Fuel selection in brown adipose tissue

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Sélection de substrats énergétiques dans la cellule: le tissu adipeux brun

RÉSUMÉ

Le tissu adipeux brun (TAB) est le principal site de production de chaleur adaptive par des mécanismes sans frissons chez les mammifères. Ce tissu est particulièrement important chez le nouveau-né d'un certain nombre d'espèces, chez les hibernants, et chez les rongeurs adultes adaptés au froid. La chaleur est générée dans le TAB par une voie de conductance des protons dans les mitochondries qui dissocie l'oxydation des substrats de la synthèse d'ATP. Ce découplage contrôlé dans la mitochondrie est régulé par une protéine spécifique du tissu, la protéine de découplage (PD), Mr 32 000. La quantité de PD est modifiée selon les besoins thermogéniques d'un animal, augmentant au cours de l'exposition prolongée au froid, et diminuant à la chaleur. La PD est aussi soumise à une régulation nutritionnelle, s'élevant en participant à la réponse thermogénique à la suralimentation (en particulier à un régime 'cafétéria'), et s'abaissant au jeûne. Le TAB stocke des quantités considérables de lipides, bien que la teneur de ce tissu en lipides soit généralement inférieure à celle de la graisse blanche (40 et 80% respectivement). Les acides gras sont les substrats énergétiques les plus important dans la thermogenèse, et ils sont produits à la fois par la circulation, par l'action de la lipase lipoprotéine (*EC* 3.1.1.34), et par la synthèse *de novo* à partir du glucose. L'activité lipoprotéine lipase et l'expression du gène codant pour l'enzyme sont rapidement stimulées par le froid, en parallèle avec l'expression du gène de la PD induite par le froid. Le taux de lipogenèse dans le TAB est élevé, en particulier chez les animaux acclimatés au froid. La plus grande partie du glucose est utilisée par le TAB pour la lipogenèse, l'absorption étant stimulée à la fois par l'insuline et par le système nerveux sympathique. Deux transporteurs facilitants du glucose sont présents dans le TAB: GLUT1 et GLUT4. Le transporteur sensible à l'insuline, GLUT4, est cependant l'isoforme la plus importante pour l'absorption du glucose par le tissu. Le TAB est un organe inhabituel, en ce qu'il ne se trouve pas chez tous les homéothermes, ou à tous les stades du cycle de la vie. Néanmoins, il apparaît que la plupart des mammifères contiennent du TAB d'après l'identification immunologique du TAB dans les tissus adipeux, une exception étant le porc domestique. Chez les espèces précoces (agneaux, chèvres), le TAB ne se trouve que sur une période limitée après la naissance, une rapide transition postnatale intervenant dans le tissu vers la graisse blanche.

The maintenance of a constant body temperature by homeotherms and their survival in a cold environment depend on the ability both to conserve and to generate heat. Thermoregulatory heat is produced by two general mechanisms, shivering (involving

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contraction of the muscles) and non-shivering thermogenesis. A number of metabolic systems for non-shivering thermogenesis have been proposed (see Trayhurn, 1994), including Na^+ pumping across plasma membranes, substrate cycles in specific metabolic pathways, and more recently 'hot pipes' in the vasculature (Colquhoun & Clark, 1991). The best documented mechanism for generating heat by non-shivering thermogenesis is, however, that associated with brown adipose tissue (BAT), or brown fat (see Nicholls & Locke, 1984; Cannon & Nedergaard, 1985; Himms-Hagen, 1989).

BAT is the only organ in mammals specialized for heat production. The tissue is particularly evident in hibernating species (such as the ground squirrels, e.g. Richardson's ground squirrel (*Spermophilus richardsonii*)), the newborn of a number of mammals (e.g. lambs and goats), and adult rodents adapted to cold environments (Trayhurn, 1993*a*). BAT is, by definition a lipid storage organ, although the fat content of the tissue is generally lower than that of white adipose tissue (300–400 *v.* 700–850 mg/g wet weight). BAT is highly vascularized, and is extensively innervated by sympathetic nerves (see Himms-Hagen, 1989).

