MECHANISMS IN ENDOCRINOLOGY

White, brown and pink adipocytes: the extraordinary plasticity of the adipose organ

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Abstract

In mammals, adipocytes are lipid-laden cells making up the parenchyma of the multi-depot adipose organ. White adipocytes store lipids for release as free fatty acids during fasting periods; brown adipocytes burn glucose and lipids to maintain thermal homeostasis. A third type of adipocyte, the pink adipocyte, has recently been characterised in mouse subcutaneous fat depots during pregnancy and lactation. Pink adipocytes are mammary gland alveolar epithelial cells whose role is to produce and secrete milk. Emerging evidence suggests that they derive from the transdifferentiation of subcutaneous white adipocytes. The functional response of the adipose organ to a range of metabolic and environmental challenges highlights its extraordinary plasticity. Cold exposure induces an increase in the 'brown' component of the organ to meet the increased thermal demand; in states of positive energy balance, the 'white' component expands to store excess nutrients; finally, the 'pink' component develops in subcutaneous depots during pregnancy to ensure litter feeding. At the cell level, plasticity is provided not only by stem cell proliferation and differentiation but also, distinctively, by direct transdifferentiation of fully differentiated adipocytes by the stimuli that induce genetic expression reprogramming and through it a change in phenotype and, consequently function. A greater understanding of adipocyte transdifferentiation mechanisms would have the potential to shed light on their biology as well as inspire novel therapeutic strategies against metabolic syndrome (browning) and breast cancer (pinking).

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The adipose organ is made up of white and brown adipocytes…

In experimental animals, gross anatomy demonstrates that the adipose organ has a multi-depot organisation (1, 2, 3), consisting of two large subcutaneous depots and of numerous visceral depots (intended here as fat in close apposition to viscera, regardless of their portal or caval venous drainage) located in the visceral cavities of the trunk (Fig. 1). Dissection studies show that the depots have a fairly consistent shape throughout life and that similar shapes are found in different mouse strains. The subcutaneous depots are located in the upper portion of the

Invited Author's profile

Prof. Saverio Cinti is a specialist in internal medicine and anatomic pathology. He has been Professor of Anatomy, School of Medicine, Ancona University, Italy since 1984. Having started work in the field of adipose tissues since 1980 in the lab of Prof. Björntorp (Gothenburg University), he developed the concept of the adipose organ focusing on the plastic properties of adipose tissues and outlined the importance of this plasticity for the future treatment of obesity and related disorders. He described the CLS as the cause of low-grade chronic inflammation in the obese adipose organ. He has authored more than 250 peer reviewed articles. In 2008, he received the Blaise Pascal medal of the European Academy of Science for biology and in 2013 the Wasserman Prize for senior scientist of the European Society of Obesity.

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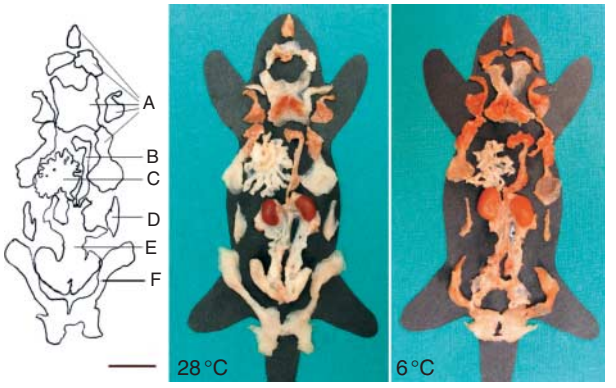

Figure 1

Gross anatomy of the adipose organ of adult female 129Sv mice. The subcutaneous and visceral depots were dissected and positioned on a mouse template to show their respective location in the body. The mouse on the left was maintained at temperatures close to thermoneutrality (28 °C for 10 days), whereas the mouse on the right was acclimated to cold (6 °C for 10 days). Browning of the adipose organ is visually evident in the cold-acclimated mouse. The adipose organ is made up of two subcutaneous depots: (A) anterior (deep cervical, superficial cervical, interscapular, subscapular, axillo-thoracic) and (F) posterior (dorso-lumbar, inguinal, gluteal), and of several visceral depots: (B) mediastinal, (C) mesenteric, (D) retro-peritoneal and (E) abdomino-pelvic (perirenal, periovarian, parametrial, perivescical). Scale bar: 1 cm. Reproduced with permission. Murano I, Zingaretti CM & Cinti S. The adipose organ of Sv129 mice contains a prevalence of brown adipocytes and shows plasticity after cold exposure. *Adipocytes* 2005 **1** 121–130.

thorax close to the forelimbs and the neck (anterior) and in the lower part of the abdomen, close to the hind legs (posterior). In animals kept at an environmental temperature close to thermoneutrality (about 28 °C), the adipose organ is predominantly white, with a few brownish areas in the anterior subcutaneous depot at the interscapular, subscapular, axillary, perisucclavian and pericarotid levels; brownish areas in the visceral depots are found mainly around the heart, the aorta and its main branches (mediastinal and perirenal sites). By light microscopy the adipose organ contains two different cell types: brown and white adipocytes (4, 5, 6). 'Brownish' areas are those where brown adipocytes are the predominant parenchymal cell type: they correspond to brown adipose tissue (BAT), which is richly innervated and vascularised. 'White' areas, where white adipocytes are the predominant cell type, are white adipose tissue (WAT) and show fewer nerves and a lower number of blood vessels. Depot colour is determined by the relative amount of the two cell types and the degree of vascularisation (7). The relative amount of BAT and WAT in the adipose organ is variable, depending on several factors of which age, diet and environmental temperature are the most important. Quantitative histological studies have shown that the vast majority of depots have a mixed composition also in warm-acclimated mice (see below).

