Biology of upper-body and lower-body adipose tissue—link to whole-body phenotypes

Fredrik Karpe and Katherine E. Pinnick

Abstract | The distribution of adipose tissue in the body has wide-ranging and reproducible associations with health and disease. Accumulation of adipose tissue in the upper body (abdominal obesity) is associated with the development of cardiovascular disease, insulin resistance, type 2 diabetes mellitus and even all-cause mortality. Conversely, accumulation of fat in the lower body (gluteofemoral obesity) shows opposite associations with cardiovascular disease and type 2 diabetes mellitus when adjusted for overall fat mass. The abdominal depots are characterized by rapid uptake of predominantly diet-derived fat and a high lipid turnover that is easily stimulated by adrenergic receptor activation. The lower-body fat stores have a reduced lipid turnover with a capacity to accommodate fat undergoing redistribution. Lower-body adipose tissue also seems to retain the capacity to recruit additional adipocytes as a result of weight gain and demonstrates fewer signs of inflammatory insult. New data suggest that the profound functional differences between the upper-body and lower-body tissues are controlled by site-specific sets of developmental genes, such as *HOXA6*, *HOXA5*, *HOXA3*, *IRX2* and *TBX5* in subcutaneous abdominal adipose tissue *and HOTAIR*, *SHOX2* and *HOXC11* in gluteofemoral adipose tissue, which are under epigenetic control. This Review discusses the developmental and functional differences between upper-body and lower-body fat depots and provides mechanistic insight into the disease-protective effects of lower-body fat.

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Introduction

The distribution of body fat is an important factor that determines metabolic health. Although central obesity is closely related to the development of cardiovascular disease and type 2 diabetes mellitus, lower-body fat is paradoxically associated with improved cardiovascular and metabolic profiles.[1,2] These opposing relationships probably reflect the unique intrinsic characteristics of individual fat depots. The functional differences between multiple regional fat depots, including visceral (omental and mesenteric), subcutaneous (upper body and lower body) and brown fat depots have been reviewed elsewhere.[3] In this Review, we focus on lower-body (gluteal and femoral) fat and discuss the 'protective' properties of these tissues, with a particular focus on their capacity for lipid storage and turnover in comparison with the upper-body depots. Although rodent models have been useful in the study of visceral (epididymal) and subcutaneous (inguinal) fat, it is difficult to translate findings from rodents when examining upper-body and lower-body depots in humans as a result of the absence of comparable lower-body depots in rodents. For this reason, this Review focuses on human studies and provides a detailed link between cellular and physiological studies.

The mechanisms governing the distribution of body fat are complex and poorly understood. As well as the apparent contribution of sex hormones[4] and glucocorticoids,[5] twin studies have repeatedly identified a strong heritable component of both overall obesity and fat distribution.[6–8] Rare monogenic disorders, such as familial partial lipodystrophy, are characterized by substantial and selective loss of lower-body adipose tissue.[9] The functional significance of mutations in genes causing familial partial lipodystrophy (*LMNA*, *PPARG*, *AKT2*, *PLIN1* and *CIDEC*) has been reviewed elsewhere.[10] Furthermore, genome-wide association studies have identified multiple genetic loci associated with the distribution of body fat, independent of overall obesity.[11,12] However, the combined contribution of these genetic loci to the distribution of body fat is small, which suggests the involvement of other determinants, such as regulation at an epigenetic level. Robust data suggest that site-specific expression of early developmental genes direct adipose tissue development and form the mechanistic basis of variant adipocyte function in the different fat depots.[3,13–15] We also review the literature on the role of early developmental genes in adipose tissue in the context of the strikingly divergent functional properties of upper-body and lower-body adipose tissue.

Metabolically healthy obesity

Over the past three decades, a subgroup of individuals with metabolically healthy obesity (MHO) has been clinically recognized.[16] Individuals with this clinical phenotype do not exhibit typical obesity-associated metabolic features, such as insulin resistance, dyslipidaemia and

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Key points

- Upper-body and lower-body fat accumulation exhibits opposing associations with risk of cardiovascular disease and type 2 diabetes mellitus; lower-body fat seems to have a protective role
- The abdominal fat depots have high lipid turnover and demonstrate a vigorous lipolytic response to stress hormones
- The gluteofemoral fat depots sequester lipids that would otherwise be destined for ectopic fat depots
- The characteristic functional differences between adipocytes in the upper body and lower body are probably regulated by site-specific expression of a set of developmental genes that are under epigenetic control

hypertension.[17] Why some individuals with obesity are protected from metabolic complications is unknown; however, the pattern of body fat distribution, rather than overall adiposity, might be one important component.[18] Studies of fat distribution in individuals with MHO and metabolically unhealthy obesity (MUO) have focused on abdominal obesity and employed waist circumference as a surrogate marker of abdominal fat; some studies have also included measurements of intra-abdominal fat that were obtained by single slice CT or MRI.[18] The amount of lower-body fat might also be higher in individuals with MHO than in those with MUO.[19,20] However, the contribution of lower-body fat to MUO has been largely ignored and it was concluded in 2013 that fat distribution *per se* is not sufficient to distinguish individuals with MHO from those with MUO.[18] Although this conclusion is probably a fair assessment of the role of fat distribution, exploratory data from the Oxford BioBank[21] help to explain the opposing relationship between lower-body fat and the MUO phenotype.

The Oxford BioBank is a population-based cohort of apparently healthy men and women aged 30–50 years, the majority of whom have undergone detailed body composition analysis by dual-energy X-ray absorptiometry (DXA) and assessment of metabolic markers, not least including measurement of fasting levels of insulin in serum as a marker of insulin resistance. Women with a BMI in the range of 30–35 kg/m^2 (class I obesity) were assigned to quintiles on the basis of fasting serum levels of insulin; women within this BMI range with low levels of insulin (bottom quintile) were defined as MHO, whereas women with high levels of insulin (top quintile) were defined as MUO (Figure 1).[21] In support of the MUO and MHO phenotype concept, the MHO group had distinctly lower fasting levels of blood glucose and lower fasting levels of plasma triglycerides than the MUO group (F. Karpe and K. E. Pinnick, unpublished work). In accord with previous observations,[18] waist circumference, android fat mass and visceral fat mass were higher in the MUO group than in the MHO group; however, gynoid fat mass and leg fat mass were also lower in the MUO group than in the MHO group. No correlation between visceral fat mass and leg fat was evident in the overall cohort, indicating that lower-body fat might independently influence metabolic health in obesity compared with the visceral fat content (F. Karpe and K. E. Pinnick, unpublished work). Although visceral fat mass was previously shown to associate with MUO independent of levels of abdominal subcutaneous adipose tissue (ASAT), the mass of lower-body fat was not examined in that study.[22] In conclusion, lower-body fat accumulation could, at least to some extent, be an overlooked independent factor in the distinction between MHO and MUO, in addition to abdominal fat accumulation.

