Implications of nonshivering thermogenesis for energy balance regulation in humans

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van Marken Lichtenbelt WD, Schrauwen P. Implications of nonshivering thermogenesis for energy balance regulation in humans. Am J Physiol Regul Integr Comp Physiol 301: R285–R296, 2011. First published April 13, 2011; doi:10.1152/ajpregu.00652.2010.—The incidence of the metabolic syndrome has reached epidemic levels in the Western world. With respect to the energy balance, most attention has been given to reducing energy (food) intake. Increasing energy expenditure is an important alternative strategy. Facultative thermogenesis, which is the increase in energy expenditure in response to cold or diet, may be an effective way to affect the energy balance. The recent identification of functional brown adipose tissue (BAT) in adult humans promoted a renewed interest in nonshivering thermogenesis (NST). The purpose of this review is to highlight the recent insight in NST, general aspects of its regulation, the major tissues involved, and its metabolic consequences. Sustainable NST in adult humans amounts to 15% of the average daily energy expenditure. Calculations based on the limited available literature show that BAT thermogenesis can amount to 5% of the basal metabolic rate. It is likely that at least a substantial part of NST can be attributed to BAT, but it is possible that other tissues contribute to NST. Several studies on mitochondrial uncoupling indicate that skeletal muscle is another potential contributor to facultative thermogenesis in humans. The general and synergistic role of the sympathetic nervous system and the thyroid axis in relation to NST is discussed. Finally, perspectives on BAT and skeletal muscle NST are given.

brown adipose tissue; mild cold; skeletal muscle

Components of Energy Expenditure

The energy balance consists on the one hand of energy intake as food and on the other hand of energy expenditure as heat and work. In humans, of the energy ingested, about 4–8% is lost during digestion (feces), and another 3–5% leaves the body with urine and via the skin (13). This leaves about 90% of the ingested energy as metabolizable energy. Some of this is used for external work (increase potential energy in the environment) and, depending on the physiological state of the body, for tissue production or repair (growth and reproduction). This means that for a weight-stable individual, by far the

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THE RECENT IDENTIFICATION of functional brown adipose tissue (BAT) in healthy adult humans boosted a revitalized interest in facultative and adaptive thermogenesis. Many editorials and review papers have appeared recently on BAT. Most of these reviews focus on possible pharmacological treatment of obesity. Here, we focus on cold-induced nonshivering thermogenesis (NST) in humans, its metabolic implications, and the role of the major tissues involved, i.e., BAT and skeletal muscle.

Obesity and related disorders are a major health issue worldwide. The treatment of obesity has concentrated on reducing energy (food) intake. Increasing energy expenditure, however, is an important alternative strategy. Most attention in this respect has been given to physical activity. Although exercise, in general, promotes healthier outcomes in relation to the metabolic syndrome (diabetes type II, cardiovascular diseases), the role of exercise training in weight loss is limited (50, 90, 136). A generally overlooked strategy is the use of facultative and adaptive thermogenesis. Facultative thermogenesis occurs in response to cold exposure or dietary intake and is regulated by hypothalamic centers that integrate skin and internal temperatures and visceral cues (75). Small increases in facultative thermogenesis can significantly affect the long-term energy (weight) balance, because its effect can be continuous (day and night). The terms facultative thermogenesis and adaptive thermogenesis are interchanged in many scientific articles. Here, we conform to the original meaning of the terms and use facultative thermogenesis in cases in which heat production is turned on when needed (e.g., in the cold). Adaptive thermogenesis means that the capacity for heat production becomes larger when the organism stays for a prolonged time (days, weeks, months) in the cold. These definitions are in line with Cannon and Nedergaard (17).

Therefore, the purpose of this review is to highlight the recent insights into cold-induced nonshivering thermogenesis and its metabolic consequences.
The greatest portion of metabolizable energy is turned into heat. Heat production plus external work account for the average daily metabolic rate (ADMR). ADMR varies between individuals, being largely dependent on age and body composition. ADMR can be divided into components. Classically, the components consist of basal metabolic rate (BMR; normally 55–65% of ADMR), physical activity energy expenditure (normally 25–35% of ADMR), and diet-induced thermogenesis (DIT, about 10% of ADMR). BMR is measured in the morning in a thermoneutral environment in supine position, in postabsorptive state. On average, BMR amounts to about 80 W for an adult male of 80 kg and 65 W for a female of 70 kg. BMR can be subdivided into sleeping metabolic rate and energy expenditure related to arousal. The frequently used term resting metabolic rate (RMR) refers to BMR under less strict measurement conditions.

An alternative division uses the obligatory energy expenditure, required for basal body functions, and facultative thermogenesis (Fig. 1). Obligatory energy expenditure is relatively fixed and refers to the heat produced for normal functions of cells and organs, including heat generated due to “vital” physical activity that is not aimed at extra heat production. It also includes the obligatory part of DIT, i.e., the energy costs of digestion and metabolism of the ingested nutrients. Obligatory energy expenditure varies greatly between phyla. Endotherms have 5–8 times higher standard obligatory energy expenditure (i.e., BMR corrected for body temperature) than ectotherms of the same size (94), indicating a phylogenetic adaptation necessary for the warm-blooded state. In addition, BMR per gram of body weight varies inversely with body size between species within a phylum (63), indicating a scaling adaptation. Indeed, the metabolic rate of a mouse on a per weight basis is more than 10 times greater than that of an elephant.

