1 The Adipose Organ

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Abstract

Mammals are provided with an organ that has been neglected by scientists in the past: the adipose organ. This organ is formed by a series of well-defined depots mainly located at two corporal levels: superficial (subcutaneous depots) and deep (visceral depots). In adult rodents, two main depots are the anterior and posterior subcutaneous depots. The first consists of a central body located in the area between the scapulae and several elongated projections abutting toward the cervical region and the axillae. The second is extended from the dorsolumbar area to the gluteal, with an intermediate region located in the inguinal area.

The main visceral depots are tightly connected with viscera. In adult rodents, the main visceral depots are mediastinic, perirenal, perigonadal, mesenteric, and retroperitoneal. The weight of the adipose organ is about 20% of the body weight and therefore it is one of the biggest organs in the body. Its color is mainly white but some areas are brown. In young-adult rodents, maintained in standard conditions, the interscapular region and parts of the cervical and axillary projections of the anterior subcutaneous depot, as well as parts of the mediastinic and perirenal depots, are brown. These two colors correspond to the two tissues: white and brown adipose tissues. The relative amount of the two tissues varies with age, strain, environmental and metabolic conditions, and subsequently, the distribution of the two colors is also variable and implies the ability of reversible transdifferentiation of the two types of adipocytes. During pregnancy and lactation, the subcutaneous depots are transformed into mammary glands.

Each depot of the organ receives its own neurovascular peduncle that is specific for the subcutaneous depots and is usually dependent on the peduncle related to the connected organ in the case of visceral depots. The vascular and nerve supply is much more dense in the brown areas than in the white areas. Their density changes in conjunction with the number of brown adipocytes.

It has been shown that the white adipose tissue of obese mice and humans is infiltrated by macrophages and that the level of infiltration correlates with body mass index and mean size of adipocytes.

This infiltration seems to be an important cause for the insulin resistance associated with obesity. We recently observed that macrophages are mainly located at the level of dead adipocytes in white adipose tissue of obese mice, obese humans, and in transgenic mice, which are lean but have hypertrophic adipocytes (HSL knockout mice). The suggested function of these macrophages is mainly to reabsorb the lipid droplet from dead adipocytes.

Key Words: Adipose tissue; obesity; metabolism; anatomy; adipocytes.

1. INTRODUCTION

Adipose tissues have been neglected by scientists until recently. However, adipose tissues are now emerging as collaborative tissues into an active organ, the adipose organ, that significantly contributes to the regulation of body's homeostasis. This chapter will

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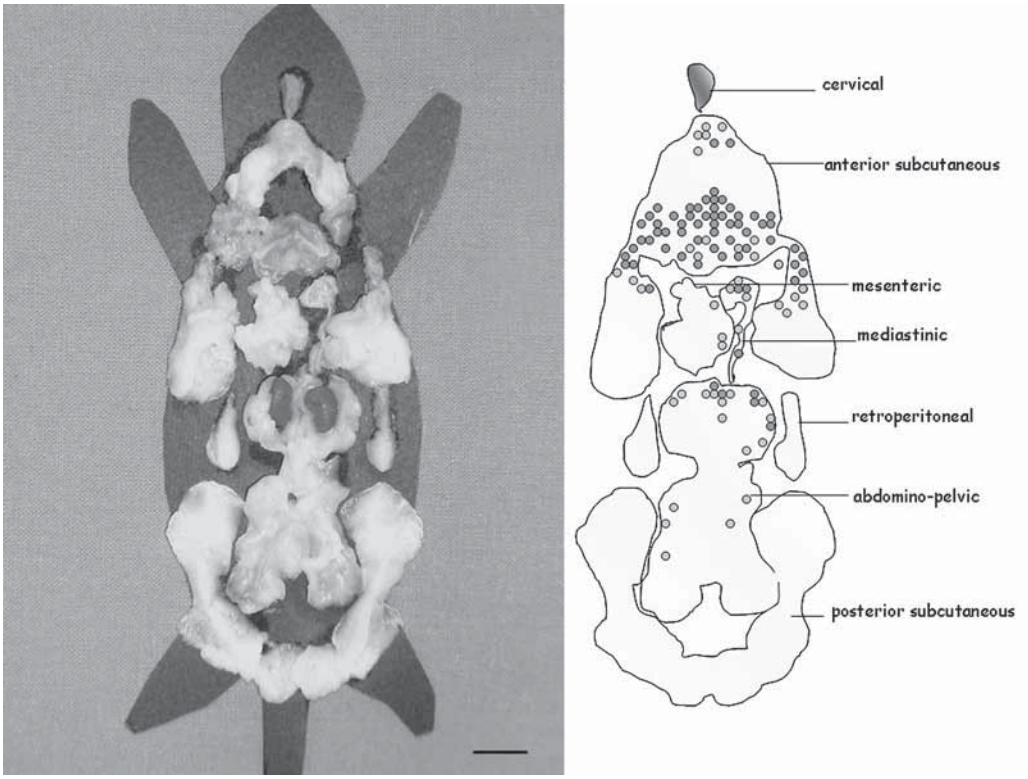

Fig. 1. Gross anatomy of the adipose organ. Lean Sv 129 female mouse maintained at 28 to 29°C. The organ is composed of two subcutaneous depots and several visceral depots. The most representative of these visceral depots are visible. Kidneys and ovaries were dissected together with the depots. White areas are made mainly by white adipose tissue and brown areas are made mainly by brown adipose tissue. Both are visible and indicated in the scheme. Circles indicate brown areas. Bar = 2 cm.

discuss the anatomy and physiology of white and brown adipose tissues in rodents and humans, with an emphasis on two novel concepts. The first concerns the anatomy of the adipose organ. The second is related to a developmental property of adipocytes: transdifferentiation.

2. GROSS ANATOMY

The adipose organ is a multidepot organ with a complex shape (Fig. 1) *(1–4)*.

In small mammals there are two main subcutaneous depots (anterior and posterior) and several visceral depots located inside the thorax (mediastinic) and abdomen (omental, mesenteric, perirenal, retroperitoneal, parametrial, periovaric, epididymal, perivescical). Discrete subcutaneous depots are also dissectable at the level of the major joints in the limbs.

The colors of the organ are white and brown. The white parts are made mainly by white adipocytes. The brown parts are made mainly by brown adipocytes. The relative amounts of white and brown parts are genetically determined and depend on several factors (mainly age, sex, environmental temperature, and nutritional status).

