 60%:

Interval time (min:sec)

Week	80%	60%
5-7	0:30	1:30
8-10	1:00	1:00
11-13	1:30	0:30
14-16	2:00	2:00
17-19	3:00	1:00
20-22	4:00	2:00
23-24	5:00	1:00

Appendix 5. Published flow cytometry protocol

Analytical Biochemistry 613 (2021) 113951

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Technical note

A reliable, reproducible flow cytometry protocol for immune cell quantification in human adipose tissue

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ARTICLE INFO

Keywords:
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Adipose tissue
Immune cells
Humans
Inflammation
Obesity

ABSTRACT

The ability to accurately identify and quantify immune cell populations within adipose tissue is important in understanding the role of immune cells in metabolic disease risk. Flow cytometry is the gold standard method for immune cell quantification. However, quantification of immune cells from adipose tissue presents a number of challenges because of the complexities of working with an oily substance and the rapid deterioration of immune cell viability before analysis can be performed. Here we present a highly reproducible flow cytometry protocol for the quantification of immune cells in human adipose tissue, which overcomes these issues.

1. Introduction

Adipose tissue has a complex microenvironment containing a wide array of immune cells [1–4]. In obesity, the expansion of adipose tissue causes a shift from a balanced anti/pro-inflammatory to a predominantly pro-inflammatory environment [5]. The resulting inflammatory response has been linked to the development of metabolic diseases such as type 2 diabetes and cardiovascular disease [6–9]. However, the exact role and mechanisms by which adipose tissue immune cells increase metabolic disease risk remains unclear. Optimizing the accurate identification and quantification of immune cells in adipose tissue is key to developing a better understanding of their role in metabolic disease risk.

Flow cytometry is the gold standard method for the examination of immune cells within tissue samples. The flow cytometry method allows for the quantification of cells by size and granularity, as well as the quantification of specific cells of interest through the detection of multiple cell surface markers via antibody staining. With regard to adipose tissue, there are several challenges to performing a successful flow cytometry experiment such as isolating a stromovascular fraction (SVF) that is pure and residue free, while maintaining cell viability and eliminating high rates of auto-fluorescence. Additionally, there has yet to be a study confirming the reproducibility of a flow cytometry protocol in human adipose tissue. The protocol presented here has been adapted from a series of protocols meant for use on murine tissue [10–13] and optimized for human adipose tissue through a number of trouble-shooting trials. In refining our protocol, we have overcome the aforementioned challenges which have plagued several of the previous studies that have used flow cytometry to analyze immune cell populations within human adipose tissue. The objective of this manuscript is to present and show the reproducibility of an optimized protocol for the quantification of immune cells by flow cytometry in human adipose tissue.

2. Methods

2.1. Participants

Fourteen women were recruited from the CIUSSS-NIM bariatric surgery clinic. Average age of participants was 40.4 ± 9.0 y, weight was 128.4 ± 14.5 kg, and BMI was 48.0 ± 6.4 kg/m^2. Four of the fourteen

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women had diagnosed type 2 diabetes. Women were premenopausal, non-smokers, weight stable, and free of renal failure and uncontrolled hypothyroidism. Women were excluded if they had hypertension, uncontrolled hyperlipidemia, or if they were on any medications that interfered with lipid metabolism and inflammation. Informed consent was obtained from all study participants. The study received ethical approval from the Comité d'éthique de la recherche du CIUSSS du Nord-de-l'Île-de-Montréal, Hôpital du Sacré-Coeur de Montréal and Comité central d'éthique de la recherche du ministre de la Santé et des Services sociaux.

2.2. Study protocol

In all participants, subcutaneous adipose tissue biopsies were obtained from the lateral thigh area, in an outpatient setting [14]. For this procedure, the incision site was first sterilized then frozen with lidocaine. A fan-shaped subcutaneous area was numbed with a solution of lactate ringer and lidocaine. A small incision was made and the subcutaneous adipose tissue was aspirated using a 12-gauge tri-eye cannula. After removal, tissue was first placed on a 100 μm mesh screen under which there was a large weight boat on ice. The tissue was washed thoroughly with saline solution to remove any blood residue, and then stored on ice in a 50 ml conical tube containing plating media for transport to our laboratory (~15–20 min after removal). To examine protocol reproducibility, cell markers CD206, CD68 and CD45 were selected because they are known to be present in relatively large quantities in human adipose tissue. Samples were processed and analyzed on two separate panels with a tri-laser BD FACSVerse (BD Biosciences, San Jose, CA, USA) and FlowJo software (v9.3.2). To demonstrate protocol versatility, we have additionally provided results for the quantification of T cells (CD3, CD4, CD8, CD45RA), dendritic cells (CD11c, CD11b), B cells (CD19) and NK cells (CD56, CD16), in the supplementary materials. Dose compensation values were calculated by quantifying cell number on respective frequency minus one (FMO) channels. Dose compensation values were then subtracted from corresponding values to control for background noise (Dose compensation = # of cells - # of FMO cells).

2.3. Statistics

Paired sample t-tests were used to determine if a difference existed between duplicate measures of CD206, CD68, and CD45. Bland-Altman plots were also used to examine how well the two measures correspond to each other. Additionally, Pearson's correlations were used to determine the relationship between duplicate measures. Data was analyzed using IBM SPSS Statistics v22 (Armonk, NY) and are reported as mean ± SEM. *P*-values of <0.05 were considered statistically significant.

Table 1
Comparison and association between duplicate measures of cell markers.

	Panel 1	Panel 2	Paired t-tests	Bland-Altman	Pearson's correlations
	Cells per g tissue	Cells per g tissue	p value	Mean difference of means	
CD206+	1075 ± 340	1090 ± 332	0.87	−15.0 ± 87.5	r = 0.996**
CD68+	2671 ± 1094	1941 ± 618	0.36	730.4 ± 760.2	r = 0.74**
CD45+	29,082 ± 5694	27, 653 ± 5290	0.47	746.3 ± 1989.0	r = 0.94**

CD206 n = 10, CD68 n = 10, CD45 n = 14. Data are presented as mean ± SEM, **p ≤ 0.01.

3. Results

No differences were observed between duplicate measures of CD206, CD68 and CD45 (Table 1, Fig. 1). Additionally, Bland Altman plots indicate good agreement between duplicate measures (Fig. 1). Strong positive correlation ($r = 0.996$, $p < 0.01$; $r = 0.74$, $p < 0.01$; $r = 0.94$, $p < 0.01$, respectively) were also observed among duplicate measures of CD206, CD68 and CD45.

Bland-Altman plots showing the mean versus the mean difference of means per gram of initial adipose tissue for CD68, CD45 and CD206.

4. Discussion

To our knowledge, this is the first study to examine the reproducibility of a flow cytometry protocol in human adipose tissue. We found that our optimized flow cytometry protocol provides highly reproducible results in samples taken at the same time from the same region that are processed separately. The protocol presented here has been adapted from a series of protocols meant for use on murine tissue [10–13] and refined through numerous troubleshooting trials. At each stage of the protocol there are vital steps that can be implemented to drastically improve the overall quality and reproducibility of results.

Proper adipose tissue digestion is an important step in a flow cytometry experiment. A fine balance must be achieved between over digestion, which will lead to excessive SVF cell death, and under digestion, which will not separate SVF cells from the adipose tissue. Adipose tissue digestion with collagenase II was first detailed by Rodbell in 1964 [15] and has remained the dominant method for the separation of SVF cells from adipocytes. To minimize noise and produce clean flow cytometry results, maintaining the viability of SVF cells during adipose tissue digestion is essential. To improve the maintenance of cell integrity during digestion, both a digestion buffer and HEPES buffer solution are used with the collagenase to provide a physiologically neutral environment for cells during digestion. Currently, published protocols either do not use any buffers or only use one [10–13]. We have found that the simultaneous use of these two buffers results in a significantly greater live cell population when compared to previously published protocols by Brake et al. [10] and Cho et al. [12]. In addition, to improve overall cell yield, adipose tissue samples should be minced into fine pieces, exposing as much surface area as possible to the digestive agents. Regular vortexing and vigorous shaking of the samples during digestion also helps to separate cells, speed digestion, and increase the final cell yield. The addition of a cell check in the microscope by Cho et al. [12] greatly facilitates proper digestion by helping to avoid over or under digestion. Lastly, in our protocol we have added DNase to our digestive solution. DNase cleaves DNA allowing for improved protein accessibility during our staining phase. We have found that the addition of DNase results in clearer, more distinct populations. This can be observed when comparing our results to those previously published by Orr et al. [11] and Cho et al. [12].

Though blood contamination is of concern, we have previously shown that both needle aspirated and excised adipose tissue yields similar results when processed adequately [14]. When examining immune cells within adipose tissue samples it is important to remove all blood cells and debris from lysed cells to prevent contamination of the final sample. Removing visible blood residue revand thorough rinsing with saline helps to prevent contamination. Importantly, in the described protocol, two lysing phases have been added. The use of RBC lysing buffer and ACK (ammonium chloride potassium) lysing buffer is key in further removing potential contamination and cellular debris. After these two incubations, a second filtration has also been added, which helps to remove the debris from the lysed cells that could otherwise interfere with protein staining. As seen in Supplementary Material - Fig. 5 these added steps result in a large reduction in the autofluorescence that often plagues current flow cytometry protocols.

Minimizing autofluorescence is a great challenge when working with

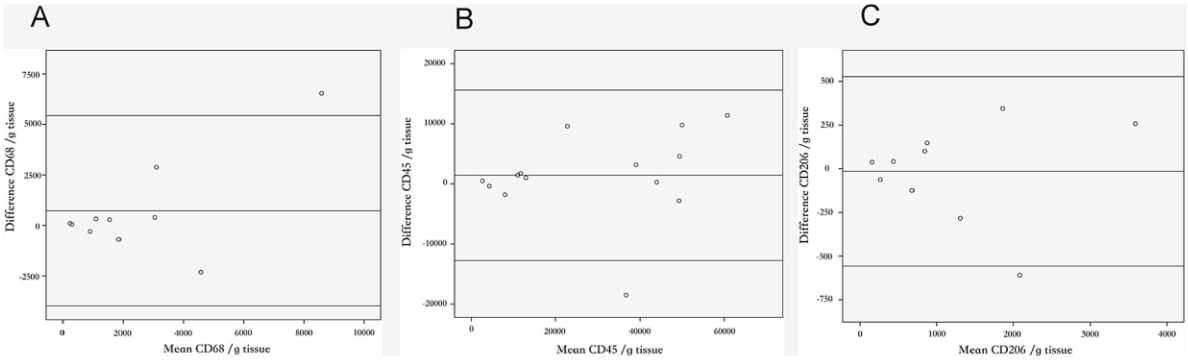

Fig. 1. Bland-Altman plots.

adipose tissue samples in flow cytometry. The presence of autofluorescence in final results reduces resolution and sensitivity of immune cell quantification [13]. Decreasing the amount of fatty and oily residue that is left on immune cells can greatly aid in reducing autofluorescence. We prevent this residual contamination in our protocol at several steps designed to minimize the carry-over of any remaining adipose tissue remnants. In the first filtration phase (step 10 in digestion), the supernatant is filtered before the fat cake. This not only allows for SVF cells to pass through the filter without being impeded by the fat cake but also reduces the amount of fatty residue that passes through the filter. After each centrifugation stage, the supernatant is additionally aspirate by circling the outside of the conical tube (step 12 of digestion and step 2 of isolation and purification). This technique ensures that the supernatant containing oil and fatty residues is removed and additionally ensures that no residue clings to the side of the tube, which would otherwise be brought forward to the next step of the protocol. To further reduce residual contamination, we pre-chill our 50 ml conical tubes to prevent the sticking of fat and oil residues to the sides of the tube. When comparing our results with the Brake et al. (10) protocol, to the results we obtain from current protocol presented here, the reduction in autofluorescence in the current protocol is especially evident (Supplementary Materials – Fig. 5). Autofluorescence can also be reduced by choosing appropriate antibody to fluorochrome pairings. For instance, it has been widely observed that the FITC channel should be avoided for macrophage staining because adipose tissue macrophages stained on FITC often produce a large amountof autofluorescence [10–12].

Throughout the protocol there are general principles that are followed to improve both the quantity and quality of our final cell yield. At all times, unless otherwise specified, samples are kept on ice. This allows for a reduction in cell death. Cell viability can be further preserved through timely sample analysis. Performing a well practiced protocol and completing as much preparation as possible before the start of the experiment can greatly help reduce protocol time and prevent cell death.

We have presented a highly reproducible flow cytometry protocol for measurement of immune cells in human adipose tissue. The use of this protocol for examining adipose tissue immune cells facilitates an area of study that is becoming increasingly important with the expansion of immunometabolism research. As the field of immunometabolism continues to grow, so too does the importance of developing protocols for accurate immune cell quantification.

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Author Contributions

K.Z.D. wrote the manuscript and was involved in protocol optimization. V.D. was involved with protocol optimization and manuscript review. J.M. was involved with protocol optimization and manuscript review. J.A.M. was involved with conceptualization and manuscript review. R.D. provided fat biopsies for protocol optimization. H.A. provided fat biopsies for protocol optimization. R.P. provided fat biopsies for protocol optimization P.Y.G. provided fat biopsies for protocol optimization. S.S. was involved with conceptualization, oversaw protocol optimization, writing, and editing of the manuscript.

Declaration of competing interest

There are no conflicts of interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ab.2020.113951.

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Supplementary Methods

2.4. Materials and Reagents

I. Adipose tissue digestion

Materials

1. Large weight boats
2. 100 μm & 250 μm screen mesh
3. Tweezers
4. Scissors
5. Ice
6. 50 ml conical tubes
7. Microscope slide
8. Centrifuge set to 1600 rpm (300g) at 4 ^{O}C
9. Rocking water bath set to 37 ^{O}C and 100 rpm
10. Vortex

Reagents

1. Digestion Buffer
   - DMEM containing 10% FBS
2. Collagenase Sigma Type II _C-6885 (Stock 10 mg/ 1 ml)
   - 10X Collagenase solution (10 mg/ml in digestion buffer)
   - Dilute 1g of collagenase with 100 ml H_2O (filter-sterilized for optimal results – 0.22 μm)
   - Aliquot & freeze
3. HEPES Buffer Solution (500 ml)
4. DNase1 2500 units/ml (Thermo Scientific 90083)
5. EDTA

II. Isolation and purification of the SVF

Materials

1. Ice
2. 50mL conical tubes
3. Microfuge tubes
4. Hemocytometer for counting cells
5. Centrifuge set to 1600 rpm (300g) at $4^{O}C$
6. Rocking water bath set to $37^{O}C$ and 100 rpm

Reagents

1. RBC Lysing Buffer

2. FACS Buffer (1500ml)

- 150 ml 10X PBS
- 15 ml 5% NaN_3
- 7.5 g BSA
- 1335 ml doubly distilled H_2O

3. ACK (500ml 10X Stock)

- 41.45 g NH_4Cl
- 5.0 g $KHCO_3$
- 0.185 g EDTA

Top up to 500 ml with doubly distilled H_2O. Use at 1X concentration.

4. PBS
5. Trypan Blue Solution

III. Staining SVF cell surface markers

Materials

1. 5 ml FACS tubes
2. Flow cytometer

Reagents

1. Human serum/plasma (heparinized)
2. Antibodies of choice (see Table 1)

2.5. Protocol

I. Adipose tissue digestion

1. Immediately after collection, rinse at least 0.5 g of adipose tissue with saline placed on a 100 µm nylon mesh screen over a large weight boat on ice. Wash the sample thoroughly to remove any blood residue. Additionally, remove any visible blood vessels and connective tissue with tweezers. Work quickly to prevent excessive cell death.
2. Place tissue into a pre-weighed 50 ml conical tube. Re-weigh tube with adipose tissue to determine the sample mass. For best results a tube should not contain more than 2 g of adipose tissue.
3. Add 7 ml of cold (~4 ^{O}C) digestion buffer to the conical tube and keep on ice to preserve cells.
4. While on ice add 3 ml/g tissue of both HEPES buffer solution and collagenase II, & 5 µl of DNase.
5. To optimize the digestion process, samples should be minced into small pieces (~1-3 mm in size) using scissors.
6. Vortex sample and place in 37 °C water bath at 100 rpm so that the entire sample is submerged.

7. Samples should be manually shaken vigorously, and vortexed thoroughly for a few seconds every 5 min. Samples should remain in the water bath until all adipose tissue pieces appear homogeneously digested *but not so long that a clear oil supernatant layer appears indicating over digestion*. Digestion time will vary, ~ 20-60 min, but should remain under 60 min.
8. Once the sample appears visually digested take 10 µl of the digestion mixture, below the fat cake, and place on a microscope slide. Examine microscopically under 10x lens. Fat cells will appear as large circles and stromovascular cells (SVC) will appear as smaller circles. If fat cells still have a large number of SVC attached to them, continue digestion. If the majority (~80%) of fat cells and SVC appear to be separate proceed to the next step.
9. Once the sample appears digested, add EDTA to a final concentration of 10mM and incubate at 37^0C for an extra 5-10 min, depending on how well the sample is digested (ie. longer if the sample could be digested more and shorter if there is risk of over digestion). This allows for full dissociation of SVCs.
10. Place a 100 µm mesh screen over top a new chilled 50 ml conical tube. Pre-wet the mesh with PBS. From the sample tube, pipette up from the bottom of the sample avoiding the fat cake resting at the top. Once all the fluid has been filtered, pour the fat cake onto the mesh, rinse with PBS and manually squeeze all remaining liquid into the new conical tube. Pipetting from the bottom of the sample upwards prevents the fat cake from clogging the mesh screen and blocking the passage of SVF cells.
11. Spin at 1600 rpm (300g) at 4^0C for 5 min. You will see a pellet at the bottom of the tube, liquid in the middle, and a fat layer on top.
12. Aspirate the liquid starting with the top fat layer ensuring to circle the walls of the conical tube to reduce amount of fatty residue carried forward. Continue to aspirate while moving around the wall of the conical tube until only the pellet is left. Be careful to not disturb the pellet.

II. Isolation and purification of the SVF

1. Add 5 ml of RBC lysing buffer, mix by gently pipetting up and down, and incubate at room temperature in the dark for 5-7 min. Time is dependent on how bloody the sample is. If there was a large amount of blood residue in the sample, even after initial cleaning, incubate for 7 minutes. Be careful to not over incubate as lysing buffer can affect the vitality of SVF cells.
2. Neutralize lysing buffer with 5 ml of FACS buffer. Spin at 1600 rpm (300g) for 5 min. Aspirate supernatant with the same circling technique as before, eliminating as much fatty and oily residue as possible.
3. Resuspend with 3 ml ACK and incubate for 5 min at room temperature in the dark.
4. Add 7 ml PBS buffer and filter through a 250 μm screen place over a new chilled 50 ml conical tube. Spin at 1600 rpm (300g) for 5 min.
5. Aspirate supernatant and bring cells up to a total volume of 1 ml PBS buffer. Put on ice and in the dark while counting cells (step 6).
6. Take 10 μl of the sample and place in a microfuge tube. Immediately prior to microscopic examination, add 10 μl trypan blue. Limit the samples exposure to trypan blue as it will cause cell death leading to inaccurate cell counts.
7. Count SVC using a hemocytometer and calculate the total number of cells in your sample.
8. Label a 5 ml FACS tubes for each analysis/ panel that will be run. Each experiment should have a negative control sample along with the positive stained samples. Divide cells equally into each tube.
9. Add 1 ml of PBS to each tube. Cap each tube and spin at 1600 rpm (300g) for 5 min. Dump supernatant. Proceed to staining.

III. Staining SVF cell surface markers

All antibodies should be previously titrated to determine the amount required for accurate staining. Our titration tables can be viewed below (Table 1).

1. Stain positively labeled cells with viability dye per manufacturer instructions.

2. Resuspend pellets in positive and negative control stained tubes with 47.5µl blocking serum or plasma. Incubate for 10min, in the dark, on ice.

1. Cell surface marker staining:

i. *On positive stain tubes only*, add antibodies in the volumes previously determined by titration (Table 2). Incubate for 20 min, in the dark, on ice.

ii. Add 1 ml PBS buffer to both positive and negative stained tubes. Spin 1600 rpm (300g) for 5 min. Dump supernatant. Repeat step twice.

iii. After the final wash bring the pellet up in 1 ml of PBS and leave on ice in the dark until the sample is ready for analysis.

Alternatively if samples cannot be analyzed for an extended period of time they can be fixed;

iv. After last wash, dump supernatant and bring cells up in **400 ul 2% PFA** to fix

III. Data acquisition

1. Tube settings should be created ahead of time to speed up the analysis time.
2. Run the unstained negative control sample and adjust forward scatter and side scatter so that all the populations of interest are appearing within range.
3. Copy and paste the tubes settings that were established when running the unstained sample so that they are used for the rest of the data acquisition for that sample.
4. Run all single stain controls (Table 2) and fluorescence minus one (FMO) samples (Table 2).
5. Run all positively stained samples.

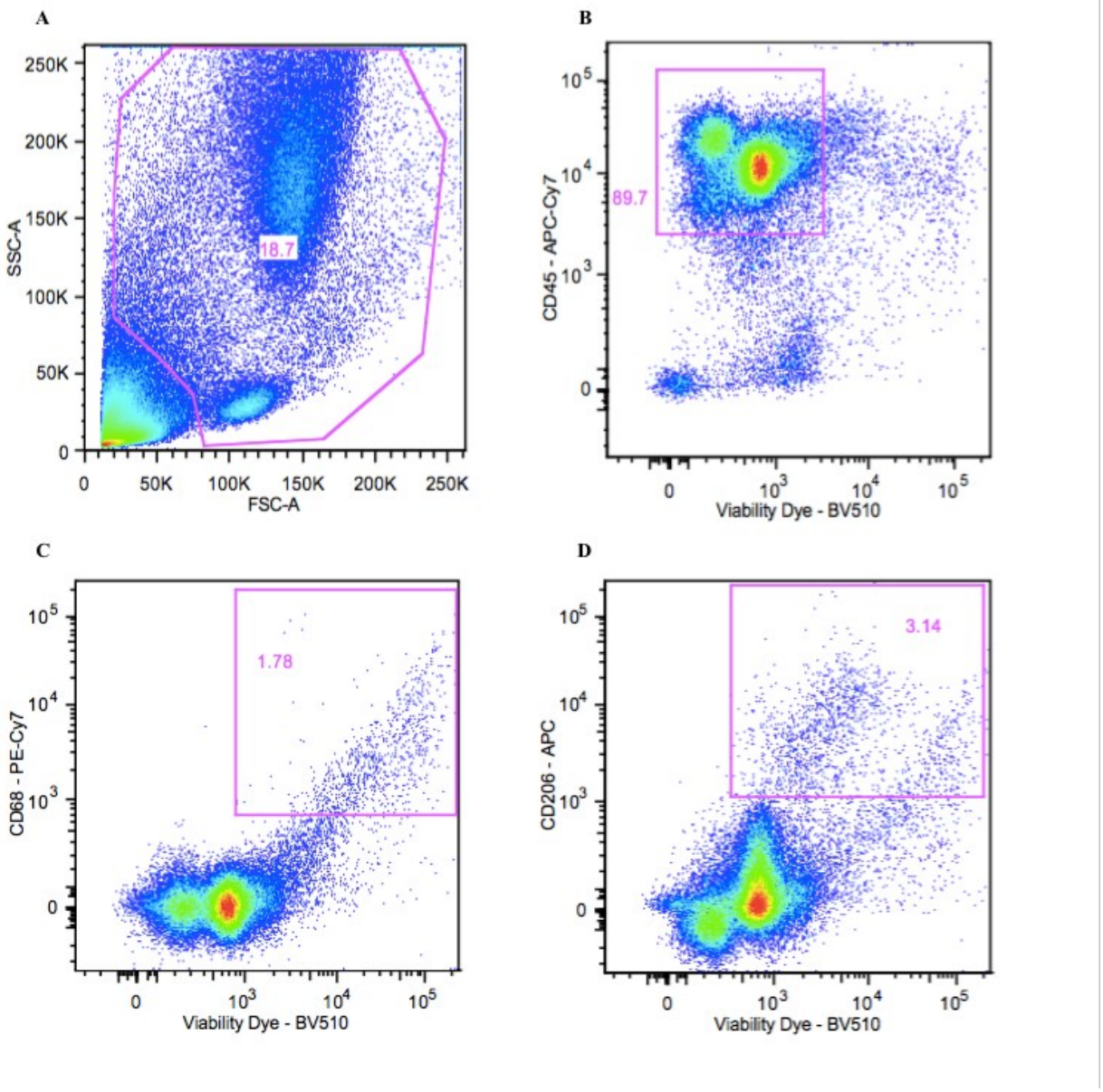

Figure 1. Gating strategy used for reproducibility measures

A. Live cells gated to remove fluorescent dead cells and cell debris. B. Viable CD45+ cells gated. C. Viable CD68+ cells gated. D. Viable CD206+ cells gated.

Table 1. Antibody and fluorochrome pairings as well as supplier information and clone that was utilized.

Antibody*	Fluorochrome	Supplier	Clone	Titrated concentration (µl/10^6 cells)
CD206	APC	eBioscience	19.2	1.5
CD68	Pe-Cy7	eBioscience	815CU17	1.5
CD45	APC-H7	BD Pharmingen	2D1	1.5
Viability Dye	BV510	BioLegend		1

*Antibodies were titrated on human adipose tissue to determine the optimal staining volume per 10^6 cells.

Table 2. Controls for flow cytometry experiment

	APC	PE-Cy7	APC-H7	BV510	Compensation beads
SS APC	CD206	---	---	---	Positive/negative
SS PE-Cy7	---	CD68	---	---	Positive/negative
SS APC-H7	---	---	CD45	---	Positive/negative
SS BV510				Viability Dye	Positive/negative
FMO APC	---	CD68	CD45	Viability Dye	---
FMO PE-Cy7	CD206	---	CD45	Viability Dye	---
FMO APC-H7	CD206	CD68	---	Viability Dye	---
FMO BV510	CD206	CD65	CD45	---	---

Single stain (SS) cocktails for each antibody used and fluorescence minus one (FMO) samples.

Appendix 6. Supplementary first-authored or co-first-authored publications

METABOLISM CLINICAL AND EXPERIMENTAL 67 (2017) 31–40

Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

Reviews

Factors associated with adipocyte size reduction after weight loss interventions for overweight and obesity: a systematic review and meta-regression

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ABSTRACT

Aims. Enlarged adipocytes are a prime feature of adipose tissue dysfunction, and may be an appropriate target to decrease disease risk in obesity. We aimed to assess the change in adipocyte size in response to lifestyle and surgical weight loss interventions for overweight or obesity; and to explore whether certain participant and intervention characteristics influence this response.

Methods. We systematically searched MEDLINE, EMBASE, CINAHL and Cochrane electronic databases to identify weight loss studies that quantified adipocyte size before and after the intervention. Using meta-regression analysis, we assessed the independent effects of weight loss, age, sex, adipocyte region, and intervention type (surgical vs. lifestyle) on adipocyte size reduction. We repeated the model as a sensitivity analysis including only the lifestyle interventions.

Results. Thirty-five studies met our eligibility criteria. In our main model, every 1.0% weight loss was associated with a 0.64% reduction in adipocyte size ($p = 0.003$); and adipocytes from the upper body decreased 5% more in size than those in the lower body ($p = 0.009$). These relationships were no longer significant when focusing only on lifestyle interventions. Moreover, age, sex and intervention type did not independently affect adipocyte size reduction in either model.

Conclusions. Weight loss in obese individuals is consistently associated with a decrease in adipocyte size that is more pronounced in upper-body adipocytes. It remains to be clarified how biological differences and intervention characteristics influence this relationship, and whether it corresponds with reductions in other aspects of adipose tissue dysfunction and disease risk.

1. Introduction

One of the proposed biological explanations for adverse obesity outcomes is dysfunctional adipose tissue, also known as adiposopathy. Named for its pathologic potential, it is characterized by both anatomic and functional abnormalities including enlarged adipocytes, immune cell infiltration, impaired adipogenesis, and deranged inflammatory responses [1].

Abbreviations: LCD, Low-calorie diet; VLCD, Very low-calorie diet.

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Treating adiposopathy in obesity may therefore be more important for restoring metabolic health and reducing disease risk than losing weight alone [2]. A 2006 narrative review outlined the mechanisms through which diet, exercise, and pharmacological therapies may target adiposopathy [3], but the magnitude of its improvement after weight loss interventions has not been comprehensively evaluated.

One challenge in studying adiposopathy is that its concept has grown to encompass several defects that can be assessed in terms of morphology, cell composition, secretory patterns, metabolic responses, and gene expression. This vast number of adiposopathy outcomes and measurement techniques greatly limits the comparisons that can be made among weight loss interventions from different studies. Fortunately, one indicator of adipose tissue dysfunction that is easy to quantify and compare across studies is adipocyte size. Enlarged adipocytes have abnormal expression and secretion of many adipokines [4,5], and on a clinical level, are associated with systemic inflammation, insulin resistance, and a host of cardiometabolic diseases [6]. Though it is generally understood that adipocytes decrease in size during negative energy balance, factors that influence this response remain unexplored.

The objective of this systematic review and meta-regression was to examine the linear relationship between weight loss and change in adipocyte size during lifestyle and surgical interventions for overweight or obesity; and to assess the effects of age, sex, adipocyte region, and type of weight loss intervention on this response.

2. Methods

2.1. Eligibility Criteria

Only articles reporting original research published in English were considered for this review. All types of weight loss studies conducted in overweight or obese adults (≥18 years old) with at least one arm that was a lifestyle or surgical intervention were eligible. This included observational prospective longitudinal studies, randomized controlled trials, and also studies where weight loss was part of a secondary analysis.

The studies must have reported body weight and any parameter of adipocyte size as an absolute or percent change during the intervention period, or as pre- and post-intervention values. We excluded studies that expressed body weight and/or adipocyte size changes graphically without providing numerical values.

2.2. Search Strategy

We searched MEDLINE, EMBASE, CINAHL and Cochrane electronic databases up to January 2016 using the following key words and index terms: ("weight loss" or "weight reduction program") and ("overweight" or "obesity") and ("adipocytes" or "fat cell" or "adipose tissue"). The search was limited to humans and to publications in English. The MEDLINE search strategy via Ovid is presented in Appendix A, and was adapted to each of the other three databases based on their unique indexing for subject headings. We also conducted a hand search that included the reference lists of eligible publications as well as all previous published reviews on the topic. References were managed and screened in EndNote X7.4 (Thomson Reuters).

2.3. Study Selection

The studies were selected following the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement. Two authors (JM, SS) examined the eligibility of relevant studies separately, based on the consecutive examination of the titles, abstracts, and full texts. The results were then discussed in committee, and disagreements between authors were resolved by discussion. When more than one article published on the same data, the most comprehensive one was selected. The same procedure was conducted to identify additional studies from our hand search.

2.4. Data Extraction

From each of the selected studies, we extracted information about study design, setting, adipose tissue biopsy region, participant characteristics, intervention details, weight-related data, and adipocyte size data. We extracted information about statistical analyses, whether changes in weight and adipocyte size were significantly different from baseline, and when assessed, whether there were any group differences in these responses. We also intended to extract any measures of association between the changes in body weight and adipocyte size, as well as information about factors that may predict or influence this association. In accordance with the Cochrane model, we assessed the risk of selection bias, performance bias, attrition bias, detection bias and reporting bias for each study using the design-specific criteria outlined in Viswanathan et al. [7].

2.5. Data Synthesis and Analysis

Each weight loss intervention arm was classified as lifestyle or surgical. The arms in the lifestyle category were further divided into the following intervention types: exercise, low-calorie diet (LCD), low-calorie diet with exercise (LCD + exercise), and very low-calorie diet (VLCD). We considered a calorie intake of less than 800 kcal/day to be a VLCD, and any other degree of calorie restriction to be a LCD.

We reported the change in body weight and change in adipocyte size in absolute terms and as percentages. When percent (%) changes were not reported, they were calculated as 100 × (post-value − pre-value)/pre-value or as 100 × the change value/pre-value, based on the data available. We anticipated that different measurement units for adipocyte size would be used across studies – most commonly mass, lipid content (μg lipid/cell), diameter and volume – making their absolute changes incomparable. For quantitative analysis, we therefore converted all measurement units to volume (expressed in pL) using methods frequently implemented in the literature. We assumed adipocyte size expressed as lipid content was equivalent to adipocyte mass. Volume was calculated from adipocyte mass assuming a density of 0.915 g/mL (triolein). We used the formula derived by

Erikkson-Hogling et al. [8] to calculate volume from adipocyte diameter as $\pi/6 \times (3\sigma^2 \times d + d^3)$, where d is the mean diameter and σ is the standard deviation of the diameter. The results of studies using other indices of adipocyte size were not included in the quantitative analysis, but all study findings were summarized narratively.

Using the Comprehensive Meta-Analysis (CMA) software (version 3.3.070) [9], we first calculated the pooled effects estimate of the weight loss interventions on adipocyte size reduction using differences in means with 95% confidence intervals. This common effect estimate was generated using a random effects model because the studies varied greatly in participant characteristics and assessment methods. Forest plots were also generated using CMA software. Heterogeneity of estimates was assessed using the I^2 statistic [10].

Potential sources of heterogeneity in the estimate of adipocyte size reduction were examined by performing a series of subgroup and bivariate meta-regression analyses for the following variables: age, sex (percent women), adipocyte region (upper-body vs. lower-body subcutaneous adipose tissue), and intervention type (surgical vs. lifestyle). We then ran a mixed model meta-regression analysis using R version 3.1.1 [11] to assess the independent relationship between weight loss (key predictor) and adipocyte size reduction (outcome) adjusting for all the above-cited potentially confounding variables. We ran the model expressing the changes in body weight and adipocyte size in absolute terms (*Model 1a*), and as percentages (*Model 1b*). The latter model allowed us to account for baseline adipocyte size, which has been shown to differ with sex and adipocyte region. Due to the inconsistent and variable reporting of measurement uncertainty, we were unable to estimate the standard error for the percent change in adipocyte size for many studies. We used study as our random effects variable to account for the heterogeneity across studies. For the studies that reported adipocyte size change for more than one adipose tissue region within the same participant, each region was considered as a separate data point.

Additionally, we conducted a sensitivity analysis (*Models 2a & 2b*) to assess the models including only the lifestyle weight loss interventions. As such, the intervention variable was coded as follows: VLCD, LCD + exercise, or LCD (reference category).

3. Results

3.1. Study Selection

The search identified a total of 893 possibly eligible articles (see Fig. 1). This number fell to 788 after duplicates were removed. Based on the titles and abstracts, 617 were excluded. The full texts of the remaining 171 articles were screened, and 35 studies published between 1986 and 2015 met all the inclusion criteria. Of these, 32 studies were included in the meta-regression analysis.

Indeed, one study [12] was not suitable for quantitative comparison because it expressed adipocyte size data separately for men and women, but reported weight as an overall average. Two additional studies [13,14] were excluded because they did not measure adipocyte size as a mass, diameter or volume. This left 57 data points available for analysis. Eleven intervention groups were represented twice as adipocyte size was measured from two different adipose tissue regions.

3.2. Study Characteristics and Main Outcomes

The 35 weight loss studies, meeting all our eligibility criteria, had a combined total of 50 lifestyle or surgical intervention groups that included 1011 participants. Our risk-of-bias assessment (Appendix B) identified selection bias as the most common type of bias (13/35 studies) among the studies. Appendix C shows the details for each intervention group including the baseline participant characteristics. Ten studies were randomized clinical trials, one was a non-randomized trial [15], and the remaining were single arm trials or prospective cohort studies. Of the lifestyle intervention groups, 15 were classified as a LCD, 10 as a LCD with an exercise component, and 11 as a VLCD. Their intervention lengths ranged from 4 to 26, 8–52, and 2–54 weeks, respectively. There was one exercise-only study [16] that was 13 weeks in duration, and for the 12 surgical interventions, the follow-up time ranged from 13 to 193 weeks.