The obligatory component is not necessarily fixed, i.e., the BMR can vary depending on the cold acclimatization, e.g., the thyroid status (see Thyroid hormone axis). Moreover, thyroid status, in its turn, facilitates facultative thermogenesis. Thus, the obligatory and facultative thermogeneses are not independent processes.

As stated above, the facultative thermogenesis occurs in response to cold exposure or dietary intake and is regulated by hypothalamic centers. Facultative thermogenesis is highly variable. In humans, facultative part of DIT varies with the composition and amount of food (5–10% of ADMR). Cold-induced thermogenesis consists of shivering [3–5 times RMR (37)], nonexercise activity thermogenesis (NEAT, 5–30% of RMR), and NST (0–30% of RMR) (139). Also, exercise with the purpose of producing heat can be included. Shivering is uncomfortable and cannot be maintained over prolonged periods. High levels of NEAT can also not be sustained over prolonged periods. Moreover, it is shown that NEAT is not necessarily increased upon cold exposure (52). In climate room conditions, physical activity even decreases during mild cold exposure (137, 138). NST can be maintained over prolonged periods (44). The NST occurs in BAT (most information from rodent studies) and possibly in other tissues as well, such as skeletal muscle. From rodent studies, it has been surmised that BAT is likely the only place for NST (38). However, studies in humans indicate that skeletal muscle can be involved as well (80, 140). For more details, see Skeletal muscle below.

The most frequently studied mechanism of nonshivering thermogenesis is mitochondrial uncoupling in BAT, as it accounts for a major portion of thermogenesis upon cold exposure in cold-acclimatized rodents (17). This uncoupling process is executed by uncoupling protein (UCP1), a unique inner-membrane mitochondrial protein for BAT. UCP1 causes a reflux of protons into the mitochondrial matrix, bypassing the ATP synthase. F(0)F(1)-ATPase uses the energy stored in the proton gradient to produce ATP, which is the energy intermediate in the organism. Instead, mitochondrial uncoupling leads to proton leakage, which induces heat dissipation by the uncoupling itself plus the other parts of the phosphorylation process (17, 64, 83). Though described in detail in BAT, a regulated increase in mitochondrial uncoupling may also occur in other tissues, such as skeletal muscle. The protein UCP1 is a member of the mitochondrial carrier protein family. Other members of this family are UCP2 and UCP3.

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Fig. 1. Components of human energy expenditure (not growing and no reproduction).
are 73% identical to each other and even 56% identical to UCP1 (20), but they do not share the same uncoupling abilities. UCP1 is the only protein from this family that is shown to mediate nonshivering thermogenesis (82) in BAT, at least in rodents. UCP2 is usually found in most tissues in rodents and humans at varying levels, and UCP3 is mostly found in skeletal muscle cells and BAT (75), although its role as a regulator for mitochondrial uncoupling for facultative thermogenesis is unclear (106, 140).

Finally, other tissues, such as liver and other processes than mitochondrial uncoupling, such as futile cycling, transmembrane leaking, calcium cycling, may also be involved (139). Especially transmembrane leaking and calcium cycling are, besides mitochondrial uncoupling, very relevant in skeletal muscle (9). However, these mechanisms are out of the scope of the present review.

Cold-Induced Thermogenesis

In a cold environment, humans have two physiological responses: they can increase their energy expenditure to produce extra heat, or change the blood perfusion (heat transport) to the skin and other distally located tissues. Therefore, the insulative properties of the skin, subcutaneous fat layer, and skeletal muscle tissue can be used to protect the core temperature. In most cases, a combination of thermogenesis and insulation is used (105, 126). As mentioned above, cold-induced thermogenesis is subdivided into NST, shivering thermogenesis, and NEAT. For the purposes of this review, we restrict ourselves to NST. Shivering in both animals and humans generally is measured by means of electromyography in both animals and humans. In humans, volunteers are also asked to report shivering. Shivering and nonshivering thermogenesis were also reported to occur simultaneously in both animals and humans [see among others the “classical” studies of Davis et al. (30) and Davis (31)]. NST is described in cold acclimatization experiments in rats (30, 33). During daily cold exposure, shivering gradually decreased toward zero intensity in 20 days, but oxygen consumption remained elevated (30). This indicates the gradual increase of the NST component. This experiment was repeated later in men, indicating a nonshivering component in human adults, although not as large as shown in rodents (31). Human newborns are able to increase their energy expenditure more than twofold. According to Brück (14) and Himms-Hagen (56), shivering does not occur in newborns, and the increase has thus been attributed to nonshivering thermogenesis. However, it is well known that cold induces restlessness and crying in infants and thus a form of NEAT is likely to be involved as well. In adult humans, the cold-induced nonshivering thermogenesis varies from 0 to 14% of resting metabolic rate in studies that last one to several days and up to 30% in short-term (hours) studies (18, 21, 129, 133, 137). The difference between the short-term and longer-term studies can be caused by the fact that in studies lasting more than 24 h, subjects sleep in beds and create their own microclimate. Moreover, longer-term studies take place under less strict conditions, and thus behavioral changes are possible.

