

The adipose organ at a glance

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The main parenchymal cells of the adipose organ are adipocytes. White adipocytes store energy, whereas brown adipocytes dissipate energy for thermogenesis. These two cell types with opposing functions can both originate from endothelial cells, and co-exist in the multiple fat depots of the adipose organ – a feature that I propose is crucial for this organ's plasticity. This poster review provides an overview of the adipose organ, describing its anatomy, cytology, physiological function and histopathology in obesity. It also highlights the remarkable plasticity of the adipose organ, explaining theories of adipocyte transdifferentiation during chronic cold exposure, physical exercise or lactation, as well as in obesity. White-to-brown adipocyte transdifferentiation is of particular medical relevance, because animal data indicate that higher amounts of brown adipose tissue are positively associated with resistance to obesity and its co-morbidities, and that 'browning' of the adipose organ curbs these disorders.

Introduction

Adipose tissues are generally regarded as connective tissues without a specific anatomy. However, accumulating data support the idea that adipose tissues are organized to form a large organ with discrete anatomy, specific vascular and nerve supplies, complex cytology, and high physiological plasticity (Cinti, 1999; Cinti, 2011). The organ is made up of several depots located in two compartments of the body: some are below the skin (subcutaneous depots) and some are in the trunk (visceral depots). Adipose tissues can thus be considered a multi-depot organ (Cinti, 2001; Cinti, 2005). This organ contributes to many of an organism's crucial survival needs: thermogenesis, lactation, immune responses and fuel for metabolism. In this article and the accompanying poster, I provide a brief overview of the adipose organ in mice and humans, touching on cytology, physiological function and dynamics during health and disease. Because most of the data available derive from mouse studies, the poster illustrates the adipose organ of mice; however, many of the anatomical, physiological and pathological

aspects are common to the adipose organ of humans. The anatomy of female mice is shown to emphasize changes in the adipose organ that occur during pregnancy and lactation.

Cytology

The main parenchymal cells of the adipose organ are called adipocytes. There are two main types of adipocytes, which are easy to distinguish by morphology: white adipocytes [see top scanning electron microscopy (SEM) image in blue panel of poster] are leptin- and S100-B-immunoreactive spherical cells with ~90% of their volume comprising a single cytoplasmic lipid droplet and a 'squeezed' nucleus, whereas brown adipocytes (see bottom SEM image in blue panel of poster) are polygonal cells with a roundish nucleus and several cytoplasmic lipid droplets. Brown adipocytes are also characterized by numerous large mitochondria packed with cristae. Mitochondria in brown adipocytes are marked by the expression of uncoupling protein 1 (UCP1), a unique protein that uncouples oxidative phosphorylation from ATP synthesis and thereby results in the production of heat (thermogenesis) (Cannon and Nedergaard, 2004; Ricquier, 2005; Frontini et al., 2007). Thus, white and brown adipocytes are quite different in their morphology and physiology: white adipocytes store energy for the metabolic needs of the organism, whereas brown adipocytes burn energy for thermogenesis. Both cell types are contained in the multiple depots of the adipose organ (Murano et al., 2005; Murano et al., 2009; Vitali et al., 2012). White adipocytes of different sizes are present in subcutaneous depots (mainly large adipocytes) and visceral depots (mainly small adipocytes) (Murano et al., 2008; Barbatelli et al., 2010). Brown adipocytes in visceral depots are mainly found near the aorta. Paucilocular adipocytes, which are cells with intermediate morphology between that of white and brown adipocytes, are also present in the adipose organ. It should be noted that some groups also refer to 'beige' (Ishibashi and Seale, 2010) or 'brite' (brown in white) (Petrovic et al., 2010; Waldén et al., 2012) regions of white adipose tissue, containing brown or brown-like adipocytes.

