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SYMPOSIUM ON 'BROWN ADIPOSE TISSUE—ROLE IN NUTRITIONAL ENERGETICS'

Brown adipose tissue and nutritional energetics—where are we now?

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The discovery that brown adipose tissue plays an important role in energy balance and energy metabolism in certain species represents one of the most interesting and dynamic developments in the recent history of bioenergetics. In the past decade brown adipose tissue has moved from relative obscurity to become a focus for several key areas of biology. Members of the Nutrition Society have been at the forefront of research on brown adipose tissue, a number of presentations in the field having been made at meetings of the Society. It is, therefore, particularly appropriate that a Symposium concerned exclusively with the role of brown adipose tissue in nutritional energetics should take place under its auspices.

In this brief overview several issues relating to the function of brown adipose tissue are presented. It is emphasized that some of the concepts which are now reasonably well established were regarded as radical, almost heretical, only a few years ago.

Brown adipose tissue v. *white adipose tissue*

In any discussion on brown adipose tissue it is necessary to consider the primary features distinguishing it from the more familiar white adipose tissue. The main differences between the types of adipose tissue have recently been summarized (Trayhurn & Ashwell, 1987). The two tissues clearly have very different functions in energy metabolism, the primary role of brown adipose tissue being to oxidize substrates to produce heat. The central role of white adipose tissue, on the other hand, is to serve as the primary energy store in an animal. At the histological level the two tissues have usually been distinguished on the basis of the presence of multiple fat droplets (multilocular structure) in the brown adipocyte, while only a single large fat droplet (unilocular structure) is present in the white fat cell.

There are, however, sufficient exceptions to this simple histological distinction for it to be an inappropriate basis for determining whether an adipose tissue is 'white' or 'brown'. For example, in obese (*ob/ob*) mice brown adipocytes appear unilocular because of the abnormally high amount of triacylglycerol which is stored. Functionally, however, the cells are thermogenic, albeit less so than in lean siblings (see Trayhurn, 1986). Even the presence of more mitochondria, with a highly developed cristae structure, in brown than

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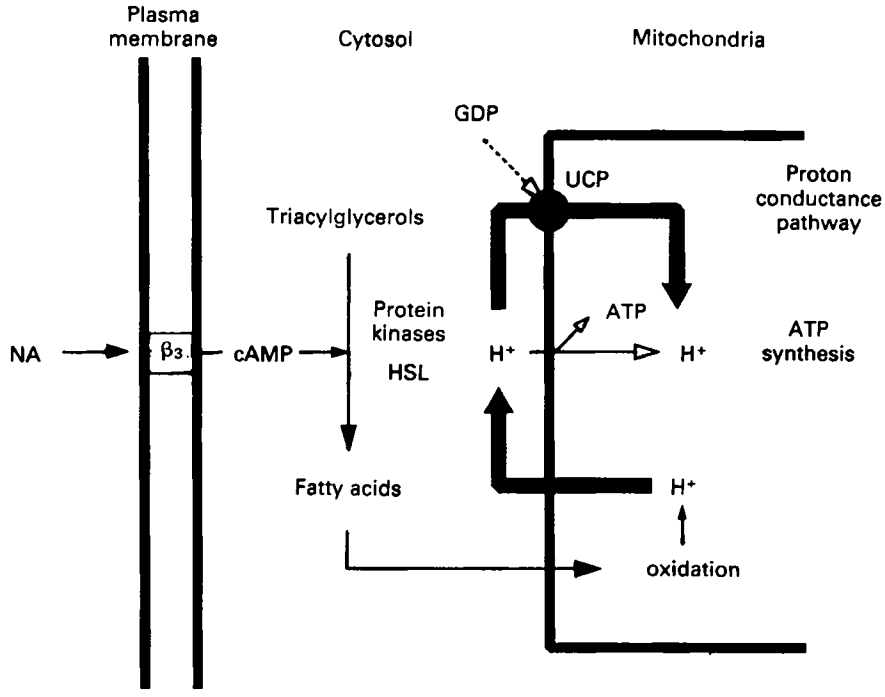

Fig. 1. A schematic representation of the mechanism for thermogenesis in brown adipose tissue. NA, noradrenaline; HSL, hormone-sensitive lipase; UCP, uncoupling protein.

in white adipocytes is too inexact a distinction to form a satisfactory basis for differentiating the two adipose tissues.