At a functional level, the distinction between MHO and MUO has been linked to the concept of adipose tissue expandability.[23,24] The expansion of adipose tissue can occur either by the enlargement of existing adipocytes (hypertrophy) or by the proliferation and adipogenic differentiation of resident adipocyte progenitors (hyperplasia). The overall view is that adipocyte numbers in humans are determined during childhood and remain constant thereafter.[25,26] However, regional adipose depots seem to respond differently to weight gain in adulthood; lower-body fat shows a proliferative response to weight gain whereas the adipocytes in ASAT demonstrate a hypertrophic response.[27] Healthy individuals of normal weight generated 2.6 billion new adipocytes in the femoral adipose tissue depot after 8 weeks of overfeeding. In contrast, a 2 kg increase in ASAT was accounted for by adipocyte enlargement without an increase in cell number.[27] Incorporation of atmospheric ^{14}C into adipocyte DNA has enabled the assessment of the rate of turnover of human abdominal adipocytes *in vivo*. Similarly, radiocarbon dating has estimated the lifespan of the abdominal adipocyte at ~10 years, with 10% of the total number of adipocytes being replaced each year by new cells—a rate not substantially altered by loss or gain of weight.[28] Considering the potential for adipocyte hyperplasia in gluteofemoral subcutaneous adipose tissue (GSAT), studies of adipocyte turnover in this tissue are warranted.

Developmental gene expression

ASAT versus GSAT

Adipocytes are derived from mesenchymal stem cells (MSCs) through a process that initially involves the generation of committed adipocyte precursors or preadipocytes, which is then followed by adipogenic differentiation that results in the formation of mature adipocytes. MSCs are thought to originate from the mesoderm during embryonic development, with different regions producing separate adipose tissue depots.[29] Lineage tracing studies in mice seem to support this view by demonstrating that visceral, but not subcutaneous, adipocytes originate from *Wt1*-expressing cells that are derived from the lateral plate mesoderm.[20] However, early stages in the generation and commitment of preadipocytes from MSCs are poorly characterized and other potential sources of preadipocytes require consideration, including the neural crest,[31] pericytes associated with the blood vasculature[32] and haematopoietic cells originating from myeloid progenitors.[33] It remains uncertain whether the embryonic origins of ASAT and GSAT differ; however, the inherently different expression patterns of early developmental genes in these depots suggests that they have different embryonic origins.[15]

Preadipocytes, isolated from different adipose tissue depots and cultured and differentiated under the same

