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The pathophysiology of abdominal adipose tissue depots in health and disease

Abstract: Obesity is currently the most important contributor to ill health and expenditure worldwide. More alarming is the fact that the pediatric population parallels adults, with obesity closely associated to type 2 diabetes mellitus (T2D), cardiovascular disease, hypertension, non-alcoholic fatty liver disease, vitamin D deficiency (VDD) and certain types of cancer. The observation in the early 1950s that android or truncal adipose tissue (AT) distribution compared to gynoid had a greater association with metabolic dysfunction, in particular T2D and cardiovascular disease risk, led to the hypothesis that obesity-associated complications are not associated with fat mass per se, but the pattern of fat distribution. This concept was further supported by groups of individuals with metabolic dysfunction despite a lean phenotype, and healthy obese people protected from metabolic dysfunction. It is now well recognized that an increase in visceral AT is an independent risk factor for the development of obesity-associated comorbidities with AT depot distribution, their anatomic, cellular and molecular features defining their role. The differences and the plasticity of subcutaneous, visceral and ectopic ATs to store and release fatty acids and to synthesize and secrete adipokines, defines the metabolic outcomes. The present review will examine the phenotypic and pathophysiological differences between the different AT depots, with a particular focus on the abdominal depots and their link to metabolic complications.

Keywords: adipose tissue; obesity; subcutaneous adipose tissue; visceral adipose tissue.

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Introduction

According to the World Health Organization, obesity is now the most important contributor to ill health and expenditure worldwide, with the pediatric population paralleling adults [1]. Based on latest available OECD survey data, more than half (52.6%) of the adult population of the European Union are overweight or obese, with levels exceeding 50% in 20 out of 34 OECD countries, while rates based on height and weight measurements are approximately 23% and 21% for 15-year-old boys and girls, respectively, http://www.oecd.org/els/health-systems/Health-at-a-Glance-2013. The impact of these statistics highlights a more important issue – the stress to healthcare systems – in that obesity is closely associated with type 2 diabetes mellitus (T2D), cardiovascular diseases, hypertension, non-alcoholic fatty liver disease (NAFLD), vitamin D deficiency and certain types of cancer [2], with childhood obesity most strongly associated with insulin resistance [3]. Most alarming is the likelihood that a child who is obese at puberty will remain obese into adulthood.

Based on this picture of obesity, it is important to examine the cause of a disproportionate accumulation of adipose tissue (AT). Originally viewed as a connective tissue with a storage capacity, presently AT is considered an organized endocrine organ, both vascularized and innervated, with a clear anatomy and a high degree of plasticity responding to both corporeal and environmental changes. In addition to storing excess triglycerides (TGAs) and releasing free fatty acids (FFAs) for fuel, AT in the form of white AT (WAT) or brown/beige AT (BAT) are crucial for immune responses, thermogenesis, fertility and lactation [4–6]. In the human body, AT can range from 5% to 60% of total body weight, with a clear association evident between severe obesity and increased rates of cardiovascular disease and T2D (reviewed by [7]). In the 1950s, Vague was the first to suggest that the regulation of the endocrine and metabolic functions of abdominal AT were controlled, in part, by the anatomical distribution of fat with "android or male-type" obesity associated with T2D and atherosclerosis [8]. It is now

well accepted that AT is not a single homogeneous tissue, but rather a tissue comprised of regional depots, each with a clear metabolic diversity [9–11]. Importantly, it is these individual adipose tissue depots that are more closely related to the obesity-associated comorbidities than the total AT mass, reinforcing the concept of depot-specific metabolic functions [9–11]. For example, an increase in abdominal AT is a risk factor for the development of metabolic diseases, while an increase in gluteo-femoral AT has been described to have a clear, protective role (reviewed by [12]). This concept is not specifically related to an obese state, with lean subjects who have a high ratio of central to peripheral AT found to be insulin-resistant (reviewed by [13]) and the existence of "metabolically obese but normal weight" subjects [14]. Here we will review the characteristics of the AT depots, with a clear focus on abdominal AT depots, highlighting the characteristics that link their depot-specific biology to normal health and to the obesity-associated complications.

Adipose tissue distribution

Adipose tissue depots

While well used in a clinical setting to define obesity, the body mass index (BMI; kg/m^2) does not clearly represent the underlying complexity of obesity. Large-scale association studies in adults and children have demonstrated that individuals with the same BMI can have quite different metabolic disease risks, emphasizing the importance of the development of novel predictors of long-term risks, particularly with respect to the pediatric population [15, 16]. One explanation for this, as Vague eluded to in 1956, is the variation in the anatomical distribution of AT depots. In fact, AT is not a homogenous organ, but includes numerous and quite distinct anatomical depots.

On the broadest of scales, the principal depots of WAT include visceral AT (VAT), centrally located and enclosed by the peritoneum, the subcutaneous AT (SAT) located directly below the skin, and finally ectopic AT, which consists of depots in localities not directly associated with storage (reviewed by [7, 17]). The SAT compartment comprises >80% of the total body fat and consists of gluteal, femoral and abdominal AT, with the abdominal AT further sub-divided by Scarpa's fascia into superficial SAT (sSAT) located below the epidermis and deep-SAT (dSAT) adjacent to the peritoneum visible with computed tomography (CT) and ecography [18] (Figure 1). Between 10% and 20% of total body fat, a proportion that is lower in both adult and pediatric females [19, 20], is intra-abdominal or VAT that is found to be associated to the internal organs. VAT is located within the abdominal cavity packed around the internal organs, in particular the digestive organs, and includes the omental AT, which is associated with the stomach, mesenteric to the intestine and epiploic to the