Apart from the large individual variation in NST, it is interesting to note that some individuals also may show a reduction in energy expenditure in mild cold. This is probably not a coincidence, since this is described in many experimental studies (18, 21, 129, 133, 137). It can most likely be attributed to the so-called $Q_{10}$ effect in the tissues (92). The $Q_{10}$ temperature coefficient is a measure of the rate of change of biological or chemical processes as a consequence of changing the temperature by 10°C. In biological systems, the $Q_{10}$ generally varies between 2 and 3. Upon cooling, the $Q_{10}$ effect thus causes a temperature-related drop in all chemical reactions and, thus, cell and tissue metabolism. When individuals use insulation to protect the body core temperature, whole body energy expenditure may drop as a result of the cooled (distal) body parts. This can be counteracted by the regulated increase in energy expenditure of specific tissues, resulting in (whole body) facultative thermogenesis. In fact, a small amount of facultative thermogenesis can be obscured by a $Q_{10}$ effect due to locally cooled tissues.

Cold acclimatization and cold-induced thermogenesis. The study by Davis (31) showed that cold acclimatization increased NST in humans. A comparison between 3-h mild exposure between summer and winter seasons in the same subjects showed an adaptive response, i.e., the average NST (at 15°C) was significantly higher in winter (11.5% of RMR) compared with summer (7.0% of RMR) (128). It was concluded that in winter, the subjects showed less heat debt in the course of these 3 h of cooling. Such adaptations have been described earlier, but under more extreme cold conditions, including shivering responses (11). The higher metabolic response in winter compared with summer indicates cold acclimatization. Interestingly, the metabolic response in winter was significantly related to the response in summer. Although the magnitude of the cold response varies, the relative contribution of metabolic and insulative response was individual-specific and consistent throughout the seasons. This indicates that the relative magnitude of the cold response is an individual trait.

In conclusion, adult humans show nonshivering thermogenesis and adaptive thermogenesis, i.e., cold acclimatization increases NST. There are indications that the magnitude of NST is specific to the individual.

Obesity and cold-induced thermogenesis. Can overweight people be characterized by an “overweight”-specific cold response? There are not many studies in overweight subjects on NST. A study showed that the overnight metabolic response upon mild cold exposure was significantly increased in lean, while reduced in obese diabetic women (71). More recently, we showed that NST in response to 1-h mild cold exposure and 1-h rewarming was significantly larger in the lean group (17%) compared with the overweight group (6%) (21). This blunted NST in overweight people was confirmed in a longer-term study in respiration chambers, where the subjects were engaged in daily physical activities (137). Here, lean subjects generally counteracted the cold by increasing energy expenditure, while in the obese subjects, increasing tissue insulation was more prominent (137). This shows that interindividual and group (lean vs. obese) differences exist with respect to the relative contribution of metabolic (energy expenditure) and insulative (decrease in temperature) adaptations to cold. We recently showed that age may also affect NST. Thus, the reduction could partly be attributed to the larger fat content (tissue insulation in elderly), but age itself also significantly contributed to the reduced NST (Kingma BRM, Frijns AJH, Saris WHM, van Steenhoven AA, van Marken Lichtenbelt WD, unpublished data).
Overall, obese subjects tend to show a blunted NST under the same mild cold situation compared with lean subjects. This may imply that nonshivering thermogenesis is impaired in obese subjects, but it may also just indicate that obese subjects need a cooler environment to provoke nonshivering thermogenesis.

Differences in the level of NST between overweight and lean people might be attributable to the insulation properties of body fat (8, 21, 101), surface-to-volume ratio (66), or skin vasoconstrictive reaction to cold (123). These three properties are involved in reducing body heat loss from the skin and may determine to a yet unknown extent a reduction in facultative thermogenesis. From a study in lean subjects, we reported that the individual variation in 24 h thermogenic response to mild cold (nonshivering) was negatively related to changes in (distal) skin body temperatures (126). A reduction in heat loss in obese persons may also be due to differences in cold-induced autonomic responsiveness (77, 78) and to a reduced TSH and thyroid hormone response (71). Whether reduced hormonal or neural responses are a consequence of obesity or a causal factor for the risk of weight gain, is still unsolved.

In conclusion, there exist large and physiologically significant individual (and group-)specific variations in thermogenic responses to mild cold. The range is from 5% decrease to 14% increase of RMR, with short-term increases up to 30% of RMR. The individual variation in nonshivering thermogenesis can, to some extent, be explained by individual differences in thermal tissue insulation.

Link Between Nonshivering Thermogenesis and Diet-Induced Thermogenesis

DIT and NST share some characteristics, such as the regulation by the sympathetic nervous system (SNS), and animal UCP1 knockout-studies show that UCP1 mediates both NST and DIT (35, 38). Thus, in case of DIT, the purpose is that uncoupled respiration can waste food energy and, as such, can serve as a defense mechanism against excess energy intake. However, it is still being disputed whether it is physiologically employed in such a way (39, 65).

Diet-induced thermogenesis can be ascribed to obligatory and facultative components. The obligatory part consists of heat generated by digestion, absorption, and processing. The facultative part consists of the regulated heat production to dissipate food energy. Several factors can potentially influence the facultative part of DIT, including glucose tolerance, insulin sensitivity, body fat (distribution), and SNS activity (16, 124). The SNS activity increases in response to feeding (144), especially to carbohydrate intake (108) or very low or very high protein diets (113). In response to starvation, the activity of the SNS is reduced (144). The SNS response to feeding significantly contributes to DIT (93).