Brown adipocytes are present in all the aforementioned subcutaneous (including the limbic ones) and visceral depots of the adipose organ, but the areas where they are most

constantly found in young/adult mice maintained at standard conditions are the interscapular, subscapular, axillary, and cervical areas of the subcutaneous anterior depot. Also, brown adipocytes are found in the inguinal part of the subcutaneous posterior depot and the periaortal part of the mediastinic depot, and the interrenal part (near the hilus of both kidneys) of the perirenal depot. It must be outlined that clear anatomical boundaries between brown and white adipose tissues do not exist. Many other parts of the adipose organ are mixed with brown adipocytes widespread within the white depot. We have recently described quantitative data of the adipose organ of Sv129 adult mice maintained at different environmental temperatures *(5)*.

Most of the adipocytes are located in the depots of the adipose organ described above, but white adipocytes are also found in the skin, thymus, lymph nodes, bone marrow, parotid, parathyroid, pancreas, and other tissues.

3. LIGHT AND ELECTRON MICROSCOPY

3.1. White Adipose Tissue

As described under the previous heading, in the areas where the adipose organ is white (or pale) the parenchymal element is the white adipocyte. These spherical cells have a diameter from a minimum of 30 to 40 µm to a maximum of 150 to 160 µm (lean, mammary subcutaneous) and from a minimum of about 20 to 30 µm to a maximum of about 90 to 100 µm (lean, visceral perirenal) (by light microscopy of fixed, but not embedded, human white adipose tissue [WAT]). In white adipocytes, most of the cytoplasm is occupied by the lipid droplet and only a thin rim of cytoplasm is visible (Fig. 2). Here, elongated mitochondria (Fig. 3), Golgi complex, rough and smooth endoplasmic reticulum, vesicles, and other organelles are usually visible by transmission electron microscopy.

Many pinocytotic vesicles are present in the proximity of the plasma membrane and an external lamina surrounds the cell.

3.2. Brown Adipose Tissue

Although these cells share the name "adipocyte," they differ greatly in their anatomy and, consequently, in their physiology. The common part of the name is due to the fact that they both accumulate lipids (triglycerides) into the cytoplasm. However, white adipocytes form only one big vacuole (unilocular cell), whereas brown adipocytes form numerous small vacuoles (multilocular cell; Fig. 4). The shape of brown adipocytes is polygonal or ellipsoid, with a maximum diameter between a minimum of 15 to 20 µm and a maximum of 40 to 50 µm. The most important organelles are the mitochondria. They are numerous, big, and rich in transverse cristae (Fig. 5). Peroxisomes, Golgi complex, rough and smooth endoplasmic reticulum, vesicles, and other organelles are also visible by transmission electron microscopy. Pinocytotic vesicles and external lamina are also present in this cell. Brown adipocytes are joined by gap junctions *(6)*.

4. VASCULAR SUPPLY

The adipose organ is diffuse into the organism. Most of its depots receive vascular supply by regional visceral or parietal nerve–vascular bundles. Specific bundles are present in the two main subcutaneous depots (murine adipose organ). The best studied is the anterior subcutaneous depot: two symmetrical bundles reach the depot at the lateral extremities. In the superior lateral bundles, four big and two small nerves are

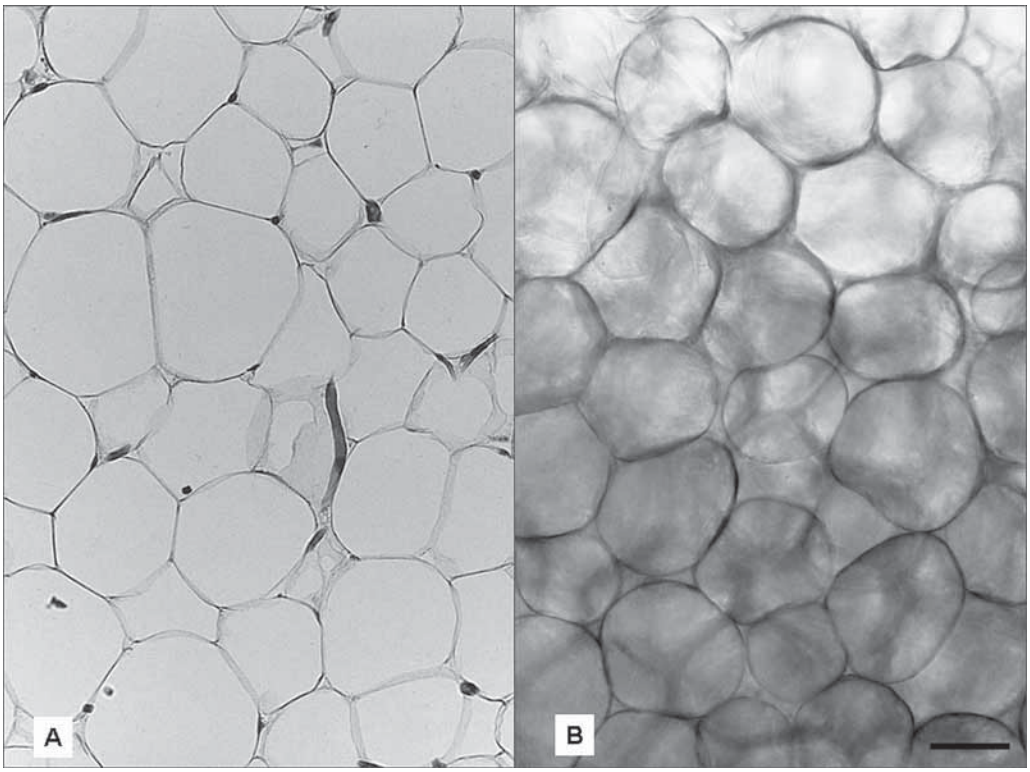

Fig. 2. Light microscopy of human white adipose tissue. **(A)** subcutaneous adipose tissue (formaldehyde-fixed and paraffin-embedded). **(B)** subcutaneous adipose tissue (formaldehyde-fixed and not embedded). Bar = 30 μm.

present. The same bundle also contains an artery and a vein. The lateral inferior bundle does not contain nerves, but only has an artery and a vein. The main vein of the depot is located at the apex of the deep part of the interscapular region and is directly connected to the azygos vein.

The posterior subcutaneous depot is reached by two main nerve–vascular bundles. The first is collateral of the femoral nerve–vascular tract and reaches the depot in the inguinal part; the second is a parietal bundle peduncle and reaches the depot in the dorsolumbar part.

The extension of the capillary network is quite different in the white and brown parts of the organ. In the brown areas the density of the capillaries is much higher than in the white areas.