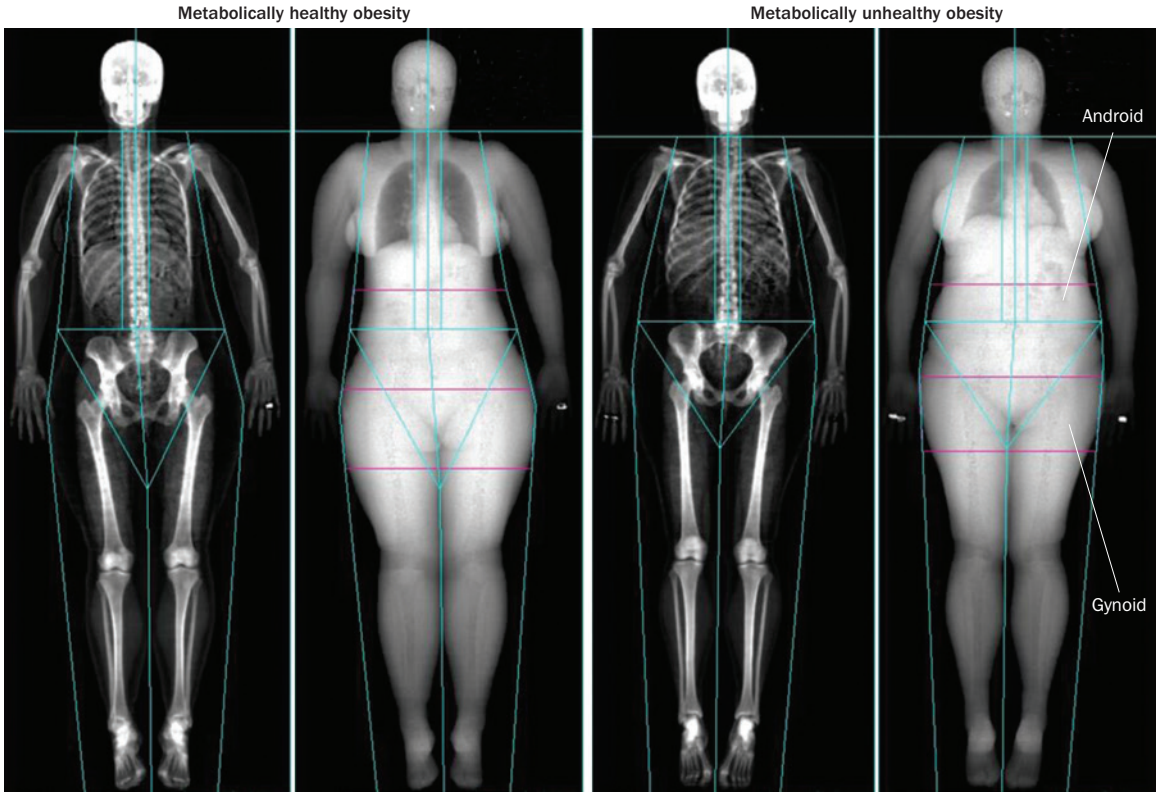

Figure 1 | Opposing association between lower-body fat accumulation and metabolically healthy obesity. Body composition analysis by dual-energy X-ray absorptiometry of a representative pair of women with obesity who are matched for age, lean body mass and total fat mass but different for insulin sensitivity (metabolically healthy versus metabolically unhealthy obesity). Women with obesity (30–35 kg/m^2) aged 30–50 years (n = 234) who were participants in the Oxford BioBank[21] were assigned to quintiles on the basis of fasting serum levels of insulin. Each quintile contained 46 women and 25 pairs matched for age (within 5 years), lean body mass (within 1 kg) and total fat mass (within 1 kg) were selected. Figure shows a representative matched pair of women. An obvious gynoid fat distribution is evident in the metabolically healthy woman (low levels of insulin) compared with the android fat distribution evident in the metabolically unhealthy woman (high levels of insulin). The women whose scans are shown gave consent for their data to be used for research and dissemination of research purposes.

experimental conditions, exhibit distinct differences in their functional characteristics.[34–38] The observation that regional characteristics are retained *in vitro* strongly indicates that adipocyte heterogeneity is inherent rather than a consequence of the local microenvironment. Multiple studies have examined the transcriptional profile of different adipose tissues, both in humans (Table 1)[15,39–43] and in rodents.[44–46] These studies have repeatedly identified marked depot-specific differences in the expression of developmental transcription factors that are involved in organ development and body patterning. Notably, depot-specific differences are not just observed between overtly different fat depots such as brown and white depots,[46] but are also observed between apparently similar depots such as mesenteric and omental depots[44] and upper-body subcutaneous and lower-body subcutaneous depots.[15,43] Considering the importance of body fat distribution in determining metabolic health, and the opposing associations that are observed between metabolic disease risk factors and upper-body and lower-body fat accumulation,[1,2,15] there is a strong need to utilize functional cellular studies to examine the roles of genes that are differentially expressed in upper-body and lower-body adipose tissue depots.

Transcriptional profiling of GSAT and ASAT has identified an extensive list of differentially-expressed developmental genes,[15,43] including members of the homeobox (HOX) family, HOX-domain encoding genes (for example, *SHOX2* and *IRX2*) and T-box genes (for example, *TBX15* and *TBX5*). These genes can be broadly classified as transcriptional regulators that are involved in early embryonic development, body patterning and cell specification. However, it remains to be unambiguously defined whether these regional developmental gene signatures are merely residual markers of differing developmental origins or whether they have an active role in shaping the functional characteristics of adipose tissue. To date there has been only limited investigation of the functional roles of several developmental genes (*TBX15*, *SHOX2*, *TBX5* and *HOTAIR*) in adipose tissue.[14,15,47–49]

HOX genes and adipose tissue function

The HOX gene family consists of 39 genes that are clustered in four chromosomal regions (*HOXA, HOXB, HOXC*

Table 1 | HOX gene expression in human adipose tissue

Study (details)	HOX gene expression	Reference
Gluteal vs subcutaneous abdominal		
Comparison of ASAT with GSAT in 31 men and 18 women (BMI range: 21.4–45.5 kg/m^2)	ASAT: ↑ *HOXA5*, *HOXA7*, *HOXA3*, *HOXA6*, *HOXA9*, *HOXB8*, *HOXB7*, *HOXA1*, *HOXA2*, *IRX2*, *IRX1*, *MSX2* GSAT: ↑ *HOTAIR*, *SHOX2*, *HOXC10*, *HOXC11*, *HOXC10*, *HOXA11*	Pinnick *et al.* (2014)[15]
Comparison of ASAT and GSAT in 21 men (BMI: 27.2±4.5 kg/m^2) and 14 women (BMI: 27.3±6.2 kg/m^2)	ASAT (both sexes): ↑ *HOXA3*, *HOXA5*, *HOXB8*, *HOXC8*, *IRX2*, *PBX3** ASAT (men only): ↑ *HOXA2*, *HOXAS*, *HOXA9*, *HOXB7*, *PBX1**, *MEIS1** GSAT (both sexes): ↑ *HOXA10*, *HOXC13*	Karastergiou *et al.* (2013)[43]
Comparison of ASAT and GSAT in 14 female individuals (BMI: 21.0–26.2 kg/m^2)	ASAT: ↑ *HOXA2*, *HOXA3*, *HOXA4*, *HOXA5*, *HOXB6*, *HOXD8* GSAT: ↑ *HOXC10*, *HOXC11*, *HOXC12*	Gehrke *et al.* (2013)[61]
Subcutaneous abdominal vs visceral		
Comparison of ASAT with OAT in 10 men with obesity (BMI: 59.1±12.8 kg/m^2)	ASAT: ↑ *HOXA10*, *HOXC6*	Vohl *et al.* (2004)[39]
Comparison of extraperitoneal, intraperitoneal and dermic adipose tissues (no information available on donors)	Expression of all 39 HOX genes analysed; most genes in HOX network were expressed in one or more adipose tissue depots	Cantile *et al.* (2003)[114]
Confirmation of HOX gene expression in ASAT and VAT in 22 men and 31 women (BMI <25.0) by quantitative PCR	ASAT: ↑ *SHOX2*, *HOXC9* VAT: ↑ *HOXA5*‡, *HOXC8*	Gesta *et al.* (2006)[44]
Comparison of ASAT with OAT in 32 women: lean (n=8), overweight (n=8), with obesity (n=8), with the metabolic syndrome (n=8)	ASAT: ↑ *HOXC9* OAT: ↑ *HOXA5*	Klimcakova *et al.* (2011)[111]
Comparison of preadipocytes isolated from ASAT with preadipocytes isolated from OAT	ASAT: ↑ *HOXC6*, *HOXC8*, *HOXC10*, *HOXA10*, *HOXA9* OAT: ↑ *HOXA4*, *HOXA5*, *HOXA2*	Tchkonia *et al.* (2007)[40]
Other comparisons		
Comparison of ASAT and DAT in patients with HIV-1 who were treated with cART with (n=21) and without lipodystrophy (n=11)	ASAT: ↑ *HOXA10*, *HOXC9*, *HOXC8* DAT: ↑ *SHOX2* Differences in HOX gene expression were observed between the depots irrespective of lipodystrophy	Sevastianova *et al.* (2011)[41]
Comparison of ASAT (from the anterior chest wall) with PAT in 19 individuals undergoing cardiac operations	ASAT: ↑ *IRX5*, *HOXC9*, *HOXA10*, *IRX3*, *IRX1*, *HOXB7*, *HOXA9*, *HOXC6*, *SHOX2*, *HOXC10* PAT: ↑ *HOXA2*	Lau *et al.* (2011)[42]
Comparison of ASAT before and 1 year after bariatric surgery in 16 individuals with obesity	ASAT (after fat loss): ↑ *IRX3*, *IRX5*, *HOXA5*, *HOXA9*, *HOXB5*, *HOXC6*	Dankel *et al.* (2010)[52]