White and brown adipocytes display distinctive features by transmission electron microscopy (1, 2, 3). White adipocytes contain a single large lipid droplet occupying about 90% of the cell volume. The nucleus is squeezed to the cell periphery and the cytoplasm forms a very thin rim. The organelles are poorly developed; in particular mitochondria are small, elongated and have short, randomly organised cristae (Fig. 2). Because of these ultrastructural characteristics, these cells are also called unilocular adipocytes.

Brown adipocytes are smaller than white adipocytes, their cytoplasm contains several lipid droplets, a roundish nucleus and numerous, large, generally spherical mitochondria with laminar cristae (Fig. 3). These mitochondria contain a unique protein, uncoupling protein 1 (UCP1), that supports the thermogenic function of brown adipocytes (8, 9). These cells are also called multilocular adipocytes.

The different morphology of white and brown adipocytes underpins their different functional roles. The white adipocyte stores energy (in the form of lipids) that is released between meals: its lipid droplet is spherical because this is the geometrical shape maximising volume and minimising space occupation. The brown adipocyte burns lipids to produce heat: its multilocularity maximises the cytoplasmic–lipid interface, making large amounts of fatty acids available quickly for mitochondrial uncoupling and consequently thermogenesis (8).

Notably, WAT and BAT do not exhibit distinct anatomical boundaries, but rather are found as a seamless continuum in all depots at both the macroscopic and microscopic levels. Indeed, in the areas between WAT and BAT, we described adipocytes with an intermediate morphology between white and brown adipocytes and designated them as paucilocular adipocytes (Fig. 4). Recent data from our laboratory have suggested that paucilocular adipocytes, which are found in all adipose depots, are the population showing the greatest proneness to transdifferentiate into brown adipocytes upon environmental or pharmacological stimulation (see below).

In conclusion, both white and brown adipocytes harbour a large amount of lipids in their cytoplasm. However, they serve two almost opposite functions that are essential for survival, to store and to dissipate energy respectively.

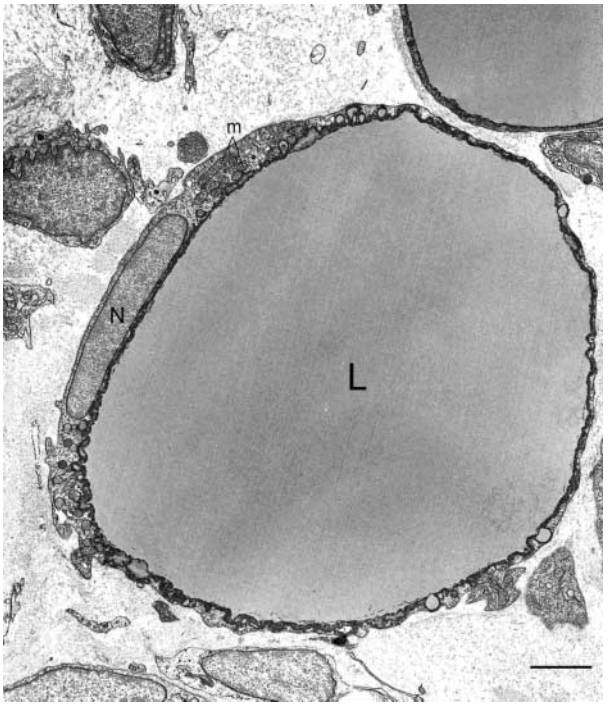

Figure 2
Transmission electron microscopy of a epididymal WAT white adipocyte from a 3-week-old rat. N, nucleus; m, mitochondria; L, lipid droplet. Scale bar: 3 μm. Reproduced with permission. Reproduced with permission. Cinti S. The Adipose Organ. Milan: Kurtis, 1999.

…but 'pink' adipocytes arise during pregnancy

During pregnancy and lactation the anterior and posterior subcutaneous depots of the female adipose organ turn into an organ whose function is to produce and secrete milk: the mammary gland (1, 10) (Fig. 5). Such transformation involves mainly the parenchyma through the development of milk-secreting lobulo-alveolar glandular structures. This dynamic process is generally referred to as alveologenesis.