Within the intervention groups, 18 contained both men and women, 3 of which were equally distributed [14,17,18], while 23 contained only women and 8 only men. One study did not report the sex of the participants [19]. Average age across intervention groups ranged from 21 to 62 years, and average BMI from 25 to 51 kg/m^2, with two studies having only overweight participants [17,20]. Most studies were conducted in obese adults who were otherwise healthy; only 9 intervention groups had a disease or specific metabolic impairment. These included participants with type 2 diabetes [21–23], insulin resistance [24], features of the metabolic syndrome [12,19], and polycystic ovarian syndrome [15].

Appendix D summarizes the changes in weight and adipocyte size in the intervention groups of all studies, as well as the results of group comparisons. Five studies compared men and women who took part in the same weight loss intervention, two compared disease states, and six compared more than one type of intervention. Adipocyte size was predominantly assessed from abdominal subcutaneous adipose tissue, with 7 studies reporting measures from both upper-body and lower-body regions. None of the included studies evaluated the independent effect of specific factors on the change in adipocyte size during weight loss, and in those that made comparisons between groups, the reporting of differences in terms of absolute or percent change, was highly variable.

The overall percent weight loss in the included studies ranged from 3% to 36%, and the percent reduction in adipocyte volume from −13% (non-significant increase) to 54%. In absolute terms, weight loss ranged from 2.7 to 41.6 kg, and adipocyte size reduction from −73 (non-significant increase) to 522 pL. In the surgical interventions, the percent weight loss ranged from 18% to 36% and the percent reduction in adipocyte mass or volume from 16% to 54%. Furthermore, the percent weight loss ranged from 7% to 17% in the LCD, 4%–13% in the LCD + exercise, and 8%–17% in the VLCD intervention groups. Their corresponding ranges for percent reduction in adipocyte mass or volume ranged from 5% to 32%, 7%–32%, and 10%–44%. The lone exercise-only intervention group [16] had a 3% weight

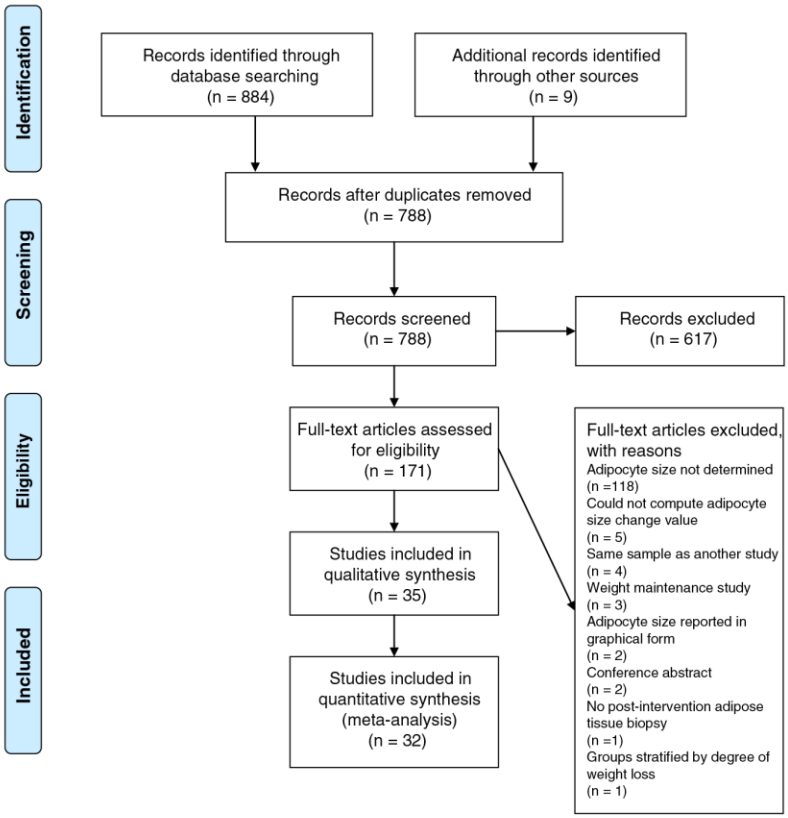

Fig. 1 – PRISMA flow diagram.

loss, and a −13% and 8% reduction in abdominal and gluteal adipocyte mass, respectively.

3.3. *Pooled Effects Estimate*

Fig. 2 shows the forest plot for the overall pooled effects estimate of the weight loss interventions on adipocyte size change. The pooled estimate of the adipocyte size mean difference was −202.46 (95% CI: −237.96, −166.95) pL with high heterogeneity ($Q_{(31)} = 1226$, $p < 0.001$; $I^2 = 97$). The forest plots for our subgroups of interest are shown in Appendix E.

3.4. *Subgroup and Bivariate Analyses*

Table 1 and Fig. 3 show the relationships between adipocyte size change (outcome) and our continuous and categorical predictors, respectively. There was a strong positive linear relationship between weight change (key predictor) and adipocyte size change ($p < 0.001$). Surgical interventions were associated with greater weight loss and adipocyte size reduction than lifestyle interventions ($p < 0.001$). There was no significant relationship between adipocyte size change and sex, age, or adipocyte region.

3.5. *Mixed Model Meta-Regression*

The results of our mixed model meta-regression are shown in Table 2. When expressing the changes in weight and adipocyte size in absolute terms (*Model 1a*), weight loss was the only independent predictor of adipocyte size reduction. For every 1 kg weight loss, adipocyte size decreased by 146 pL ($p = 0.001$) when adjusting for age, sex, adipocyte region and intervention type. This relationship was reduced to a trend ($p = 0.059$) when running the model only with the studies evaluating the lifestyle interventions (*Model 2a*). Moreover, lifestyle intervention type was a significant predictor of adipocyte size reduction. Compared to LCD interventions, LCD + exercise was independently associated with a 63 pL greater reduction in adipocyte size. Age, sex and adipocyte region did not independently predict adipocyte size reduction in either model.

When focusing on the relative change (% weight loss as the key predictor, and % adipocyte size reduction as the outcome), every 1.0% weight loss was associated with a 0.64% reduction in adipocyte size ($p = 0.003$) in our main model (*Model 1b*). In addition, the size of adipocytes from the upper body decreased 5% more than those in the lower body ($p = 0.009$).

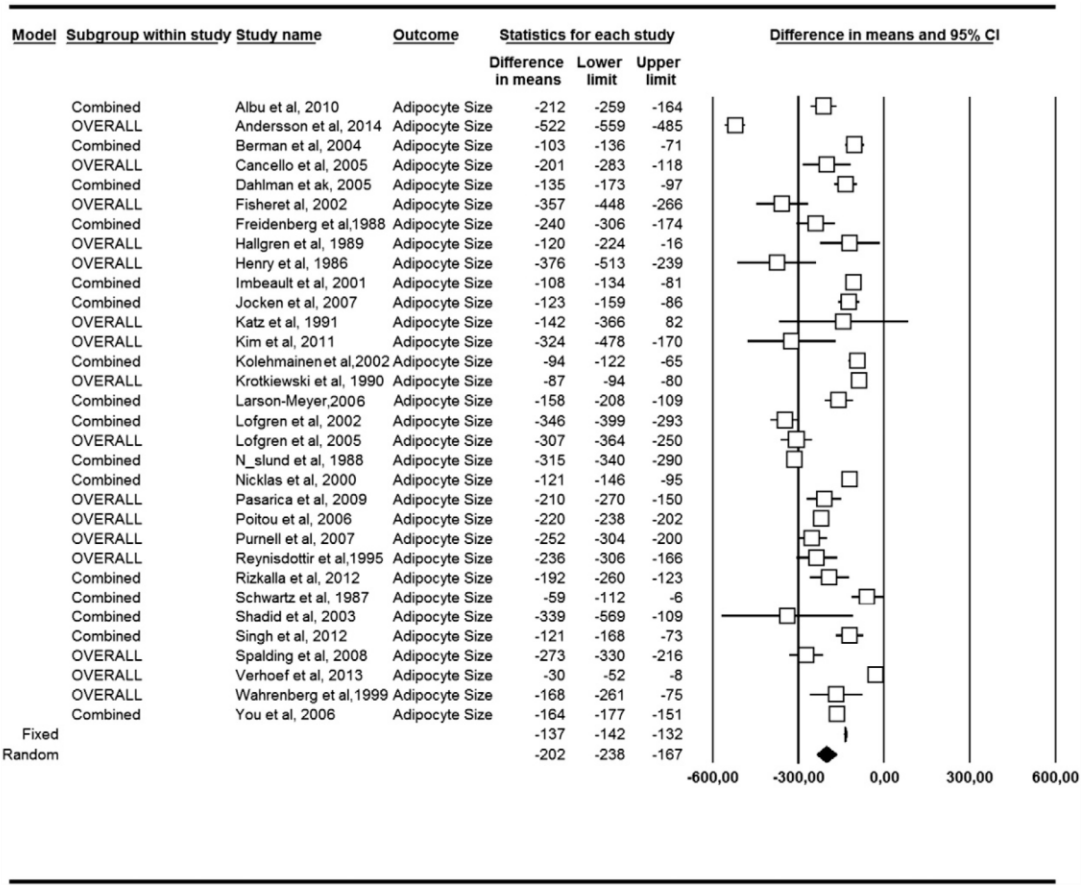

Fig. 2 – Forest plot: pooled effects estimates (random-effect model) of weight loss interventions on adipocyte size change.

These relationships did not persist in our sensitivity analysis (*Model 2b*) as % weight loss only showed a trend to predict the % reduction in adipocyte size ($p = 0.065$). Unlike our analysis in absolute terms, lifestyle intervention type was not independently associated with % adipocyte size reduction; nor were the other covariates.

Table 1 – Relationships between categorical predictors and adipocyte size reduction (outcome).

Predictor	Adipocyte size change (pL) Mean (95% CI)	*p*-value
Overall intervention type		
Lifestyle	−152.11 (−177.68, −126.53)	<0.001
Surgical	−294.85 (−375.58, −214.13)	
Adipocyte region		
Lower-body	−166.38 (−199.70, −133.06)	0.106
Upper-body	−205.85 (−240.30, −171.41)	
Intervention type		
Surgical	−291.71 (−350.19, −233.24)	<0.001
Exercise	9.81 (−107.81, 127.44)	
LCD	−139.22 (−165.70, −112.76)	
LCD + exercise	−153.43 (−173.19, −133.67)	
VLCD	−165.99 (−216.94, −115.03)	

Abbreviations: CI = confidence interval; LCD = low-calorie diet; VLCD = very low-calorie diet.

4. Discussion

Managing adipose tissue dysfunction is an emerging goal of obesity treatment that goes beyond simply losing weight. Before treatments can be properly targeted to realize this goal, it is essential to understand what factors affect indicators of dysfunctional adipose tissue – such as enlarged adipocytes – in response to weight loss. We therefore systematically reviewed the literature for studies that assessed the change in adipocyte size during lifestyle and surgical weight loss interventions for overweight and obesity. We then conducted a meta-regression analysis to determine whether age, sex, adipocyte region and intervention type predicted adipocyte size reduction, independently of weight loss. Our results showed that after adjusting for all variables of interest, every 1.0% weight loss was associated with a 0.64% greater reduction in adipocyte size. The addition of exercise to a LCD also led to a greater reduction in adipocyte size compared

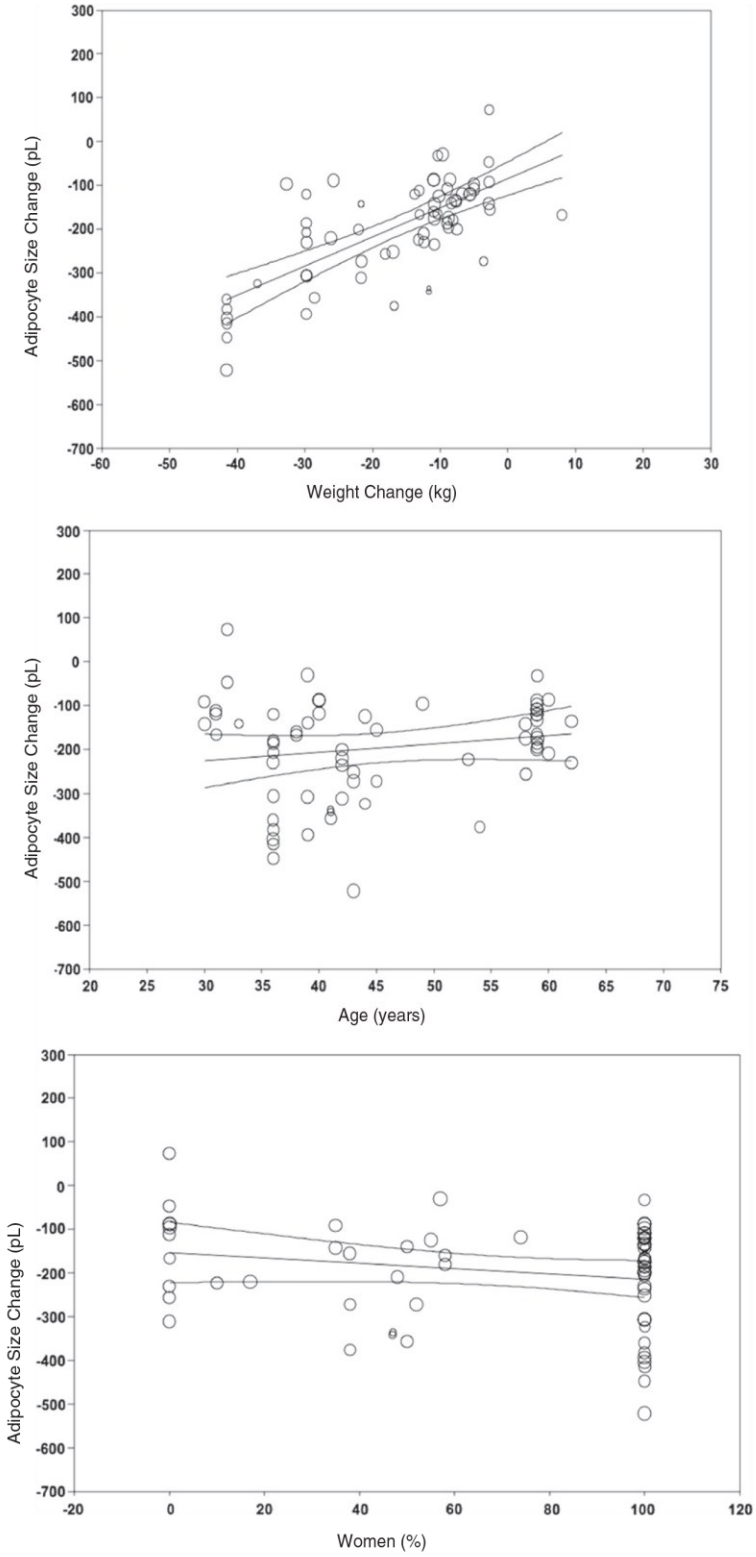

Fig. 3 – Bivariate relationships between continuous predictors and adipocyte size reduction (outcome). Graphs are scatter plots with linear regression lines and 95% confidence intervals. Circles are proportional to study weights.

Table 2 – Mixed model meta-regression of the change in adipocyte size with weight loss.

Outcome: Adipocyte size reduction (pL)

Model 1a			*Model 2a*		
Predictor	Coefficient (95% CI)	*p*-value	Predictor	Coefficient (95% CI)	*p*-value
(Intercept)	16.37 (−213.58, 246.33)	0.885	(Intercept)	39.45 (−142.46, 221.37)	0.657
Weight loss (kg)	11.08 (7.22, 14.94)	<0.001	Weight loss (kg)	7.40 (−0.31, 15.11)	0.059
Women (%)	0.49 (−0.10, 1.09)	0.102	Women (%)	0.17 (−0.47, 0.80)	0.585
Age (years)	−0.86 (−5.32, 3.61)	0.698	Age (years)	0.82 (−2.63, 4.27)	0.618
Adipocyte region			Adipocyte region		
Lower-body [reference]	–	–	Lower-body [reference]	–	–
Upper-body	3.50 (−31.65, 38.64)	0.840	Upper-body	−15.40 (−52.69, 21.90)	0.391
Overall intervention type			Lifestyle intervention type		
Lifestyle [reference]	–	–	LCD [reference]	–	–
Surgical	41.7 (−64.84, 148.28)	0.430	LCD + exercise	61.82 (10.15, 113.49)	0.022
			VLCD	4.28 (−68.41, 76.98)	0.901

Outcome: Adipocyte size reduction (%)

Model 1b			*Model 2b*		
Predictor	Coefficient (95% CI)	*p*-value	Predictor	Coefficient (95% CI)	*p*-value
(Intercept)	15.95 (−1.07, 32.98)	0.065	(Intercept)	11.20 (−7.96, 30.35)	0.238
Weight loss (%)	0.64 (0.24, 1.04)	0.003	Weight loss (%)	0.82 (−0.06, 1.70)	0.065
Women (%)	−0.02 (−0.08, 0.03)	0.374	Women (%)	-0.04 (−0.10, 0.03)	0.279
Age (years)	0.03 (−0.28, 0.33)	0.861	Age (years)	0.01 (−0.34, 0.36)	0.936
Adipocyte region			Adipocyte region		
Lower-body [reference]	–	–	Lower-body [reference]	–	–
Upper-body	5.06 (1.36, 8.76)	0.009	Upper-body	1.02 (−3.29, 5.32)	0.621
Overall intervention type			Lifestyle intervention type		
Lifestyle [reference]	–	–	LCD [reference]	–	–
Surgical	−6.24 (−14.08, 1.59)	0.114	LCD + exercise	3.36 (−2.34, 9.05)	0.227
			VLCD	0.49 (−7.22, 8.21)	0.893

Abbreviations: CI = confidence interval; LCD = low-calorie diet; VLCD = very low-calorie diet.

to LCD alone. Moreover, adipocyte region – but not age, sex or intervention type (surgical vs. lifestyle) – independently affected adipocyte size reduction.

When adjusting for percent weight loss, age, sex and intervention type, subcutaneous adipocyte size decreased 5% more in the abdominal than gluteal and femoral regions. This linear relationship was not significant when expressing weight loss and adipocyte size reduction in absolute terms likely due to the smaller average size of upper-body versus lower-body adipocytes at baseline. Adipocyte size is ultimately dependent on the balance between fatty acid uptake for storage and fatty acid release through lipolysis. The enhanced ability for upper-body adipocytes to shrink is consistent with the body composition changes typically seen during weight loss. The highly lipolytic visceral and subcutaneous abdominal adipose tissue depots are usually the first to decrease in size, with the loss of gluteal/femoral adipose tissue proceeding more slowly [21,25]. We know, however, that regional variations in body fat distribution are accounted for by differences in lipoprotein lipase-mediated and direct fatty acid storage – but not lipolysis [26]. Furthermore, after weight loss in individuals with upper-body obesity, it has been shown that meal-derived fatty acid storage is increased in abdominal subcutaneous adipose tissue implying a propensity to retain body fat distribution [27]. We could not assess the impact of obesity phenotype; however, a greater reduction in the size of abdominal adipocytes in our sample as a whole, suggests that other kinetics of fatty acid uptake may be regulated to favor a larger net release of fatty acids from abdominal versus gluteal/femoral adipose tissue during weight loss. Though we cannot draw mechanistic conclusions from our study results, there is strong evidence that the net free fatty acid release driving adipocyte size reduction with weight loss is mediated by improvements in insulin sensitivity [17,28] and adipokine profile [29].

Age and sex did not independently affect the change in adipocyte size in our meta-regression analysis. This is intriguing given that sex differences have been reported in many fatty acid kinetics including meal-derived and direct fatty acid uptake; very low-density lipoprotein-triacyclglycerol secretion and clearance; and lipolysis [30]. Though age has not been studied as extensively, we know that estrogen deficiency typical of postmenopausal women leads to more alterations in lipid metabolism than does testosterone deficiency in men [30]. Despite these reported differences, our results show that the sum of changes in fatty acid kinetics in response to weight loss gives a similar adipocyte size reduction, regardless of age and sex. The role that aging and sex-associated differences in body fat distribution plays in adipocyte size reduction is unclear. Interestingly, the studies done in post-menopausal women suggest a unique region-specific trend. You et al. [31] showed that after an 11% weight loss on a LCD, gluteal but not abdominal adipocytes significantly decreased in size. The LCD + exercise groups in this study, however, had significant reductions in adipocyte size from both regions, despite a similar

energy balance and weight loss as the LCD-only group. Likewise, Berman et al. [32] and Nicklas et al. [33] showed that both abdominal and gluteal adipocytes decreased in size during LCD + exercise interventions for postmenopausal women. Given the redistribution of adipose tissue that often occurs in menopause, with a preferential increase in both the visceral and subcutaneous abdominal compartments [34], these findings provide further support for the role of aerobic exercise training in reducing this unfavorable body composition change [35]. Unfortunately, there were no intervention groups in our review that were comprised solely of healthy middle-aged men with both upper-body and lower-body adipocyte size measurements to evaluate sex differences in these responses.

Our meta-regression analysis showed that though intervention type (surgical vs. lifestyle) and caloric intake did not independently affect the change in adipocyte size during weight loss, the addition of exercise further reduced adipocyte size. This finding is in line with recent research which suggests that at the same caloric deficit, diet plus exercise improves some indicators of adipose tissue dysfunction more than diet alone [36]. Hence, further investigation on the relationship between exercise and adipose tissue dynamics is warranted.

The broad intervention classification in our meta-regression likely did not allow us to capture the variability across study arms, especially for the dietary protocols. Of the studies that compared two different LCD protocols matched for energy intake, variations in carbohydrate or fat intake did not influence the change in adipocyte size during weight loss [37], but participants on a high-protein diet had a greater reduction in adipocyte size than those following a conventional diet [38]. This corresponds with the finding that high-protein diets are most effective at maximizing fat mass loss while preserving lean body mass [39,40].

All of the intervention groups with an exercise component encompassed aerobic-based training. Only one study compared the effect of adding low- versus high-intensity aerobic exercise to a LCD [31], and showed no difference in adipocyte size reduction between groups. Notably, the lone exercise-only group included in this review experienced a 3% weight loss and no change in abdominal or gluteal adipocyte size [16]. As the only intervention of its kind, it is difficult to ascertain whether this lack of change is primarily due to the small percent weight loss, or the ineffectiveness of exercise alone to impact adipocyte size. It is worth mentioning, however, that the LCD + exercise intervention in Singh et al. [20] significantly decreased both abdominal and femoral adipocyte size despite only a 4% weight loss.

The inconsistent results between our main model and sensitivity analysis questions whether there are certain biological characteristics unique to individuals taking part in surgical versus lifestyle interventions that influence how adipocytes respond to weight loss. The lack of an independent, significant association between weight loss and adipocyte size reduction in the lifestyle interventions suggests that other factors besides weight loss are driving the decrease in adipocyte size. Though most of our lifestyle studies showed weight loss with a concomitant reduction in adipocyte size, none related the two parameters. One study that was not eligible for our review showed that a weight loss greater than 5% is required to see reductions in both visceral and subcutaneous abdominal adipocyte size after a 3-week VLCD in severely obese women [29]. All but two of our studies met this weight loss criterion. It seems plausible that at the lower range of weight loss seen in the lifestyle interventions, hormonal and metabolic changes associated with negative energy balance may be more predictive of adipocyte size reduction than weight loss itself. Moreover, the lack of regional differences in subcutaneous adipocyte size reduction in our lifestyle intervention groups could relate to the preferential mobilization of visceral adipose tissue during earlier phases of voluntary weight loss that is attenuated with greater weight loss [41].

Our review of the literature identified several limitations that we were able to overcome by pooling the data in a meta-regression model. Use of the meta-regression model allowed us to overcome the small sample sizes in some of the studies and assess the independent effects of specific factors on adipocyte size. Though the randomized controlled trials were well-designed, they were very few in number and only one compared surgical interventions. There was also little variety in the exercise interventions limiting our ability to compare the change in adipocyte size across several intervention types. As a result we classified the interventions into broader categories for our quantitative analysis. Furthermore, we categorized adipocyte region into upper-body and lower-body, the latter comprising both gluteal and femoral adipocytes. We acknowledge that it is not entirely clear whether gluteal and femoral adipose tissue respond similarly to weight loss; however, they have been shown to share many biological characteristics [42]. There was also a low representation of male-only intervention groups, and no studies were conducted in elderly individuals. Future studies should be designed to examine the effects of aging on adipocyte size and function.

Our analysis demonstrates that weight loss in obese individuals is consistently associated with a reduction in adipocyte size that is more pronounced in upper-body adipocytes. Sex, age, and taking part in a surgical versus lifestyle intervention did not predict adipocyte size reduction independent of weight loss. These findings contribute an initial understanding of factors that influence adipose tissue dysfunction in response to weight loss. A more detailed investigation is necessary to clarify the effects of both non-modifiable and modifiable factors – namely biological and intervention characteristics. From a clinical standpoint, our results suggest that weight loss, regardless of approach, is an important component of adiposopathy treatment in both obese men and women throughout early to middle adulthood. Hence, prescribing a weight loss intervention that will maximize adherence and comfort for the individual must be a priority. Future research should examine the long-lasting effects of both lifestyle and surgical weight loss interventions on upper- and lower-body adipocyte size. Additionally, it remains to be seen how a decrease in adipocyte size corresponds to improvements in other aspects of adipose tissue dysfunction, and whether it is associated with reduced disease risk independent of weight loss.

Disclosure Statement

The authors report no conflicts of interest.

Author Contributions

J. Murphy assisted with study design, conducted the systematic review and wrote the manuscript. G. Moullec assisted with study design, performed the data analyses, wrote part of the manuscript, and edited the final version. S. Santosa assisted with study design and the conduct of the systematic review, and edited the final version for important intellectual content. All authors approved the final version of the manuscript.

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Supplementary Material

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Appendix A. MEDLINE Search Strategy

1. Weight Loss/
2. Weight Reduction Programs/
3. Obesity/ or obes*.mp.
4. Overweight/
5. Adipocytes/ or fat cell.mp.
6. Adipose Tissue/cy, me [Cytology, Metabolism]
7. 1 or 2
8. 3 or 4
9. 5 or 6
10. 7 and 8 and 9
11. limit 10 to (english language and humans)
12. 11 not review/
13. remove duplicates from 12

Appendix B. Risk-of-Bias Assessment of Studies included in the Systematic Review

Reference	Selection Bias	Performance Bias	Attrition Bias	Detection Bias	Reporting Bias
Studies Comparing Men and Women in One Adipocyte Region					
Albu et al, 2010[17]	Low Risk	Low Risk	Low Risk	Low Risk	Low Risk
Lappalainen et al, 2008[8]	Unclear Risk	High Risk	Low Risk	Low Risk	Low Risk
Kolehmainen et al, 2002[39]	Low Risk	Low Risk	Low Risk	Low Risk	Low Risk
Löfgren et al, 2002[40]	Low Risk	Low Risk	Low Risk	Low Risk	Low Risk
Studies Comparing Men and Women in Two Adipocyte Regionss					
Imbeault et al, 2001[26]	Low Risk	Low Risk	Low Risk	Low Risk	Low Risk
Studies Comparing Disease States in One Adipocyte Region					
Freidenberg et al, 1988[18]	Low Risk	Low Risk	Low Risk	Low Risk	Low Risk
Jocken et al, 2007[20]	Low Risk	Low Risk	Low Risk	Low Risk	Low Risk
Studies Comparing Disease States in One Adipocyte Region					
Dahlman et al, 2005[33]	Unclear Risk	Low Risk	Low Risk	Low Risk	Low Risk
Larson-Meyer et al, 2006[13]	Low Risk	Low Risk	Low Risk	Low Risk	Low Risk
Rizkalla et al, 2012[34]	Low Risk	Low Risk	Low Risk	Low Risk	Low Risk
Näslund et al, 1988[41]	Unclear Risk	Low Risk	Low Risk	Low Risk	Low Risk
Studies Comparing Intervention Types in Two Adipocyte Regions					
Schwartz et al, 1987[12]	Unclear Risk	Low Risk	Low Risk	Low Risk	Low Risk
You et al, 2006[27]	Unclear Risk	Low Risk	Low Risk	Low Risk	High Risk
Single Arm Studies in One Adipocyte Region					
Hallgren et al, 1989[42]	Unclear Risk	Low Risk	Low Risk	High Risk	Low Risk
Henry et al, 1986[43]	Low Risk	Low Risk	Low Risk	High Risk	Low Risk

Katz et al, 1991[44]	Low Risk	Low Risk	Low Risk	High Risk	Low Risk
Kern et al, 1995[9]	Unclear Risk	Low Risk	Low Risk	High Risk	Low Risk
Krotkiewski et al, 1990[45]	Low Risk	Low Risk	Low Risk	Low Risk	Low Risk
Pasarica et al, 2009[19]	Low Risk	Low Risk	Low Risk	Low Risk	Low Risk
Purnell et al, 2007[46]	Unclear Risk	Unclear Risk	Low Risk	Low Risk	Low Risk
Rasmussen et al, 2005[10]	Unclear Risk	Low Risk	Low Risk	Low Risk	Low Risk
Reynisdottir et al, 1995[47]	Low Risk	Low Risk	Low Risk	Low Risk	Low Risk
Verhoef et al, 2013[48]	Low Risk	Low Risk	Low Risk	Low Risk	Low Risk
Wahrenberg et al, 1999[11]	High Risk	Low Risk	Low Risk	Low Risk	Low Risk
Andersson et al, 2014[49]	Low Risk	High Risk	Low Risk	Low Risk	Low Risk
Cancello et al, 2005[50]	Low Risk	Unclear Risk	Low Risk	Unclear Risk	High Risk
Fisher et al, 2002[14]	Unclear Risk	Unclear Risk	Unclear Risk	Low Risk	Low Risk
Kim et al, 2011[15]	Low Risk	Unclear Risk	Unclear Risk	Low Risk	Low Risk
Löfgren et al, 2005[51]	High Risk	High Risk	Low Risk	High Risk	Low Risk
Poitou et al, 2006[52]	Low Risk	Unclear Risk	Low Risk	Unclear Risk	High Risk
Spalding et al, 2008[53]	Unclear Risk	Unclear Risk	Low Risk	Low Risk	Low Risk
Singe Arm Studies in Two Adipocyte Regions					
Berman et al, 2004[28]	Low Risk	Low Risk	Low Risk	Low Risk	Low Risk
Nicklas et al, 2000[29]	Low Risk	Low Risk	Low Risk	Low Risk	Low Risk
Shadid et al, 2003[25]	Low Risk	Low Risk	High Risk	Low Risk	Low Risk
Singh et al, 2012[16]	Low Risk	Low Risk	High Risk	Low Risk	Low Risk

Appendix C. Characteristics and Baseline Data of Studies included in the Systematic Review

Study Information			Intervention Details				Baseline Patient Data		
Reference	**Location**	**Type**	**Type**	**Group**	**Description**	**Length[a]** (weeks)	**Age[a]** (years)	***N* (M/F)**	**BMI[a]** (kg/m^2)
Studies Comparing Men and Women in One Adipocyte Region									
Albu et al, 2010[17]	USA	RCT[c]	LCD + Ex	1: T2D Men; Intensive lifestyle intervention	Hypocaloric diet (< 30 % fat; 1200 – 1500 kcal/d if < 114 kg; 1500 – 1800 kcal/d if ≥ 114 pounds); Physical activity (progressing to ≥ 175 min/week)	52	62	26 (26/0)	32
			LCD + Ex	2: T2D Women; Intensive lifestyle intervention	Hypocaloric diet (< 30 % fat; 1200 – 1500 kcal/d if < 114 kg; 1500 – 1800 kcal/d if ≥ 114 pounds); Physical activity (progressing to ≥ 175 min/week)	52	59	32 (0/32)	35
Lappalainen et al, 2008[8]	Finland	RCT[c]	LCD	1: Men with IFG or IGT + ≥ 2 features of the metabolic syndrome[f]; Weight loss program	Detailed instructions provided by a dietitian; Usual physical activity level	12	60	12 (12/0)	33
			LCD	2: Women with IFG or IGT + ≥ 2 features of the metabolic syndrome[f]; Weight loss program	Detailed instructions provided by a dietitian; Usual physical activity level	12	60	16 (0/16)	33
Kolehmainen et al, 2002[39]	Finland	Prospective Cohort	Bariatric Surgery	1: Men; Gastric banding		52	49	8 (8/0)	51
			Bariatric Surgery	2: Women; Gastric banding		52	40	8 (0/8)	50
Löfgren et al, 2002[40]	Sweden	Prospective Cohort	Bariatric Surgery	1: Men; Gastric banding		103	42	10 (10/0)	40
			Bariatric Surgery	2: Women; Gastric banding		103	39	13 (13/0)	45
Studies Comparing Men and Women in Two Adipocyte Regions									
Imbeault et al, 2001[26]	Canada	Single arm trial	LCD	1: Men; Moderate calorie restriction	Hypocaloric diet (700 kcal/d deficit): 38 – 39% fat; 18 – 19% protein, 41 – 46% carbohydrate, 1 – 2% alcohol as per usual intake	15	36 – 50[b]	17 (17/0)	34

			LCD	2: Women; Moderate calorie restriction	Hypocaloric diet (700 kcal/d deficit): 38 – 39% fat; 18 – 19% protein, 41 – 46% carbohydrate, 1 – 2% alcohol as per usual intake	15	36 – 50[b]	20 (0/20)	36
Studies Comparing Disease States in One Adipocyte Region									
Freidenberg et al, 1988[18]	USA	Single arm trial	VLCD	1: Non-diabetic adults; Very low-calorie diet	Hypocaloric formula diet (420 – 600 kcal/d): 3% fat, 42% protein, 55% carbohydrate; Supplemented with 200 – 300 kcal/d of high-fibre fruits and vegetables	2 – 4[b]	58	5 (5/0)	39
			VLCD	2: T2D adults; Very low-calorie diet	Hypocaloric formula diet (420-600 kcal/d): 3% fat, 42% protein, 55% carbohydrate; Supplemented with 200-300 kcal/d of high-fibre fruits and vegetables	2 – 4[b]	53	10 (9/1)	37
Jocken et al, 2007[20]	Netherlands	Single arm trial	LCD	1: Insulin sensitive adults; Hypocaloric diet	Hypocaloric diet (600 kcal/d deficit); choice of medium fat (40 – 45% fat; 15% protein, 40 – 45% carbohydrate or low fat (20 – 25% fat, 15% protein, 60 – 65% carbohydrate) diet	10	40	19 (5/14)	32
			LCD	2: Insulin resistant adults; Hypocaloric diet	Hypocaloric diet (600 kcal/d deficit); choice of medium fat (40 – 45% fat; 15% protein, 40 – 45% carbohydrate or low fat (20 – 25% fat, 15% protein, 60 – 65% carbohydrate) diet	10	44	20 (9/11)	36
Studies Comparing Disease States in One Adipocyte Region									
Dahlman et al, 2005[33]	Sweden	Randomized trial	LCD	1: Moderate-fat diet	Hypocaloric diet (600 kcal/d deficit): 40 – 45% fat, 15% protein, 40 – 45% carbohydrate	10	21 – 49[b]	13 (0/13)	37
			LCD	2: Low-fat diet	Hypocaloric diet (600 kcal/d deficit): 20 – 25% fat, 15% protein, 60 – 65% carbohydrate	10	21 – 49[b]	10 (0/10)	38
Larson-Meyer et al, 2006[13]	USA	RCT[c]	LCD	1: Calorie restriction	Hypocaloric diet (25% deficit)	12	39	12 (6/6)	28
			LCD + Ex	2: Calorie restriction and exercise	Hypocaloric diet (12.5% deficit); Structured exercise (12.5% increase in energy expenditure)	12	36	12 (5/7)	28
			LCD	3: Low-calorie diet	Hypocaloric diet until a 15% reduction in body weight followed by weight maintenance	12	38	12 (5/7)	28