↑ Denotes increased gene expression. *HOX cofactor expression was also depot specific. ‡Expression levels of *HOXA5* in ASAT and VAT correlate with obesity. Abbreviations: ASAT, abdominal subcutaneous adipose tissue; cART, combination antiretroviral therapy; DAT, dorsocervical adipose tissue; GSAT, gluteofemoral subcutaneous adipose tissue; OAT, omental adipose tissue; PAT, pericardial adipose tissue; VAT, visceral adipose tissue.

and *HOXD*).[50] Accumulating evidence suggests that certain HOX genes have active roles in adipose tissue function and distribution. Firstly, single nucleotide polymorphisms in *HOXC13* and *HOXB5* have been associated with body fat distribution in genome-wide association studies[11] and in studies of childhood obesity.[51] Secondly, extreme weight loss in humans is associated with the upregulation of multiple members of the HOX gene family,[52] which implicates these transcriptional regulators in the remodelling of adipose tissue. Studies utilizing the rodent 3T3-L1 cell line have also shown that the expression of certain HOX gene family members (*Hoxa4, Hoxa7* and *Hoxd4*) is regulated as a function of adipocyte development.[53] Furthermore, adipose-tissue-specific deletion of *Shox2* protects mice against high-fat-diet-induced obesity. Adipocytes isolated from *Shox2*-deficient mice exhibit increased expression of the β3 adrenergic receptor (*Adrb3*) and display an increased rate of lipolysis, although preadipocyte differentiation is not affected.[14] In humans, *SHOX2* is more highly expressed in GSAT-derived preadipocytes than in ASAT-derived preadipocytes.[15] It is unclear whether differences in the expression of *SHOX2* are directly involved in the regional adipose tissue differences in lipolytic regulation that are described later.

T-box genes and adipose tissue function

TBX15, a member of the Brachyury (T) family of transcription factors that share a common (T-box) DNA-binding domain, has also been implicated as an important depot-specific regulator of adipocyte development and function. *TBX15* was first identified by its markedly higher expression in visceral adipose tissue (VAT) than in ASAT in both humans and rodents.[44] Interestingly, in states of obesity, *TBX15* expression is strongly downregulated in VAT compared with ASAT, such that the direction of differential expression is reversed.[44,54] Higher expression levels of

TBX15 in GSAT than in ASAT have been reported;[11] however, the depot differences were considerably less marked than those observed between VAT and ASAT. Cellular studies performed in 3T3-L1 cells have shown that overexpression of *Tbx15* impairs adipocyte differentiation, which results in reduced lipid accumulation and mitochondrial dysfunction.[47] *Tbx15* also regulates the differentiation of brown and beige adipocytes in mice,[48] which implicates this developmental gene as a depot-specific regulator of adipose tissue development.

A second member of the T-box family of genes that also displays depot-specific expression in ASAT and GSAT is *TBX5*.[15] In fact, *TBX5* exhibits a far more marked difference in expression levels between the two depots than *TBX15*, and is almost exclusively expressed in ASAT preadipocytes. A role for *TBX5* in the development of human adipose tissue had not previously been suggested; however, we hypothesized that *TBX5* might be an abdominal-specific regulator of adipose tissue development as mutations in *TBX5* are responsible for the developmental disorder Holt–Oram syndrome, which is characterized by upper-body organ defects that affect the heart, arms and hands.[55] Silencing of *TBX5* expression in preadipocytes isolated from ASAT reduced the cells' proliferative capacity and prevented differentiation along the adipogenic lineage, which demonstrates an important functional role for *TBX5* in the development of ASAT.[15]

Cumulatively, these cellular studies provide evidence that depot-specific expression of developmental genes (*SHOX2*, *TBX15* and *TBX5*) might have a very important role in interdepot functional differences. Further studies are required to characterize the numerous genes that are differentially expressed between ASAT and GSAT to improve understanding of the regulation of these distinct adipose tissue depots.

Intrinsic depot-of-origin memory

The mechanisms that regulate the intrinsic differences between adipose tissue depots are still uncertain. Regional developmental gene signatures are not only apparent in whole adipose tissue but also in isolated preadipocytes and/or stromovascular cells cultured *ex vivo*.[15,40,56] A key study supporting the view that different preadipocyte populations are developmentally distinct reported the generation of preadipocyte cell lines from ASAT and omental and mesenteric adipose tissues by stably expressing human telomerase reverse transcriptase (hTERT).[57] After 40 population doublings, the cell lines retained depot-specific characteristics. The cell lines exhibited differences in replication rates, sensitivity to apoptotic stimuli, lipid accumulation, and also the expression levels of adipogenic transcription factors.[57] This depot-of-origin 'memory' effect, which was passed down through subsequent cell generations, lucidly demonstrates intrinsic functional differences between cells from different depots. Furthermore, GSAT and ASAT human preadipocytes that have been differentiated *in vitro* (and therefore exposed to the same experimental microenvironment) display depot-specific differences in the *de novo* fatty acids that are synthesized during adipogenesis.[58] Specifically, GSAT preadipocytes produce a higher proportion of stearoyl-CoA-desaturase-derived fatty acids (such as palmitoleic acid and vaccenic acid) than ASAT preadipocytes. In addition, *in vivo* release of palmitoleic acid is also greater from GSAT than from ASAT. Palmitoleic acid is proposed to act as an insulin-sensitizing lipokine that enables adipocytes to communicate with other cells and tissues.[59] Intrinsic differences in lipid metabolism between ASAT and GSAT preadipocytes could create different local microenvironments in the two tissues. Depot-specific differences in the local microenvironments might also have a role in maintaining and reinforcing intrinsic regional features; various hormones and signalling molecules have been shown to influence the expression of certain HOX genes.[13]

