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Review

Origins and early development of the concept that brown adipose tissue thermogenesis is linked to energy balance and obesity

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ABSTRACT

Brown adipose tissue (BAT) was identified as a thermogenic organ in 1961, and in 1978 shown to be the major site of thermoregulatory non-shivering thermogenesis in rats acclimated to the cold. Investigations in the mid-late 1970s established the uncoupling of oxidative phosphorylation through a proton conductance pathway across the mitochondrial inner membrane as the mechanism for heat production in BAT, this being regulated by UCP1 which was first discovered as a 32,000 M_r cold-inducible protein. These developments came when those concerned with nutritional energetics were proposing that thermogenesis is a significant factor in energy balance and the aetiology of obesity. A link with BAT was first demonstrated in obese *ob/ob* mice, which were shown to have decreased thermogenic activity in the tissue, and in rats exhibiting diet-induced thermogenesis (DIT) during overfeeding on a cafeteria diet where an activation of brown fat was evident. These pioneering observations led to extensive studies on BAT in different animal models of obesity, both genetic (particularly *ob/ob* and *db/db* mice, *fa/fa* rats) and experimentally-induced. In each case, indices of BAT activity and capacity (mitochondrial content, GDP binding, amount of UCP1) indicated that the tissue plays a role in DIT and that obesity is characterised by reduced thermogenesis. Links between BAT and whole-body energetics were also made in physiological situations such as lactation and fasting. Studies in the 1980s also provided clear evidence for the presence of BAT in adult humans, particularly through the detection of UCP1, and its activation in patients with phaeochromocytoma. Interest in BAT in energetics and obesity waned by the 1990s; the current major renewal of interest has undoubtedly been contingent on the pioneering developments that emerged some 40 years ago.

Keywords:

Brown fat; Diet-induced thermogenesis; Energy expenditure; GDP binding; Non-shivering thermogenesis; Uncoupling protein-1

Abbreviations:

BAT, brown adipose tissue; BMI, body mass index; DIT, diet-induced thermogenesis; FDG-PET, fluorodeoxyglucose positron emission tomography; GDP, guanosine diphosphate; NST, non-shivering thermogenesis; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide electrophoresis; UCP1, uncoupling protein-1; VMH, ventromedial hypothalamus.

1. Introduction

Brown adipose tissue, or brown fat, was first formally described in 1551 by the Swiss naturalist Conrad Gessner. It was originally termed the 'hibernating gland' and over the following 400 years multiple different functions were attributed to the tissue – as part of the thymus, as an endocrine gland (active in the formation of blood), as a modified form of fat serving as a reservoir of food substances, and again as an endocrine gland [1]. It was only in 1961 that brown adipose tissue (BAT) was firmly identified as a thermogenic organ – the key site of thermoregulatory non-shivering thermogenesis [2]. A decade later there was considerable interest, particularly centred on Lindberg's group in Stockholm, in the mechanisms by which heat is generated in the tissue. Heat is, of course, a by-product of metabolic processes in general, but in brown fat it is the *required* product.

The search for the primary thermogenic mechanism in BAT resulted in the identification, following the application of Mitchell's chemiosmotic theory by Nicholls and colleagues, of the uncoupling of oxidative phosphorylation through a regulated proton leakage across the inner mitochondrial membrane; this is the central means by which heat is produced in the tissue [3]. The discovery of the proton conductance pathway was accompanied by the observation from Ricquier's group that the amount of a 32,000 Mr protein band on sodium dodecyl sulphate-polyacrylamide electrophoresis (SDS-PAGE) gels was markedly increased in rats acclimated to the cold [4]. This protein band was subsequently identified as the mitochondrial uncoupling protein (UCP) - the key factor in regulating the proton conductance of brown fat mitochondria. With the identification of further UCPs in the late 1990's [5-7], UCP was renamed UCP1.

In addition to the focus on unravelling the molecular mechanisms of heat production in BAT mitochondria, a key question in the mid 1970's was the quantitative contribution of brown fat to the total capacity for non-shivering thermogenesis (NST). At the time, there was continuing interest in skeletal muscle as a site of NST, reflecting in part the large size of this organ [8]. A pivotal development came from blood flow studies showing that brown fat accounts for approximately two-thirds of the capacity for NST in rats acclimated to the cold [9-10]. The technique used in these studies, which at the time had only recently been introduced, measured the distribution of the cardiac output by injecting radioactively-labelled microspheres of a defined size (~15 μM diameter) which lodge in the microcirculation thereby becoming entrapped in tissues. This followed a report by the same authors demonstrating that the previously employed approach to measure blood flow based on the fractional distribution of ^{86}Rb gave erroneous results, seriously underestimating in particular the proportion of the cardiac output channelled to BAT [11].

The demonstration that BAT is the main site of NST in rats acclimated to the cold had immediate impact not only on thermal physiology, but also on a quite different area – nutritional energetics and the aetiology of obesity. The origin of this link to energetics and obesity, and its subsequent initial development in the early-mid 1980s are described in the present article.

2. Energetics of obesity

The fundamental law of the energetics of obesity is that the condition can only develop when energy intake is greater than energy expenditure, i.e. following a period of positive energy balance. Energy intake means, of course, metabolisable intake and not simply the gross energy in the food consumed, there being caloric losses in both faeces and urine. Energy expenditure is the totality of several different components – customarily divided into basal metabolic rate, physical activity and thermogenesis (Fig. 1). The prevailing attitude to obesity in the 1970s was that it is a consequence of 'gluttony and sloth' – over-eating and under-exercising. This essentially puritan perspective was evident despite the concept of 'luxuskonsumption' having been introduced at the beginning of the last century, this proposing essentially that excess dietary energy can be dissipated as heat. Several studies in the 1960s and 70s on rats, pigs and humans provided distinct support for this proposition [12-14].

The concept of dissipating excess energy intake as heat is complicated by the varying terminology that has been used to describe the phenomenon. Apart from 'luxuskonsumption' - which is not widely used – the expression 'specific dynamic action' which refers to an apparent specific stimulatory effect of protein on heat production, and the generic terms 'thermic effect of food', 'post-prandial thermogenesis' and 'diet-induced thermogenesis' have each been employed. Diet-induced thermogenesis (DIT) is now the most commonly used expression, and it is divided into two components – obligatory and facultative. Obligatory DIT reflects the basic energy costs of digesting, processing and metabolising food. Around 5% of the energy contained in dietary fat is expended to digest, absorb and directly deposit that lipid in the white adipose tissue depots, while in the case of dietary carbohydrate approximately 25% of the energy potential is used to process and deposit this macronutrient as lipid.

It is the facultative, or adaptive, component of DIT that is directly implicated in the regulation of energy balance; this is the component that is genuinely energy dissipative. One particular animal model emerged in the late 1970s which stimulated widespread interest in facultative DIT – the so-called 'cafeteria-fed' rat. In this model, rats given a mixed, palatable human-type diet voluntary overfeed, exhibiting substantial hyperphagia. Much of the additional energy intake is dissipated as heat, however, rather than being deposited as lipid, as energy

balance and metabolic rate measurements demonstrated, i.e. there is a marked stimulation of DIT [15-16]. This is illustrated in Fig. 2.

At the same time, energy balance studies on *ob/ob* (Lep^{ob}/Lep^{ob}) obese mice demonstrated that young mutants pair-fed to the *ad libitum* intake of their lean (+/+, *ob*/+) siblings deposit energy at more than twice the rate of the normal mice with a corresponding increase in gross efficiency (kJ gain/kJ energy intake; Fig. 3) [17]. Such an outcome can only result from a reduction in energy expenditure and subsequent studies linked this to decreased expenditure on NST [17]. This was based in part on the cold-sensitivity of *ob/ob* mice and the decreased capacity for NST as assessed from the increase in metabolic rate following the administration of noradrenaline [18]. Similar observations were made on the *db/db* ($Lepr^{db}/Lepr^{db}$) mouse [19].

3. A link between brown adipose tissue, diet-induced thermogenesis and obesity

Several mechanisms were proposed, or were under active consideration, as the source of DIT and of the reduced thermogenesis of obese mice. These included protein turnover [20], the pumping of Na+ pump across the plasma membrane through the Na^+-K^+-ATPase [21], and so-called futile cycles such as that between fructose-6-phosphate and fructose-1,6,-bisphosphate [22-23]. Despite observations which included reduced Na^+-K^+-ATPase activity in several tissues (liver, kidney and skeletal muscle) of *ob/ob* mice [24-25], these mechanisms were considered to have two key disadvantages. First, there was little evidence that they would make more than a minor contribution to thermogenesis, and in the case of protein turnover, could be rapidly switched on and off without wider implications for metabolic regulation. Secondly, the tissue localisation of these putative mechanisms was non-specific with processes such as Na+ transport and protein turnover being essentially universal.