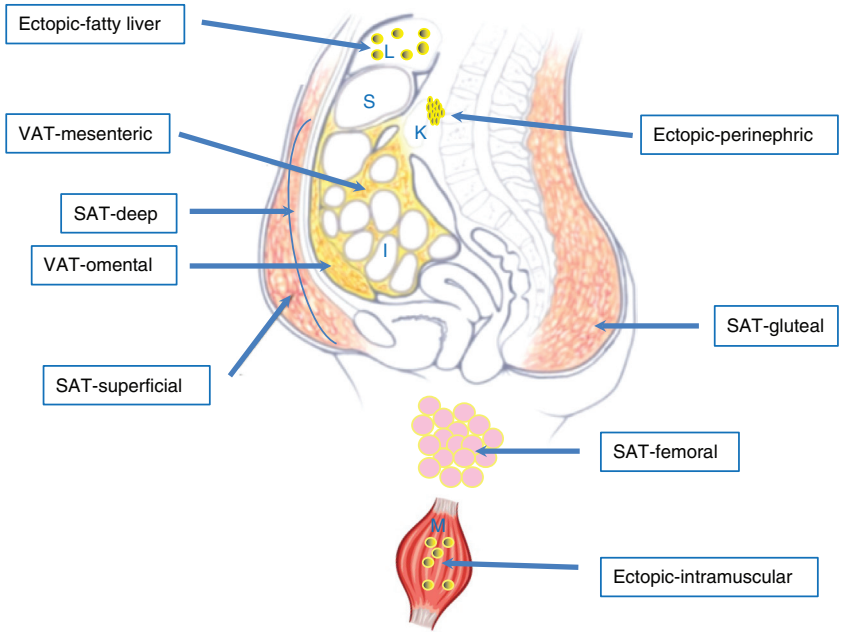

Figure 1 The major adipose tissue depots in humans. L, liver; K, kidney; S, stomach; I, intestine; M, muscle. The figure is an extensively modified line diagram from the Mayo Foundation for Medical Education and Research.

colon. Posteriorly in the retroperitoneum are deposits of retroperitoneal AT delineated by the dorsal borderline of the intestine and ventral borderline of the kidney, which tend to be more evident and influential in adult males making up 42% and 31% of the intra-abdominal fat mass in adult obese and lean males, respectively [21]. Finally, the depots that comprise ectopic AT depots include intrahepatic or "fatty liver", epicardial AT localized around the heart, and perinephric or renal sinus linked to the kidney, pancreatic, intramuscular and perivascular AT, which is a sheath of AT surrounding most blood vessels. Ectopic AT depots are believed to arise as a result of the metabolic disarrangement of VAT, however the principal hypothesis is that these depots occur as a result of the inability of SAT to store excess lipids; in effect a "spillover" from SAT to VAT and eventually to other organs and tissues [17].

Methods of abdominal fat assessment

The non-invasive assessment of body fat is an important component in obesity research and fat measures are useful biomarkers for stratifying patients and evaluating the efficacy of therapies. Assessing body composition in obese people is challenging, however, because obesity is characterized by an increase in body fat and changes in body composition different from that of normal weight individuals. In particular, an increase in total body hydration and a relative expansion of the extracellular water component are typical of obesity.

Numerous techniques are available for body fat assessment, with a different validity in obesity and specific populations; the strengths and limitations being due to the accuracy, costs, safety and portability of the device. Apart from anthropometry, the most frequently used in research and clinical practice are hydrodensitometry, air displacement plethysmography, bioelectric impedance and dual energy X-ray absorptiometry (DXA). All of these, however, are indirect techniques that measure body density or resistance, with body fat percentage derived from specific equations (for review see [22–24]). These indirect methods are not able to differentiate between SAT and VAT or identify ectopic fat in the liver, muscle, epicardial tissue and so forth.

Starting with anthropometry, which is the least expensive, BMI is the most frequently used index in epidemiological studies. It is a surrogate index of overall adiposity and many studies have shown its validity in the prediction of body fatness in age-, sex- and ethnic-matched populations, as well as for disease risk and mortality. Despite this, in obesity BMI does not show a strong correlation with body fat, supporting the use of corrections such as leptin concentrations to increase the accuracy of BMI [25]. Further, BMI does not identify fat compartments, in particular VAT [24]. Diversely, waist circumference, hip circumference, waist-to-hip ratio and waist-to-height ratio are indirect indexes for VAT or SAT. The first two have been validated against measures derived by DXA, CT scan and magnetic resonance imaging (MRI) [24, 26, 27]. It is thought that waist circumference represents VAT and SAT, while hip circumference reflects SAT only, with the biological significance of the last being less clear [28]. Their correlations, however, with fat mass vary by sex, ethnicity, life stages and other as-yet unknown factors [29]. It seems that waist-to-hip ratio provides no advantage over waist circumference alone [24]. Moreover, the accuracy of these measurements is relatively low, being dependent on the training of the person taking the measurements, with high intra- and inter-individual variability often observed. There is also evidence that fat distribution measurements have an added value in predicting morbidity and mortality among patients who are of normal weight or who are moderately overweight, but not in those who are obese, particularly the morbidly obese. The waist-to-height ratio is adjusted for frame size (represented by height) and is simple to use. There is, however, no evidence that it is a better predictor of morbidity and mortality than waist circumference or the waist-to-hip ratio, and because it is composed of the ratio of two complex variables, its interpretation is quite complicated. In fact, for these measurements, corrections using blood biomarkers for the prediction of VAT and SAT and the stratification of patients are ongoing [30].

CT and MRI are the most accurate techniques available for the measurement of fat sub-compartments, however they remain highly expensive, are not portable and have huge limitations in clinical practice. Both CT and MRI provide high-resolution cross-sectional scans of selected tissues or organs and can be used to measure the volume and distribution of SAT, VAT, muscle mass, and organ composition, including ectopic fat. A CT scan uses X-rays and the reconstruction of total body mass and separate organ masses based on scans along the length of the body at 10-cm intervals and has been shown to have excellent accuracy and precision (<1% for both) [22, 31]. While single-slice images are often used in research studies to predict whole body compartments to reduce costs and radiation exposure, they are less accurate than the multislice because soft-tissue structures are continuously moving and may negatively affect the reliability of the VAT measurement. Furthermore, intra-subject variability and the fact that fat loss in the abdomen is not uniform

reduce the accuracy of single-slice images [22, 24, 32]. A computer-aided non-contrast CT scan is able to measure pericardial and thoracic fat [33]. Due to the high radiation exposure, CT scans are limited in research studies and are not suggested or allowed by ethical committees for specific populations, such as children.

In contrast to a CT scan, MRI does not use X-rays and as such is safe and more applicable for all populations, including neonates. Along with the CT scan, multi-slice volume MRI is considered the gold standard for measuring total and regional AT depots, as demonstrated by a comparison with dissection in human cadavers [34]. The single-slice imagines with the prediction of VAT have the same limitations as discussed above for the CT scan. Although X-ray-free, MRI cannot be used for individuals who suffer from claustrophobia or who are morbidly obese (BMI >40 kg/m^2) and cannot fit within the field-of-view. This is also a notable problem for CT scanning.