Epithelial ducts ending in symmetrical nipples (three in anterior and two in posterior subcutaneous fat) develop before puberty and branch through the subcutaneous depot parenchyma, where in normal conditions white and brown adipocytes account for about 90% of depot volume. In terms of anatomical organisation, the mouse mammary glands are five symmetrical structures, each ending with a nipple; in terms of adipose organ organisation, the subcutaneous depot contains two glands, one anterior and one posterior (corresponding to the anterior and posterior subcutaneous depots respectively), each provided with symmetrical nipples (three anterior and two posterior). During pregnancy, which in mice lasts 21 days, alveoli gradually replace adipose tissue. Well-developed alveoli formed by epithelial cells devoid of lipid droplets, likely deriving from the proliferation of ductal stem cells, are visible already 12–15 days from conception (11, 12). Alveologenesis continues with the appearance of lipid-laden epithelial cells and culminates on days 18–21. Our data suggest that these cells derive from transdifferentiation of subcutaneous white adipocytes (13). We have proposed the name of 'pink adipocytes' for these adipocyte-derived milk-producing cells, because: i) they meet the definition of adipocyte, i.e. a parenchymal cell capable of storing large amounts of lipids; ii) they arise exclusively in female subcutaneous depots during pregnancy and lactation; and iii) the pregnant mammary gland is pink at the macroscopic level.

Thus, the adipose organ parenchyma contains three cell types characterised by a discrete morphology and function: i) the white adipocyte stores and secretes lipids; ii) the brown adipocyte produces heat; and iii) the pink adipocyte produces milk (Fig. 6). Each function is critical for individual and species survival. Interestingly, despite

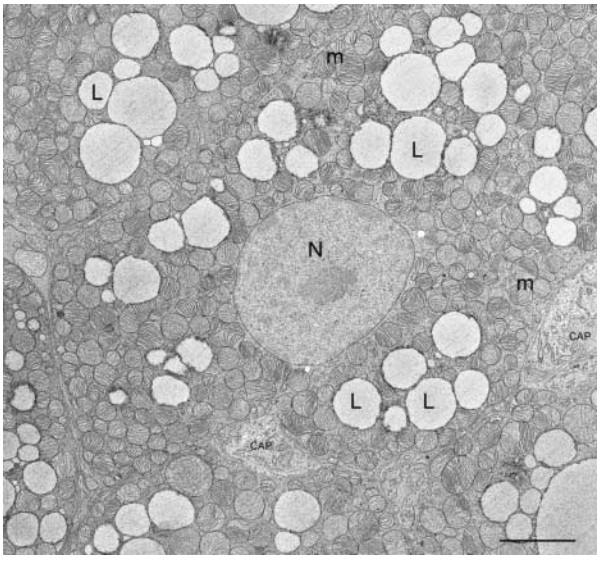

Figure 3
Transmission electron microscopy of a interscapular BAT brown adipocyte from a 10-day-old rat. Note the numerous, large and generally spherical mitochondria with laminar cristae (m). N, nucleus; L, lipid droplet; CAP, capillary. Scale bar: 3 μm. Reproduced with permission. Cinti S. The Adipose Organ. Milan: Kurtis, 1999.

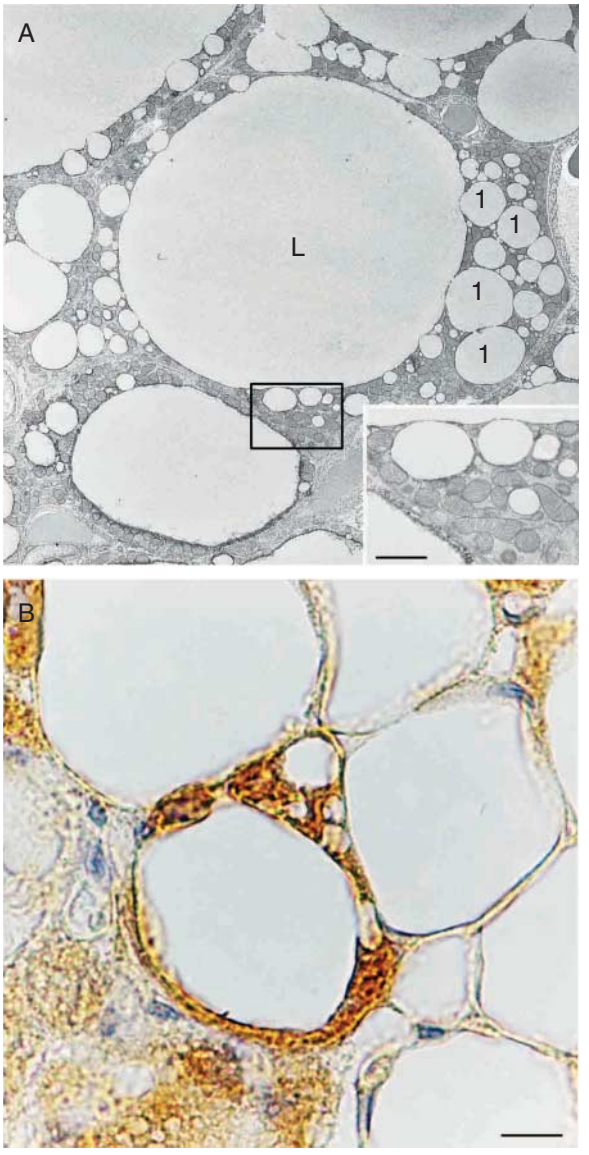

Figure 4
Paucilocular adipocytes. (A) Transmission electron microscopy of subcutaneous fat of a cold acclimated adult mouse (6 °C for 5 days) showing a paucilocular adipocyte with an intermediate morphology between white and brown adipocytes. Note the predominant large central lipid droplet (L) and several small cytoplasmic lipid droplets (l). Mitochondria are numerous and exhibit an intermediate morphology between those typical of white and brown adipocytes (inset: enlargement of squared area in (A). Scale bar: A = 5 μm and inset = 0.5 μm. (B) UCP1-immunoreactive paucilocular adipocyte found in omental fat from a patient suffering for pheochromocytoma. Note the morphology corresponding to that described in (A). Surrounding (upper and right) white adipocytes are unilocular and UCP1 negative. Scale bar: 10 μm.