The search for the mechanisms involved in adaptive DIT and the reduced expenditure on thermogenesis in *ob/ob* mice was taking place at the same time as the developments in brown fat physiology described above. The blood flow studies demonstrating the quantitative importance of BAT in NST [9-10] had considerable resonance with those working on nutritional energetics and obesity. Two key observations resulted in brown fat rapidly becoming a central focus for those working in these areas. In the first, measurement of mitochondrial GDP binding, a key index of thermogenic activity (see below), demonstrated that BAT thermogenic activity is reduced in *ob/ob* mice and that there is an attenuated response to cold relative to lean siblings [26]. In the second pivotal observation, increases in the mass, temperature and lipolytic response to noradrenaline of BAT were evident in cafeteria-fed rats and it was proposed that the tissue is directly involved in DIT [15].

3.1 Tools for assessing brown adipose tissue thermogenesis

Before describing in detail the development of the initial proposals that brown fat is implicated in energy balance and obesity, it is appropriate to comment on the measures of BAT thermogenic activity and capacity that were employed at that period. Oxygen utilisation from blood flow measurements and the arterial-venous difference in oxygen tension is the most direct approach to assessing the thermogenic activity of BAT. However, this necessitates highly demanding *in vivo* physiological procedures that are not appropriate as a routine, or general, tool. Consequently, proxies of BAT activity of a biochemical or molecular nature conducted on tissue and its isolated mitochondria were widely employed from the beginning, both in studies relating to thermoregulation and to energetics and obesity [27]; see Table 1.

Tissue weight is commonly assessed, but in the case of obesity it primarily reflects the deposition of additional lipid in a manner that parallels the overall expansion of the white fat depots. While tissue mass is rarely a useful indicator of BAT activity or thermogenic state, the total protein content was frequently taken in early studies to provide a simple, albeit crude, index. More clearly linked to thermogenesis is the mitochondrial content of a BAT depot, the recruitment of mitochondria resulting in the augmentation of oxidative and thermogenic capacity. Mitochondrial content was widely assessed through measurement of tissue cytochrome *c* oxidase activity [27].

The most potent index of thermogenic activity, which was the method of choice in many early studies on BAT thermogenesis in energy metabolism and obesity, is the mitochondrial GDP binding assay. This measures the extent to which freshly isolated BAT mitochondria bind [^{3}H]GDP, and provides an index of the activity of the proton conductance pathway – the greater the level of binding, binding being to UCP1, the greater the proton conductance and the thermogenic activity of the mitochondria. Mitochondrial GDP binding can differ between treatments, such as cold and warm-acclimation, by up to 10-fold; thus, the assay provides a high level of discrimination [27]. Direct measurements of proton conductance or GDP-sensitive respiration in BAT mitochondria were conducted in a small number of studies, but GDP binding was customarily the technique of choice.

A further key tool is, and was, the measurement of the amount of UCP1 per unit of mitochondrial protein. From the total mitochondrial content, the amount of UCP1 per BAT depot can be obtained and this defines the thermogenic capacity of the tissue. Radioimmunoassays and ELISAs with high specificity and good species cross-reactivity were developed in the early part of the 1980s and were extensively exploited thereafter [28-30]. The

cloning of the UCP1 gene in the mid 1980s provided the ability to measure mRNA levels, enabling the factors that regulate the expression of the gene to be investigated [31-32]. One of the first observations was that cold exposure induces a rapid stimulation of UCP1 gene expression [33].

3.2 BAT and diet-induced thermogenesis

Following the initial proposition - based on tissue mass, temperature, and lipolytic sensitivity - that BAT is involved in DIT, in a follow-up study molecular indices of thermogenesis were assessed in cafeteria-fed and normal rats. The BAT of animals exhibiting facultative DIT had increased total protein, mitochondrial content and oxidative capacity (cytochrome *c* oxidase and α-glycerophosphate dehydrogenase activities); critically, they also had increased mitochondrial GDP binding and exhibited an increase in GDP-sensitive respiration [34]. Later studies, employing either an ELISA for UCP1 or densitometric analysis of SDS-PAGE gels, demonstrated a marked recruitment of UCP1 in BAT of cafeteria-fed rats [35-36]. The levels of the protein increased both 'per mg of mitochondrial protein' and 'per depot', confirming that the capacity for BAT thermogenesis is increased in DIT. UCP1 gene expression, from measurements of the mRNA level, was also soon shown to be elevated in cafeteria-fed rats [36].

Collectively, these changes indicate extensive activation of BAT and substantial increases in thermogenic capacity in animals exhibiting high levels of DIT; such changes parallel those that take place following adaptation, or acclimation, to the cold [37]. Further early studies documented additional changes in the metabolic activity of BAT in cafeteria-diet rats, including reduced lipogenesis which is likely to reflect the high fat content that characterises such diets [38]. It should be noted that in practise not all studies in the 1980's supported a role for BAT in DIT [39]; however, in the past decade reports involving the genetic ablation of UCP1 have provided unequivocal evidence for a central role for the tissue in this form of thermogenesis [40].

3.3 BAT in obese animals

The initial observations of a reduction in the thermogenic activity of BAT in *ob*/*ob* mice were followed by further studies on these leptin-deficient obese mutants as well as on several other animal models of obesity. A blood flow study employing radioactively labelled microspheres to map regional blood flow in young (5 week-old) mice found that BAT was a major site of noradrenaline-stimulated NST in normal, lean mice consistent with the observations on cold-acclimated rats [41]. Importantly, it also demonstrated that the reduced thermogenic capacity of

ob/ob mice in response to noradrenaline was almost entirely due to decreased heat production by BAT. Blood flow studies on Zucker *fa/fa* ($Lepr^{fa}/Lepr^{fa}$) rats resulted in a similar conclusion, the effect being attenuated by adrenalectomy [42].

Among the reported changes in BAT of mature *ob/ob* mice was a reduced concentration of UCP1 in the mitochondria relative to lean siblings [43]. However, the UCP1 concentration was found to be normal in *ob/ob* mice during the suckling period and shortly after weaning, indicating that the reduced thermogenic capacity of the older mutant animals is not an intrinsic defect [43]. Since GDP binding is reduced even in suckling *ob/ob* mice, activity is nevertheless reduced. Again, similar results were evident in *fa/fa* rats [43] with a decrease in GDP binding being evident from the early days (day 2) of postnatal life [44]. Reduced GDP binding under basal conditions and a lack of activation by diet, but a normal activation by cold, was additionally reported for *fa/fa* rats [45]. Decreased GDP binding was also shown in both adult and suckling *db/db* mice which, like the *fa/fa* rat, are characterised by a mutation in the leptin receptor [46]. Further studies on obese mutants demonstrated lower sympathetic activity in BAT, based on noradrenaline turnover studies, when the animals are housed under normal environmental conditions, but both acute cold-exposure and cold-acclimation induce a similar activation in obese mice as in their lean siblings [47-48].

In addition to extensive studies on the single gene obese mutants, the properties of BAT were widely explored in several non-genetic models of obesity. The attractions of the obese mutants are, of course, the combination of the extreme obesity together with its early onset. In general, the obesity exhibited by non-genetic models is less dramatic, and unlike the main obese mutants does not relate specifically to abnormalities in the leptin system – whether of the hormone itself (Lep^{ob}/Lep^{ob}) or of the receptor ($Lepr^{db}/Lepr^{db}$, $Lepr^{fa}/Lepr^{fa}$). Among the experimentally-derived obesities that were initially investigated are those in which obesity is induced by surgical lesioning of the ventromedial hypothalamus (VMH) – at the time a classical model of obesity – or by the administration gold thioglucose (which also results in a lesion of the VMH). In both these models, increased BAT mass, reflecting the deposition of excess lipid, and reduced GDP binding were evident, together with a normal response to cold in terms of the acute activation of thermogenesis [49-50]. Decreased mitochondrial content was observed in the VMH-lesioned rats [51], though not in gold thioglucose-induced obese mice [52].