Rizkalla et al, 2012[34]	France	Randomized cross-over trial	LCD	1: Low-calorie conventional diet	Hypocaloric conventional diet (~1200 kcal/d: 31% fat, 25% protein, 44% carbohydrate	4	45	13 (8/5)	32
			LCD	2: Low-calorie, high-protein, low glycemic index diet	Hypocaloric high-protein (-1200 kcal/d) diet with a low glycemic index and enriched with soluble fibre: 25% fat, 35% protein, 40 % carbohydrate	4	45	13 (8/5)	32
Näslund et al, 1988[41]	Sweden	Randomized trial	Bariatric Surgery	1: Gastric bypass		52	36	26 (0/26)	42
			Bariatric Surgery	2: Gastroplasty		52	36	25 (0/25)	43
Studies Comparing Intervention Types in Two Adipocyte Regions									
Schwartz et al, 1987[12]	USA	Randomized trial	LCD	1: Diet	Hypocaloric diet (1200 kcal/d): 30% fat, 20% protein, 50% carbohydrate; Follow-up with a dietitian 3 times/wk; Usual physical activity level	13	31	12 (12/0)	NR[j]
			Ex	2: Exercise	Progressive walk/jog program under direct supervision of an exercise physiologist 3 times/wk; After 2 – 3 wks, given 2 additional home sessions/wk	13	32	14 (14/0)	NR[j]
You et al, 2006[27]	USA	Randomized trial	LCD	1: Diet	Hypocaloric diet (2800 kcal/wk deficit = 400 kcal/d deficit) with meals provided	20	50 – 70[b]	12 (0/12)	33
			LCD + Ex	2: Diet and low-intensity exercise	Hypocaloric diet (2400 kcal/wk deficit) + progressive low-intensity treadmill walking program 3 days/wk (400 kcal/wk increase in energy expenditure)	20	50 – 70[b]	14 (0/14)	33
			LCD + Ex	3: Diet and high-intensity exercise	Hypocaloric diet (2400 kcal/week deficit) + progressive high-intensity treadmill walking program 3 days/wk (400 kcal/wk increase in energy expenditure)	20	50 – 70[b]	15 (0/15)	33
Single Arm Studies in One Adipocyte Region									
Hallgren et al, 1989[42]	Sweden	Single arm trial	VLCD	1: Very low-calorie diet	Hypocaloric high-protein formula diet (365 kcal/d)	NR[d]	NR	5 (0/5)	35
Henry et al, 1986[43]	USA	Single arm trial	VLCD	1: T2D adults; Very low-calorie diet	Hypocaloric formula diet (330-600 kcal/d): 3% fat, 42% protein, 55% carbohydrate	9 – 54	54	8 (5/3)	34
Katz et al, 1991[44]	USA	Single arm trial	VLCD	1: Formula diet	Hypocaloric formula diet (400 kcal/d): 2 g fat, 70 g protein, 30 g carbohydrate; Supplemented with a potassium supplement and multivitamin	12	33	5 (0/5)	46
Kern et al, 1995[9]	USA	Single arm trial	VLCD	1: Weight loss program	Hypocaloric diet (520 – 800 kcal/d) and behaviour modification	NR[e]	44	7 (6/3)	41

Krotkiewski et al, 1990[45]	Sweden	Single arm trial	VLCD	1: Formula diet	Hypocaloric high-protein formula diet (544 kcal/d) in the form of four meals per day	4	40	25 (0/25)	37
Pasarica et al, 2009[19]	USA	RCT[c]	LCD + Ex	1: T2D Adults; Calorie restriction and exercise	Hypocaloric diet (< 30 % fat; 1200 – 1500 kcal/d if < 114 kg; 1500 – 1800 kcal/d if ≥ 114 pounds)); Physical activity (progressing to ≥ 175 min/week)	52	60	44 (23/21)	34
Purnell et al, 2007[46]	USA	Single arm trial	LCD	1: Low-calorie diet	Hypocaloric formula diet (1000 kcal/d)	13	43	13 (5/8)	35
Rasmussen et al, 2005[10]	Denmark	Single arm trial	VLCD	1: Very low-calorie diet	Hypocaloric formula diet (800 kcal/d) supervised by a dietitian every other week	8	40	18 (9/9)	32
Reynisdottir et al, 1995[47]	Sweden	Single arm trial	VLCD	1: Very low-calorie formula diet	Hypocaloric high-protein formula diet administered 5 times/d: 6% fat, 63% protein, 31% carbohydrate; Supplemented with vitamins and minerals	8 – 12[b]	42	14 (0/14)	36
Verhoef et al, 2013[48]	Netherlands	Single arm trial	VLCD	1: Very low-calorie diet	Hypocaloric high-protein formula diet (500 kcal/d): 7 g fat, 52 g protein, 50 g carbohydrate; Vegetable consumption permitted	9	39	28 (12/16)	32
Wahrenberg et al, 1999[11]	Sweden	Non-randomized trial[i]	VLCD	1: Women with PCOS; Weight loss program	Hypocaloric high-protein formula diet (544 kcal/d) in the form of 4 meals/day	8 – 12[b]	38	9 (0/9)	38
Andersson et al, 2014[49]	Sweden	Prospective Cohort	Bariatric Surgery	1: RYGB		104	43	62 (0/62)	43
Cancello et al, 2005[50]	France	Prospective Cohort	Bariatric Surgery	1: RYGB		13	42	10 (0/10)	48
Fisher et al, 2002[14]	Sweden	Prospective Cohort	Bariatric Surgery	1: Gastric banding		103	41	12 (6/6)	42
Kim et al, 2011[15]	France	Prospective Cohort	Bariatric Surgery	1: Adults with ≥ 1 comorbidity[g]; RYGB		52	44	71 (NR)	48
Löfgren et al, 2005[51]	Sweden	Prospective Cohort	Bariatric Surgery	1: Gastric banding or lifestyle modification (increased motivation, altered eating habits, increased exercise and regular exercise)[h]		89 – 193[b]	39	25 (0/25)	43
Poitou et al, 2006[52]	France	Prospective Cohort	Bariatric Surgery	1: Gastric banding or gastric bypass		52	42	60 (50/10)	50
Spalding et al, 2008[53]	Sweden	Prospective Cohort	Bariatric Surgery	1: Gastric banding		103	43	21 (9/11)	42
Singe Arm Studies in Two Adipocyte Regions									
Berman et al, 2004[28]	USA	Single arm trial	LCD + Ex	1: Post-menopausal women; Weight loss program	Hypocaloric diet (250 – 350 kcal/d deficit); Low-intensity walking 3 d/wk for 30 – 45 min; Weekly dietetic consultations; Focused on improving eating behaviours	26	59	34 (0/34)	33

Nicklas et al, 2000[29]	USA	Single arm trial	LCD + Ex	1: Post-menopausal women; Weight loss program	Hypocaloric diet (250 – 350 kcal/d deficit); Low-intensity walking 3d/wk for 30 – 45 min; Weekly dietetic consultations; Focused on improving eating behaviours	26	59	36 (0/36)	33
Shadid et al, 2003[25]	USA	Randomized trial[i]	LCD + Ex	1: Diet and exercise program	Hypocaloric diet (500 kcal/d deficit); Progressive aerobic exercise program from 3 – 4 times/wk; Biweekly behavioural modification program; Dietetic consultations every 4 wks	18 – 20[b]	41	19 (10/9)	32
Singh et al, 2012[16]	USA	Single arm trial	LCD + Ex	1: Weight loss program	Dietary counselling and encouragement to increase activity	8	30	23 (15/8)	25

Abbreviations: BMI = body mass index; Ex = exercise; IFG = impaired fasting glucose; IGT = impaired glucose tolerance; LCD = low-calorie diet; M/F = male/female; NR = not reported; Ob = observational study; PCOS = polycystic ovarian syndrome; RCT = randomized controlled trial; RYGB = roux-en-y gastric bypass; T2D: type 2 diabetic; VLCD = very low-calorie diet

a: reported as mean unless otherwise indicated; b: range; c: control group not included; d: until a 14 kg weight loss was achieved; e: lost weight and then maintained it for 3 months; f: waist circumference > 102 cm in males or > 88 cm in females, fasting serum triacylglycerol concentration ≥ 1.7 mmol/L, fasting serum HDL cholesterol < 1.0 mmol/L in males or <1.3 mmol/L in females, or blood pressure ≥ 130/80 mmHg; g: hypertension, type 2 diabetes, dyslipidemia, or obstructive sleep syndrome; h: n = 18 received gastric banding and n = 7 lifestyle modification; i: study had another treatment arm that was not a lifestyle or surgical weight loss intervention; j: relative weights ranged from 111-185 % of ideal body weight.

Appendix D. Changes in Body Weight and Adipocyte Size for each Study Arm and Summary of Group Comparisons

Intervention				Body Weight				Adipocyte Size					
Reference	**Type**	**Group**	***N***	**Baseline** (kg)	**Change[f]** (kg)	**Change[f]** (%)	**Group Comparisons Reported**	**SAT Region**	**Size Parameter (units)**	**Baseline** (units)	**Change[f]** (units)	**Change[f]** (%)	**Group Comparisons Reported**
Studies Comparing Men and Women in One Adipocyte Region													
Albu et al, 2010[17]	LCD + Ex	1: T2D Men; Intensive lifestyle intervention	26	101.2	-12.4	-12.3	T_1: NR ↓ (Abs): 1 > 2 ↓ (%:): 1 > 2	Abdominal	Volume (pL)	730	-230	-31.5	T_1: NR ↓ (Abs): 1 = 2 ↓ (%:): NR
	LCD + Ex	2: T2D Women; Intensive lifestyle intervention	32	91.4	-7.5[h]	-8.2[h]		Abdominal	Volume (pL)	960	-200	-20.8	
Lappalainen et al, 2008[8]	LCD	1: Men with IFG or IGT + ≥ 2 features of the metabolic syndrome; Weight loss program	12	92.8[c]	-4.6[c]	-5.0[c]	T_1: NR ↓ (Abs): NR ↓ (%:): NR	Abdominal	Volume (pL)	533	247[g]	46.3[g]	T_1: 2 > 1 ↔: 1 & 2 T_2: 1 = 2
	LCD	2: Women with IFG or IGT + ≥ 2 features of the metabolic syndrome; Weight loss program	16					Abdominal	Volume (pL)	865	-146[g]	-16.9[g]	
Kolehmainen et al, 2002[39]	Bariatric Surgery	1: Men; Gastric banding	8	156.8	-32.8	-20.9	T_1: NR ↓ (Abs): NR ↓ (%:): 1 = 2	Abdominal	Volume (pL)	459	-97	-21.1	T_1: 2 > 1 ↓ (Abs): NR ↓ (%): 1 = 2
	Bariatric Surgery	2: Women; Gastric banding	8	134.8	-25.8	-19.1		Abdominal	Volume (pL)	545	-89	-16.3	
Löfgren et al, 2002[40]	Bariatric Surgery	1: Men; Gastric banding	10	NR	NR	-18.5[d]	T_1: NR ↓ (Abs): NR ↓ (%:): NR	Abdominal	Volume (pL)	841	-310	-36.9	T_1: NR ↓ (Abs): NR ↓ (%): 1 = 2
	Bariatric Surgery	2: Women; Gastric banding	13	NR	NR	-29.2[d]		Abdominal	Volume (pL)	911	-394	-43.2	
Studies Comparing Men and Women in Two Adipocyte Regions													

Imbeault et al, 2001[26]	LCD	1: Men; Moderate calorie restriction	17	105.0	-11.0	-10.5	T_1: 1 > 2 ↓ (Abs): 1 = 2 ↓ (%:): NR	Abdominal	Lipid Mass (µg lipid/cell)	0.60	-0.08	-13.3	<u>Abdominal</u> T_1: 2 > 1 ↓ (Abs): 1 = 2 ↓ (%:): NR <u>Femoral</u> T_1: 2 > 1 ↓ (Abs): 1 = 2 ↓ (%:): NR *No comparisons between regions
								Femoral	Lipid Mass (µg lipid/cell)	0.56	-0.08	-14.3	
	LCD	2: Women; Moderate calorie restriction	20	91.0	-9.0	-9.9		Abdominal	Lipid Mass (µg lipid/cell)	0.72	-0.10	-13.9	
								Femoral	Lipid Mass (µg lipid/cell)	0.81	-0.17	-21.0	
Studies Comparing Disease States in One Adipocyte Region													
Freidenberg et al, 1988[18]	VLCD	1: Non-diabetic adults; Very low-calorie diet	5	117.3	-18.2	-15.5	T_1: NR ↓ (Abs): NR ↓ (%:): NR	Abdominal	Volume (pL)	1021	-256	-25.1	T_1: NR ↓ (Abs): NR ↓ (%:): NR
	VLCD	2: T2D adults; Very low-calorie diet	10	112.3	-13.2	-11.8		Abdominal	Volume (pL)	1076	-224	-20.8	
Jocken et al, 2007[20]	LCD	1: Insulin sensitive adults; Hypocaloric diet	19	91.0	-6.8	-7.5	T_1: 2 > 1 ↓ (Abs): NR ↓ (%:): NR	Abdominal	Mass (µg)	0.71	-0.11	-15.4	T_1: 2 > 1 ↓ (Abs): NR ↓ (%:): NR
								Abdominal	Volume (pL)	755	-100	-13.2	
	LCD	2: Insulin resistant adults; Hypocaloric diet	20	106.4	-10.2	-9.6		Abdominal	Mass (µg)	0.80	-0.11	-14.3	
								Abdominal	Volume (pL)	869	-125	-14.4	
Studies Comparing Intervention Types in One Adipocyte Region													
Dahlman et al, 2005[33]	LCD	1: Moderate-fat diet	13	103.2	-7.8	-7.6	T_1: 1 = 2 ↓ (Abs): 1 = 2 ↓ (%:): 1 = 2	Abdominal	Volume (pL)	836	-134	-16.0	T_1: 1 = 2 ↓ (Abs): 1 = 2 ↓ (%:): NR
	LCD	2: Low-fat diet	10	102.8	-7.6	-7.4		Abdominal	Volume (pL)	845	-136	-16.1	

Larson-Meyer et al, 2006[13]	LCD	1: Calorie restriction	12	81.0	-8.4	-10.4	T_1: NR ↓ (Abs): NR ↓ (%:): NR	Abdominal	Volume (pL)	650	-140	-21.5	T_1: NR ↓ (Abs): NR ↓ (%:): NR
	LCD + Ex	2: Calorie restriction and exercise	12	82.0	-8.1	-9.9		Abdominal	Volume (pL)	700	-180	-25.7	
	LCD	3: Low-calorie diet	12	81.0	-11.0	-13.6		Abdominal	Volume (pL)	670	-160	-23.9	
Rizkalla et al, 2012[34]	LCD	1: Low-calorie conventional diet	13	89.8	-2.7	-3.1	T_1: NR ↓ (Abs): 1 = 2 ↓ (%:): NR	Abdominal	Diameter (μm)	102.1	-10.4	-10.2	T_1: NR ↓ (Abs): 2 > 1 ↓ (%:): NR
	LCD	2: Low-calorie, high-protein, low glycemic index diet	13	90.4	-3.6	-3.9		Abdominal	Diameter (μm)	109.9	-17.7[h]	-16.1[h]	
Näslund et al, 1988[41]	Bariatric Surgery	1: Gastric bypass	26	115.7	-41.6	-36.0	T_1: 1 = 2 ↓ (Abs): 1 > 2 ↓ (%:): NR	Average[e]	Mass (μg)	0.79	-0.37	-46.8	T_1: 1 = 2 ↓ (Abs): 1 > 2 ↓ (%:): NR
	Bariatric Surgery	2: Gastroplasty	25	116.2	-29.8[h]	-25.6[h]		Average[e]	Mass (μg)	0.76	-0.21[h]	-27.6[h]	
Studies Comparing Intervention Types in Two Adipocyte Regions													
Schwartz et al, 1987[12]	LCD	1: Diet	12	102.7	-13.1	-12.8	T_1: 1 = 2 ↓ (Abs): 1 > 2 ↓ (%:): NR	Abdominal	Lipid Mass (μg lipid/cell)	0.60	-0.15	-25.5	<u>Abdominal</u> T_1: 1 = 2 ↓ (Abs): 1 > 2 ↓ (%:): NR <u>Gluteal</u> T_1: 1 = 2 ↓ (Abs): 1 = 2 ↓ (%:): NR *No comparisons between regions
								Gluteal	Lipid Mass (μg lipid/cell)	0.58	-0.10[g]	-17.5[g]	
	Ex	2: Exercise	14	99.9	-2.8[h]	-2.8[h]		Abdominal	Lipid Mass (μg lipid/cell)	0.53	0.07[g]	12.7[g]	
								Gluteal	Lipid Mass (μg lipid/cell)	0.54	-0.04[g]	-7.9[g]	

You et al, 2006[27]	LCD	1: Diet	12	91.2	-10.4	-11.4	T_1: 1 = 2 = 3 ↓ (Abs): 1 = 2 = 3 ↓ (%:): 1 = 2 = 3	Abdominal	Mass (µg)	0.83	-0.04[g]	-4.8[g]	<u>Abdominal</u> T_1: 1 = 2 = 3 ↓ (Abs): 2 & 3 > 1 ↓ (%): 2 & 3 > 1 <u>Gluteal</u> T_1: 1 = 2 = 3 ↓ (Abs): 2 & 3 > 1 ↓ (%): 2 & 3 > 1 *No comparisons between regions
								Gluteal	Mass (µg)	0.96	-0.15	-15.6	
	LCD + Ex	2: Diet and low-intensity exercise	14	86.6	-10.9	-12.6		Abdominal	Mass (µg)	0.82	-0.16[h]	-19.5[h]	
								Gluteal	Mass (µg)	0.88	-0.13	-14.8	
	LCD + Ex	3: Diet and high-intensity exercise	15	85.8	-8.8	-10.3		Abdominal	Mass (µg)	0.89	-0.16[h]	-18.0[h]	
								Gluteal	Mass (µg)	0.95	-0.19	-20.0	
Single Arm Studies in One Adipocyte Region													
Hallgren et al, 1989[42]	VLCD	1: Very low-calorie diet	5	96.4	-13.8	-14.3	N/A	Abdominal	Mass (µg)	0.72	-0.11[g]	-15.3[g]	N/A
Henry et al, 1986[43]	VLCD	1: T2D adults; Very low-calorie diet	8	102.9	-16.8	-16.3	N/A	Abdominal	Volume (pL)	851	-376	-44.2	N/A
Katz et al, 1991[44]	VLCD	1: Formula diet	5	130.5	-21.7	-16.6	N/A	NR	Lipid Mass (µg lipid/cell)	0.57	-0.13[g]	-22.8[g]	N/A
Kern et al, 1995[9]	VLCD	1: Weight loss program	7	NR	-34.7	-26.6	N/A	Abdominal	g DNA/g adipose tissue[a]	476	460	97	N/A
Krotkiewski et al, 1990[45]	VLCD	1: Formula diet	25	103.1	-8.6	-8.3	N/A	Average[b]	Mass (µg)	0.82	-0.08	-9.8	N/A
Pasarica et al, 2009[19]	LCD + Ex	1: Calorie restriction and exercise	44	98.1	-12.5	-12.6	N/A	Abdominal	Volume (pL)	850	-210	-24.7	N/A
Purnell et al, 2007[46]	LCD	1: Low-calorie diet	13	99.0	-17.0	-17.2	N/A	Gluteal	Lipid Mass (µg lipid/cell)	0.73	-0.23	-31.5	N/A

Rasmussen et al, 2005[10]	VLCD	1: Very low-calorie diet	18	99.6	-12.8	-12.9	N/A	Abdominal	ng DNA/mg adipose tissue[a]	37.6	12.7	33.8	N/A
Reynisdottir et al, 1995[47]	VLCD	1: Very low-calorie formula diet	14	NR	NR	-16.8[d]	N/A	Abdominal	Volume (pL)	891	-236	-26.5	N/A
Verhoef et al, 2013[48]	VLCD	1: Very low-calorie diet	28	96.9	-9.7	-10.0	N/A	Abdominal	Diameter (µm)	66.9	-3.7	-5.5	N/A
								Abdominal	Volume (x105 µm^3)	1.8	-0.3	-16.7	
Wahrenberg et al, 1999[11]	VLCD	1: Women with PCOS; Weight loss program	9	NR	-8.0	-14.8d	N/A	Abdominal	Volume (pL)	874	-168	-19.2	N/A
Andersson et al, 2014[49]	Bariatric Surgery	1: RYGB	62	NR	NR	-33.0	N/A	Abdominal	Volume (pL)	972	-522	53.7	N/A
Cancello et al, 2005[50]	Bariatric Surgery	1: RYGB	10	128.2	-22.1	-17.2	N/A	Abdominal	Diameter (µm)	98.7	-15.4	-15.6	N/A
Fisher et al, 2002[14]	Bariatric Surgery	1: Gastric banding	12	128.9	-28.6	-22.2	N/A	Abdominal	Volume (pL)	900	-357	-39.7	N/A
Kim et al, 2011[15]	Bariatric Surgery	1: Adults with ≥ 1 comorbidity; RYGB	71	130.0	-37.0	-28.5	N/A	Abdominal	Diameter (µm)	115.5	-17.6	-15.2	N/A
Löfgren et al, 2005[51]	Bariatric Surgery	1: Gastric banding or lifestyle modification	25	NR	NR	-23.7[d]	N/A	Abdominal	Volume (pL)	830	-307	-37.0	N/A
Poitou et al, 2006[52]	Bariatric Surgery	1: Gastric banding or gastric bypass	60	124.0	-26.2	-21.1	N/A	Abdominal	Volume (pL)	970	-0.28	-28.9	N/A
Spalding et al, 2008[53]	Bariatric Surgery	1: Gastric banding	21	NR	NR	-18.0	N/A	Abdominal	Volume (pL)	890	-273	-30.7	N/A
Singe Arm Studies in Two Adipocyte Regions													
Berman et al, 2004[28]	LCD + Ex	1: Post-menopausal women; Weight loss program	34	86.0	-5.0	-5.8	N/A	Abdominal	Lipid Mass (µg lipid/cell)	0.89	-0.09	-10.1	T_1: Gluteal > Abdominal ↓ (Abs): NR ↓ (%): Gluteal = Abdominal
								Gluteal	Lipid Mass (µg lipid/cell)	0.84	-0.10	-11.9	

Nicklas et al, 2000[29]	LCD + Ex	1: Post-menopausal women; Weight loss program	36	86.6	-5.6	-6.5	N/A	Abdominal	Lipid Mass (µg lipid/cell)	0.85	-0.11	-12.9	*No comparisons between regions
								Gluteal	Lipid Mass (µg lipid/cell)	0.9	-0.11	-12.2	
Shadid et al, 2003[25]	LCD + Ex	1: Diet and exercise program	19	97.5	-11.7	-12.0	N/A	Abdominal	Lipid Mass (µg lipid/cell)	0.84	-0.16	-19.0	*No comparisons between regions
								Femoral	Lipid Mass (µg lipid/cell)	0.85	-0.06[g]	-7.1[g]	
Singh et al, 2012[16]	LCD + Ex	1: Weight loss program	23	75.9	-2.8	-3.7	N/A	Abdominal	Lipid Mass (µg lipid/cell)	0.56	-0.08	-15.1	*No comparisons between regions
								Femoral	Lipid Mass (µg lipid/cell)	0.71	-0.13	-18.4	

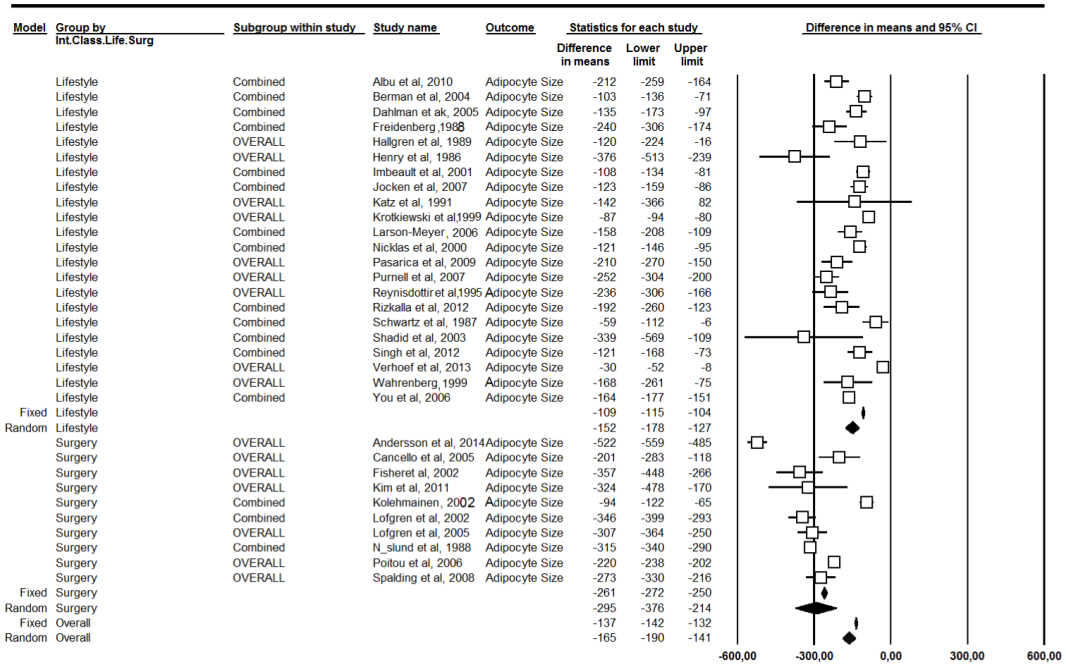
Data are presented as means

Abbreviations: ↓ (Abs): absolute decrease; ↓ (%): percent decrease; ↔: no change; Ex = exercise; IFG = impaired fasting glucose; IGT = impaired glucose tolerance; LCD = low-calorie diet; N/A = not applicable; NR = not reported; PCOS = polycystic ovarian syndrome; RYGB = roux-en-y gastric bypass; SAT = subcutaneous adipose tissue; T_1 = baseline; T2D = type 2 diabetic; VLCD = very low-calorie diet

a: a positive change indicates a decrease in adipocyte size; b: abdominal and gluteal regions; c: for intervention group 1 and 2 combined; d: derived from the change in body mass index; e: femoral, gluteal, epigastric and hypogastric regions; f: statistically significant change unless other wise indicated ($p < 0.05$); g: not a statistically significant change ($p > 0.05$) h: significantly different from intervention group 1 ($p \leq 0.05$)

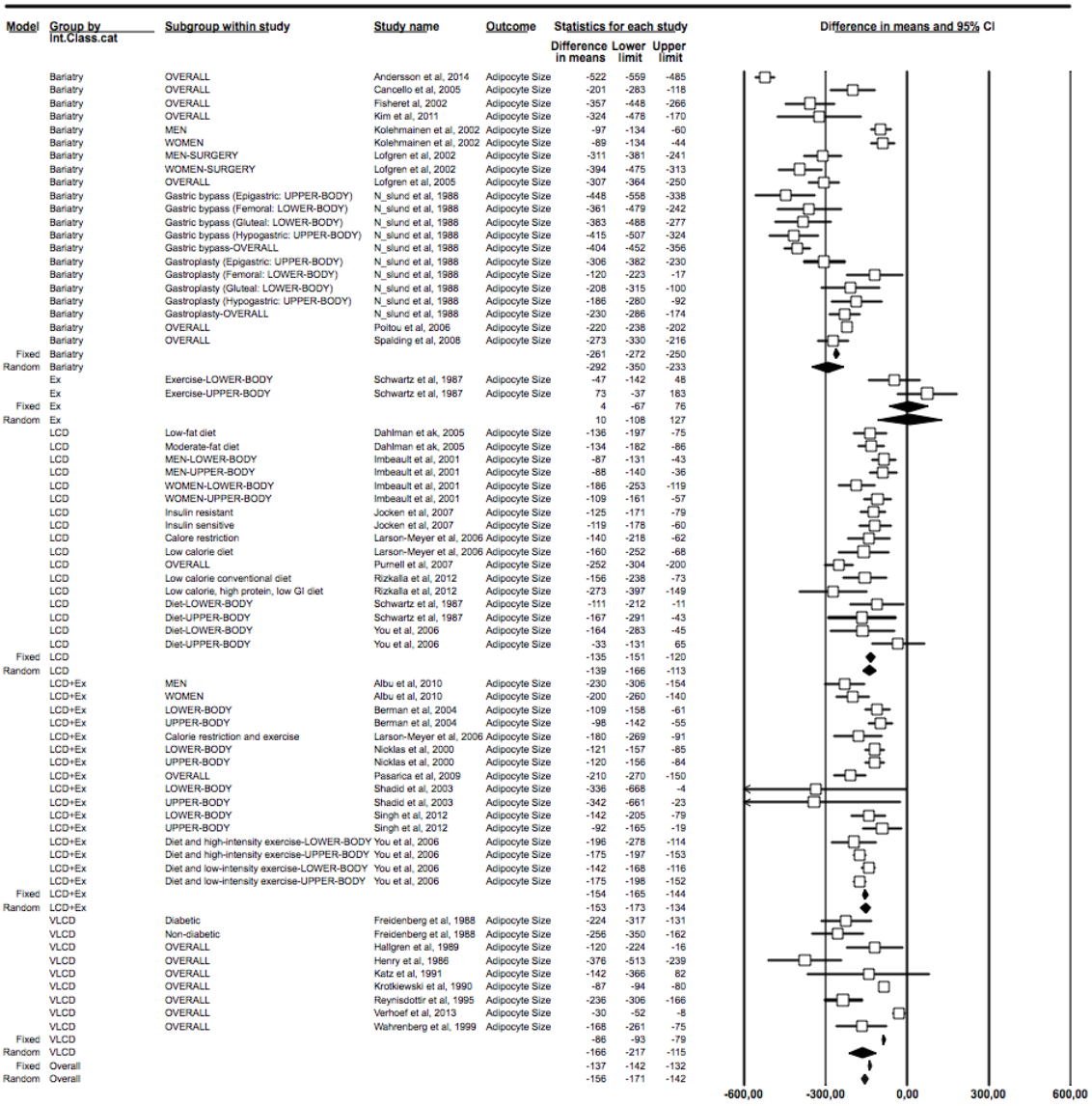
Appendix E. Forest Plots for Subgroups

Broad Intervention Type: Surgical and Lifestyle



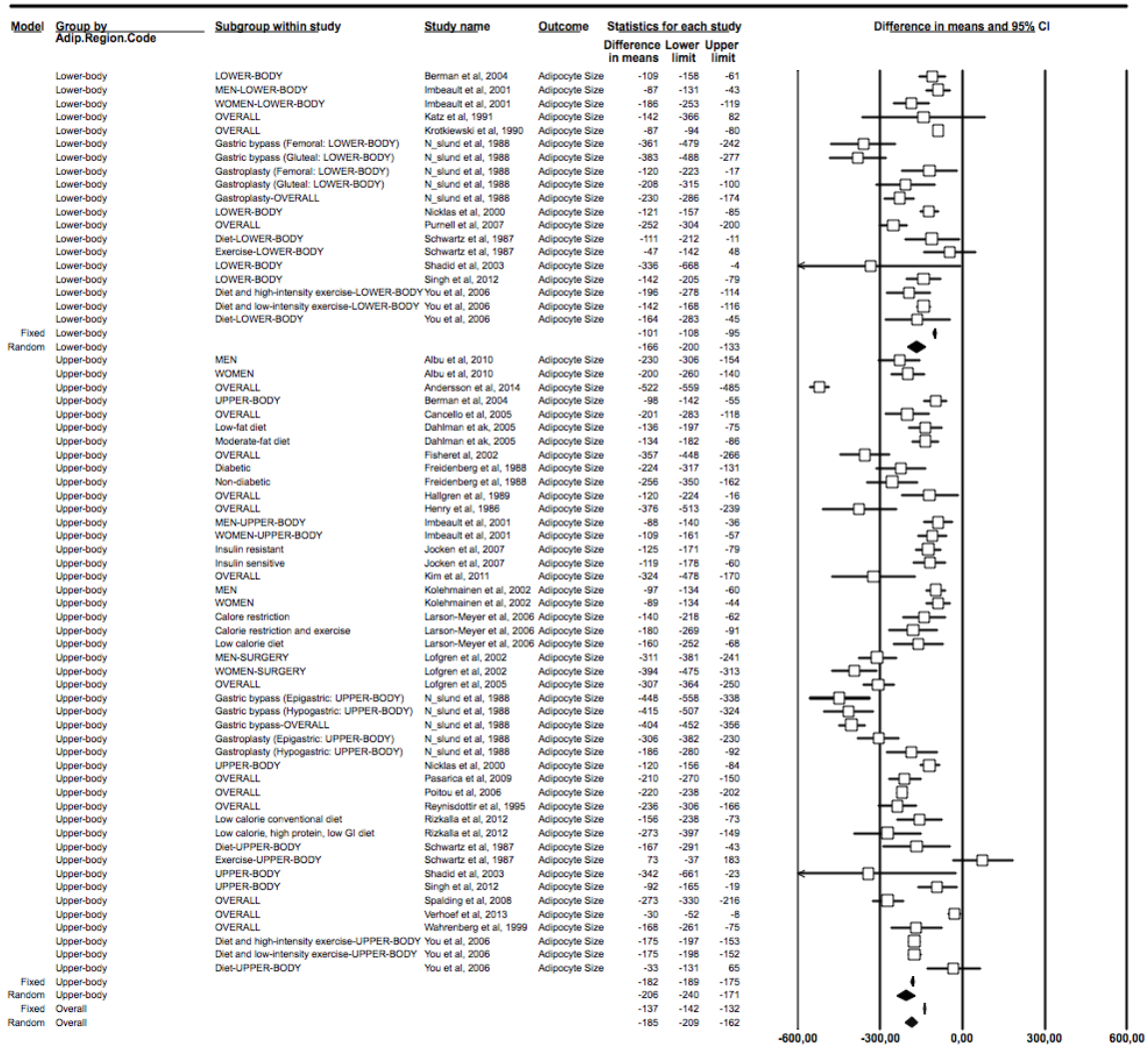
Model	Group by Int.Class.Life.Surg	Subgroup within study	Study name	Outcome	Difference in means	Lower limit	Upper limit
	Lifestyle	Combined	Albu et al, 2010	Adipocyte Size	-212	-259	-164
	Lifestyle	Combined	Berman et al, 2004	Adipocyte Size	-103	-136	-71
	Lifestyle	Combined	Dahlman et ak, 2005	Adipocyte Size	-135	-173	-97
	Lifestyle	Combined	Freidenberg,1988	Adipocyte Size	-240	-306	-174
	Lifestyle	OVERALL	Hallgren et al, 1989	Adipocyte Size	-120	-224	-16
	Lifestyle	OVERALL	Henry et al, 1986	Adipocyte Size	-376	-513	-239
	Lifestyle	Combined	Imbeault et al, 2001	Adipocyte Size	-108	-134	-81
	Lifestyle	Combined	Jocken et al, 2007	Adipocyte Size	-123	-159	-86
	Lifestyle	OVERALL	Katz et al, 1991	Adipocyte Size	-142	-366	82
	Lifestyle	OVERALL	Krotkiewski et al,1999	Adipocyte Size	-87	-94	-80
	Lifestyle	Combined	Larson-Meyer, 2006	Adipocyte Size	-158	-208	-109
	Lifestyle	Combined	Nicklas et al, 2000	Adipocyte Size	-121	-146	-95
	Lifestyle	OVERALL	Pasarica et al, 2009	Adipocyte Size	-210	-270	-150
	Lifestyle	OVERALL	Purnell et al, 2007	Adipocyte Size	-252	-304	-200
	Lifestyle	OVERALL	Reynisdottir et al,1995	Adipocyte Size	-236	-306	-166
	Lifestyle	Combined	Rizkalla et al, 2012	Adipocyte Size	-192	-260	-123
	Lifestyle	Combined	Schwartz et al, 1987	Adipocyte Size	-59	-112	-6
	Lifestyle	Combined	Shadid et al, 2003	Adipocyte Size	-339	-569	-109
	Lifestyle	Combined	Singh et al, 2012	Adipocyte Size	-121	-168	-73
	Lifestyle	OVERALL	Verhoef et al, 2013	Adipocyte Size	-30	-52	-8
	Lifestyle	OVERALL	Wahrenberg,1999	Adipocyte Size	-168	-261	-75
	Lifestyle	Combined	You et al, 2006	Adipocyte Size	-164	-177	-151
Fixed	Lifestyle				-109	-115	-104
Random	Lifestyle				-152	-178	-127
	Surgery	OVERALL	Andersson et al, 2014	Adipocyte Size	-522	-559	-485
	Surgery	OVERALL	Cancello et al, 2005	Adipocyte Size	-201	-283	-118
	Surgery	OVERALL	Fisheret al, 2002	Adipocyte Size	-357	-448	-266
	Surgery	OVERALL	Kim et al, 2011	Adipocyte Size	-324	-478	-170
	Surgery	Combined	Kolehmainen, 2002	Adipocyte Size	-94	-122	-65
	Surgery	Combined	Lofgren et al, 2002	Adipocyte Size	-346	-399	-293
	Surgery	OVERALL	Lofgren et al, 2005	Adipocyte Size	-307	-364	-250
	Surgery	Combined	N_slund et al, 1988	Adipocyte Size	-315	-340	-290
	Surgery	OVERALL	Poitou et al, 2006	Adipocyte Size	-220	-238	-202
	Surgery	OVERALL	Spalding et al, 2008	Adipocyte Size	-273	-330	-216
Fixed	Surgery				-261	-272	-250
Random	Surgery				-295	-376	-214
Fixed	Overall				-137	-142	-132
Random	Overall				-165	-190	-141