From the above, we can conclude that both NST and DIT are mediated by the sympathetic nervous system. It should be noted, however, that there is a functional difference between NST and DIT. Heat produced by the facultative part of DIT is used to dissipate energy from the body to prevent the body weight to rise, while NST prevents a drop in (core) temperature.

There are hardly any studies in humans that combine cold exposure and dietary interventions. Because of the reported large individual differences in energy response to cold and to overfeeding, we compared this variation in thermogenesis in subjects exposed to both mild cold (3 days) and overfeeding (3 days, 160% of normal energy intake) (138). The individual changes in energy expenditure during mild cold exposure and overfeeding appeared to be significantly correlated. These results suggest that both overfeeding-induced and mild cold-induced thermogenesis are specific to the individual and may share common regulating mechanisms, confirming results from animal studies (38). Indeed, fasting norepinephrine plasma concentrations correlated significantly to energy expenditure in both situations. Although fasting catecholamine levels may not be the best indicator, this result is in line with SNS involvement. Now that we know that BAT is present and active in healthy humans (81, 99, 127, 131), it is likely that this variation in thermogenesis is, at least in part, caused by the presence and/or activity of BAT.

Regulation of Facultative Nonshivering Thermogenesis

Sympathetic nervous system. Low environmental temperatures are detected by the skin. Information from the skin and internal temperatures are integrated by the hypothalamus. Thermosensitive neurons of the preoptic anterior hypothalamus are considered to be the most important for triggering autonomic thermoeffector responses, including the SNS (for more details on thermoregulation, among others, see Ref. 95). Overfeeding studies in rats clearly demonstrate that carbohydrates and fat stimulate SNS activity, while proteins do not. Low-protein diets are markedly stimulatory of SNS activity (68). The SNS innervates thermogenic targets, such as the brown adipose tissue (BAT) and skeletal muscle (75). Several studies have shown that rodents with a blocked SNS or lacking catecholamines cannot maintain body temperature during cold exposure (69, 117). The sympathetic control of facultative thermogenesis is mediated by β-adrenoceptors and not by α-adrenoceptors (12). There are three β-adrenoceptors that could mediate sympathetically driven thermogenesis. Of the three subtypes of rodent β-adrenergic receptors, the β3-adrenoceptor is the most significant in mature brown adipocytes (17). β3-adrenergic receptors are also present in white adipocytes. β1-Adrenoceptors are expressed in mature brown adipocytes, and although related to cAMP production (57), they are not coupled to any significant extent to signaling processes in these cells (17). β2-Adrenoceptors are not expressed in the brown adipocytes themselves, predominantly localized to the vascular system (17). Mice lacking all three β-ARs show complete failure of diet-induced thermogenesis (6).

In humans, both β1- (heart, adipose tissue) and β2- (skeletal muscle, adipose tissue) adrenergic receptors are present and may lead to an increase in energy expenditure when pharmacologically stimulated (102, 103). The β3-adrenoceptors are expressed in human brown and white adipocytes, and recently, it has been shown that they are expressed in many other tissues as well (122), including skeletal muscle (19).

The role of the human sympathetic nervous system in controlling metabolic rate, heart rate, and vasomotion in cold-exposed humans has been demonstrated repeatedly (for review, see Ref. 60). Nonshivering thermogenesis is accompanied by increases in serum norepinephrine (18, 138). Infusion of nor-
adrenaline and adrenaline caused similar metabolic responses as mild cold exposure (24–36% increase in BMR) (73). It should be noted that these injections also likely activate tissues that are not specifically those involved in NST. Propranolol (β1/β2-antagonist, and to a much lesser extent β3-antagonist) administration after glucose infusion induced a decrease in glucose-induced energy expenditure from 2.3 to 1.7 MJ/day (1). With respect to human SNS innervation in brown adipose tissue, not much information is available. Pretreatment of patients with propranolol blocks 18F-2-fluoro-2-deoxyglucose (FDG) uptake in brown adipose tissue (112). These results may indicate the involvement of the SNS in human BAT activation. However, other studies indicate that propranolol is relatively ineffective at blocking the β3-adrenoceptor (141), due to its low affinity for this β-receptor subtype (7).

Thyroid hormone axis. For basal metabolic rate, thyroid hormones (THs) are essential, as with severe hypothyroidism, energy expenditure can drop as much as 50%. NST is stimulated by the SNS but also requires TH (110). Mainly from animal studies, it is known that in stimulating thermogenesis, TH works synergistically with the SNS at all levels. TH is essential to maximize the responsiveness to catecholamines acting at the adrenergic receptor level, as well as at several postreceptor steps in the catecholamine signaling pathways, particularly those initiated in the β-ARs (110). The synergistic interaction is best described in BAT-adaptive thermogenesis. Here, TH and SNS work in concert to increase thermogenesis by means of mitochondrial uncoupling. Cold-induced adrenergic-receptor stimulation has both acute and chronic effects on BAT (75). UCP1 activity increases within seconds of stimulation, while chronic stimulation over hours and days results in increased amounts of UCP1 protein, mitochondrial biogenesis, and both hyperplasia and hypertrophy of brown adipose tissue. Acute stimulation of UCP1 activity is due to increased amounts of cyclic AMP, which activates lipolysis (17). This results in an increase in free fatty acids, which stimulate UCP1.