5. NERVE SUPPLY

The nerve supply to the adipose organ is different in the brown and white areas. The former are more innervated than the latter *(7,8)*. In brown areas, numerous noradrenergic fibers are also found in fat lobules (parenchymal nerves), running with blood vessels (until the level of precapillary and postcapillary structures) and directly in contact with adipocytes.

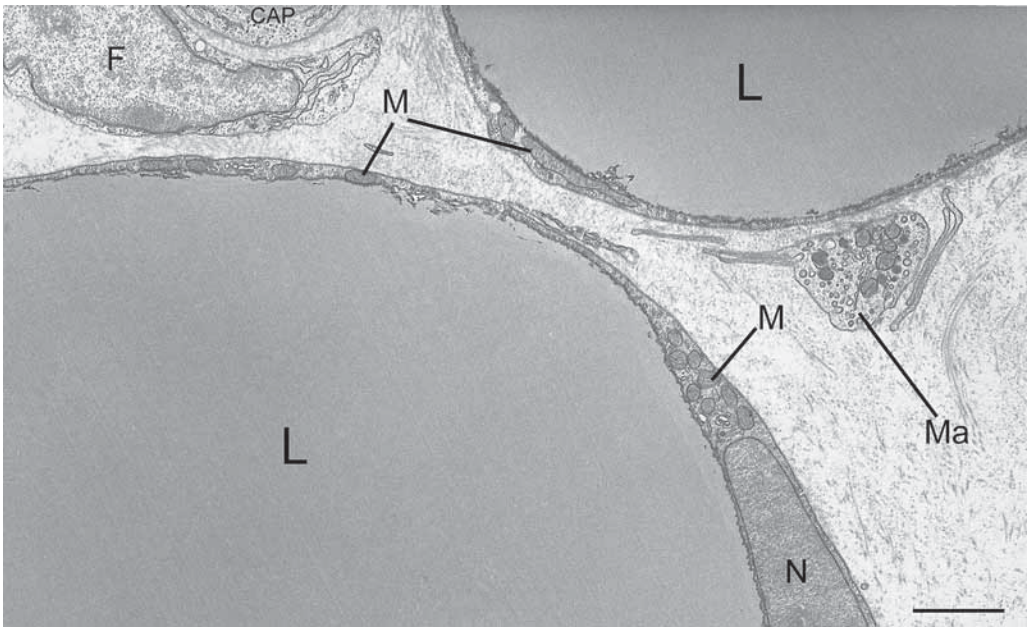

Fig. 3. Electron microscopy of murine white adipose tissue. Note the small and elongated mitochondria in the perinuclear area and in the thin rim of cytoplasm surrounding the large unilocular lipid droplet. F, fibroblast; CAP, capillary lumen; Ma, macrophage; M, mitochondria; N, nucleus; L, liquid droplet. Bar = 2 μm.

Adrenergic receptors (α1, 2 and β1, 2, and 3) are present in the adipose organ.

The density of parenchymal fibers varies according to the functional status of the organ. During cold exposure the noradrenergic parenchymal fibers increase their number in the brown part of the organ *(8)*. During fasting, the noradrenergic parenchymal fibers increase their number in the white part of the organ *(9)*.

Vascular noradrenergic fibers are also immunoreactive for neuropeptide Y. The vast majority of these nerves also contain noradrenaline *(9,10)*, suggesting that they belong to the sympathetic nerve supply to WAT blood vessels.

Brown and white areas also have a provision of sensory nerves *(11)* that are capsaicin-sensitive and are immunoreactive for calcitonin gene-related peptide and substance P. The functional significance of these sensory nerves is not precisely known, although in the rat periovarian adipose depot they affect the recruitment of brown adipocytes during cold acclimation *(12)*.

6. HISTOPHYSIOLOGY

White adipocytes' main purpose is to be a depot of highly energetic molecules (fatty acids) that can supply fuel to the organism during intervals between meals. When the interval is prolonged for weeks, WAT represents the survival tissue.

Brown adipocytes use the same highly energetic molecules to produce heat (non-shivering thermogenesis). This function is fulfilled by the activity of a unique protein, uncoupling protein 1 (UCP1), exclusively expressed by brown adipocytes in mitochondria (and therefore representing a molecular marker of brown adipocytes) *(13–19)*.

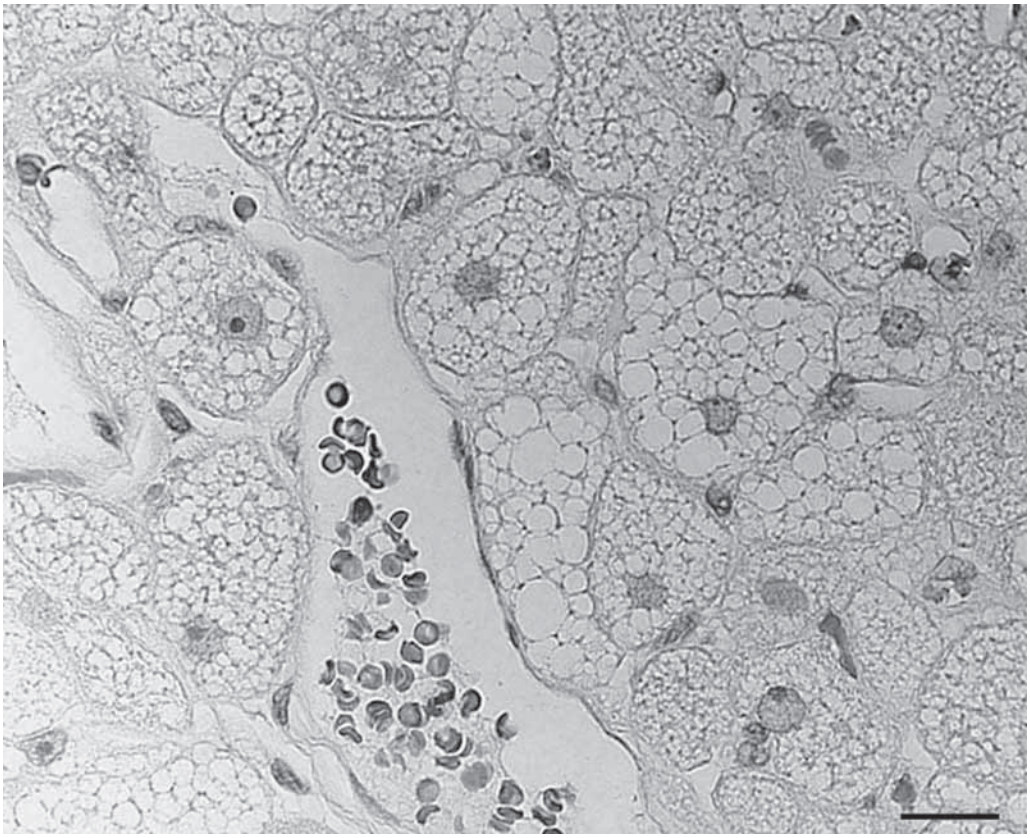

Fig. 4. Light microscopy of human brown adipose tissue. Note the characteristic multilocular lipid organization of the cytoplasm of adipocytes. Hibernoma removed from the skin of a 16-yr-old male patient. Bar = 15 μm.