A role for glucocorticoids in the impaired thermogenic activity of obesity was a common focus in several early studies and this followed particularly from the notable effects of adrenalectomy on energy balance in both *ob/ob* mice and *fa/fa* rats; adrenalectomy leads to the attenuation of hyperphagia and obesity itself, as well as of insulin resistance and other metabolic

abnormalities [53-54]. The effect of adrenalectomy on BAT in the obese mutants is to normalise the reduced GDP binding and the mitochondrial content, responses that are rapid and which are reversed by the administration of corticosterone [55-56]. It was noted that adrenalectomy had no effect on lean animals [56]. Two other key observations were part of the early reports; adrenalectomy was shown to lead to a normalisation of sympathetic activity in BAT of *fa/fat* rats [57], and both the response to overfeeding and the immediate thermic effect of a single meal were restored in brown fat of the obese animals [58].

A second approach to examining the role of glucocorticoids came from studies in which corticosterone was directly administered to lean mice. Administration of the hormone leads to a moderate obesity, with a reduction in mitochondrial content in BAT and a fall in GDP binding [59]. This inhibition of BAT activity by corticosterone was consistent with the studies on adrenalectomy in obese rodents.

3.4 Further models of altered energetics

In addition to investigations on overt obese models, one of the alternative strands in early studies on the role of BAT in nutritional energetics came from the exploration of changes in the tissue in physiological situations where there are substantial alterations in energy flux and/or energy balance. These included pregnancy and lactation, photoperiod, seasonally-induced changes in body fat, and marked alterations in the quantity or composition of the diet (Fig. 4). In each case, changes in BAT consistent with the concept that the tissue is an important component in energy balance and its regulation were observed (see [60-61]). Two specific examples will be summarised here which were of particular interest to the present author: lactation and fasting.

Lactation in small animals is characterised by a very substantial increase in food intake in order to fuel the high energy costs of milk production, intake in mice being at least twice that of the pre-pregnant state [62]. The amount of lipid stored in rodents in pregnancy is small and its subsequent mobilisation during lactation makes only a very limited contribution to the energy costs of milk production – in contrast to larger mammals. Lactation in mice was shown to lead to a major atrophy of BAT, the total protein and mitochondrial contents being markedly decreased, as is GDP binding and GDP-sensitive respiration [63]. Later studies demonstrated that there is a marked fall in UCP1 concentration in lactation [64] and the decrease in thermogenic activity and capacity in BAT reflects a fall in the sympathetic drive to the tissue [65]. The functional atrophy of BAT in lactating rodents begins in late pregnancy, peaks in mid-late lactation, and is reversed following weaning [63-64].

The scale of the atrophy of BAT in lactation is such that the thermogenic activity and capacity of the tissue is similar to that in mice acclimated to thermoneutrality [62]. The energetic implications of the near total suppression of BAT thermogenesis during lactation are considerable; it has been estimated that at an environmental temperature of 21°C some 40% of the maintenance energy expenditure of non-pregnant animals is 'saved' in lactating mice, this reflecting the energy costs of thermoregulatory NST. In effect, the additional heat generated by the obligatory DIT associated with the increased food intake in lactation, together with that consequent to the synthesis of milk, negates the requirement for NST through BAT; heat from rapid foetal growth would have a similar effect in late pregnancy [66]. It is also noteworthy that the hyperphagia of lactating rodents does not lead to the stimulation of facultative DIT in BAT.

Fasting represents the most extreme of nutritional manipulations and several early studies examined the effects of total food deprivation in rats and mice. Although some differences were observed which are likely to be due to variations in the length of the period of food deprivation, the caging conditions (number of animals per cage) and whether the animals were able to undergo coprophagy, a reduction in mass (due to loss of lipid) and GDP binding was widely observed which is reversible on re-feeding [67-71]. Fasting was also found to lead to a reduction in mitochondrial content and in UCP1 (per unit of mitochondrial protein and per depot), and thus of thermogenic capacity – effects that were again reversed on re-feeding [70-71].

Despite these observations, with prolonged starvation thermoregulatory needs begin to predominant. While fasting rats for 24-48 h was shown to lead to a fall in UCP1 mRNA level, which was reversed on refeeding, longer fasting resulted in increases in UCP1 mRNA [72]. This rise in UCP1 mRNA on prolonged fasting was not observed, however, if the rats were at thermoneutrality (28°C) rather than room temperature (23°C), suggesting that in starved animals the drive to maintain body temperature through NST ultimately counteracts the short-term loss of BAT resulting from food-deprivation [72].

Overall, the changes in BAT in the specific situations illustrated, as well as in a number of other physiological conditions, were consistent with the core concept of a central role of the tissue in nutritional energetics and in the aetiology of obesity in experimental animals (Fig. 4). In the early 1990s the link between decreased BAT thermogenesis and obesity was further established through genetic ablation studies in which the knockdown of UCP1 in transgenuc mice was shown to result in obesity [40, 73].

4. Early evidence for active BAT in humans

A critical question was - and in part still is - the extent to which the animal data on BAT is applicable to human energy metabolism, and to obesity in man in particular. This has encompassed two distinct issues: (i) whether facultative thermogenesis is more than a very minor component of energy expenditure in adults, and (ii) whether active BAT is present in humans beyond the early years of life. The first question has been a matter of continuing debate, indeed controversy, as has the extent to which reduced facultative thermogenesis may play a role in the aetiology of obesity. The energy expenditure of mice at room temperature (21°C) is up to twice that thermoneutrality (32°C), while at 4°C it is some 3 times higher, these differences reflecting the energy costs of thermoregulation (primarily NST) [18]. There is no doubt that the energy expenditure of adult humans on thermogenesis is proportionately considerably less, even on exposure to low environmental temperatures.

Studies by Hull and colleagues, in particular, in the 1960s identified plentiful amounts of BAT in newborn infants, the earliest unequivocal description of BAT in humans being at the beginning of the last century [74-76]. The general consensus at the time was that BAT disappears from humans over the first few years of postnatal life, though there was some evidence for the persistence of the tissue in adults [77] and that when present it can be activated by stimuli such as cold. However, these investigations were anatomical and histological in nature, largely centring on the visualisation of multilocular fat cells. Given that they were made prior to the discovery of UCP1, the identification of BAT was not based on the presence of the critical diagnostic feature of a thermogenic adipocyte – whether brown or brite (beige).

UCP1 was soon identified and isolated from human adipose tissue and antibodies raised against it to provide a tool for the subsequent exploration of the extent to which BAT is present in adult humans [78-79]. A radioimmunoassay based on these antibodies demonstrated that immunoreactive UCP1 is present in specific adipose tissue depots (perirenal and axillary) of many adults, albeit at lower amounts than in children [80]. Patients with phaeochromocytoma, in which there are high circulating levels of noradrenaline, have much higher UCP1 concentrations in perirenal adipose tissue as well as a high mitochondrial content and GDP-sensitive respiration [81]. These observations followed an earlier study on phaeochromocytoma patients in which adipose tissue from around the adrenals and the kidneys was found to be rich in mitochondria with a well-developed cristae structure and which exhibited GDP-sensitive respiration [82]. The human UCP1 gene was subsequently cloned and found to be expressed in perirenal adipose tissue of phaeochromocytoma patients [83].

Despite this clear evidence for BAT in adult humans and its capacity for activation following an appropriate stimulus, there was little recognition that the tissue is present and a potential component of energy expenditure in adults. As a consequence, a decade after a link between brown fat and obesity was unequivocally established in rodents, interest in the idea that impaired BAT thermogenesis plays a role in the aetiology of human obesity faded sharply. Indeed, interest in brown fat at all levels waned during the 1990s.

5. The past decade: 2007 to 2016

In the late 2000s there was a major renaissance of interest in brown fat. This was partly because of two key discoveries relating to the fundamental biology of the tissue – that brown adipocytes, in contrast to white fat cells, are derived from myogenic precursors in skeletal muscle [84-85], and that there is a third type of adipocyte, the brite (or beige) fat cell, which expresses UCP1 together with other, though not all, of the molecular markers of brown adipocytes [86-87]. However, the critical factor underlying the renewed focus on BAT has come from the application of a procedure - fluorodeoxyglucose positron emission tomography (FDG-PET) - which is employed in cancer investigations to track the metastasis of tumours by localising areas that exhibit a high rate of glucose uptake. FDG-PET studies found high levels of glucose uptake in fat tissue sites which had a distribution pattern similar to the presumptive pattern of BAT in adult humans based on earlier anatomical observations [77].

