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Nonshivering thermogenesis and its adequate measurement in metabolic studies

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Summary

Alterations in nonshivering thermogenesis are presently discussed as being both potentially causative of and able to counteract obesity. However, the necessity for mammals to defend their body temperature means that the ambient temperature profoundly affects the outcome and interpretation of metabolic experiments. An adequate understanding and assessment of nonshivering thermogenesis is therefore paramount for metabolic studies. Classical nonshivering thermogenesis is facultative, i.e. it is only activated when an animal acutely requires extra heat (switched on in minutes), and adaptive, i.e. it takes weeks for an increase in capacity to develop. Nonshivering thermogenesis is fully due to brown adipose tissue activity; adaptation corresponds to the recruitment of this tissue. Diet-induced thermogenesis is probably also facultative and adaptive and due to brown adipose tissue activity. Although all mammals respond to injected/infused norepinephrine (noradrenaline) with an increase in metabolism, in non-adapted mammals this increase mainly represents the response of organs not involved in nonshivering thermogenesis; only the increase after adaptation represents nonshivering thermogenesis. Thermogenesis (metabolism) should be expressed per animal, and not per body mass [not even to any power (0.75 or 0.66)]. A 'cold tolerance test' does not examine nonshivering thermogenesis capacity; rather it tests shivering capacity and endurance. For mice, normal animal house temperatures are markedly below thermoneutrality, and the mice therefore have a metabolic rate and food consumption about 1.5 times higher than their intrinsic requirements. Housing and examining mice at normal house temperatures carries a high risk of identifying false positives for intrinsic metabolic changes; in particular, mutations/treatments that affect the animal's insulation (fur, skin) may lead to such problems. Correspondingly, true alterations in intrinsic metabolic rate remain undetected when metabolism is examined at temperatures below thermoneutrality. Thus, experiments with animals kept and examined at thermoneutrality are likely to yield an improved possibility of identifying agents and genes important for human energy balance.

Key words: brown adipose tissue, norepinephrine, adaptive thermogenesis, facultative thermogenesis, cold tolerance.

Introduction

Probably mainly as an effect of the global obesity epidemic, the scientific interest in thermogenesis has increased dramatically in recent years. The questions asked are principally: are (some) forms of obesity due to decreased metabolism? – and can (some) forms of obesity be treated by an increased thermogenesis?

Although these questions are rather clear, evaluation of the thermogenesis data collected in these contexts is not always straightforward. In particular, it is important to realize that thermogenesis is functionally linked to the maintainance of body temperature and is therefore also closely linked to ambient temperature. Therefore, the detailed conditions under which thermogenesis is estimated can profoundly affect the experimental outcome and the interpretation of the data.

In this review, we discuss the phenomenon of nonshivering thermogenesis (in a broad sense) and the problems and pitfalls associated with the quantitative evaluation of nonshivering thermogenesis, not least in connection with metabolic studies.

The thermoneutral zone and cold-induced metabolism

The body temperatures of the majority of organisms closely follow the ambient temperature, and the metabolic rates of these organisms are exponentially related to ambient temperature; most animals are thus ectotherms. Although many higher organisms try to maintain their body temperatures at a relatively high level and as constant as possible under any particular circumstance, only birds and mammals have an endogenous capacity to do this constantly, i.e. they are endotherms. Birds and mammals also show considerably higher levels of insulation than ectothermic organisms. This insulating barrier has considerable consequences for their thermal balance. Although there are several studies of thermogenesis-related issues in birds [as e.g. summarized in Nedergaard et al. (Nedergaard et al., 1986)], these phenomena have been characterized much more in mammals. In the following review, we will restrict the discussion to mammals – mainly mice. This is because, although most classical studies of thermogenesis have been performed on rats, the present ability to generate transgenic mice has directed almost all current efforts to mouse systems. The differences between these two rodents may appear minimal, and many will think of a mouse as just 'a small rat'. However, exactly the point that a mouse is much smaller than a rat turns out to have profound effects on its metabolism; in particular it means that it has to devote a large fraction of its metabolism to defence of its body temperature, as will be clarified below.

For all mammals, there is a range of ambient temperatures within which the general metabolism of the organism, in the absence of any physical activity, generates sufficient heat as a byproduct of the continually ongoing metabolism so that its predetermined body temperature can be maintained. This temperature range is known as the thermoneutral zone, and at this temperature the organism demonstrates its basal metabolic rate (Fig. 1) [for a deeper overview of thermoregulation than that presented below and for further references, see e.g. Schmidt-Nielsen (Schmidt-Nielsen, 1990)]. On mild cold exposure, an animal will initially attempt to defend its

