Molecular signatures of brown, white and brite adipose tissues

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Preliminary manuscript

Abstract

Mainly from cell culture studies, a series of genes have been identified that have been suggested to be characteristic of different types of adipocytes. Here we have examined gene expression patterns in nine defined adipose depots: interscapular BAT, cervical BAT, axillary BAT, mediastinic BAT, cardiac WAT, inguinal WAT, retroperitoneal WAT, mesenteric WAT and epididymal WAT. We found that each depot displayed a distinct gene expression fingerprint, but that three major types of depots were identifiable: the brown, the brite and the white. Although differences in gene expression pattern were generally quantitative, some gene markers showed, even in-vivo, remarkable depot specificities: Zic1 for the classical brown adipose tissue depots, Hoxc9 for the brite depots, Hoxc8 for the brite and white in contrast to the brown, and Tcf21 for the white depots. The significance of these gene expression patterns both for understanding the developmental background of the depots and as possible master regulators is discussed.
Introduction

The last few years have seen a paradigm shift with regard to the understanding of the origin of and relationship between adipose tissues. A series of investigations have demonstrated that brown and white adipocytes are not sister cells but rather that brown adipocytes are closely related to myocytes and both originate from a common “adipomyocyte” precursor\[^{1-3}\]. Further, even among classical white adipocytes, it would seem that two types may exist: the “genuine” white adipocytes but also “brite” adipocytes: a type of adipocytes that possess the ability to express the uncoupling protein 1 (UCP1) (until recently believed to be a unique marker for brown adipocytes) but do not possess the molecular characteristics of brown adipocytes\[^{4}\].

Based on investigations by us\[^{2, 4, 5}\] and others\[^{6-10}\], a number of genes have been identified that are suggested to characterize (or possibly determine) the different adipocyte lineages. However, most of the characterization of these markers has been performed in adipocyte cell cultures, or only in a limited number of adipose tissues. Although there are clearly interpretation advantages of studying cell cultures, the underlying question must with time be approached: to which degree can the conclusions from the in-vitro studies be extrapolated to in-vivo conditions.

In the present investigation, we have therefore undertaken to examine all the definable adipose depots of mice in order to extend the information concerning cell lineage markers to the physiology of the intact animal, allowing for the regulatory systems of the intact animal to influence the development of the adipose cells. Particularly we have examined conditions where the brown adipose depots would be minimally stimulated (i.e. the mice are maintained at thermoneutrality and on a chow diet), as well as conditions where the brown adipose tissue depots would be maximally adrenergically stimulated, i.e. under conditions of cold acclimation.

We found that each adipose depot examined was characterized by a unique marker gene expression fingerprint. However, despite this, we conclude that it is possible to divide the depots into three main types: the classical brown adipose tissue depots, the brite adipose tissue depots and the (“genuine”) white adipose tissue depots, and to associate particular gene expressions patterns with these depots.

Materials and Methods

Sampling of tissues. The experiments were approved by the Animal Ethics Committee of North Stockholm. To avoid the problems associated with differential gene expressions in inbred strains like C57/Bl6\[^{11}\], and in continuation of our earlier studies, we used outbred NMRI mice. Male NMRI mice 3-4 weeks old from a local supplier (B&K, Stockholm, Sweden) were housed at room temperature for at least 48 h after arrival and then divided into two groups: one was transferred to 30 ° and one to 4 °C; both with free access to food (chow: R70 Lactamin) and water. After 3 weeks at the acclimation temperatures, the mice were killed, and samples from the brown and white adipose tissue depots (and muscle)
detailed below were dissected out and immediately frozen in liquid nitrogen. The samples were taken from the central parts of each depot.

**Adipose tissue depots examined**

Based on visual impression (and principally later confirmed by gene expression patterns), we identified the following adipose depots.

**Brownish depots**

Interscapular BAT (iBAT) – the largest brown adipose depot (≈ 200 mg), found subcutaneously above the shoulder blades.

Cervical BAT (cBAT) - a sausage-like depot (≈ 50 mg) submerged into a cavity of surrounding muscle tissue in the neck. This depot is defined as the deep-cervical since there is also a superficial cervical depot conjoint with the iBAT[12].