The present article considers metabolic fuel selection in BAT, the theme of the Lavoisier Bicentenary Symposium. Some background to the physiology of the tissue is also presented; review articles are quoted where possible.

BROWN ADIPOSE TISSUE AND ENERGY EXPENDITURE

The impact of BAT on the total energy expenditure of an animal is highly variable. It is dependent on factors such as body size (being greater in small animals than in large animals), environmental temperature, diet (amount and composition), length of exposure to cold, developmental stage, reproductive state (reduced during lactation), and species (Rothwell & Stock, 1986; Himms-Hagen, 1989; Trayhurn, 1993*b*). At one extreme, where BAT is either absent from a species or at a particular developmental stage, self-evidently the tissue plays no role in energy expenditure. Similarly, when present, its contribution to overall energy expenditure is negligible in animals adapted to thermoneutral temperatures. However, in small animals adapted to the cold, such as mice at 0–4°, energy expenditure by the tissue may be equivalent to some three to four times the BMR. Thus, during maximal rates of thermogenesis, BAT may make a very substantial contribution to total energy expenditure.

The considerable energy costs of thermoregulation for small animals in the cold can be illustrated from data on the food intake of mice acclimated at either thermoneutrality (32°) or 4°. Intake in the cold is three times that under thermoneutral conditions (Trayhurn, 1981), the difference in intake essentially being the costs of thermogenesis. Although on acute cold exposure thermogenesis is derived in part from shivering, during long-term adaptation non-shivering heat production gradually replaces the heat generated by muscle contraction (Foster & Frydman, 1979). Measurements of blood flow and tissue oxygen utilization *in vivo* have documented the quantitative importance of BAT to non-shivering thermogenesis in fully cold-adapted small rodents (Foster & Frydman, 1978, 1979; Foster, 1986). Those organs, such as the heart, lungs, and respiratory muscles, which play a supporting role in non-shivering thermogenesis also contribute to the energy costs of thermoregulation in cold-adapted animals. In addition to its role in thermoregulatory thermogenesis, BAT is involved in the adaptive response to overfeeding, through diet-induced thermogenesis (Rothwell & Stock, 1986).

BAT is similar to skeletal muscle in its impact on overall energy expenditure being highly variable, in contrast to organs such as the brain. However, in a well-nourished animal high rates of energy expenditure can be sustained indefinitely by BAT, while fatigue develops in skeletal muscle after prolonged exercise.

MECHANISM OF HEAT PRODUCTION

Apart from lipid droplets, the major histological feature of active BAT is the presence of a large number of mitochondria, which are characterized by a well-developed cristae structure (see Néchad, 1986). The mitochondria are, of course, central to the ability of BAT to generate heat (Nicholls & Locke, 1984). There is a substantial oxidative capacity in the tissue, but in contrast to mitochondria in other tissues, the proton gradient that is generated during oxidation is not linked to the synthesis of ATP, but to the direct generation of heat (Nicholls & Locke, 1984; Nicholls *et al.* 1986). This occurs through the presence of an alternative pathway for the passage of protons across the inner mitochondrial membrane, the proton conductance pathway. In essence, this represents a short circuiting of the proton current through the membrane. The proton conductance pathway is due to the presence in the inner membrane of brown fat mitochondria of a unique uncoupling protein (Nicholls & Locke, 1984; Ricquier & Bouillaud, 1986). This protein, also termed thermogenin (Cannon & Nedergaard, 1985), has a molecular weight of approximately 32 000 Da, and is a member of the family of mitochondrial carrier proteins, which includes the ADP-ATP translocase and the phosphate carrier (Klingenberg, 1990).