Anatomy

In mice, the adipose organ is made up of two subcutaneous depots and several visceral depots. The anterior subcutaneous depot is quite complex (Frontini and Cinti, 2010; Vitali et al., 2012). Its main volume is located in the upper dorsal area at the level of the scapulae. Several parts of it have been described in literature as distinct depots: interscapular, subscapular, axillary and cervical. The posterior subcutaneous depot is located mainly in the lower ventral part of the body, and is formed of three parts (also often described as distinct depots): dorso-lumbar, inguinal and gluteal. One or two lymph nodes are present in the dorso-lumbar and inguinal parts. The truncal depots are contained in the mediastinum and abdomen. All truncal depots are closely associated with the aorta and its main collaterals. In females, perirenal, periovarian, parametrial and perivesical fat form a single anatomical structure called the

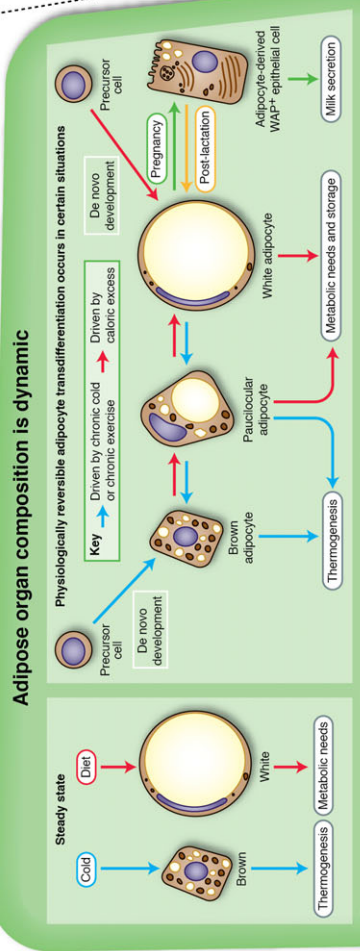
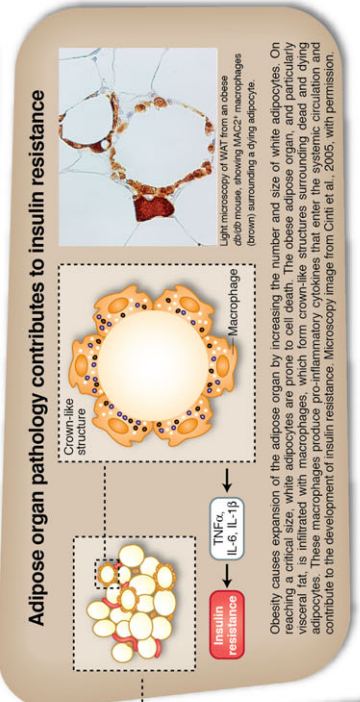
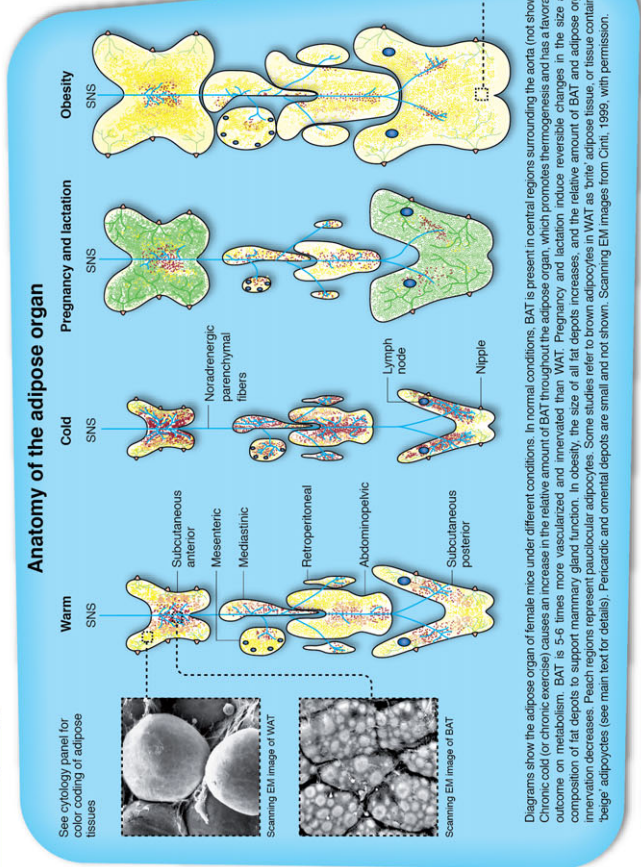
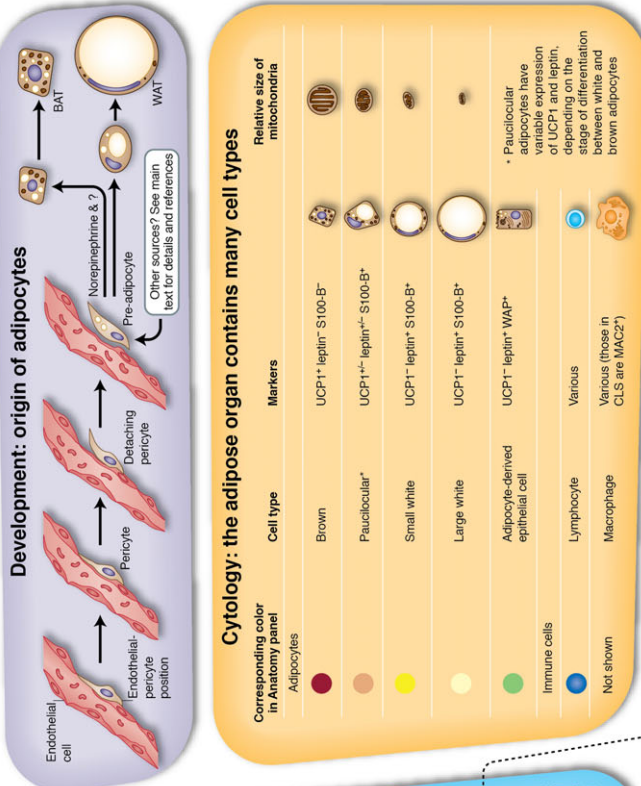
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abdominopelvic depot (Murano et al., 2005; Frontini and Cinti, 2010; Vitali et al., 2012). Furthermore, subcutaneous depots in female mice are infiltrated by ramified epithelial ducts ending in five symmetrical pairs of nipples; thus, in female mice, all of the subcutaneous tissue can be considered as part of ten mammary glands (see Cinti, 1999; Masso-Welch et al., 2000).

