Development of thermogenic adipose tissue

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ABSTRACT Besides having a metabolic and insulatory-supporting function, adipose tissue in endotherms also performs a thermogenic function. Thermogenic adipocytes contain specific UC-mitochondria with uncoupling protein (UCP) and produce heat. Thermogenic adipose tissue has two forms: brown adipose tissue (BAT) and convertible adipose tissue (CAT). Brown adipocytes have UC-mitochondria and express UCP throughout the entire life of small rodents, chiropterans, and insectivores. However, in other endotherms and in humans CAT participates as thermogenic tissue only during early postnatal period. Both BAT and CAT start to develop *in utero*, although in some animals (hamsters, marsupials) or in some particular areas (thoraco-periaortal and medio-perirenal areas in rats) development of thermogenic adipose tissue starts after birth. Postnatal development of BAT in small endotherms is characterized by quantitative changes (the amount of UC-mitochondria, UCP, and lipids). Postnatal development of CAT causes qualitative changes during which UC-mitochondria in convertible adipocytes are replaced by common, nonthermogenic C-mitochondria; vascularization of adipocytes drops to a low level and, with lipid accumulation, convertible adipocytes appear as lipid-store cells. Postnatal development of CAT can be modulated or reversed by the environmental temperature. The duration of postnatal changes varies between species; *i.e.*, cats, rabbits and sheep, change their thermogenic form of CAT into the lipid-store form within the first postnatal month, while in humans the same process takes up to 15-20 years. In maturity all these large endotherms have CAT in lipid-store form. In light of these results, the question of participation of thermogenic adipose tissue in the regulation of human obesity needs to be answered.

KEY WORDS: *thermogenic adipose tissue, brown adipose tissue, convertible adipose tissue, white adipose tissue, mitochondria, uncoupling protein, obesity*

Introduction

In spite of its simple structure, adipose tissue and its main constituents, the adipocytes, perform very different functions in the body of endotherms. These functions are in strong correlation with the anatomical location (Williams and Warwick, 1980; Greenwood and Johnson, 1983; Krstic, 1984; Fawcett, 1986; Cormack, 1987). For example, the adipose tissue in the orbit, in the major joints, and on the palms of the hands and soles of the feet has a supportive function. This adipose tissue together with insulatory adipose tissue does not participate in the everyday lipid metabolism. It can be classified as non-metabolic adipose tissue and in this way distinguished from the metabolically active adipose tissue, which exists in deeper parts of the body. By releasing triglycerides during the period when the organism has a shortage of glucose (periods between meals) the metabolic adipose tissue becomes the main «battery» maintaining the body functions (Fig. 1) (Guyton, 1991). Because of their macroscopic appearance, both «supporting» and «metabolic» adipose tissue are described as white adipose tissue (WAT), the name introduced by Malpighi (1688). In small endotherms (not birds) whose metabolic rate cannot maintain the constant body temperature in a cold environment, adipose tissue appears also as a direct source of heat (Fig. 1). Because of its brown color, this thermogenic adipose tissue has been described as brown adipose tissue. As recent comparative studies performed on different species have shown, the thermogenic adipose tissue has two forms: the classical brown adipose tissue (BAT) – present in particular anatomical areas of small rodents, insectivores and chiropterans, and the form of convertible adipose tissue (CAT) present in particular areas of bigger mammals (Fig. 1) (Loncar *et al.*, 1986; Loncar, 1989, 1990a; Loncar and Afzelius, 1989). While BAT keeps its specific structure and function throughout the life of the host (Afzelius, 1970; Néchad, 1986), CAT appears as thermogenic adipose tissue during the perinatal period (Alexander *et al.*, 1970;

Abbreviations used in this paper: BAT, Brown adipose tissue; CAT, convertible adipose tissue; UCP, uncoupling protein; WAT, white adipose tissue.

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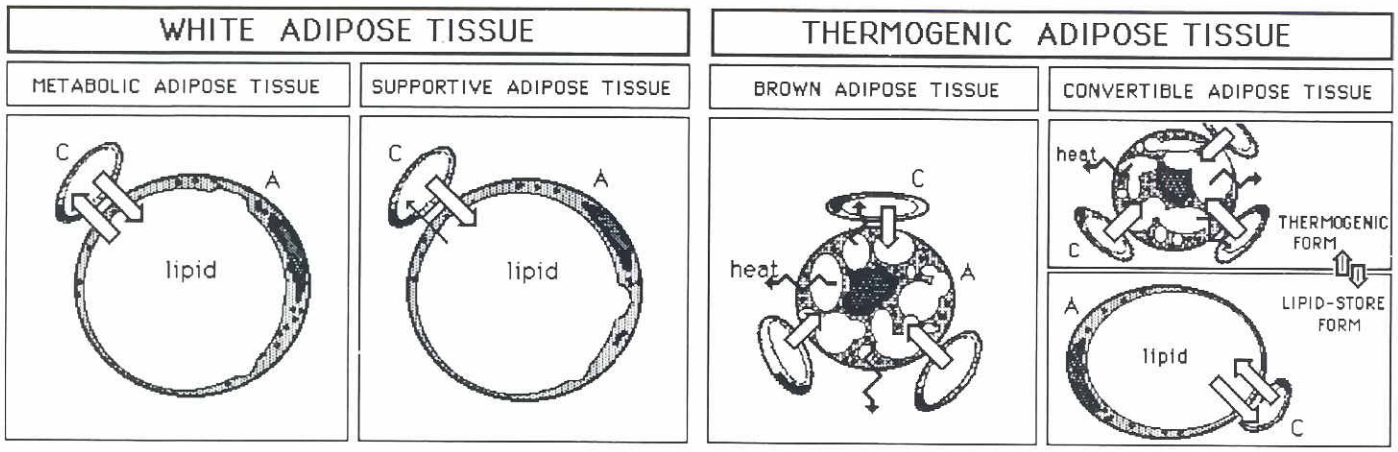


Fig. 1. Adipose tissue is present in mammals as white and thermogenic adipose tissue. *Metabolic adipose tissue contains adipocytes (A) which during food supply accumulate lipids from surrounding capillaries (C). Adipocytes release accumulated lipids in the period between meals. Differing from these, the turnover of lipids in adipocytes of supportive adipose tissue is neglected. Accumulated lipids in supportive adipocytes serve as the body's mechanico-insulatory support. Lipids from supportive adipocytes are mobilized only in the case of pathological starvation. Thermogenic adipose tissue comes in two forms, as brown and convertible adipose tissue. Mitochondria of brown and convertible adipocytes (thermogenic form) produce heat by burning lipids. A highly developed vascular system (C) disseminates heat throughout the body. While brown adipocytes retain a thermogenic function even in maturity, maturing convertible adipocytes change from a thermogenic into lipid-store form. This conversion is reversible.*

Bruck, 1970; Hull and Hardman 1970; 1975; Alexander, 1979, 1981; Loncar, 1989, 1991a; Loncar and Afzelius, 1989), during exposure of the animal to low temperatures (Bruck, 1970; Cox *et al.*, 1978; Alexander, 1979; Huttunen *et al.*, 1981; Loncar *et al.*, 1986; Loncar, 1990a), or in some pathological cases like phaeochromocytoma or cachexia (Ricquier and Mory, 1984; Lean and James, 1986; Lean *et al.*, 1986, 1987; Bouillaud *et al.*, 1988). However, simultaneously with growth and increase of body mass and metabolic rate, the demand for an additional heat source declines and the thermogenic adipose tissue converts into a lipid store adipose tissue which resembles white adipose tissue (Fig. 1) (Aherne and Hull, 1966; Bruck, 1970; Smalley, 1970; Rowlatt *et al.*, 1971; Derry *et al.*, 1972; Alexander, 1979; Ashwell *et al.*, 1987; Loncar, 1989, 1990a; Loncar and Afzelius, 1989).