The signal for brown adipocyte activation is a temperature below thermoneutrality (34°C for mice, 28°C for rats, 20–22°C for humans), that induces activation of the sympathetic nervous system. These neurons of the sympathetic chain directly reach brown adipocytes in the adipose organ *(14)*.

These two functions of the two tissues of the adipose organ (WAT and BAT) are therefore balanced between them, because the intrinsic energy of lipids can be accumulated (WAT) or dissipated (BAT). The total volume of the adipose organ is also dependent on the equilibrium between WAT and BAT activities. Of note, genetic ablation of BAT induces obesity in mice *(20)*, although mice lacking UCP1 are cold-sensitive but not obese *(21)*.

In 1994, another primary function of white adipocytes was discovered: production of leptin, a hormone able to influence animal behavior concerning food intake *(22)*. This hormone also induces energy dispersion (via BAT activation) and has gonadotrophic properties. Leptinemia is positively correlated to fat mass; therefore most obese patients are leptin-"resistant," but rare human cases of leptin or leptin receptor congenital absence have been found. Recombinant leptin administration has reversed cases of human massive obesity caused by congenital leptin absence *(23)*. Brown adipocytes in

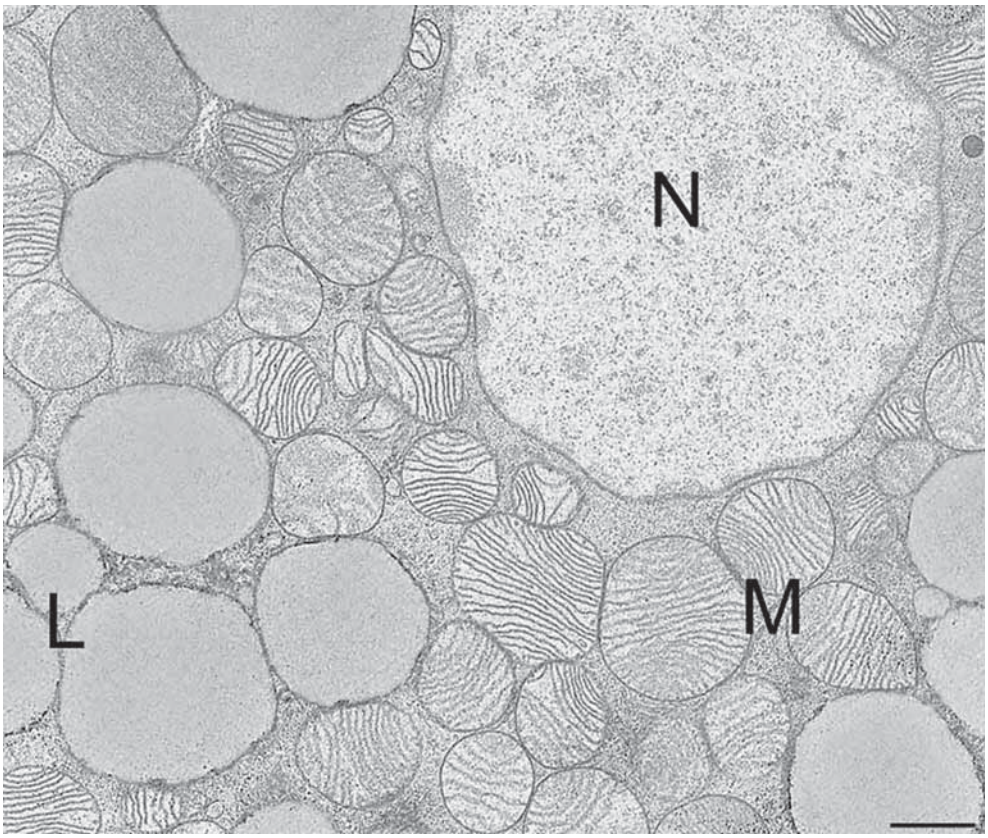

Fig. 5. Electron microscopy of mouse brown adipose tissue. Note the typical mitochondria (abundant, large, and rich in cristae). L, lipid droplets; N, nucleus of the adipocyte; M, mitochondria. Bar = 1 μm.

their classic multilocular configuration (i.e., during thermogenic activity) are not immunoreactive for leptin *(24,25)*.

In addition to these primary functions, the two cell types have many other "secondary" functions. Among them we should remember the activity as a thermo-insulator of subcutaneous white adipose tissue and the regulation of hydric compartments of the organism by production of receptors C and ANP and angiotensinogen II *(26)*. Many other functions have been recognized for WAT, as a growing body of evidence suggests production of several factors known as adipokines, controlling several important functions such as glucose and lipid metabolism, blood coagulation, blood pressure, and steroid hormone modulation. Brown adipocytes produce and secrete many substances, such as autocrine, paracrine, and endocrine factors. The production of all these adipokines raised the recent concept of the adipose organ as an endocrine organ *(27,28)*. In this context, it must be outlined that adipocytes are not the only cell type present in the adipose organ. It has been calculated that only about 50% of its cells are adipocytes *(29)*. Vascular elements, preadipocytes, fibroblasts, mast cells, macrophages, nervous elements, and mesenchymal cells with unknown functions *(1)* are usually found in all depots of the adipose organ.