their different morphology and physiology, the three cell types nonetheless share the expression of some genes. For example, most of the genes related to lipid metabolism are expressed in all three cells: leptin is expressed in both white (14, 15) and pink adipocytes (16), S-100b is expressed in white (17) and pink adipocytes (18) and perilipin A is expressed in white and brown adipocytes (19). From a physiological point of view, all three types of adipose cells have endocrine properties. White adipocytes secrete a number of adipokines that affect eating behaviour (leptin) (20) and metabolism (e.g. adiponectin, resistin, adipsin) (21). Brown adipocytes also secrete hormones and growth factors (e.g. betatrophin and FGF21) (22, 23, 24). Pink adipocytes, besides milk

Figure 5
Gross anatomy of the adipose organ of a lactating female mouse. Both anterior and posterior subcutaneous depots are transformed into mammary glands. Scale bar: 1.5 cm. Reproduced with permission. Cinti S. The Adipose Organ. Milan: Kurtis, 1999.

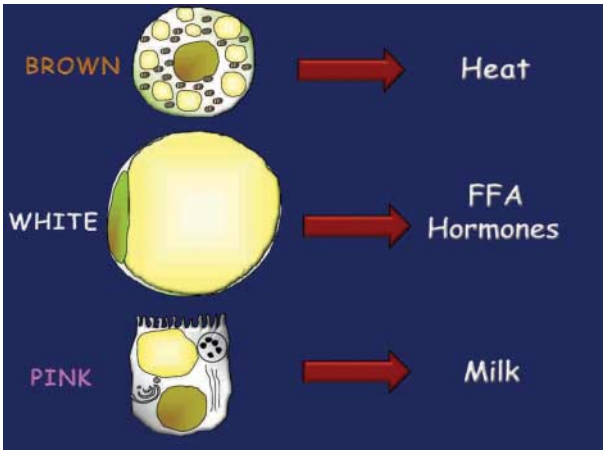

Figure 6
Scheme of the three adipocytes forming the parenchyma of the adipose organ.

components, also secrete leptin, which seems to have an important role in preventing obesity in pups (25, 26).

Plasticity of the adipose organ

The anatomical, cytological and physiological aspects reviewed above prompt the question of why three different cell types, each playing discrete physiological roles, should all be found in the same organ, the adipose organ. Similarities in morphology and gene expression can to some extent explain their coexistence. Yet distinctive phenotypic aspects underlie striking morphological and functional differences. For instance, some important genes appear to be specific and crucial for the function exerted by each cell type: *UCP1* underpins thermogenesis and is unique to brown adipocytes (8, 9), leptin is not found in classic multilocular brown adipocytes (14) and perilipin B is found in pink adipocytes along with a number of epithelial and milk-related genes (27, 28).

White–brown plasticity

Over the last three decades our and other laboratories have collected a large body of evidence documenting that fully differentiated adipocytes have the outstanding physiological ability to transdifferentiate. In particular, mature adipocytes undergo genome reprogramming and turn into a different cell type, serving different physiological roles (15, 29, 30, 31, 32, 33, 34, 35); crucially, the process is reversible. White-to-brown transdifferentiation is essential to meet increased heat production requirements during chronic cold exposure. Cold exposure activates BAT by acting on the sympathetic nervous fibres that directly innervate brown adipocytes at the parenchymal level (36, 37, 38, 39); chronic cold exposure results in branching of noradrenergic parenchymal fibres, significantly increasing BAT sympathetic innervation, a phenomenon that appears to be closely related to white-to-brown transdifferentiation (40, 41). β3-adrenoceptors (AR) are specifically expressed by brown adipocytes. When activated by noradrenaline they drive brown adipocyte thermogenic activation, but are also likely responsible for white-to-brown transdifferentiation. 'Browning', i.e. an increase in the brown component of the organ, is detectable even at the macroscopic level in the adipose organ of a mouse kept at 6 °C compared with one acclimated to 28 °C (Fig. 1). The tissue remodelling is partly due to recruitment of precursor cells, especially in interscapular and inguinal subcutaneous depots (42), and partly to direct conversion of a subpopulation of unilocular/paucilocular adipocytes (6, 31, 32, 33, 35, 43). The two processes, which most likely coexist, are driven by the same physiological stimulus through β-AR activation. Cold-exposed mice lacking β3-AR do not undergo browning (32, 43), but precursor development, probably driven by β1-AR, is not hampered in these animals, because preadipocyte development has been documented after administration of β1-AR agonists and lack of development has been demonstrated after administration of β3-AR agonists (43). As also suggested by *in vitro* findings (44), β3-AR could thus be responsible for white-to-brown transdifferentiation and β1-AR for precursor proliferation and differentiation.