The purpose of this review was to summarize the works that have led to the establishment of a new subgroup of thermogenic adipose

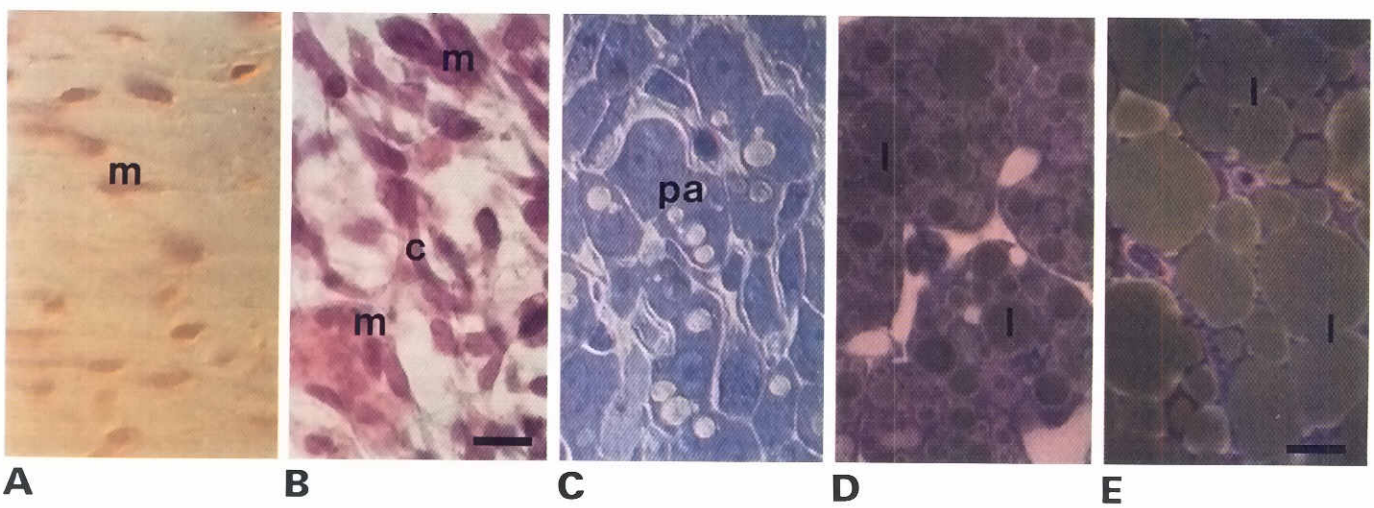
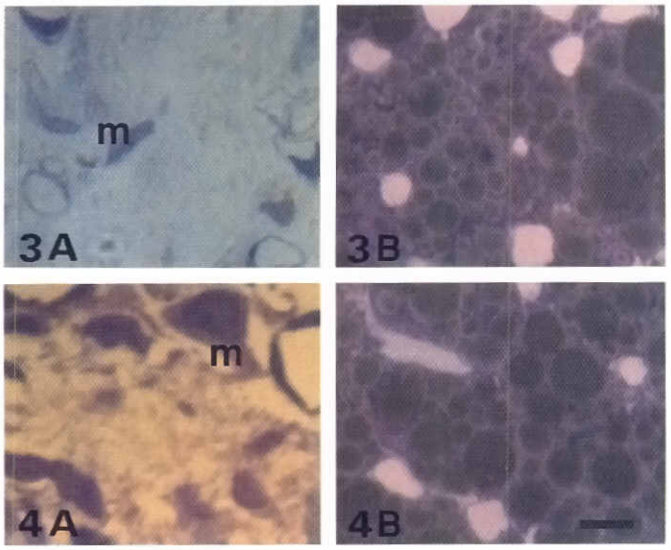


Fig. 2. Development of interscapular BAT of rat. (A) *Interscapular area in a 17-day-old embryo contains rare mesenchymal cells (m).* **(B)** *One day later, well defined islands of mesenchyme contain numerous mesenchymal cells (m) and developing capillaries (c).* **(C)** *Twenty-day-old embryo in the interscapular area has developing preadipocytes (pa) which generate specific UC-mitochondria and accumulate lipid droplets.* **(D)** *Two weeks after birth there are roundish brown adipocytes with numerous lipid droplets (l).* **(E)** *Brown adipocytes of a 15-month-old rat have a multilocular appearance with a mean maximal diameter approaching 40 μ m. These adipocytes contain specific UC-mitochondria. (see Barnard, 1969; Suter, 1969b; Barnard and Skala, 1970; Nnodim and Lever, 1985; Loncar, 1991b). (A and B) Paraffin embedded section, 7 μ m, hematoxylin-eosin, Nomarski optics, Bar represents 15 μ m. (C to E) Epon embedded section, 2 μ m, toluidine blue, Nomarski optics, Bar represents 10 μ m.*



Figs. 3 and 4. Periaortal area (Fig. 3A) and medio-perirenal area (Fig. 4A) of a newborn rat *have mesenchymal cells (m), which in another two weeks would develop into typical BAT (Figs. 3B and 4B). Epon embedded section, 2 μ m, toluidine blue, Nomarski optics. Bar 10 μ m.*

tissue, named convertible adipose tissue (Loncar and Afzelius, 1989), and its importance for further research, especially in obesity research in humans. In addition, developmental characteristics of typical brown adipose tissue will be discussed and compared to developmental characteristics of convertible adipose tissue.

Time and duration of thermogenic adipose tissue development

The appearance of anlage for thermogenic adipose tissue is species and space specific. An extensive review of literature has shown that intrauterine development of both BAT and CAT show the same developmental pattern (Hammar, 1895; Barnard and Lindberg, 1969; Barnard and Skala, 1970; Loncar, 1984a,b; Nnodim and Lever, 1985; Néchad, 1986; Nnodim, 1987). The only recorded difference is the time when thermogenic adipose tissue starts to develop. Thus, for example, in some rodents (interscapular BAT in rats and mice), the first trace of thermogenic adipose tissue appears 5-6 days before birth (Fig. 2) (Barnard and Skala, 1970; Loncar, 1984a,b; Nnodim and Lever, 1985), while in humans the first traces of thermogenic adipose tissue appear around the 28th week of gestation (Fig. 8) (about 12 weeks before delivery) (Aherne and Hull, 1966; Hull, 1974; Merklin, 1974). In both cases, the first appearance of thermogenic adipose tissue occurs within the last quarter of pregnancy. However, in some animals development of thermogenic tissue appears post partum (Figs. 3, 4 and 6) (hamsters, marsupials, see below) (Néchad and Barnard, 1979; Loudon *et al.*, 1985; Houstek *et al.*, 1990). In hamsters the first traces of BAT in the interscapular area appear by the end of the first postnatal week (Fig. 6). With birth, rats have morphologically developed BAT in interscapular, subscapular and axillary areas (Fig. 2), whereas the anlage for thermogenic adipose tissue in thoraco-periaortal (Fig. 3) and medio-perirenal areas (Fig. 4) appears post partum, resulting in the development of BAT in these areas and achieving structural-functional maturity during the second postnatal week only (Loncar, 1991b).

Pattern of thermogenic adipose tissue development

Prenatal development

Development of either BAT or CAT starts with the penetration of a vascular bud in a well-characterized particular mesenchymal area (Figs. 2A and 8A) (Simon, 1965; Wassermann, 1965; Barnard and Skala, 1970; Loncar, 1984a,b; Nnodim and Lever, 1985). This penetration parallels the differentiation of mesenchymal cells. Clusters of mesenchymal cells together with penetrating vessels form primitive fat lobules (Fig. 2B). In these lobules one group of mesenchymal cells participates in establishing the vascular network while the rest of them become preadipose cells. Further ramification of vessels together with the very high mitotic activity of mesenchymal cells results in the growth of lobules (Fig. 2C) so that upon birth of rats or mice BAT in the intrascapular area is completely shaped (Barnard and Skala, 1970; Loncar, 1984a,b; Nnodim and Lever, 1985).

Postnatal development

Postnatal development of interscapular BAT is characterized mainly by adipocyte growth (Figs. 2D, E, 3 and 4) (Suter, 1969a; Barnard and Skala, 1970; Lindgren and Barnard, 1972). Although with further maturation brown adipocytes accumulate more lipids, the mature brown adipocytes still have the structural and functional characteristic of BAT (Figs. 2E, 3B and 4B) (Suter, 1969b; Afzelius, 1970; Nnodim and Lever, 1985; Néchad, 1986; Loncar, 1989).