The composition of the adipose organ varies in different anatomical locations, and under different conditions, as represented by different colors in the blue panel of the poster. In normal animals in a warm environment, regions close to the aorta and its main collaterals are shown in brown, representing brown adipose tissue (BAT). Note that brown adipocytes are normal components of several subcutaneous and visceral depots and are not exclusive to interscapular fat. BAT is mainly formed of UCP1-expressing brown adipocytes, a rich capillary network and a high density of noradrenergic parenchymal fibers (shown as blue lines in the poster). The majority of the adipose organ is shown in yellow in the poster, representing white adipose tissue (WAT), which is mainly formed of leptin- and S100-B-expressing white adipocytes (Cinti et al., 1989; Barbatelli et al., 1993; Cinti et al., 1997) and a network that is five- to six-times less vascularized and innervated than BAT (Nechad, 1986). The areas shown in peach are mainly composed of paucilocular cells that can be positive for UCP1 and S100-B, depending on their differentiation stage (Barbatelli et al., 1993). On the basis of electron microscopy, paucilocular cells show features that are intermediate between white and brown adipocytes, including mitochondrial pleomorphism (Barbatelli et al., 2010), as illustrated in the green panel of the poster.

Pericardic, omental (which are very small in mice and not represented in the poster), mesenteric and subcutaneous depots are characterized by the presence of lymphatic tissue (Cinti, 2011). Moreover, mesenteric, omental and pericardic fat contain lymphocytes that are in close contact with adipocytes. Of note, lymphocytes express the leptin receptor, and visceral fat lymphocytes have been discovered to have specific functional properties (Fantuzzi and Faggioni, 2000; Moro et al., 2010). Furthermore, there are one or two lymph nodes in the posterior subcutaneous depots as well as in other anatomical sites such as the popliteal fat depots (Almind et al., 2007). Thus, it is reasonable to assume a functional relationship between adipocytes and lymphocytes, and this could be altered in obesity.