All Intervention Types: Surgical, Exercise (Ex), Low-Calorie Diet (LCD), LCD + Ex, and Very Low-Calorie Diet (VLCD)



Model	Group by Int.Class.cat	Subgroup within study	Study name	Outcome	Statistics for each study: Difference in means	Lower limit	Upper limit
	Bariatry	OVERALL	Andersson et al, 2014	Adipocyte Size	-522	-559	-485
	Bariatry	OVERALL	Cancello et al, 2005	Adipocyte Size	-201	-283	-118
	Bariatry	OVERALL	Fisheret al, 2002	Adipocyte Size	-357	-448	-266
	Bariatry	OVERALL	Kim et al, 2011	Adipocyte Size	-324	-478	-170
	Bariatry	MEN	Kolehmainen et al, 2002	Adipocyte Size	-97	-134	-60
	Bariatry	WOMEN	Kolehmainen et al, 2002	Adipocyte Size	-89	-134	-44
	Bariatry	MEN-SURGERY	Lofgren et al, 2002	Adipocyte Size	-311	-381	-241
	Bariatry	WOMEN-SURGERY	Lofgren et al, 2002	Adipocyte Size	-394	-475	-313
	Bariatry	OVERALL	Lofgren et al, 2005	Adipocyte Size	-307	-364	-250
	Bariatry	Gastric bypass (Epigastric: UPPER-BODY)	N_slund et al, 1988	Adipocyte Size	-448	-558	-338
	Bariatry	Gastric bypass (Femoral: LOWER-BODY)	N_slund et al, 1988	Adipocyte Size	-361	-479	-242
	Bariatry	Gastric bypass (Gluteal: LOWER-BODY)	N_slund et al, 1988	Adipocyte Size	-383	-488	-277
	Bariatry	Gastric bypass (Hypogastric: UPPER-BODY)	N_slund et al, 1988	Adipocyte Size	-415	-507	-324
	Bariatry	Gastric bypass-OVERALL	N_slund et al, 1988	Adipocyte Size	-404	-452	-356
	Bariatry	Gastroplasty (Epigastric: UPPER-BODY)	N_slund et al, 1988	Adipocyte Size	-306	-382	-230
	Bariatry	Gastroplasty (Femoral: LOWER-BODY)	N_slund et al, 1988	Adipocyte Size	-120	-223	-17
	Bariatry	Gastroplasty (Gluteal: LOWER-BODY)	N_slund et al, 1988	Adipocyte Size	-208	-315	-100
	Bariatry	Gastroplasty (Hypogastric: UPPER-BODY)	N_slund et al, 1988	Adipocyte Size	-186	-280	-92
	Bariatry	Gastroplasty-OVERALL	N_slund et al, 1988	Adipocyte Size	-230	-286	-174
	Bariatry	OVERALL	Poitou et al, 2006	Adipocyte Size	-220	-238	-202
	Bariatry	OVERALL	Spalding et al, 2008	Adipocyte Size	-273	-330	-216
Fixed	Bariatry				-261	-272	-250
Random	Bariatry				-292	-350	-233
	Ex	Exercise-LOWER-BODY	Schwartz et al, 1987	Adipocyte Size	-47	-142	48
	Ex	Exercise-UPPER-BODY	Schwartz et al, 1987	Adipocyte Size	73	-37	183
Fixed	Ex				4	-67	76
Random	Ex				10	-108	127
	LCD	Low-fat diet	Dahlman et ak, 2005	Adipocyte Size	-136	-197	-75
	LCD	Moderate-fat diet	Dahlman et ak, 2005	Adipocyte Size	-134	-182	-86
	LCD	MEN-LOWER-BODY	Imbeault et al, 2001	Adipocyte Size	-87	-131	-43
	LCD	MEN-UPPER-BODY	Imbeault et al, 2001	Adipocyte Size	-88	-140	-36
	LCD	WOMEN-LOWER-BODY	Imbeault et al, 2001	Adipocyte Size	-186	-253	-119
	LCD	WOMEN-UPPER-BODY	Imbeault et al, 2001	Adipocyte Size	-109	-161	-57
	LCD	Insulin resistant	Jocken et al, 2007	Adipocyte Size	-125	-171	-79
	LCD	Insulin sensitive	Jocken et al, 2007	Adipocyte Size	-119	-178	-60
	LCD	Calore restriction	Larson-Meyer et al, 2006	Adipocyte Size	-140	-218	-62
	LCD	Low calorie diet	Larson-Meyer et al, 2006	Adipocyte Size	-160	-252	-68
	LCD	OVERALL	Purnell et al, 2007	Adipocyte Size	-252	-304	-200
	LCD	Low calorie conventional diet	Rizkalla et al, 2012	Adipocyte Size	-156	-238	-73
	LCD	Low calorie, high protein, low GI diet	Rizkalla et al, 2012	Adipocyte Size	-273	-397	-149
	LCD	Diet-LOWER-BODY	Schwartz et al, 1987	Adipocyte Size	-111	-212	-11
	LCD	Diet-UPPER-BODY	Schwartz et al, 1987	Adipocyte Size	-167	-291	-43
	LCD	Diet-LOWER-BODY	You et al, 2006	Adipocyte Size	-164	-283	-45
	LCD	Diet-UPPER-BODY	You et al, 2006	Adipocyte Size	-33	-131	65
Fixed	LCD				-135	-151	-120
Random	LCD				-139	-166	-113
	LCD+Ex	MEN	Albu et al, 2010	Adipocyte Size	-230	-306	-154
	LCD+Ex	WOMEN	Albu et al, 2010	Adipocyte Size	-200	-260	-140
	LCD+Ex	LOWER-BODY	Berman et al, 2004	Adipocyte Size	-109	-158	-61
	LCD+Ex	UPPER-BODY	Berman et al, 2004	Adipocyte Size	-98	-142	-55
	LCD+Ex	Calorie restriction and exercise	Larson-Meyer et al, 2006	Adipocyte Size	-180	-269	-91
	LCD+Ex	LOWER-BODY	Nicklas et al, 2000	Adipocyte Size	-121	-157	-85
	LCD+Ex	UPPER-BODY	Nicklas et al, 2000	Adipocyte Size	-120	-156	-84
	LCD+Ex	OVERALL	Pasarica et al, 2009	Adipocyte Size	-210	-270	-150
	LCD+Ex	LOWER-BODY	Shadid et al, 2003	Adipocyte Size	-336	-668	-4
	LCD+Ex	UPPER-BODY	Shadid et al, 2003	Adipocyte Size	-342	-661	-23
	LCD+Ex	LOWER-BODY	Singh et al, 2012	Adipocyte Size	-142	-205	-79
	LCD+Ex	UPPER-BODY	Singh et al, 2012	Adipocyte Size	-92	-165	-19
	LCD+Ex	Diet and high-intensity exercise-LOWER-BODY	You et al, 2006	Adipocyte Size	-196	-278	-114
	LCD+Ex	Diet and high-intensity exercise-UPPER-BODY	You et al, 2006	Adipocyte Size	-175	-197	-153
	LCD+Ex	Diet and low-intensity exercise-LOWER-BODY	You et al, 2006	Adipocyte Size	-142	-168	-116
	LCD+Ex	Diet and low-intensity exercise-UPPER-BODY	You et al, 2006	Adipocyte Size	-175	-198	-152
Fixed	LCD+Ex				-154	-165	-144
Random	LCD+Ex				-153	-173	-134
	VLCD	Diabetic	Freidenberg et al, 1988	Adipocyte Size	-224	-317	-131
	VLCD	Non-diabetic	Freidenberg et al, 1988	Adipocyte Size	-256	-350	-162
	VLCD	OVERALL	Hallgren et al, 1989	Adipocyte Size	-120	-224	-16
	VLCD	OVERALL	Henry et al, 1986	Adipocyte Size	-376	-513	-239
	VLCD	OVERALL	Katz et al, 1991	Adipocyte Size	-142	-366	82
	VLCD	OVERALL	Krotkiewski et al, 1990	Adipocyte Size	-87	-94	-80
	VLCD	OVERALL	Reynisdottir et al, 1995	Adipocyte Size	-236	-306	-166
	VLCD	OVERALL	Verhoef et al, 2013	Adipocyte Size	-30	-52	-8
	VLCD	OVERALL	Wahrenberg et al, 1999	Adipocyte Size	-168	-261	-75
Fixed	VLCD				-86	-93	-79
Random	VLCD				-166	-217	-115
Fixed	Overall				-137	-142	-132
Random	Overall				-156	-171	-142

Adipocyte Region: Upper-Body and Lower-Body



Model	Group by Adip.Region.Code	Subgroup within study	Study name	Outcome	Difference in means	Lower limit	Upper limit
	Lower-body	LOWER-BODY	Berman et al, 2004	Adipocyte Size	-109	-158	-61
	Lower-body	MEN-LOWER-BODY	Imbeault et al, 2001	Adipocyte Size	-87	-131	-43
	Lower-body	WOMEN-LOWER-BODY	Imbeault et al, 2001	Adipocyte Size	-186	-253	-119
	Lower-body	OVERALL	Katz et al, 1991	Adipocyte Size	-142	-366	82
	Lower-body	OVERALL	Krotkiewski et al, 1990	Adipocyte Size	-87	-94	-80
	Lower-body	Gastric bypass (Femoral: LOWER-BODY)	N_slund et al, 1988	Adipocyte Size	-361	-479	-242
	Lower-body	Gastric bypass (Gluteal: LOWER-BODY)	N_slund et al, 1988	Adipocyte Size	-383	-488	-277
	Lower-body	Gastroplasty (Femoral: LOWER-BODY)	N_slund et al, 1988	Adipocyte Size	-120	-223	-17
	Lower-body	Gastroplasty (Gluteal: LOWER-BODY)	N_slund et al, 1988	Adipocyte Size	-208	-315	-100
	Lower-body	Gastroplasty-OVERALL	N_slund et al, 1988	Adipocyte Size	-230	-286	-174
	Lower-body	LOWER-BODY	Nicklas et al, 2000	Adipocyte Size	-121	-157	-85
	Lower-body	OVERALL	Purnell et al, 2007	Adipocyte Size	-252	-304	-200
	Lower-body	Diet-LOWER-BODY	Schwartz et al, 1987	Adipocyte Size	-111	-212	-11
	Lower-body	Exercise-LOWER-BODY	Schwartz et al, 1987	Adipocyte Size	-47	-142	48
	Lower-body	LOWER-BODY	Shadid et al, 2003	Adipocyte Size	-336	-668	-4
	Lower-body	LOWER-BODY	Singh et al, 2012	Adipocyte Size	-142	-205	-79
	Lower-body	Diet and high-intensity exercise-LOWER-BODY	You et al, 2006	Adipocyte Size	-196	-278	-114
	Lower-body	Diet and low-intensity exercise-LOWER-BODY	You et al, 2006	Adipocyte Size	-142	-168	-116
	Lower-body	Diet-LOWER-BODY	You et al, 2006	Adipocyte Size	-164	-283	-45
Fixed	Lower-body				-101	-108	-95
Random	Lower-body				-166	-200	-133
	Upper-body	MEN	Albu et al, 2010	Adipocyte Size	-230	-306	-154
	Upper-body	WOMEN	Albu et al, 2010	Adipocyte Size	-200	-260	-140
	Upper-body	OVERALL	Andersson et al, 2014	Adipocyte Size	-522	-559	-485
	Upper-body	UPPER-BODY	Berman et al, 2004	Adipocyte Size	-98	-142	-55
	Upper-body	OVERALL	Cancello et al, 2005	Adipocyte Size	-201	-283	-118
	Upper-body	Low-fat diet	Dahlman et ak, 2005	Adipocyte Size	-136	-197	-75
	Upper-body	Moderate-fat diet	Dahlman et ak, 2005	Adipocyte Size	-134	-182	-86
	Upper-body	OVERALL	Fisheret al, 2002	Adipocyte Size	-357	-448	-266
	Upper-body	Diabetic	Freidenberg et al, 1988	Adipocyte Size	-224	-317	-131
	Upper-body	Non-diabetic	Freidenberg et al, 1988	Adipocyte Size	-256	-350	-162
	Upper-body	OVERALL	Hallgren et al, 1989	Adipocyte Size	-120	-224	-16
	Upper-body	OVERALL	Henry et al, 1986	Adipocyte Size	-376	-513	-239
	Upper-body	MEN-UPPER-BODY	Imbeault et al, 2001	Adipocyte Size	-88	-140	-36
	Upper-body	WOMEN-UPPER-BODY	Imbeault et al, 2001	Adipocyte Size	-109	-161	-57
	Upper-body	Insulin resistant	Jocken et al, 2007	Adipocyte Size	-125	-171	-79
	Upper-body	Insulin sensitive	Jocken et al, 2007	Adipocyte Size	-119	-178	-60
	Upper-body	OVERALL	Kim et al, 2011	Adipocyte Size	-324	-478	-170
	Upper-body	MEN	Kolehmainen et al, 2002	Adipocyte Size	-97	-134	-60
	Upper-body	WOMEN	Kolehmainen et al, 2002	Adipocyte Size	-89	-134	-44
	Upper-body	Calore restriction	Larson-Meyer et al, 2006	Adipocyte Size	-140	-218	-62
	Upper-body	Calorie restriction and exercise	Larson-Meyer et al, 2006	Adipocyte Size	-180	-269	-91
	Upper-body	Low calorie diet	Larson-Meyer et al, 2006	Adipocyte Size	-160	-252	-68
	Upper-body	MEN-SURGERY	Lofgren et al, 2002	Adipocyte Size	-311	-381	-241
	Upper-body	WOMEN-SURGERY	Lofgren et al, 2002	Adipocyte Size	-394	-475	-313
	Upper-body	OVERALL	Lofgren et al, 2005	Adipocyte Size	-307	-364	-250
	Upper-body	Gastric bypass (Epigastric: UPPER-BODY)	N_slund et al, 1988	Adipocyte Size	-448	-558	-338
	Upper-body	Gastric bypass (Hypogastric: UPPER-BODY)	N_slund et al, 1988	Adipocyte Size	-415	-507	-324
	Upper-body	Gastric bypass-OVERALL	N_slund et al, 1988	Adipocyte Size	-404	-452	-356
	Upper-body	Gastroplasty (Epigastric: UPPER-BODY)	N_slund et al, 1988	Adipocyte Size	-306	-382	-230
	Upper-body	Gastroplasty (Hypogastric: UPPER-BODY)	N_slund et al, 1988	Adipocyte Size	-186	-280	-92
	Upper-body	UPPER-BODY	Nicklas et al, 2000	Adipocyte Size	-120	-156	-84
	Upper-body	OVERALL	Pasarica et al, 2009	Adipocyte Size	-210	-270	-150
	Upper-body	OVERALL	Poitou et al, 2006	Adipocyte Size	-220	-238	-202
	Upper-body	OVERALL	Reynisdottir et al, 1995	Adipocyte Size	-236	-306	-166
	Upper-body	Low calorie conventional diet	Rizkalla et al, 2012	Adipocyte Size	-156	-238	-73
	Upper-body	Low calorie, high protein, low GI diet	Rizkalla et al, 2012	Adipocyte Size	-273	-397	-149
	Upper-body	Diet-UPPER-BODY	Schwartz et al, 1987	Adipocyte Size	-167	-291	-43
	Upper-body	Exercise-UPPER-BODY	Schwartz et al, 1987	Adipocyte Size	73	-37	183
	Upper-body	UPPER-BODY	Shadid et al, 2003	Adipocyte Size	-342	-661	-23
	Upper-body	UPPER-BODY	Singh et al, 2012	Adipocyte Size	-92	-165	-19
	Upper-body	OVERALL	Spalding et al, 2008	Adipocyte Size	-273	-330	-216
	Upper-body	OVERALL	Verhoef et al, 2013	Adipocyte Size	-30	-52	-8
	Upper-body	OVERALL	Wahrenberg et al, 1999	Adipocyte Size	-168	-261	-75
	Upper-body	Diet and high-intensity exercise-UPPER-BODY	You et al, 2006	Adipocyte Size	-175	-197	-153
	Upper-body	Diet and low-intensity exercise-UPPER-BODY	You et al, 2006	Adipocyte Size	-175	-198	-152
	Upper-body	Diet-UPPER-BODY	You et al, 2006	Adipocyte Size	-33	-131	65
Fixed	Upper-body				-182	-189	-175
Random	Upper-body				-206	-240	-171
Fixed	Overall				-137	-142	-132
Random	Overall				-185	-209	-162

Appendix F. References of Excluded Studies

Reason 1: Adipocyte size data reported separately for men and women but weight data reported overall average (n = 1):

8. Lappalainen T, Kolehmainen M, Schwab U, Pulkkinen L, Laaksonen DE, Rauramaa R, et al. Serum concentrations and expressions of serum amyloid A and leptin in adipose tissue are interrelated: the Genobin Study. Eur J Endocrinol. 2008;158(3):333-41.

Reason 2: Did not measure adipocyte size as a mass, volume or diameter (n = 2):

9. Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest. 1995;95(5):2111-9.

10. Rasmussen M, Belza A, Almdal T, Toubro S, Bratholm P, Astrup A, et al. Change in beta1-adrenergic receptor protein concentration in adipose tissue correlates with diet-induced weight loss. Clin Sci (Lond). 2005;108(4):323-9.

Appendix G. PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	4-5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	App. A
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6-7

Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Fig. 1/App. F
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	App. C/App. D
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	App. B
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Fig. 2/App. E
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	17
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	N/A
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	17
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	11-12
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	15

Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	15-16
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	18

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Original Article
OBESITY BIOLOGY AND INTEGRATED PHYSIOLOGY

Obesity

Intra-Abdominal Adipose Tissue Quantification by Alternative Versus Reference Methods: A Systematic Review and Meta-Analysis

Jessica Murphy [1,2,3], *Simon L. Bacon* [1,3,4], *José A. Morais*[1,5,6], *Michael A. Tsoukas*[6], *and Sylvia Santosa* [1,2,3]

Objective: This meta-analysis aimed to assess the agreement between intra-abdominal adipose tissue (IAAT) quantified by alternative methods and the reference standards, computed tomography (CT) and magnetic resonance imaging (MRI).

Methods: MEDLINE and EMBASE electronic databases were systematically searched to identify studies that quantified IAAT thickness, area, or volume by a comparator method and CT or MRI. Using an inverse variance weighted approach (random-effects model), the mean differences and 95% limits of agreement (LoA) were pooled between methods.

Results: The meta-analysis included 24 studies using four comparator methods. The pooled mean differences were −0.3 cm (95% LoA: −3.4 to 3.2 cm; $P = 0.400$) for ultrasound and −11.6 cm^2 (95% LoA: −43.1 to 19.9 cm^2; $P = 0.004$) for bioelectrical impedance analysis. Dual-energy x-ray absorptiometry (DXA) quantified both IAAT area and volume with mean differences of 8.1 cm^2 (95% LoA: −98.9 to 115.1 cm^2; $P = 0.061$) and 10 cm^3 (95% LoA: −280 to 300 cm^3; $P = 0.808$), respectively.

Conclusions: Ultrasound and DXA measure IAAT with minimal bias from CT or MRI, while bioelectrical impedance analysis systematically underestimates IAAT. However, with the exception of DXA for IAAT volume, the wide LoA caution against clinical or research use of the comparator methods and emphasize the need to optimize alternatives to the reference standards.

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Introduction

The excess accumulation of visceral or intra-abdominal adipose tissue (IAAT) is a well-established risk factor for cardiometabolic disease (1). However, BMI, the crude population measure of obesity (2), fails to characterize IAAT. A recent review elegantly depicted how, throughout the BMI continuum, both body fat distribution and cardiometabolic disease risk vary tremendously (3). While anthropometric measures such as waist circumference are easily employed as surrogates for visceral adiposity, they do not differentiate between subcutaneous and visceral depots. Experts have therefore urged the implementation of visceral fat measurement in clinical practice to ensure successful monitoring of disease risk and treatment responses in obesity (3).

The reference methods for IAAT quantification are computed tomography (CT) and magnetic resonance imaging (MRI). However, the radiation exposure associated with CT and the inaccessibility, time, and cost inherent to MRI have led to the development of alternative methods. A 2012 narrative review (4) discussed the clinical utility of many of these techniques, including ultrasound and bioelectrical impedance analysis (BIA). Dual-energy x-ray absorptiometry (DXA) is limited by its inability to distinguish the different abdominal fat compartments, but software has evolved to estimate IAAT (5).

When IAAT measurement is indicated, the choice of method often involves weighing cost, safety, and feasibility without compromising accuracy. With the view of determining which alternative methods may

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Additional Supporting Information may be found in the online version of this article.

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have a place in both research and clinical settings, the aim of this systematic review and meta-analysis was to assess their agreement with CT or MRI to quantify IAAT. Our secondary objective was to determine whether reference method (CT vs. MRI), reference IAAT location (i.e., L2-L3 vs. L4-L5), and participant characteristics (namely, age, sex, ethnicity, adiposity, and disease status) influence the agreement between methods.

Methods

Eligibility criteria

Literature on original research published in English was eligible for this review. We considered all studies that quantified IAAT in humans by CT or MRI (reference method) and one of the following comparator methods: DXA, ultrasound, air displacement plethysmography, or BIA. Both the reference and comparator method had to quantify IAAT at the same anatomical location as a thickness, area, volume, or mass in the same unit of measurement. Prediction equations and combination techniques that included a measure of adipose tissue or fat from at least one of the above comparator methods were also eligible. Prediction equations based solely on anthropometric and demographic factors, however, were excluded.

The studies were required to report the mean differences and SDs of the differences in IAAT quantity between the two methods or enough information from which they could be computed.

Search strategy

We searched MEDLINE and EMBASE electronic databases via Ovid up to September 2018 using Medical Subject Headings and Emtree terms, respectively. The search was limited to humans and to publications in English. The complete search strategies are shown in the online Supporting Information. We also hand searched reviews on the topic and the reference lists of included studies. References were managed and screened in EndNote X7.4 (Thomson Reuters, Eagan, Minnesota).

Study selection

The studies were selected following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (6). Supporting Information Table S1 shows the PRISMA checklist. Two authors (JM and SS) independently examined the titles and abstracts of the references identified through the search strategy and then reviewed the full-text articles of studies that were potentially eligible. Consensus on the included studies was reached through discussion. When more than one article was published on the same data, the most comprehensive one was selected. The same procedure was conducted to identify additional studies from our hand search.

Data extraction and quality assessment

For all included studies, we extracted information about study design, setting, participant characteristics, and IAAT assessment methods. We retrieved the necessary data and statistics to record or compute the mean differences (comparator − reference) in IAAT quantity and 95% limits of agreement (LoA) (mean difference ± 1.96 × SD of the difference). IAAT thickness, area, and volume measurements were recorded in centimeters, centimeters squared, and centimeters cubed, respectively. Appropriate conversions were made if necessary (i.e., liters to centimeters cubed). IAAT mass was converted to volume assuming an adipose tissue density of 0.94 g/cm^3 if a conversion factor was not indicated in the article (7). We also extracted information on subgroup comparisons and Bland-Altman analyses (8), including author comments and conclusions. Study quality was assessed using select items from the Guidelines for Reporting Reliability and Agreement Studies (9).

Data synthesis and analysis

We considered all studies for quantitative synthesis except for those that used prediction equations as the comparator method. Studies reporting on a comparator method that was represented in at least three studies were included in the meta-analysis.

We used a random-effects model (inverse variance weighted approach) to pool the mean differences (bias) (10) and 95% LoA (prediction interval) (11) between IAAT measurements by comparator and reference methods. Separate analyses were conducted for each technique that quantified IAAT thickness, area, or volume. If the technique was compared with multiple reference locations (i.e., L2-L3 and L4-L5), the one most highly represented in the other studies was used in the pooled analysis. When studies reported results for subgroups and the total sample, those of the total sample were used in the pooled analysis. When only subgroup results were reported, they were combined to keep the study as the unit of analysis. Heterogeneity of estimates was assessed using the I^2 statistic (12). Analyses were conducted using Comprehensive Meta-Analysis software (version 3.3.070; Biostat, Englewood, New Jersey), and statistical significance was set at $P \leq 0.05$. Forest plots were customized in Microsoft Excel (2011; Microsoft Corp., Redmond, Washington) to display 95% LoA rather than 95% CI (13). We identified outliers using the methods outlined in Viechtbauer and Cheung (14). Analyses were rerun omitting these studies.

Based on our secondary objective, we planned to conduct subgroup analyses for reference method (CT vs. MRI), reference IAAT location (i.e., L2-L3 vs. L4-L5), age, sex, ethnicity, adiposity, and disease status if sufficient data were available for a given comparator method.

Results

Study selection

Figure 1 shows the PRISMA flow diagram of study selection. The search identified 1,477 unique records, of which 1,272 were excluded because they had nothing to do with method comparison based on the screen of titles and abstracts. The full texts of the remaining 205 were assessed for eligibility, and 37 met all criteria for inclusion in the systematic review. Eleven of these studies evaluated the agreement between prediction equations and CT or MRI and, therefore, were excluded from quantitative synthesis. Two additional studies were not suitable because their comparator methods were not used in any other study. Accordingly, 24 studies using four comparator methods were selected for meta-analysis.

Study characteristics

Supporting Information Table S2 shows the characteristics and method comparison results (reported or computed) for each of the included studies. Our quality assessment (Supporting Information Table S3) showed that explanation of sample size selection, description of sampling method, description of measurement process, and discussion on practical relevance of results were the least met criteria on the Guidelines for Reporting Reliability and Agreement Studies (9).

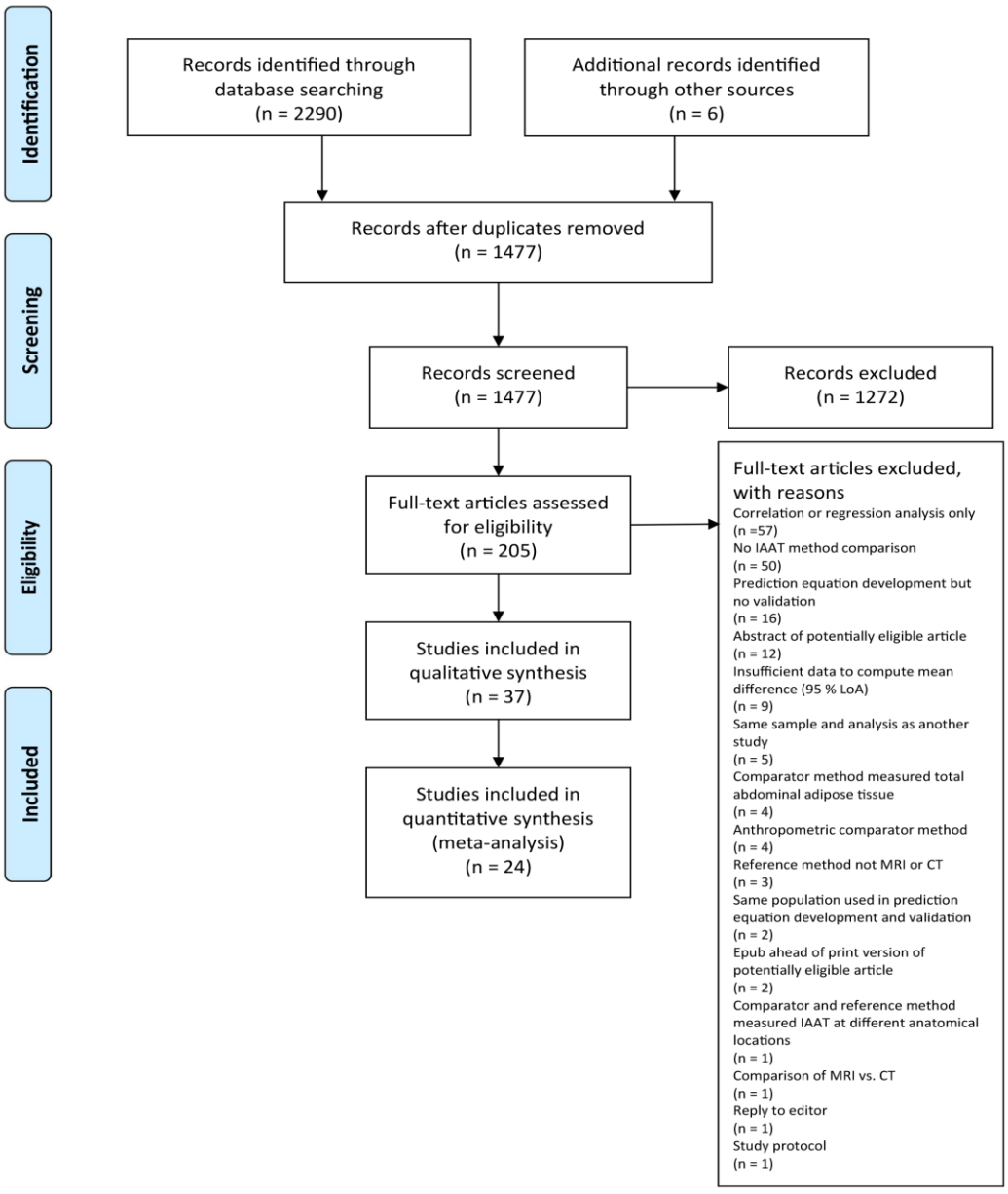

Figure 1 PRISMA flow diagram. [Colour figure can be viewed at wileyonlinelibrary.com]

Four studies (15-18) with a total of 186 participants compared agreement between ultrasound and MRI or CT to quantify IAAT thickness. Of the studies that estimated IAAT area, comparator methods included DXA (19-21) and several BIA techniques (22-29). The BIA estimation of IAAT was based on unpublished equations derived from the instrument manufacturers. IAAT volume was estimated

using DXA enCORE software (GE Healthcare, Madison, Wisconsin) or related modeling software in eight studies, totaling 3,505 participants (31-38). The Lunar android region of interest was used in six out of the eight studies. Another study (39) used BIA to quantify IAAT volume, and one additional study (40) used a method combining single-slice CT and DXA. By applying the visceral to subcutaneous fat ratio measured by a L2-L3 CT slice to the DXA total abdominal fat region, IAAT volume was computed. Furthermore, 11 studies compared IAAT estimated from prediction equations with CT or MRI (41-51).

Pooled mean differences and 95% LoA

Figures 2–4 show the forest plots for the mean differences and 95% LoA between comparator methods and CT or MRI for the quantification of IAAT thickness, area, and volume, respectively. The pooled mean IAAT thickness difference between ultrasound and CT or MRI was −0.32 cm (95% LoA: −3.38 to 3.17 cm; $P=0.400$) with high heterogeneity ($Q_{(3)}=41$; $P<0.001$; $I^2=93$).

For the studies that compared methods of measuring IAAT area with one of the reference standards, the overall pooled mean differences were 8.08 cm^2 (95% LoA: −98.88 to 115.07 cm^2; $P=0.061$) for DXA and −11.63 cm^2 (95% LoA: −43.12 to 19.85 cm^2; $P=0.004$) for BIA. These studies also presented with high heterogeneity (DXA: $Q_{(2)}=31$; $P<0.001$; $I^2=98$; BIA: $Q_{(11)}=544$; $P<0.001$; $I^2=94$).

Lastly, among the eight studies comparing IAAT volume measured by DXA versus CT or MRI, the pooled mean difference was −124 cm^3 (95% LoA: −479 to 230 cm^3; $P=0.013$) with high heterogeneity ($Q_{(7)}=773$; $P<0.001$; $I^2=99$). However, we identified the study of Cheung et al. (31) as an outlier. When this study was removed, the heterogeneity remained high ($Q_{(6)}=458$; $P<0.001$; $I^2=99$), and the pooled mean difference was 10 cm^3 (95% LoA: −280 to 300 cm^3; $P=0.808$).

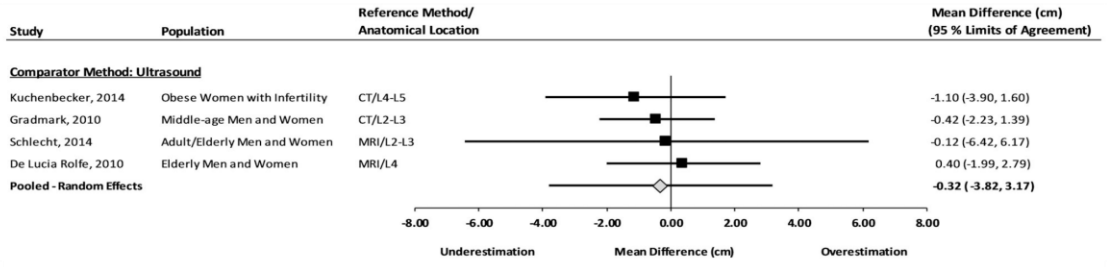

Figure 2 Forest plot: mean differences (centimeters) and 95% limits of agreement between comparator methods and CT or MRI to quantify IAAT thickness.