Axillary BAT (aBAT) – an intermuscular depot (≈ 100 mg) found directly beneath the scapulae (shoulder blades).

Mediastinic BAT (mBAT) - a visceral depot (≈10 mg) found in the mediastinum. The largest mass of the depot, which is used in this study, is in the arch of the aorta.

**Whitish depots**

Cardiac white adipose tissue (cWAT) – a thin visceral white depot (≈ 10 mg) lining the left ventricle of the heart down to the apex.

Retro-peritoneal WAT (rWAT) – a depot (≈ 200 mg) attached on the dorsal wall of the back, encapsuled in a thin membrane.

Inguinal WAT (iWAT) – a depot attached dorsally along the pelvis and skewed ventrally down on to the thigh of the hindlimb. This depot is the largest subcutaneous depot (≈ 500 mg) and is the depot routinely considered to represent subcutaneous adipose tissue in mice.

Mesenteric WAT (mWAT) – A visceral depot (≈ 500 mg) found embedded in the mesenterium lining the surface of the intestines; these mice showed no clear omental depot.

Epididymal WAT (eWAT) – The largest visceral depot (≈ 700 mg) in male rodents. This depot embeds the vas deferens, testicular arteries and the epididymus. Most reports discussing visceral adipose tissue in mice refer to this depot.

We have not sampled the subdermal depot (the thin layer of fat on the inside of the skin).

As a representative muscle sample we used the gastrocnemius from the hind leg.

**RNA isolation and Real-Time qPCR.**

Total RNA was extracted from frozen adipose tissue with Ultraspec (BiotecX, Houston, TX) according to the manufacturer’s protocol, and RNA concentrations were measured on a Nanodrop™ nd-1000 spectrophotometer (Thermo-Scientific, Wilmington, DE). To synthesize cDNA, 200 ng RNA from each sample were reverse-transcribed with a High Capacity cDNA kit (Applied Biosystems, Foster City, CA) in a total volume of 10 µl and diluted to 200 µl. To measure each gene of interest (GOI), 4 µl of each cDNA sample were loaded in duplicate in SybrGreen® Jumpstart™ Taq Ready-mix™ (Sigma-Aldrich), together with pre-validated, designed primers (Suppl. Table 1) obtained from the Universal Probe Library (Roche Applied Biomedical).
Science) on a 7900HT Real Time PCR System (Applied Biosystems, Foster City, CA). All mRNA levels were normalized to TFIIB mRNA, according to the comparative threshold method (Ct-method) ($\Delta \text{Ct}$). The equation $2^{-\Delta \text{Ct}}$ was applied to convert the logarithmic $\Delta \text{Ct}$ values to linear values.

For miR-206 microRNA measurements, total RNA was diluted to 5 ng/µl. In the microRNA-cDNA synthesis, 2.5 µl of these dilutions was reverse-transcribed in 3.5 µl reaction mix and 1.5 µl of the miR-206-specific reverse-transcription primer (Suppl. Table 1) provided with the MicroRNA Assay kit (Applied Biosystems, Foster City, CA). 1.2 µl of miRNA-specific cDNA from this reaction was amplified in duplicate with the TaqMan Gene Expression mastermix and the probe/primer mix provided in the MicroRNA Assay kit (Applied Biosystems, Foster City, CA) in the TaqMan qPCR system as above. TFIIB mRNA was used as an endogenous control; log to lin-transformed microRNA/TFIIB mRNA expression ratios were calculated as above for the GOI mRNA.

In addition to the results presented here, an initial experiment performed on a number of adipose depots from another mouse strain (FVB/N) showed results qualitatively similar to those shown here.

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Table 1. Primer sequences and miRNA assay.

For determination of markers in the adipose depots, we used tissues sampled from mice at thermoneutrality (and with a chow diet), i.e. conditions where the innate characteristics of the depots will be most markedly displayed, as the tissues are then

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Table 1. qPCR primer sequences. Before the measurements, the intron-spanning primer pairs for each mRNA, designed and obtained from the Universal Probe Library, were validated by a standard curve to verify the amplification efficiency and the linear range of amplification (not shown). The miRNA assay from Applied Biosystems consisted of a miRNA-specific RT primer and a miRNA-specific qPCR probe/primer mix.