The adrenergic responsiveness of BAT and many other tissues is dependent on the presence of high tissue concentrations of the most active form of TH, T3 (110). The main product of thyroid secretion, however, is the less active prohormone T4. In the cells, T4 is converted by deiodinase II into T3. B-adrenergic receptor stimulation increases the expression of deiodinase II (34). T3 in the BAT cell increases thermogenesis by increasing UCP1 expression.

Normally, serum T3 is constant and such that the contribution from serum T3 alone results in ~50% saturation of the thyroid receptors of the cells in most tissues (9). This is the case during thermoneutral and fasting conditions. However, tissues expressing deiodinase II can reach thyroid receptor saturation as high as 100% with more than half of this T3 produced locally (9). In mice, it has been shown that deiodinase II plays a critical role in the adaptive T3 production (10).

Brown adipocyte tissue. Animal studies. Mammals possess roughly two types of fat cells: white and brown adipocytes. The most well known are white fat cells. Their most prominent function is energy storage. Unlike white adipose tissue, the main function of brown adipose tissue (BAT) is heat production. Contrasting the white to yellow appearance of white fat cells, brown adipocytes are characterized by a light pink to dark red tone, which is caused by the high vascularization and the more granular appearance of the cytoplasm. The latter is caused by the small fat-filled vacuoles and the large amount of mitochondria in the cell. Another important trait of BAT cells is the rich innervation by the sympathetic nervous system. Recent studies in animals have suggested that brown fat and skeletal muscle may share a common developmental ancestry (118). Brown-fat progenitors have also been identified in human skeletal muscle (BRUSCLE, brown in muscle) (26). Animal studies also show that brown-fat cells emerging in white fat (BRITE, brown in white) develop from different precursor cells (109) and express molecular characteristics different from the distinct brown-fat depots. The brown-fat cells found in white fat and between muscle bundles appear to be more abundant in obesity-resistant strains of mice [among others: (47)], suggesting a potential role for these systemic brown adipocytes in protection against obesity.

For a more extensive overview of the differential characteristics between the different adipose tissue types, see Refs. 17, 20, 40, 119.

BAT is well known from small mammals, hibernators, but it is also present in larger mammals, including human newborns. As mentioned above, BAT mitochondrial respiration is being uncoupled by UCP1 to dissipate energy as heat (17). UCP1 knockout mice indeed rely on shivering thermogenesis in the cold (17, 35, 44) and lack the ability to recruit adrenergic thermogenesis during acclimatization to cold (43). However, UCP1-ablated mice failed to demonstrate an obesogenic effect. The reason for this is that in most laboratories, mice are kept below the thermal neutral zone and thus burned excess energy by shivering thermogenesis. When housed at thermoneutrality, UCP1 ablation actually showed diet-induced obesity, while wild-type mice did not and showed diet-induced thermogenesis (38).

Human studies. Newborns are known to have substantial amounts of BAT, especially in the axillary, cervical, perirenal, and periadrenal regions (70). It has been generally agreed that soon after birth, most of the brown fat disappears. Therefore, it was assumed that, under normal circumstances, adult humans do not have significant amounts of BAT.

On the other hand, early studies already showed the occurrence of BAT in adults (25, 53, 115, 116, 135), and significant amounts of BAT have for long been demonstrated in adults living in cold environments from samples obtained by nec-
In addition to the anatomical detection of BAT in humans, genetic studies have also suggested a role for BAT in human thermogenesis. Thus, part of the intersubject variation in facultative thermogenesis can be explained by the influence of the UCP1 polymorphisms. A-3826 A/G UCP1 polymorphism is associated with fat gain (86), weight gain (22), resistance to weight loss after a low-energy diet (41), increased susceptibility to obesity (55), less pronounced recovery from overfeeding (120), and lower expression of UCP1 mRNA in intraperitoneal depots (36, 85). Moreover, this polymorphism of UCP1 and one in the β3-AR (Trp64Arg) have been shown to have additive effects since individuals with both variants have a significantly increased risk of weight gain (22). In contrast, several other studies could not demonstrate an association between UCP1 polymorphisms and obesity-related phenotypes or weight gain over time (42, 49, 121).

Despite the anatomical and genetic observations, the general consensus was still that BAT does not play a major role in thermogenesis in adult humans. One reason for this was an elaborate physiological study on perirenal BAT thermogenesis. Whole body BAT activity was calculated by extrapolation from activity of this perirenal depot. It was concluded that the contribution of BAT in ephedrine-induced thermogenesis was marginal (4). However, we now know from PET/computer tomography (CT) studies that the perirenal depot in adults is small compared with other depots; thus, the extrapolation revealed an underestimation.

Indeed, recently, unrelated pursuits within nuclear medicine, using PET/CT scans, indicated that BAT is present and active in adult patients (24, 51, 81, 142). Thus, for localization of tumors, markers for cellular activity are used in nuclear medicine. Because tumors in general are glycolytic, increased glucose uptake may indicate the presence of a tumor. With a PET scan performed 45–60 min after intravenous injection of FDG, glucose uptake can be visualized. FDG is taken up via members of the sodium-independent glucose transporter family (such as GLUT1, GLUT3, and GLUT4). For a long time, symmetrical uptake of FDG in the neck and upper chest on a 18F-FDG PET scan had been recognized, but it was only until recently that the tissue involved in this uptake was identified as brown adipose tissue (51, 107, 134). BAT activity in adult humans is most commonly seen in the supraclavicular and neck region (Fig. 2A), but also paravertebral, mediastinal, para-aortic, and suprarenal localizations (81, 127).