The level and activity of uncoupling protein change according to the physiological requirements for heat production of an animal (Himms-Hagen, 1989; Trayhurn, 1993*b*). Acutely, this occurs through activation of pre-existing uncoupling protein, this appearing as an increase in mitochondrial GDP binding (unmasking of GDP-binding sites), which is a widely used index *in vitro* of thermogenic activity (Trayhurn & Milner, 1989). Following a chronic thermogenic stimulus the total uncoupling protein content of BAT increases by two separate mechanisms; the concentration of the protein in the mitochondria increases (i.e. concentration per mg mitochondrial protein), and there is an increase in the total mitochondrial content of the tissue (Trayhurn & Milner, 1989).

Thermogenesis is initiated by the release of noradrenaline from the sympathetic nervous system, the sympathetic system being critical to the regulation of many of the processes that occur in brown fat (Himms-Hagen, 1991). The noradrenaline binds to a β_3-receptor on the plasma membrane, which is a novel type of receptor found in BAT (Arch, 1989). Although there has been considerable emphasis on the importance of the β_3-adrenoceptor, β_1- and α-mediated responses in the tissue have also been characterized (Mohell *et al.* 1987; Arch, 1989; Rehnmark *et al.* 1990).

FUELS FOR THERMOGENESIS

Central to the activation of thermogenesis is the noradrenergic stimulation of lipolysis, through hormone-sensitive lipase (*EC* 3.1.1.3), with the production of fatty acids from the stored triacylglycerol (Bukowiecki, 1986). Fatty acids are the main fuel for thermogenesis in BAT. Although glucose is oxidized by the tissue, its direct contribution

as a fuel is small relative to that of fatty acids (Ma & Foster, 1986; Cawthorne, 1989). Other substrates, including lactate, ketones, and amino acids may also provide fuel, but again their role is minor (Williamson, 1986).

The fatty acids oxidized by BAT as substrates for thermogenesis can be derived by two different routes, uptake from circulating chylomicrons and lipoproteins, or *de novo* synthesis. BAT contains a high lipoprotein lipase (*EC* 3.1.1.34) activity, which allows the tissue to import fatty acids from circulating triacylglycerols (Carneheim *et al.* 1984); this enzyme, therefore, plays a central role in the provision of fuel for non-shivering thermogenesis. Lipoprotein lipase activity in brown fat is stimulated by noradrenaline, as well as by insulin, in contrast to the situation pertaining in white adipose tissue (Carneheim *et al.* 1984); in white fat, noradrenaline does not lead to any stimulation of lipoprotein lipase activity. The expression of the gene encoding lipoprotein lipase in BAT is also stimulated by noradrenaline (Mitchell *et al.* 1992).

LIPOGENESIS

Studies with tritiated water *in vivo* have indicated that the capacity of BAT to synthesize fatty acids is very high, relative to the other major lipogenic organs, the liver and white adipose tissue (McCormack & Denton, 1977; Trayhurn, 1981). Not surprisingly, the rate of lipogenesis in brown fat varies considerably with the environmental temperature to which an animal is adapted. For example, in mice fed on a high-carbohydrate, low-fat diet the rate of lipogenesis (per whole tissue) in interscapular BAT is about 16-fold higher in animals acclimated at 4° than that in those maintained at thermoneutrality (Trayhurn, 1981). The liver, on the other hand, shows no increase in lipogenic rate in the cold, while in white fat there is a modest (relative to BAT) cold-induced stimulation (Trayhurn, 1981).

Lipogenesis in BAT can be quantitatively important on a whole-body basis. In mice acclimated at 4°, it has been estimated that approximately one-third of whole-body lipogenesis takes place in the tissue (Trayhurn, 1981). This contrasts with a whole-body contribution of 11% for the liver. However, in mice acclimated at thermoneutrality, BAT accounts for only 5% of whole-body lipogenesis and the liver one-third. Thus, the relative importance of different organs in lipogenesis varies markedly with environmental temperature. Under conditions of low temperature, BAT represents a major site of the conversion of carbohydrate to lipid in rodents consuming a high-carbohydrate, low-fat diet (McCormack & Denton, 1977; Trayhurn, 1981).