Firm evidence that the fat tissue identified as having high glucose uptake is indeed BAT was presented in 2009; the tissue exhibited clear immunostaining for UCP1 [88-89]. These studies thus confirmed what the pioneering work in the 1980s had indicated – that BAT is clearly present and functional in adult humans. The continuing application of FDG-PET has demonstrated that not only is BAT in adults stimulated by cold and by insulin [89-92], but that it is less active in older subjects - and importantly, the activity is lower in obese than in lean individuals, being inversely proportional to BMI (body mass index) [88, 90, 93-94]. Thus, some forty years after the initial proposal that BAT thermogenesis is impaired in the obese, the tissue has again become a focus of research into the causes of obesity. There is correspondingly a renewed interest in the activation and/or recruitment of BAT as a therapeutic route for the treatment of obesity [95-97].

Important metabolic roles for BAT have also been recently suggested, specifically in triglyceride clearance, insulin sensitivity, and in glucose homeostasis where the tissue has been proposed as a major organ in glucose disposal [98-100]. As a consequence, reduced activity in BAT has now been linked to the development of the metabolic syndrome [99, 101]. An

important role for brown fat in glucose removal was in practise first suggested in the early 1980s - on the basis of the high activity of key glycolytic enzymes [102], together with high rates of 2-deoxyglucose uptake in the tissue, uptake being stimulated by both insulin and noradrenaline [103]. It is noteworthy that in the late 1970s very high rates of lipogenesis were also documented in rodent BAT, particularly following cold-acclimation where the tissue is the major site of fatty acid synthesis in whole-body terms [104-106].

The importance of insulin sensitivity in BAT thermogenesis was also noted at that time, with the development of insulin resistance in the tissue in *ob/ob* mice being associated with a loss in the ability to stimulate thermogenic activity on exposure to cold [107]. The reversal of insulin resistance through administration of ciglitazone, the prototype thiazolidinedione, was further shown to restore the normal cold-induced increase in GDP binding in the obese mutants [108]

6. **Conclusions**

The studies that were initiated on BAT thermogenesis towards the end of the 1970s resulted in a paradigm shift in the understanding of nutritional energetics and the development of obesity. They also resulted in a substantial shift in our comprehension of the physiological functions of brown fat itself. Although the involvement of BAT in energy balance and obesity was incidental to the exploration of the fundamental cellular and molecular mechanisms by which heat is generated in brown adipocytes, considerable attention was drawn to the tissue because of this link and interest in the more basic aspects of how these particular adipocytes function was heightened. Certainly, the later discovery of a family of UCPs based on UCP1 would in all probability have taken rather longer.

The demonstration in the 1980s that UCP1 is present in adipose tissue depots of adult humans and that human BAT can be activated, at least in the present of the hypersecretion of noradrenaline in phaeochromocytoma, was an important backdrop to the studies on human BAT that emerged in the late 2000s following the application of the technique of FDG-PET. In the case of human BAT, there is still uncertainty as to whether the tissue can make more than a minor contribution to overall energy expenditure in adults. There is, nonetheless, renewed interest in BAT as a therapeutic target for the treatment of obesity – whether by activating existing brown adipocytes, recruiting new brown fat cells, or by the 'beiging' of specific adipose tissue depots [97]. Some of the proposed routes by which these options might be achieved are extremely challenging, and are in all probablility unrealistic.

Adult humans are not the only species where there has been uncertainty as to the quantitative importance of BAT to energy expenditure. Despite the early demonstration that

BAT is activated in rats exhibiting DIT, the extent to which the tissue accounts for the expenditure associated with this facultative process even in rodents is debated. Indeed, it has been argued that this is not a function of the tissue [109]; nevertheless, genetic ablation studies would seem to provide unequivocal evidence that BAT is central to DIT in rodents [40].

7. Coda – A Personal Note

Between 29 July and 3 August 1979, the Fourth International Symposium on the Pharmacology of Thermoregulation was held at St Catherine's College, Oxford. One of the organisers, Dr Eduard Schönbaum, had become aware of the emerging interest in brown fat as a factor in the regulation of energy balance and the development of obesity. He then suggested that some of us involved should attend the conference and present our work. When the meeting finished on the Friday afternoon, four of us – Jean Himms-Hagen, Nancy Rothwell, Michael (Mike) Stock and I - adjourned to the 'Turf Tavern', an iconic Oxford pub, for beer and talk. I remember vividly the intensity and excitement of that afternoon as we shared thoughts and speculations on the possible relationship between brown fat and obesity. For me, there has been nothing quite like that afternoon throughout my scientific career, neither before nor since.

One of the immediate outcomes of our discussions was that Mike Stock, Nancy Rothwell and I agreed to collaborate on examining whether the mitochondrial proton conductance pathway was activated in rats exhibiting DIT when fed a cafeteria diet. Their iconic paper proposing a role for brown fat in DIT was to appear in *Nature* just a month later (6 September 1979) [15], and my PhD student Anne Goodbody and I had recently set-up at the MRC Dunn Nutrition Laboratory in Cambridge the GDP binding assay to assess thermogenic activity. Mike and Nancy subsequently transported rats in the boot of a car (something that would not now be permitted) from London to Cambridge where we undertook the GDP binding measurements and showed that DIT was indeed associated with an activation of the proton conductance pathway in BAT mitochondria. On 20 February 1980 we submitted the findings as a '*Letter to Nature*', and this was published just 4 months later on 17 July [34].

This collaboration featured in the BBC Horizon science documentary on brown fat - 'The Fat in the Fire' - which was broadcast in the UK on 10 December 1979.

Conflicts of interest

The author declares that he has no conflicts of interest.

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References

[1] B.A. Afzelius, Brown adipose tissue: Its gross anatomy, histology , and cytology, In Brown Adipose Tissue Edited by O Lindberg; American Elsevier, New York (1970) 1-31.

[2] R.E. Smith, B.A. Horwitz, Brown fat and thermogenesis, Physiol. Rev. 49 (1969) 330-425.

[3] D.G. Nicholls, R.M. Locke, Thermogenic mechanisms in brown fat, Physiol. Rev. 64 (1984) 1-64.

[4] D. Ricquier, J.C. Kader, Mitochondrial protein alterations in active brown fat: A sodium dodecyl sulfate-polyacrylamide gel electrophoretic study, Biochem. Biophys. Res. Commun. 73 (1976) 577-583.

[5] C. Fleury, M. Neverova, S. Collins, S. Raimbault, O. Champigny, C. Levi Meyrueis, F. Bouillaud, M.F. Seldin, R.S. Surwit, D. Ricquier, C.H. Warden, Uncoupling protein-2: A novel gene linked to obesity and hyperinsulinemia, Nature Genet. 15 (1997) 269-272.

[6] O. Boss, S. Samec, A. Paoloni-Giacobino, C. Rossier, A. Dulloo, J. Séydoux, P. Muzzin, J.P. Giacobino, Uncoupling protein-3: A new member of the mitochondrial carrier family with tissue-specific expression, FEBS Lett. 408 (1997) 39-42.

[7] D. Ricquier, F. Bouillaud, The mitochondrial uncoupling proteins, M S-Medecine Sciences 14 (1998) 889-897.

[8] L. Jansky, Non-shivering thermogenesis and its thermoregulatory significancce, Biol. Rev. 48 (1973) 85-132.

[9] D.O. Foster, M.L. Frydman, Nonshivering thermogenesis in the rat. II. Measurements of blood flow with microspheres point to brown adipose tissue as the dominant site of the calorigenesis induced by noradrenaline, Can J Physiol Pharmacol 56 (1978) 110-122.

[10] D.O. Foster, M.L. Frydman, Tissue distribution of cold-induced thermogenesis in conscious warm- or cold-acclimated rats re-evaluated from changes in tissue blood flow: The dominant role of brown adipose tissue in the replacement of shivering by non-shivering thermogenesis, Can. J. Physiol. Pharmacol. 57 (1979) 257-270.

[11] D.O. Foster, M.L. Frydman, Comparison of microspheres and $^{86}Rb+$ as tracers of the distribution of cardiac output in rats indicates invalidity of $^{86}Rb+$-based measurements, Can. J. Physiol. Pharmacol. 56 (1977) 97-109.

[12] D.S. Miller, P. Mumford, Gluttony (1): An experimental study of overeating low- or high-protein diets, Am. J. Clin. Nutr. 20 (1967) 1212-1222.

[13] D.S. Miller, P. Mumford, M.J. Stock, Gluttony. 2. Thermogenesis in overeating man, Am. J. Clin. Nutr. 20 (1967) 1223-1229.