The most relevant criterion for determining whether an adipose tissue is 'brown' is the presence of the mitochondrial proton conductance pathway. This pathway is regulated by a 32 000 M_r protein, generally termed uncoupling protein, located in the inner mitochondrial membrane (Nicholls & Locke, 1984). The presence of this protein in mitochondria isolated from an adipose tissue provides the definitive criterion for identifying the tissue as 'brown'. Correspondingly, the absence of the protein indicates that the tissue is 'white'. Uncoupling protein can be detected immunologically using antisera raised against the pure protein (Cannon *et al.* 1982; Lean *et al.* 1983; Ricquier *et al.* 1983).

Mechanism of thermogenesis in brown adipose tissue

The proton conductance pathway described by Nicholls and his colleagues is the central mechanism for thermogenesis in brown adipose tissue, and it has been extensively reviewed in recent years (Nicholls & Locke, 1984; Cannon & Nedergaard, 1985; Nicholls *et al.* 1986). Only a brief outline of the salient features will, therefore, be given here, and these are illustrated schematically in Fig. 1.

Noradrenaline from the sympathetic nerves which extensively innervate brown adipose tissue is the main acute stimulus for thermogenesis (see Girardier & Seydoux, 1986). The β-adrenoceptor on the plasma membrane of the brown adipocyte, to which noradrenaline binds, is increasingly considered to be of a novel subtype, i.e. β_3 (see

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Arch *et al.* 1984; this symposium). Following noradrenergic stimulation, adenylcyclase (*EC* 4.6.1.1) is activated, presumably through the G-binding protein system, with the formation of cAMP. The cAMP activates a hormone-sensitive lipase, via one or more of a series of protein kinases (Skala, 1984). Fatty acids mobilized from the stored triacylglycerol are then transported to the mitochondria and oxidized. Fatty acids appear to play a dual role in thermogenesis in that in addition to being the primary substrates they are also considered to provide the key intracellular signal activating the proton conductance pathway (Nicholls *et al.* 1986).

The proton conductance pathway acts as a proton short circuit across the inner mitochondrial membrane, with the result that substrate (fatty acid) oxidation is uncoupled from the synthesis of ATP. Thus the potential energy associated with the proton gradient is dissipated as heat (Nicholls *et al.* 1986). As indicated previously, the pathway is regulated by the tissue-specific uncoupling protein. Uncoupling protein has a high affinity for purine nucleotides, such as GDP and ADP, which on binding to the protein inhibit the proton short circuit leading to a recoupling of the mitochondria (Nicholls & Locke, 1984).

Thermogenesis: what are we measuring?

A central consideration in all studies on thermogenesis in brown adipose tissue is assessment of the most appropriate measurements to make, together with the interpretation of these measurements. This seemingly obvious point is made in the light of the confusion which has surrounded the precise meaning of the widely used indices of the thermogenic activity of the tissue. Table 1 summarizes the main measurements that have been made; these are essentially biochemical. The most direct measure of thermogenesis, oxygen utilization in vivo, has not been widely employed because of the considerable technical difficulties in conducting such studies on free-living animals. In addition, there is some uncertainty associated with the effects of anaesthesia and surgery, especially when performed immediately before measurements of blood flow and tissue O_2 utilization. Nevertheless, such studies have been crucial, not only for defining the quantitative importance of brown adipose tissue to thermogenesis (Foster, 1986), but also as a reference point for the scale of changes that occur with the in vitro biochemical measurements.

Tissue weight is a reflection of body fat in the animal rather than an indicator of thermogenic activity. Thus obese animals generally show tissue hypertrophy in terms of weight, while thermogenic activity is reduced (see Trayhurn, 1986). Weight is also increased on overfeeding with a cafeteria diet, although in this case thermogenesis is increased (see Rothwell & Stock, 1986). The protein content of the tissue provides a crude index of active tissue mass. Purine nucleotide, particularly GDP, binding studies on isolated mitochondria are the most commonly used measure of thermogenesis in brown adipose tissue. Binding values are usually expressed on a per mg mitochondrial

Table 1. *Measurements on brown adipose tissue*

Weight
Protein content
Cytochrome *c* oxidase (*EC* 1.9.3.1) activity (mitochondrial content)
Mitochondrial GDP binding
Mitochondrial swelling; proton conductance
Uncoupling protein
mRNA for uncoupling protein

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protein basis. Thus in order to assess thermogenesis in terms of the whole tissue it is necessary to have an index of the total mitochondrial content, and this is customarily done through measurements of cytochrome *c* oxidase (*EC* 1.9.3.1) activity.