**Results**

For determination of markers in the adipose depots, we used tissues sampled from mice at thermoneutrality (and with a chow diet), i.e. conditions where the innate characteristics of the depots will be most markedly displayed, as the tissues are then
under minimal adrenergic stimulation. Additionally, we examined the depots from animals exposed to chronic cold, i.e. to a chronic adrenergic stimulation, conditions expected to most markedly display the brown-fat-like characteristics of the tissues.

Whereas the results below thus clarify gene expression under physiological conditions, the setup will necessarily add complexity to the analysis. In tissues, the cells are not homogeneous and the samples will not consist purely of parenchymal cells; rather, other cell types must unavoidably be present, including endothelial cells, blood cells, cells from the immune system etc. Additionally, even the parenchymal cells will be in different phases of development. Nonetheless, the dominant expressions should be from mature parenchymal cells.

With real-time PCR techniques, extremely low levels of mRNAs can be detected. We therefore found it of importance to report gene expression levels in semi-absolute terms (i.e. all expressed as the number of mRNAs of the gene of interest (GOI) per mRNA coding for the reference gene TFIIB). Although there is not necessarily a simple relationship between mRNA and final protein level (and protein activity), the levels stated in this way will give some indication of the quantitative significance of each gene.

A primary classification of adipose tissues
To make an initial classification of the different adipose tissue depots, we first examined the expression levels of two genes well accepted as being associated with brown adipose tissue: UCP1 and Prdm16.

![Figure 1](image.png)

**Fig. 1.** Expression levels of UCP1 (A) and Prdm16 (B) and of the transcription factor II B TFIIB (C). Abbreviations here and in subsequent figures are iBAT, cBAT, aBAT and mBAT: interscapular cervical, axillary and mediastinal brown adipose tissue, and cWAT, iWAT, rWAT, mWAT and eWAT: cardiac, inguinal, retroperitoneal, mesenteric and epididymal white adipose tissue. Data here and in subsequent figures are means ± SE from six to twelve mice in each group. Significant effects of cold acclimation here and in subsequent figures are indicated as *, ** or *** (P < 0.05, 0.01 and 0.001, respectively; Student’s unpaired t-test); no symbol indicates no statistically significant effect. “0” indicates that the mRNA was not detectable at all with qPCR techniques. UCP1 mRNA levels in thermoneutral (30 °C) mice in the brite depots were 3 in cWAT, 0.7 in iWAT and 0.06 in rWAT. UCP1 mRNA levels in the white depots mWAT and eWAT at thermoneutral (30 °C) were both at 0.03 and were increased to approximately 0.2 at 4 °C.
Uncoupling protein 1 (UCP1). As expected, UCP1 was not found in muscle (Fig. 1A). Under thermoneutral conditions, UCP1 was expressed in only four depots of adipose tissue: the interscapular, the cervical, the axillary and the mediastinal depots. We will therefore refer to these depots as brown adipose tissue depots: iBAT, cBAT, aBAT and mBAT. Acclimation to cold led to highly significant increases in the expression levels in these depots. However, the increase was only about 2-fold. This may perhaps be considered less than expected but is in accordance with earlier observations under similar conditions[13]. It should be understood that despite this apparently marginal relative increase in UCP1 gene expression, the total amount of UCP1 in the brown adipose tissue is totally about 50-fold higher in cold-acclimated than in thermoneutral mice, due to a combination of increased mitochondriogenesis and hyperplasia of the tissue[14].

In a further three depots of adipose tissue, the cardiac, the inguinal and the retroperitoneal depots, UCP1 mRNA was not detectable under thermoneutral conditions but became fairly well expressed in cold-acclimated mice. We will refer to these depots, that are apparently white at thermoneutrality, as cWAT, iWAT and rWAT, and we classify these depots as “brite” depots in that they have the ability to express UCP1 but do not always do so. That these depots have a potential capacity to express UCP1 under conditions of high stimulation has earlier been observed[11, 15].