[14] A. Djazayery, D.S. Miller, M.J. Stock, Energy balances in obese mice, Nutr. Metab. 23 (1979) 357-367.

[15] N.J. Rothwell, M.J. Stock, A role for brown adipose tissue in diet-induced thermogenesis, Nature 281 (1979) 31-35.

[16] N.J. Rothwell, M.J. Stock, Regulation of energy balance, Ann. Rev. Nutr 1 (1981) 235-256.

[17] P.L. Thurlby, P. Trayhurn, The role of thermoregulatory thermogenesis in the development of obesity in genetically obese (*ob/ob*) mice pair-fed with lean siblings, Br. J. Nutr. 42 (1979) 377-385.

[18] P. Trayhurn, W.P.T. James, Thermoregulation and non-shivering thermogenesis in the genetically obese (*ob/ob*) mouse, Pflügers Archiv Eur. J. Physiol. 373 (1978) 189-193.

[19] P. Trayhurn, L. Fuller, The development of obesity in genetically diabetic-obese (*db/db*) mice pair-fed with lean siblings. The importance of thermoregulatory thermogenesis, Diabetologia 19 (1980) 148-153.

[20] B.G. Miller, R.F. Grimble, T.G. Taylor, Liver protein metabolism response to cold in genetically obese (*ob/ob*) mice, Nature 266 (1977) 184-186.

[21] G.A. Bray, D.A. York, Y. Yukimara, Activity of (Na^+ + K^+)-ATPase in the liver of animals with experimental obesity, Life Sci. 22 (1978) 1637-1642.

[22] E.A. Newsholme, B. Crabtree, Substrate cycles in metabolic regulation and in heat generation, Biochem. Soc. Symp. 41 (1976) 61-109.

[23] E.A. Newsholme, Substrate cycles: Their metabolic, energetic and thermic consequences in man, Biochem. Soc. Symp. 43 (1978) 183-205.

[24] D.A. York, G.A. Bray, Y. Yukimura, An enzymatic defect in the obese (*ob/ob*) mouse; loss of thyroid-induced sodium - and potassium-dependent adenosinetriphosphatase, Proc. Natl. Acad. Sci. U.S.A. 75 (1978) 477-481.

[25] M.H. Lin, D.R. Romsos, T. Akera, G.A. Leveille, Na^+; K^+-ATPase enzyme units in skeletal muscle and liver of 14-day-old lean and obese (*ob/ob*) mice, Proc. Soc. Exp. Biol. Med. 161 (1979) 235-238.

[26] J. Himms-Hagen, M. Desautels, A mitochondrial defect in brown adipose tissue of the obese (*ob/ob*) mouse: Reduced binding of purine nucleotides and a failure to respond to cold by an increase in binding, Biochem. Biophys. Res. Commun. 83 (1978) 628-634.

[27] P. Trayhurn, R.E. Milner, A commentary on the interpretation of invitro biochemical measures of brown adipose tissue thermogenesis, Can J. Physiol. Pharmacol. 67 (1989) 811-819.

[28] D. Ricquier, J.P. Barlet, J.M. Garel, G.M. Combes, M.P. Dubois, An immunological study of the uncoupling protein of brown adipose tissue mitochondria, Biochem. J. 210 (1983) 859-866.

[29] M.E.J. Lean, W.J. Branch, W.P.T. James, G. Jennings, M. Ashwell, Measurement of rat brown-adipose tissue mitochondrial uncoupling protein by radioimmunoassay. Increased concentration after cold acclimation, Biosci. Rep. 3 (1983) 61-71.

[30] E.S. Hansen, J. Nedergaard, B. Cannon, J. Knudsen, Enzyme-linked immunosorbent assay (ELISA) studies of the interaction between mammalian and avian anti-thermogenin antibodies and brown-adipose tissue mitochondria from different species, Comp. Biochem. Physiol. 79B (1984) 44l-445.

[31] F. Bouillaud, D. Ricquier, J. Thibault, J. Weissenbach, Molecular approach to thermogenesis in brown adipose tissue: cDNA cloning of the mitochondrial uncoupling protein, Proc. Natl. Acad. Sci. USA 82 (1985) 445-448.

[32] S. Klaus, L. Casteilla, F. Bouillaud, D. Ricquier, The uncoupling protein UCP - a membraneous mitochondrial ion carrier exclusively expressed in brown adipose tissue, Int. J. Biochem. 23 (1991) 791-801.

[33] D. Ricquier, G. Mory, F. Bouillaud, J. Thibault, J. Weissenbach, Rapid increase of mitochondrial uncoupling protein and its mrna in stimulated brown adipose tissue, FEBS Lett. 178 (1984) 240-244.

[34] S.L. Brooks, N.J. Rothwell, M.J. Stock, A.E. Goodbody, P. Trayhurn, Increased proton conductance pathway in brown adipose tissue mitochondria of rats exhibiting diet-induced thermogenesis, Nature 286 (1980) 274-276.

[35] J. Nedergaard, A. Raasmaja, B. Cannon, Parallel increases in amount of (^{3}H)GDP binding and thermogenin antigen in brown-adipose-tissue mitochondria of cafeteria-fed rats, Biochem. Biophys. Res. Commun. 122 (1984) 1328-1336.

[36] R. Falcou, F. Bouillaud, G. Mory, M. Apfelbaum, D. Ricquier, Increase of uncoupling protein and its mRNA in brown adipose tissue of rats fed on 'cafeteria' diet, Biochem. J. 231 (1985) 241-244.

[37] P. Trayhurn, M. Ashwell, G. Jennings, D. Richard, D.M. Stirling, Effect of warm or cold exposure on GDP binding and uncoupling protein in rat brown fat, Am. J. Physiol. Endocrinol. Metab. 252 (1987) E237-E243.

[38] N.J. Rothwell, M.J. Stock, P. Trayhurn, Reduced lipogenesis in cafeteria-fed rats exhibiting diet-induced thermogenesis, Biosci. Rep. 3 (1983) 217-224.

[39] S. Ma, D.O. Foster, B.E. Nadeau, J. Triandafillou, Absence of increased oxygen consumption in brown adipose tissue of rats exhibiting "Cafeteria" diet-induced thermogenesis, Can J. Physiol. Pharmacol 66 (1988) 1347-1354.

[40] H.M. Feldmann, V. Golozoubova, B. Cannon, J. Nedergaard, UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality, Cell Metab. 9 (2009) 203-209.

[41] P.L. Thurlby, P. Trayhurn, Regional blood flow in genetically obese (*ob/ob*) mice: The importance of brown adipose tissue to the reduced energy expenditure on non-shivering thermogenesis, Pflügers Archiv Eur. J. Physiol. 385 (1980) 193-201.

[42] S.J. Wickler, B.A. Horwitz, J.S. Stern, Blood flow to brown fat in lean and obese adrenalectomized Zucker rats, Am. J. Physiol. Reg. Integr. Comp. Physiol. 25l (1986) R85l-R858.

[43] M. Ashwell, S. Holt, G. Jennings, D.M. Stirling, P. Trayhurn, D.A. York, Measurement by radioimmunoassay of the mitochondrial uncoupling protein from brown adipose tissue of obese (*ob/ob*) mice and Zucker (*fa/fa*) rats at different ages, FEBS Lett. 179 (1985) 233-237.

[44] R. Bazin, D. Eteve, M. Lavau, Evidence for decreased gdp binding to brown-adipose-tissue mitochondria of obese zucker (*fa/fa*) rats in the very first days of life, Biochem. J. 221 (1984) 241-245.

[45] J. Triandafillou, J. Himms-Hagen, Brown adipose tissue in genetically obese (fa/fa) rats: Response to cold and diet, Am. J. Physiol. Endocrinol. Metab. 244 (1983) E145-E150.

[46] A.E. Goodbody, P. Trayhurn, GDP binding to brown-adipose-tissue mitochondria of diabetic-obese (*db/db*) mice. Decreased binding in both the obese and pre-obese states, Biochem. J. 194 (1981) 1019-1022.

[47] A.W. Knehans, D.R. Romsos, Reduced norepinephrine turnover in brown adipose tissue of *ob/ob* mice, Am. J. Physiol. Endocrinol. Metab. 242 (1982) E253-E26l .

[48] G. Zaror-Behrens, J. Himms-Hagen, Cold-stimulated sympathetic activity in brown adipose tissue of obese (*ob/ob*) mice, Am. J. Physiol. Endocrinol. Metab. 244 (1983) E361-E366.