Epigenetic marks such as DNA methylation and histone modification are probably important in maintaining intrinsic differences between depots. Although it has been shown that overall adiposity (assessed by BMI) is associated with changes in DNA methylation in adipose tissue,[60] only a few studies have examined adipose tissue depot-specific differences in DNA methylation. DNA methylation profiling of ASAT and GSAT has identified a set of differentially expressed genes that are associated with depot-specific differential DNA methylation and that are highly enriched with members of the HOX gene family.[61] HOX gene transcription is known to be under complex epigenetic control that involves DNA methylation and histone modification;[62] however, the contribution of epigenetic mechanisms to depot-specific HOX gene expression in adipose tissue has received little attention. Preadipocyte cells lines (expressing hTERT) that were generated from both ASAT and GSAT exhibited a transcriptional depot-of-origin 'memory' of differentially expressed developmental genes.[15] Examination of the degree of promoter DNA methylation in two of these genes (*HOTAIR* and *TBX5)* by use of bisulphite sequencing revealed that, across the CpG sites examined, there were marked depot-specific differences in the degree of DNA methylation that were consistent with the observed depot-specific differences in gene expression.[15] These findings strongly suggest the involvement of epigenetic regulation in the maintenance of depot-specific adipose tissue features. More epigenetic research in adipose tissue is needed to further elucidate what factors and/or mechanisms govern depot-specific gene expression profiles.

HOTAIR is of added interest as it is a long noncoding RNA that has previously been described as an epigenetic regulator of HOX gene transcription. *HOTAIR* is transcribed from within the *HOXC* gene cluster and downregulates the expression of genes in the *HOXD* gene cluster.[63] This regulation involves an interaction between *HOTAIR* and the Polycomb repressive complex 2, which results in a 'silent' histone mark on the chromatin. Comparison of ASAT with GSAT revealed that *HOTAIR* displays marked gluteal-specific expression and is only detected at very low levels in the ASAT depot.[15,49] Ectopic expression of *HOTAIR* in abdominal preadipocytes increased both adipogenic differentiation and expression of key adipogenic genes.[49] Thus, it would seem that

HOTAIR might be a gluteal-specific adipogenic regulator that contributes to the expansion of this depot.

MicroRNAs (miRNAs) have important roles in the regulation of various processes in adipose tissue, including adipogenesis.[64] miRNAs are small noncoding RNAs, typically 22 nucleotides in length, that regulate gene expression at the post-transcriptional level. Of particular interest is the finding that the global miRNA expression profiles of ASAT and GSAT are distinct,[65] which suggests that regional differences in miRNA expression contribute to upper-body and lower-body adipose tissue characteristics.

Adipose tissue triglyceride content

Isotopic dilution studies and direct measurements of tissue flux in humans have demonstrated that the *in vivo* rate of nonesterified fatty acid (NEFA) release in the overnight fasted state is consistently less from the lower body than from the upper body.[66–70] The difference between the upper body and lower body is particularly noticeable if expressed as per unit of fat mass, which shows ~50% lower release rates from the lower-body adipose tissue than from the corresponding upper-body tissue. The observed reduced release rates of NEFA from the lower body agree well with the reduced overall turnover of this adipose tissue triglyceride pool. The turnover of regional adipose triglyceride stores has been investigated by a study protocol that involved oral ingestion of radiolabelled glucose, which becomes incorporated into the glycerol backbone of the triglyceride molecule, followed by serial biopsies that enabled calculation of the rate of decline of radiolabel incorporation into the tissue triglyceride pool.[71] In a group of women with and without obesity ($n = 17$), the half-life of tissue triglycerides was ~12.0 months in ASAT but ~50% longer (18.9 months) in femoral adipose tissue.[71] The turnover rate of triglycerides in adipose tissue depends on both the absolute rates of lipid storage and lipid release (assuming a eucaloric balance), as well as a possible imbalance depending on either positive or negative energy balance. The routes for regional adipose tissue lipid storage and release and how they are regulated by normal physiological stimuli are now discussed.

Methodological considerations

Arterio–venous techniques

Although direct assessment of arterio–venous concentration differences or the use of tracer dilution techniques across tissues has proven extremely useful in understanding regional adipose tissue fluxes of lipids, it is important to recognize some limitations in these approaches. The leg fat depots can be accessed by studying the arterio–venous difference across the femoral and/or inguinal artery and/or vein; however, the positioning of the venous sampling point is critical to the evaluation of the results. A classic position often used in exercise physiology is to place the catheter tip distal to the entry of the saphenous vein into the femoral vein.[72] In terms of the assessment of lower-body fat, this positioning excludes a major proportion of the flow through the superficial leg structures, which has proven to be quite specific for leg adipose tissue;[73] the validity of this positioning for leg adipose tissue is therefore questionable. However, if the catheter is positioned in the other direction (to ensure capturing of the venous blood from the saphenous vein), above the inguinal ligament,[74] this positioning might also capture the venous blood from the inferior superficial epigastric vein entering the femoral vein and thus delivering a major proportion of ASAT venous drainage. With this positioning, there is a distinct risk of admixture with blood from nonleg adipose tissue. A different approach is to establish arterio–venous gradients across adipose tissue without any notable admixture with other tissues.[73,75] This direct technique is unfortunately not possible to apply to intra-abdominal fat. Similar to the venous drainage of the ASAT depot, this technique would sample the entire depot without separating the contributions of the distinct deep and superficial layers that have been demonstrated to be functionally different.[76]