New techniques have been developed and are being validated. Chemical shift imaging and magnetic resonance spectroscopy separate water and fat signals and are applied to an MRI. They are used to detect ectopic fat and integrate MRI information on body fat distribution [35]. Although expensive, they open new perspectives to better explore body fat compartments in relation to diseases and biomarkers. More recently, the quantitative MRI has been developed for body composition assessment in humans after validation in animal studies. It is able to detect small changes in fat mass and is superior to the other techniques, although some discrepancies are present with respect to the gold standards. It is also very fast (<3 min) and overall seems promising [24, 36]. In addition to these methods, studies to validate ultrasound techniques in the measuring of SAT and VAT in the abdomen and for muscle thickness are ongoing [37]. New devices with specific software are needed to increase their accuracy and reduce operator variability in order to provide a method that is reliable in both clinical practice and epidemiological studies.

Cellular composition and remodeling of SAT and VAT

Cellular composition of SAT and VAT

The AT is divided broadly into two principal categories of cells: adipocytes, the main parenchymal cell type; and the remaining cellular components collectively termed the stromal vascular fraction (SVF). The SVF includes preadipocytes, fibroblasts, endothelial cells, multipotent stem cells and immune cells such as macrophages, neutrophils, lymphocytes and T-cells [38]. While conceptually it seems that adipocytes make up the majority, in fact the SVF outnumber the adipocytes with 4–6×10^6 cells/g of AT vs. 1–2×10^6 adipocytes, applicable to both healthy and obese subjects. This is explained by the fact that adipocytes occupy a larger space, making up approximately >90% of the tissue volume. This is due to their unique morphology, in that they contain a single large lipid droplet which occupies up to 90% of the cell volume, with the cytoplasm comprising a thin rim, the nucleus squeezed into the periphery, and poorly formed mitochondria [38]. It is the size of the lipid droplet that determines the adipocyte cell size, which can range from 20 μM to 200 μM. Interestingly, adipocytes are the only cell type whose size may vary significantly in response to physiological conditions.

Depot-specific differences in the cellular composition of AT are evident and demonstrate obesity-dependent influences. Due to the limitation of reproducible isolation methods and standardized cell size measurements, inter- and intra-individual data regarding depot-specific cellular characteristics are few [39]. Adipocytes in general are smaller in VAT as opposed to the SAT of lean and healthy overweight subjects, with the size differences and cell numbers less apparent in overweight and obese populations, independent of gender [40]. While SAT is considered the safer AT, large subcutaneous fat cells have been shown to be associated with insulin resistance and a high risk of developing T2D, with recent studies following Roux-en-Y gastric bypass in women, demonstrating that a reduction in SAT fat cell size correlates to improved insulin sensitivity [41]. In dSAT, with respect to sSAT, adipocytes are smaller, highlighting the presence of a gradual decrease in adipocyte diameter moving from the skin to the abdomen [42]. At an individual level, Garaulet et al. [40], observed that SAT adipocytes were larger than perivisceral adipocytes, while no differences were observed between SAT and omental adipocytes.

With respect to the SVF, the number of SVF cells is higher in VAT as opposed to SAT, with 10% of omental SVF endothelial cells, while for SAT the levels are almost negligible and not distinguishable between sSAT or dSAT [42, 43]. Of the SVF, preadipocytes that give rise to new fat cells occupy between 15% and 50% of AT, with the preadipocyte cell population said to be higher in SAT than VAT [44, 45], with no differences observed between sSAT and dSAT [42]. The VAT has a larger accumulation of lymphocytes, including B, alphabetaT, gammadeltaT, natural killer and natural killer T cells, due to the presence of lymph nodes. It has been proposed that VAT-specific lymphocytes are

representative of innate immunity, while SAT lymphocytes are part of adaptive immunity (reviewed by [46]). Interestingly comparing sSAT and dSAT, dSAT had a higher CD3+ T lymphocyte infiltration, which the authors propose could be the motive for a decreased adipogenic activity and decreased adipocyte size in dSAT [42].

An alteration in lymphocyte number, type and activity is said to precede macrophage infiltration and subsequent depot-specific AT inflammatory state, with the number of macrophages present coincidental to the presence of insulin resistance [47]. Notably, the number of macrophages is significantly higher in VAT as opposed to SAT, with no differences observed between sSAT and dSAT in healthy overweight subjects [42, 48]. With progression to an obese or morbidly obese state, an increase in macrophage infiltration has been observed, with higher infiltration in dSAT as opposed to sSAT [49]. Macrophage infiltration has also been demonstrated to be positively correlated to the size of the adipocytes in both VAT and SAT depots independent of obesity, with a higher presence of hypertrophic adipocytes as opposed to hyperplastic adipocytes. This has been demonstrated in lean mice deficient for hormone-sensitive lipase that have a predominance of hypertrophic adipocytes and a greater monocyte infiltration [50]. Macrophages, along with neutrophils also migrate in response to the mesothelial cell population, which have a large presence in omental AT as opposed to SAT, being found surrounding the fat lobules. Unlike SAT, omental AT contains mesothelial cells that have also been identified in epicardial AT [51].

Differences in growth and differentiation

An accumulation of AT is dependent on the proliferation of preadipocytes, their differentiation into new adipocytes and the hypertrophy of existing adipocytes. Early studies defined obese phenotypes in terms of AT cellularity, introducing the terms “hyperplastic” and “hypertrophic” obesity [52] with each being influenced by fasting insulin and insulin sensitivity, diet, genetics, physical activity and the environment [53, 54]. Hyperplasia, an increase in cell number, and hypertrophy, an increase in cell size, are two growth mechanisms for AT. The hyperplastic growth of AT occurs in the early stages of AT development with a genetic foundation [55, 56], with hypertrophy occurring prior to hyperplasia to meet the need of for additional fat storage capacity [56, 57].