[49] S. Hogan, D.V. Coscina, J. Himms-Hagen, Brown adipose tissue of rats with obesity-inducing ventro-medial hypothalamic lesions, Am. J. Physiol. Endocrinol. Metab. 243 (1982) E338-E344.

[50] J. Seydoux, D. Ricquier, F. Rohner-Jeanrenaud, F. Assimacopoulos-Jeannet, J.P. Giacobino, B. Jeanrenaud, L. Girardier, Decreased guanine nucleotide binding and reduced

equivalent production by brown adipose tissue in hypothalamic obesity: Recovery after cold acclimation, FEBS Lett. 146 (1982) 161-164.

[51] J. Seydoux, F. Rohner-Jeanrenaud, F. Assimacopoulos-Jeannet, B. Jeanrenaud, Functional disconnection of brown adipose tissue in hypothalamic obesity in rats, Pflügers Archiv Eur. J. Physiol. 390 (1981) 1-4.

[52] S. Hogan, J. Himms-Hagen, Brown adipose tissue of mice with gold thioglucose-induced obesity: Effect of cold and diet, Am. J. Physiol. Endocrinol. Metab. 244 (1983) E581-E588.

[53] G.A. Bray, D.A. York, Hypothalamic and genetic obesity in experimental animals: An autonomic and endocrine hypothesis, Physiol. Re.v 59 (1979) 719-809.

[54] G.A. Bray, Integration of energy intake and expenditure in animals and man: The autonomic and adrenal hypothesis, Clinics Endocrinol. Metab. 13 (1984) 521-546.

[55] S. Holt, D.A. York, The effect of adrenalectomy on GDP binding to brown-adipose-tissue mitochondria of obese rats, Biochem. J. 208 (1982) 819-822.

[56] S.J. Holt, D.A. York, Effect of adrenalectomy on brown adipose tissue of obese (*ob*/*ob*) mice, Horm. Metab. Res. 16 (1984) 378-379.

[57] D.A. York, D. Marchington, S.J. Holt, J. Allars, Regulation of sympathetic activity in lean and obese zucker (*fa*/*fa*) rats, Am. J. Physiol. Endocrinol. Metab. 249 (1985) E299-E305.

[58] D. Marchington, N.J. Rothwell, M.J. Stock, D.A. York, Energy balance; diet-induced thermogenesis and brown adipose tissue in lean and obese (f*a*/*fa*) Zucker rats after adrenalectomy, J. Nutr. 113 (1983) 1395-1402.

[59] K.S. Galpin, R.G. Henderson, W.P.T. James, P. Trayhurn, GDP binding to brown-adipose-tissue mitochondria of mice treated chronically with corticosterone, Biochem. J. 214 (1983) 265-268.

[60] P. Trayhurn, Brown adipose tissue and energy balance, Brown Adipose Tissue (edited by P Trayhurn and DG Nicholls) Edward Arnold, London (1986) 299-388.

[61] J. Himms-Hagen, Brown adipose tissue thermogenesis and obesity, Prog. Lipid Res. 28 (1989) 67-115.

[62] P. Trayhurn, Brown adipose tissue thermogenesis and the energetics of lactation in rodents, Int. J. Obesity 9 Suppl 2 (1985) 81-88.

[63] P. Trayhurn, J.B. Douglas, M.M. McGuckin, Brown adipose tissue thermogenesis is 'suppressed' during lactation in mice, Nature 298 (1982) 59-60.

[64] P. Trayhurn, G. Jennings, Functional atrophy of brown adipose tissue in mice: Effects of lactation and weaning on mitochondrial gdp binding and uncoupling protein, Biochem. J. 248 (1987) 273-276.

[65] P. Trayhurn, M.C. Wusteman, Sympathetic activity in brown adipose tissue in lactating mice, Am. J. Physiol. Endocrinol. Metab. 253 (1987) E515-E520.

[66] B. Cannon, J. Nedergaard, Brown adipose tissue: Function and physiological significance, Physiol. Rev. 84 (2004) 277-359.

[67] M. Hayashi, T. Nagasaka, Suppression of norepinephrine-induced thermogenesis in brown adipose tissue by fasting, Am. J. Physiol. Endcorinol. Metab. 245 (1983) E582-E586.

[68] N.J. Rothwell, M.E. Saville, M.J. Stock, Brown fat activity in fasted and refed rats, Biosci. Rep. 4 (1984) 35l-357.

[69] M. Desautels, Mitochondrial thermogenin content is unchanged during atrophy of bat of fasting mice, Am. J. Physiol. Endcorinol. Metab. 249 (1985) E99-E106.

[70] P. Trayhurn, G. Jennings, Evidence that fasting can induce a selective loss of uncoupling protein from brown adipose tissue mitochondria of mice, Biosci. Rep. 6 (1986) 805-810.

[71] P. Trayhurn, G. Jennings, Nonshivering thermogenesis and the thermogenic capacity of brown fat in fasted and/or refed mice, Am. J. Physiol. Reg. Integr. Comp. Physiol. 254 (1988) R11-R16.

[72] O. Champigny, D. Ricquier, Effects of fasting and refeeding on the level of uncoupling protein mRNA in rat brown adipose tissue - evidence for diet-induced and cold-induced responses, J. Nutr. 120 (1990) 1730-1736.

[73] B.B. Lowell, V. S-Susulic, A. Hamann, J.A. Lawitts, J. Himms-Hagen, B.B. Boyer, L.P. Kozak, J.S. Flier, Development of obesity in transgenic mice after genetic ablation of brown adipose tissue, Nature 366 (1993) 740-742.

[74] W. Aherne, D. Hull, Brown adipose tissue and heat production in the newborn infant, J. Path. Bact. 91 (1966) 223-234.

[75] D. Hull, The structure and function of brown adipose tissue, Br. Med. Bull. 22 (1966) 92-96.

[76] M.E.J. Lean, W.P.T. James, Brown adipose tissue in man, Brown Adipose Tissue (edited by P Trayhurn and DG Nicholls) Edward Arnold, London (1986) 339-365.

[77] J. Heaton, The distribution of brown adipose tissue in the human, J. Anat. ll2 (1972) 35-39.

[78] F. Bouillaud, G.M. Combes, D. Ricquier, Mitochondria of adult human brown adipose tissue contain a 32,000-Mr uncoupling protein, Biosci. Rep. 3 (1983) 775-780.

[79] M.E.J. Lean, W.P.T. James, Uncoupling protein in human brown adipose tissue mitochondria: Isolation and detection by specific antiserum, FEBS Lett. 163 (1983) 235-240.

[80] M.E.J. Lean, W.P.T. James, G. Jennings, P. Trayhurn, Brown adipose tissue uncoupling protein content in human infants, children and adults, Clin. Sci. 7l (1986) 29l-297.

[81] M.E.J. Lean, W.P.T. James, G. Jennings, P. Trayhurn, Brown adipose tissue in patients with phaeochromocytoma, Int. J. Obesity l0 (1986) 2l9-227.

[82] D. Ricquier, M. Néchad, G. Mory, Ultrastructural and biochemical characterization of human brown adipose tissue in pheochromocytoma, J. Clin. Endocrinol. Metab. 54 (1982) 803-807.

[83] F. Bouillaud, F. Villarroya, E. Hentz, S. Raimbault, A.M. Cassard, D. Ricquier, Detection of brown adipose tissue uncoupling protein mrna in adult humans by a genomic probe, Clin. Sci. 75 (1988) 21-27.

[84] J.A. Timmons, K. Wennmalm, O. Larsson, T.B. Walden, T. Lassmann, N. Petrovic, D.L. Hamilton, R.E. Gimeno, C. Wahlestedt, K. Baar, J. Nedergaard, B. Cannon, Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages, Proc. Natl. Acad. Sci. USA 104 (2007) 4401-4406.

[85] P. Seale, B. Bjork, W. Yang, S. Kajimura, S. Chin, S. Kuang, A. Scime, S. Devarakonda, H.M. Conroe, H. Erdjument-Bromage, P. Tempst, M.A. Rudnicki, D.R. Beier, B.M. Spiegelman, PRDM16 controls a brown fat/skeletal muscle switch, Nature 454 (2008) 961-967.

[86] N. Petrovic, T.B. Walden, I.G. Shabalina, J.A. Timmons, B. Cannon, J. Nedergaard, Chronic peroxisome proliferator-activated receptor γ (PPARγ) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes, J. Biol. Chem. 285 (2010) 7153-7164.