Development in hamsters and marsupials

In most species, development of the thermogenic adipocyte cell line is characterized by the appearance of specific thermogenic mitochondria (UC-mitochondria) (Figs. 10, 11 and 15) and by the simultaneous accumulation of lipids in the form of numerous small

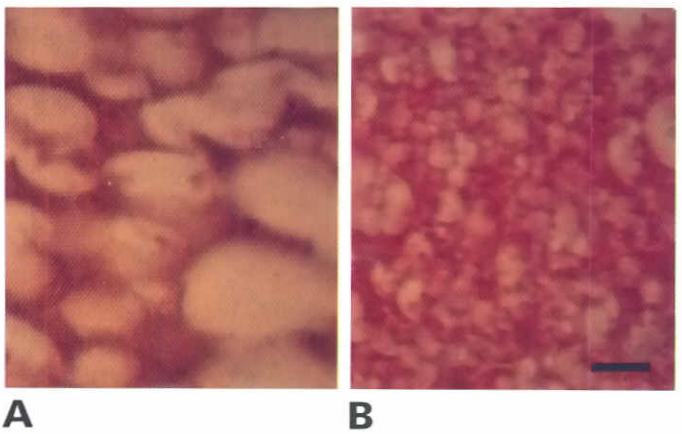


Fig. 5 (A) *Interscapular BAT from a rat which when 5-days-old had been transplanted into the thoraco-subcutaneous area. It has developed mostly into unilocular cells which resemble white adipocytes.* **(B)** *However, in some cases, the transplanted tissue remains of the specific structure, where small multilocular brown adipocytes are present. (see Loncar, 1985). Paraffin embedded section, 7 μ m, hematoxylin-eosin, Nomarski optics. Bar 15 μ m.*

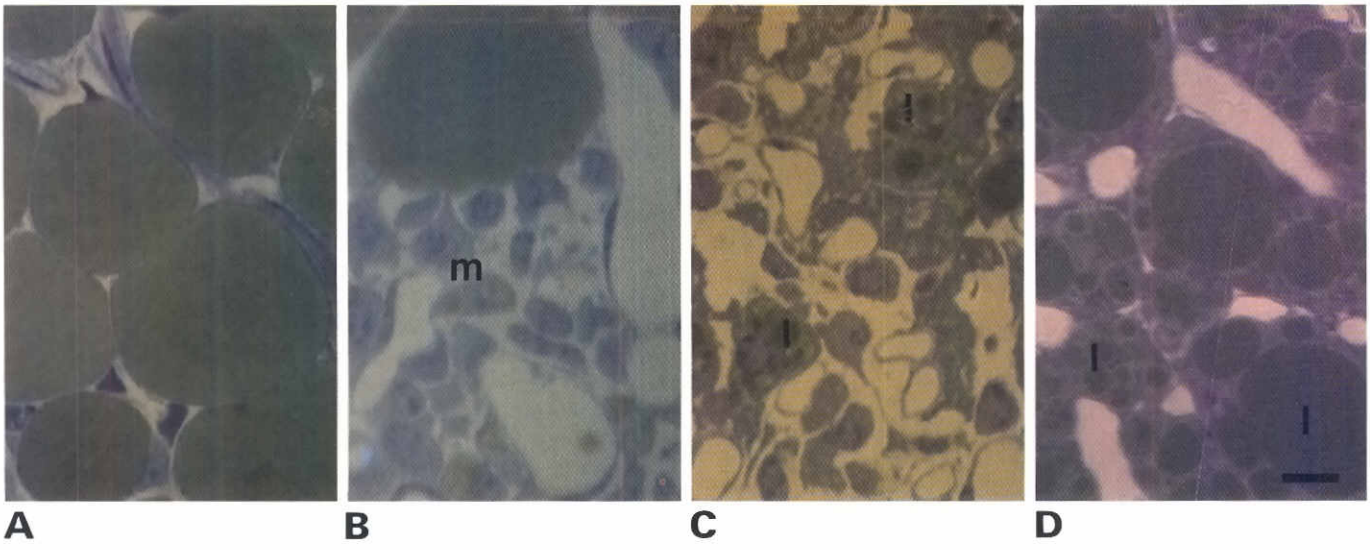


Fig. 6. Development of interscapular BAT of the hamster. (A) *Fourth day after birth; the interscapular area contains unilocular adipocytes.* **(B)** *Two days later, numerous mesenchymal cells (m) appear between the unilocular adipocytes.* **(C)** *Twelve days post partum, the interscapular area contains numerous preadipocytes which contain mitochondria and accumulate lipids (1).* **(D)** *Interscapular area of a one-year-old hamster with specific brown adipocytes having UC-mitochondria and numerous lipid droplets (1) of different sizes. (see Néchad and Barnard, 1979). Epon embedded section, 2 μ m, toluidine blue, Nomarski optics. Bar 10 μ m.*

droplets (Figs. 2-4 and 9). This results in thermogenic adipocytes having both the thermogenic machinery (UC-mitochondria) and the fuel (lipids) ready for the moment when nonshivering thermogenesis starts. In hamsters and marsupials (Néchad and Barnard, 1979; Loudon *et al.*, 1985; Houstek *et al.*, 1990) the BAT anlage appears postnatally (Figs. 6 and 9). Development and differentiation of their mesenchymal cells into thermogenic adipocytes occurs in two ways (Fig. 9). In the first phase developing adipocytes accumulate lipids without genesis of UC-mitochondria (Figs. 6A and 9). This results in the interscapular (or perirenal) area in hamsters containing unilocular adipocytes at the end of the first postnatal week (Fig. 6A). During the next week, these unilocular adipocytes lose most of their lipids (Fig. 6B). This is probably due to the intensive lipid mobilization which causes the adipocytes to have a multilocular appearance during that period. It seems that «delipidization» is also a trigger for intensive mitochondriogenesis as is shown in kittens which were exposed to severe cold stress (Loncar *et al.*, 1986) and in cold-exposed rats (Loncar *et al.*, 1988a,b, 1989). During the second postnatal week, thermogenic adipocytes start to generate specific UC-mitochondria and accumulate lipids in the form of numerous droplets, resulting in the development of specific brown adipocytes (Figs. 6D and 9).

The second way in which thermogenic adipocytes develop in hamsters takes place during the second postnatal week when differentiating mesenchymal cells and pericytes generate UC-mitochondria and accumulate small lipid droplets (Figs. 6C and 9) (Néchad and Barnard, 1979). Thus, this second way, described in hamsters, is identical to the intrauterine development of thermogenic adipocytes described in other species.

As mentioned above, the periaortal (Fig. 3) and medio-perirenal (Fig. 4) development of BAT in rats appears also after birth by the same mechanism of development as interscapular BAT (Figs. 2 and 9) (Loncar, 1991b). Therefore, the appearance of unilocular cells as a transitory type of cells during differentiation of mesenchymal cells into brown adipocytes (Figs. 6A and 9) seems to be specific to certain species and distinct from the postnatal development of convertible adipose tissue.

Differentiation of mesenchymal cells into thermogenic adipocytes

Influence of specific vessels

The mechanisms which initiate the process of differentiation of mesenchymal cells into cells of thermogenic adipose tissue are unknown. Early observations (Wassermann, 1926; Hammar, 1895) suggested that adipose tissue develops in predetermined areas. Newer findings (Simon, 1965) have suggested that the penetration of specific (neuro-) vascular elements in a particular area is the crucial event for development and differentiation of mesenchymal cells into adipose tissue cells. The results with transplantation of BAT anlage below the kidney capsule (Hausberger, 1938, 1939, 1955) were differently interpreted; *i.e.*, although mesenchymal cells appear undifferentiated, they are determined and their further development is independent of penetration of space specific neuro-vascular elements. These conclusions are ambiguous, since it has been demonstrated that the adipogenic environment below the kidney capsule facilitates development of BAT even from nine-day-old embryonic mesoderm (Skreb *et al.*, 1976), probably through the neuro-vascular elements from BAT around the kidney hilus. The fact that differentiation of mesenchymal cells into adipocytes can be achieved without permanent supervising of specific neuro-vascular elements is implied from two other grafting experiments. Transplantation of hamster's interscapular adipose tissue in the anterior eye chamber demonstrated that preadipocytes can differentiate into brown adipocytes without direct support of specific neuro-vascular elements (Néchad and Olson, 1983). Autografted interscapular BAT of 5-day-old rats in the thoraco-subcutaneous area developed into white adipose tissue (Fig. 5A), as well as into