Finally we identified two depots in which no UCP1 was detectable at the resolution given here: the mediastinal and the epididymal adipose tissues (mWAT and eWAT); these tissues will be referred to as white adipose tissues. It may be noted, however, that even within the adipocyte precursor population that can be harvested from the epididymal depots, adipocytes with “brite” characteristics can be observed[4].

PR domain containing 16 (Prdm16) has been identified as a master regulator in the bifurcation between the myocyte and the brown adipocyte fates in the developmental pathway of the adipomyocyte, principally turning off the myogenic pathway[3, 16, 17]. The expression characteristics of Prdm16 were not as distinct as those for UCP1: at thermoneutrality there was some expression even in muscle, a 10-fold higher expression in the BAT depots, but also some expression in the brite depots (that are suggested not to originate from an adipomyocyte precursor) and even in the white depots (Fig. 1B). Further, in the BAT depots, cold acclimation led to a systematic decrease in Prdm16 gene expression, the significance of which is not known; such a depression was not seen in the brite and white depots.

The less distinct pattern of Prdm16 versus UCP1 gene expression may be partially understood by realizing that although Prdm16 is a master regulator in the myocyte/brown adipocyte bifurcation, Prdm16 is not a factor exclusively expressed in brown adipocytes. Prdm16/MEL1 is also expressed in the lung, heart, placenta and kidney, as well as in leukocytes and bone-marrow cells (CD34-positive)[18].

Transcription factor IIB (TFIIB). As is customary in gene expression studies, all mRNA levels have been expressed in relation to a reference gene, expected to be equally expressed in all cells under all
circumstances. The choice of reference gene is not trivial, as such genes may not be as stable in their expression as anticipated. We have chosen TFIIB as reference gene, and in Fig. 1C, we have examined the minimal requirement for this choice. We observed that the expression level is fairly similar between the depots and is not markedly altered due to chronic cold (with the possible exception of the levels in eWAT but this is of marginal significance in the analysis).

**The sirtuins**

The sirtuins are a family of seven class-III histone/protein deacetylases (H/PDAC). The sirtuins function as energy sensitizers by responding to shifts in NAD$^+/NADH$ levels. In different contexts, some of the sirtuins have been suggested to be of relevance with respect to adipocyte precursor fate.

*Sirtuin1 (Sirt1).* Based on a comparison of the altered gene expression caused by Sirt1 overexpression in myoblasts$^{[19]}$ and that occurring during brown adipocyte differentiation, we have pointed to Sirt1 as a potential master regulator in the bifurcation of the adipomyocyte precursor into a brown adipocyte$^{[2]}$. Sirt1 could function as a repressor of myogenic factors. However, we observed here similar Sirt1 mRNA levels in all adipose depots and in muscle, and the expression did not change as an effect of acclimation to cold (Fig. 2A). Thus, if Sirt1 is involved in repression of the myogenic genes, this effect is caused by alterations in the expression level of the Sirt1 gene.

*Sirtuin 3 (Sirt3).* Sirt3 mRNA levels are increased during brown adipocyte differentiation$^{[2]}$. This is of particular interest because Sirt3 mRNA levels have been reported to be low in WAT but high in BAT and to be increased in response to cold exposure (and, somewhat paradoxically, during caloric restriction), and Sirt3 was therefore suggested to be important for non-shivering thermogenesis$^{[20]}$.

![Fig. 2. Expression levels of members of the sirtuin family: Sirt1 (A), Sirt3 (B) and Sirt5 (C). For details, see legend to Fig. 1.](image-url)
Overexpression of Sirt3 also enhanced the expression of UCP1, PGC-1α and mitochondrial genes. However, Sirt3 KO mice were described as having normal energy expenditure and unaltered UCP1 protein levels. Here we confirm that Sirt3 levels were somewhat higher in BAT depots than in all other adipose depots, but the levels were unaltered by cold acclimation in all depots (Fig. 2B). A very significant role for Sirt3 in brown adipocyte differentiation is not supported by these observations.

Sirtuin 5 (Sirt5). Sirt5 gene expression was also reported to be up-regulated by cold-exposure in BAT, but similarly to the case for Sirt3, we found rather similar levels of Sirt5 mRNA in all depots examined, and the expression was not altered by cold acclimation (Fig. 2C).