Regional adipose tissue and lipolysis

Regulation by adrenaline

Regional differences and whole-body differences in NEFA mobilization from adipose tissue as a result of increased systemic concentrations of adrenaline were first described in 1996.[77] Although adrenaline increased upper-body lipid mobilization in both men and women, there was no increase in lipid mobilization from lower-body fat stores in women and only a small increase in men.[77] A later study utilizing catheterization of a vein directly assessed specific drainage of the lower-body (femoral) adipose tissue in comparison with ASAT, and showed a complete absence of lipid mobilization by adrenaline from lower-body fat in both men and women.[70] However, β-adrenergic stimulation (by use of isoproterenol) markedly increased NEFA mobilization from ASAT and GSAT in both men and women, which suggests a different balance between α-adrenergic (antilipolytic) and β-adrenergic (lipolytic) receptors in upper-body and lower-body adipose tissues. Furthermore, these differences have been unambiguously demonstrated *in vitro*.[78,79] Although adrenergic control of fat depots is intrinsically different, it is unlikely to be the main explanation for sex-specific differences in fat distribution.[68]

Regulation by cortisol

Owing to the distinct redistribution of fat stores in relation to hypercortisolism (which results in increased abdominal fat and reduced gluteofemoral fat), it is reasonable to postulate that cortisol has a differential effect on either lipid storage or lipid mobilization in upper-body and lower-body adipose tissue; however, the available data are inconsistent. Examination of regional lipolysis by use of microdialysis monitoring of glycerol in response to a week of exposure to prednisolone demonstrated increased abdominal lipolysis, but the data were difficult to interpret as there was no effect on systemic NEFA concentrations.[80] More direct evidence was obtained by acutely increasing the systemic concentration of cortisol, which increased lipolysis in both abdominal and femoral adipose tissue concomitant with an increase in systemic NEFA concentrations.[81] The considerably higher overall turnover of adipose tissue triglycerides in ASAT than

in GSAT suggests that an equivalent cortisol stimulus produces a stronger effect in the lower body than in the upper body, which is in agreement with the concept that hypercortisolism leads to depletion of lower-body fat and redistribution to upper-body fat. The combined use of cortisol and growth hormone resulted in an obvious augmentation of the cortisol effect,[82] again supporting the idea that imbalance in the hypothalamic–pituitary–adrenal and growth hormone–IGF-1 axes could lead to specific depletion of lower-body fat stores. A clinical trial using the 11β-HSD1 inhibitor R05093151 to test the concept of local generation of cortisol showed equal loss of VAT and ASAT, but also showed an unexpected total reduction in body weight that made it difficult to interpret the results in terms of fat redistribution.[83]

Regional adiposity and weight loss

Detailed examination of regional fat masses during a weight loss intervention revealed that the proportionally greatest weight loss was from VAT, whereas the smallest weight loss was from thigh fat.[84] This result suggests that long-term negative energy balance has the greatest effect on the area of fat mass that has the highest turnover of triglycerides. Weight loss had the expected positive associations with improvements in glycaemic control and liver fat content.[84] Consistent with this observation, loss of lower-body fat has been described as being disadvantageous for metabolic health.[85] It is not unreasonable to hypothesize that intentional weight loss (particularly in women) that leads to reduced fat mass in people with a distinct gynoid fat distribution is of little or no benefit for the major health outcomes associated with obesity. Loss of 'beneficial' fat mass might be one of many underlying reasons for the paradoxical and negative health outcomes sometimes observed in population studies in relation to weight loss.[86]

Exercise and fatty acid mobilization

Aerobic exercise increases the demand for provision of NEFAs to working muscles. NEFA release from leg fat increases during long-term exercise (approximately fourfold compared with the fasting state) and is directly proportional to the systemic NEFA rate of appearance with little evidence of a difference in the mobilization of NEFA between upper-body and lower-body fat stores.[87] The absence of a specific regional effect on fat mobilization during exercise is also supported by a subsequent study.[88] Considering the absence of an increase in lipolytic stimulus by adrenaline in the lower-body,[70] it is reasonable to postulate that lipolysis from lower-body fat stores is stimulated by alternative routes during exercise; for example, by exercise-induced atrial natriuretic peptide.[89] Unfortunately, studies on lipolysis induction by atrial natriuretic peptide in the gluteal and/or femoral region have not been conducted.

On the basis that the intra-abdominal fat depot is particularly sensitive to adrenergic stimulation and has a high turnover rate, it has been suggested that physical exercise specifically reduces the size of this fat depot. However, the aggregate result of all studies that investigated this relationship is that no direct evidence exists that VAT is more depleted than total fat mass in response to physical exercise.[90]

Regulation of regional lipid storage

Uptake of fatty acids via chylomicrons

The removal of chylomicron triglycerides as they pass through the capillary bed in adipose tissue can be quite considerable, with a single pass removing ~30% of triglycerides. Differential fatty acid uptake in the upper body and lower body following a meal was first reported in 1990.[91] Administration of a meal tagged with radiolabelled oleate followed by serial biopsies of abdominal and femoral adipose tissue showed ~50% higher incorporation of diet-derived fatty acids in the abdominal depot than in the femoral depot; however, with time, substantial recirculation of fatty acids was evident. Subsequent studies employing a range of different techniques uniformly demonstrated higher relative uptake of chylomicron-derived fatty acids in abdominal adipose tissue or VAT than in femoral adipose tissue.[67,74,92] Considering the higher turnover of triglycerides in upper-body adipose tissue than in lower-body adipose tissue, this result is probably an expected finding as the abdominal fat depots are the first destination of diet-derived fat.

Uptake of fatty acids via VLDL triglycerides

Very few studies have assessed specific VLDL-derived triglyceride fatty acid uptake in adipose tissue. However, a study published in 2010 utilized two different fatty acid labels simultaneously to quantify diet-derived fatty acids (chylomicrons) and VLDL-derived fatty acids in upper-body and lower-body adipose tissue.[92] In contrast to chylomicron-derived fatty acids that were more efficiently removed by ASAT, there was no difference for VLDL-derived fatty acids, which indicates a specific and enhanced route for uptake of these fatty acids in the lower body. The mechanistic basis for this observation is not obvious, but the implications could be of considerable interest. Of note, lipodystrophic syndromes that are characterized by a specific absence of GSAT are almost invariably associated with increased concentrations of VLDL triglycerides. There exists a distinct possibility that GSAT has the specific ability to sequester fatty acids from VLDL-derived triglycerides, which are composed of fatty acids secreted by the liver and thus could be referred to as ectopic fat.