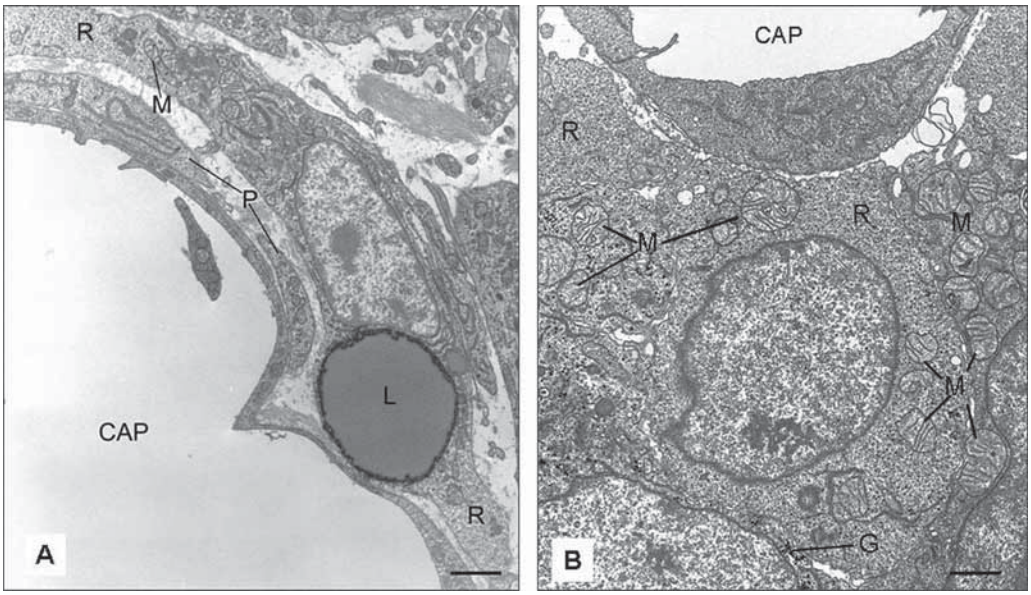

Fig. 6. Electron microscopy of white **(A)** and brown **(B)** adipocyte precursors. Note the different morphology of mitochondria (M). At this early stage of differentiation (note areas indicated by R, occupied only by ribosomes and polyribosomes, typical of poorly differentiated cells), small lipid droplets are visible in the white precursor. Glycogen (dark particles and "G)" and numerous pretypical M (compare with Fig. 5) are visible in the brown precursor. P, pericytes (probably an earlier stage of adipocyte precursor development); CAP, capillary lumen; L, liquid droplets; G, glycogen. Bar in **A** = 2 µm; bar in **B** = 1 µm.

7. DEVELOPMENT AND PLASTICITY

The origin of adipocytes is still unknown, but the in vivo and in vitro steps of their development have been described.

7.1. White Adipocytes

In the first week of postnatal development, the most "white" depot of murine adipose organ (epididymal depot) shows a high number of poorly differentiated cells with minimum adipose differentiation; these are usually referred to as white preadipocytes.

These cells are always tightly connected to the wall of capillaries. Electron microscopy can easily detect in these "blast-like cells" minimal signs of adipose differentiation—i.e., clusters of glycogen and small lipid droplets (Fig. 6A).

Preadipocytes can be distinguished from other cell types in the tissue because they are surrounded by a distinct basal membrane (external lamina).

Often there is a predominant lipid droplet surrounded by numerous small lipid droplets. Very soon the growing white adipocyte assumes the characteristic aspect of a unilocular cell. In adult human adipocytes we showed that the external lamina is immunoreactive for laminin, collagen IV, and heparan sulfate, but not for fibronectin, which is present in the external lamina of adipocyte precursors *(30)*. In vitro studies by electron microscopy confirmed these steps and showed that precursors of adipocytes in adult rats do not reach a complete differentiation *(31,32)*.

7.2. Brown Adipocytes

Brown adipocyte precursors—similarly to white precursors—are always tightly associated with the walls of capillaries. The early steps of development are characterized by pretypical mitochondria, a morphological marker that appears earlier than the molecular marker UCP1 (Fig. 6B). The second step of development is characterized by mitochondrial proliferation and lipid accumulation. Lipid droplets are mainly small and similar in size; therefore lipid accumulation is quite different from that of white precursors and these droplets tend to form multilocular cells. Pretypical mitochondria gradually assume the morphology of the mature adipocyte; this coincides with expression of the molecular marker UCP1. In vitro studies have confirmed the ultrastructure of the developmental steps described in vivo and demonstrated the importance of noradrenaline for mitochondriogenesis *(33–36)*.

7.3. Reversible Transdifferentiation of White Adipocytes Into Brown Adipocytes

Transdifferentiation is a process of direct transformation of a differentiated cell into another cell type with different morphology and physiology. We are convinced that a significant amount of adipocytes in the adipose organ can reciprocally transdifferentiate.

The concept of adipose organ discussed here implies that all depots have variable amount of both types of adipocytes, whose proportion depends on several factors (species, age, strain, environmental temperature, etc.) (Fig. 7). Therefore, in theory, all depots should have both types of precursors, whose morphological characteristics are quite different (*see* Subheadings 6.1. and 6.2.). The description of the developmental steps of the two types of precursors made above refers to the ontogenetic development into two depots that are quite characteristic for WAT (epididymal) and BAT (interscapular part of the anterior subcutaneous). In other words, the depot developed from adipocyte precursors of that specific area in the adipose organ became predominantly WAT (epididymal) or BAT (interscapular) in adult animals. Detailed morphological studies of ontogenesis in depots becoming mixed in adult animals are lacking, but, in our experience, both types of adipocyte precursors with the morphological characteristics of those reported in Figs. 1 and 2 are found during the ontogenesis of these depots *(1)*.

Furthermore, it is well-known that white adipocytes develop in brown areas of the organ (i.e., interscapular area) in genetic or diet-induced obese animals, as well as that brown adipocytes develop in white areas of the organ during cold exposure or treatment with β3-adreneregic (AR) agonists.

We have studied mainly the phenomenon of brown adipocyte development in white areas of the adipose organ, because this phenomenon is associated with amelioration of obesity and diabetes *(37–40)*.