Myogenic factors

It was our observation of myogenic markers in undifferentiated brown adipocytes (and their absence in undifferentiated white adipocytes) that allowed us to suggest that brown and white adipocytes originate from distinct cells lineages. It should be noted that the myogenic markers were just that, i.e. markers: we did not observe the products of muscle-associated genes at the protein level. Characteristically, the expression level of the myogenic markers decreased with advancing differentiation of the brown adipocytes, and had disappeared in fully differentiated brown adipocytes.

Myosin regulatory light chain (Mylpf) is such a muscle-associated gene, earlier reported to be expressed in both brown adipose tissue and even in cultured brown adipocytes. We here confirmed Mylpf gene expression in the BAT depots. It should be noticed that although Mylpf expression was some 10-fold higher in the BAT depots than in the other adipose depots, the level was still some 1000-fold lower than in muscle. However, as the expression of the myogenic genes disappears in maturing brown adipocytes, low levels would be expected in the tissue, and the Mylpf mRNA observed a correspond to a small fraction of undifferentiated brown precursors found in the tissue.

Based on previous work we chose not to measure the myogenic regulatory factor Myf5, since this gene was practically undetectable in interscapular BAT.
MicroRNA 206 (miR-206) is a “myomir” (i.e. a miRNA normally associated with muscle cells) that we observed also to be expressed in brown adipocytes (but not in white), and therefore miR-206 could be considered to be part of the myogenic signature of brown adipocytes. In contrast to what is the case for those myogenic markers that are mRNAs, miR-206 levels do not decline during brown adipocyte differentiation\cite{4, 5}. miR-206 levels were clearly higher in BAT depots than in the other adipose depot – but were some 100-fold lower than in muscle (Fig. 3B).

Thus, based on myogenic markers, it was possible to quantitate differences between brown adipose tissues of adipomyocyte origin and the brite and white depots – but the differences were not of the qualitative character observed in cell cultures\cite{2, 4, 5}.

**Lineage-specific gene markers that were not depot-specific**

In our characterization of differential gene expression in brown and white adipocyte cultures, a series of genes could qualitatively be associated with each cell type. However, as detailed below, two of these markers did not demonstrate a corresponding depot specificity, one gene that was brown-specific in-vitro, and one that was white-specific.

*Mesenchyme homeobox 2 (Meox2)* is well expressed in brown adipocytes in cultures, but not in white adipocytes\cite{2, 4}. However, Meox2 showed no differential expression between the different adipose depots (Fig. 4A). It may, however, be noted that in cold-acclimated mice the expression was systematically repressed in all brown and brite depots but unaffected in white depots. The even Meox2 levels were not due to contamination by muscle, as muscle Meox2 levels were not higher than adipose tissue levels.

![Fig. 4. Expression levels of lineage-specific gene markers that were not depot-specific markers Meox2 (A) and Igfbp3 (B). For details, see legend to Fig. 1.](image)

*Insulin-like growth factor binding protein 3 (Igfbp3)* is specifically expressed in cultured white pre-adipocytes and differentiated white adipocytes, as compared to brown adipocytes (and C2C12 muscle cell lines)\cite{2, 4}. However, also for this gene we found no depot specificity (Fig. 4B).

Amongst the genes examined here which were selected based on earlier in-vitro characterizations, only these two genes fully lost their cell lineage characteristic when examined in-vivo. We
propose no simple explanation for this. There is a possibility that the genes are expressed in other cell types than the depot parenchymal cells, but considering e.g. the differences in vascularization of the different depots and the large alterations induced in vascularization by cold acclimation\textsuperscript{[15]}, this is not a very likely explanation.

**A brown adipocyte gene marker that may be brown/brite-depot-specific**

*T-box 15* (*Tbx15*) was presented in our microarray as a brown adipocyte-specific gene compared to white adipocytes\textsuperscript{[2]}. As it was expressed at an even higher level in C2C12 cells, *Tbx15* may be regarded as a muscle-gene and further underlines the adipomyocyte origin of the brown adipocyte.

![Tbx15 expression levels](image)

**Fig. 5.** Expression levels of the brown and brite adipose tissue-specific gene marker Tbx15. For details, see legend to Fig. 1.