Lipogenesis in BAT, as in other tissues, is sensitive to the level of dietary lipid, and is stimulated by insulin (McCormack & Denton, 1977; McCormack, 1983). The provision of a high-fat diet to cold-acclimated animals results in much lower rates of lipogenesis in BAT than when a low-fat diet is given (van den Brandt & Trayhurn, 1981). The suppressive effect of dietary lipid on BAT lipogenesis appears to be particularly marked with diets rich in linoleic acid (or polyunsaturated fatty acids in general). Low rates of lipogenesis in BAT are also evident in sucking rodents, where the thermogenic capacity of the tissue is high (Trayhurn, 1981). Sucking animals consume, of course, a high-fat diet in the form of milk. Weaning of rodents onto the customary low-fat high-carbohydrate laboratory diets leads to a rapid rise in lipogenesis (Mercer & Trayhurn, 1983). This is accompanied by increased expression of the genes coding for lipogenic enzymes such as acetyl-CoA carboxylase (*EC* 6.4.1.2) and fatty acid synthetase (*EC*

2.3.1.85), together with increases in enzyme activity (Perdereau *et al.* 1992). Overall, the relative importance of fatty acids derived from the circulation (i.e. from other tissues), and by *de novo* synthesis within BAT, as fuels for thermogenesis is dependent on the lipid content of the diet.

BAT contains an intracellular fatty acid-binding protein (FABP), and this is probably similar to the aP2 protein identified in white fat (Dutta-Roy *et al.* 1993). FABP is likely to be important in the transport of fatty acids within the brown adipocyte. In practice, there appear to be two FABP isoforms in rat brown fat, and we have speculated that one may be associated with the transport of fatty acids as fuel for oxidation. The other may interact directly with uncoupling protein, since fatty acids (or fatty acyl CoA) act as a signal for the activation of the protein in BAT mitochondria (Nicholls *et al.* 1986).

GLUCOSE UTILIZATION

Although, as discussed previously, glucose is not an important oxidative fuel in brown fat, the rate of glucose uptake by the tissue can be considerable. BAT is a highly-insulin-sensitive organ (Ferré *et al.* 1986), glucose uptake and insulin sensitivity increasing in the cold (see Cawthorne, 1989). Two facilitative glucose-transporter isoforms, GLUT1 and GLUT4, have been identified in BAT (Slot *et al.* 1991; Nikami *et al.* 1992). GLUT4, the insulin-sensitive transporter, is likely to be responsible for most of the glucose taken up by the tissue (Slot *et al.* 1991; Nikami *et al.* 1992); this transporter thus plays, together with lipoprotein lipase, a critical role in fuel provision to brown fat. Both noradrenaline and insulin are known to stimulate glucose uptake by BAT (Cawthorne, 1989). It is probable, therefore, that in the brown adipocyte the translocation of GLUT4 from intracellular pools to the plasma membrane is stimulated by the sympathetic system, as well as by insulin.

The glycolytic capacity of BAT is substantial, the tissue containing high activities of glycolytic enzymes (Cooney & Newsholme, 1982) and of pyruvate dehydrogenase (*EC* 1.2.4.1; McCormack & Denton, 1977). Cold-acclimation leads to increases in the maximal activities of both hexokinase (*EC* 2.7.1.1) and phosphofructokinase (*EC* 2.7.1.11), consistent with a cold-induced augmentation of glycolytic flux (Cooney & Newsholme, 1984). Apart from acting as a key substrate for lipogenesis, glucose may be particularly important to BAT by providing substrate to prevent the depletion of citric acid cycle intermediates during high rates of thermogenesis (Cannon & Nedergaard, 1979). The development of insulin resistance in BAT results in a loss of the acute thermogenic response to cold (see Cawthorne, 1989).

WHERE AND WHEN IS BROWN ADIPOSE TISSUE PRESENT?

BAT occupies a quite unusual position among tissues. In contrast to other organs, its species distribution is a matter of continuing uncertainty and debate (Trayhurn, 1993*a*). The issue of species distribution, and developmental stage at which BAT is present, is of critical importance not only for the physiology of thermogenesis, but also in relation to the variable impact that brown fat has on energy expenditure and fuel selection in an animal.