Adipocyte number – while an important determinant of fat mass – remains constant in adulthood, with adipocyte cell numbers increasing and being set during childhood and adolescence with a 10% turnover annually for all adult ages and BMIs [55, 58]. Even with extreme weight loss, fat cell numbers remain constant, confirming that the differences observed in fat cell number between lean and obese subjects is set during childhood [55]. Interestingly though, depot-specific differences in the proliferation of primary culture preadipocytes have been observed, with SAT preadipocytes proliferating faster (4±1 days) than those from the omentum (5±1 days), independent of BMI and gender [43]. It has been noted, however, that the proliferation rate of SAT declines with the age of the individual, while VAT demonstrates no correlation, supporting the concept that there is an age-associated preference for the proliferation of VAT with time [43, 44] as opposed to BMI. The importance of hypertrophy versus hyperplasty with fat mass accumulation remains conflictual however, with recent observations of patients who underwent an omentectomy and bariatric surgery demonstrating that omental weight are primarily determined by adipocyte number and – to a lesser degree – by adipocyte size, suggesting that increased VAT mass in obesity is predominantly dependent on adipocyte proliferation [54].

Key to the accumulation of AT is the differentiation of preadipocytes to adipocytes. Studies regarding depot-specific differences in differentiation are conflictual. Consistent is the concept that VAT preadipocytes are fewer [44, 45, 59], however some believe that VAT with respect to SAT has a lower ability to differentiate [59, 60], in our case even in the presence of thiazolidinediones such rosiglitazone [61], while others observed no differences between SAT and VAT [43]. Studies examining femoral preadipocyte differentiation compared to SAT claim that femoral preadipocytes exhibit a lower differentiation capacity [62]. Despite the fact that these studies use primary culture cells in their first passages, it is important to remember when interpreting the data that differentiation models require a clear endocrine interaction and most often an in vitro environment may not truly reflect in vivo events as they exclude the cellular composition, vascularization and innervation that are actual and may vary according to individual subjects.

Adipose tissue has come a long way with respect to its earlier definition of being strictly a storage organ. Recent evidence highlights a high degree of AT plasticity, where adipocytes have the ability to transdifferentiate as a result of extreme cold and physical activity, turning from a storage tissue to one that burns energy [63, 64]. It has been demonstrated in rat models that the sympathetic activation via treatment with 3-adrenergic receptor agonists, an extreme cold stress or the knockdown of orexigenic neuropeptide Y in the dorsomedial hypothalamus,

causes transdifferentiation in various WAT depots in rats, including the retroperitoneal, mesenteric, epididymal and inguinal depots [65, 66]. These findings are interesting in the sense that adult humans have increased BAT activity during cold exposure, but decreased BAT activity when they are overweight or obese, with animals lacking a functional BAT prone to developing obesity and T2D [67, 68]. In humans, BAT depots are found above the cervical-supraclavicular, perirenal and paravertebral regions, yet these classic BAT adipocytes are considered to be ontogenetically different from transdifferentiated WAT adipocytes [69, 70]. As such, white-to-brown adipocyte transdifferentiation has become of therapeutic interest in a biomedical setting. Animal model studies have demonstrated that a higher BAT content is positively associated with resistance to obesity and its comorbidities [71] and that the "browning" of WAT, in particular VAT, is able to reduce these comorbidities in both animal models [72–74] and humans [4, 75]. In the search for BAT activators for metabolic benefits, very recent reports have highlighted the cold-activated cytokine irisin in combination with fibroblast growtn factor 21, which are able to up-regulate human adipocyte brown fat gene/protein expression and thermogenesis in a depot-specific manner [76].

Turnover

As described, fat cell numbers are set in late childhood/early adulthood, with changes in fat mass in adulthood attributed to fat cell volume. Spalding et al. [55], in pioneering experiments used the integration of ^{14}C into genomic DNA from nuclear bomb tests to demonstrate that in fact 10% of fat cells are renewed every year for all adult ages and BMIs, suggesting that the generation of adipocytes may be balanced by adipocyte cell death, with the total number tightly regulated and constant. It has been demonstrated from a lipoaspirate of AT that 1% of adipocytes isolated show signs of necrosis and 20% apoptosis [77]. The concept of adipose tissue apoptosis, a programmed cell death designed to maintain tissue homeostasis, is not a new one. Prins et al. [78] were first to show that following growth factor deprivation or mild heat injury, adipocytes undergo apoptosis. At a depot-specific level, between human omental and SAT preadipocytes treated in serum-free medium or with tumor necrosis factor (TNF)-α, the omental preadipocytes were described to be more susceptible to apoptosis [60, 79]. This was also observed in the VAT of a bovine model of lactation treated with a diet high in conjugated linoleic acids [80].

A TNF-α receptor, TNFR1, is a known death receptor that once bound activates the apoptotic cascade via its cytoplasmic death domain, to assemble pro-caspae-8 that when converted, activates other downstream caspases as well as other proteins including FLICE-like inhibitory protein, whose down-regulation is central to SAT-related differentiated adipocyte apoptosis [81]. TNFR1, as well as other death receptors such as CD95 and TNF-related apoptosis-inducing ligand receptors 1 and 2, are expressed in human AT (reviewed by [82]). This differential depot-specific apoptotic susceptibility of preadipocytes [60, 79], which highlights a preferential loss of VAT, suggests that targeting VAT adipocyte apoptosis could be a novel strategy for treating obesity [83]. This targeted induction of VAT apoptosis could, however, be of concern due to increasing the circulating levels of released lipids, increased ectopic lipid storage and the resulting overall metabolic effects including an effect on WAT transdifferentiation, and the use of BAT in treating obesity [4, 75].

Determinants of abdominal fat distribution

Abdominal fat distribution is strictly linked to several dynamic and fixed determinants. The principal determinants are age, gender and ethnicity. Despite the long-term knowledge of these determinants beginning with Vague et al. [8] in the early 1950s, their mechanisms remain to be fully clarified.

Age

Humans are quite unique among the known mammals in that they are born with relatively large levels of AT. The fat content at term has been estimated to be 15% of the total body weight, as opposed to other mammals in which it is lower than 2% [84]. Cross-sectional studies have shown that the adiposity peak in is attained at 4–6 months of age, when the fat mass is about 25% of whole body weight. This increase is mainly located in the arms with respect to the trunk, with a predominance of SAT that comprises 89% of the total fat mass [85]. Moreover, there is some evidence to suggest that accelerated growth may lead to increased body adiposity in this period [86]. Adiposity in newborns, in contrast, is also modulated by maternal BMI, ethnicity, gestational age and sex [87]. Due to the risks associated with CT scans and the high costs of MRI, there are few data available on the distribution of AT in newborns and infants, including the deposition of ectopic fat.