Dynamics of adipose organ composition Innervation and adaptation to temperature changes

The adipose organ is innervated (Bartness and Bamshad, 1998; Giordano et al., 2008; Bartness et al., 2010), enabling it to interface with the nervous system and respond to physiological and environmental cues. Nerve endings in adipose tissue reach the vasculature and adipocytes (Cannon et al., 1986). Most of the parenchymal fibers (i.e. nerve fibers in contact with adipocytes) express tyrosine hydroxylase (TH), an enzyme that is widely considered to be a marker of noradrenergic fibers (Giordano et al., 2008). Thermogenesis is required when animals are exposed to temperatures below thermoneutrality and implies sympathetic nervous system activation (Himms-Hagen, 1986). Norepinephrine acts on beta3 adrenoceptors, which promote the molecular pathway for thermogenesis in brown adipocytes (Cannon and Nedergaard, 2004). Our own data show that the density of TH-

expressing parenchymal fibers is much higher in the brown parts of the adipose organ than in white parts and, during cold exposure, the density of these fibers increases in parallel with the increase in number of brown adipocytes (Murano et al., 2009; Vitali et al., 2012). Thus, the adipose organ of cold-exposed mice is browner and more densely innervated than the adipose organ of mice in a warm environment, as shown in the blue panel of the poster.

The adipose organ of humans behaves similarly to the adipose organ of mice. Metabolically active BAT is detectable by positron emission tomography (PET) (Nedergaard et al., 2007; Cypess et al., 2009; Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). Similarly to mouse BAT, human BAT is composed of UCP1-expressing adipocytes that are densely innervated by TH-immunoreactive fibers (Zingaretti et al., 2009). Interestingly, cold-exposed normal subjects and patients suffering from pheochromocytoma (a norepinephrine-secreting tumor of the adrenal gland) show increased BAT by PET with an anatomic distribution similar to that of mice – that is, closely associated with the aorta and its main collaterals (Kuji et al., 2008; Saito et al., 2009).

Adipose organ plasticity

The origin of newly formed brown adipocytes in the adipose organ of cold-exposed animals – a phenomenon referred to as ‘browning’ – is under debate (Timmons et al., 2007; Seale et al., 2008; Barbatelli et al., 2010). Notably, data obtained from three independent groups using different experimental approaches support the idea that brown-like adipocytes arising in predominantly white fat depots in response to cold exposure are ontogenically different from those in the classic interscapular BAT (Atit et al., 2006; Seale et al., 2008; Petrovic et al., 2010).

Our own data support the idea that most of the brown adipocytes responsible for the browning phenomenon observed after adrenergic stimulus or cold exposure (with the exception of the interscapular fat) derive from a direct transformation of white adipocytes into brown adipocytes (white-to-brown transdifferentiation) (Himms-Hagen et al., 2000; Barbatelli et al., 2010). The recent discovery that both brown and white adipocytes derive from vascular endothelial cells of the adipose organ brings strong new support to the transdifferentiation theory (Tran et al., 2012; Gupta et al., 2012). However, it should be noted that other routes of adipocyte differentiation have been proposed (Crossno et al., 2006; Maumus et al., 2011; Lee et al., 2012). The transdifferentiation theory explains why these two different cell types are contained together in the same organ: in special cases (such as chronic cold exposure) the white part of the organ might ‘help’ the brown part by forming new BAT (Cinti, 2009a). The many reasons supporting our theory of transdifferentiation are discussed in more detail elsewhere (Cinti, 2009a; Cinti, 2009b; Cinti, 2011). Notably, WAT also expresses beta3 adrenoceptors (De Matteis et al., 2002), and the browning phenomenon is blunted in beta3 adrenoceptor knockout mice (Jimenez et al., 2003; Barbatelli et al., 2010). In addition, a wide variety of other mechanisms have been reported to be involved in BAT activation and browning of the adipose organ (see Box 1).