[87] J. Wu, P. Boström, Lauren M. Sparks, L. Ye, Jang H. Choi, A.-H. Giang, M. Khandekar, Kirsi A. Virtanen, P. Nuutila, G. Schaart, K. Huang, H. Tu, Wouter D. van Marken Lichtenbelt, J. Hoeks, S. Enerbäck, P. Schrauwen, Bruce M. Spiegelman, Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human, Cell 150 (2012) 366-376.

[88] A.M. Cypess, S. Lehman, G. Williams, I. Tal, D. Rodman, A.B. Goldfine, F.C. Kuo, E.L. Palmer, Y.H. Tseng, A. Doria, G.M. Kolodny, C.R. Kahn, Identification and importance of brown adipose tissue in adult humans, New Engl. J. Med. 360 (2009) 1509-1517.

[89] K.A. Virtanen, M.E. Lidell, J. Orava, M. Heglind, R. Westergren, T. Niemi, M. Taittonen, J. Laine, N.J. Savisto, S. Enerback, P. Nuutila, Functional brown adipose tissue in healthy adults, New Engl. J. Med. 360 (2009) 1518-1525.

[90] W.D. van Marken Lichtenbelt, J.W. Vanhommerig, N.M. Smulders, J.M. Drossaerts, G.J. Kemerink, N.D. Bouvy, P. Schrauwen, G.J. Teule, Cold-activated brown adipose tissue in healthy men, New Engl. J. Med. 360 (2009) 1500-1508.

[91] J. Orava, P. Nuutila, Martin E. Lidell, V. Oikonen, T. Noponen, T. Viljanen, M. Scheinin, M. Taittonen, T. Niemi, S. Enerbäck, Kirsi A. Virtanen, Different metabolic responses of human brown adipose tissue to activation by cold and insulin, Cell Metab. 14 (2011) 272-279.

[92] V. Ouellet, S.M. Labbe, D.P. Blondin, S. Phoenix, B. Guerin, F. Haman, E.E. Turcotte, D. Richard, A.C. Carpentier, Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans, J. Clin. Invest. 122 (2012) 545-552.

[93] C. Pfannenberg, M.K. Werner, S. Ripkens, I. Stef, A. Deckert, M. Schmadl, M. Reimold, H.-U. Häring, C.D. Claussen, N. Stefan, Impact of age on the relationships of brown adipose tissue with sex and adiposity in humans, Diabetes 59 (2010) 1789-1793.

[94] T. Yoneshiro, S. Aita, M. Matsushita, T. Kameya, K. Nakada, Y. Kawai, M. Saito, Brown adipose tissue, whole-body energy expenditure, and thermogenesis in healthy adult men, Obesity 19 (2011) 13-16.

[95] M. Harms, P. Seale, Brown and beige fat: Development, function and therapeutic potential, Nature Med. 19 (2013) 1252-1263.

[96] F. Villarroya, A. Vidal-Puig, Beyond the sympathetic tone: The new brown fat activators, Cell Metab. 17 (2013) 638-643.

[97] P. Trayhurn, J.S. Arch, New physiological aspects of brown adipose tissue, Curr. Obesity Rep. 3 (2014) 414-421.

[98] A. Bartelt, O.T. Bruns, R. Reimer, H. Hohenberg, H. Ittrich, K. Peldschus, M.G. Kaul, U.I. Tromsdorf, H. Weller, C. Waurisch, A. Eychmuller, P.L.S.M. Gordts, F. Rinninger, K. Bruegelmann, B. Freund, P. Nielsen, M. Merkel, J. Heeren, Brown adipose tissue activity controls triglyceride clearance, Nature Med. 17 (2011) 200-205.

[99] A. Bartelt, J. Heeren, The holy grail of metabolic disease: Brown adipose tissue, Curr. Opin. Lipidol. 23 (2012) 190-195.

[100] K.I. Stanford, R.J.W. Middelbeek, K.L. Townsend, D. An, E.B. Nygaard, K.M. Hitchcox, K.R. Markan, K. Nakano, M.F. Hirshman, Y.-H. Tseng, L.J. Goodyear, Brown adipose

tissue regulates glucose homeostasis and insulin sensitivity, J. Clin. Invest. 123 (2013) 215-223.

[101] J. Nedergaard, T. Bengtsson, B. Cannon, New powers of brown fat: Fighting the metabolic syndrome, Cell Metab. 13 (2011) 238-240.

[102] G.J. Cooney, E.A. Newsholme, The maximum capacity of glycolysis in brown adipose tissue and its relationships to control of the blood glucose concentration, FEBS Lett. 148 (1982) 198-200.

[103] G.J. Cooney, I.D. Caterson, E.A. Newsholme, The effect of insulin and noradrenaline on the uptake of 2-[1-14c}deoxyglucose in vivo by brown adipose tissue and other glucose-utilizing tissues of the mouse, FEBS Lett. 188 (1985) 257-261.

[104] J.G. McCormack, R.M. Denton, Evidence that fatty acid synthesis in the inter-scapular brown adipose tissue of cold-adapted rats is increased in vivo by insulin by mechanisms involving paralleled activation of pyruvate dehydrogenase and acetyl-coenzyme a carboxylase, Biochem. J. 166 (1977) 627-630.

[105] P. Trayhurn, Fatty acid synthesis in vivo in brown adipose tissue liver and white adipose tissue of the cold-acclimated rat, FEBS Lett. 104 (1979) 13-16.

[106] P. Trayhurn, Fatty acid synthesis in brown adipose tissue in relation to whole body synthesis in the cold-acclimated golden hamster (mesocricetus auratus), Biochim. Biophys. Acta 620 (1980) 10-17.

[107] S.W. Mercer, P. Trayhurn, The development of insulin resistance in brown adipose tissue may impair the acute cold-induced activation of thermogenesis in genetically obese (*ob*/*ob*) mice, Biosci. Rep. 4 (1984) 933-940.

[108] S.W. Mercer, P. Trayhurn, Effects of ciglitazone on insulin resistance and thermogenic responsiveness to acute cold in brown adipose tissue of genetically obese (*ob*/*ob*) mice, FEBS Lett. 195 (1986) 12-16.

[109] L.P. Kozak, Brown fat and the myth of diet-induced thermogenesis, Cell Metab. 11 (2010) 263-267.

Table 1.

In vitro measures of the thermogenic activity and capacity of brown adipose tissue

Measure	**Significance**	**Utility**
Tissue mass	primarily reflects lipid content	minimal
Tissue total protein	crude index of active tissue mass	limited
Cytochrome *c* oxidase activity	index of tissue mitochondrial content	high
Mitochondrial GDP binding	index of activity of proton conductance pathway	high
UCP1	provides measure of thermogenic capacity	high
GDP-sensitive mitochondrial respiration	index of uncoupled respiration	high
Mitochondrial swelling (GDP sensitive)*	indirect measure of proton permeability	medium
Proton conductance*	direct measure of proton conductance pathway	high
UCP1 mRNA	gene expression	high

These specific measures were employed to varying extents in the decade from 1978. Modified from [27]. *little used.

Legends to Figures

Fig. 1. A schematic representation of energy flux and energy expenditure in mammals, with the buffering role of white adipose tissue. BMR, basal metabolic rate; NEAT, non-exercise activity thermogenesis (which has been considered a component of expenditure.

Fig. 2. Illustration of the stimulation of facultative diet-induced thermogenesis in rats during voluntary overfeeding on a cafeteria diet. The metabolisable energy intake, energy deposited and energy expenditure of the control (Con) animals fed a standard laboratory diet and the cafeteria-fed (Cafe) rats is shown. Data adapted from Rothwell and Stock [15].

Fig. 3. Development of obesity in young *ob/ob* mice pair-fed to the ad libitum food intake of lean *(+/+, ob/+)* siblings. (a) energy gain, (b) gross efficiency (kJ energy gain/kJ energy intake). The difference between lean and *ob/ob* in energy gain on precisely the same intake is due to the reduced energy expenditure of the obese mutants. Data taken from [17].

Fig. 4. Schematic representation of how different physiological and pathological conditions in which energy flux and balance alter are characterised by increased or decreased brown adipose tissue thermogenesis in experimental animals. The diagram summarises the major situations in which BAT thermogenesis changes, which in most cases reflect long-term adaptations.