The interpretation of GDP binding results has been a matter of some debate and uncertainty. Some authors have argued that GDP binding is a measure of the amount of uncoupling protein (Rial & Nicholls, 1984; Cannon & Nedergaard, 1985), while others consider it to be an index of thermogenic activity (Himms-Hagen, 1986; Trayhurn, 1986). The issue has now been clarified, however, several recent studies employing an independent measure of uncoupling protein having shown that the level of GDP binding can be dissociated from the amount of the protein (Gribskov *et al.* 1986; Trayhurn *et al.* 1987; Milner *et al.* 1988; Peachey *et al.* 1988). Thus GDP binding is an index of the *activity* of the proton conductance pathway, and not a measure of uncoupling protein. The concentration of the protein does, however, set the upper limit to the amount of GDP that can be bound. Other functional indices of mitochondrial proton conductance include swelling in acetate or chloride buffers in the presence of the potassium ionophore, valinomycin, and direct measurements of proton conductance. In general, each appears to be highly correlated with the level of GDP binding (Nicholls & Locke, 1984; Rial & Nicholls, 1984).

Uncoupling protein can be quantitated immunologically, either by radioimmunoassay (Lean *et al.* 1983), ELISA (Cannon *et al.* 1982; Desautels, 1985) or by immunoblotting techniques (Henningfield & Swick, 1987; Milner *et al.* 1989). cDNA probes for uncoupling protein are now available, which enables the mRNA for the protein to be detected and measured (Bouillaud *et al.* 1984; Jacobsson *et al.* 1985; Ridley *et al.* 1986). The presence of the mRNA indicates that the gene coding for uncoupling protein is being expressed, and like the immunological detection of the protein itself, provides a potent tool for differentiating between brown and white adipose tissues.

Uncoupling protein in brown adipose tissue

The concentration of uncoupling protein in mitochondria determines the *capacity* of the proton conductance pathway, while the total amount in a brown adipose tissue depot sets the thermogenic capacity of that particular depot. The uncoupling protein content of brown adipose tissue is generally modulated by variations in both the specific mitochondrial concentration and the mitochondrial mass of the tissue. However, the pre-hibernation changes in Richardson's ground squirrel (*Spermophilus richardsonii*) appear to be an exception to this rule (Milner *et al.* 1989).

The effect on the uncoupling protein content of brown adipose tissue of several different treatments and conditions has now been reported. Cold-acclimation produces profound changes in the amount of the protein. In rats, for example, the total amount of uncoupling protein in the interscapular pad following acclimation at 4° is approximately 100-fold greater than at thermoneutrality (29°), as illustrated in Fig. 2. Overfeeding on a cafeteria diet also leads to an increase in the uncoupling protein content of the tissue (Fig. 3), while fasting induces a decrease (Fig. 4). Reductions in the amount of the protein have also been documented during lactation (Trayhurn & Jennings, 1987), and in obesity (Ashwell *et al.* 1985).

The relative changes in uncoupling protein in these other situations are smaller than those occurring during the response to extreme variations in acclimation temperature. However, in interpreting the information in Figs 2–4, and comparing between studies, it is important to note that a different reference point is being used in each case. In focusing on uncoupling protein, thermogenic capacity rather than activity is being stressed. GDP binding studies show, however, changes which would be predicted from the measure-