Browning is of remarkable pathophysiological interest, because it could be harnessed to tackle obesity and metabolic syndrome (45, 46, 47, 48). Indeed, ectopic UCP1 expression (49) and expression in white adipocytes of key molecules involved in brown adipocyte differentiation, such as *Prdm16* (50), induce obesity resistance and ameliorate insulin sensitivity. Obesity-prone mouse strains have less BAT than obesity-resistant strains (3, 4, 51); mice lacking brown fat (52) and β3-AR (34) are prone to diet-induced obesity; and specific β3-AR agonists curb obesity in obese rats (53, 54, 55).

Warm exposure, ageing and obesity lead to 'whitening', which involves a significant reduction in the density of parenchymal noradrenergic nerve fibres in the adipose organ (1, 2, 3). Different gene expression profiles are found in brown adipocytes from different depots (56) and even in *UCP1*-expressing adipocytes (57). Furthermore, a different gene expression has been found during development in interscapular

(anterior subcutaneous depot) and perirenal (visceral depot) brown adipocytes as compared with those found predominantly in white depots, such as the posterior subcutaneous depot (58). These findings have prompted terms such as 'brite' and 'beige' for a population of UCP1-containing multilocular adipocytes (57, 59). However, we feel that the notion of 'brown' should be maintained until different functions are demonstrated for these cells. The different gene expression profiles described in interscapular brown adipocytes and in other depots could merely depend on differences in the extracellular hormonal milieu, the degree and type of innervation, and/or the cell development stage. As mentioned earlier, paucilocular adipocytes, i.e. adipocytes with an intermediate morphology, are found in the areas between BAT and WAT. We documented a varied morphology and degree of UCP1 expression in these adipocytes (43). Some were UCP1-negative, others were UCP1-positive; accordingly, mitochondrial morphology spanned from that typical of white adipocytes to that typical of brown adipocytes, thus accounting for the variable immunoreactivity of these cells for the brown marker UCP1. It is conceivable that UCP1 immunoreactivity is acquired only upon achievement of a given degree of mitochondrial differentiation. The distinctive gene expression profile of brite/beige adipocytes may reflect the density of multilocular adipocytes and their level of differentiation. A true, functional difference between interscapular brown adipocytes and those found in other areas of the organ has not yet been documented *in vivo* and at single cell level.

A recent paper seems to confirm the direct, reversible transdifferentiation of white adipocytes into brown adipocytes (35). Cold-induced UCP1-expressing adipocytes from posterior subcutaneous fat turned into unilocular adipocytes, expressing genuine white phenotype genes when the animals were exposed to a warm environment, and re-exposure to cold involved a return to the multilocular UCP1 phenotype. Not all white adipocytes seem to have the ability to turn into brown adipocytes, and some white adipocytes might never be able to undergo the phenotype change, possibly because of their distance from noradrenergic fibres. In this connection, we found a positive correlation between the density of brown adipocytes and the density of noradrenergic fibres in the adipose organ of two different mouse strains (5).

The plasticity of the adipose organ raises the question of the origin of adipocytes. Despite contrasting findings from different laboratories (60) our data, including lineage-tracing studies, seem to indicate that endothelial cells of adipose tissue capillaries are able to turn into pericytes and then into either white and brown adipocytes (61, 62, 63, 64, 65, 66). Such unique origin could support their ability to undergo multiple changes before achievement of the adult phenotype.

White–pink plasticity

During pregnancy and lactation, all subcutaneous depots of the adipose organ turn into mammary glands (1, 10). Our morphological studies of the transforming subcutaneous depots seem to suggest that mammary gland alveoli develop in two stages through two different mechanisms. In the first stage of pregnancy, alveoli are constituted of epithelial cells lacking cytoplasmic lipid droplets that could derive from stem cell proliferation (Fig. 7), but in the second stage of pregnancy they are constituted of lipid-laden epithelial cells while subcutaneous fat shrinks progressively (Fig. 8). Ultrastructural data support the astonishing possibility that in the second part of pregnancy subcutaneous adipocytes progressively acquire epithelial-like features, likely under hormonal stimuli, aggregating with similarly committed pink adipocytes and with myoepithelial cells to form adipose tissue-derived milk-secreting alveoli (13) (Fig. 9). To document the striking transdifferentiation of adipocytes into milk-producing glands and establish whether the opposite process occurs during mammary gland involution, we carried out lineage-tracing studies using aP2-cre/R26R and WAP-cre/R26R double transgenic mice respectively. In aP2-cre/R26R mice, about 70% of alveolar epithelial cells expressed the reporter gene in late pregnancy, whereas in WAP-cre/R26R mice most of the adipocytes found in subcutaneous fat post-lactation were positive for the reporter gene. Notably, about 30% of alveolar epithelial cells never stained for the gene during pregnancy, in line with the origin of a large number of alveolar cells from the well-characterised ductal alveolar progenitor cells (10, 11, 12). The transdifferentiation of white adipocytes into pink cells was also confirmed by explants experiments, where both adipose tissue and isolated adipocytes from *Rosa26* (*Gt(ROSA)26Sor*) mice implanted in pregnant WT female mice gave rise to donor-derived glands (68).

Reversible white-to-pink transdifferentiation could shed light on breast cancer biology, as suggested by recent data showing that loss of *PPARγ* expression by mammary secretory epithelial cells creates a pro-breast tumourigenic environment (69). Notably, *PPARγ* seems to be a key factor for pink-to-white transdifferentiation *in vitro* (70).