The potential to manipulate the inherent plasticity of the adipose organ is important because it is widely accepted that animals with more BAT are more resistant to obesity and type 2 diabetes (T2D)

Box 1. Mechanisms reportedly involved in BAT activation and browning of the adipose organ

- Enhancement of the activity of the regulatory subunit R1 α of the cAMP-dependent protein kinase A (PKA) (Cummings et al., 1996)
- Activation of peroxisome proliferator-activated receptor- γ (PPAR γ) (Toseland et al., 2001)
- Inhibition of the activity of 4E-BP1, which represses translation of PPAR γ coactivator-1 α (PGC1 α) (Tsukiyama-Kohara et al., 2001)
- Activation of the activity of forkhead box protein C2 (FOXC2), which increases the sensitivity of the β -adrenergic cAMP-PKA signaling pathway (Cederberg et al., 2001)
- Inhibition of retinoblastoma (Rb) protein activity (Hansen et al., 2004)
- Inhibition of RIP140 [also known as nuclear receptor-interacting protein 1 (NRIP1)] (Leonardsson et al., 2004)
- Activation of the zinc-finger protein PRDM16 (Seale et al., 2007; Seale et al., 2011)
- Activation of bone morphogenetic protein 7 (BMP7) (Tseng et al., 2008)
- Activation of cyclooxygenase 2 (COX2) (Madsen et al., 2010; Vegiopoulos et al., 2010)
- Activation of the microRNA cluster comprising miR-193b-365 (Sun et al., 2011)
- Inhibition of the TGF β -SMAD3 system (Yadav et al., 2011)
- Activation of the evolutionarily conserved PLAC8 protein (Jimenez-Preitner et al., 2011)
- Inhibition of the gender-sensitive α -arrestin domain-containing 3 (ARRDC3) protein (Patwari et al., 2011)
- Activation of the growth factor FGF21 (Hondares et al., 2010; Fisher et al., 2012)
- Induction of myokine irisin (Boström et al., 2012)
- Activation of microRNA 196a (Mori et al., 2012)
- Inhibition of retinaldehyde dehydrogenase 1 (ALDH1A1) (Kiefer et al., 2012)

(Kopecky et al., 1996; Collins et al., 1997; Guerra et al., 1998; Almind et al., 2007; Vitali et al., 2012). Conversely, animals without functional BAT are prone to obesity and T2D (Lowell et al., 1993; Bachman et al., 2002; Feldmann et al., 2009). Furthermore, obesity in rats is curbed when they are treated with beta3 adrenoceptor agonists, which induce the browning phenomenon in WAT (Ghorbani et al., 1997; Ghorbani and Himms-Hagen, 1997). These data also seem to be valid for humans (Oberkofler et al., 1997; Cyppess and Kahn, 2010).

Very recently, a newly identified hormone named irisin, which is produced by skeletal muscle during physical exercise, has been shown to induce browning of WAT in mice (Boström et al., 2012). Administration of irisin to mice with diet-induced obesity and insulin resistance reduced these conditions. This hormone is also produced in humans, suggesting that it could be manipulated to modulate plasticity of the adipose organ. Thus, both physiological stimuli (such as cold or physical exercise) or pharmaceutical derivatives could be used for the treatment of obesity and related disorders.

Lactation: a special case for adipose organ plasticity

During pregnancy and lactation, all subcutaneous depots of female mice transform into milk-secreting glands. This hormone-regulated phenomenon suggests that there is a progressive reduction in the number of adipocytes in parallel with an increase in the number of epithelial cells that form the functional adenomeres of the mammary glands (shown as green lines in the blue panel of the poster). Data from our laboratory support the idea that these newly formed epithelial cells derive from a direct transformation of adipocytes into milk-secreting epithelial cells (adipo-epithelial transdifferentiation). The process seems to be reversible and the epithelial cells, which are marked by the expression of whey acidic protein (WAP; a milk protein expressed only in milk-secreting epithelial cells in mammary glands), revert into adipocytes in the post-lactation period (Morrioni et al., 2004; De Matteis et al., 2009).