After exposure to cold, the increase in the number of brown adipocytes in white areas of the adipose organ is accompanied by the appearance of brown adipocyte precursors *(41)*. Treatment with β3AR agonists induces development of brown adipocytes in white areas of the adipose organ. This is accompanied by the appearance of cells with morphological characteristics intermediate between white and brown adipocytes and quite different from those of brown adipocyte precursors. These cells show a multilocular lipid depot, usually with a predominant central vacuole and numerous small ones at the periphery of the cell. Their mitochondria are more numerous than in white adipocytes, but less numerous than in brown adipocytes. The mitochondrial morphology is intermediate

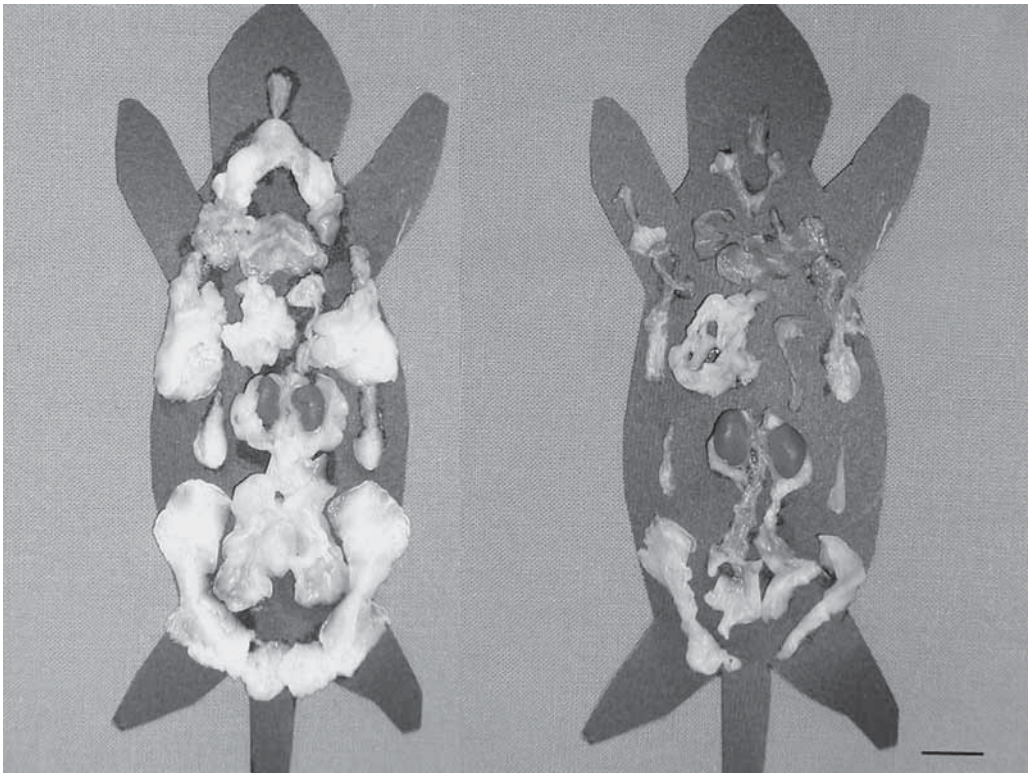

Fig. 7. Adipose organs of adult Sv129 mice maintained at 28 to 29°C (left) or 6°C (right) for 10 d. Note the evident reduction in size of the organ and the increased brown areas in the cold-acclimated mice. Bar = 2 cm.

between that of "white" mitochondria and that of "brown" mitochondria. We think that this type of multilocular adipocyte rich in mitochondria is the morphological equivalent of a white adipocyte transdifferentiating into a brown adipocyte. Some of these cells, also found in white areas of the adipose organ of cold-exposed animals, are immunoreactive for UCP1. Of note, 80 to 95% of these cells are BrdU negative, suggesting that their development is independent from mitotic processes *(42,43)*.

Recently, it has been shown that Sv129 mice are quite resistant to obesity and diabetes in comparison with B6 mice *(44)*. A recent morphometric investigation of the cellular composition of the adipose organ of Sv129 mice showed that the most numerous cells in the organ were multilocular (brown) adipocytes (60% of all adipocytes in the adipose organ of controls and 80% in cold-acclimated mice). In addition, in line with the transdifferentiation concept, cold acclimation did not significantly affect overall adipocyte number, but induced a significant increase in the number of brown adipocytes and an equivalent, significant reduction in the number of white adipocytes *(45)*.

White-into-brown transdifferentiation is also suggested by in vitro studies using primary cultures from human subcutaneous adipose tissue; in these studies, UCP1 expression was induced by treatment with peroxisome proliferator-activated receptor (PPAR)γ agonists *(46)* or PGC1 transfection *(47)*.

All together, these data suggest a possible role for β3AR agonists in the treatment of human obesity and diabetes. Human WAT is immunoreactive to β3AR monoclonal highly specific antibodies *(48)*, but a β3AR agonist producing curative effects for human obesity has not yet been identified *(49)*.

Energy expenditure via the sympathetic system seems to be essential for energy balance; mice lacking all three subtypes of β-adrenoceptors develop massive obesity in the absence of alterations in food intake and locomotion. These mice show a precocious and massive transformation (we think transdifferentiation) of brown into white adipose tissue *(50)*. This is in line with the obese phenotype of mice lacking brown adipose tissue *(20)* and with the obesity resistance of transgenic mice that express UCP1 ectopically *(51)*, but it must be remembered that mice lacking UCP1 do not develop obesity *(21)*.

Studies of genetically manipulated models seem to suggest plasticity of the adipose organ, with white-into-brown adipocyte transdifferentiation. In this context, it is interesting to note that mice lacking the subunit RIIβ (one of the regulatory subunits of cAMP-dependent proteinphosphokinase A, abundant in adipose tissues) have a compensatory hyperexpression of the RIα subunit, with an increase in phosphokinase A sensitivity to cAMP in white adipose tissue and activation of UCP1. These mice have a brown phenotype of abdominal fat and resistance to obesity *(52)*.

FOXC2 is a gene for a transcription factor that is exclusively expressed in adipose tissue. Its transgenic expression in the adipose tissue in mice results in a lean, obesity-resistant, and insulin-sensitive phenotype. The adipose organ of these mice has a browner phenotype than that of controls *(53)*. Of note, humans with insulin resistance have a reduction of *FOXC2* expression in biopsies from the abdominal subcutaneous adipose tissue, together with a reduction of other genes of brown adipocyte phenotype *(54)*.

WAT has high expression of 4E-BP1, a protein important for the posttranscriptional regulation of protein synthesis. Mice lacking 4E-BP1show a reduction of the total fat mass and a brown phenotype of their adipose organ, suggesting that a posttranscriptionally regulated protein can be responsible for the white phenotype. The author suggests that PGC1 (a cofactor of PPARγ) is the protein whose posttranscriptional synthesis is blocked by 4E-BP1 *(55)*.