The qualitative distinction between the brown and white adipocyte expression was paralleled by the presence of *Tbx15* in the brown depots and its total absence in the white depots (Fig. 5). However, *Tbx15* was also well expressed in the inguinal brite depot (although not in the retroperitoneal depot). In this context it may be observed that it has earlier been described as being specifically expressed in mouse subcutaneous adipose tissue (apparently the inguinal depot) in contrast to the visceral, intraabdominal adipose tissue depot (the epididymal depot) \textsuperscript{[7]}. Thus, whereas Gesta et al. considered *Tbx15* a subcutaneous versus abdominal marker, we would rather consider it a common marker for brown and brite depots.

**Novel brown gene markers that are brown adipose tissue depot-specific**

*Lim homebox 8* (*Lhx8*) is specifically expressed in cultured brown adipocytes compared to both white adipocytes and the C2C12 muscle cell line\textsuperscript{[2, 4]}.

![Lhx8 expression levels](image)

**Fig. 6.** Expression levels of the novel brown adipomyocyte markers Lhx8 (**A**) and Zic1 (**B**). For details, see legend to Fig. 1.
Fig. 7. Genes expressed at a high levels in both brite and white adipose tissues Hoxc8 (A), Tcf21 (B), Inhbb (C) and Dpt (D). For details, see legend to Fig. 1.

The present analysis shows that it is absent from muscle but well expressed in iBAT and cBAT and less expressed in the other adipose depots, except in eWAT where it is not observable at all (Fig. 6A).

Zinc fingers in the cerebellum 1 (Zic1) is specifically expressed in cultured brown adipocytes, found at similar levels in the C2C12 muscle cell line but is absent in white adipocytes \cite{2, 4}. Zic1 shows a remarkable distinct expression pattern in-vivo: it is markedly expressed in the classical BAT depots but is absent from brite and white adipose depots and is not found in muscle either. It thus displays a brown-fat specificity exceeding that of Prdm16 (and even UCP1).

**White adipocyte specific gene markers that are white/brite depot specific**

A series of genes were observed to be white adipocyte-specific in Timmons et al. \cite{2}. When examined in the different depots, these markers could broadly be described as being well expressed in the white depots, also well expressed in the brite depots but poorly or not expressed in the brown depots. These genes include (Fig. 7A-D) Homeobox C8 (Hoxc8), Transcription factor 21 (Tcf21), Inhibin B (Inhbb) and Dermatopontin (Dpt). Hoxc8 has earlier been described to be specifically expressed in abdominal (i.e. epididymal) in comparison to the subcutaneous adipose tissue (probably the inguinal depot)\cite{7}. We cannot confirm such a qualitative difference (Fig. 7A).

**Carbonic anhydrase 3**

Carbonic anhydrase 3 (Cat3) protein is down-regulated in mouse BAT by acute and chronic cold exposure\cite{9}. As seen (Fig. 8), we confirm this effect and demonstrate also that the repression is specific for brown and brite depots. Carbonic
anhydrase 3 protein constitutes ~24% of the total protein content\textsuperscript{[22]}. In agreement, only Ca3 levels reached the mRNA levels observed for UCP1, i.e. hundreds of mRNAs per TFIIB mRNA.

![Fig. 8. Expression levels of carbonic anhydrase 3.](image)

**Brite adipocyte gene markers**

Two of the examined genes display remarkable brite depot specificity (Fig. 9).

*Homeobox C9 (Hoxc9)* was described by us as a white adipocyte-specific mRNA\textsuperscript{[2, 4]}. However, Hoxc9 mRNA levels were significantly up-regulated by rosiglitazone treatment (that promotes brite adipocytes)\textsuperscript{[4]} and could thus indicate the emergence of another cell population, possibly the brite adipocytes. A similar but even more marked expression pattern was observed for *short stature homeobox 2* (Shox2).

Both Hoxc9 and Shox2 have been reported to be specifically expressed in mouse subcutaneous (inguinal) in comparison to epididymal depots \textsuperscript{[7]}. We can principally confirm this (Fig. 9) but would rather formulate it that Hoxc9 and Shox2 are brite depot markers. The expression levels of these markers did not change due to cold acclimation.