After this first period, total fat progressively decreases, with a nadir of 15% at 5–7 years of age. Along with the progression to puberty, although there is a more prominent fat-free mass deposition, children increase their fat mass, in particular SAT on the trunk; however the data regarding this are imprecise due to the lack of consistent longitudinal studies [87, 88]. In young women and men and in middle-aged women increase in weight is dependent on SAT accrual [89], and an approximate doubling of body fat from 20 to 50 years has been recorded. VAT in the abdomen also progressively increases, with more in men and in post-menopausal women [90, 91]. Aging is characterized by a redistribution of body fat: an increase in fat mass, with a specific increase in VAT and ectopic fat and a reduction in SAT has been shown, while with extreme old age this process is reversed [88, 92]. The increase in fat mass can be unassociated with weight changes due to a contemporary reduction in lean mass.

Gender

The amount and distribution of body fat is sexually dimorphic. This is already detectable in newborns and infants, with females at birth presenting a higher total fat mass, primarily constituted by SAT [93]. With advancing puberty, SAT increases slightly more in girls than in boys, while males deposit more VAT in late-stage adolescence. These gender differences are missing in juvenile obesity [87, 88]. Young women have 34% more body fat than young men, after adjusting for height, with the interpretation that this is an adaptive mechanism for reproduction [88]. It has to noted that during pregnancy, more than half of the weight accumulated up until the second trimester is fat, and this is mainly SAT that will be used during the lactation period [94]. In *Maturitas*, it is stated that men tend to have a central fat distribution, whereas women tend to have a peripheral fat distribution, deposited in the limbs and hips. As previously discussed, young and premenopausal women have less VAT even though they have higher total body fat and abdominal SAT [91]. The VAT is higher in men than in women with similar waist circumferences and starts to increase in females when they reach the post-menopausal period [90]. Women also seem to have more sSAT and men more dSAT [95]. With aging, the period of the increase of VAT is more pronounced in females than in males, however this sexual dimorphism has not been fully elucidated [96].

Racial differences

Significant ethnic differences in AT deposition have been observed independent of sex and age. South Asian newborn babies have a higher fat mass, in particular VAT, compared to their Caucasian or Afro-Caribbean counterparts [87]. Despite this, data regarding the accumulation of total and ectopic fat in children and adolescents with respect to ethnicity are contrasting. The risk of obesity and cardiovascular disease, also in adolescence, seem more closely linked to a higher inflammatory state of the AT, both from SAT and VAT [87, 97, 98]. These results should be confirmed with more studies on minority populations. In adulthood, Asian, particularly Indian individuals seem to accumulate more VAT, despite lower adiposity values with respect to other ethnic backgrounds [99]. The increase in fat mass with aging seems to be more pronounced in African-American people than in Caucasians. Conversely, for both African-American and Asian individuals the aging decrease is seen earlier than their Caucasian counterparts, independent of weight and BMI, although these data should be confirmed [96]. It is likely that genetics in combination with environment plays a substantial role in these inter-racial AT-deposition profiles.

Abdominal adipose tissue depots and metabolic complications

While overall fat mass is important for the development of insulin resistance and obesity-associated comorbidities, it is evident that specific fat depots are more closely linked to disease-risk factors than others [7]. These principal VAT and SAT depots show inter-individual variation with respect to age, nutrition and race, as discussed above. Of the two, however, VAT accumulation is central to android obesity and is an independent risk factor for obesity-related metabolic and cardiovascular disorders, in particular insulin resistance, T2D, high TGAs, low HDL cholesterol, hypertension, metabolic syndrome and cancer [100]. In agreement with these observations, the surgical removal of VAT in rats was followed by an improvement in insulin sensitivity and a delay in the onset of T2D [101]. Premenopausal women in which SAT is predominant have a higher insulin sensitivity and are protected by the risk of diseases cited above [102, 103]. It has to be noted that the association reported between VAT and diseases in numerous epidemiological studies used surrogate indexes of VAT, such as BMI, waist circumference, waist-to-hip ratio or waist-to-height ratio. It is necessary in the future to confirm these results using more specific methods of fat depot measurements (reviewed by [103]). Despite this, however, the Health ABC Study conducted in more than 3000 adults of VAT measurements using CT

scans showed that VAT is associated with the metabolic syndrome in both normal-weight and obese individuals [104]. Interestingly this study also reported a different role for SAT dependent on its localization: SAT in the abdomen was directly associated with the metabolic syndrome in normal-weight and obese men; while larger gluteo-femoral SAT was inversely associated with metabolic syndrome in both obese men and women [105]. The results observed for abdominal SAT could be dependent on the fact that SAT is composed of sSAT and dSAT, where dSAT but not sSAT has been demonstrated to correlate with metabolic impairment similar to that reported for VAT [9, 11]. Some mechanisms have been advocated to explain the causative role of VAT on these diseases, in particular lipolysis and the increased sensitivity to catecholamines, the flow of FFAs to the liver through the portal vein, the accumulation of inflammatory cells, an alteration in the production of adiponectin and in the function of PPAR-γ signaling, a lower angiogenetic capability and hypoxia [17, 100].

More recently, the role of ectopic fat has been widely discussed in relation to its contribution to the development of the cited diseases. In particular, ectopic fat in the liver and in the muscle seem to be two of the main and precocious contributors to insulin resistance and T2D in adults, children and adolescents. Furthermore, an association between a specific fat depot and the derangement in its organ role has been hypothesized, in particular for pericardial, periaortic, intramyocardial and renal fat [17]. It is clear that the systemic and local regulation of fat, divided into subgroups of depots, is still in its infancy, with many points remaining obscure including the role omental fat. Epidemiological studies supported by more precise techniques to measure the different depots will be crucial to dissect the role of each type of fat and their relationship.