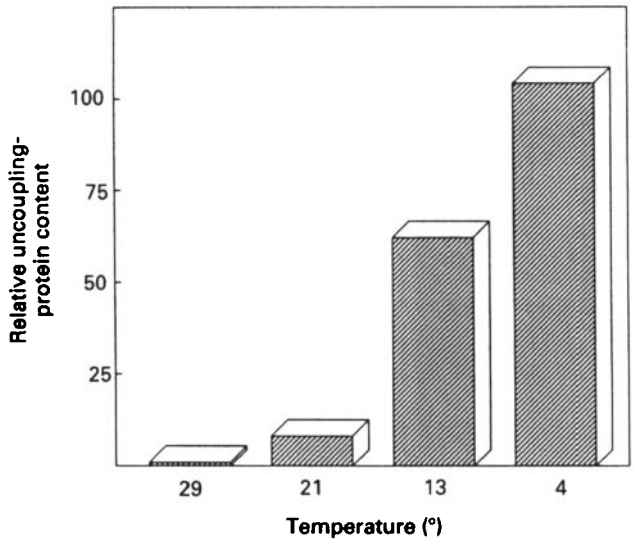

Fig. 2. Relative uncoupling protein content of interscapular brown adipose tissue of rats acclimated at different environmental temperatures. Rats at 29° = 1. Adapted from Trayhurn *et al.* (1987).

ments of uncoupling protein, especially when mitochondrial mass is taken into account. Although changes in GDP binding can occur without alterations in the concentration of uncoupling protein (Desautels *et al.* 1978; Gribskov *et al.* 1986; Trayhurn *et al.* 1987; Peachey *et al.* 1988), long-term adaptations in the amount of the protein invariably lead to corresponding changes in binding.

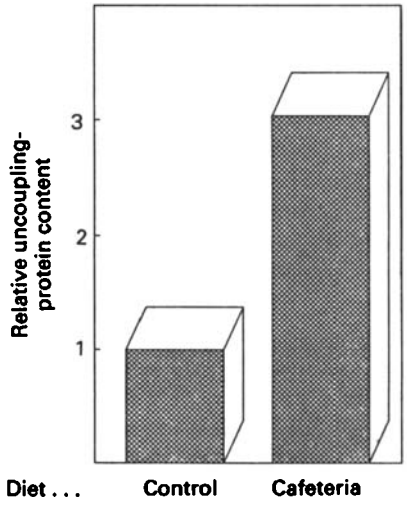

Fig. 3. Effect of giving a cafeteria diet on the relative uncoupling protein content of interscapular brown adipose tissue of rats. Control rats = 1. Adapted from Ashwell *et al.* (1984).

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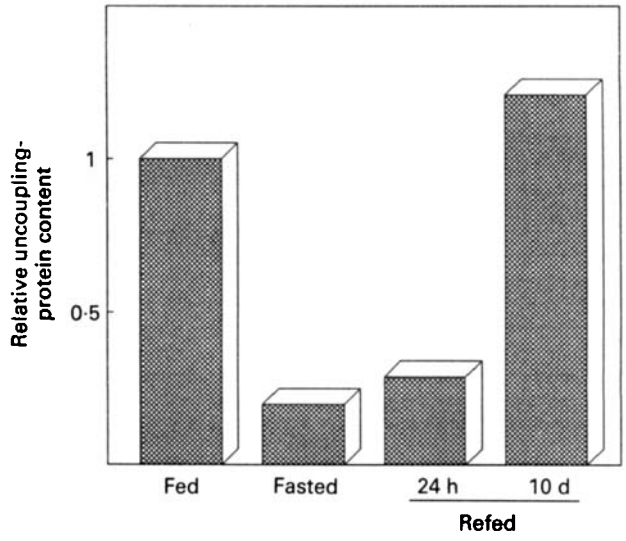

Fig. 4. Effect of fasting on the relative uncoupling protein content of interscapular brown adipose tissue of mice. Fed mice = 1. Adapted from Trayhurn & Jennings (1988).

Neural and hormonal control of brown adipose tissue

The importance of the sympathetic nervous system in the acute stimulation of both lipolysis and the proton conductance pathway has been discussed previously. Noradrenaline from the sympathetic system has a number of other short-term effects on brown adipose tissue. Glucose transport is stimulated (Cooney *et al.* 1985), as is the activity of lipoprotein lipase (*EC* 3.1.1.34) (Carneheim *et al.* 1984). Together these changes lead to an increase in the provision of substrates for thermogenesis, although only a small proportion of the glucose is likely to be oxidized directly, the major part being used for lipogenesis (Ma & Foster, 1986; Isler *et al.* 1987; Wilson *et al.* 1987). The enzyme iodothyronine 5′-deiodinase (*EC* 3.8.1.4) is present at high activity in brown adipose tissue, and is stimulated by noradrenaline (Silva & Larsen, 1983). The presence of this enzyme provides a local supply of triiodothyronine to brown adipose tissue, but the tissue also appears to be a major source of the hormone for other organs (Fernandez *et al.* 1987).