Uptake of fatty acids from the NEFA pool

Although lipoprotein lipase-mediated release of fatty acids from chylomicrons and VLDL constitutes the major source of fatty acids that are re-esterified in adipose tissue, fatty acids are also directly taken up by adipose tissue from the NEFA pool.[93,94] This delivery route is augmented in the postprandial state, when the outward flux of NEFA from the tissue is low, but is also detectable in the fasted state. Direct uptake of fatty acids from the NEFA pool is greater in women than in men. Under certain circumstances, such as moderate exercise, there seems to be enhanced uptake via this route in lower-body fat in women. Consequently, it has been suggested that this route of fatty acid uptake has a role in human fat redistribution.[95,96]

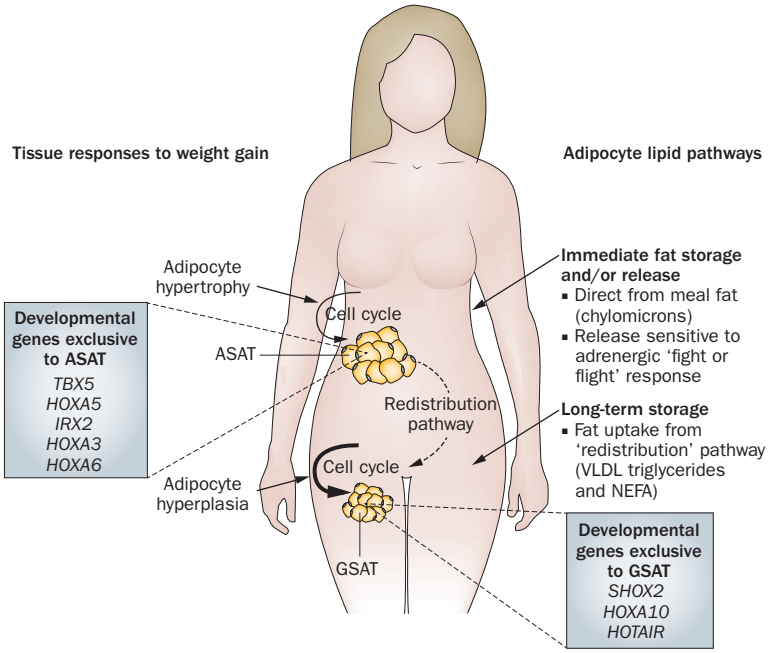

Figure 2 | Functional differences between adipose tissue depots. Abdominal adipose tissue (in ASAT and VAT depots) is the primary site for immediate storage of diet-derived fat. These depots have a high lipid turnover and undergo lipolysis in response to adrenergic stress stimuli. During weight gain, the abdominal depots undergo adipocyte hypertrophy whereas the GSAT depots show evidence of adipocyte hyperplasia. GSAT (in both men and women) is qualitatively different from the upper-body depots: direct uptake of chylomicron triglyceride-derived fatty acids is less active; turnover of cellular triglycerides is slower; and the tissues are insensitive to adrenergic stimulation of lipolysis. The net result is a tissue that retains fatty acids well and results in diminished exposure of lipids to ectopic fat depots. A greater proportion of fatty acids entering GSAT come from a redistribution pathway consisting of recirculated fatty acids (from VLDL triglycerides or directly from the NEFA pool). The functional characteristics of ASAT and GSAT are controlled by site-specific expression of different sets of developmental genes (mostly under epigenetic control) that direct both the degree of adipocyte proliferation and aspects of adipocyte differentiation.[15] Abbreviations: ASAT, abdominal subcutaneous adipose tissue; GSAT, gluteofemoral adipose tissue; NEFA, nonesterified fatty acid; VAT, visceral adipose tissue.

Contribution of *de novo* lipogenesis

Human adipose tissue has an obvious potential for *de novo* lipogenesis; human preadipocytes isolated from adipose tissue samples readily produce lipid droplets *in vitro* when provided with glucose as their only metabolic substrate. Quantification of the extent to which *de novo* lipogenesis takes place in tissues *in vivo* has been performed by use of long-term labelling of glycerol and palmitate with ^2H_2O, and has revealed a substantial amount of *de novo* lipogenesis.[97] However, the interpretation of this study is difficult as the contribution of *de novo* lipogenesis-derived fatty acids generated within the tissue was not separated from the contribution of those fatty acids formed in other tissues and then transported and taken up by adipose tissue. A subsequent study provided unambiguous evidence of metabolic conversion of fatty acids (elongation and desaturation, consistent with the *de novo* lipogenesis pathway) within adipose tissue and also revealed regional differences with a distinctly higher level of production of palmitoleic fatty acids in GSAT than in ASAT.[58]

Regional effects of PPAR-γ agonists

Rare mutations in *PPARG*, which encodes the adipogenic nuclear receptor peroxisome proliferator-activated receptor γ, are associated with a mild form of partial lipodystrophy that is characterized by loss of peripheral subcutaneous fat, particularly from the legs and buttocks.[98] Such mutations also markedly increase the risk of type 2 diabetes mellitus.[99] Thus, it seems probable that regional differences in PPAR-γ action might contribute to differences in adipogenesis and fat storage between upper-body and lower-body adipose tissues. Studies that have examined the effects of administering PPAR-γ agonists (for example, thiazolidinediones) lend further *in vivo* support to this association. The DREAM trial followed-up patients with impaired fasting levels of glucose or impaired glucose tolerance for 3 years, during which time they received rosiglitazone.[100] Among individuals who were given rosiglitazone, a 70–80% increase in levels of normoglycaemia was reported compared with those given placebo. This normalization was accompanied by a marked increase in body weight but a reduction in the waist:hip ratio; in fact, hip circumference increased by 2 cm whereas waist circumference was unaffected, which suggests that the beneficial effects of thiazolidinediones are exerted through expansion of lower-body adipose tissue.