Differences in the metabolic activity of abdominal fat depots

Lipolysis

Lipolytic dysregulation is associated with the progression of obesity and is key in the development of obesity-associated comorbidities, with depot-specific differences a major contributor to the overall circulating FFA levels. Lipolysis involves the hydrolysis of TGAs stored in adipocytes, into 1 glycerol and 3 FFAs by lipases that include adipose tissue TGA lipase and hormone sensitive lipase (HLS) (reviewed by [106]). The FFAs bind to albumin, where they are transported to the muscle, liver or AT where they undergo oxidation, TGA synthesis or re-esterification depending on the tissue destination, while glycerol is used for hepatic glucose production. Lipolysis can be induced by a drop in insulin levels, or by epinephrine, norepinephrine, ghrelin, growth hormone, testosterone and cortisol binding activating adenylate cyclase, increasing the production of cyclic-adenosine monophosphate, which activates protein kinase A and eventually hormone sensitive lipase within the AT [106]. It is well documented that FFA release increases with an increasing VAT mass, with in vivo lipolytic activity demonstrated to be greater in the upper body SAT as opposed to the lower body SAT [107]. In fact the adipocytes of VAT are lipolytically more active than SAT in both lean and obese subjects, and with VAT draining directly to the portal vein system it contributes more to the plasma FFA levels, providing an important link between VAT and the development of insulin resistance [108]. Further, a pathogenic role for VAT adipocyte lipolytic function in NAFLD, independent of total body fat as well as abdominal fat distribution, has been observed in morbidly obese women, emphasizing further the key role VAT lipolytic activity [109]. In considering the division of VAT, it has been demonstrated that the depot-specific lipolytic role is more important for the mesenteric depot as opposed to omental in diabetic obese individuals, with mesenteric adipocytes showing high basal glycerol release and impaired isoproterenol stimulated glycerol release [110]. Likewise, the division of SAT has highlighted that anterior dSAT has a 20% higher lipolytic rate than anterior sSAT following isoproterenol stimulation and glycerol release [111]. Despite these data, however, it is important to note that the omental basal lipolytic rate is similar to SAT. It is its response to adrenergic agonists that is significantly higher [112], likely due to the presence of a higher number of lipolytic β-adrenergic receptors and lower α2-adrenergic receptors [113, 114]. This is further coupled by a weak response to the anti-lypolytic actions of insulin in VAT, which is due to a lower receptor affinity and hence downstream actions [115, 116]. While the response to catecholamine-induced lipolysis in VAT is dependent on the presence of obesity, gender and race as a result of altered adrenergic receptor expression, the anti-lypolytic effects of insulin in VAT are not [115, 117]. Likewise, the response to catecholamine-induced lipolysis in SAT is also dependent on the presence of obesity and gender, however with obesity SAT presents a “protective” lipolytic pattern that contrasts with VAT, with a depletion of β2-adrenergic receptors and subsequent lower catecholamine response to lipolysis and an increased sensitivity to insulin [115, 118]. Despite the protective role of SAT with

the progression of obesity, it is unable to compensate for VAT lipolytic activities, and as a result current studies are focusing their efforts on the normalization of VAT insulin sensitivity and adrenergic receptor lipolytic cascades with novel mechanistic and therapeutic approaches [119–121].

FFA uptake and lipogenesis

Fatty acids (FAs) are energy-rich molecules that play important metabolic roles at the cellular and systemic levels. They are essential components of cell membranes, linked to channel and receptor functions, and there is a demonstration of hormone-like effects that influence preadipocyte cell growth and differentiation [122]. As described, FAs are central to the main function of AT, i.e., the storage of TGA with the unilocular lipid droplets determining adipocyte cell size and the regional growth of AT. At the in vivo level, the regulation of adipocyte cell size and number by FA's in overweight and obese populations has been demonstrated, with depot-specific effects on both cell size and cell number being dependent on the FA composition [40, 123]. Such an increase in fat cell volume occurs primarily by FFA uptake, but also by *de novo* TGA synthesis, with *de novo* synthesis accounting for up to 20% of lipid turnover [124]. Lipogenesis describes both the process of FA synthesis and TGA synthesis, with TGAs formed when FAs are esterified with glycerol.

FA uptake consists of two distinct mechanisms: dissociation from albumin and transport across the membrane. Adipose tissue depot-specific uptake is more evident with small SAT adipocytes having a higher avidity for FFAs and TGA. This preference has been shown in abdominal SAT as opposed to femoral AT [125, 126]. These small, more insulin-sensitive adipocytes act as a "sink" for FFAs and TGAs, however once saturated the spillover effect passes to VAT and eventually ectopic deposits.

Circulating TGAs, which can include very-low-density lipoproteins or chylomicrons, are broken down by lipoprotein lipase (LPL) that deliver FFAs to adipocytes. LLP was originally considered a master regulator of AT lipid accumulation, and has been shown to have clear depot-specific activities. In women, whose omental adipocytes are smaller, the LPL activity is lower than in the larger SAT adipocytes, demonstrating that LPL activity reflects fat cell size [127, 128]. In both normal and overweight/obese men, omental adipocytes are larger and LPL activity greater, which has led to the hypothesis that LPL is a mechanism by which there is a preferential accumulation of VAT in men [128]. Likewise, women, who accumulate more femoral/gluteal fat than men, demonstrate a higher mRNA expression and LPL activity in this region compared to their male counterparts [129]. While no distinction has been given to gender, it has also been demonstrated at the AT cellular level in individuals that there is a gradual decrease in the mRNA expression of LPL from sSAT, through to dSAT and VAT preadipocytes with parallel results following adipocyte differentiation, with dSAT showing an intermediary expression to sSAT and VAT [61]. While hyperinsulinemia has been excluded as a regulator of LPL expression/activity (reviewed by [130]), depot-specific LPL expression has been shown to be regulated by sex steroids, glucocorticoids and catecholamines (reviewed by [7]).