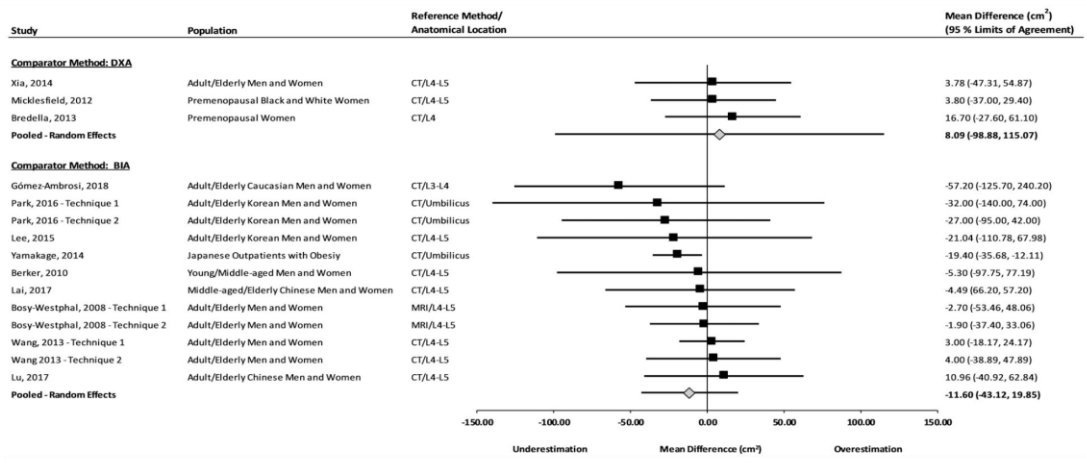

Figure 3 Forest plot: mean differences (centimeters squared) and 95% limits of agreement between comparator methods and CT or MRI to quantify IAAT area.

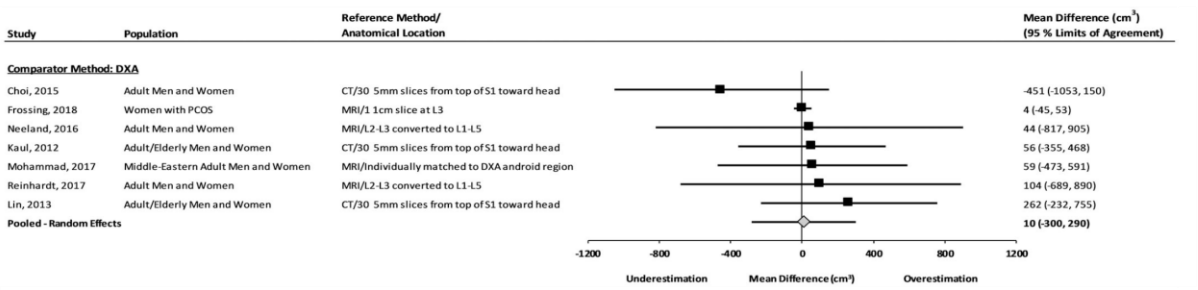

Figure 4 Forest plot: mean differences (centimeters cubed) and 95% limits of agreement between comparator methods and CT or MRI to quantify IAAT volume. Outlier has been removed.

TABLE 1 Subgroup analyses

Comparator method (IAAT assessment)	Subgroup	Number of comparator methods	I^2	Pooled mean difference[a] (95% limits of agreement)	*P* for subgroup comparison
BIA	Sex				
(IAAT area)	Men	6	99	6.92 cm^2 (–58.94 to 72.79)	0.160
	Women	6	85	–9.67 cm^2 (–63.52 to 44.18)	
BIA	BMI				
(IAAT area)	<25 kg/m^2	4	96	–5.23 cm^2 (–57.40 to 46.94)	0.864
	≥ 25 kg/m^2	4	99	–2.95 cm^2 (–78.44 to 72.53)	
DXA	Reference method				
(IAAT volume)	CT	3	100	–41.15 cm^3 (–881.96 to 930.25)	0.311
	MRI	4	90	49.52 cm^3 (–498.42 to 586.23)	
	Sex				
	Men	5	95	144.04 cm^3 (–512.29 to 800.38)	0.042
	Women	6	90	59.96 cm^3 (–381.08 to 492.99)	

[a]Comparator minus reference method.
BIA, bioelectrical impedance analysis; CT, computed tomography; DXA, dual-energy x-ray absorptiometry; IAAT, intra-abdominal adipose; MRI, magnetic resonance imaging.

Subgroup analyses

Data availability permitted a limited number of subgroup analyses on our factors of interest (Table 1). For BIA assessment of IAAT area, the pooled mean difference from the reference methods was not affected by sex or BMI (<25 vs. ≥ 25 kg/m^2). The bias in DXA-derived IAAT volume was not affected by the reference method (CT vs. MRI) but was significantly higher in men than women. Of note, the I^2 statistic was high in many of the subgroups, suggesting that a large proportion of the variability in the estimate was due to heterogeneity rather than sampling error. Hence, it is possible that the sex effect observed for DXA-derived IAAT volume may have been confounded by other factors. Furthermore, the subgroups of men and women may not have been entirely representative of the total population in the pooled analysis (Figure 4), as the LoA was considerably larger.

Discussion

Quantifying visceral adipose tissue or IAAT can be useful in the evaluation and monitoring of cardiometabolic disease risk. Because reference techniques are costly and not always accessible, we systematically searched the literature for alternative methods that have been compared with CT or MRI. We then pooled the mean differences (bias) and 95% LoA between methods.

Our meta-analysis indicated no statistically significant bias between ultrasound and CT or MRI in the measurement of IAAT thickness. Similarly, IAAT volume was not different between DXA and the reference standards after the removal of an outlier. For IAAT area, we were able to assess the agreement between the reference standards and DXA and BIA. While DXA was similar to the reference standards, BIA systematically underestimated IAAT area. Despite the minimal bias between most comparator methods and the reference standards, the 95% LoA was considerably wide.

In Bland-Altman analysis (8), interpretation of these values requires that clinically meaningful limits of agreement be defined a priori (52,53). Our quality assessment showed that no study defined these limits and only a few discussed the clinical relevance of their findings. However, they centered their explanation on the overall bias rather than

the LoA or visual inspection of the Bland-Altman plot. Furthermore, conclusions regarding "poor," "good," or "very good" agreement were arbitrary among studies.

To guide our own interpretation, we consulted the generally acceptable margins of error for the reference methods. The coefficients of variation (CV) of IAAT quantification are ~9% to 18% for MRI (54-56) and ~1% to 2% for CT (57,58). We would therefore expect the error associated with the comparator methods to exceed these margins. When we average reference quantities of IAAT across studies and apply the possible error in measurement, we can have a better indication of what the pooled LoA represent.

Given an average reference IAAT thickness across studies of ~7 cm, our pooled 95% LoA suggest that this value can range from 3.2 to 10.2 cm when measured by ultrasound. This represents a possible 52% underestimation and 43% overestimation, which many would deem clinically unacceptable. Moreover, researchers have failed to recommend ultrasound for IAAT assessment on the basis of its CV of 64% (59).

The pooled 95% LoA for BIA can underestimate the average ~100 cm^2 reference IAAT area by 43% and overestimate it by 20%. Regardless of its degree of error, BIA's suitability for IAAT quantification is an important topic for discussion. BIA predicts body fat quantity from impedance as well as other factors such as age, sex, height, and weight. Of the BIA techniques included in this meta-analysis, only the ViScan device (Tanita AB-140; Tanita Corp., Tokyo, Japan) (30) attempted to measure impedance across the abdomen. The other techniques used hand-to-foot or foot-to-foot electrode configurations, quantifying impedance across the total or lower body, respectively. This questions their superiority to quantify IAAT over prediction equations derived from the combination of BIA-estimated total or trunk fat mass and anthropometric indices. Moreover, the demographic and anthropometric factors included in BIA equations can often have greater predictive power than impedance itself. Unfortunately, details about the manufacturers' equations used to predict IAAT were seldom reported. The variable BIA electrode configurations (bipolar vs. tetrapolar vs. abdominal) and frequencies (single vs. multiple) employed across studies also challenge the appropriateness of pooling BIA data. However, the unclear descriptions of BIA techniques across studies made it impossible to create a narrower classification for analysis.

The percentage error was most problematic for the DXA assessment of IAAT area but least concerning for DXA-estimated IAAT volume. Applying the LoA to the average reference IAAT area of ~100 cm^2 shows that DXA can differ from CT or MRI by more than 100%. Notably, because there were only three studies (degrees of freedom = 1) included in this comparison, the pooled 95% LoA were much wider than 2 SDs from the mean. More studies are needed to confirm the result. For IAAT volume, DXA has the potential to over- and underestimate the pooled 1,145 cm^3 reference value by 25%. This margin of error is not far from the CV of 18% reported for MRI in a similar population with varying degrees of adiposity (54).

Indeed, the percentage errors resulting from the pooled LoA depend on the population being studied. A population with a lower mean IAAT quantity would have a greater percentage error. Assuming an adipose tissue density of 0.94 g/cm^3, IAAT volume can be converted to mass for a more practical interpretation. Total IAAT mass ranges from ~1 to 3.5 kg (1,064-3,723 cm^3) in healthy individuals, depending on age and sex (60), and can reach as high as 10 kg in those living with obesity (61). When accounting for DXA's LoA (−0.28 to 0.27 kg), 1 kg of IAAT can range from 0.72 to 1.27 kg, a 27% over- and underestimation. At the other extreme, DXA could over- and underestimate 10 kg of IAAT by only 3%. However, DXA typically estimates IAAT volume or mass at the android region of interest rather than the entire abdomen, so it is common to have estimates approaching 0 kg in lean individuals. Moreover, a recent study calculated a CV of 42% for DXA-estimated IAAT in weight-stable individuals (BMI = 19-32 kg/m^2) over 3 months and 16% for duplicate scans on the same day, both much higher than any other body compartment (62). Hence, caution must be executed when measuring IAAT at low quantities because of DXA's poor precision and reliability. IAAT quantification, nonetheless, is mainly indicated in individuals with obesity in whom the margins of error may not be as clinically significant.

While IAAT quantities do not typically guide critical clinical decisions as in measurements like blood values, specific IAAT area cut points have been established to determine metabolic risk and define visceral obesity based on single-slice CT scans (63-65).

The wide LoA between comparator methods and single-slice CT in our meta-analysis suggest that DXA- and BIA-estimated IAAT area should not be used for these purposes.

Our meta-analysis is not without limitations. We were able to pool no more than 12 results from 9 studies for any given comparator method. Moreover, there was extensive heterogeneity among the studies. Most were conducted across wide age and BMI ranges in generally healthy populations. While this potentially broadens the applicability of findings, it makes it difficult to explore the sources of heterogeneity. We were able to conduct few subgroup analyses on a very small number of studies, warranting caution in interpretation. It is noteworthy that children were highly underrepresented in our systematic review, and while elderly populations were studied, they were seldom separated from young and middle-aged adults. Future research is needed to investigate how age, ethnicity, adiposity, and disease status impact the agreement between alternative and reference methods.

Another limitation of our meta-analysis was that we could not assess whether the anatomical landmark of the reference method affected the accuracy of alternative methods that measured IAAT thickness or cross-sectional area. The slice most representative of total IAAT has been highly debated (66-68), which makes the quantification of IAAT volume more appealing. Our results showed that DXA-derived IAAT volume had low bias compared with reference methods, and the 95% LoA were the most clinically acceptable. This software, however, is still evolving to improve the segmentation of visceral from subcutaneous fat.

We acknowledge that our review does not include every study that has attempted to compare the agreement between alternative and reference methods for IAAT quantification. We excluded many studies that solely assessed the linear relationship between methods, many of which deemed a high correlation coefficient indicative of validity. A strong linear relationship may result from consistent under- or over- estimation and, therefore, does not guarantee a lack of bias between measurements. The misconduct of method comparison studies and erroneous conclusions drawn from them, however, is nothing new (69,70).

Our results show that ultrasound and DXA measure IAAT with minimal bias from the reference methods, while BIA systematically

underestimates IAAT. DXA-estimated IAAT volume had the lowest degree of error and may therefore be an appropriate method to quantify IAAT in individuals with obesity. The wide LoA for the other techniques caution their use at the level of the individual and to measure changes over time. Our review also highlights the need to define acceptable LoA for IAAT quantification in both research and clinical settings. Future directions should focus on optimizing alternatives to the reference methods in order to better manage the health risks associated with excess adipose tissue accumulation in this depot.O

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Supporting Information

Intra-abdominal Adipose Tissue Quantification by Alternative versus Reference Methods: A Systematic Review and Meta-Analysis

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Search Strategies

MEDLINE

1 exp Intra-Abdominal Fat/
2 ((intra-abdominal or intraabdominal or intra abdominal or visceral) adj2 (fat or adipose tissue)).tw.
3 1 or 2
4 Magnetic Resonance Imaging/
5 ((magnetic resonance or MR or NMR) adj2 (imag* or tomograph* or scan*)).tw.
6 (MRI or MRIs or NMRI).tw.
7 4 or 5 or 6
8 Tomography, X-Ray Computed/
9 (comput* adj3 tomograph*).tw.
10 (CT or CAT).tw.
11 8 or 9 or 10
12 Ultrasonography/
13 ultraso*.tw.
14 Absorptiometry, Photon/
15 (DXA or DEXA or absorptiometry).tw.
16 Electric Impedance/
17 (impedance or bioimpedence).tw.
18 Plethysmography/
19 (Plethysmography or BodPod).tw.
20 7 or 11
21 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19
22 3 and 20 and 21
23 limit 22 to (english language and humans)
24 remove duplicates from 23

EMBASE

1 exp intraabdominal fat/
2 ((intra-abdominal or intraabdominal or intra abdominal or visceral) adj2 (fat or adipose tissue)).tw.
3 1 or 2
4 nuclear magnetic resonance imaging/
5 ((magnetic resonance or MR or NMR) adj2 (imag* or tomograph* or scan*)).tw.
6 (MRI or MRIs or NMRI).tw.
7 4 or 5 or 6
8 computer assisted tomography/
9 (comput* adj3 tomograph*).tw.
10 (CT or CAT).tw.
11 8 or 9 or 10
12 echography/
13 ultraso*.tw.
14 dual energy X ray absorptiometry/
15 (DXA or DEXA or absorptiometry).tw.
16 impedance/
17 (impedance or bioimpedence).tw.
18 Plethysmography/
19 (Plethysmography or BodPod).tw.
20 7 or 11
21 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19
22 3 and 20 and 21
23 limit 22 to (english language and humans)
24 remove duplicates from 23

Table S1. PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	1
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	1-2
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	2
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	2
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Suppl. Info.
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	3-4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	2
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	2
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	2
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	2

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	2
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	N/A
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	2
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	2/Fig. 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Suppl. Info.
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Suppl. Info.
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Fig. 2-4
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	4/Fig. 2-4
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	N/A
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	5/Table 1
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	5-6
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	6
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	6-7
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	N/A

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Table S2. Study Characteristics and Main Findings

Study	Group	n	Age (years): Mean (Range)	BMI (kg/m²): Mean (Range)	Reference Method/ Anatomical Location		Comparator Method Quantification Details	Unit	Reported or Computed Mean IAAT Difference* (95 % Limits of Agreement)
Studies Comparing IAAT Thickness (cm)									
Comparator Method: Ultrasound									
De Lucia Rolfe, 2010	Elderly Men and Women	74	71 (67, 76)	27.1 (20.7, 40.4)	MRI	L4	L4: Distance from peritoneal boundary to corpus of lumbar vertebra	cm	0.4 (-2.0, 2.8)
	Elderly Men	41	71 (NR)	27.4 (NR)					0.5 (-2.2, 2.9)
	Elderly Women	33	71 (NR)	26.7 (NR)					0.3 (-1.8, 2.5)
Gradmark, 2010	Middle-age Men and Women	29	49.1 (41.9, 62.3)	26.5 (21.3, 32.0)	CT	L2-L3	L2-L3: Distance from inside bowel wall to spine	cm	-0.4 (-2.2, 1.4)
Kuchenbecker, 2014	Women with Obesity and Infertility	53	29.4 (NR)	37 (NR)	CT	L4-L5	Smallest waist: Distance from anterior boundary of the lumbar vertebrae to peritoneal boundary of anterior abdominal wall	cm	-1.1 (-3.9, 1.6)
Schlecht, 2014	Adult/Elderly Men and Women	30	43.5 (20, 70)	24.8 (19.48, 36.02)	MRI	L2-L3	Cut-point between left and right midpoint of lower rib and iliac crest on median line of abdomen: distance from linear alba to lumbar vertebra corpus	cm	-0.1 (-6.4, 6.2)
Studies Comparing IAAT Area (cm²)									
Comparator Method: DXA									
Bredella, 2013	Premenopausal Women	135	32.4 (19, 45)	29.4 (15.6, 47.5)	CT	L4	5 cm region above iliac crest (~ L4); using modelling of Kelly, 2010	cm²	16.7 (-27.6, 61.1)
	Premenopausal Women with Anorexia Nervosa	19	24.8 (19, 33)	17.9 (15.6, 20.3)					6.9 (-10.2, 24.1)
	Normal Weight Premenopausal Women	27	30.1 (19, 45)	22.0 (18.1, 24.8)					11.7 (-14.6, 37.9)
	Premenopausal Women with Overweight/Obesity	89	34.8 (19, 45)	34.0 (25.1, 47.5)					20.4 (-30.6, 77.5)
Micklesfield, 2012	Premenopausal Black and White Women	131	28.1 (NR)	28.7 (NR)	CT	L4-L5	5 cm region above iliac crest (~ L4); using modelling of Kelly, 2010	cm²	3.8 (-37.0, 29.4)
Xia, 2014	Adult/Elderly Men and Women	115	NR (21, 85)	NR (20.0, 39.7)	CT	L2-L3	Lunar Android ROI converted to single-slice using a regression equation; enCORE software	cm²	5.0 (-44.6, 54.6)
						L4-L5			3.8 (-47.3, 54.9)
Comparator Method: BIA									
Berker, 2010	Young/middle-aged Men and Women	104	37.3 (19, 58)	31.2 (18.3, 52.8)	CT	L4-L5	X-Scan Body Composition Analyzer manufacturer's equation based on age and sex outputs visceral fat area (electrodes on metal footpads and handles)	cm²	-5.3 (-97.8, 77.2)
	Young/middle-aged Men	21	36.4 (23, 50)	28.2 (18.3, 44.0)					13.5 (81.0, 107.9)
	Young/middle-aged Women	83	37.4 (19, 58)	32.1 (18.5, 52.8)					3.2 (76.1, 82.5)

	Young/middle-aged Men and Women BMI < 25	31	NR (NR)	NR (18.3, 25)					-9.1 (-42.4, 24.2)
	Young/middle-aged Men and Women BMI >=35	34	NR (NR)	NR (35.0, 52.8)					9.7 (-92.8, 112.1)
	Young/middle-aged Men and Women BMI 30.0-34.9	16	NR (NR)	NR (30, 34.9)					-30.9 (-137.9, 76.1)
	Young/middle-aged Men and Women BMI 25-29.9	23	NR (NR)	NR (25.0, 29.9)					-4.4 (-63.9, 55.1)
Bosy-Westphal, 2008	Adult/Elderly Men and Women	106	54.2 (33, 78)	25.8 (16.8, 40.2)	MRI	L4-L5	Tanita BC-532 manufacturers's equation outputs visceral fat (VF_{level}) in $cm^2/10$; Foot-to-foot electrodes;	cm^2	-2.7 (-53.5, 48.1)
							Omron BF-500 manufacturer's equation outputs visceral fat (VF_{level}) in $cm^2/10$; 8 electrode arrangement on metal footpads and handles		-1.9 (-37.4, 33.6)
Gómez-Ambrosi, 2018	Adult/Elderly Caucasian Men and Women	140	55 (18, 77)	29.8 (17.7, 50.4)	CT	L3-L4	Tanita AB-140 ViScan; uses a wireless electrode belt that is placed on the supine subject's midriff. Outputs visceral fat in arbitrary units ranging from 1 – 59 (equal to $cm^2/10$)	cm^2	-57.2 (-125.7, 240.2)
Lai, 2017	Middle-aged/Elderly Chinese Men and Women	100	68.5 (NR)	24.4 (NR)	CT	L4-L5	Tanita BC-305 manufacturer's equation based on height, sex and age outputs visceral fat area at the lumbar level of L4-L5; four-plate standing leg-to-leg BIA	cm^2	-4.5 (-66.2, 57.2)
	Middle-aged/Elderly Chinese Men	48	71.2 (NR)	25.1 (NR)					14.4 (-38.3, 67.1)
	Middle-aged/Elderly Chinese Women	52	66.1 (NR)	23.7 (NR)					-22.0 (-70.4, 31.7)
	Middle-aged/Elderly Chinese Men and Women BMI < 25	58	70.4 (NR)	21.9 (NR)					-11.2 (-67.3, 44.9)
	Middle-aged/Elderly Chinese Men and Women BMI >= 25	42	66.0 (NR)	27.8 (NR)					4.7 (-60.4, 69.8)
	Middle-aged/Elderly Chinese Men BMI < 25	24	77.0 (NR)	22.4 (NR)					5.4 (-45.8, 56.6)
	Middle-aged/Elderly Chinese Men BMI >= 25	24	65.5 (NR)	27.8 (NR)					23.5 (-25.5, 72.5)
	Middle-aged/Elderly Chinese Women BMI < 25	34	65.8 (NR)	21.5 (NR)					-21.8 (-70.0, 26.4)
	Middle-aged/Elderly Chinese Women BMI >= 25	18	66.6 (NR)	27.8 (NR)					-20.4 (-70.4, 29.6)
Lee, 2015	Adult/Elderly Korean Men and Women	1006	55.2 (19, 87)	26 (NR)	CT	Umbilicus	InBody720 manufacturer's equation manufacturer's equation based on age and sex	cm^2	-21.4 (-110.8, 68.0)
	Adult/Elderly Korean Men	492	53.7 (NR)	26.1 (NR)					-38.2 (-128.2, 51.8)

	Adult/Elderly Korean Women	514	56.7 (NR)	25.8 (NR)			outputs visceral fat area (electrodes on metal footpads and handles)		-5.4 (-82.2, 71.4)
	Adult/Elderly Korean Men and Women Age 19-39	95	NR (19, 39)	NR (NR)					-8.5 (-110.8, 93.8)
	Adult/Elderly Korean Men and Women Age 40-49	205	NR (40, 49)	NR (NR)					-21.7 (-100.9, 57.5)
	Adult/Elderly Korean Men and Women Age 50-59	314	NR (50, 59)	NR (NR)					-24.4 (-112.0, 63.2)
	Adult/Elderly Korean Men and Women Age 60-69	291	NR (60, 69)	NR (NR)					-21.2 (-110.0, 67.6)
	Adult/Elderly Korean Men and Women Age >=70	101	NR (70, 87)	NR (NR)					-24.6 (-125.3, 76.1)
	Adult/Elderly Korean Men and Women BMI < 20	23	NR (NR)	NR (17.0, 19.9)					14.1 (-43.1, 71.3)
	Adult/Elderly Korean Men and Women BMI 20-22.9	161	NR (NR)	NR (20.0, 22.9)					-3.2 (-68.5, 62.1)
	Adult/Elderly Korean Men and Women BMI 23-24.9	232	NR (NR)	NR (23.0, 24.9)					-15 (-92.0, 62.0)
	Adult/Elderly Korean Men and Women BMI 25-26.9	248	NR (NR)	NR (25.0, 26.0					-29.7 (-115.0, 55.6)
	Adult/Elderly Korean Men and Women BMI 27-29.9	223	NR (NR)	NR (27.0, 29.9)					-32.8 (-128.4, 62.8)
	Adult/Elderly Korean Men and Women BMI >=30	119	NR (NR)	NR (30.0, 46)					-27.2 (-141.7, 87.3)
	Adult/Elderly Korean Men Age < 50	175	NR (NR, 49)	NR (NR)					-33.5 (-118.6, 51.6)
	Adult/Elderly Korean Men Age >= 50	317	NR(50, NR)	NR (NR)					-40.8 (-132.9, 51.3)
	Adult/Elderly Korean Women Age < 50	125	NR (NR, 49)	NR (NR)					4.9 (-66.4, 76.2)
	Adult/Elderly Korean Women Age >= 50	389	NR (50, NR)	NR (NR)					-8.7 (-86.3, 68.9)
	Adult/Elderly Korean Men BMI < 25	188	NR (NR)	NR (NR, 24.9					-21.2 (-95.3, 52.9)
	Adult/Elderly Korean Men BMI >= 25	304	NR (NR)	NR (25, NR)					-48.8 (-141.5, 43.9)
	Adult/Elderly Korean Women BMI < 25	228	NR (NR)	NR (NR, 24.9)					1.4 (-64.8, 67.8)
	Adult/Elderly Korean Women BMI >= 25	286	NR (NR)	NR (25, NR)					-10.7 (-93.6, 72.2)

Lu, 2017	Adult/Elderly Chinese Men and Women	381	NR (NR)	24.7 (NR)	CT	L4-L5	Tanita BC-305 manufacturer's equation based on height, sex and age outputs visceral fat area at the lumbar level of L4-L5; four-plate standing leg-to-leg BIA	cm^2	NR (-44.0, 68.0)
	Adult/Elderly Chinese Men	240	34.9 (20.3, 81.5)	24.8 (16.3, 41.7)					NR (NR, NR)
	Adult /Elderly Chinese Women	141	34.2 (18.8, 74.8)	24.6 (17.2, 39.1)					NR (NR, NR)
	Adult /Elderly Chinese Men and Women BMI < 25	235	NR (NR)	NR (NR)					8.3 (-39.3, 55.9)
	Adult /Elderly Chinese Men and Women BMI >= 25	146	NR (NR)	NR (NR)					18.2 (-43.7, 80.1)
	Adult /Elderly Chinese Men BMI < 25	148	NR (NR)	NR (NR)					13.3 (-42.6, 69.2)
	Adult /Elderly Chinese Men BMI >= 25	92	NR (NR)	NR (NR)					22.4 (-48.9, 93.7)
	Adult /Elderly Chinese Women BMI < 25	87	NR (NR)	NR (NR)					-10.0 (-37.2, 17.2)
	Adult /Elderly Chinese Women BMI >= 25	54	NR (NR)	NR (NR)					-10.9 (-47.9, 26.1)
Park, 2016	Adult/Elderly Korean Men and Women	102	44.2 (21, 76)	23.9 (18, 35)	CT	Umbilicus	Umbilicus: Dual BIA-IAAT = $\alpha_1 A + \alpha_2 B^2 - \alpha_3(A2+B2)1/2Zs - \alpha_4/Zt + \varepsilon$, where A = abdominal transverse diameter; B = anteroposterior abdominal diameter; Zs = surface impedance, Zt = truncal impedance; and ε = residual constant; and the constants α_i(i = 1-4) were previously determined by regression analysis (Yoneda, 2007)	cm^2	-27.0 (-95.0, 42.0)
							InBody720 manufacturer's equation manufacturer's equation based on age and sex outputs visceral fat area (electrodes on metal footpads and handles)		-32.0 (-140.0, 74.0)
Wang, 2013	Adult/Elderly Men and Women	200	48.0 (18, 80)	NR (NR)	CT	L4-L5	Tanita BC-532 manufacturers's equation outputs visceral fat (VF_{level}) in $cm^2/10$; Foot-to-foot electrodes	cm^2	4.0 (-39.9, 47.9)
							Omron HBF-359 manufacturer's equation outputs visceral fat (VF_{level}) in $cm^2/10$; 2 hand and 2 foot electrodes; measures hand-to-hand and hand-to-foot bioelectrical impedance		3.0 (-18.2, 24.2)
	Adult/Elderly Men	100	48.3 (18, 80)	25.6 (19.1, 34.3)			Tanita BC-532 manufacturers's equation outputs visceral fat (VF_{level}) in $cm^2/10$; Foot-to-foot electrodes		22.0 (-25.3, 69.3)
							Omron HBF-359 manufacturer's equation outputs visceral fat (VF_{level}) in $cm^2/10$; 2 hand and 2 foot electrodes; measures hand-to-hand and hand-to-foot bioelectrical impedance		15.0 (-38.4, 68.4)

	Adult/Elderly Women	100	47.7 (18, 80)	24.2 (16.5, 34.8)			Tanita BC-532 manufacturers's equation outputs visceral fat (VF_{level}) in $cm^2/10$; Foot-to-foot electrodes		-15.0 (-62.6, 32.6)
							Omron HBF-359 manufacturer's equation outputs visceral fat (VF_{level}) in $cm^2/10$; 2 hand and 2 foot electrodes; measures hand-to-hand and hand-to-foot bioelectrical impedance		-7.0 (-55.9, 41.9)
Yamakage, 2014	Japanese Outpatients with Obesity	100	55.5 (NR)	30.2 (NR)	CT	Umbilicus	Umbilicus: Dual BIA-IAAT = $\alpha_1 A + \alpha_2 B^2 - \alpha_3(A2+B2)1/2Zs - \alpha_4/Zt + \varepsilon$, where A = abdominal transverse diameter; B = anteroposterior abdominal diameter; Zs = surface impedance, Zt = truncal impedance; and ε = residual constant; and the constants α_i($_i$ = 1-4) were previously determined by regression analysis (Yoneda, 2007)	cm^2	-19.4 (-35.7, -12.1)
Comparator Method: Prediction Equations using Ultrasound									
Armellini, 1997	Adult/Elderly Women (Inpatients)	98	41 (18, 72)	37 (24, 55)	CT	L4	Model B: VAT = -172 + 13.5 (sagittal diameter - subcutaneous thickness by US)	cm^2	2.0 (-105.9, 109.9)
							Model C: VAT = -213 + 11.7 (sagittal diameter - subcutaneous thickness) + 2 (age)		0.9 (-85.4, 87.2)
							Model D: VAT = -283 + 11.4(sagittal diameter - subcutaneous thickness) + 1.4 (age) - 1.6 (weight) + 2.6 (waist)		0.2 (-61.5, 61.9)
De Lucia Rolfe, 2011	Black South African Adolescent Boys	48	18.9 (18, 19)	21 (NR)	MRI	L4	De Lucia Rolfe: VAT = (3.2 x BMI kg/m^2) + (1.7 x waist cm) + (14.6 x US VAT thickness cm) - 184.9); developed in older white men	cm^2	28.0 (-29.9, 85.9)
	Black South African Adolescent Girls	52	18.8 (18, 19)	23.6 (NR)			De Lucia Rolfe: VAT = (-1.8 x BMI kg/m^2) + (1.8 x waist cm) + (15.9 x US VAT thickness cm) - 96.1); developed in older white women		23.6 (-14.3, 61.5)
Comparator Method: Prediction Equations using DXA									
Ball, 2003	Peri- and post-menopausal obese women	35	47 (35, 67)	32.2 (21.1, 46.3)	CT	L4-L5	Treuth: IAAT = -208.2 + 4.62 (sagittal diameter, cm) + 0.75 (age, y) + 1.73 (waist, cm) + 0.78 (DXA trunk fat, %)	cm^2	58.2 (-44.6, 161.0)
Bertin, 2000	Late Teenage/Adult/Elderly Women	44	42 (16, 70)	36.9 (27.5, 51.6)	CT	L4	Treuth: IAAT = -208.2 + 4.62 (sagittal diameter, cm) + 0.75 (age, y) + 1.73 (waist, cm) + 0.78 (DXA trunk fat, %)	cm^2	31.0 (-83.4, 145.4)

Treuth, 1995	Healthy Late Teenage/Adult/Elderly Women	55	46 (23, 76)	23.8 (18.0, 35.7)	CT	L4-L5	IAAT = -208.2 + 4.62 (sagittal diameter, cm) + 0.75 (age, y) + 1.73 (waist, cm) + 0.78 (DXA trunk fat, %)	cm^2	-3.6 (-64.0, 39.0)
							IAAT = -211.2 + 4.76 (sagittal diameter, cm) + 0.76 (age, y) + 1.73 (waist, cm) + 0.74 (DXA pelvic fat, %)		-3.9 (-64.0, 38.8)
							Svendsen 1: Log (sum SF) + 47.6 (DXA Abdominal fat) - 55.4; developed in postmenopausal women		-41.1(-212.8, 110.7)
							Svendsen2: 423.3 (WHR) + 35.8 (DXA Abdominal fat) - 310.9; developed in postmenopausal women		-4.0 (-75.5, 46.2)
							Svendsen 3: 36.3(Log (sum SF)) + 508.2(WHR) + 23.4 (DXA Abdominal fat) - 503.0; developed in postmenopausal women		-3.3 (-77.4, 47.8)
Hill, 2007	Postmenopausal Women with Overweight/Obesity	41	55.4 (49, 66)	31.3 (25.7, 37.1)	CT	L3-L4	IAAT area = 51.844 + DXA 10 cm ROI (0.031) + Abdominal skinfold (1.342)	cm^2	0.5 (-44.5, 45.5)
Goran, 1998	Pre-pubertal Children:11 boys, 1 girl	12	8.1 (5.6, 10.4)	21.7 (16.0, 35.7)	CT	Umbilicus	IAAT = 10.0 x trunk fat - (3.1 x total fat) + (0.77 x Abdominal skinfold) + 11.2	cm^2	-3.7 (-27.4, 20.0)
Demura, 2007B	Japanese Adult/Elderly Men and Women	60	46.1 (21, 71)	24.5 (17.1, 34.6)	CT	Umbilicus (L4-L5)	VFA = -195.564 - 42.068 x X1 + 0.360 x X2 + 312.032 x X3 + 3.215 x X4, where X1 = sex (male = 1, female = 2); X2 = age; X3 = WHR; X4 = IFM by DXA (kg); IFM = total fat mass (kg) - subcutaneous fat mass (kg); subcutaneous fat mass (kg) = body surface (m^2) x mean skinfold of 14 sites (mm) x density of fat (kg/m^3) x proportion of fat in adipose tissue	cm^2	-2.7 (-52.0, 46.6)
Demura, 2007A	Japanese Adult/Elderly Men and Women	60	46.1(21, 60)	24.5 (NR)	CT	Umbilicus (L4-L5)	VFA = -41.26 - 47.68 x X1 = 0.49 x X2 +1.52x X3 + 3.46 x X4, where X1 = sex (male = 1, female = 2); X2 = age; X3 = WC; X4 = DXA trunk fat mass (kg)	cm^2	0.7 (-38.5, 39.8)
Comparator Method: Prediction Equations using BIA									
Demura, 2007B	Japanese Adult/Elderly Men and Women	20	NR (NR)	NR (NR)	CT	Umbilicus (L4-L5)	VFA = -195.564 - 42.068 x X1 + 0.360 x X2 + 312.032 x X3 + 3.215 x X4, where X1 = sex (male = 1, female = 2); X2 = age; X3 = WHR; X4 = IFM by BIA (kg); IFM = total fat mass (kg) - subcutaneous fat mass (kg); subcutaneous fat mass (kg) = body surface (m^2) x mean skinfold of 14 sites (mm) x density of fat (kg/m^3) x proportion of fat in adipose tissue	cm^2	-2.9 (-55.4, 49.7)