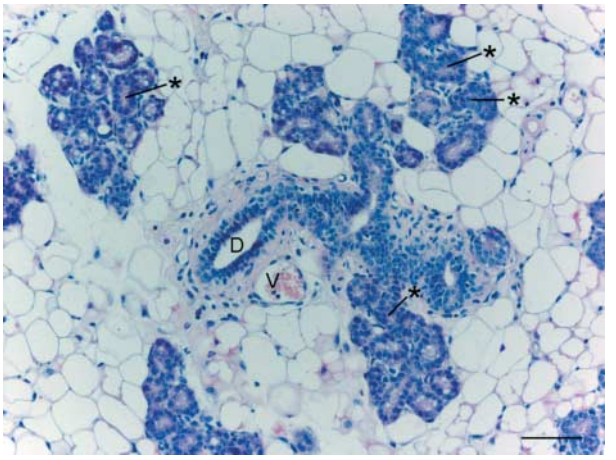

Figure 7
Haematoxylin–eosin staining of the subcutaneous adipose depot (mammary gland) from a female mouse at day 10 of pregnancy. In the first stage of pregnancy, the alveoli (asterisk) are constituted of epithelial cells lacking cytoplasmic lipid droplets. D, duct; V, blood vessel. Scale bar: 60 μm.

The obese adipose organ

In 2003, two independent groups in the United States showed that the adipose organ of obese animals and humans is infiltrated by macrophages; the infiltration was found to relate to adipocyte size and to the development of insulin resistance (71, 72). The majority of cytokines with key roles in inducing insulin resistance are expressed by the stroma-vascular fraction of fat (including macrophages) and a minority by the floating fraction formed by mature adipocytes, reflecting the importance of macrophage infiltration in the development of insulin resistance and subsequently type 2 diabetes. Our group found that most of the macrophages infiltrating obese fat are arranged around dead adipocyte remnants into distinctive figures that we denominated crown-like-structures (CLS) (73). In a subsequent paper, we described ultrastructural abnormalities (such as calcium build-up and cholesterol crystals), signs of oxidative stress and *NLRP3* inflammasome activation with formation of active caspase 1 in hypertrophic adipocytes from obese mice and suggested that these cells die of pyroptosis, a proinflammatory programmed cell death (74).

Resorption of dead adipocyte remnants, especially the large lipid droplets, is an extended process characterised by a chronic low-grade inflammation similar to that seen in foreign body reactions. Accordingly, CLS may also contain syncytial giant macrophages. To assess whether CLS originate from adipocyte debris, we used Philip Scherer's transgenic model, where white adipocyte apoptotic death is specifically induced by administration of a dimeriser that activates caspase 8 (75). All dead adipocytes gave rise to CLS, in line with our hypothesis (76). The time course of fat histopathology in this model disclosed that CLS form after adipocyte death, demonstrating that this event may be sufficient to recruit macrophages and induce CLS formation (76). Altogether, we think that the death and degeneration of hypertrophic adipocytes, with the consequent exposure to the extracellular milieu of nuclear and cytoplasmic (mainly, lipid droplets) determinants that are normally segregated into the cell, represent the primary events triggering the inflammatory and immune reactions in the obese adipose tissue. This view has been recently reinforced by data from Xu *et al*. (77), showing the importance of lipid catabolism in the macrophages infiltrating the obese adipose tissue. Interestingly, this

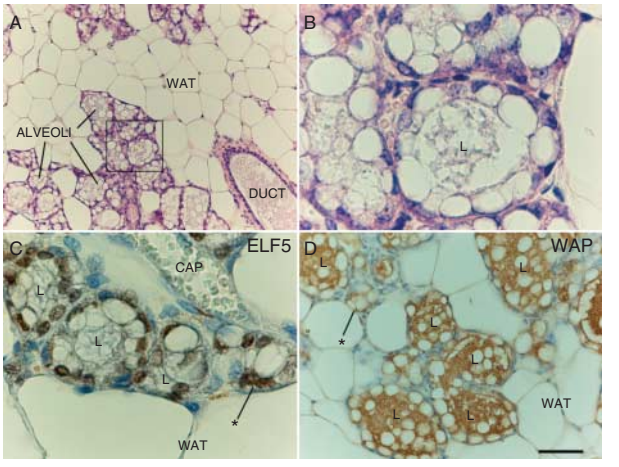

Figure 8
Subcutaneous adipose depot (mammary gland) from a female mouse at day 17 of pregnancy. Haematoxylin–eosin staining (A and B) shows the appearance of alveoli constituted of lipid-laden epithelial cells (pink adipocytes). B is the enlargement of the area framed in A: most of the alveolar cells are pink adipocytes with large cytoplasmic lipid droplets. Pink adipocytes of well-developed alveoli (with central lumen: L) and of early alveoli (without central lumen: *) show strongly immunoreactive nuclei for the transcription factor *Elf5* (C), a master regulator of alveologenesis (67), and are immunoreactive for the milk whey acidic protein (WAP) (D). CAP, capillary; WAT, white adipose tissue. Scale bar: A = 50 μm; B = 15 μm; C and D = 30 μm.