Although LPL has been described as the metabolic gatekeeper for TGA uptake and appears to distribute TGA among tissues [131], humans with genetic defects in LPL and mice with exclusively muscle-specific LPL expression (MCK/L0) have been reported to have a normal body weight and fat distribution [132]. These data support a tissue- and AT depot-specific role for *de novo* lipogenesis, which involves the carboxylation of acetyl-CoA by acetyl-CoA carboxylase, which in a multistep process is converted to palmitate by fatty acid synthetase [133]. Studies regarding AT-depot-specific *de novo* lipogenesis are an exploding area. A recent mRNA evaluation of key lipogenic enzymes in the SAT and VAT have demonstrated that a lower expression of SAT-specific acetyl-CoA carboxylase 2 correlates to higher circulating FFA and that following bariatric surgery on 23 morbidly obese individuals, lower SAT and VAT mRNA levels were associated with an improvement in anthropometric variables [134]. Glucocorticoids and insulin have opposite roles in regulating AT *de novo* lipogenesis in a contrasting fashion [135]. While the effects were similar between VAT and SAT, demonstrating a key role for 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) which is responsible for the cortisone:cortisol shuttle, insulin was unable to inhibit the dexamethasone down-regulation of lipogensis in VAT, suggesting glucocorticoids are central to VAT-associated insulin resistance [135]. A contribution is likely from the depot-specific expression of 11β-HSD1; in lean subjects there is a progressive decrease in expression from sSAT, through to dSAT and VAT, with obesity specifically modulating sSAT 11β-HSD1 with respect to dSAT [10, 11]. Studies using a transgenic mouse model with a humanized lipoprotein profile exposed to a high-fat diet demonstrated a down-regulation in SAT and mesenteric AT *de novo* lipogenesis, highlighting a potential role for the androgen receptor in this regulation [136].

TGAs are also derived from glucose, which is transported into adipocytes by glucose transporters (GLUTs).

Of the now 13 members of the GLUT family, the insulin-responsive GLUT4, translocates to the cell membrane enabling an increase glucose uptake [137]. The expression of GLUT4 has been shown to be AT-specific, with dSAT showing independent intracellular protein expression with respect to sSAT and VAT in lean subjects and with sSAT and dSAT expression decreasing significantly in the presence of an obese state [10, 11]. The expression of GLUT4 is highest in VAT, and in combination with a greater blood flow to this tissue there is a likely greater uptake of glucose. In fact, there is a greater glucose uptake per kg of AT in VAT as opposed to SAT, with no observation that VAT is resistant to insulin-stimulated glucose uptake [138]. There is, however, recent evidence demonstrating that VAT can be influenced in animal models of extreme stress [139].

Adipokine production

The finding in the early 1990s that AT synthesizes and secretes leptin established that AT does not simply store and mobilize TGAs but is also an endocrine organ that can communicate with the central nervous system [140, 141]. It is now well accepted that AT secretes numerous hormones and bioactive molecules, with the list continually growing. These adipokines act in an endocrine, autocrine and paracrine fashion to regulate AT metabolism and other key metabolic organs including liver, muscle, the central nervous system and pancreas (reviewed by [142]). This intricate network, in turn, is able to regulate appetite, energy balance, immunity, insulin sensitivity, angiogenesis, blood pressure, lipid metabolism and homeostasis [142]. As a consequence, dysfunction in the adipokine pathways results in impaired organ communications that in turn effect multiple tissues, and lead to the development of metabolic diseases (reviewed by [143]). Depot-specific differences in adipokine secretion are evident in both the lean and obese states, adding to the complexity of AT endocrine regulation, with secretion profiles depending not only on the activity of adipocytes but also other cellular components of AT, such as monocytes.

Adipocytes produce a large array of proteins, with the most notable among these being leptin and adiponectin, which regulate appetite, AT metabolism, innate immune function and reproduction [143]. Serum leptin levels are positively correlated with the amount of body fat [144, 145], with greater synthesis and secretion seen in SAT that is more pronounced for women, explained by the predominant SAT deposition in females and the larger SAT adipocytes [146, 147]. In a division of sSAT and dSAT in lean and obese subjects, significantly higher endogenous protein expression was found in sSAT in both groups, with the presence of obesity having a greater influence on leptin production sSAT, with the sSAT production most likely accounting for the positive correlation of obesity and circulating leptin concentrations [10, 11]. Adiponectin, which is exclusively and abundantly produced by the adipocytes in AT, follows a contrary pattern to leptin with the appearance of obesity. Circulating adiponectin levels have been shown to be inversely correlated with fasting insulin, TGA, blood pressure, waist-to-hip ratio, VAT and SAT. Circulating levels are more strongly influenced by VAT, unlike leptin, which is more influenced by SAT, in particular sSAT [11, 145]. In the division of the anterior abdominal SAT depots, adiponectin protein levels were 16-fold higher in sSAT with respect to dSAT in the lean population, with sSAT adiponectin production predominantly being modified by obesity with a seven-fold down-regulation across the obese subjects studied [11]. Further, between sSAT, dSAT and VAT in lean subjects, the sSAT adiponectin production was the most responsive to the presence of thiazolidinediones with preadipocyte differentiation [61]. Adiponectin with its anti-inflammatory properties, including the ability to reduce TNF-α and coordination of the clearance of apoptotic cells by macrophages, makes adiponectin a focus for obesity-related therapeutic strategies [148, 149], with AT depot-specific studies highlighting the importance of sSAT for the plasticity of adiponectin expression [11].

Adipocytes are key to the increased pro-inflammatory state seen in obesity and T2D, synthesizing both pro-inflammatory and anti-inflammatory proteins. As expected, the expression and synthesis of the proinflammatory cytokines such as interleukin-6, interleukin-8, monocyte chemoattractant-1, plasminogen activator inhibitor-1 and release of regulated on activation normal T cell expressed and secreted protein [150–153] are higher in VAT with a positive association to obesity, while interferon γ-inducible protein-10 is higher in SAT [154]. Results regarding the depot-specific production of TNF-α synthesis and secretion are conflicting [10, 155]. The dSAT-specific mRNA expression of TNF-α has been shown to correlate with insulin resistance, suggesting that dSAT may in fact be a critical point in altered insulin sensitivity [10]; however, due to its spontaneous activation under stress, TNF-α results remain difficult to interpret.

As described, AT secretes an array of bioactive molecules, with the list continually expanding. Bearing this in mind, it is impossible to cover the entire literature in the present review, despite its eminent importance. Recent and potentially key AT-derived proteins relevant

for obesity and its comorbidities include omentin, a VAT-specific adipokine that increases insulin signal transduction through the activation of protein kinase Akt/protein kinase B and enhancing insulin-stimulated glucose transport in adipocytes [156]. Omentin levels decrease in proportion to obesity and the level of insulin resistance, and as such omentin has been proposed to play a protective role for VAT [157]. Lipocalin-2, another novel adipokine that transports small hydrophobic molecules in circulation, is positively associated with obesity and has a strong correlation with pro-inflammatory factors; however, this up-regulation is apparent in both SAT and VAT depots, with the contribution of other AT depots remaining to be investigated [158]. Visfatin, a novel protein in circulation with potentially important insulin-like effects, has serum concentrations that increase with T2D, gestational diabetes and impaired glucose tolerance [159]. Visfatin is secreted by VAT tissue, as well as leucocytes and hepatocytes [160]. To date, however, no differences between SAT and VAT have been identified in either normal or disease states, with the contribution of other depots remaining unknown [161, 162]. Adipose tissue has also been shown to be a site of synthesis for resistin, a highly debated protein that shows a dSAT- and VAT-specific depot preference in the lean state [10] as well as a dependence on monocyte infiltration [163]. While there are many more adipokines than those we have discussed in the present review, understanding whether there is a depot-specific production that correlates with the appearance of obesity is key for their potential therapeutic value in the treatment of obesity and comorbidities.