							VFA = -128.427 - 53.2438 x X1 + 0.864 x X2 +235.284 x X3 + 3.547 x X4, where X1 = sex (male = 1, female = 2); X2 = age; X3 = WHR; X4 = IFM by BIA (kg); IFM = total fat mass (kg) - subcutaneous fat mass (kg); subcutaneous fat mass (kg) = body surface (m^2) x mean skinfold of 14 sites (mm) x density of fat (kg/m^3) x proportion of fat in adipose tissue		-0.6 (-109.5, 108.3)
Demura, 2007A	Japanese Adult/Elderly Men and Women	19	NR (NR)	NR (NR)	CT	Umbilicus (L4-L5)	VFA = -41.26 - 47.68 x X1 = 0.49 x X2 +1.52 x X3 + 3.46 x X4, where X1 is the sex (male = 1, female = 2); X2 = age; X3 = WC; X4 = BIA trunk fat mass (kg)	cm^2	5.3 (-30.1, 40.6)
							VFA = 74.94 - 63.93x X1 + 1.01 x X2 + 5.712x X3, where X1 is the sex (male = 1, female = 2); X2 = age; X3 = BIA trunk fat mass (kg)		4.0 (-38.9, 46.8)
Demura 2010	Japanese Adult/Elderly Men and Women Baseline	42	42.7 (NR)	25.2 (NR)	CT	Umbilicus (L4-L5)	VFA = -128.427 - 53.243 x X1 + 0.864 x X2 +235.284 x X3 + 3.547 x X4, where X1 = sex (male = 1, female = 2); X2 = age; X3 = WHR, X4 = IFM by BIA (kg); IFM = total fat mass (kg) - subcutaneous fat mass (kg); subcutaneous fat mass (kg) = body surface (m^2) x mean skinfold of 14 sites (mm) x density of fat (kg/m^3) x proportion of fat in adipose tissue	cm^2	-3.5 (-63.1, 56.1)
							VFA = 74.94 - 63.93 x X1 + 1.01 x X2 + 5.712 x X3, where X1 = sex (male = 1, female = 2); X2 = age; X3 = BIA trunk fat mass (kg)		1.9 (-50.9, 54.7)
	Japanese Adult/Elderly Men Baseline	22	NR (NR)	25.6 (NR)			VFA = -128.427 - 53.243 x X1 + 0.864 x X2 +235.284 x X3 + 3.547 x X4, where X1 = sex (male = 1, female = 2); X2 = age; X3 = WHR; X4 = IFM by BIA (kg); IFM = total fat mass (kg) - subcutaneous fat mass (kg); subcutaneous fat mass (kg) = body surface (m^2) x mean skinfold of 14 sites (mm) x density of fat (kg/m^3) x proportion of fat in adipose tissue		-5.7 (-70.8, 59.4)
							VFA = 74.94 - 63.93 x X1 + 1.01 x X2 + 5.712 x X3, where X1 = sex (male = 1, female = 2); X2 = age; X3 = BIA trunk fat mass (kg)		5.8 (-54.9, 66.5)
	Japanese Adult/Elderly Women Baseline	20	NR (NR)	24.7 (NR)			VFA = -128.427 - 53.243 x X1 + 0.864 x X2 +235.284 x X3 + 3.547 x X4, where X1 = sex (male = 1, female = 2); X2 = age; X3 = WHR; X4 = IFM by BIA (kg)		-1.0 (-35.1, 33.1)

							VFA = 74.94 - 63.93x X1 + 1.01 x X2 + 5.712x X3 , where X1 = sex (male = 1, female = 2); X2 = age; X3 = BIA trunk fat mass (kg)		-2.4 (-39.8, 35.0)
	Japanese Adult/Elderly Men and Women Post-8 weeks of exercise training	42	42.7 (NR)	24.6 (NR)			VFA = -128.427 - 53.243 x X1 + 0.864 x X2 +235.284 x X3 + 3.547 X X4, where X1 = sex (male = 1, female = 2); X2 = age; X3 = WHR, X4 = IFM by BIA (kg); IFM = total fat mass (kg) - subcutaneous fat mass (kg); subcutaneous fat mass (kg) = body surface (m^2) x mean skinfold of 14 sites (mm) x density of fat (kg/m^3) x proportion of fat in adipose tissue		-1.7 (-42.6, 39.2)
							VFA = 74.94 - 63.93x X1 + 1.01 x X2 + 5.712x X3 , where X1 = sex (male = 1, female = 2); X2 = age; X3 = BIA trunk fat mass (kg)		-0.6 (-43.5, 42.3)
	Japanese Adult/Elderly Men Post-8 weeks of exercise training	22	NR (NR)	24.9 (NR)			VFA = -128.427 - 53.2438 x X1 + 0.864 x X2 +235.284 x X3 + 3.547 X X4, where X1 = sex (male = 1, female = 2); X2 = age; X3 = WHR, X4 = IFM by BIA (kg); IFM = total fat mass (kg) - subcutaneous fat mass (kg); subcutaneous fat mass (kg) = body surface (m^2) x mean skinfold of 14 sites (mm) x density of fat (kg/m^3) x proportion of fat in adipose tissue		-0.8 (-42.0, 40.4)
							VFA = 74.94 - 63.93x X1 + 1.01 x X2 + 5.712 x X3 , where X1 = sex (male = 1, female = 2); X2 = age; X3 = BIA trunk fat mass (kg)		-2.7 (-42.6, 37.2)
	Japanese Adult/Elderly Women Post-8 weeks of exercise training	20	NR (NR)	24.7 (NR)			VFA = -128.427 - 53.2438 x X1 + 0.864 x X2 +235.284 x X3 + 3.547 X X4, where X1 = sex (male = 1, female = 2); X2 = age; X3 = WHR, X4 = IFM by BIA (kg); IFM = total fat mass (kg) - subcutaneous fat mass (kg); subcutaneous fat mass (kg) = body surface (m^2) x mean skinfold of 14 sites (mm) x density of fat (kg/m^3) x proportion of fat in adipose tissue		5.9 (-44.1, 55.9)
							VFA = 74.94 - 63.93x X1 + 1.01 x X2 + 5.712x X3 , where X1 = sex (male = 1, female = 2); X2 = age; X3 = BIA trunk fat mass (kg)		-7.7 (-41.4, 26.0)
Studies Comparing IAAT Volume (cm^3)									
Comparator Method: BIA									
Bosy-Westphal, 2017	*Caucasian Adult Men and Women*	32	42.9 (18, 65)	26.0 (18.7, 34.4)	MRI	Total body	mBCA 525 equation developed again MRI from $R_{50\ trunk}$, age, sex, height, and waist circumference; 4 electrodes places on each side of the body while participant is supine	cm^3	-100 (-1178, 978)
	Asian Adult Men and Women	31	38.9 (18, 65)	22.9 (18.7, 34.4)					-700 (-1778, 378)

	Afro-American Adult Men and Women	30	38.7 (18, 65)	25.3 (18.7, 34.4)					100 (-1174, 1374)
	Hispanic Adult Men and Women	30	40.0 (18, 65)	27.5 (18.7, 34.4)					-900 (-3350, 1550)
Comparator Method: DXA									
Cheung, 2016	Older Men	95	61.6 (NR)	32.3 (NR)	MRI	3 4 cm slices above and 1 below L4	Lunar Android ROI; enCORE software	cm^3	-1285.0 (-2678.8, 108.8)
Choi, 2015	Adult Men and Women	123	NR (22, 73)	NR (17.1, 36.0)	CT	30 5mm slices from top of S1 toward head	Lunar Android ROI; enCORE software	cm^3	-451.4 (-1052.6, 149.8)
Kaul, 2012	Adult/Elderly Men and Women	109	NR (18, 90)	NR (18,5, 40)	CT	30 5mm slices from top of S1 toward head	Lunar Android ROI; enCORE software	cm^3	56.0 (-355.0, 468.0)
	Adult/Elderly Men	48	50.8 (NR)	26.7 (NR)					-43.0 (-379.0, 465.0)
	Adult/Elderly Women	61	48.5 (NR)	26.7 (NR)					67.0 (-339.0, 472.0)
Frossing, 2018	Women with PCOS	67	29.2 (NR)	32.8 (NR)	MRI	1-cm slice at L3	APEX software measured a 5-cm transverse section at L4-L5; Volume was divided by a scaling factor (3.76) to make ROIs comparable between DXA and MRI	cm^3	4 (-45, 53)
Lin, 2013	Adult/Elderly Men and Women	145	NR (19, 84)	NR (18.5, 39.3)	CT	30 5mm slices from top of S1 toward head	Lunar Android ROI; enCORE software	cm^3	262.0 (-232.0, 755.0)
	Adult/Elderly Men	73	52 (19.2, 83.7)	26.4 (20.2, 32.3)					379.0 (-128.0, 886.0)
	Adult/Elderly Women	72	50.6 (20.5, 82.6)	27.1 (18.5, 39.3)					143.0 (-210.0, 495.0)
Mohammed, 2017	*Middle-Eastern Adult Men*	130	38.8 (18, 65)	28.4 (NR)	MRI	Achieved on an individual basis as slices matching the same android region as DXA	Lunar Android ROI; enCORE software	cm^3	79.7 (-565.3, 724.7)
	Middle-Eastern Adult Women	107	43.1 (18, 65)	29.6 (NR)					46.8 (-406.4, 498.5)
Neeland, 2016	Male all	1212	44.3 (NR)	28.4 (NR)	MRI	L2-L3 converted to L1-L5	APEX software estimation of L4-L5 converted to L1-L5	cm^3	97.9 (-839.9, 1035,7)
	Female all	1477	44.5 (NR)	30.8 (NR)					10.9 (-799.0, 820.8)
	Male black	533	NR (NR)	NR (NR)					119.6 (-839.5, 1078.7)
	Male Hispanic	198	NR (NR)	NR (NR)					32.6 (-883.9, 949.1)
	Male white	423	NR (NR)	NR (NR)					97.9 (-797.3, 993.0)
	Female black	741	NR (NR)	NR (NR)					43.5 (-809.0, 896.0)
	Female Hispanic	446	NR (NR)	NR (NR)					10.9 (-735.1, 756.9)
	Female white	270	NR (NR)	NR (NR)					-43.5 (-789.5, 702.5)

	High waist girth male all	484	NR (NR)	NR (NR)					326.3 (-760.8, 1413.2)
	Low waist girth male all	728	NR (NR)	NR (NR)					-43.5 (-725.5, 638.5)
	High waist girth female all	979	NR (NR)	NR (NR)					108.7 (-722.5, 940.0)
	Low waist girth female all	489	NR (NR)	NR (NR)					-195.7 (-749.9, 358.4)
	High waist girth Black Men	209	NR (NR)	NR (NR)					369.7 (-781.2, 1520.7)
	Low waist girth Black Men	344	NR (NR)	NR (NR)					-32.6 (-693.3, 628.1)
	High waist girth Hispanic Men	73	NR (NR)	NR (NR)					239.2 (-805.1, 1283.6)
	Low waist girth Hispanic Men	125	NR (NR)	NR (NR)					-97.9 (-843.8, 648.1)
	High waist girth White Men	192	NR (NR)	NR (NR)					293.6 (-708.1, 1295.3)
	Low waist girth White Men	231	NR (NR)	NR (NR)					-65.2 (-726.0, 595.5)
	High waist girth Black Women	554	NR (NR)	NR (NR)					130.5 (-743.4, 1004.3)
	Low waist girth Black Women	187	NR (NR)	NR (NR)					-206.6 (-760.8, 347.5)
	High waist girth Hispanic Women	175	NR (NR)	NR (NR)					76.1 (-755.1, 907.4)
	Low waist girth Hispanic Women	95	NR (NR)	NR (NR)					-163.1 (-696.0, 369.7)
	High waist girth White Women	240	NR (NR)	NR (NR)					108.7 (-679.9, 897.3)
	Low waist girth White Women	206	NR (NR)	NR (NR)					-217.5 (-771.6, 336.7)
Reinhardt, 2017	Adult Men and Women	40	36.6 (18.6, 64.7)	29.1 (19.1, 57.6)	MRI	Individually sized ROI based on respective VAT ROIs from DXA	Lunar Android ROI; enCORE software	cm^3	104.1 (-681.9, 890.0)
	Adult Men	20	35.8 (18.6, 64.7)	28.6 (19.1, 57.6)					104.6 (-713.7, 923.0)
	Adult Women	20	37.4 (18.6, 64.7)	29.5 (19.1, 57.6)					103.6 (-669.9, 877.0)
Comparator Method: Prediction Equations using DXA									
Karelis, 2012	Postmenopausal Women with Overweight/Obesity - Pre-weight loss	92	58.1 (48.8, 70.5)	31.8 (26.1, 45.8)	CT	L4-L5	Bertin Index: (SD - SFW) X (TID)/height (cm)	cm^3	-4.2 (-39.3, 43.7)
	Postmenopausal Women with Overweight/Obesity - Post-weight loss (10% 6-mo RCT: CR with or without RT		NR (NR)	NR (NR)					-3.2 (-40.2, 44.7)

Comparator Method: Combined Techniques									
Jensen, 1995	Adult Men and Women	21	38 (19, 60)	26.6 (18.8, 35.8)	CT	Top of diaphragm to top of femur	DXA Total abdominal fat (top of diaphragm to top of femur) + Single CT L2-L3 slice; ratio of VAT to SAT in CT slice applied to DXA: VAT = Total abdominal fat (kg) * [(VAT (cm^2) from CT / Total L2-L3 slice AT (cm^2)	cm^3	-400.0 (-4726.4, 3926.4)

Abbreviations: BIA = bioelectrical impedance analysis; BMI = body mass index; CT = computed tomography; DXA: dual-energy x-ray absorptiometry; IAAT = intra-abdominal adipose tissue; IFM = internal fat mass; L (i.e. L4) = lumbar; MRI = magnetic resonance imaging; NR: not reported; ROI = region of interest; S (i.e. S1) = sacral; SAT = subcutaneous adipose tissue; SD: sagittal diameter; SF = skinfold; SFW = subcutaneous fat width; = TID = transverse internal diameter; US = ultrasound; VAT = visceral adipose tissue VF = visceral fat; WHR = waist-to-hip ratio

Table S3. Quality Assessment

	Select Items from the Guidelines for reporting reliability and agreement studies (GRRAS)											
Reference	2. Name and describe the diagnostic or measurement device of interest explicitly.	3. Specify the subject population of interest.	5. Describe what is already known about reliability and agreement and provide a rationale for the study (if applicable)	6. Explain how the sample size was chosen. State the determined number of raters, subjects/objects, and replicate observations.	7. Describe the sampling method.	8. Describe the measurement/rating process (e.g. time interval between repeated measurements, availability of clinical information, blinding).	10. Describe the statistical analysis.	11. State the actual number of raters and subjects/objects which were included and the number of replicate observations which were conducted.	12. Describe the sample characteristics of raters and subjects (e.g. training, experience).	13. Report estimates of reliability and agreement including measures of statistical uncertainty.	14. Discuss the practical relevance of results.	15. Provide detailed results if possible (e.g. online)
Studies Comparing IAAT Thickness (cm)												
Comparator Method: Ultrasound												
De Lucia Rolfe, 2010	Yes	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	No	Yes
Gradmark, 2010	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	No
Kuchenbecker, 2014	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Schlecht, 2014	Yes	No	Yes	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Studies Comparing IAAT Area (cm^2)												
Comparator Method: DXA												
Bredella, 2013	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	No	Yes
Micklesfield, 2012	Yes	No	Yes	No	No	No	Yes	Yes	Yes	Yes	No	Yes
Xia, 2014	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Comparator Method: BIA												
Berker, 2010	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	No
Bosy-Westphal, 2008	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Gómez-Ambrosi, 2018	Yes	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes

Lai, 2017	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Lee, 2015	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	No	Yes
Lu, 2017	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes
Wang, 2013	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	No
Park, 2016	Yes	Yes	Yes	No	No	No	Yes	Yes	No	Yes	No	Yes
Yamakage, 2014	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes	No	No	No
Comparator Method: Prediction Equations using Ultrasound												
Armellini, 1997	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	No	Yes
De Lucia Rolfe, 2011	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Comparator Method: Prediction Equations using DXA												
Ball, 2003	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Bertin, 2000	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	No	Yes
Treuth, 1995	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	No	No
Hill, 2007	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	No	No
Goran, 1998	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	Yes
Demura, 2007B	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	No	Yes
Demura, 2007A	Yes	Yes	Yes	No	No	No	No	Yes	Yes	Yes	No	No
Comparator Method: Prediction Equations using BIA												
Demura, 2007B	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	No	Yes
Demura, 2007A	Yes	Yes	Yes	No	No	No	No	Yes	Yes	Yes	No	No
Demura 2010	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	No	No	Yes
Studies Comparing IAAT Volume (cm^3)												
Comparator Method: **BIA**												
Bosy-Westphal, 2017	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	Yes	No	No
Comparator Method: DXA												
Cheung, 2016	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	No	Yes
Choi, 2015	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	Yes
Kaul, 2012	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Frossing, 2018	Yes	Yes	Yes	No	No	No	Yes	No	No	No	Yes	No
Lin, 2013	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes
Mohammed, 2017	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	No	No
Neeland, 2016	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Reinhardt, 2017	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	No
Comparator Method: Prediction Equations using DXA												
Karelis, 2012	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	No	No
Comparator Method: Combined Techniques												
Jensen, 1995	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	No	Yes	Yes

Brief Cutting Edge Report
OBESITY BIOLOGY AND INTEGRATED PHYSIOLOGY

Obesity

Sex Affects Regional Variations in Subcutaneous Adipose Tissue T Cells but not Macrophages in Adults with Obesity

Jessica Murphy [1,2,3], *Kerri Z. Delaney* [1,2,3], *Vi Dam* [1,2,3], *Bjorn T. Tam* [1,2,3], *Natalie Khor* [2], *Michael A. Tsoukas* [4], *José A. Morais* [1,5], *and Sylvia Santosa* [1,2,3]

Objective: The inflammatory environment in lower-body subcutaneous adipose tissue (SAT) has been largely unexplored. This study aimed to examine the effects of region (upper body vs. lower body) and sex on SAT immune cell profiles in young adults with obesity.

Methods: Abdominal (AB) and femoral (FEM) SAT was collected from 12 males (mean [SEM] age = 30.8 [1.4] years; mean [SEM] BMI = 34.1 [1.1] kg/m^2) and 22 females (mean [SEM] age = 30.6 [0.6] years; mean [SEM] BMI = 34.0 [0.7] kg/m^2) with obesity via needle aspiration. Flow cytometry was used to quantify macrophage (CD68+) and T-cell (CD3+) subpopulations in the stromovascular fraction of each SAT region.

Results: Females had a greater proportion of most T-cell types (CD3+CD4+CD45RA+, CD3+CD4+CD45RA−, and CD3+CD8+CD45RA+) in FEM compared with AB SAT, while males had similar proportions in both regions. Regardless of sex, the M1-like macrophage population (CD68+CD206−) was proportionally higher in AB SAT than in FEM SAT.

Conclusions: Results showed that T-cell populations vary by SAT region in females but not males. Both sexes, however, have proportionately more proinflammatory macrophages in upper-body than in lower-body SAT. It remains to be seen how these unique immune cell profiles in males and females with obesity contribute to adipose tissue inflammation and metabolic disease risk.

Obesity (2020) **0**, 1-5.

Study Importance

What is already known?

- Immune cell populations have been well characterized in visceral and upper-body subcutaneous adipose tissue but not lower-body subcutaneous adipose tissue.

What does this study add?

- T-cell populations vary by subcutaneous adipose tissue region in females but not males with obesity.
- Both sexes have proportionately more proinflammatory macrophages in upper-body than lower-body subcutaneous adipose tissue.

How might these results change the direction of research?

- Our results indicate that sex should be considered when investigating adipose tissue immune cell profiles and their implications in disease risk.

Introduction

Body fat distribution has been central to the discussion on obesity and metabolic disease. A plethora of studies have sought to uncover the causes and consequences of excess upper-body versus lower-body fat accumulation (reviewed by Tchkonia and colleagues, Lee and colleagues, and Santosa and Jensen) (1-3)). Central adiposity, in both the visceral and subcutaneous depots, is a well-established risk factor for insulin resistance and related diseases. Conversely, fat stored predominantly in the gluteofemoral region is associated with metabolic protection. These observations are partially explained by depot differences in secretory profiles and the capacity for fat storage and lipolysis. Attention has shifted to understanding how these endocrine and metabolic functions, many of which are sex dependent, relate to the adipose tissue (AT) microenvironment.

Given the chronic, low-grade inflammation associated with obesity, the AT immune landscape has been a topic of particular interest. Macrophages and T cells represent the most abundant immune cells in AT (4). However, regional variability has been chiefly investigated between visceral and abdominal (AB) subcutaneous depots in humans, and no studies have clearly assessed sex differences. Accordingly, we

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aimed to determine the effects of region (upper body vs. lower body) and sex on subcutaneous AT (SAT) macrophage and T-cell subpopulations in young adults with obesity.

Methods

Participants

We recruited healthy, nonsmoking males ($n=12$; mean [SEM] age = 30.8 [1.4] years; mean [SEM] BMI = 34.1 [1.1] kg/m^2) and females ($n=22$; mean [SEM] age = 30.6 [0.6] years; mean [SEM] BMI = 34.0 [0.7] kg/m^2) with obesity (BMI = 29.5-39.9 kg/m^2) from Montreal, Quebec, Canada. Participants were sedentary or lightly active, weight stable (±2 kg) for at least 2 months, and not taking any medications that could affect metabolism. Women who were pregnant or breastfeeding were excluded. The study was approved by the University Human Research Ethics Committee of Concordia University, and all participants provided written informed consent.

Body composition and anthropometric assessment

After an overnight fast, total and regional body composition was assessed using dual-energy x-ray absorptiometry (Lunar Prodigy Advance; GE Healthcare, Madison, Wisconsin) with Encore software (version 14.10; GE Healthcare). Dual-energy x-ray absorptiometry calibration was performed using manufacturer-supplied phantoms. Waist and hip circumferences were measured using the National Institutes of Health protocol (5).

AT biopsies and immune cell analysis

AB and femoral (FEM) SAT was collected via needle aspiration after an overnight fast. A detailed description of the biopsy procedure, AT processing, and immune cell analysis has been reported elsewhere (6). The stromovascular cells from approximately 1 g of SAT were isolated by collagenase digestion. The cells were purified; stained with CD68, CD206, CD3, CD4, CD8, and CD45RA antibodies (Supporting Information Table S1); and analyzed using an 8-color BD FACSVerse (BD Bioscienes, San Jose, California) and FlowJo software version 9.3.2 (Treestar Inc., Ashland, Oregon). Supporting Information Table S2 shows our single-stain and fluorescence-minus-one controls. We classified macrophages (CD68+) into M2-like (CD206+) and M1-like (CD206-) populations. In the T-cell population (CD3+), we identified T helper (Th) cells (CD4+), cytotoxic T cells (CD8+), and their naïve (CD45RA+) subsets. Our gating strategy for immune cell identification is displayed in Supporting Information Figure S1. Immune cells were expressed as a percentage of live stromovascular cells and, in a subset of participants, as the number of cells per gram of SAT.

Gene expression analysis

To complement our immune cell data, we analyzed the relative mRNA expression of CD11c (proinflammatory macrophage marker), forkhead box P3 (FOXP3) (regulatory T-cell marker), and the proinflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in SAT. Detailed methods are described in the online Supporting Information.

Statistical analyses

We conducted a mixed model ANOVA with Tukey post hoc analysis to determine the effects of sex, SAT region (AB vs. FEM), and their interaction on the proportion of each AT immune cell. We used Levene's test to evaluate equality of variances and the Shapiro-Wilk test to assess the normality of residuals for all models. Appropriate transformations (natural log or square root) of the dependent variable were made when statistical assumptions were not met. To facilitate interpretation, means and 95% confidence intervals (CI) of transformed immune cell data were back-transformed to the original scale. All analyses were conducted using SAS software version 9.2 (SAS Institute Inc., Cary, North Carolina), and $P \leq 0.05$ was considered statistically significant.

Results

Males and females had typical sex differences in total adiposity and body fat distribution (Table 1). Females had a lower waist to hip ratio and greater percent body fat, and they stored more fat in their gynoid region and legs. Subcutaneous and total android fat mass was similar between groups.

Figures 1 and 2 show the regional SAT immune cell populations by sex. For all T-cell proportions, there was a significant sex-by-region interaction. Females had a greater proportion of CD3+CD4+CD45RA+, CD3+CD4+CD45RA−, and CD3+CD8+CD45RA+ T cells in FEM SAT than in AB SAT (post hoc analysis for CD3+CD8+CD45RA− did not reach statistical significance), whereas males had similar proportions in both regions. Independent of sex, the CD68+CD206− M1-like macrophages were proportionally higher in AB SAT than in FEM SAT. There were no significant sex, region, or interaction effects on the CD3+CD4+/CD3+CD8+ and CD68+CD206+/CD68+CD206− ratios (data not shown) or on the absolute immune cell numbers per gram of SAT. Of note, the results for the immune cell proportions did not differ for the participants with or without absolute immune cell data.

The gene expression of CD11c and FOXP3 was detectable in both SAT regions for only 48% ($n=12$ females and $n=4$ males) and 30% ($n=8$ females and $n=2$ males) of participants, respectively. Sex and region

TABLE 1 Participant characteristics

	Males ($n=12$)	Females ($n=22$)	*P* value
Age (y)	30.8 (1.4)	30.6 (0.6)	0.93
BMI (kg/m^2)	34.1 (1.1)	34.0 (0.7)	0.92
Weight (kg)	106.4 (5.0)	94.1 (2.2)	0.01
Waist circumference (cm)[a]	109.5 (3.0)	104.1 (1.7)	0.10
Hip circumference (cm)[a]	116.3 (2.6)	120.7 (1.7)	0.15
Waist to hip ratio[a]	0.94 (0.02)	0.86 (0.01)	<0.01
Total body fat (kg)	39.4 (2.6)	42.1 (1.5)	0.35
Total body fat (%)	37.2 (1.5)	45.1 (0.9)	<0.01
Total android fat (kg)	4.3 (0.3)	3.8 (0.2)	0.11
Subcutaneous android fat (kg)	2.4 (0.2)	2.8 (0.1)	0.06
Gynoid fat (kg)	6.0 (0.5)	7.7 (0.3)	0.01
Android to gynoid fat ratio	0.74 (0.04)	0.50 (0.02)	<0.01
Leg fat (kg)	11.1 (0.9)	15.2 (0.8)	<0.01
Leg to total fat ratio	0.28 (0.01)	0.36 (0.01)	<0.01

Results are means (SEM).
[a]$n=11$ males.

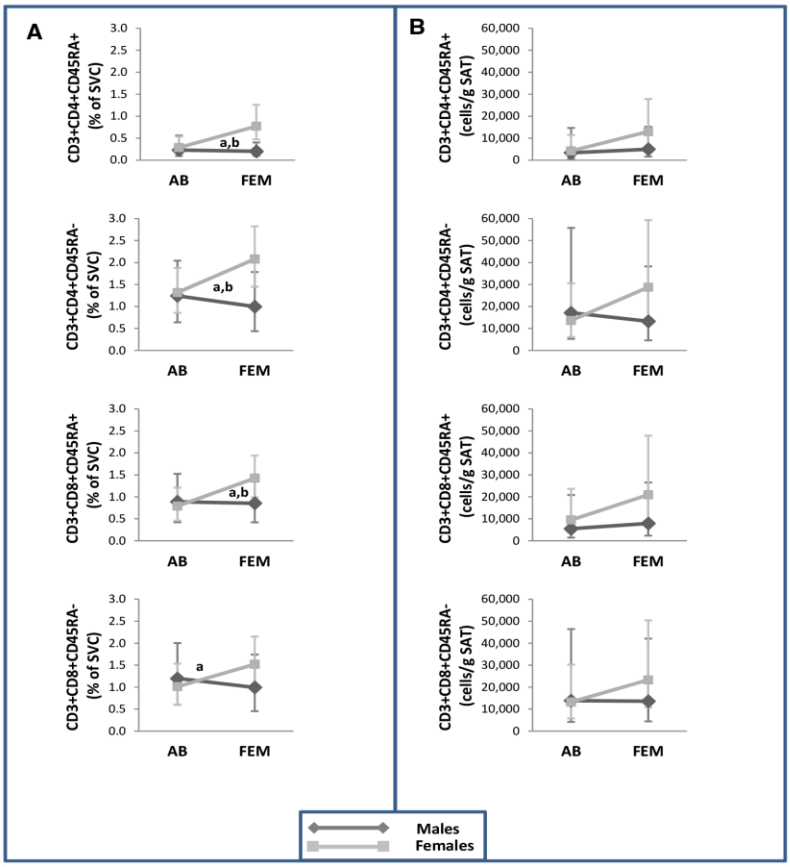

Figure 1 Regional SAT T-cell populations by sex. Immune cells are expressed as (**A**) percentage of SVC (CD4+ cells: $n=11$ males, $n=22$ females; CD8+ cells: $n=11$ males, $n=21$ females) and (**B**) number of cells per gram of SAT ($n=7$ males, $n=15$ females). In panel A, CD3+CD4+CD45RA+ was natural-log transformed, and the other outcomes were square root transformed prior to analysis in order to meet statistical assumptions. In panel B, all outcomes were natural-log transformed prior to analysis. Results are presented as back-transformed means and 95% CIs. [a]$P < 0.05$ for sex-by-region interaction; [b]FEM>AB in females ($P<0.05$ post hoc test). AB=abdominal; FEM=femoral; SAT=subcutaneous adipose tissue; SVC=stromovascular cells.

did not affect whether CD11c or FOXP3 expression was detectable or not (data not shown). There were no sex, region, or interaction effects on the gene expression of IL-6 or TNF-α (Supporting Information Figure S2).

Discussion

Here we showed that females but not males have a higher proportion of T-cell populations in FEM SAT than in AB SAT, while both sexes have proportionately more M1-like macrophages in AB SAT than in FEM SAT. When expressed in absolute terms, both T-cell and macrophage populations did not vary by sex or region. These findings are significant because the inflammatory environment is a key orchestrator of AT dysfunction yet remains largely unexplored in lower-body SAT.

There is no consensus on whether immune cell proportions or absolute numbers have a greater impact on the AT inflammatory environment. Data expression is variable across the literature, and few studies have reported both proportions and absolute numbers. Establishing best practices for AT immune cell data expression is crucial to improving data interpretation in this evolving field.

Sex differences in AT T-cell populations have not been well studied. Though Zeyda et al. (7) found that gene expression of various AB

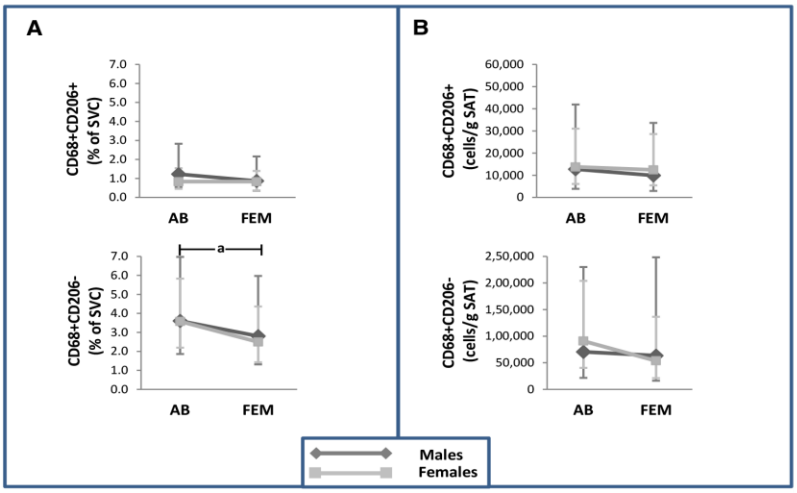

Figure 2 Regional SAT macrophage populations by sex. Immune cells are expressed as (**A**) percentage of SVC ($n=12$ males; $n=22$ females) and (**B**) number of cells per gram of SAT ($n=7$ males, $n=15$ females). All outcomes were natural-log transformed prior to analysis in order to meet statistical assumptions. Results are presented as back-transformed means and 95% CIs. $^{a}P<0.05$ for overall region effect. AB=abdominal; FEM=femoral; SAT=subcutaneous adipose tissue; SVC=stromovascular cells.

SAT T-cell populations was higher in individuals with BMI more than 40 kg/m^2 compared to those with BMI less than 30 kg/m^2, they did not detect any sex effects. However, the sample included 16 females and only 4 males and so it was not likely to have been sufficiently powered to detect sex differences. In another predominantly female sample, flow cytometry data confirmed a rise in the proportions of SAT CD4+ and CD8+ T cells (regions unspecified) with increasing adiposity (8). Conversely, studies conducted exclusively in males have shown that CD4+ and CD8+ T-cell proportions in AB SAT do not vary with degree of adiposity (9,10). Taken together, the emerging patterns from the aforementioned studies may suggest that T-cell recruitment is more responsive to SAT accumulation in females than in males. In our study, females had a predominantly lower-body fat distribution, which may explain (if this response is localized) the increased T-cell proportions in their FEM region.

It is unclear how the elevated T-cell proportions in female FEM SAT relate to the metabolically protective nature of lower-body SAT. Although we could not distinguish the activation states of our CD45RA− T-cell populations, Duffault et al. (8) showed that, in SAT from females, CD4+ memory and CD8+ effector T cells composed the majority of CD4+CD45RA− and CD8+CD45RA− T cells, respectively. Moreover, Fabbrini et al. (11) showed that increased proportions of Th17 and Th22 cells in AB SAT differentiated individuals with metabolically abnormal obesity from those who were lean or metabolically normal. It remains to be seen whether this finding translates to lower-body SAT. Regulatory T cells are another subset of CD4+ T cells that have anti-inflammatory properties but they have not been studied in lower-body SAT. They compose only around 2% of CD4+ T cells in AB SAT from individuals with overweight or obesity (12), which may explain why FOXP3 gene expression was undetectable in many of our participants.

Our cytokine gene expression results align with others (13) and they do not support the notion that lower-body SAT is metabolically protective compared with upper-body SAT. Using arteriovenous sampling, however, IL-6 release from FEM SAT was shown to be significantly lower than that from AB SAT (14). Although AT macrophages were shown to be an important source of IL-6 (15), only a few human studies have quantified them in lower-body SAT. Tchoukalova et al. (16) showed that the proportion of SAT macrophages was elevated with obesity in females and did not vary between AB and FEM regions. Another study in females reported average macrophage percentages of approximately 3% and 10% in the stromovascular cells of AB SAT and gluteal SAT, respectively (17); however, since the regional AT samples did not come from the same individuals, the macrophage proportions were not comparable. Only one study quantified M1- and M2-like macrophages in AB and FEM SAT of a predominantly female sample, but it did not report whether proportions were different between regions (18). Notably, all 3 of these studies used different laboratory techniques to quantify AT macrophages.

We used CD206 to mark for M2-like macrophages and therefore identified M1-like macrophages asCD206− cells. On this basis, our results suggest that FEM SAT is more anti-inflammatory than AB SAT. We acknowledge that the M1/M2 concept of macrophage polarization is an oversimplified concept, and that our classification does not entirely capture the inflammatory phenotype of these CD206+ and CD206− subsets. Human studies have shown that AT macrophages can simultaneously express M1- and M2-like markers (19) and that, paradoxically, M2-like macrophages have the capacity to secrete proinflammatory cytokines (20). Nevertheless, CD206− macrophages were shown to have increased gene expression of CD11c, IL-6, and TNF-α compared with CD206+ macrophages (21). We analyzed the gene expression of

CD11c in SAT to more clearly define the proinflammatory M1-like macrophage population but found undetectable expression in more than half of participants. Expression at the gene level is not always indicative of the protein level, and interestingly, most human studies that have reported CD11c gene expression in AT were conducted in individuals with severe obesity.

In summary, we showed that sex affects the regional variation of SAT T-cell populations, but not macrophages, in young adults with obesity. Our results suggest that AB SAT is more proinflammatory than FEM SAT with respect to classically defined macrophage proportions. This inflammatory gap between regions may be either widened or narrowed in females, depending on the phenotype of their elevated FEM T-cell proportions. Overall, these findings provide new insight to help explain the opposing metabolic disease risk profiles associated with upper-body versus lower-body fat distributions. Future studies in more diverse age and BMI categories should further explore the regional SAT immune landscape and its relationship with AT function without neglecting sex as an important variable.O

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Supporting information: Additional Supporting Information may be found in the online version of this article.