In addition to its role in the acute regulation of thermogenesis, noradrenaline is also the main factor controlling the long-term thermogenic capacity of brown adipose tissue, at least in rats and mice (see Trayhurn & Ashwell, 1987). The trophic effect of noradrenaline involves increases in cell number, and an increase in both mitochondrial content and the specific mitochondrial concentration of uncoupling protein (see Trayhurn & Ashwell, 1987).

Circulating hormones also influence brown adipose tissue. Corticosteroids inhibit thermogenesis (Holt & York, 1982; Galpin *et al.* 1983), while insulin is considered to be stimulatory (Rothwell & Stock, 1988). Both hormones act, at least in part, centrally by modulating the activity of the sympathetic system, insulin through an activation (Landsberg & Young, 1984; Rothwell & Stock, 1988) and corticosteroids by a suppression (Van der Tuig *et al.* 1984; York *et al.* 1985). Insulin also has direct effects on brown adipose tissue, including the stimulation of glucose transport (Czech *et al.* 1974;

Cooney *et al.* 1985; Ferre *et al.* 1986) and of lipogenesis (McCormack & Denton, 1977). The latter is the result of a parallel activation of the enzymes pyruvate dehydrogenase (*EC* 1.2.2.2, 1.2.4.1) and acetyl-CoA carboxylase (*EC* 6.4.1.2) (McCormack & Denton, 1977).

Brown adipose tissue and energy balance

The thermogenic activity and capacity of brown adipose tissue have been examined in a number of physiological and pathophysiological states where major changes in energy metabolism and energy flux take place (for reviews, see Rothwell & Stock, 1986; Trayhurn, 1986; Himms-Hagen, 1987). These include cold acclimation and overfeeding (particularly with a cafeteria diet), where thermogenesis is augmented, and fasting, lactation and several different types of obesity, where it is decreased (Table 2). In each condition brown adipose tissue thermogenesis is altered in a manner consistent with an important role for the tissue in energy balance and its regulation.

The amount of brown adipose tissue generally changes in parallel with the state of energy balance of the animal, reflecting alterations in lipid storage. Thus, during negative energy balance, such as on fasting, the weight of the brown fat depots falls (Table 2). Correspondingly, during periods of positive energy balance (overfeeding and the dynamic phase of obesity) the amount of the tissue rises. The main tool employed in the studies of brown adipose tissue in relation to energy balance is the indirect assessment of mitochondrial proton conductance from measurements of [^{3}H]GDP binding. Parallel measurements of mitochondrial mass have provided an indication of changes in thermogenic activity in terms of the whole tissue, while the quantification of uncoupling protein, discussed previously, has provided a measure of the thermogenic capacity.

Several studies have examined the effects on brown adipose tissue of feeding diets of different composition. In addition to various types of cafeteria diet, both low-protein or high-polyunsaturated-fatty-acid diets of defined composition lead to increases in thermogenic activity (Rothwell *et al.* 1983; Mercer & Trayhurn, 1987). Thermogenic capacity as such has not been reported, but the long-term nature of most dietary studies suggests that increases in thermogenic activity will reflect an increase in capacity.

A number of studies in rats and mice employing noradrenaline turnover techniques has demonstrated that changes in the thermogenic activity and capacity of brown adipose tissue in response to different conditions and stimuli are due to alterations in the level of sympathetic activity (Table 2). Thus sympathetic activity in the tissue is increased during cold exposure and cafeteria feeding (Young *et al.* 1982), and decreased during fasting

Table 2. *Summary of changes in brown adipose tissue in different conditions*

	Increased thermogenesis		Decreased thermogenesis		
	Cold adaptation	Overfeeding (cafeteria diet)	Fasting	Obesity	Lactation
Tissue wt	↑	↑*	↓	↑	↑ ↓
Protein content	↑	↑	↓	↓	↓
Mitochondrial mass	↑	↑	↓	↓	↓
GDP binding (proton conductance)	↑	↑	↓	↓	↓
Uncoupling protein concentration	↑	↑	↓	↓	↓
Total uncoupling protein content	↑	↑	↓	↓	↓
Sympathetic activity	↑	↑	↓	↓	↓

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(Young *et al.* 1982: Knehans & Romsos, 1983), lactation (Trayhurn & Wusteman, 1987) and in obesity (see Trayhurn, 1986; York, 1987). In the golden hamster (*Mesocricetus auratus*), however, changes in the thermogenic capacity of brown adipose tissue occur without any major alteration in the activity of the sympathetic system (Hamilton *et al.* 1986; McElroy & Wade, 1986).