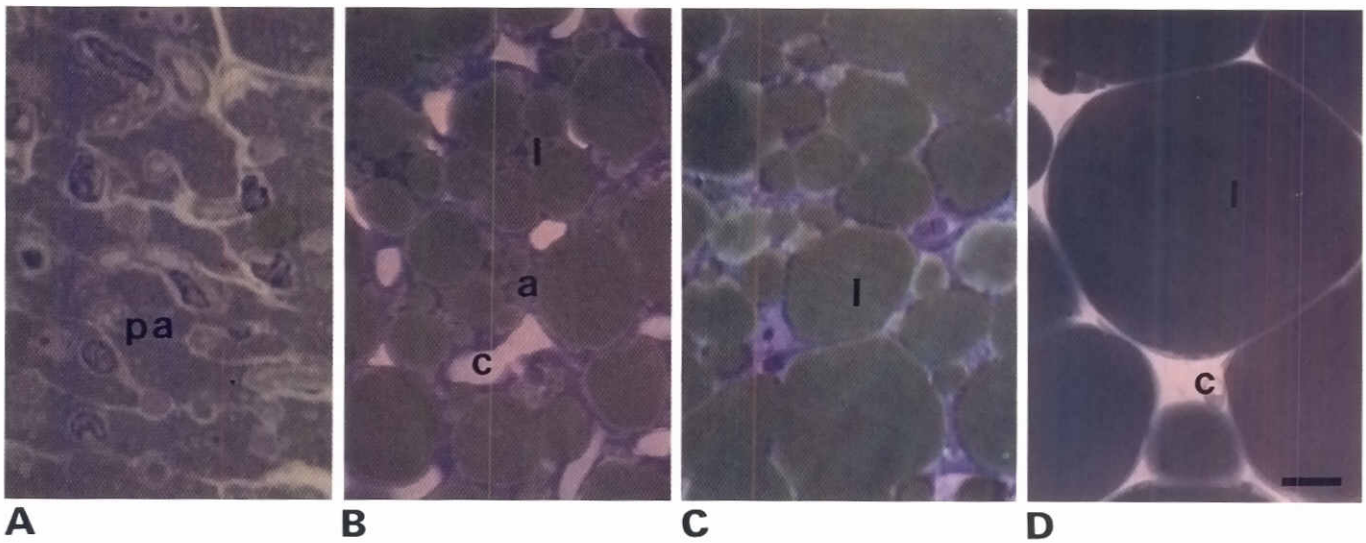


Fig. 7. Development of CAT in cervico-axillar area of rabbit. (A) *One day after birth, preadipocytes (pa) contain UC-mitochondria and accumulate lipid droplets.* **(B)** *Two weeks later, multilocular adipocytes (a) with developed capillaries (c) appear as brown adipocytes.* **(C)** *Twenty-five days after birth adipocytes contain many lipid droplets (l) which start to coalesce into one big droplet.* **(D)** *Six-month-old rabbit has round, big unilocular adipocytes with a mean maximal diameter of over 100 μ m. These cells appear as white adipocytes. (see Loncar, 1989, 1991a). Epon embedded section, 2 μ m, toluidine blue, Nomarski optics. Bar 10 μ m.*

a tissue which morphologically appears as BAT one month later (Fig. 5B) (Loncar, 1985). Nevertheless, the grafts were penetrated by «nonspecific» neuro-vascular elements. Since grafted adipose tissue in the above experiments were differentiated (hamster) or even developed (rat), the real influence of the specific neuro-vascular elements on the early commitment and differentiation of the adipose cell line should be analyzed at an earlier stage of development of adipose tissue.

Influence of local factors

The existence of some site-specific factor(s) influencing the development of adipose tissue was proved not only after grafting of embryonic mesoderm below the kidney capsule (Skreb *et al.*, 1976), but also after a cross-transplantation of adipose tissue from different anatomical areas (Ashwell, 1985, Ashwell *et al.*, 1986). In experiments in which immature white adipose tissue was transplanted in an area of interscapular brown adipose tissue and vice

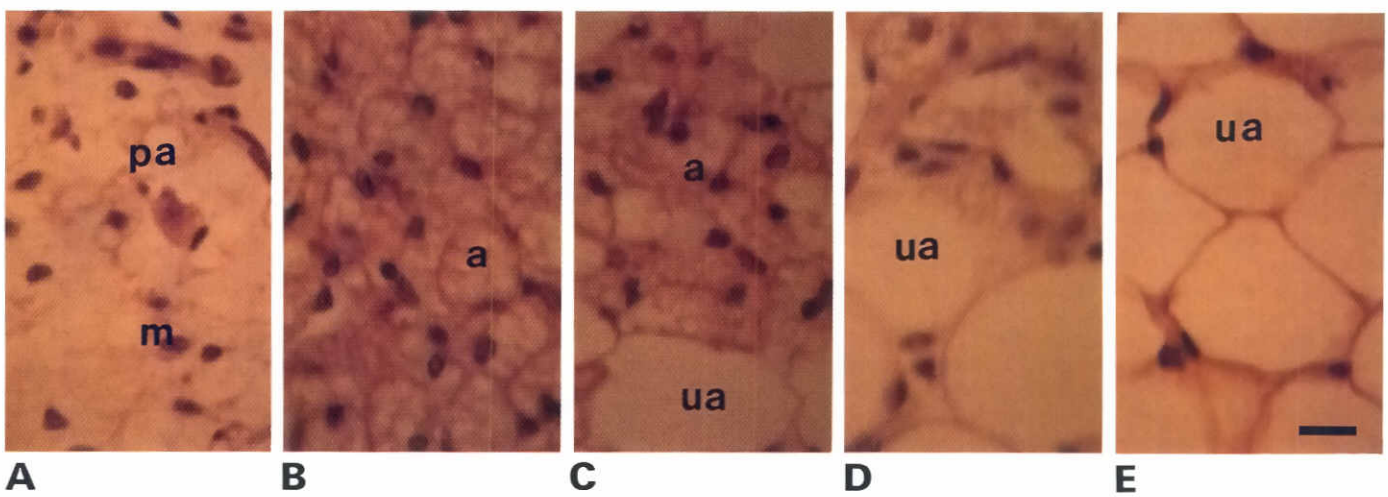


Fig. 8. Development of CAT in the cervico-axillar area in humans. (A) *During the 26th week of pregnancy, the human embryo contains a few mesenchymal cells (m) and preadipocytes (pa) which are in the phase of lipid accumulation.* **(B)** *Two weeks later, this area is full of multilocular adipose cells (a).* **(C)** *At birth, big unilocular adipose cells (ua) appear between the small multilocular cells (a).* **(D)** *Accumulation of lipids has continued in the convertible adipocytes, and in a 5-year-old child, the big unilocular adipocytes (ua) outnumbered the multilocular adipocytes.* **(E)** *In the mature stage (34-year-old person) only big, round unilovular adipocytes (ua) are present (see Hammar, 1895; Wassermann, 1965; Hull, 1974; Merklin, 1974). Paraffin embedded section, 7 μ m, hematoxylin-eosin. Bar 15 μ m.*

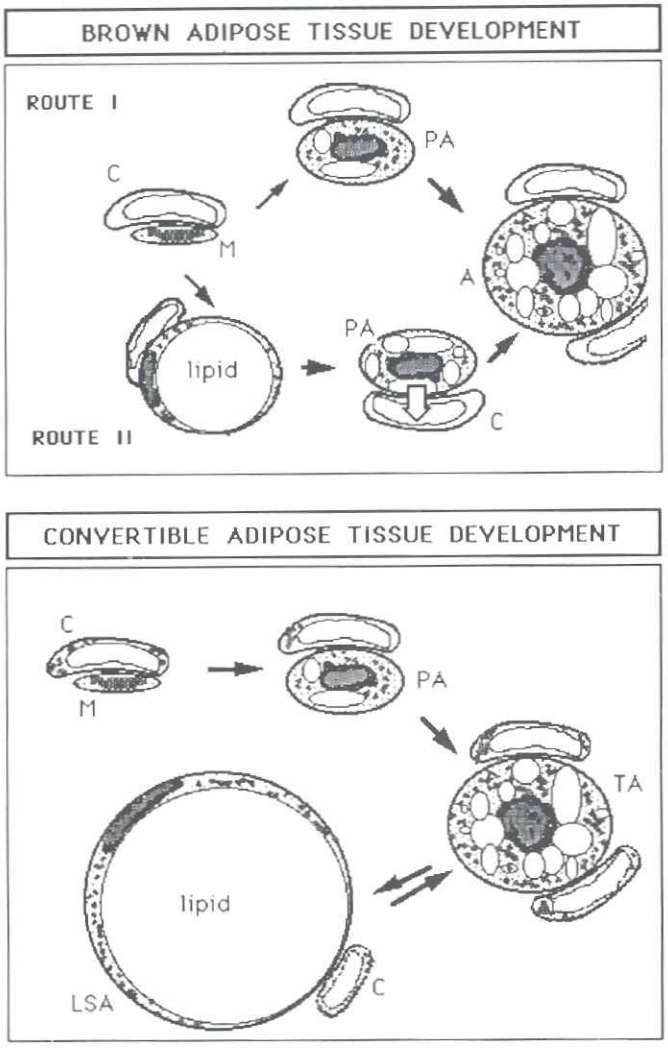


Fig. 9. Development of thermogenic adipose tissue. ***Brown adipose tissue.*** *In most species development of brown adipose tissue (BAT) follows Route I. Mesenchymal cells (M), which are in close contact with newly-established capillaries (C0), start to generate specific UC-mitochondria and accumulate lipid droplets. With further development and accumulation of UC-mitochondria and lipids preadipocytes (PA) become fully differentiated brown adipocytes (A). Development of BAT described as Route II is specific for hamsters and marsupials. Mesenchymal cells (M) first accumulate lipids in the form of one big lipid droplet. Following mobilization of lipids, preadipocytes (PA) decrease their volume and develop UC-mitochondria. Further accumulation of UC-mitochondria and lipid droplets shape brown adipocytes (A).* ***Convertible adipose tissue.*** *The thermogenic form of convertible adipocytes (TA) arises from mesenchymal cells (M) as described for brown adipocytes. With maturity, in large animals, demands for additional heat sources decline, converting the thermogenic form of convertible adipocytes into the lipid-store form (LSA). Cold stress or high noradrenaline doses cause these convertible adipocytes to regain thermogenic function.*