The presence of BAT in adult humans was confirmed in a large retrospective study (28). In 2009, several experimental studies indicated the presence of cold-activated BAT in adult humans (99, 127, 131). These studies suddenly changed the point of view concerning the role of BAT in humans. The prevalence of cold-activated BAT in these recent studies varies. In retrospective nuclear medicine studies, the prevalence ranges from 2.5% (51) to 45% (98, 114). The studies with experimentally induced cold exposure in healthy subjects show a prevalence of 53% (young adults), 8% (elderly) (99), to 95% (young adult) (127). Using a standardized cold exposure protocol, we showed that 23 of the 24 subjects had definite, albeit highly variable, amounts of FDG activity in the neck, supraclavicular region, chest, and abdomen (127). BAT presence was confirmed by the presence of BAT-specific UCP1 in tissue taken from the supraclavicular region. In line with animal studies, there was no BAT activity at thermoneutrality. Mean BAT activity in obese subjects was significantly lower compared with the lean participants. Interestingly, BAT activity was significantly negatively related to body mass index and to percent body fat. Some studies indicate that older subjects seem to have less BAT activity (99, 145).

Is BAT in adults indeed involved in facultative thermogenesis? In the case of newborns, a relation between BAT and NST is suggested (for reviews, see Refs. 2 and 70). Although large amounts of BAT exist in newborns, direct evidence on the contribution of BAT is lacking. There is limited evidence that NST in adults can be attributed to BAT in human adults. In earlier experiments, we could not show a relation with NST (127), but in more recent studies, we and others demonstrate involvement of BAT in NST. One study showed that lean subjects with BAT activity had significantly higher levels of NST than those that did not show BAT activity (143). We confirmed this in a group with a larger range of body compositions, including 15 morbidly obese patients with little or no BAT activity (BMI 21–48 kg/m2). BAT-positive subjects showed a significantly higher NST than BAT-negative subjects, indicating that BAT in humans is involved in facultative thermogenesis (130).

**BAT energetics.** What is the potential contribution of BAT thermogenesis to whole body energy expenditure in humans? From mice studies, it is known that the maximal heat-producing capacity of BAT is ~300 W/kg (17, 97). This is about two orders of magnitude of the metabolic rate of other tissues (17). It means that an amount of BAT corresponding to a few percent of the animals’ body weight can produce as much heat as all the rest of the body. On the basis of the 300 W/kg rate, Rothwell and Stock calculated that in humans 40–50 g BAT could, if maximally stimulated, account for 20% of daily energy expenditure (96). This reference is used in many recent studies on BAT volume estimates by FDG PET/CT to show the significance of BAT for whole body thermogenesis in humans (28, 40, 119).

Two assumptions, however, are made that could make this kind of estimate too high. First, it should be noted that the heat production only takes place when BAT is (maximally) activated (i.e., by cold, diet, or pharmaceuticals). Normally, this will not be the case for prolonged times. Exceptions are, for instance, outdoor workers exposed to cold for longer periods. Secondly, allometric relations should be taken into account when comparing energy expenditure in tissues or bodies of animals that differ in size. In mammals, mass-specific energy expenditure (i.e., per unit tissue mass) is negatively related to body size (104). Thus, the metabolic rate (R) for all organisms roughly follows a 3/4 power-law of the body mass (M), i.e., R ∼ M⁻³/₄ (Kleiber’s law) (63). Whole body basal metabolic rate in mice is 8 W/kg, while in humans, this amounts to 1 to 2 W/kg, following Kleiber’s law. Tissue-specific energy expenditure also follows Kleiber’s relationship to some extent: In skeletal muscle, for instance, the specific metabolic rate in mice is more than two times higher than in dogs (104). Another study on oxygen consumption in white adipose tissue showed that cell respiration is 5–10 times higher in rats compared with

**M3/4 (Kleiber’s law) (63). Whole body basal metabolic rate**

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human adipocytes (48). In liver mitochondria, it has been shown in rats that mitochondrial surface area (which is related to heat production) follows allometric relationships and is negatively related to body mass (91). It is very likely that such a relationship also holds for BAT. This is confirmed in a study on BAT mitochondrial activity in rats using Sestamibi tracer uptake (67). Although this study showed that mitochondrial activity scales allometrically, it did not provide information about actual heat production. Possibly, the cold exposure was more effective in the smaller animals, resulting in relatively high thermogenesis compared with their heavier counterparts.

There is no information available on BAT heat production in adult humans. However, in view of the tissue allometric relationships described above, active BAT thermogenesis is likely to be lower than reported for mice. Assuming the same ratio between mice and men for whole body energy expenditure and for maximal BAT activity, BAT in humans amounts to ~45 W/kg. Another calculation, using the allometric relation derived by Wang et al. (132) for skeletal muscle in rats results in BAT energy expenditure in humans of 80 W/kg. On the basis of these figures, a 20% contribution of daily energy expenditure in a 70-kg human would need 200–360 g of BAT (2.6% of body mass), 4–7 times more than the estimate of Rothwell and Stock (96). Nevertheless, the allometric estimate shows that also in humans the maximal heat production of BAT can be very large and much higher than other active tissues, such as skeletal muscle. On the basis of FDG PET/CT studies, volumes of more than 100 cm³ active BAT in human adults are common [mean BAT volume in lean subjects: 130 cm³ (127), although this volume can be a mix of brown and white adipocytes (personal observations and Ref. 145). Nevertheless, an estimate of 50 g active BAT in adult humans does not seem unrealistic. That means that activated BAT could amount to 2.2–4 W, or ~2.7–5% of BMR. Thus, even this prudent calculation shows that BAT can have a substantial impact on human body weight regulation.