Highlights:

1. BAT was shown in the late 1970s to be linked to energetics and obesity as well as thermoregulation
2. Studies in the 1980s demonstrated reduced BAT thermogenesis in a range of animal obesities
3. Active BAT was found in adult humans in the 1980s, underpinning the current interest in the tissue

Fig. 1

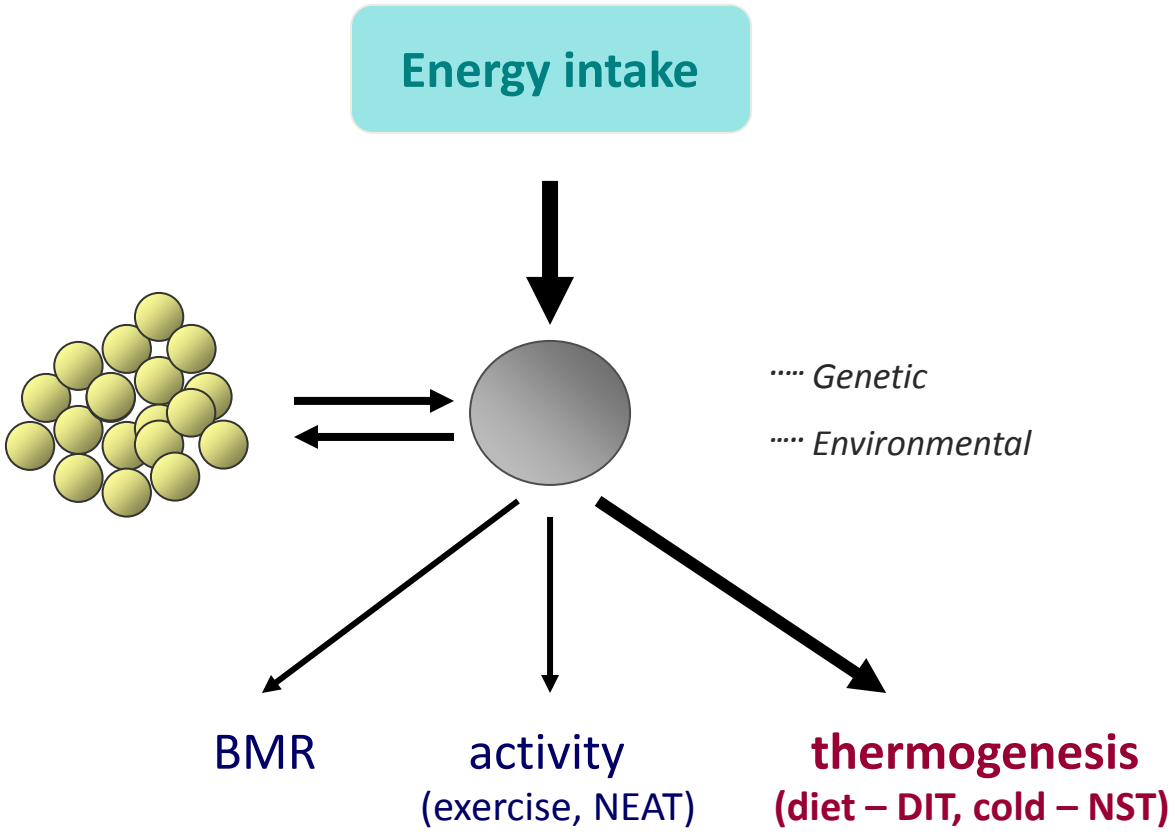
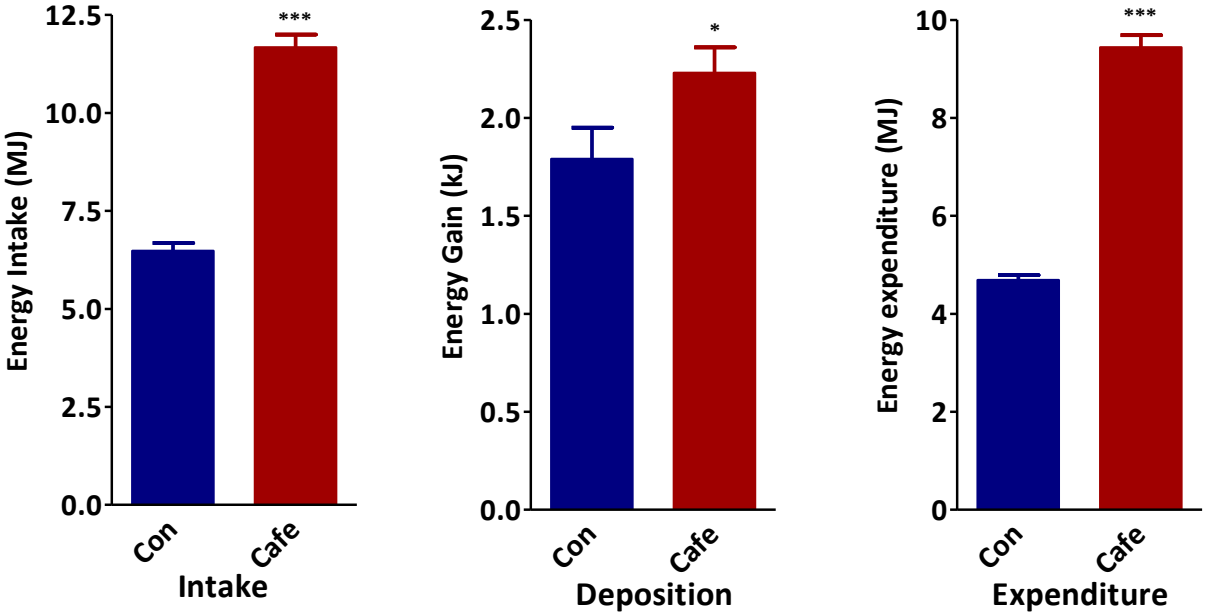
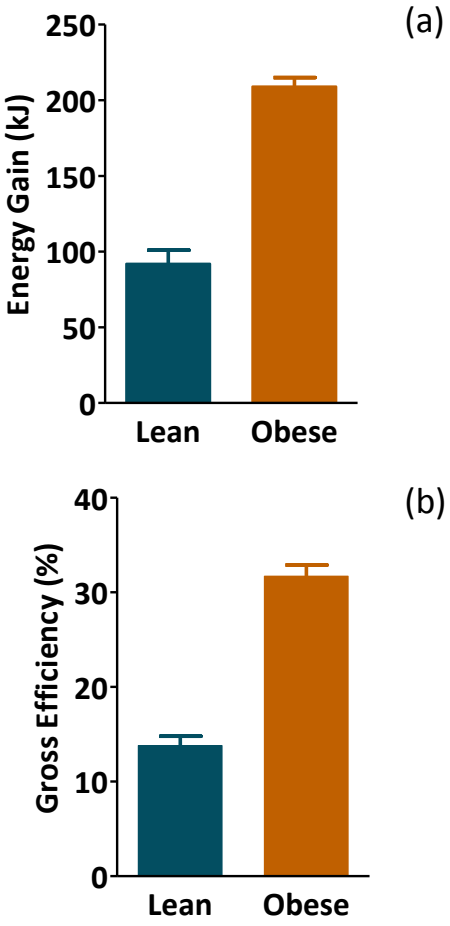

Energy intake
Genetic
Environmental
BMR
activity
(exercise, NEAT)
thermogenesis
(diet – DIT, cold – NST)

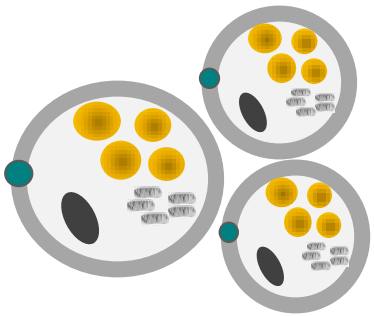
Fig. 2


Energy Intake (MJ)
12.5
10.0
7.5
5.0
2.5
0.0
***
Con
Cafe
Intake
Energy Gain (kJ)
2.5
2.0
1.5
1.0
0.5
0.0
*
Con
Cafe
Deposition
Energy expenditure (MJ)
10
8
6
4
2
0
***
Con
Cafe
Expenditure


(a)
250
200
150
100
50
0
Energy Gain (kJ)
Lean
Obese
(b)
40
30
20
10
0
Gross Efficiency (%)
Lean
Obese

Increased ↑:

Cold
Overfeeding (DIT)
Catecholamines
Arousal from hibernation
Cancer cachexia
Low protein diets



Decreased ↓:

Obesity (*ob/ob, fa/fa,* VMH)
Fasting
Lactation
Diabetes
Hibernation