versa, the type of adipose tissue present in the host always overcame the type of grafting tissue, *i.e.* local factors from the host dominated over the genetic pedigree of grafting tissue (Ashwell, 1985). Experiments with grafted mesoderm also showed that local factors from medio-perirenal BAT influenced the differentiation of the grafted mesoderm into BAT (Skreb *et al.*, 1976).

The appearance of the thermogenic adipose tissue in different anatomical areas (interscapular, subscapular-intermuscular, axillary, intercostal, periaortal, medio-perirenal, parametral, inguinal area) (Afzelius, 1970), *i.e.*, in the vicinity of different tissues (connective tissue, epidermis, bones, cartilage, skeletal, cardiac and smooth muscle, kidney, suprarenal gland) excludes the possibility that only one specific local tissue induces differentiation of mesenchymal cells into thermogenic adipose tissue cells. Any conclusions about the existence of a local trigger which would turn mesenchymal cells into thermogenic adipocytes becomes even more difficult to defend if we consider an extended period between the appearance of the first anlage in the interscapular area (17 days ante partum) and in thoraco-periaortal and medio-perirenal areas (one week post partum) (Loncar, 1991b).

Intrauterine development of CAT resembles the intrauterine development of BAT (Figs. 7-9) (Aherne and Hull, 1966; Barnard and Skala, 1970; Merklin, 1974). Upon birth, CAT morphologically appears the same as BAT (Figs. 7A and 8C). However, postnatal development causes the adipocytes of CAT to accumulate lipids and become unilocular and, with growth, the tissue will appear as a lipid-store adipose tissue, so that in mature animals CAT appears as a lipid-store white adipose tissue (Figs. 7D, 8E and 9) (Smalley, 1970; Derry *et al.*, 1972; Alexander, 1979; Loncar *et al.*, 1986; Loncar, 1989, 1990a; Loncar and Afzelius, 1989). With a permanent high concentration of noradrenaline (Ricquier and Mory, 1984; Lean *et al.*, 1986; Lean and James, 1986; Ashwell *et al.*, 1987; Lean *et al.*, 1987) or cold (Alexander *et al.*, 1970; Cox *et al.*, 1978; Alexander, 1979; Loncar *et al.*, 1986; Loncar, 1990a), this tissue and its adipocytes adopt the same structural and functional characteristics that they had during the early postnatal period (Figs. 9-14).

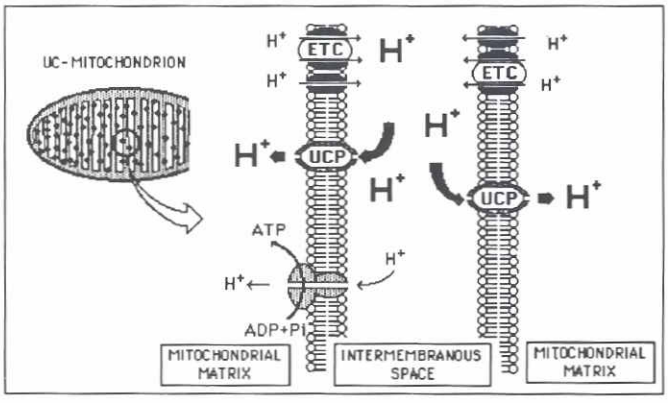


Fig. 10. Schematic drawing of heat production by the UC-mitochondria. *Enzymes of the electron transfer chain (ETC) pump protons (H^+) from the mitochondrial matrix into the intermembranous space. Numerous dimers of uncoupling protein (UCP) placed in the inner mitochondrial membrane return most of the protons into the mitochondrial matrix, allowing only a low number of protons to pass through the ADP/ATP-ase. The energy created by protons passing through the UCP-dimer is released as heat – the main product UC-mitochondria. (see Nicholls* et al., *1986; Klingenberg, 1990a, b).*

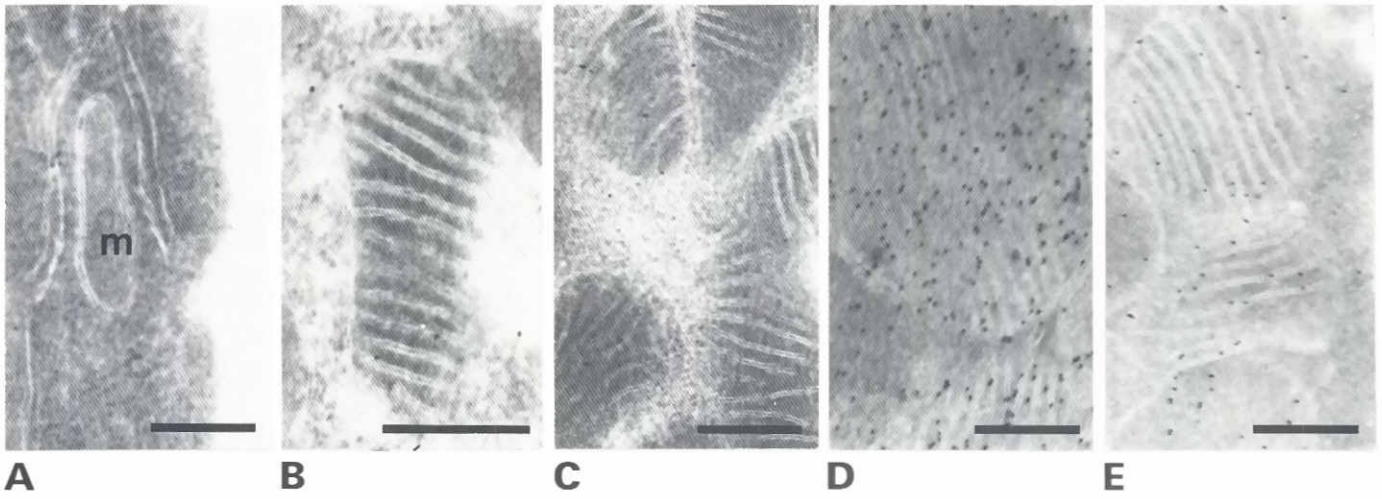


Fig. 11. Development of UC-mitochondria in interscapular brown adipocytes. (A) *Mesenchymal cells in interscapular area of 17-day-old embryo have elongated, common C-mitochondria (m) without any detectable UCP.* **(B)** *Preadipocytes in a twenty-day-old embryo have big mitochondria with numerous straight cristae in which the first molecules of UCP (arrows) are observed.* **(C)** *With birth, the amount of UC-mitochondria increases, but there is not yet any significant expression of UCP.* **(D)** *Two weeks post partum. The brown adipocytes contain big UC-mitochondria, with numerous straight cristae and a high amount of UCP.* **(E)** *Fifteen months later, the mitochondrial content is slightly declined, but the size and shape of UC-mitochondria and their cristae, and the amount of UCP remain specific for the brown adipocytes. (see Barnard, 1969; Suter 1969a; Barnard and Skala, 1970; Nnodim, 1985; Loncar, 1991b). Formaldehyde-parformaldehyde fixation, polyvinyl pyrrholidon embedding, uranyl acetate negative contrasting, anti-UCP protein-A-gold labelling. Bars 0.2 μ m.*

Ultrastructural specificity during thermogenic adipocyte development

Mitochondria during BAT development

Most of the ultrastructural features during the development of tissue are reported elsewhere (Barnard, 1969; Barnard and Lindberg, 1969; Suter, 1969a; Barnard and Skala, 1970, Lindgren and Barnard, 1972; Nnodim and Lever, 1985; Néchad, 1986; Nnodim, 1987). Since the mitochondria play a principal role in the function of the thermogenic tissue, mitochondrial changes during development of BAT and CAT will be discussed below (Figs. 10, 11-15).