We recently reported a completely new example of adipose organ plasticity, with reversible transdifferentiation of adipocytes into epithelial cells. The adipose tissue of the mammary gland is a subcutaneous adipose tissue. In female mice, all the subcutaneous part of the adipose organ (anterior and posterior subcutaneous depots; *see* Heading 1) belong to the five symmetrical mammary glands. During pregnancy and lactation almost all adipocytes in these depots disappear, in association with development of the mammary gland (mainly the milk-producing and secreting lobuloalveolar parts). This phenomenon was previously viewed as caused by a hiding of adipocytes, which undergo a delipidation process and apparently disappear. In the postlactation period the milk-secreting epithelial part of the gland disappears by massive apoptosis and the slimmed adipocytes refill and reconstruct the prepregnancy anatomy of the gland. We have brought evidence for a direct reversible transformation of adipocytes into the milk-secreting alveolar cells during pregnancy *(56)*. Of course, this example of extreme plasticity of adipocytes needs further demonstration.

7.4. Hypertrophy and Hyperplasia (Positive Energy Balance: Overweight and Obesity)

When the energy balance becomes positive, the adipose organ increases its white part. White adipocytes undergo hypertrophy followed by hyperplasia.

In fact, it has been proposed that adipocytes have a maximum volume and cannot be further expanded. This maximum volume, also referred to as "critical cell size," is genetically determined and specific for each depot *(57)*. Adipocytes with the critical cell size trigger an increase in cell numbers *(58,59)*. Not all depots have the same tendency to hypertrophy and hyperplasia: the former seems to be more characteristic of epididymal and mesenteric depots, the latter of inguinal and perirenal depots *(57)*.

Hausman et al., in a recent review *(60)*, after considering evidence supporting this theory as well as the conflicting data, conclude that not only paracrine factors but also circulating factors are involved; neural influences can also be important to regulate adipose tissue development and growth. In any case, paracrine factors seem to play a pivotal role. Adipose tissue expresses numerous factors that could be implicated in modulation of adipogenesis: insulin-like growth factor-1, transforming growth factor-β, tumor necrosis factor-α, macrophage colony-stimulating factor, angiotensin-2, autotaxin–lysophosphatidic acid, leptin, resistin, and the like *(61)*. Interestingly, it has been shown in mice that obesity induced by a high-fat diet is hypertrophic, whereas obesity induced by hypothalamic lesions caused by administration of monosodium glutamate is hyperplastic *(62)*.

It has been shown that the WAT of obese mice and humans is infiltrated by macrophages and that the level of infiltration correlates with body mass index and mean size of adipocytes *(63–65)*. This infiltration seems to be an important cause for the insulin resistance associated with obesity. We recently observed that macrophages are located mainly at the level of dead adipocytes in white adipose tissue of obese mice, obese humans, and transgenic mice that are lean but with hypertrophic adipocytes (hormone-sensitive lipase knockout mice). The suggested function of these macrophages is mainly to reabsorb by phagocytosis the lipid droplets from dead adipocytes *(66)*.

Also, the brown part of the organ is modified under this condition of positive energy balance. In obese mice, the rate of apoptosis of brown adipocytes increases; this is strongly attenuated in mice lacking tumor necrosis factor-α receptors *(67)*. The morphology of brown adipocytes gradually changes into a morphology similar to that of white adipocytes, including transformation of the multilocular lipid depot into a unilocular one. This is accompanied by activation of the leptin gene; these cells are also immunoreactive for leptin *(24,25)*.

7.5. Hypoplasia (Negative Energy Balance: Caloric Restriction, Fasting)

The morphology of the adipose organ during fasting is quite characteristic, because a variable amount of slimmed cells are present in WAT. The slimmed cell is barely visible at light microscopy but is easily recognized by electron microscopy—i.e., it has a specific ultrastructural morphology: cytoplasmatic irregular and thin projections with numerous invaginations rich in pinocytotic vesicles. These projections enlarge in proximity of the nucleus and of the residual lipid droplet. In acute fasting, completely delipidized adipocytes can be found near apparently unaffected unilocular cells (Fig. 8).