Another calculation is based on the PET/CT data. Virtanen et al. (131) used dynamic PET scanning and came to an estimate of glucose uptake of 12.2 μmol per 100 g per min. With an estimated BAT depot of 63 g and assuming that during activation of brown adipose tissue, 10% of the total metabolism of BAT is derived from glucose uptake (76), one can calculate the heat production of activated BAT is 55 W/kg. This comes up to ~4.5% of BMR. This is close to the estimate above, using rodent BAT activity and allometric scaling to calculate human BAT energy expenditure. Taken together, these calculations show that human BAT when activated could make a significant contribution to BMR. However, during cold exposure, several studies show that sustainable NST thermogenesis ranges from 0 to 15% of RMR (99, 126, 138). Although the calculations above show that at least a substantial part can be attributed to BAT, it is likely that other tissues contribute to NST. This is a somewhat controversial issue. At least in mice, only UCP1-containing BAT seems to contribute.
to adaptive thermogenesis (cold acclimation-recruited adaptive adrenergic NST) (43). Nevertheless, several studies indicate that in humans, skeletal muscle may be involved.

Skeletal Muscle

Skeletal muscle is potentially a large contributor to facultative thermogenesis in humans. Experiments using adrenaline infusion and forearm muscle measurements revealed that skeletal muscle would account for about 40% of adrenaline-induced thermogenesis (111). It should be noted, however, that the thermogenesis that can be induced by catecholamine infusion is probably of a different character than when the thermogenic target organs are directly stimulated with norepinephrine through the SNS. Results from ingestion of ephedrine, which is a sympathomimetic compound acting both centrally and peripherally, resulted in an average increase in leg oxygen consumption of 25%. This accounted for an extrapolated contribution of the skeletal muscle tissue in ephedrine-induced thermogenesis of 50% (4). Finally, it has been shown that carbohydrates induced an increased adrenaline concentration, resulting in increased muscle thermogenesis (3). The contribution of skeletal muscle to the total postprandial energy expenditure has been estimated to be within a range of 35 to 67% (125). More recently, we showed that human nonshivering thermogenesis in response to cold exposure is accompanied by and significantly related to mitochondrial uncoupling in skeletal muscle (140). Recent experiments from our group confirm these findings and additionally indicate that both BAT and skeletal muscle play a role in human NST (141). In this study, lean volunteers showed cold-induced nonshivering thermogenesis, but this was not diminished after beta-blockade by propranolol (mainly $\beta_1$ and $\beta_2$ blockade) (7). Secondly, skeletal muscle mitochondrial uncoupling was significantly related to NST in the control situation. This relation disappeared during the $\beta$-blockade. Thus, our results suggest that skeletal muscle mitochondrial uncoupling may be involved in NST and that this is regulated by $\beta_2$-receptors (muscle lacks $\beta_1$). When $\beta_1$- and $\beta_2$-receptors are blocked by propranolol, a $\beta_2$-regulated process like mitochondrial uncoupling in BAT might take over the role of skeletal muscle mitochondrial uncoupling.

The importance of skeletal muscle metabolism with respect to adaptive thermogenesis also comes from studies on thyroid hormones. It has been shown that adult humans, unlike rodents, significantly express deiodinase II in skeletal muscle (100, 110). However, no differences were observed in the expression of skeletal muscle deiodinase II mRNA between hypothyroidism and T4 treatment, although a decrease was observed after 62 h of fasting (54). On the other hand, in a study in patients with resistance to thyroid hormone, skeletal muscle mitochondrial uncoupling was a major contributor to the observed increased RMR. This was attributable to elevated thyroid hormones (80).

The mechanism by which mitochondrial uncoupling in skeletal muscle is regulated is not yet fully elucidated. Although, in general, UCP3 is not considered to be a true uncoupling protein like UCP1 and UCP3-ablated mice have normal energy expenditure (46), there are also observations that do point toward a role for UCP3 in thermogenesis. For example, in thyrotoxic human skeletal muscle, mitochondrial uncoupling was shown as evidenced by a 70% increase in the Krebs cycle without an increase in ATP production (72). Furthermore, thyrotoxicosis is accompanied by increased levels of UCP3 mRNA in rat skeletal muscle (45), which can be explained by T3-induced lipolysis, because fatty acids are potent stimulators of the UCP3 gene (5, 15). Also, it was shown that 3,4-methylenedioxyamphetamine (ecstasy)-induced thermogenesis required the presence of UCP3 (79). However, the experiments of Gong et al. (46) show that the metabolic response to thyroid hormone was normal in UCP3 knockout mice, indicating that it is unlikely that UCP3 is involved in thyroid thermogenesis. More specific human studies might be required to investigate the role of skeletal muscle mitochondrial uncoupling, UCP3, or other putative proteins involved in this process in human thermogenesis.