Mesenchymal cells in the interscapular area of rats 17 days ante partum, have poorly developed, elongated mitochondria (Fig. 11A). As the cells become more differentiated, the number of mitochondria and the amount of mitochondrial cristae increases (Fig. 11B), so that upon birth the thermogenic adipocytes have their cytoplasm crowded by round or ellipsoid mitochondria with numerous straight cristae (Fig. 11C) (Lindgren and Barnard, 1972). The postnatal period is characterized by a further increase in mitochondrial surface per mitochondrial and cytoplasmic volume. The maximal amount of mitochondria and their cristae per cytoplasm is realized in interscapular BAT 2-3 weeks after birth (Fig. 11D). Further growth of BAT is characterized by a slight decline in the amount and number of mitochondria and their cristae to a value which is about 70% of that during the postnatal peak, *i.e.*, during 2-3 weeks of postnatal life (Fig. 11E). Although developed later, BAT of periaortal and medio-perirenal areas have the same mitochondrial characteristics in maturity as do the interscapular BAT during development *in utero*.

Mitochondria during CAT development

In contrast to the adipocytes of BAT, the adipocytes of CAT change mitochondrial content qualitatively and quantitatively during postnatal life (Fig. 12) (Loncar, 1989, 1991a; Loncar and Afzelius, 1989). Mitochondria of convertible adipocytes resemble during their thermogenic phase (perinatal period and 2-3 weeks after birth) the UC-mitochondria of brown adipocytes in their amount of mitochondria and the surface of their cristae (Figs. 12A-B, 13A and 15). However, further growth and development of CAT in animals kept at room temperature, lead to the elimination of UC-mitochondria and their replacement with C-mitochondria (Figs. 12C-D, 13B and 15). In cats, rabbits, sheep, and cows this process appears about 1 month after birth, but in dogs it appears in the first fortnight after birth. Analysis of convertible adipose tissue of humans shows that the replacement of UC-mitochondria with C-mitochondria takes a longer time, up to 10-15 years.

The process of replacement of UC-mitochondria with C-mitochondria parallels the accumulation of lipids. The amount of lipids increases and cytoplasm with C-mitochondria becomes squeezed in a thin peripheral rim (Figs. 12D and 13B). At the same time, because of the enormous adipocyte growth, which is not followed by a subsequent growth of vascularization, the amount of the surface of the convertible adipocytes covered by capillaries declines from 35% to 2% (Fig. 9). These morphological changes result in a lower oxygen supply in CAT.

Although it is conceivable that a lower oxygen supply causes destruction of UC-mitochondria and their replacement with C-mitochondria, detailed analyses have suggested that the replacement process is more complex (Loncar, 1989, 1991a; Loncar and Afzelius, 1989). First, if general hypoxia causes destruction of UC-mitochondria, which are more developed and thus possibly more sensitive to hypoxic conditions, it would affect all or most of the UC-mitochondria. However, data show that the replacement of UC-mitochondria is a gradual process during which UC-mitochondria in convertible adipocytes exist in a different phase of destruction. Simultaneously with destruction of UC-mitochondria, undeveloped C-mitochondria appear in convertible adipocytes (Fig. 12C). By keeping young animals in a cold environment, the replacement of UC-mitochondria with C-mitochondria can be slowed down but not

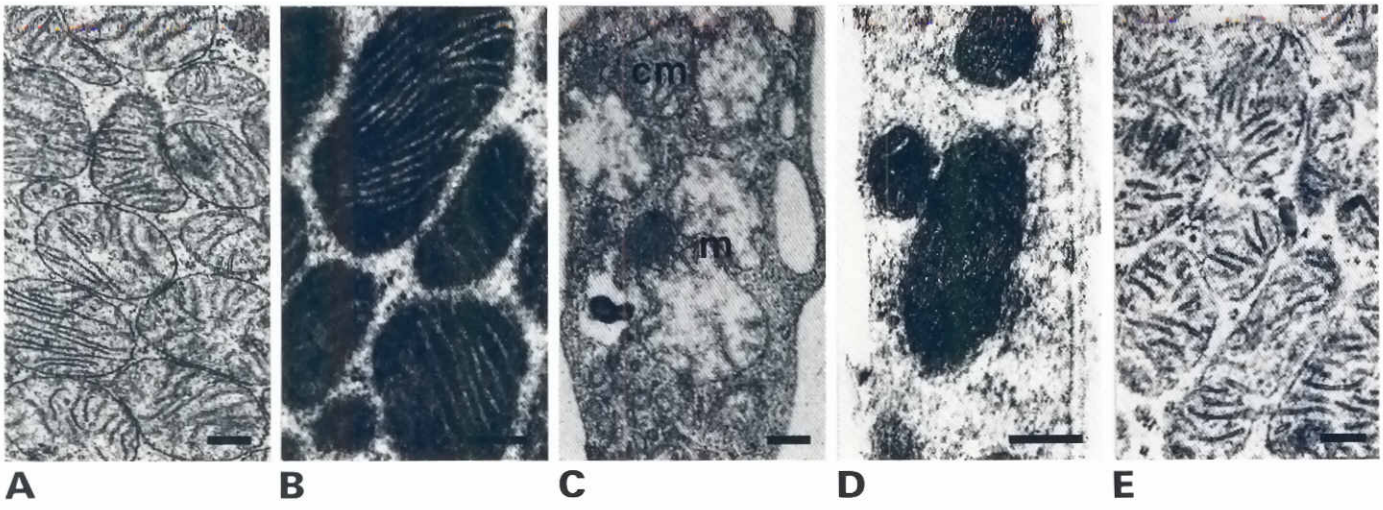


Fig. 12. Developing of mitochondria in perirenal convertible adipocytes of cat. (A) *Cytoplasm of adipocytes of newborn kitten is crowded by well-developed UC-mitochondria.* **(B)** *Fifteen days later; the mitochondrial matrix has become dark.* **(C)** *Adipocytes of a one-month-old kitten have a unilocular appearance and their developed UC-mitochondria (m) are in different stages of destruction. Simultaneously, new undeveloped C-mitochondria (cm) appear in the cytoplasm.* **(D)** *Two months later. In the thin rim of cytoplasm, the adipocytes have small, elongated C-mitochondria.* **(E)** *Severe cold stress has induced a reappearance of UC-mitochondria in convertible adipocytes of the now 10-week-old kitten. (see Loncar* et al., *1986; Loncar and Afzelius, 1989). Formaldehyde-paraformaldehyde fixation, osmium postfixation, epon embedding, uranyl acetate-lead citrate contrasting. Bars 0.2 μ m.*

stopped (Alexander *et al.*, 1970, 1975; Alexander, 1979). These data suggest that the appearance, duration, and replacement of UC-mitochondria with C-mitochondria is strictly regulated and that intracellular hypoxia, could participate in, but probably is not the main reason for, UC-mitochondria destruction (Fig. 15).

Mitochondrial content, for both UC- and C-mitochondria in convertible adipocytes, is cell-specific and intrinsically regulated; therefore, it can be distinguished from the mitochondrial content in mature brown adipocytes (Figs. 11E, 12D, 13B, 14C and 15). When kept at room or thermoneutral temperature, brown adipocytes, although still having high capillary-adipocyte surface contact, have a very low regional blood flow (15 times lower than animals exposed to 12°C (Girardier and Seydoux, 1986). In other experiments, brown adipocytes of rats delivered and reared at 33°C for one month contain UC-mitochondria although it is obvious that these animals have not had the need of brown adipocytes as a heater (Loncar, 1989). Thus, the appearance of brown and convertible adipocytes in addition to the type and the amount of their mitochondria is cell specifically regulated.