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Supplementary Methods

Gene Expression Analyses

Adipose tissue RNA was extracted using the RNeasy Lipid Tissue Mini Kit (Qiagen, Mississauga, ON), and converted to cDNA with the QuantiTect Reverse Transcription Kit (Qiagen). Quantitative PCR was performed on the CFX96 Real-Time System-C1000 ThermalCycler (Bio-Rad Laboratories, Mississauga, ON) using the PowerUp SYBR Green Master Mix (Thermo Scientific, Waltham, MA). Relative mRNA expression of IL- 6, TNF-α, CD11c, and FOXP3 were quantified using the ΔΔCt method with 18S rRNA as the housekeeping gene for normalization. Primer sequences are shown in Table S3.

Supplementary Tables and Figures

Table S1. Antibody and fluorochrome pairings with supplier and clone information

Antibody*	Fluorochrome	Supplier	Clone	Titrated concentration ($\mu l/10^6$ cells)
CD45RA	FITC	eBioscience	JS-83	1.5
CD4	PE	BioLegend	SK3	1.5
CD68	PE-Cy7	eBioscience	815CU17	1.5
CD206	APC	eBioscience	19.2	1.5
CD8	APC-Cy7	BioLegend	SK1	1.5
CD3	BV510	BioLegend	SK7	1.5

*Antibodies were titrated on human adipose tissue to determine the optimal staining volume per 10^6 cells.

Table S2. Single stain (SS) cocktails and fluorescence minus one (FMO) controls for flow cytometry experiment

	FITC	PE	PE-Cy7	APC	APC-Cy7	BV510	Compensation Beads
SS FITC	CD45RA						Positive/ negative
SS PE		CD4					Positive/ negative
SS PE-Cy7			CD68				Positive/ negative
SS APC				CD206			Positive/ negative
SS APC-Cy7					CD8		Positive/ negative
SS BV510						CD3	Positive/ negative
FMO FITC		CD4	CD68	CD206	CD8	CD3	
FMO PE	CD45RA		CD68	CD206	CD8	CD3	
FMO PE-Cy7	CD45RA	CD4		CD206	CD8	CD3	
FMO APC	CD45RA	CD4	CD68		CD8	CD3	
FMO APC-Cy7	CD45RA	CD4	CD68	CD206		CD3	
FMO BV510	CD45RA	CD4	CD68	CD206	CD8		

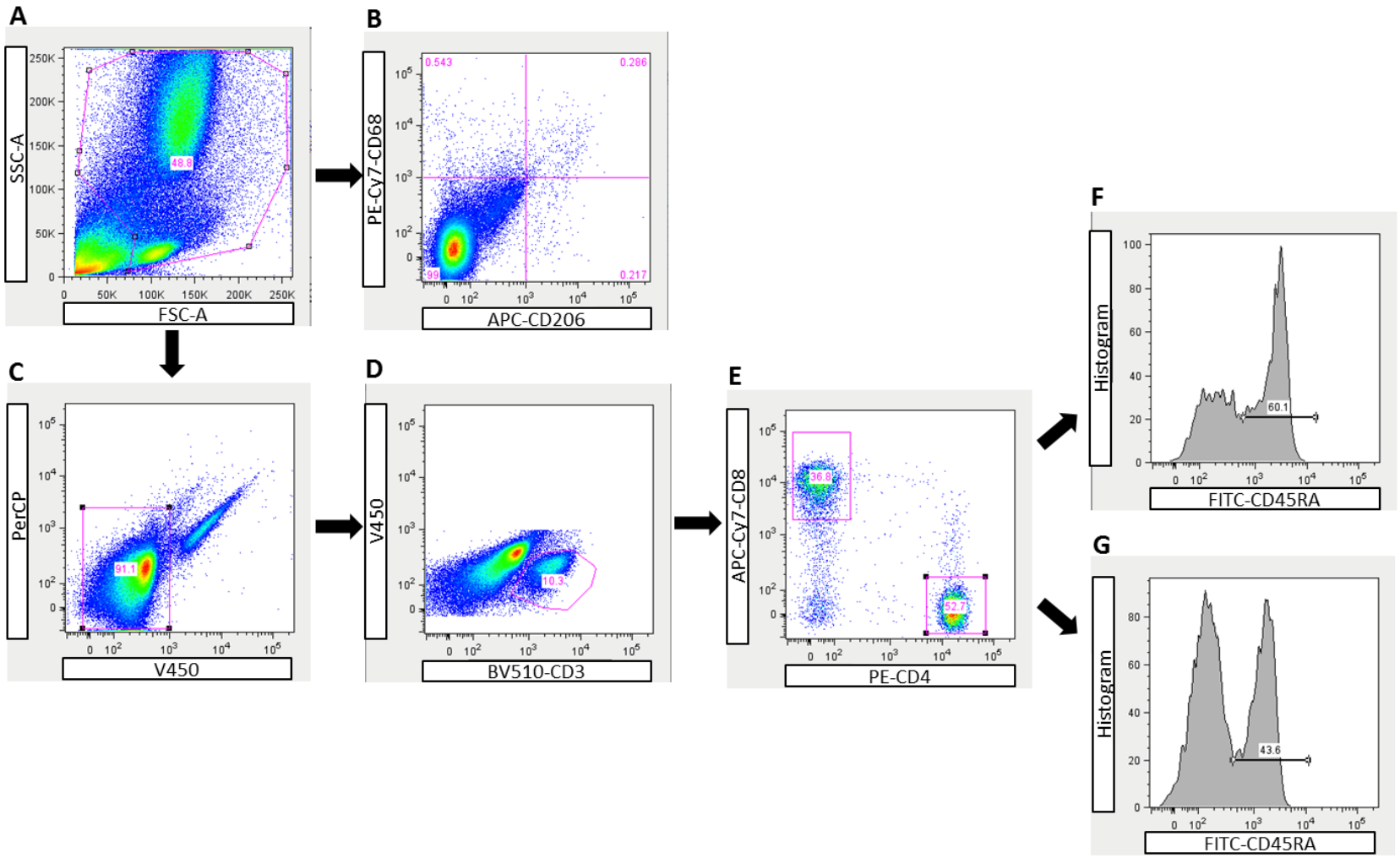

Figure S1. Representative gating strategy for the identification of immune cell populations. (**A**) Live cells were gated, and (**B**) macrophage (CD68+) populations were identified as CD206+ or CD206-. (**C**) Unwanted events were eliminated by gating out the negative cells on the two dump channels, and (**D**) T cells were identified as CD3+. (**E**) From the CD3+ gate, T cells subpopulations were identified as CD8+ or CD4+. (**F**) The naïve (CD45RA+) subset of the CD8+ T cell population and (**G**) the naïve (CD45RA+) subset of the CD4+ T cell population were further identified.

Table S3. Quantitative PCR Primer Sequences

Gene	Forward	Reverse
IL-6	5'-CCGGGAACGAAAGAGAAGCT-3'	5'-GCGCTTGTGGAGAAGGAGTT-3'
TNF-α	5'-CTCTTCTGCCTGCTGCACTTTG-3'	5'-ATGGGCTACAGGCTTGTCACTC-3'
CD11c	5'-GCACTCATCACAGCGGTACT-3'	5'-AGGGTAATGGGGAGTGGGC-3'
FOXP3	5'-CCCATGCCTCCTCTTCTTCC-3'	5'-CCATGACTAGGGGCAGTGTG-3'
18S rRNA	5’-GGCCCTGTAATTGGAATGAGTC-3’	5’-CCAAGATCCAACTACGAGCTT-3’

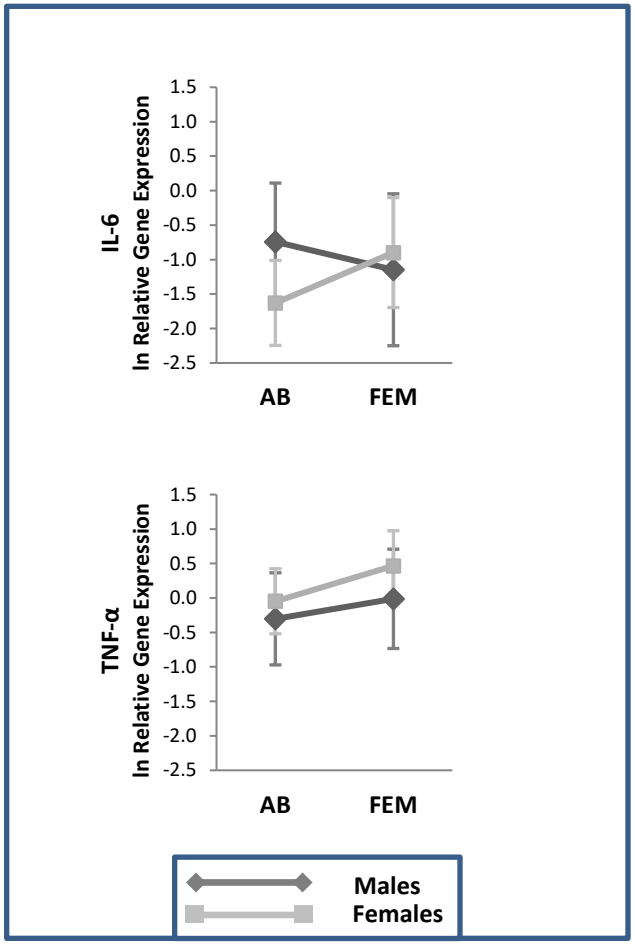

Figure S2. Proinflammatory cytokine gene expression in regional subcutaneous adipose tissue by sex
(IL-6: *n* = 11 males, *n* = 21 females; TNF-α: *n* = 11 males, *n* = 19 females)
Results are presented as means ± 95% CIs.
AB = abdominal; FEM = femoral

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Research Article

Research Article

Acetyl-CoA Regulation, OXPHOS Integrity and Leptin Levels Are Different in Females With Childhood vs Adulthood Onset of Obesity

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Abbreviations: abSAT, abdominal subcutaneous adipose tissue; ACLY, ATP citrate lyase; AO, adulthood-onset obesity; CII, complex II; CIV, complex IV; CV, complex V; CO, childhood-onset obesity; DNL, de novo lipogenesis; DXA, dual-energy x-ray absorptiometry; feSAT, femoral subcutaneous adipose tissue

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Abstract

Although childhood-onset obesity (CO) and adulthood-onset obesity (AO) are known to lead to distinctive clinical manifestations and disease risks, the fundamental differences between them are largely unclear. The aim of the current study is to investigate the fundamental differences between subcutaneous adipose tissue from CO and AO and to identify metabolic differences between abdominal (abSAT) and femoral subcutaneous adipose tissues (feSAT). Total and regional body composition was assessed using dual-energy x-ray absorptiometry (DXA) and computed tomography. Levels of acetyl-CoA, NAD+/NADH, acetyl-CoA network genes, mitochondrial complex abundance, H3 acetylation were determined in biopsied abSAT and feSAT. Serum leptin and adiponectin were measured. Our results showed that acetyl-CoA was higher in subcutaneous adipose tissue from subjects with AO compared with CO. Multiple linear regression revealed that ATP citrate lyase was the only main effect affecting the level of acetyl-CoA. Circulating leptin concentrations was higher in AO. The increased level of acetyl-CoA was strongly associated with histone H3 acetylation, *LEP* expression in adipose tissue, and circulating leptin in AO. NAD+/NADH was higher in CO; however, abundance of mitochondrial complexes, the complex II:complex V ratio, and the complex IV:complex V ratio were lower in CO, reflecting compromised mitochondrial function in subcutaneous adipose tissue from CO. Moreover, we identified differences in the level of acetyl-CoA and NAD+/NADH ratio between abSAT and feSAT, suggesting that these fat depots may possess

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different metabolic properties. The fundamental difference in the important metabolic intermediate acetyl-CoA between CO and AO may help us better understand the development of obesity and the pathogenesis of different obesity-related diseases in humans.

Key Words: obesity onset, acetyl-CoA, leptin, adipose tissue, metabolism

Compared with those who only acquire excess adiposity as adults, those with persistent adiposity since childhood have a higher risk of diabetes (1) and coronary heart disease (2). Although differences in disease risk between individuals with childhood- and adulthood-onset obesity are well recognized, the fundamental differences between them are largely unclear. Characterizing how excess adiposity developed early or later in life affects cellular and metabolic processes will give us a better understanding of how the pathogenesis obesity diverges in these individuals.

Acetyl-CoA is a central metabolite that is underappreciated. Acetyl-CoA is generated in mitochondria through pyruvate decarboxylation, β-oxidation of fatty acids, and catabolism of amino acids. When acetyl-CoA enters the citric acid cycle, NADH, FADH2, and ultimately ATP are produced. During the fed state, acetyl-CoA is condensed with oxaloacetate in mitochondria to form citrate, which is then transferred to cytoplasm. ATP citrate lyase (ACLY) catalyzes the ATP-dependent conversion of citrate and coenzyme A to oxaloacetate and acetyl-CoA. The cytosolic acetyl-CoA product is important for de novo lipogenesis (DNL) and acetylation reactions (3). As such, excess acetyl-CoA generated from macronutrients can be stored as fat and establish a nucleocytosolic pool of acetyl-CoA activating gene expression.

Recently, in those with type 2 diabetes, genes within 2 reaction steps of acetyl-CoA were observed to be dysregulated in subcutaneous fat (4). Furthermore, in 3T3-L1 adipocytes, ACLY-dependent generation of acetyl-CoA appears to contribute to increased histone acetylation (5). Therefore, the abundance of cytosolic acetyl-CoA could be a switch responsible for turning genes on and off via histone acetylation. At the same time, NAD+-dependent deacetylation may counterbalance the effects of increased acetyl-CoA (6), making overall histone acetylation dependent on both the availability or acetyl-CoA and redox state at the cellular level. The aim of the current study is to characterize the level of acetyl-CoA and redox state in regional subcutaneous adipose tissue from individuals with childhood-onset obesity (CO) and adulthood-onset obesity (AO).

Materials and Methods

Subjects

Sedentary (as assessed by FAO/WHO/UNU criteria (7)) and premenopausal women aged 25 to 40 years with body mass indexes of 30.0 to 39.9 kg/m^2 were recruited. They were then divided into 2 groups depending on the onset of obesity. Childhood-onset obesity is defined as obesity acquired pre- or peri- puberty. Adulthood-onset obesity is defined as obesity acquired after 18 years of age. Participants were required to provide a medical record and/or photographic proof of weight at childhood (8-12 years old). Additional verification of childhood weight was achieved through the administration of the Collins' Childhood Body Rating Scale (8) and the Stunkard Body Rating Scale (9). Participants were excluded if they were pregnant or planning to become pregnant, breastfeeding, used nicotine-containing products (eg, cigarettes, chewing tobacco, nicotine gum etc.), took antidepressants, antihypertensives, or any medications that may affect fatty acid (FA) metabolism. Participants were also excluded if they previously had any surgery (eg, gastric bypass, magnetic implants) or have any conditions (eg, iron deficiency anemia) or diseases (eg, diabetes, cancer, inflammatory bowel disease, history of eating disorders or mental illness) that may affect immune function and thus, study outcomes, or the ability to participate in the study. Participants must also have had stable weight for at least 2 months. Written informed consent was obtained from all participants. The study was approved by the University Human Research Ethics Committee of Concordia University.

Study design

All studies were conducted at the PERFORM Centre of Concordia University. Study measurements occurred across 2 mornings. For both morning visits participants arrived having fasted for at least 12 hours. On one morning visit participants arrived and rested for 2 hours prior to measurement of resting energy expenditure. Body composition was measured, and blood was drawn. On another morning visit participants rested upon arrival. Femoral (feSAT) and abdominal (abSAT) subcutaneous adipose tissue biopsies were conducted using sterile technique and local anesthesia, as previously described (10).

Body composition

After an overnight fast, total and regional body composition was assessed using dual-energy X-ray absorptiometry

(DXA) (Lunar Prodigy Advance; GE Healthcare; Madison, WI) with Encore Software (version 14.10; GE Healthcare; Madison, WI). Visceral adipose tissue quantification was estimated using a single-slice CT at L2-L3.

Measurement of acetyl-CoA

Intracellular acetyl-CoA in adipose tissue was measured by Pico-Probe Acetyl-CoA Fluorometric Assay Kit (BioVision, Milpitas, CA). The manufacturer's protocol for sample preparation was followed. Briefly, samples were deproteinized, homogenized, and sonicated on ice. Homogenates were spun down at 10 000*g* for 30 minutes. Neutralized supernatant was then diluted in assay buffer before transferring to wells of a 96-well plate.

Measurement of NAD+/NADH

NAD+/NADH was quantified by NAD+/NADH Quantification Colorimetric Kit (BioVision, Milpitas, CA). The manufacturer's protocol for sample preparation was followed. Briefly, adipose tissue samples were homogenized in NADH/NAD extraction buffer and then centrifuged at 12 000*g* for 15 minutes. Tubes with NAD converted to NADH by the NAD cycling buffer and enzyme mix were labeled as NADHt. Tubes with NAD decomposed by heat (60 °C) in a water bath were labeled as NADH. NAD/NADH ratio was calculated as (NADt – NADH)/NADH.

Genes associated with the acetyl CoA network

Genes within 2 reaction steps of acetyl-CoA metabolism were quantified by qPCR in adipose tissue. According to the KEGG PATHWAY Database, the following genes were included: *ACAT1*, *ACAA2*, *HADHB*, *CS*, *ACLY*, *FASN* and *ACACA*. Adipokine genes (*LEP*, *ADIPOQ*, *IL-6*, *TNF-α* and *RETN*) were also included in current study. cDNA was generated using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA) and then amplified using PowerUp SYBR Green Master Mix (Thermo Scientific, Waltham, MA). Relative expression was normalized using the expression levels of GAPDH.

Immunoblotting

Thirty µg of cytoplasmic protein from adipocytes was subjected to SDS-PAGE electrophoresis and transferred to activated PVDF membranes. After blocking for 1 hour at room temperature, membranes were incubated overnight at 4 °C with primary antibodies against Ac-Histone H3 (11) and OXPHOS (12). After the overnight incubation, membranes were incubated with the corresponding secondary antibodies (13, 14) for 1 h at room temperature. Signals were detected by SuperSignal West Pico PLUS Chemiluminescent Substrate (Life Technologies, Carlsbad, CA) and visualized by Chemidoc Image System (Biorad Laboratories, Hercules, CA).

Measurement of leptin and adiponectin in blood

Solid phase sandwich ELISA was used to determine the concentration of leptin (15) and adiponectin (16) in human blood serum. All measurements were obtained according to the manufacturer's protocol.

Measurement of estradiol

A competitive enzyme immunoassay was used to determine the concentration of estradiol in plasma (17). All measurements were obtained according to the manufacturer's protocol.

Statistical analysis

Statistical analyses were conducted using the GraphPad Prism 8 (GraphPad, La Jolla, CA). All data were expressed as means ± standard error of the mean (SEM). Kolmogorow-Smirnow test was used to check for normality. If the Kolmogorow-Smirnow test was not significant, Student's *t* test was used for statistical analysis, otherwise, the Mann-Whitney test was used. Mixed model analysis of variance (ANOVA) was used to examine the interaction and main effects of depot and onset. Sidak's post hoc test was used for multiple comparisons. Post hoc pairwise comparisons were performed using Student's *t* test. Statistical significance was defined as $P < 0.05$.

Results

Characteristics of subjects

Seventeen females with CO and 16 females with AO were recruited. Participant metabolic characteristics, body composition, and estrogen level are shown in Table 1. Other than the higher android/gynoid ratio in CO, there were no significant differences observed between the groups.

Table 1. Clinical Characteristics of Subjects With Childhood-Onset Obesity and Adulthood-Onset Obesity

	Childhood-Onset (n = 17)	Adult-Onset (n = 16)	*P*
Age (y)	30.9 ± 0.8	30.4 ± 0.7	0.604
Height (cm)	164.8 ± 1.3	164.5 ± 1.7	0.897
Weight (kg)	90.5 ± 2.5	90.6 ± 2.8	0.993
BMI (kg/m2)	33.3 ± 0.7	33.4 ± 0.7	0.916
Body fat %	44.5 ± 0.8	44.6 ± 1	0.938
Arm fat mass (kg)	4.0 ± 0.2	4.2 ± 0.2	0.430
Leg fat mass (kg)	14.1 ± 1.0	15.1 ± 0.7	0.897
Trunk fat mass (kg)	21.4 ± 0.7	20.2 ± 1.3	0.453
Android fat mass (kg)	3.8 ± 0.2	3.4 ± 0.2	0.258
Gynoid fat mass (kg)	7.1 ± 0.4	7.5 ± 0.3	0.271
Android/gynoid ratio	*0.56 ± 0.03*	*0.45 ± 0.02*	*0.001*
Total fat mass (kg)	40.4 ± 1.6	40.6 ± 1.9	0.949
Arm lean mass (kg)	4.7 ± 0.2	5.0 ± 0.3	0.466
Leg lean mass (kg)	17.6 ± 0.6	17.5 ± 0.6	0.944
Trunk lean mass (kg)	21.5 ± 0.6	21.0 ± 0.6	0.639
Total lean mass (kg)	47.0 ± 1.3	46.7 ± 1.4	0.895
VAT (cm^3)	1010 ± 121	941 ± 129	0.697
TAG (mM)	1.34 ± 0.14	1.18 ± 0.12	0.403
Cholesterol (mM)	4.65 ± 0.21	4.54 ± 0.22	0.715
HDL (mM)	1.31 ± 0.07	1.24 ± 0.06	0.478
LDL (mM)	2.74 ± 0.16	2.76 ± 0.21	0.930
Fasting glucose (mM)	4.68 ± 0.10	4.67 ± 0.11	0.927
Estradiol (pg/ mL)	87.56 ± 6.59	89.27 ± 28.46	0.734

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TAG, triacylglycerol; VAT, visceral adipose tissue.

Cytosolic acetyl-CoA level and NAD+/NADH ratio in subcutaneous adipose tissue

Acetyl-CoA is a key indicator of the cellular metabolic state. High cytosolic level of acetyl-CoA is a characteristic of a fed state. Acetyl-CoA levels were higher in AO than CO for both abSAT (P = 0.032) and feSAT (P = 0.004) (Effect of onset, P < 0.001, Fig. 1A). With respect to depot, acetyl-CoA was higher in abSAT than feSAT for both CO (P = 0.006) and AO (P = 0.036).

There was also a main effect of depot (P < 0.001) and onset (P = 0.027) on the NAD+/NADH ratio whereby the ratio was higher in feSAT than abSAT and lower in AO when compared with CO (Fig. 1B). Post hoc tests further showed that the NAD+/NADH ratio was greater in feSAT than abSAT in both CO (P < 0.001) and AO (P = 0.012) (Fig. 1B). In feSAT, NAD+/NADH ratio was higher in CO than AO (P = 0.041)

As a low NAD+/NADH ratio is associated with mitochondrial dysfunction (18), we checked the abundance of mitochondrial complexes and the ratio between complexes which indicate the integrity and function of mitochondria in AT (19, 20). In abSAT, complex II (CII; P = 0.005) and complex IV (CIV; P = 0.015) were significantly higher in AO when compared with CO (Fig. 2A). The same pattern was observed in feSAT complex II (CII; P = 0.005) and complex IV (CIV; P = 0.084), which were also higher in AO when compared with CO (Fig. 2B). When CII and CIV were normalized to complex V (CV), we found significant differences in the ratios between in CO and AO (CII:CV, P = 0.002; CIV:CV, P = 0.046), as well as between abSAT and feSAT (CII:CV and CIV:CV, P < 0.001 for both) (Fig. 2C and D). Post hoc analysis revealed that, in feSAT, the CII:CV ratio was higher in AO when compared with CO (P = 0.004, Fig. 2C). The CIV:CV ratio was higher in feSAT than abSAT in both CO and AO (P = 0.006, P < 0.001, respectively) (Fig. 2D). In feSAT, the CIV:CV ratio was higher in AO when compared with CO (P = 0.0603, Fig. 2D).

Distinctive gene expression patterns in childhood-onset and adulthood-onset obesity

Genes within 2 reaction steps of acetyl-CoA have been regarded as regulators of the level of cytosolic acetyl-CoA (4). In order to study the association between those genes and level of acetyl-CoA, we checked the genes' expression in abSAT and feSAT from our subjects. Our analyses showed that mRNA levels of *BCKD* (P = 0.037), *ACAT1* (P = 0.018), and *HADHB* (P = 0.013) were significantly lower in feSAT when compared with abSAT (Fig. 3A). Moreover, mRNA levels of *BCKD* was higher in AO than CO in both abSAT (P = 0.002) and feSAT (P = 0.047). There were no significant differences in *ACAA2* and *CS* between groups (Fig. 3A).

Compared with abSAT, mRNA expression of *ACLY* was significantly lower in feSAT (P = 0.011) (Fig. 3B). Post hoc testing revealed that mRNA expression of *ACLY* was only lower in feSAT than abSAT of AO (P = 0.009) (Fig. 3B). Post hoc tests also showed that mRNA expression of *ACLY* was higher in both abSAT (P = 0.001) and feSAT (P = 0.056) from AO vs CO (Fig. 3B). Mann-Whitney showed that *ACACA* expression was higher in feSAT from AO than that from CO (P = 0.043). A pairwise comparison revealed that *FASN* expression was significantly higher in feSAT of AO than that of CO (P = 0.046) (Fig. 3B).

The level of acetyl-CoA was positively correlated with *BCKD* (r = 0.45 P = 0.003, Fig. 4A), *ACAA2* (r = 0.34, P = 0.029, Fig. 4B), *ACAT1* (r = 0.37, P = 0.020, Fig. 4C), *HADHB* (r = 0.41, P = 0.008, Fig. 4D), *CS* (r = 0.33, P = 0.040, Fig. 4E), and *ACLY* (r = 0.56, P < 0.001, Fig. 4F). When multiple regression analysis was performed, *ACLY* expression and the level of acetyl-CoA maintained a significant association independent of the other genes (R^2 = 0.46, P = 0.0016, Fig. 4G/ Table 2). To determine whether the gene-gene interactions alter the level of acetyl-CoA, a multiple regression model with 2-way interactions was used (R^2 = 0.81, P = 0.004, Fig. 4H/ Table 3). In addition to significant gene-gene interactions, including *ACLY*ACAT1* (P = 0.003), *ACLY*HADHB* (P = 0.009), *BCKD*ACAT1*

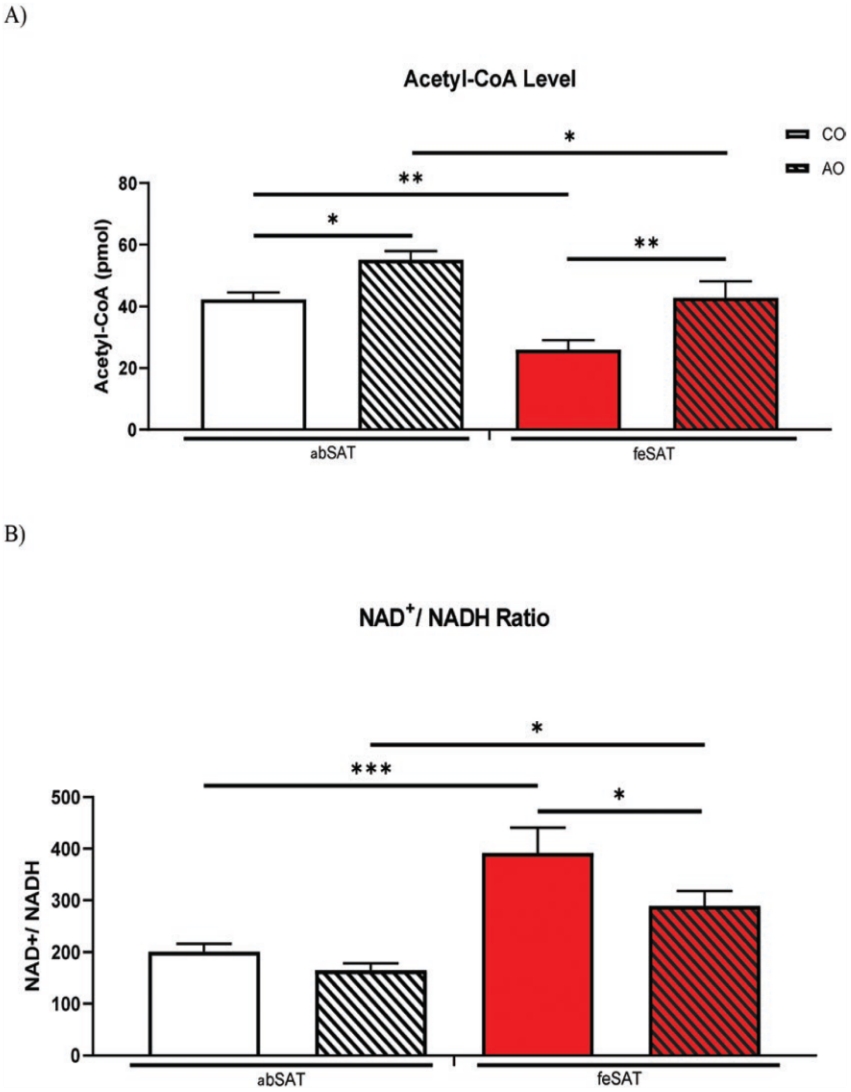

Figure 1. A) Comparison of acetyl-CoA and B) NAD+/NADH ratio in abdominal (abSAT) and femoral (feSAT) subcutaneous adipose tissue depots from individuals with childhood-onset (CO) obesity and adulthood-onset (AO) obesity. Comparisons between groups were made using mixed model ANOVA followed by post hoc tests (n = 20/ depot). Data are presented as mean ± SEM. Statistical significance was assessed by Sidak's post hoc test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

($P = 0.011$), *BCKD*HADHB* ($P = 0.003$), *ACAT1*ACAA2* ($P = 0.006$), *ACLY* remained the only main factor altering the level of acetyl-CoA ($P = 0.021$).

Expression of *LEP* and circulating leptin are different in individuals with different onsets of obesity

LEP expression was higher in AO when compared with CO ($P < 0.001$) (Fig. 5A). Post hoc test revealed that the *LEP* expression was higher in both abSAT ($P = 0.006$) and feSAT ($P = 0.007$) from AO when compared with CO (Fig. 5A). Pairwise comparison showed that individuals with AO had higher *IL-6* expression in their abSAT when compared with CO ($P = 0.036$, Fig. 5A). Post hoc comparisons showed that *IL-6* expression was higher in feSAT than in abSAT (Fig. 5A) for both CO ($P = 0.046$) and AO ($P = 0.039$). There were no significant differences in *ADIPOQ*, *TNF-α*, and *RETN* between groups.

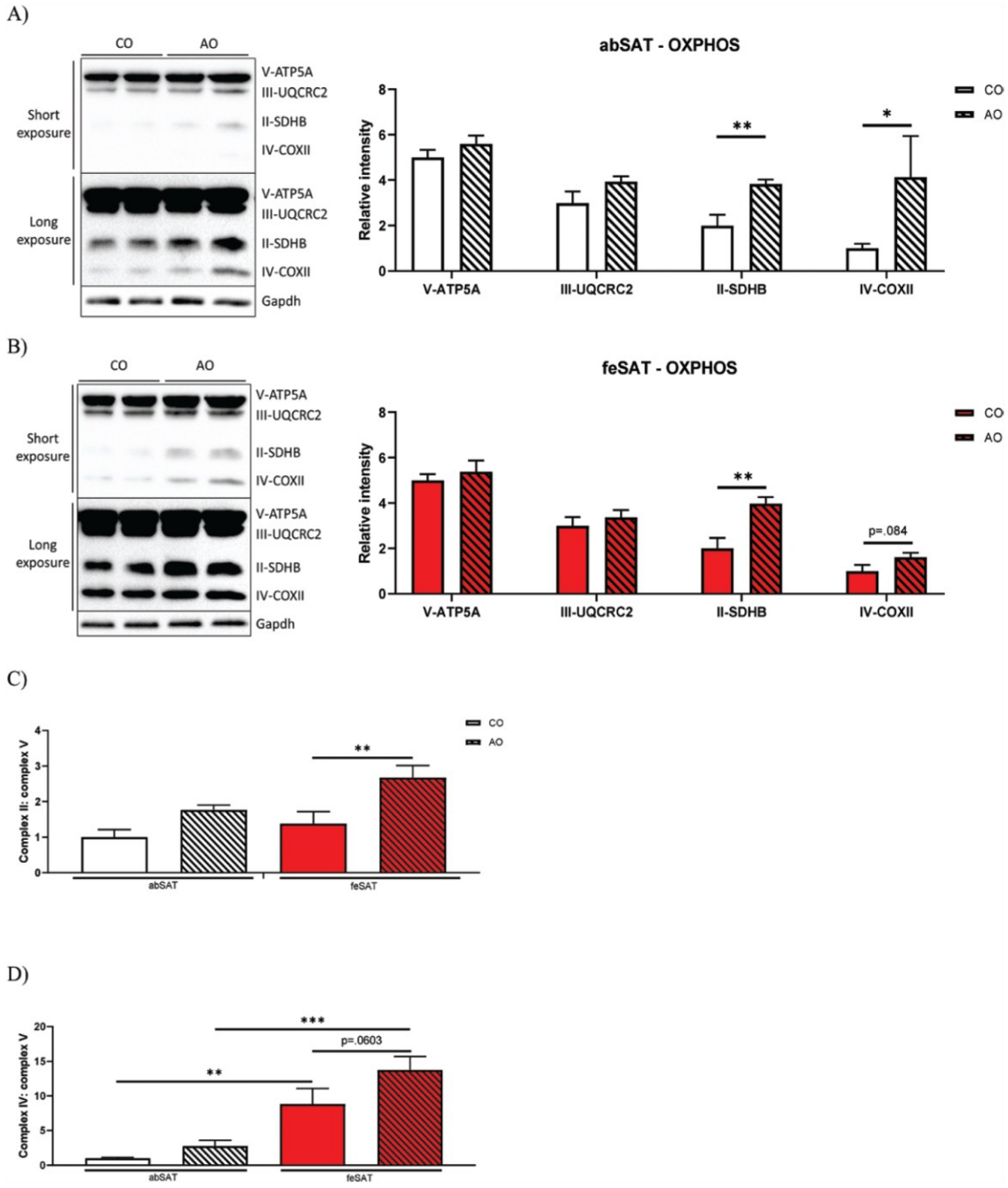

Figure 2. Immunoblot of mitochondrial complexes in isolated adipocytes from A) abdominal subcutaneous adipose tissue (abSAT) depots and B) femoral subcutaneous adipose tissue (feSAT) depots of individuals with childhood-onset (CO) obesity and adulthood-onset (AO) obesity. Statistical significance was assessed by student's *t* test (n = 16/ depot). Ratio of expression levels between C) complex II and complex V and D) complex IV and complex V in adipocytes from abdominal subcutaneous fat depots and femoral subcutaneous fat depots of individuals with childhood-onset obesity and adulthood-onset obesity. Comparisons between groups were made using mixed model ANOVA followed by post hoc tests (n = 16/ depot). Data are presented as mean ± SEM. Statistical significance was assessed by Sidak's post hoc test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Higher circulating leptin in subjects with AO was detected ($P = 0.002$; Fig. 5B). Oppositely, there was no difference in circulating adiponectin between CO and AO (Fig. 5C). Overall, the adiponectin-to-leptin ratio was lower in subjects with AO when compared with subjects with CO (Fig. 5D).