Perspectives and Significance

The demonstration of active BAT in adult humans has renewed interest in nonshivering thermogenesis. More knowledge on the facultative and adaptive responses to cold are potentially relevant in relation to the metabolic syndrome, and age-related disturbances in thermoregulation. So far, studies on mild cold exposure are limited. Not much data are available that relates NST to body composition, sex, and age. Much attention is now given to BAT and, to some extent, skeletal muscle. However, parallel to studies on these tissues, basic studies on cold responses (metabolic and insulative) comparing individuals of different groups are needed.

Mechanistic animal studies of the last few years drastically increased our insight into the mechanisms and regulation of BAT and skeletal muscle-uncoupling activity. Also, much information has been gathered on the embryonic development of BAT and skeletal muscle (109). As mentioned above, brown adipocytes from distinct depots and skeletal muscle cells share common precursors. In contrast, brown-like adipocytes appearing in white adipose tissue (“BRITE adipocytes”) appear to originate from other precursors, which are closer to white adipocytes (88). This knowledge can help us unravel the cellular mechanism of those tissues in humans.

Currently, little is even known about the basic physiology of BAT in adult humans. Retrospective studies using FDG-PET/CT suggest that BAT activity is negatively related to age and that females have higher BAT activity compared with males (28, 87, 89). However, because these patient studies did not make use of a temperature-controlled protocol to study BAT, no firm conclusions about these relations can be drawn. Only Saito et al. (99) measured cold-activated FDG uptake and showed less BAT activity in elderly compared with young volunteers (99). With respect to sex differences, we showed that 23 of the 24 men showed BAT activity (127), a fact that speaks against the idea of female supremacy in BAT activity. Possibly in the retrospective studies, more female patients were experiencing cold than the male patients during FDG uptake in the clinic environment. Therefore, more research is needed to study the basic physiology of human BAT under controlled conditions. It is also relevant to determine whether BAT physiology differs between healthy subjects and patients with type 2 diabetes.
To some extent, the same applies for skeletal muscle. Although much more is known about the physiology of human skeletal muscle than of BAT, the relations between skeletal muscle metabolism and diet and environmental temperature need further investigation. The importance of skeletal muscle in nonshivering thermogenesis has been indicated in several studies, but the actual metabolic activity of skeletal muscle during cold has not been studied. For both BAT and skeletal muscle studies, new techniques, such as magnet resonance spectroscopy, may be promising. Ideally, in the future, we will be able to study NST with the same technique in BAT and skeletal muscle, to compare the contribution of each.

Besides the pharmacological approach to increase thermogenesis, it is relevant to study which “natural” factors determine facultative and adaptive thermogenesis. How important are daily environmental temperatures and how is the interaction between temperature and dietary intake and diet composition in this respect? Can we manipulate BAT and skeletal muscle metabolism by moderate cold exposure?

Currently, it is not clear yet, whether mild cold exposure (in BAT or in skeletal muscle) prevents obesity. If so, does it prevent obesity because of its changes in basal metabolism, due to submaximal activation of BAT and/or skeletal muscle, or because of maximal BAT/skeletal muscle thermogenesis during occasional exposure to more intense cold. Additionally, diet-induced thermogenesis in relation to NST and the role of BAT and skeletal muscle deserves further attention.

It is also interesting to know whether people who experience thermal comfort, in fact physiologically are not in thermoneutral condition. In such cases, BAT and/or skeletal muscle may be submaximally activated. This will especially be the case in winter seasons. This idea is supported by the fact that the retrospective patient studies on BAT activity did not use a cold exposure protocol. Nevertheless, they show BAT activity and significantly more so in the winter season (28, 87). This indicates that those patients were not in thermoneutral conditions, even in hospitals. Besides a lack of thermoneutrality, other activators of the SNS, including nutritional status, and/or anxiety, could also be responsible for stimulating BAT in these retrospective studies. Clearly, more controlled studies are needed about the effect of indoor environmental temperatures on BAT activation.

Nowadays, we live in a much more thermal comfortable environment than in the past. There is some circumstantial evidence that remaining in the thermoneutral zone promotes adiposity (61). Indeed, in husbandry, animals are preferably kept at thermoneutral conditions, to maximize weight gain (74). Whether a cool environment with increased thermogenesis can be used to prevent body weight gain or even reduce body weight in humans still needs to be investigated. Although this is an attractive, low-cost, and environmentally friendly possibility, it is yet unknown whether such a stimulation of thermogenesis produces an adaptive response, leading to increased energy intake.

Nevertheless, it is interesting to find out whether it is healthier for humans to live under more variable temperature conditions than most buildings nowadays offer. In the built environment, most offices and dwellings are designed to provide a constant thermal comfort. Is a deviation from thermoneutrality realistic? The protocols used in the metabolic studies use mild cold temperatures of 16–19°C (18, 99, 127, 140). It is possible that even slightly higher temperatures may already activate BAT (as seen in retrospective studies). From experiments in which people are allowed to regulate their own indoor climate, it is known that in winter time, 80% of the people accept temperatures of 18–20°C (32). Therefore, it is not unrealistic to design buildings and dwellings with more variable and slightly cooler indoor climate than currently is the standard to provide a healthier, less obesogenic, environment.

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