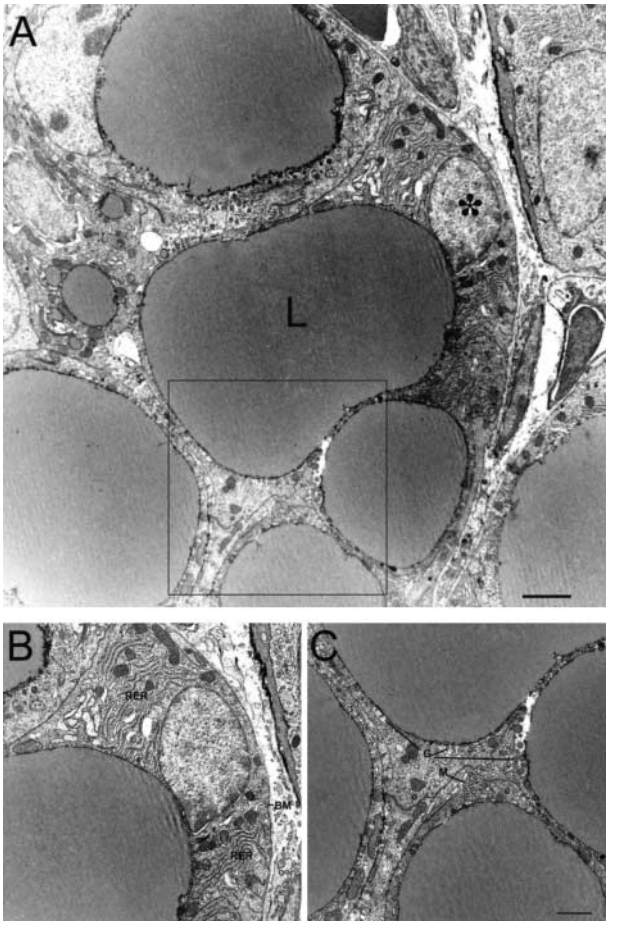

Figure 9
Transmission electron microscopy of pink adipocytes. (A) Pink adipocytes contain large cytoplasmic lipid droplets (L). (B) Enlargement of the pink adipocyte marked by * in A. Abundant stacked rough endoplasmic reticulum (RER) and a distinct basal membrane (BM) are visible. (C) Enlargement of the framed area in (A). Milk granules (G) and microvilli projecting in the early lumen are visible. Scale bars: 2 μm.

view may also predict that autoimmune reactions could develop in obesity, and be possibly involved in some physiopathological aspects of the metabolic syndrome.

Hormone-sensitive lipase knockout mice created by Grant Michell are lean but their fat is characterised by hypertrophic adipocytes (78). These animals exhibited the same CLS density as obese animals (73). Notably, we found a positive correlation between CLS density and adipocyte size both in subcutaneous and visceral fat depots; their density was lower in subcutaneous fat containing larger adipocytes (79). Collectively, these data suggest that visceral adipocytes have a smaller death critical size (size-triggering death) (41), in line with the well-known greater morbidity due to accumulation of visceral fat (80). Interestingly, we failed to detect CLS in either mice or humans with hyperplastic obesity, which is characterised by small adipocytes and the absence of secondary metabolic disorders (73, 81). The positive correlation between adipocyte size and insulin resistance has recently been confirmed in non-obese humans (82).

The plasticity of the adipose organ could be the basis for future treatment, or prevention, of obesity and type 2 diabetes. As mentioned above, white-to-brown trans-differentiation involves a reduction in adipocyte size and an increase in their mitochondrial content. Thus, 'mild' white-to-brown transdifferentiation could make white adipocytes less prone to death and turns the adipose organ parenchyma into a 'healthier' tissue.

The human adipose organ

As in experimental animals, also in humans the adipose organ is made up of subcutaneous and visceral depots (83, 84, 85, 86, 87). Whereas in rodents, dermal and subcutaneous adipose tissues are separated by a layer of skeletal muscle cells, in humans they are continuous with one another; moreover, subcutaneous adipose tissue is not confined to some areas, but forms an uninterrupted layer throughout the body with the exception of hands and feet. Importantly, mammary and gluteo-femoral subcutaneous adipose tissues are more developed in females than in males. The distribution of the visceral depots is very similar to that described in rodents, but the omental depot is particularly well developed in humans. In lean adults, the human adipose organ accounts for 8–18% of body weight in males and for 14–28% in females (~5% in monkeys) (88, 89).

Independent of total body fat, body fat distribution is a well-known important risk factor for obesity-associated diseases, with visceral obesity displaying greater morbidity (80). It should be noted, however, that the vast majority of free fatty acids, that are especially involved in the cardiovascular complications of the metabolic syndrome (90), are released by the upper body subcutaneous fat depots (91, 92).

As in small rodents, the human adipose organ contains brown adipocytes organised into typical BAT. The lower surface:volume ratio, hence thermal dispersion, of the human compared with the rodent's body involves lower heat production demands, at least in adults. On the other hand, newborns are characterised by a greater surface:volume ratio and a considerable amount of BAT

(93, 94). Human BAT thus seems to undergo an age-related morphofunctional involution (likely, brown-to-white transdifferentiation). The histological and electron microscopic features of human adipose tissue are identical to those of their murine counterpart (95). In particular, UCP1-positive brown adipocytes are found among white adipocytes (96, 97, 98). In human newborns, BAT is found in almost all the areas described in rodents, and *UCP1* gene expression has been documented in visceral adipose tissue of lean and obese adult patients. The brown:white adipocyte ratio in the visceral adipose tissue of lean adult humans has been put at 1/100–200 (99).