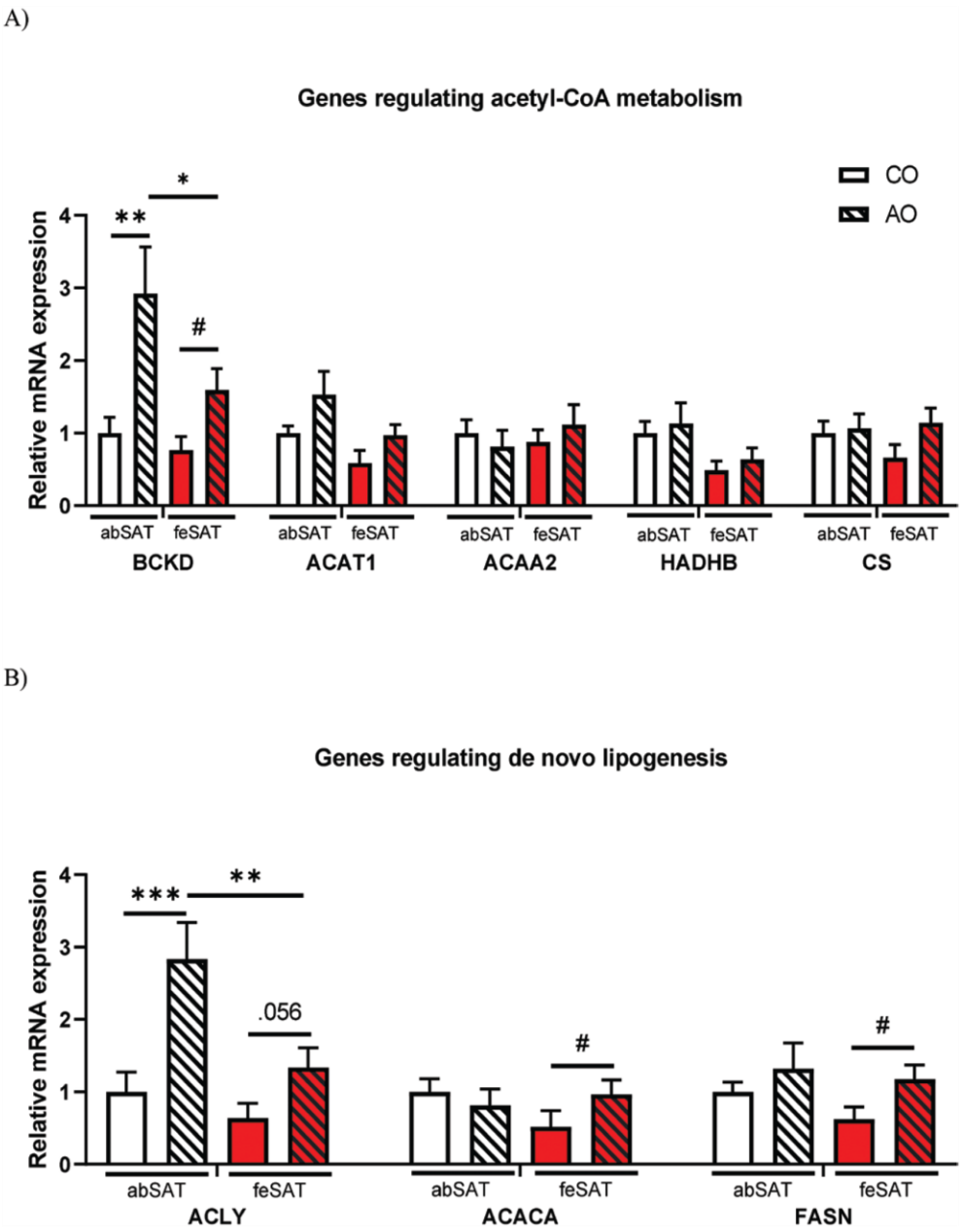

Figure 3. mRNA expressions of A) genes regulating acetyl-CoA metabolism and B) genes regulating de novo lipogenesis. Comparisons between groups were made using mixed ANOVA followed by post hoc tests (n = 20/ depot). Data are presented as mean ± SEM. Statistical significance was assessed by Sidak's post hoc test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) and post hoc pairwise comparison (#$P < 0.05$). Abbreviations: AO, adulthood-onset obesity; CO, childhood-onset obesity.

LEP expression and secretion are strongly associated with cytosolic acetyl-CoA and H3-acetylation

H3-acetylation was higher in AO for both abSAT ($P = 0.011$) (Fig. 6A) and feSAT ($P = 0.028$) (Fig. 6B). Pearson correlation indicated that the level of acetyl-CoA was associated with Ac-H3 ($r = 0.48$, $P = 0.062$ in abSAT; $r = 0.54$, $P = 0.030$ in feSAT), *LEP* expression ($r = 0.53$, $P = 0.017$ in abSAT; $r = 0.55$, $P = 0.011$ in feSAT) and circulating leptin ($r = 0.57$, $P = 0.008$ in abSAT; $r = 0.63$, $P = 0.003$ in feSAT) (Fig. 6C and D). Acetylation of histone is regarded as a major regulator of transcription, resulting in

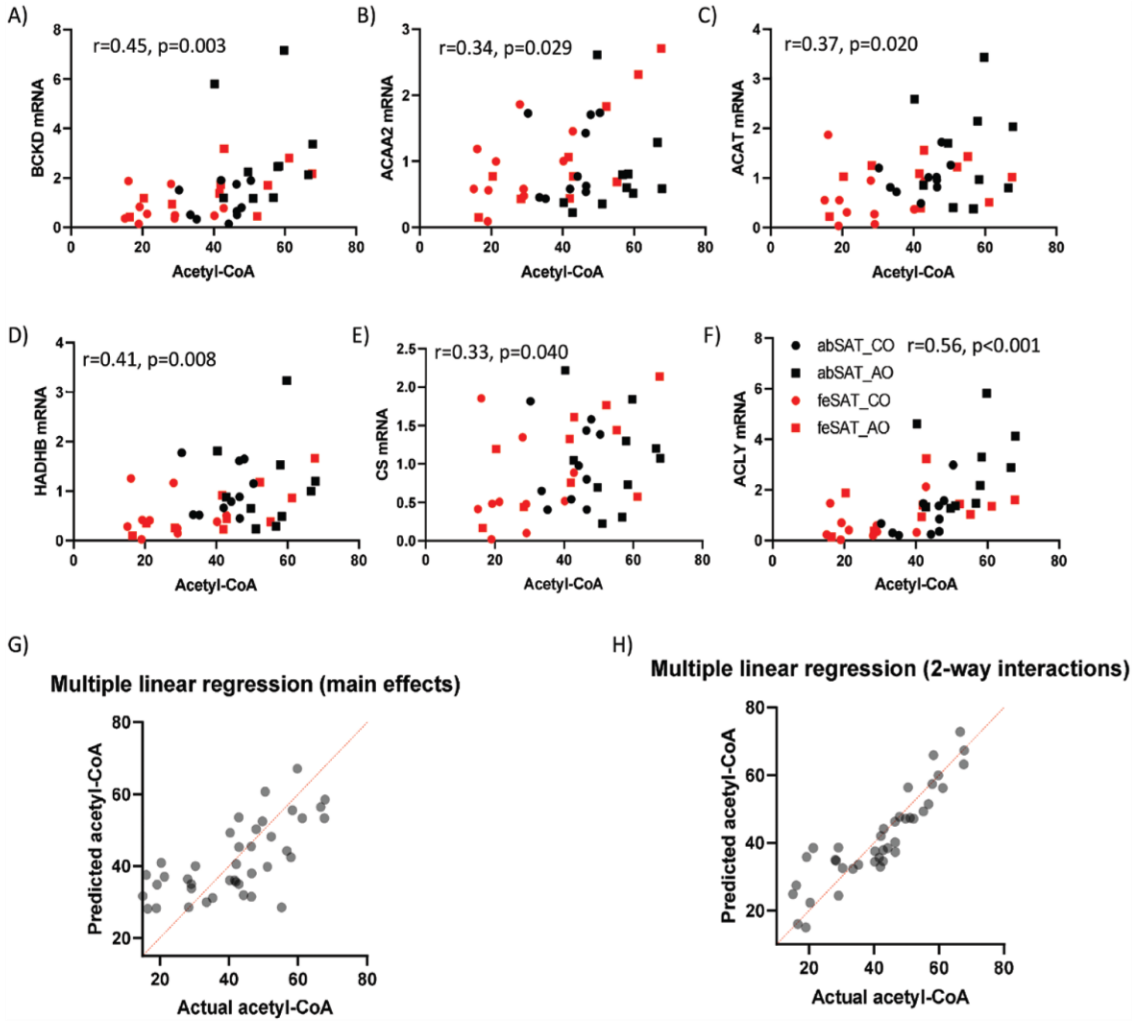

Figure 4. Pearson's correlation coefficient was used to examine the strength of associations between A-F) acetyl-CoA and mRNA expression of genes regulating acetyl-CoA in isolated adipocytes from abdominal subcutaneous adipose tissue (abSAT) and femoral subcutaneous adipose tissue (feSAT) depots of individuals with childhood-onset (CO) obesity and adulthood-onset (AO) obesity (n = 20/ depot). Linear regression model G) without 2-way interactions analysis and H) with 2-way interactions analysis showed predicted acetyl-CoA on y-axis versus actual acetyl-CoA on x-axis. Red dotted line represents the line of best fit.

increased expression of genes (21). Our results also showed that Ac-H3 was associated with *LEP* expression (r = 0.49, P = 0.056 in abSAT; r = 0.79, P < 0.001 in feSAT) and circulating leptin (r = 0.52, P = 0.039 in abSAT; r = 0.71, P = 0.002 in feSAT) (Fig. 6C and D). In line with the literature, the *LEP* expression was correlated to the circulating leptin (r = 0.54, P = 0.013 in abSAT; r = 0.63, P = 0.003 in feSAT) (Fig. 6C and D).

Discussion

Although CO and AO have been postulated to be 2 different types of obesity, little evidence has supported this claim. Here we provided the first depiction of differences between CO and AO by characterizing acetyl-CoA metabolism in regional subcutaneous adipose tissue. We show that the level of acetyl-CoA, *ACLY* expression, and H3 acetylation in subcutaneous adipose tissue are higher in AO when compared to CO. These findings indicate a potentially important role of acetyl CoA metabolism in subcutaneous adipose tissue functioning in AO. Moreover, the high level of acetyl-CoA is associated with the concentration of circulating leptin, suggesting a possible epigenetic regulation of leptin through histone acetylation.

Despite no differences in estrogen level, fat mass, and body composition, higher circulating leptin was observed

Table 2. Multiple Linear Regression Coefficients (main effects only)

	$\beta \pm$ SEM	t	Sig.
Acetyl-CoA level (n = 40)			
Intercept	27.63 ± 4.31	6.41	<0.001
ACLY	9.56 ± 3.19	3.00	0.005
BCKD	-2.63 ± 3.27	0.80	0.427
ACAT1	-12.32 ± 17.49	0.70	0.747
HADHB	50.28 ± 27.89	1.80	0.383
CS	10.6 ± 20.69	0.51	0.260
ACAA2	2.513 ± 3.517	2.51	0.170

Table 3. Multiple Linear Regression Coefficients With 2-way Interactions

	$\beta \pm$ SEM	t	Sig.
Acetyl-CoA level (n = 40)			
Intercept	17.21 ± 7.79	2.21	0.040
ACLY	42.67 ± 16.89	2.53	0.021
BCKD	-29.45 ± 16.65	1.77	0.094
ACAT1	-12.32 ± 17.49	0.70	0.490
HADHB	50.28 ± 27.89	1.80	0.090
CS	10.60 ± 20.69	0.51	0.613
ACAA2	-3.90 ± 14.42	0.27	0.790
ACLY X ACAT1	-46.49 ± 13.75	3.38	0.003
ACLY X HADHB	35.08 ± 11.97	2.93	0.009
BCKD X ACAT1	58.70 ± 20.65	2.84	0.011
BCKD X HADHB	-48.57 ± 14.10	3.45	0.003
ACAT1 X ACAA2	-32.16 ± 10.25	3.14	0.006

in individuals with AO, which were consistent with the expression of *LEP* at the transcriptional level. Such a discrepancy between CO and AO may indicate differences in leptin secretion that vary by obesity onset. However, we cannot rule out the possibility that individuals with AO are more leptin-resistant than those with CO. It has previously been suggested that those with AO have more difficulty losing weight than those with CO (22, 23). As leptin is an anorexogenic adipokine, it is plausible that the greater weight retention that has been observed in AO during weight loss is mediated, in part, by resistance to leptin signaling (24). Unlike leptin, there was no difference in adiponectin between groups, resulting in lower adiponectin: leptin ratio in AO. The adiponectin:leptin ratio has been implicated in adipose tissue dysfunction and negatively associated with atherosclerosis risk (25). A recent study proposed that patients with AO are more likely to have hypertension (26), which may be explained partly by the lower adiponectin:leptin ratio, although some studies proposed the opposite (27). A larger cohort is required to validate these findings.

The level of intracellular acetyl-CoA, under the fasted state, was higher in AT from AO when compared with CO. This observation indicates a possibility that fatty acid storage is enhanced in AO vs CO, as the greater availability of acetyl-CoA can better support fatty acid synthesis. The lower NAD+/NADH ratio and greater *ACLY* expression in AT from AO supports this view (28, 29). The expression of *ACLY* is tightly regulated by nutrient availability in adipose tissue—increased with carbohydrate and inhibited with high-fat consumption (29, 30). This regulation is crucial for turning excessive dietary carbohydrates to lipid stored in adipocytes and maintaining proper glucose and fatty acid metabolism, especially in female mice (30). In the current study, we have a similar observation in our female subjects with AO. Expression of genes regulating DNL (ie, *ACLY*, *ACACA*, and *FASN*) in feSAT were increased, making fatty acid synthesis from acetyl-CoA in feSAT possible. Despite the fact that android and gynoid fat mass distribution was not different between our groups, AO had lower android-to-gynoid ratios vs CO, indicating relatively enhanced AT mass in the lower body depot in AO. The difference in the ratio could be attributed to differences in the activation of the DNL pathway between fat depots. The activated DNL and lower android-to-gynoid ratio are also consistent with the observation that the feSAT, in females especially, is more responsive to overfeeding, which is a key feature of AO (31, 32).

We also noticed a difference in the levels of acetyl-CoA from 2 depots that strongly correlated with genes that produce acetyl CoA from other reaction intermediates in the TCA cycle indicating that the level of acetyl-CoA expression may be dependent on the expression of network genes. Indeed, the expression of *BCKD*, *ACAT1*, *HADHB*, and *ACLY* were higher in abSAT vs feSAT. Therefore, we hypothesized that the higher level of acetyl-CoA in abSAT was due to the higher expression of acetyl-CoA-regulating genes. Despite including the expression of several genes that potentially regulate acetyl-CoA in a multiple linear regression model, the only one that consistently predicted acetyl-CoA concentrations in both models was *ACLY*. Thus, the higher expression of *ACLY* in abSAT may explain the difference in the levels of acetyl-CoA in the 2 depots.

Obesity is associated with low NAD+/NADH ratio in human adipose tissue (33). However, the effects of obesity on regional NAD+/NADH ratio have not been reported up to now. Here, we found a lower NAD+/NADH ratio in abSAT. Low NAD+/NADH ratio is associated with mitochondrial disorders and aging (34). Thus, the difference in the ratio may suggest that adipose tissue from different depots age at different rates. Alternatively, the difference in NAD+/NADH could represent a fundamental difference

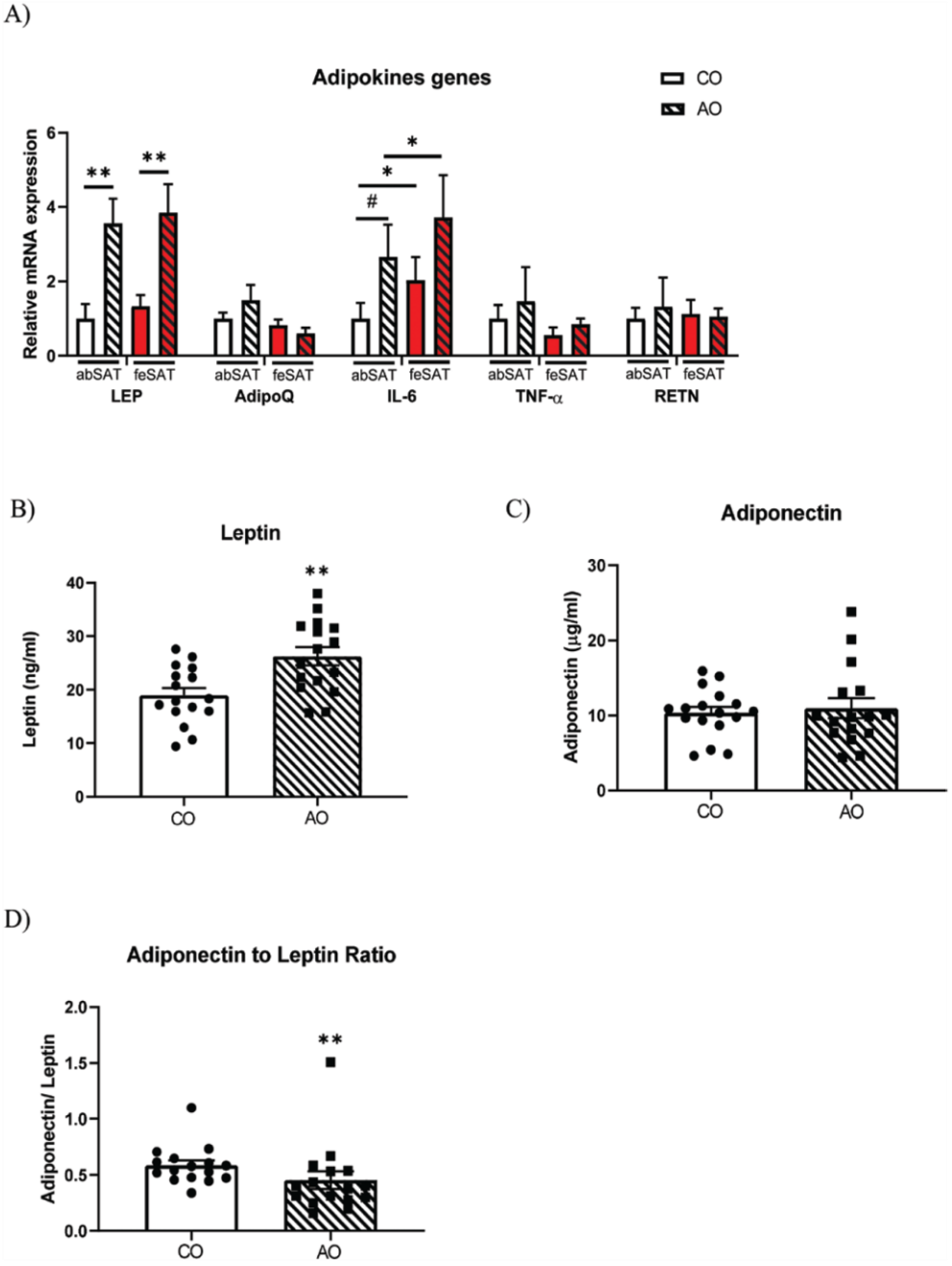

Figure 5. A) mRNA expressions of adipokine genes. Comparisons between groups were made using mixed model ANOVA followed by post hoc tests (n = 20/ depot). Data are presented as mean ± SEM. Statistical significance was assessed by Sidak's post hoc test (*$P < 0.05$, **$P < 0.01$) and post hoc pairwise comparison (#$P < 0.05$). Comparisons of B) leptin, C) adiponectin and D) adiponectin to leptin ratio in individuals with childhood-onset (CO) obesity and adulthood-onset (AO) obesity. Statistical significance was assessed by Student's *t* test (n = 16, childhood-onset obesity; n = 16, adulthood-onset obesity, **$P < 0.01$)).

between the two depots. The reduced ratio is associated with a decline in electron transport chain activity and metabolic diseases (35). It is not surprising to observe this regional difference as abSAT has a unique developmental root and is more hypertrophic in nature (36). abSAT has a higher rate of lipolysis and fatty acid uptake but is less capable of synthesizing lipid through DNL (37). The lower NAD+/NADH ratio can partially explain this regional

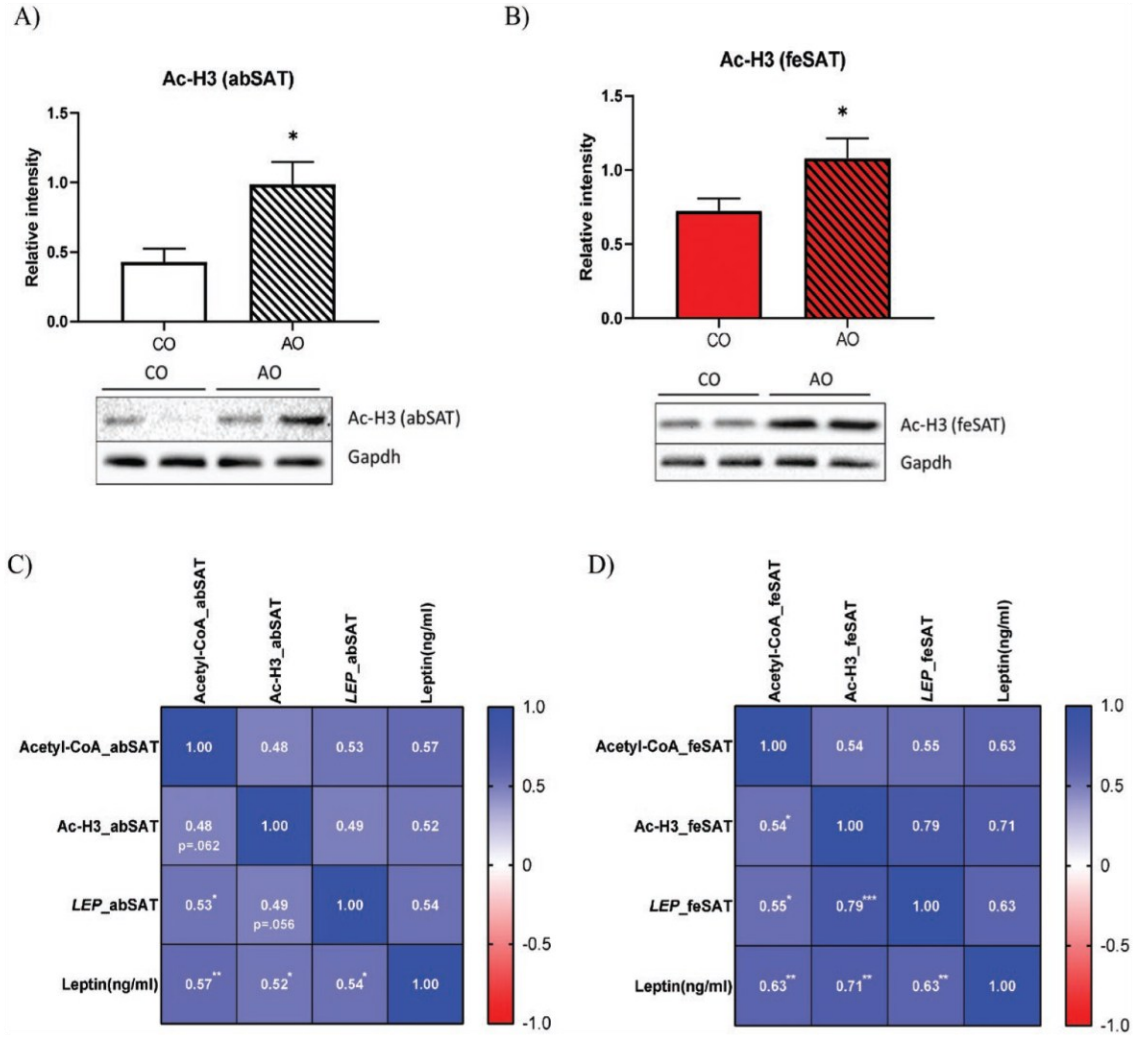

Figure 6. Level of histone H3 acetylation in isolated adipocytes from A) abdominal subcutaneous fat depots and B) femoral subcutaneous fat depots of individuals with childhood-onset obesity and adulthood-onset obesity. Statistical significance was assessed by Student's *t* test (n = 16/depot, $*P < 0.05$). Correlation matrix showing correlation coefficients between acetyl-CoA, Ac-H3, *LEP* expression and circulating leptin in isolated adipocytes from C) abdominal subcutaneous fat depots and D) femoral subcutaneous fat depots ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

difference. During DNL, the production of 1 molecule of palmitate from acetyl-CoA requires 7 molecules of ATP. Therefore, a depot with low ATP production might be less capable of sustaining the ATP-demanding DNL (38). Indeed, the lower CII:CV and CIV:CV ratios in abSAT also supports the notion that mitochondria from abSAT may neither be able to maintain high mitochondrial membrane potential ($\Delta\psi_{mt}$) (19) nor ensure energy generation in mitochondria through β-oxidation (20). Complex II is the only mitochondrial complex involved in both the TCA cycle and electron transport chain. It has been demonstrated that CII is the greatest contributor to maintain $\Delta\psi_{mt}$ in cell (19). Consistently, reduced CIV in mitochondria from adipose tissue has been regarded as a sign of adipose tissue dysfunction associated with aging and compromised β-oxidation (20). Overall, these results highlight the differences between abSAT and feSAT and suggest that ATP synthesis is less favorable in abSAT when compared with feSAT.

It has been suggested that impaired mitochondrial function leads to insulin resistance and type 2 diabetes mellitus (20, 39). Our results showed that mitochondria from individuals with CO had lower CII and CIV abundance and reduced CII:CV and CIV:CV ratio, especially in feSAT. These

results indicate that the mitochondria in adipose tissue are negatively affected when obesity develops in childhood. This is consistent with a previous observation that mitochondrial function is impaired in the adipose tissue of children with obesity (39). We speculate that mitochondrial dysfunction is an early and continuous event due to increased oxidative stress throughout the period of obesity, which eventually leads to adipose tissue dysfunction and subsequent diseases. Greater mitochondrial dysfunction in feSAT could also result in shunting of substrates to other adipose tissue depots, negatively affecting their mitochondrial function. The higher expression of CII in adipose tissue from AO also suggests elevated electron transport chain activity leading to greater fuel supply through β-oxidation. Together with the higher expression of *ACACA* and FASN in feSAT from individuals with AO, the futile lipid cycle is likely to be higher in AO, resulting in an increased triglycerides synthesis and decreased fatty acid release from adipocytes, which may represent a healthier metabolic profile when compared to CO (40).

Our results support the notion that CO and AO are two different types of obesity. When prescribing interventions for individuals with different onsets of obesity, different approaches may be considered. Exercise has been shown to alleviate mitochondrial dysfunction through PGC-1α-mediated mitochondrial biogenesis (41), but is not an effective treatment of hyperleptinemia in humans (42). Therefore, exercise interventions may better serve the improvement of mitochondrial impairment observed in CO. Likewise, diet intervention is well known for its effectiveness in managing weight and alleviating hyperleptinemia (43), but not in improving mitochondrial biogenesis in humans (44). Thus, a dietary intervention may be more beneficial to those with AO. A recent meta-analysis showed that only physical activity, but not diet, is associated with the effectiveness of weight loss interventions for children (45), but it is still unclear whether exercise is also a more important component in weight loss programs for individuals with CO. Furthermore, the impacts of interventions combined with exercise and diet on individuals with different onsets of obesity are largely unknown. It would be interesting to examine whether CO and AO respond differently to weight loss interventions. Collectively, our results suggested that individuals with AO have better mitochondrial integrity, higher DNL genes expression in feSAT, lower android:gynoid ratio, but higher leptin concentrations when compared with their CO counterparts.

Finally, we examined how the increased level of acetyl-CoA affected histone acetylation and established associations between adipokines mRNA expression in different subcutaneous adipose tissue depots. In this study, we found greater H3 histone acetylation in adipose tissue from individuals with AO. The higher level of histone acetylation in AO is in line with our hypothesis that the larger amount of acetyl-CoA generated in mitochondria is exported to cytosol, increasing the nucleocytosolic acetyl-CoA pool and histone acetylation. Of note, both a previous study (46) and our multiple linear regression model suggests that ACLY is a dominant supplier of acetyl-CoA for histone acetylation. The higher expression of *ACLY* and concentration of acetyl-CoA, and low NAD+/NADH ratio created favorable cellular conditions for histone acetylation in both abSAT and feSAT from AO. Nucleocytosolic acetyl-CoA is well known for its ability to activate genes through histone acetylation (47). In the current study, acetyl-CoA is strongly associated with H3 acetylation, *LEP* mRNA expression, and circulating leptin, implying that the acetylation of H3 may regulate the expression of *LEP* (Fig. 7). Although the expression patterns between *IL-6* and *LEP* are similar, no such association was found between acetyl-CoA and *IL-6* expression or other adipokines. How acetyl-CoA and subsequent histone acetylation regulate *LEP* expression requires further investigation at the genomic level.

There are several limitations in the current study that should be addressed. First, we only measured the intracellular acetyl-CoA in adipose tissue, so either the mitochondrial or nucleocytosolic pool of acetyl-CoA (or both) could contribute to higher level of acetyl-CoA in AO. It is generally accepted that most of the acetyl-CoA generated in mitochondria is consumed in TCA cycle (48) and the excess acetyl-CoA is directed to cytosol (47). The nucleocytosolic pool of acetyl-CoA, but not mitochondrial acetyl-CoA, is responsible for histone acetylation and the subsequent epigenetic control of gene expression (47-50). Based on the association between acetyl-CoA and leptin levels, it is speculated that the elevated histone acetylation in AO is, at least partly, mediated by the higher level of nucleocytosolic acetyl-CoA in AO; however, this hypothesis remains to be tested. Second, fresh biopsy samples were collected from our participants in the morning following an overnight fast. How feeding alters the transient fluctuation of NADH and acetyl-CoA is worth further investigation. Over the long term, obesity (or an obesogenic environment) chronically inactivates PDH due to the abundant formation of NADH and acetyl-CoA from lipid-derived fuels (51). Western-pattern diet activates ACLY-mediated generation of nucleocytosolic acetyl-CoA which may eventually induce histone and protein acetylation (30). Although we demonstrated an association between the intracellular acetyl-CoA level, histone H3 acetylation, *LEP* expression, and level of circulating leptin, a more mechanistic study

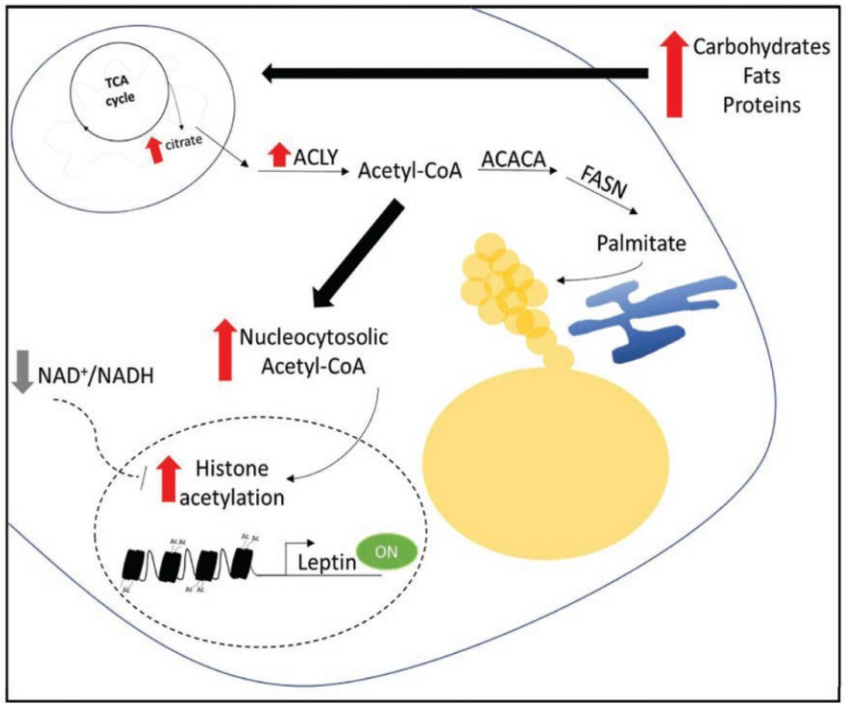

Figure 7. A schematic model proposing a possible mechanism regulating *LEP* expression through histone acetylation in adult-onset obesity. Increased food intake results in elevated uptake of glucose, fatty acid, and amino acid into adipocytes. Citrate is generated through TCA cycle and the excess citrate is exported to cytosol. Cytosolic citrate is then converted to acetyl-CoA by ACLY. The acetyl-CoA can be used for lipid synthesis or histone acetylation. Moreover, adulthood-onset obesity is associated with lower NAD+/NADH ratio in adipose tissue, possibly resulting in decreased deacetylation. Overall, enhanced histone acetylation may increase accessibility of chromatin and, hence, turn gene expression (eg, *LEP*) "on."

addressing the above causal relationship at the genomic level is warranted.

In conclusion, we have shown evidence in support of CO and AO being two different types of obesity based on the acetyl-CoA levels in adipose tissue, abundances of mitochondrial complexes, expression of genes regulating DNL, and level of circulating leptin. We also established a link between acetyl-CoA level, histone acetylation, and leptin abundance at both transcriptional and systemic levels. Differences observed between abSAT and feSAT further characterize fundamental differences between these depots. Collectively, these differences give a possible explanation for the differences in the clinical manifestations of obesity with different onsets and facilitate our understanding of adipose tissue biology.

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Data Availability: Restrictions apply to the availability of data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

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Appendix 7. List of supplementary co-authored publications

Guimarães Almeida L, Dera A, **Murphy J**, Santosa S. Improvements in cardiorespiratory fitness, muscle strength and body composition to modest weight loss are similar in those with adult- versus childhood-onset obesity. Clin Obes. 2024;14(1):e12623

Kakinami L, Plummer S, Cohen T, Santosa S, **Murphy J**. Body-composition phenotypes and their associations with cardiometabolic risks and health behaviours in a representative general US sample. Prev Med. 2022;164:107282

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Kakinami L, Danieles P, Ajibade K, Santosa S, **Murphy, J**. Adiposity and muscle mass phenotyping is not superior to BMI in detecting cardiometabolic risk in a cross-sectional study. Obesity (Silver Spring). 2021;29: 1279-1284.

Wang C, **Murphy J**, Delaney KZ, Khor N, Morais JA, Tsoukas, MA, Lowry DE, Mutch DM, Santosa S

Association between rs174537 FADS1 polymorphism and immune cell profiles in abdominal and femoral subcutaneous adipose tissue: an exploratory study in adults with obesity. Adipocyte. 2021;10: 24-130

Cooke AB, Kutate Defo A, Dasgupta K, Papaioannou TG, Lee, J, Morin S, **Murphy J**, Santosa S, Daskalopoulou, SS. Methodological considerations for the measurement of arterial stiffness using applanation tonometry. J Hypertens. 2021;39:428-436.

Delaney KZ, Dam V, **Murphy J**, Morais JA, Denis R, Atlas H, Pescarus R, Garneau PY, Santosa S. A reliable, reproducible flow cytometry protocol for immune cell quantification in human adipose tissue. Anal Biochem. 2021;613:113951

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A reliable, reproducible flow cytometry protocol for immune cell quantification in human adipose tissue

Author:
Kerri Z. Delaney,Vi Dam,Jessica Murphy,José A. Morais,Ronald Denis,Henri Atlas,Radu Pescarus,Pierre Y. Garneau,Sylvia Santosa

Publication: Analytical Biochemistry

Publisher: Elsevier

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Factors associated with adipocyte size reduction after weight loss interventions for overweight and obesity: a systematic review and meta-regression

Author: Jessica Murphy,Grégory Moullec,Sylvia Santosa

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