Outlooks

Proteomic approach to AT depot-specific roles

As highlighted in the present review, depot-dependent differences in AT physiology are evident, reflecting specialized metabolic functions not only for AT itself but also its interactions with surrounding tissues. Adipose tissue is a highly orchestrated tissue involving receptor and second messenger pathways, with steps and passes that influence hyperplasia, hypertrophy, differentiation, turnover, lipolysis, FFA metabolism and lipogenesis, and the secretome profile. Due to the limitations of the classical molecular biological methods, only pieces of the puzzle have been studied, with studies failing to consider the global, time-resolved changes that are evident in this highly plastic organ. The recent advances in chromatography and electrophoresis, as well as highly sensitive and specific analytical techniques that allow the handling of large numbers of samples with high selectivity and sensitivity, have given birth to the term 'Omics'. This is a general term for a broad discipline concerned with analyzing the interactions of biological molecular components in various 'omes' [164].

"Proteomics," first coined in 1995, is a large-scale characterization of the entire protein profile of a cell line, tissue, or organism, not only from the perspective of expression but also post-translational modifications [165]. A proteomic approach, which involves two-dimensional electrophoresis with highly sensitive protein stains such as Sypro-Ruby and/or mass spectrometry (MS), are now being used to study the different AT-depots in relation to their biochemical, morphological and functional characteristics in both normal and obese phenotypes. Including classic two-dimensional electrophoresis or difference gel electrophoresis in combination with matrix-assisted laser desorption ionization-mass spectrometry with time-of-flight detection, >250 studies from 2000 to the present day can be found in the literature, with Halvorsen et al. [166] having written the pioneering study in human AT. While most of the AT proteomic studies are animal in origin, >15% have examined specifically human AT depots including SAT [167, 168] and VAT [169, 170], epicardial depots [167] and the role of the SVF [171]. Secretome profiles have also been investigated for SAT [172] and VAT [173] tissue explants. Apart from the rapid advancement in available methodologies and instrumentation, researchers are now investigating novel approaches to examine AT plasticity in both healthy and diseased states. One example has been to introduce obesity-associated comorbidities including insulin resistance and examine the SAT secretome, in particular the post-translational modification or glycome. This recent study identified that O- and linked glycosylation modifications are involved in the regulation of adipokine secretion upon the induction of insulin resistance in human SAT adipocytes [174]. An alternative approach has been to investigate the plasmatic profiles of obesity and bring these results back to AT to determine the functional consequences. For example, an evaluation of vitamin D deficiency in pediatric obesity, highlighted the importance of adiponectin and its regulation by $1,25(OH_2)VD_3$, the bioactive form of vitamin D, at the AT level [175]. Depot-specific proteomic investigations with limited transcriptomic and lipidomic profiles have been investigated to compare SAT and mesenteric AT in transgenic mouse model [136]. In vitro studies investigating AT phosphoproteome, insulin signaling [176], the plasma membrane proteome in AT [177], extracellular

matrix proteins [178], high fat diet and caloric restriction [136, 179] have been investigated on a global scale. While still in its early stages, "adipo-proteomics" has made substantial contributions and will continue to do so with improvements and developments in the current proteomic methodologies.

Genome-wide expression profiles of AT depots

Gene expression profiling is the measurement of the expression of thousands of genes at one and the same time to create a global picture of cellular activity. The most common methodology is DNA microarray, which measures the expression of previously-identified target genes. While unlike proteomics, which is able to identify post-translational modifications and protein isoforms, genome-wide expression profiles have made substantial contributions in determining the depot-specific role of AT. A genome-wide profile identified that human mesenteric preadipocytes have an expression profile closer to SAT than omental preadipocytes [180]. An analysis of the gene expression profile of SAT and gluteal adipose tissue combined with their genome-wide DNA methylation patterns identified that the Homeobox gene family appears to be significantly different in these two depots and epigenetically-regulated in a diverse way [181]. While not offering the expansive data of proteomics, gene expression profiling is complementary. The now-exploding area of "modeling" collectively combining these data with the metabolic characteristics of patients or animal models with proteomic data will enable researchers to determine a more complete scenario of AT-depot biology and its contribution to the disease states.

Highlights

In general, with an increased energy intake and a reduced level of physical activity, we are likely to become obese and eventually unhealthy. It is well recognized that obesity is closely associated with T2D, cardiovascular diseases, hypertension, NAFLD, and certain types of cancer, with these comorbidities essentially putting worldwide public health systems under stress. Interestingly, however, the concept that "obesity is paired with being unhealthy" is not always true. There have been demonstrations of metabolically obese but normal weight individuals who can have a lean phenotype yet still exhibit an "obese-like" metabolic dysfunction with T2D and increased cardiovascular risk factors [14, 125]; likewise there of metabolically-healthy obese individuals who are relatively well protected from these complications [182]. Studies in these categories over the past decades have identified a similar key feature, that each person has a particular anatomical distribution of AT. These AT depots possess quite distinct cellular, molecular and metabolic features. The VAT depot, in the form of omental and mesenteric AT and SAT that consists of sSAT, dSAT, femoral and gluteal AT, show inter-individual variations with respect to distribution and volume, being dependent on age, gender, race, genetics, nutritional intake and the autonomic regulation of energy homeostasis. It has been well documented that VAT accumulation is central to android obesity and is the key independent risk factor for obesity-related metabolic and cardiovascular disorders, in particular insulin resistance and dyslipidemia. While we continuously accumulate evidence as to the role of these AT-depots, what remains to be understood, is how we can harbor and harness the key features of these AT depots to reduce the alarming increase in metabolic complications.

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