As in experimental animals, also in humans the adipose organ displays outstanding plasticity. An increased amount of BAT has been described in outdoor workers in northern Europe (100) and in patients with pheochromocytoma, a tumour derived from the cells of the adrenal medullary and characterised by catecholamine secretion (101, 102). Furthermore hibernoma, a rare BAT tumour, has been described in several anatomical areas, including subcutaneous and visceral fat (103).

Brown adipocytes have a strong oxidative metabolism and incorporate high levels of fluorodeoxyglucose, the tracer used in positron emission tomography (PET); this has enabled unexpectedly large amounts of BAT to be detected in adult humans (83, 84, 85, 86, 104, 105). In normal adults, BAT depots are found at the base of the neck, the root of the upper limbs, and the intercostal spaces (106). We found UCP1-positive brown adipocytes in perithyroid fat from adult biopsies. These specimens also contained parenchymal noradrenergic fibres in direct contact with brown adipocytes (Fig. 10) (87, 107), documenting a similar parenchymal innervation of the adipose organ in humans and small mammals. Electron microscopy has disclosed preadipocytes in close proximity to capillary walls in human BAT (87); interestingly, the density of preadipocytes was about five times higher in a case of hibernoma than in normal BAT (108).

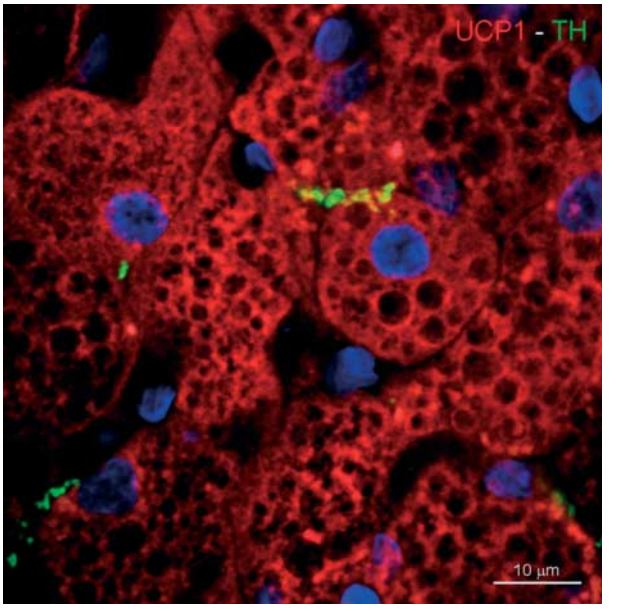

Figure 10

Double immunostaining and confocal microscopy of human BAT. The sympathetic nerve fibres (detected by thyrosine hydroxylase immunofluorescence: green) are closely apposed to the surface of *UCP1*-immunoreactive (red) brown adipocytes. Nuclei are stained by TOTO3 (blue). Scale bar: 10 µm.

Human adipose organ plasticity and therapeutic prospects

As the physiological role of BAT in adult humans continues to be explored, the possibility to expand this energy-dissipating tissue through pharmacological interventions is being hailed as a possible approach to treat obesity and related disorders (109). Functional BAT has clearly been demonstrated in adult humans and proved to contribute to the overall energy balance (83, 84, 85, 86, 110). Cold exposure can recruit BAT to produce non-shivering thermogenesis (111). Moreover, poor BAT activity correlates with ageing, BMI and measures of metabolic disease (112, 113, 114).

It is interesting to note that humans with a reduced brown phenotype of abdominal subcutaneous adipose tissue have reduced insulin sensitivity (115), and human white adipocyte precursors can be induced *in vitro* to express UCP1 through administration of drugs (116, 117). We recently reported that a white visceral depot (the omentum), which normally contains only unilocular UCP1-negative (white) adipocytes, showed multilocular UCP1-positive (brown) adipocytes in six of 12 patients with pheochromocytoma (118). In these six patients, we also detected several UCP1-positive paucilocular adipocytes, the intermediate phenotype preceding white-to-brown transdifferentiation. In such adipocytes, electron microscopy documented mitochondria with an intermediate morphology between typical white and typical brown. Similar features have been detected in transdifferentiating human brown adipocytes *in vitro* (119). Taken together, these data suggest that white-to-brown transdifferentiation also occurs in humans and might be harnessed for therapeutic purposes. The master

molecular pathways could be the noradrenergic stimulation via the β3-AR signalling, despite the unsuccessful clinical trials performed with β3-AR agonists (120, 121) before PET, and bioptic studies renewed the interest in the topic in 2009. A range of new molecular mechanisms to induce browning have recently been proposed (reviewed in (107)). Among these, secreted factors such as *ANP* (*NPPA*), *BMP8B*, irisin and *FGF21* that affect brown adipocyte activation and recruitment seem to be particularly promising for the development of new anti-obesity drugs in the near future (22, 122, 123, 124, 125).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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