Species distribution of brown adipose tissue

The studies elaborating a role for brown adipose tissue in energy balance have inevitably focused on laboratory rodents, principally rats and mice. This has led to the concern that there may be limited relevance in such studies to the energetics of other, particularly larger, mammals. Even if this were so, studies on brown adipose tissue would continue to be of value as a reflection of a 'model' thermogenic system. In addition, the tissue is prominent in the thermoregulation of the newborn of a number of mammalian species, although it is widely considered to atrophy after the first few weeks of life. This assumption is, however, based largely on the gross appearance of the tissue rather than any systematic investigation of function.

As discussed earlier, anatomical and histological appearance is an inadequate basis for determining whether or not an adipose tissue is functionally brown. Nevertheless, such studies have suggested that brown adipose tissue is widely distributed in mammals, from rodents through to primates (Smith & Horwitz, 1969; Rothwell & Stock, 1985; Nechad, 1986). GDP-binding studies to isolated mitochondria suggest that the tissue is also present in marsupials (Loudon *et al.* 1985). Histological examination of chickadees (*Parus atricapillus*) and ruffed grouse (*Bonasa umbellus*) suggest that brown adipose tissue may even be present in birds (Oliphant, 1983). However, using the more rigorous and conservative criterion of the identification of uncoupling protein, or its mRNA, a smaller number of species have been clearly identified as containing brown adipose tissue. The list includes laboratory rodents, hibernating species, domestic animals (young) and primates (Table 3). Uncoupling protein has been reported in young pigs (Henningfield & Swick, 1987), but we have so far failed to replicate this finding (P. Trayhurn and J. Van Aerde, unpublished results). Importantly, the tissue has now been firmly identified in man, both in the newborn and in adults (Lean *et al.* 1986; this symposium).

It is emphasized that the restricted list of species in which brown adipose tissue has been clearly identified on the basis of the presence of uncoupling protein (Table 3) reflects the limited number of animals that have been examined. There is every reason to

Table 3. *Species distribution of brown adipose tissue*

Laboratory:	
	Mouse, rat, guinea-pig, rabbit
Hibernators:	
	Golden hamster (*Mesocricetus auratus*), European hamster (*Cricetus cricetus*), ground squirrels (*Richardson's, Columbian*) (*Spermophilus richardsonii, S. columbianus*)
Domestic:	
	Dog, sheep, cattle, pig(?)
Primates:	
	Monkey, human

Based on the immunological identification of uncoupling protein (see Ricquier *et al.* 1983; Lean *et al.* 1986; Casteilla *et al.* 1987; Henningfield & Swick, 1987; Milner *et al.* 1989).

suppose that the list will expand considerably as more species are investigated. While the mere presence of brown adipose tissue does not itself indicate that the tissue plays a significant role in energy metabolism, its identification is, of course, a prerequisite for any such consideration.

Coda

In view of the large body of information that has now been accumulated on how brown adipose tissue functions, it is important to identify the areas where considerable progress still needs to be made. The outstanding problems range from the realm of molecular biology to neurophysiology and whole-body energetics. Prominent questions requiring a solution include the following. By what mechanism is the activity of uncoupling protein modulated acutely? What factors regulate the gene coding for uncoupling protein, particularly during early development? How wide is the species distribution of brown adipose tissue? What are the mechanisms by which the tissue is reactivated, particularly in those species where it undergoes a rapid functional atrophy soon after birth; is the answer simply changes in sympathetic activity? What are the neural networks and signals (neuropeptides) involved in the central control of thermogenesis? Which hormones are involved in the regulation of the thermogenic activity and capacity of brown adipose tissue, and do they do so by direct effects on the tissue or through a central modulation of sympathetic activity?

Finally, and critically from the perspective of nutritional energetics, there is the continuing question of the importance of brown adipose tissue to energy balance and its control in species other than laboratory rodents.

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