![Fig. 9. Expression levels of the brite adipocytes markers Hoxc9 and Shox2 (B). For details, see legend to Fig. 1.](image)

**Discussion**

In the present study, we have investigated the depot-specific expression of a series of adipocyte markers, earlier identified in in-vitro studies as being particularly associated with brown, white or brite adipocytes. Although we find that expression patterns in-vivo are not as distinctive as those earlier observed in cell culture systems, we can point to several markers as being particularly informative, and although all investigated depots present their own fingerprints, they can reasonably be divided into three main types: the brown, the brite and the white depots. The marker genes characterized here point to separate lineages for the different depots, and understanding the lineages and characteristics of the different
depots may both advance experimental design in coming studies and lead to the ability to differentially affect different depots.

Certain gene expression observations stand out
Concerning most of the genes examined here, mainly quantitative differences were observed between the depots. However, a few genes displayed expression patterns with a qualitative difference between depots.

Zic1 gene expression was found to be extremely specific for the three classical brown adipose tissue depots, much more so than Prdm16, and Zic1 was also expressed at a reasonably high level (1/10th the level of Prdm16). These observations would clearly point to Zic1 having a functional role in the determination of the classical brown adipocytes, but there are presently no functional studies relating Zic1 expression to brown adipocyte determination, differentiation or function.

Hoxc9 is remarkably specific for the brite adipose tissue depots, being fully absent from the brown adipose tissue depots.

Hoxc8 is found both in white and brite adipose depots but is down-regulated when the animal is cold-acclimated.

Tcf21 is the gene closest to being a true white adipose depot marker.

Particularly for the hox genes, it is not presently possible to distinguish as to whether the expression of these genes should only be seen as (a memory of) positional markers (which would mean that all white and brite adipose tissue depots may originate from a narrow range of segments during development) or whether they still have functions in determination of the phenotype of the cells.

The classification of depots
A thorough examination of the data presented here clearly indicates that the initial division into brown, brite and white tissues does not imply that the different depots within these divisions are identical. Concerning each gene examined, differences in expression level may be discussed. However, in order to present a simplified view of the differences between the different depots, we have created tissue fingerprints in Fig. 10. These fingerprints were made in the way that the level of gene expression in interscapular brown adipose tissue (iBAT) of each gene was set to 1, and the expression in all other depots expressed in relation to this and depicted on a logarithmic scale. As seen, in the mature stage there is not much similarity between muscle and brown adipose tissue. Within the traditional brown adipose tissue depots, the similarity between iBAT and cBAT is very high, and the similarity to aBAT is not much different.

However, even mBAT displays a fingerprint quite different from the classical brown adipose tissue depots. – When these depots are examined under conditions where the tissues have been chronically adrenergically stimulated, they tend all to become more similar in gene expression.

Even the “brite” depots show fingerprints far from that of brown adipose tissue, but particularly the iWAT and the rWAT, tend to move towards a brown-fat fingerprint in the cold-acclimated mice.

The white depots are clearly different from the brown and there is no tendency
that the expression pattern moves towards the brown in the cold-acclimated mice.

**Conclusions**
Perhaps to a higher extent than expected, the gene expression patterns established from studies of adipose cells in primary culture remain as distinctions when the cells are found in their natural environment. It is, however, clearly so that it is not realistic to think of adipose tissues

![Diagram](image)

**Fig. 10.** Fingerprints of gene expression in different BAT and WAT depots at thermoneutrality and 4°C. The gene expression level in interscapular brown adipose tissue (iBAT) was set to 1 for each gene and the expression level in the other depots expressed relative to this. The genes fingerprinted were (top to bottom): UCP1, Prdm16, Sirt1, Sirt3, Sirt5, Mylpf, miR-206, Zic1, Lhx8, Mox2, Tbx15, Hoxc9, Shox2, Igfbp3, Hoxc8, Tcf21, Inhbb, Dpt and Ca3.
of being either brown or white, or even brown, brite and white, but rather each depot displays gene expression patterns which characterize that particular depot and which probably determine its functional character as well.

Aknowledgegements
The work was supported by grants from the Swedish Research Council and the BBSRC, UK

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