Differentiation from white adipose tissue

Anatomical and histological appearance is not a satisfactory basis for determining whether or not brown fat is present in a given species, or at a particular stage of development. The classical structural appearance of the tissue, namely a multilocular arrangement of the stored fat droplets, together with large numbers of mitochondria (with a well-developed cristae structure), is an inappropriate basis for distinguishing BAT from white adipose tissue. Relatively inactive BAT, such as in obese animals, can appear similar to normal white adipose tissue, while white fat in cold-exposed or fasted animals may appear histologically similar to brown fat (see Trayhurn, 1993*a*).

Other, molecular, criteria for differentiating between the two forms of adipose tissue are, therefore, necessary. The criterion now used is the presence or absence of the BAT-specific mitochondrial uncoupling protein (Klaus *et al.* 1991; Trayhurn, 1993*a*), and this can be determined immunologically (generally by Western blotting), using antibodies raised against the protein. An alternative approach is the detection of the mRNA for uncoupling protein, using an oligonucleotide based on a highly conserved region of the uncoupling protein gene (Trayhurn, 1994; Trayhurn & Duncan, 1994).

Species distribution

On the basis of the immunological identification of uncoupling protein, it is now evident that BAT is widely distributed in mammals (Klaus *et al.* 1991; Trayhurn, 1993*a*). In addition to the traditional laboratory animals (mice, rats, rabbits), the species in which uncoupling protein has been detected include hibernators (ground squirrels, hamsters), bats (pipistrelle bat (*Pipistrellus pipistrellus*)), carnivores (dogs), ruminants (lambs, cattle, goats, red deer (*Cervus elaphus*)), and primates (monkeys, humans). One species from which uncoupling protein, and thus BAT, appears to be absent is the pig, based on studies with domesticated animals (Trayhurn, 1993*a*). Whether brown fat is absent only from domesticated strains, which have been subject to intensive selection over a large number of generations, requires the analysis of feral pigs.

To date, uncoupling protein has not been directly identified in marsupials, nor in the several species of birds that have been examined (Trayhurn, 1993*a*). Members of the class *Avies* investigated include several adapted to the cold of the subarctic winter (Saarela *et al.* 1991), as well as the only species of bird for which there is evidence of true hibernation, the common poorwill (*Phalaenoptilus nuttallii*; Brigham & Trayhurn, 1994).

Developmental changes

The identification of BAT in a given species is made more complicated by the fact that in some cases the tissue may not be present throughout life, occurring only during a restricted period in development. This is very evident in the case of precocial agricultural animals, such as goats and lambs. Recent studies have indicated that uncoupling protein is present in adipose tissues of newborn lambs, deer, cattle and goats (Casteilla *et al.* 1987; Trayhurn *et al.* 1993*a*); indeed in neonatal lambs and goats, all fat depots appear to be functionally brown (Trayhurn *et al.* 1993*a*,*b*). However, within the first few weeks of postnatal life, immunoreactive uncoupling protein is no longer detectable, there being a transition of BAT to white fat (Casteilla *et al.* 1987; Trayhurn *et al.* 1993*a*). Whether this

is an interconversion between fat cell types, or a replacement of brown adipocytes by white adipocytes, has not been established.

CONCLUSIONS

BAT is specialized for facultative heat production by non-shivering mechanisms. The tissue can be the major contributor to overall energy expenditure in an animal, with considerable impact on the flux of nutrients, particularly in small rodents adapted to the cold. Fatty acids are the primary fuel for heat production in BAT, and these are obtained both from exogenous sources and by *de novo* synthesis from glucose and other substrates. Lipoprotein lipase and the insulin-sensitive glucose transporter, GLUT4, play a pivotal role in facilitating the provision of fuels for BAT. Noradrenaline from the sympathetic nervous system, and insulin, are central to the regulation of substrate uptake and utilization by the tissue. BAT is an unusual organ in that it is not present in all mammalian species (or other homeotherms), nor at all stages of development.

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