Assessment of the effect of thiazolidinediones on body fat distribution by use of more advanced technologies (such as CT and DXA) has led to the accepted view that PPAR-γ agonists mediate redistribution of fat away from visceral depots to subcutaneous depots. However, not all studies have produced concordant results and these studies mainly focused on the abdominal depots,[101–104] with few studies directly assessing changes in lower-body adiposity.[105–107] In individuals with obesity, treatment with pioglitazone for 8 weeks resulted in a redistribution of body fat to ASAT and femoral depots.[105] Histological examination of adipose tissue biopsy samples obtained from ASAT depots showed an increased number of small adipocytes after pioglitazone treatment, which suggests increased recruitment of preadipocytes; no data were reported for the femoral depot. Increased lower-body adipose tissue has also been reported independent of a loss of visceral fat after pioglitazone administration.[106] Furthermore, a substudy of the DREAM trial reported a 2 kg increase in leg fat in patients who had received rosiglitazone; however, this increase was attenuated after adjustment for total fat mass, which suggests that the effects of rosiglitazone are not depot specific.[107]

Regional differences

Inflammation signalling

Few studies have directly compared inflammation signalling in lower-body and upper-body adipose tissue.[15,108] No differences in the expression levels of 17 candidate macrophage and cytokine genes were found between GSAT and ASAT, either before or after a low-calorie weight-loss intervention in 14 premenopausal women.[108] Consistent with this result, global transcriptional profiling of 49 men and women failed to identify differentially expressed clusters of inflammation-specific genes in abdominal and gluteal

tissues; however, the associations with obesity-related metabolic consequences (such as hyperlipidaemia and insulin resistance) were considerably weaker with inflammatory and/or cytokine genes in the gluteal region than in the abdominal region.[15] More importantly, quantification of tissue release of IL-6 in an arterio–venous model showed a substantially lower rate of release from femoral adipose tissue than from the corresponding abdominal tissue.[15] Inflammatory changes in ASAT and omental adipose tissue seem to be related to the infiltration of M1 proinflammatory adipose tissue macrophages;[109,110] however, whether macrophage number or polarization differs between lower-body and upper-body adipose tissue depots in states of obesity is yet to be established. The adipose tissue inflammatory response in states of obesity is closely associated with dysfunctional lipid metabolism[111] and a number of putative mechanisms have been proposed in which lipid signalling intermediates might mediate the inflammatory response.[112] These mechanisms include activation of the inflammasome and Toll-like receptors, and the induction of cell death via endoplasmic reticulum stress and reactive oxygen species. As discussed earlier, GSAT and ASAT differ in their capacity for lipid metabolism and it is possible that this variation contributes to regional differences in cytokine release. However, further studies are required to establish the importance of these and other mechanisms, such as hypoxia in lower-body adipose tissue.

Release of adipokines and lipokines

Levels of leptin correlate positively with total fat mass, but the plasma concentration of leptin is substantially higher in women than in men, which suggests that there is a greater release of leptin from lower-body adipose tissue than from upper-body adipose tissue. In addition, levels of leptin were higher in adipose tissue interstitial fluid (assessed by microdialysis) in femoral adipose tissue than in ASAT in one study.[113] However, tissue release of leptin can also be monitored by the specific arterio–venous sampling technique; a study using this technique found no difference in the release rate of leptin from leg fat compared with ASAT.[15] Owing to the low release rate and long half-life of adiponectin, release of this molecule cannot be monitored by use of the arterio–venous technique. No difference in interstitial adiponectin concentrations was found when comparing femoral adipose tissue with ASAT. Palmitoleic fatty acid has been reported to have insulin-sensitizing properties.[59] The release of palmitoleic acid is considerably greater from leg adipose tissue than from abdominal adipose tissue and, unlike other fatty acids, the resulting plasma enrichment of palmitoleic fatty acid in the NEFA pool negatively correlates with systemic insulin resistance.[58]

Conclusions

Different metabolic pathways regulate lipid metabolism in ASAT and GSAT and there are also alternative mechanisms through which these tissues respond to weight gain (Figure 2). It seems that ASAT and VAT have a high capacity for the rapid uptake and immediate storage of diet-derived fat. These compartments are also the first to release fatty acids for bodily needs. The upper-body 'immediate' fat storage pathway involves the uptake of fatty acids from chylomicrons, whereas the lower body favours a 'redistribution' pathway that involves the uptake of fatty acids via VLDL and NEFA. The impairment of lower-body fat expansion could provide a specific impediment to the removal of plasma VLDL triglycerides.

Under eucaloric conditions the rapid cycles of storage and release of fatty acids in the upper-body adipocytes are unlikely to trigger any signals demanding expansion of the tissue. However, in conditions of positive-energy balance, the first response is an increase in adipocyte cell size, which probably triggers the recruitment of new adipocytes. The capacity to recruit new adipocytes seems limited in ASAT, but not in GSAT. Thus, ASAT is reliant on hypertrophy and has a finite lipid storage capacity. Beyond the initial demand for fat storage, the change in tissue adipocyte cellularity is under complex genetic and epigenetic control, probably governed by unique sets of developmental genes with tissue-specific expression. Additionally, tissue adipocyte cellularity is influenced by levels of hormones, not least those of the sex steroids. It can be postulated that the recruitment of new cells from adipocyte progenitors generates additional adipocytes in a process controlled by a site-specific epigenetic programme of developmental gene expression, which also controls the functional characteristics of differentiated adipocytes.

The obvious functional differences between upper-body and lower-body adipose tissue, underpinned by the distinct characteristics of adipocytes in the respective tissues, provide compelling evidence for a mechanistic basis for causality between lower-body fat accumulation and reduced risk of cardiovascular disease or type 2 diabetes mellitus.

Review criteria

A search for original articles published between 1950 and June 2014 and focusing on human adipose tissue distribution in relation to regional adipocyte function and overall metabolic features was performed in PubMed. The following search terms were used alone or in combination: "adipose", "adipocyte", "femoral", "gluteal" and "abdominal".

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Author contributions
Both authors researched data for the article, provided substantial contributions to discussions of the content, wrote the article and reviewed and/or edited the manuscript before submission.