Uncoupling protein during BAT development

The described changes in histological appearance and mitochondrial content are in agreement with the expression of the specific protein of thermogenic tissue – uncoupling protein (UCP). Although Smith and co-workers (Smith, 1961, 1964; Smith and Hock, 1963) in the early sixties showed that BAT produces heat, it was almost twenty years before we started to understand molecular mechanisms on how mitochondria of thermogenic adipocytes produce heat. Applying a hemiosmotic model about the proton circuit through the inner mitochondrial membrane, Nicholls and co-workers proposed that the presence of molecules of UCP make thermogenic mitochondria uncoupled (Fig. 10) (see Nicholls *et al.*, 1986; Rial and Nicholls, 1989; Klingenberg, 1990a,b). Protons, which are pumped into intermembranous space by enzymes of the respiratory chain, return into the mitochondrial matrix mostly through the UCP dimer rather than through the ADP/ATP-ase. By recycling the H+ into the matrix, neutralization energy in the form of heat is generated (Fig. 10). The highly developed vascular system (Afzelius, 1970) disseminates such released heat throughout the body.

Analysis of UCP performed at different levels (the expression of UCP mRNA, the biochemical determination of UCP as well as the immuno-electron microscopical determination of UCP, confirmed previous histological and ultrastructural observations about the existence of two types of thermogenic tissue.

The first expression of UCP mRNA in interscapular BAT of rats appears at the end of intrauterine life (Obregon *et al.*, 1987, 1989; Loncar, 1991b), one or two days before birth. Interscapular BAT starts the expression of UCP mRNA intensively with birth and during the first 2 weeks after the amount of UCP mRNA reaches a peak (Loncar, 1991b). With the first month of life, the expression of UCP mRNA in interscapular BAT starts to decline and, in mature animals, the standard expression drops to 20-25% (rat, mice) of the expression from the first fortnight.

A time-curve of the amount of UCP is similar to the time-curve of the expression of the UCP mRNA (Sundin and Cannon, 1980; Sundin *et al.*, 1981; Bazin *et al.*, 1984; Peachey *et al.*, 1988). The first traces of UCP are detected on the first days after birth in rats, after which time the amount of UCP reaches its highest peak; after the first fortnight there is a recorded decline in the amount of UCP. Henceforth, rats one-month-old or older, have about 25-30% of the amount that UCP has at the end of the first fortnight.

The very sensitive immuno-electron microscopical technique reveals UCP-molecules in thermogenic mitochondria first around the perinatal period (Fig. 11C) (Houstek *et al.*, 1988; Loncar, 1990c, 1991b). Upon birth and postnatal development and growth of brown adipocytes, the amount of UCP molecules per mitochondrial cristae increases rapidly and reaches a peak 2-3 weeks after birth (Fig. 11D). After this period, the amount of UCP molecules per

mitochondrial cristae declines and mature animals kept at room temperature have a lower, but significant, specific level of UCP molecules per mitochondrial cristae (Fig. 11E) (King and Lean, 1987; Loncar *et al.*, 1988c; Cinti *et al.*, 1989; Houstek and Kopecky, 1989; Loncar, 1990b,c, 1991b).

Uncoupling protein during CAT development

The expression of UCP mRNA and the amount of UCP in mitochondrial cristae of CAT of large mammals have a different pattern from that of BAT (Figs. 13-15). Results from several laboratories on adipose tissue from different species have shown that convertible adipocytes have the highest amount of both UCP mRNA and UCP immediately after birth and during early postnatal development, respectively (Fig. 13A). With growth, both the expression of UCP mRNA and the amount of UCP declines and finally become undetectable (Fig. 13B and 14A) (Lean *et al.*, 1986; Ashwell *et al.*, 1987; Casteilla *et al.*, 1987, 1989; Balogh *et al.*, 1989; Rozon *et al.*, 1989; Thompson *et al.*, 1989). Large adult endotherms are devoid of UCP. However, cold stress (Fig. 14B) or some pathological conditions induce again an expression of UCP mRNA with the subsequent appearance of detectable UCP (Rafael and Heldt, 1976; Holloway *et al.*, 1984, 1985; Rial and Nicholls, 1984; Lean et al., 1986; Lean and James, 1986; Ashwell *et al.*, 1987; Loncar, 1990a).

Concluding remarks

Appearance of BAT and CAT

From the presented data it is clear that thermogenic adipose tissue comes in two forms: as a typical brown adipose tissue and as a convertible adipose tissue (Figs. 1 and 9). Intrauterine development seems the same for both types. During intrauterine development, CAT reaches maturity and becomes effective soon after birth. Postnatal development in CAT causes structural and functional changes which result in the loss of thermogenic capacity (Figs. 9 and 15). Thereafter the convertible adipocytes will serve as a lipid-store during the rest of the life of the adult animals. Severe cold stress or prolonged high concentrations of noradrenaline again will convert lipid-store adipocytes into thermogenic adipocytes (Figs. 9, 12E, 14, 15). After cessation of these thermogenic stimuli, the convertible adipose tissue again becomes a fat-store adipose tissue (Fig. 14C). Parallel studies with white adipose tissue showed that thermogenic function cannot be adopted in these types of adipose tissue which have not expressed thermogenic capability during early development (Loncar *et al.*, 1988a,b; Loncar, 1990a).

BAT, contrary to CAT, reaches full development in most animals at the end of the first fortnight. After this period, a certain regression appears which quantitatively diminishes the thermogenic capacity of BAT, although BAT is present during the rest of the life in small endotherms as a thermogenic adipose tissue. Thus, BAT exists as a specific structural-functional entity (Figs. 9 and 15).

Metabolic rate and distribution of BAT and CAT among endotherms

In analyzing the metabolic rate of different animals, it is possible to find out why some endotherms have BAT whereas others have CAT (Fig. 16). Furthermore, it is possible to predict which type of thermogenic adipose tissue is present in some unknown endotherms. The metabolic rate (kcal per day) as a collective measure of the total metabolism of an animal is proportional to the size of the animal. Because of their greater surface-to-volume ratios, smaller endotherms lose heat more rapidly than larger endotherms; *i.e.*,

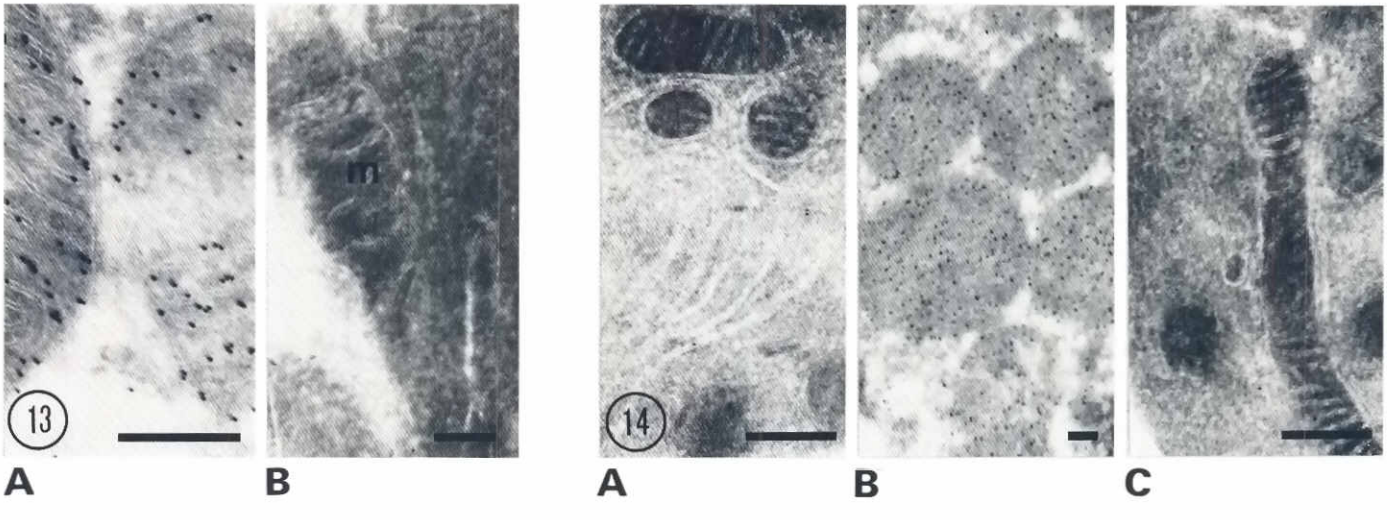


Fig. 13. Development of mitochondria in cervico-axillar convertible adipocytes of the rabbit. (A) *In a five-day-old rabbit, adipocytes have big, developed UC-mitochondria with cristae containing numerous UCP molecules (black dots).* **(B)** *Six months later, convertible adipocytes are in the lipid-store form and contain undeveloped C-mitochondria (m) without detectable UCP. (see Loncar, 1989, 1991a). Formaldehyde-paraformaldehyde fixation, polyvinyl pyrrholidon embedding, uranyl acetate negative contrasting, anti-UCP protein-A-gold labeling. Bars 0.1 μ m.*