Pathology

When the energy balance is positive (i.e. when food intake is greater than energy expenditure), the white part of the adipose organ expands. This expansion occurs via an increase in the volume of

existing adipocytes as well as the formation of new white adipocytes (Hausman et al., 2001). These newly formed white adipocytes develop from pre-adipocytes, but also from a direct transformation of brown into white adipocytes (Cinti et al., 1997; Bachman et al., 2002). It was recently discovered that knockdown of SMAD3 (a component of the TGF β signaling pathway) in mice induces browning of the adipose organ and protects from diabetes and obesity (Yadav et al., 2011), suggesting that TGF β could be involved in transdifferentiation. Notably, levels of TGF β are also correlated with body mass index. This completes the hypothesis of adipose organ plasticity: energy accumulation induces BAT to 'help' WAT store more energy (i.e. induces brown-to-white adipocyte transdifferentiation).

Storing of excess energy over time can lead to obesity and associated conditions, such as insulin resistance. These conditions are associated with adipose tissue pathology, to which many different factors probably contribute. In particular, macrophages seem to contribute to insulin resistance and other conditions associated with obesity. The obese adipose organ is infiltrated by macrophages, and macrophage infiltration of fat is coincident with the appearance of insulin resistance (Weisberg et al., 2003; Xu et al., 2003; Strissel et al., 2007). The number of macrophages is much higher in visceral than in subcutaneous fat, both in diet-induced and genetic obesity (Strissel et al., 2007; Murano et al., 2008). Macrophages [$>90\%$ of which are immunoreactive for galactose-specific lectin 3 (also known as MAC2)] form crown-like structures (CLSs) surrounding dead adipocytes, which are phagocytosed by the macrophages (see bottom-right panel of the poster) (Cinti et al., 2005). Tumor necrosis factor- α (TNF α), interleukin-6 (IL-6), interleukin-1 α (IL-1 α) and other pro-inflammatory cytokines are produced by macrophages, and the ability of such cytokines to interfere with the physiology of insulin receptor signaling is well known (Gregor and Hotamisligil, 2011). CLSs are also present in the fat of obese humans (Cinti et al., 2005). Macrophage infiltration is positively correlated with the size of adipocytes both in visceral and subcutaneous fat, but can occur independently of obesity per se, because lean mice deficient for hormone-sensitive lipase have hypertrophic adipocytes and the same amount of CLSs as obese mice (Cinti et al., 2005). Notably, obese mice and humans without

adipocyte hypertrophy (hyperplastic obesity) do not show CLSs in fat and are insulin sensitive (Cinti et al., 2005; Hoffstedt et al., 2010). Visceral adipocytes in obese mice are smaller than subcutaneous adipocytes, but visceral fat has a higher amount of CLSs, suggesting a different 'critical death size' (CDS) for adipocytes in visceral fat (Murano et al., 2008; Virtue and Vidal-Puig, 2010). Thus, visceral adipocytes die at a smaller CDS, which might explain the well-known dangerous metabolic consequences of visceral fat accumulation (Cinti, 2009a).

On the basis of the current data, the sequence of events linking obesity with insulin resistance could be:

- adipocyte hypertrophy due to obesity;
- adipocyte stress, possibly involving hypoxia (Wood et al., 2009) and the production of chemoattractants;
- chemoattraction and infiltration of macrophages;
- death of adipocytes on reaching the CDS (visceral adipocytes die first);
- chronic reabsorption of adipocyte remnants by macrophages, accompanied by a massive production of cytokines by macrophages;
- increased levels of circulating cytokines contributes to insulin resistance in peripheral tissues.

Conclusion

In conclusion, the adipose organ is a complex structure with highly plastic properties that include the ability of its parenchymal cells (the adipocytes) to reprogram their genes and transdifferentiate into cells with a different morphology and physiology (a state that is physiologically reversible). It is hoped that this plasticity of the adipose organ can be exploited in the next generation of therapeutic strategies to combat the increasing incidence of metabolic diseases, including obesity and T2D.

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COMPETING INTERESTS

The authors declare that they do not have any competing or financial interests.

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