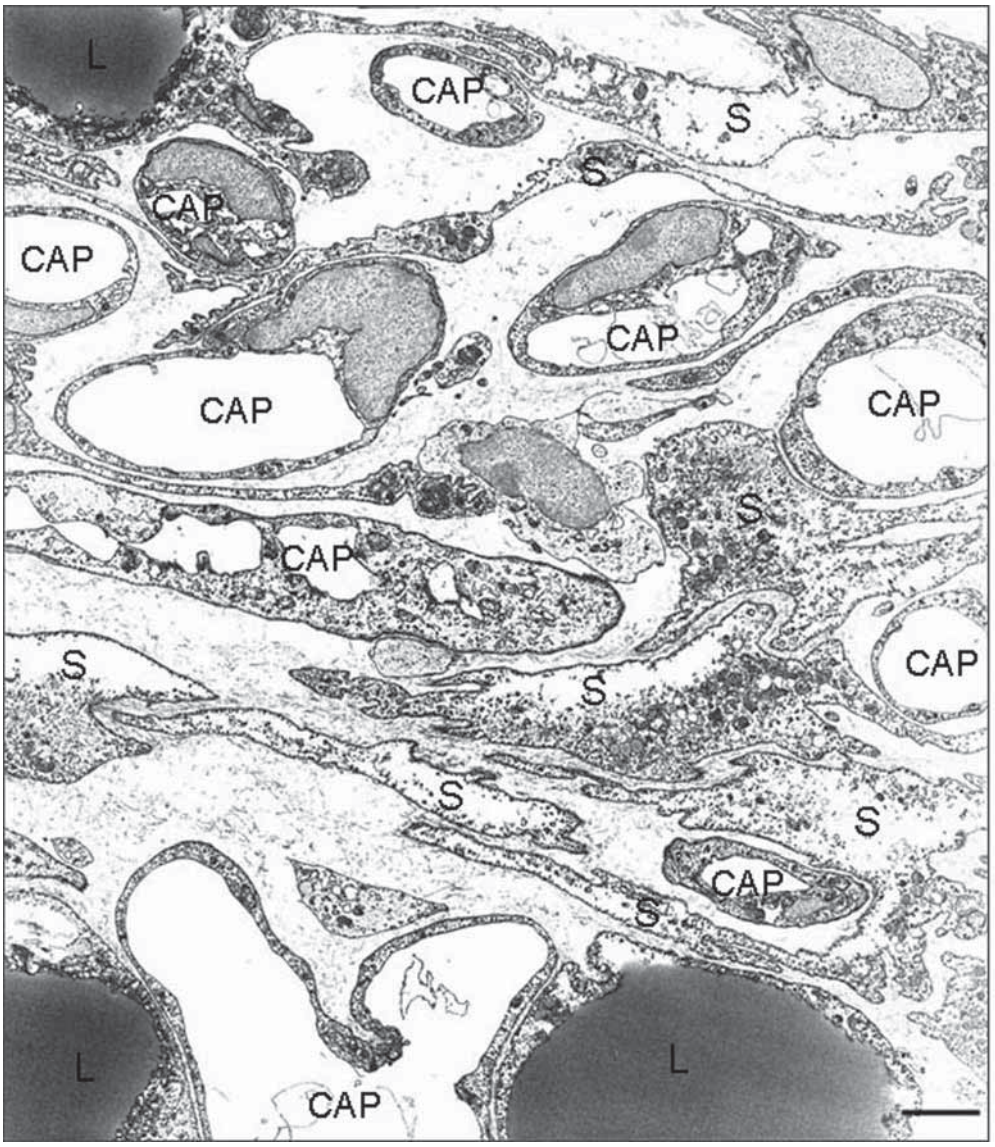

Fig. 8. Electron microscopy of white adipose tissue of fasted mouse. Numerous slimmed adipocytes are visible (S), showing a characteristic morphology different from that of other cell types found in the tissue. Note the dense vascular supply. CAP, capillary lumen. Bar = 4 μm.

Vasculogenesis and neurogenesis is also found in white adipose tissue of fasted animals. Neurogenesis is mainly supported by an increase of noradrenergic fibers *(9)*.

In chronic caloric restriction, the reduction in size of adipocytes is homogeneously distributed *(68)*.

8. THE ADIPOSE ORGAN OF HUMANS

The basic concepts of the adipose organs of small mammals reported above are applicable also to the adipose organ of humans. In fact, white, brown, and mixed adipose

tissues are also present in the adipose organ of humans, with all the morphological and physiological characteristics described for the murine adipose organ.

Although a detailed description of the gross anatomy of the human adipose organ has never been performed, it is well-known that it is composed of subcutaneous and visceral depots. In humans, the subcutaneous adipose tissue is in continuity with the dermal adipose tissue (in rodents, dermal adipose tissue is separated from subcutaneous adipose tissue by a smooth muscle layer) and it is not limited to defined areas but is present as a continuous layer beneath the skin. Mammary and gluteofemoral subcutaneous adipose tissue is more developed in females than in males.

Visceral depots correspond to those described previously for the rodent adipose organ, but the omental depot is particularly well developed in humans.

The weight of the human adipose organ of lean adults is about 8 to 18% of body weight in males and 14 to 28% in females (and about 5% in monkeys) *(69)*.

Light and electron microscopy of human adipose tissues is identical to that of murine adipose tissues, but the size of adipocytes is about 30 to 40% bigger than that of mice and rats.

Development of the human adipose organ extends for a long period, until puberty, mainly through proliferation *(70)*. During the first year of age there is mainly an increase in size. In line with these data, the number of adipocytes, total fat mass, and the percentage of body fat correlate positively with age in both sexes. Instead, adipocyte size does not seem to positively correlate with age, but it seems to be correlated to the amount of fat mass and percentage in both sexes *(71)*. In massively obese humans, the adipose organ can increase four times and reach 60 to 70% of body weight *(46,60)*.

In case of negative energy balance, the adipose organ reduces its volume and the size of adipocytes. The reduction in size of adipocytes is important because the size of adipocytes correlates with insulin sensitivity *(72)*. Not all depots react in the same way to negative energy balance. Subcutaneous adipose tissue from the gluteofemoral region of premenopausal women is more resistant to slimming than subcutaneous abdominal adipose tissue, but after menopause the slimming process is similar. This seems to be due to a combination of increased lipoproteinlipase activity and reduced lipolytic activity in the gluteofemoral adipose tissue. The reduced lipolytic activity seems to be due to a relative preponderance of antilipolytic activity of α2-adrenoceptors over the lipolytic β-adrenoceptors *(73)*. In general, α2-adrenoceptors are more abundant in human adipose tissue than in murine adipose tissue. In genetically modified mice lacking β3- and expressing human α2-adrenoceptors, obesity induces hyperplasia (but not hypertrophy) of adipose tissue and mice are insulin-sensitive. These experiments are in line with the importance of α2 AR for adipocyte hyperplasia and with the relationship of their size with insulin sensitivity *(74)*.

Like the murine adipose organ, the human adipose organ contains brown adipose tissue. It is easy to understand that the relationship between surface and volume of the human body is quite different from that of small mammals; therefore, human thermodispersion is much lower than that in rodents. This alone justifies a reduced need for brown adipose tissue in adult humans. Newborns have a different surface/volume relationship and a considerable amount of brown adipose tissue is present at that age. Nevertheless, brown adipocytes dispersed among white adipocytes have been described in several histological studies (including studies showing the presence of UCP1) *(75,76)*

and in our own studies of a case series of 100 consecutive perirenal biopsies of adult humans, brown adipocytes were found in 24% of cases (all ages) and in 50% of cases after exclusion of patients over 50 yr old (unpublished data).

BAT in human newborns has been described in almost all the same sites described for rodents, and *UCP1* gene expression was found in biopsies from visceral adipose tissue of adult lean and obese patients. In the same paper, the authors calculated the presence of one brown adipocyte every 100 to 200 white adipocytes in the visceral adipose tissue of adult lean humans *(77)*.

BAT has also been described as increased in outdoor workers in northern Europe *(78)* and in patients with feocromocitoma (a noradrenalin-secreting tumor). Furthermore, rare cases of hibernoma—BAT tumors occurring in several anatomical sites, including subcutaneous and visceral fat—have been described (about 100 cases have been described in the literature *[79]* and we recently observed a case [Fig. 4] in which brown adipocytes expressed UCP1 and had the classic electron microscopy with typical mitochondria).

The physiological role of BAT in humans is debated, but the possibility to artificially increase it in order to treat obesity and related disorders cannot be excluded. On this matter, it is interesting to note that human adults with reduced brown phenotype of abdominal subcutaneous adipose tissue have reduced insulin sensitivity and that human white adipocyte precursors can be induced in vitro to express UCP1 by administration of drugs *(46)*.

9. CONCLUSIONS

This chapter described in detail the anatomy of adipose tissue and introduced the novel concept of the adipose organ. Both white and brown adipose tissues are organised into a real organ, with a complex multi-depot organisation. Each depot has its own discrete vascular and nerve supply. The characteristics of the organ can be adapted to functional requirements in relation to the energy balance of the organism. The two tissues seem to derive from precursors with different morphological and functional characteristics, but with possibilities of reciprocal conversion, with an important role played by the nervous system. Both white and brown adipocytes produce factors that can influence the tissue pattern, adapting it to the functional needs.

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