Fig. 14. Convertible adipocyte in the inguinal area of the mouse. (A) *Adipocytes appear in lipid-store form and its cytoplasm contains C-mitochondria.* **(B)** *However, after severe cold stress, inguinal adipocytes adopt the thermogenic form with recently generated UC-mitochondria containing high amounts of UCP.* **(C)** *One month after cold stress; the adipocytes have adopted the lipid-store form and their C-mitochondria (which have replaced the UC-mitochondria) are devoid of UCP molecules. (see Loncar, 1990a). Formaldehyde-paraformaldehyde fixation, polyvinyl pyrrholidon embedding, uranyl acetate negative contrasting, anti-UCP protein-A-gold labeling. Bars 0.4 μ m.*

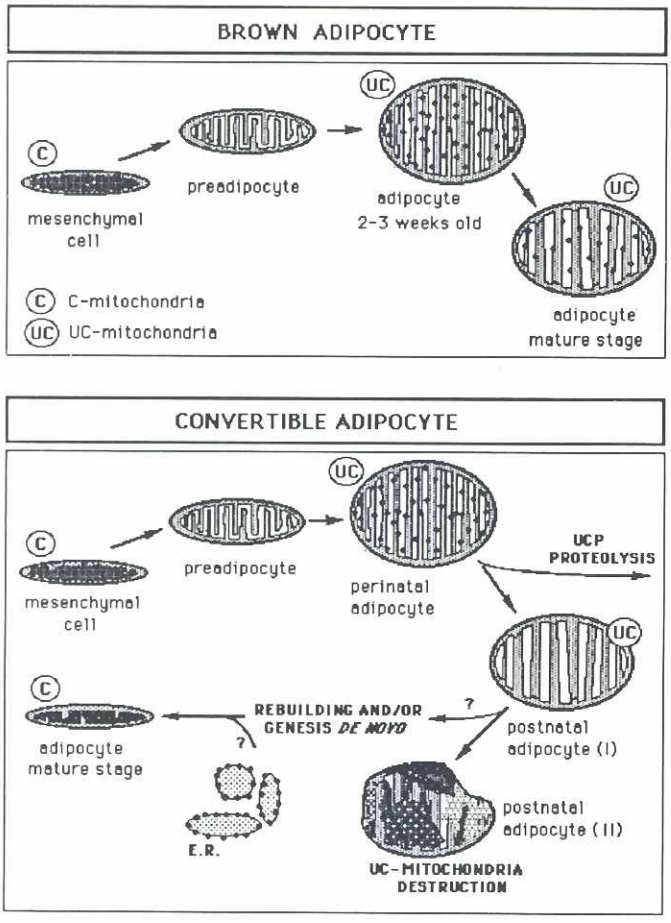


Fig. 15. Development of mitochondria brown and convertible adipocytes. ***Brown adipocyte.*** *Mesenchymal cells have elongated, common C-mitochondria while preadipocytes contain large UC-mitochondria with numerous cristae. Two-three weeks postnatal; UC-mitochondria in brown adipocytes have reached a maximal volume and surface of mitochondrial cristae and a maximal amount of uncoupling protein (UCP) (black dots). In maturity, brown adipocytes still have mitochondria in which size, shape, amount of cristae, and UCP are specific for UC-mitochondria.* ***Convertible adipocyte.*** *UC-mitochondria of perinatal convertible adipocytes arise in the same way as UC-mitochondria of brown adipocytes. With growth, the amount of UCP in UC-mitochondria declines due to UCP-proteolysis and stopped UCP synthesis, resulting in UC-mitochondria that are devoid of UCP (postnatal adipocyte I, 3-5 weeks-old in cats and rabbits). With further growth (postnatal adipocytes II), UC-mitochondria are destroyed and C-mitochondria remain in the cytoplasm of mature convertible adipocytes. Cold stress or high concentration of noradrenaline causes the reappearance of UC-mitochondria. The mechanism of mitochondria replacement is still unknown. E.R. endoplasmic reticulum.*

small endotherms must generate heat more rapidly in order to maintain the body temperature (Purves and Orians, 1987). As a result the small endotherms have a relatively high metabolic rate (kcal per kg per day) (Fig. 16). Thus, in all endotherms with a low metabolic rate but with a high relative metabolic rate there exists a need for an additional source of heat. Although these endotherms produce relatively more heat than larger endotherms, the small endotherms also lose more heat. This situation persists in all endotherms of low body mass (Rodentia, Chiroptera, Insectivora) and these endotherms have BAT- *i.e.*, an active form of thermogenic adipose tissue which has the capability of heat production (Himms-Hagen, 1985, 1986). Large endotherms, during early postnatal life, have thermogenic characteristics similar to the small endotherms; low body weight, low metabolic rate, great surface-to-volume ratio, and a high relative metabolic rate (Fig. 16). Thus, during early postnatal life large endotherms must also have active thermogenic adipose tissue. With growth, the large endotherms have a higher metabolic rate and lower surface/volume ratio, and there is no need for an additional source of heat (Purves and Orians, 1987). Convertible adipocytes, which serve as a heater during the early postnatal period, are converted into lipid store adipocytes. Thus, larger endotherms have thermogenic tissue which can be present in the thermogenic or lipid-store form (Fig. 1). These conversions cannot take place in small rodents even if they grow in thermoneutral conditions (Loncar, 1989). This implies that the environmental temperature has only a limited influence on the structural-functional appearance of thermogenic adipose tissue. The presence of a type of thermogenic adipose tissue (brown or convertible) is directed genetically.

Thermogenic adipose tissue and regulation of obesity in humans

The presence and distribution of BAT and CAT have more than just terminological meanings. Besides the interest in the intrinsic mechanisms which conduct the conversion of thermogenic into lipid store adipocytes (Fig. 9), mitochondriogenesis and the replacement

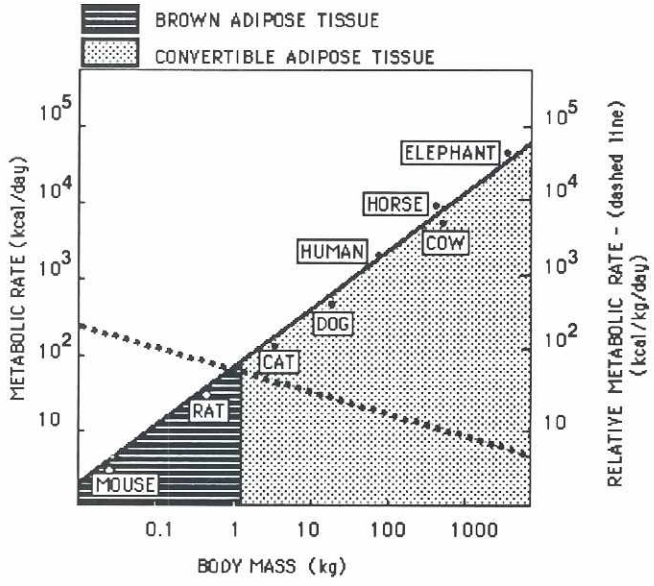


Fig. 16. Distribution of thermogenic adipose tissue among mammals in conjunction with metabolic rate. *Mammals of low body mass also have a low metabolic rate, and they have an additional heater in the form of brown adipose tissue. An increase in the body mass also increases the metabolic rate and simultaneously decreases the relative metabolic rate. A body mass of about 1 kg (in the adult stage) becomes critical for the appearance of convertible adipose tissue. The high metabolic rate in these animals excludes needs for an additional heater. Therefore, in adult large mammals, the thermogenic adipose tissue appears as convertible adipose tissue in its lipid-store form. (For the metabolic rate and body mass data see Purves and Orians, 1987).*

of UC- with C-mitochondria (Fig. 15), the question about direct participation of thermogenic adipose tissue (both BAT and CAT) in regulation of obesity needs to be answered.

The idea that BAT participates directly and probably conducts the control of obesity in endotherms was proposed after research on rats (Rothwell and Stock, 1979, 1986). Even without extensive morphological and functional studies, this hypothesis will be unacceptable to anyone who has spent some time in the autopsy room analyzing human adipose tissue. It is even clear macroscopically that the adult human body, like the body of any other larger endotherm, has no BAT, and that its CAT has unilocular adipocytes with nonthermogenic C-mitochondria (Fig. 8E). Mature large endotherms evidently have only fat-store adipocytes (Figs. 7D and 8E). New studies are necessary to investigate whether and how convertible adipocytes might be involved in the regulation of human obesity.

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