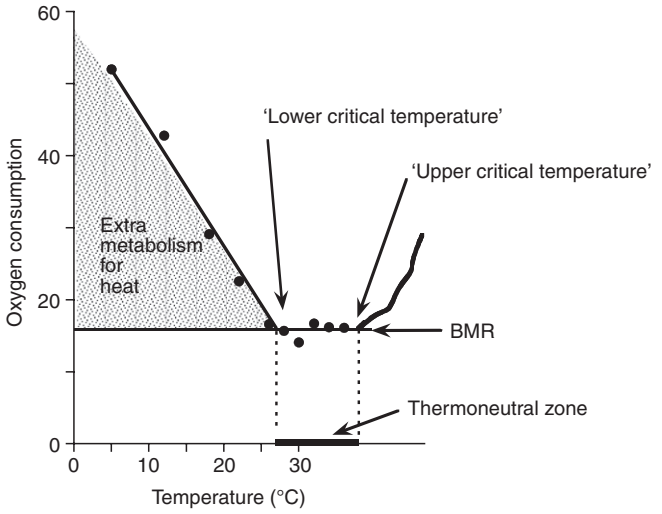

Fig. 1. The energetic consequences of different ambient temperatures. Metabolism is fully governed by intrinsic factors only within a narrow zone of ambient temperatures – the thermoneutral zone (indicated by the area between the dashed lines, i.e. the lower and upper 'critical' temperatures) (calling these temperatures 'critical' may be considered misleading but this is the current nomenclature in thermal physiology). In mice, the thermoneutral zone lies at approximately 30°C. At ambient temperatures outside the thermoneutral zone, a large fraction of total energy is used for thermoregulation; already at normal animal house conditions (18–22°C), this fraction is an additional 50–100% above the basal metabolic rate. The shaded area indicates the extra metabolism required for body temperature defence. BMR, basal (or resting) metabolic rate. Oxygen consumption rates are arbitrary units. Based on data on wild-type mice published previously (Golozoubova et al., 2004).

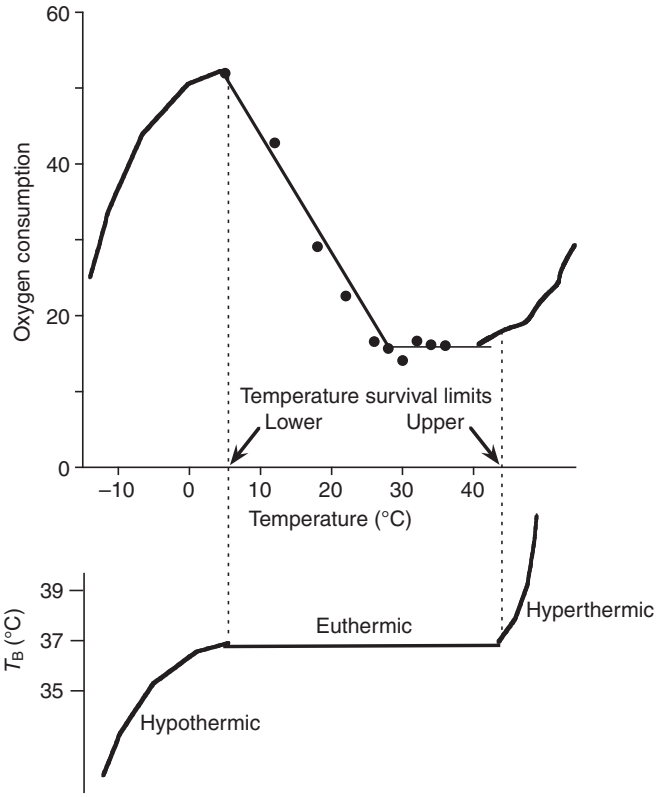

Fig. 2. The effects of exceeding the ambient temperature survival limits. Principal figure extended from data in Fig. 1. Dashed lines indicate the lower and upper temperature survival limits. T_B, body temperature.

body temperature by energetically inexpensive means, such as vasoconstriction and piloerection, and by changes in posture to decrease surface area. If such measures prove insufficient, because the cold challenge is stronger, the animal will increase its endogenous heat production. This increase will occur initially through shivering thermogenesis. By these involuntary muscle contractions, ATP will be hydrolyzed without useful work being done on the environment, and heat will be evolved. When an animal is returned to its thermoneutral temperature, its metabolism immediately returns to the basal level. Thus, these cold-induced increases in metabolism are facultative. Time delays are in the order of minutes, at the most.

Fig. 2 expands this picture to a wider range of ambient temperatures and demonstrates principally the relationship between metabolism and body temperature. It is evident that any animal can only defend its body temperature over a limited range of ambient temperatures, i.e. from the lower to the upper temperature survival limit. Experimental tests that exceed these limits and impose challenges that fall outside the range of control provide no information on the metabolism of the animal under study. This is particularly apparent in neonatal and young animals tested under conditions appropriate for the adults of the species, but may also be evident in animals with compromised physiology, e.g. due to genetic modifications. Thus, if these limits are crossed, the animal will successively cool down or heat up until its body temperature becomes identical to that of the surroundings – i.e. it dies.

Particularly at high ambient temperatures, high demands are placed on thermoregulation in mammals. Thus, at temperatures above the upper critical temperature, metabolism increases because of the metabolic cost of the mechanisms for cooling that the animal must utilize here (sweating and panting). Thus, even more heat is generated by the animal in order to dispose of the external heat inflow. Therefore, most mammals are in a problematic situation when ambient temperatures become high and there is little margin before the upper temperature survival limit is reached. We will not discuss defence against overheating further here.

At the opposite end of the thermoneutral zone, at the lower critical temperature, the animal initiates heat production to defend its body temperature. The increase in heat production that is necessary to counteract heat lost to the surroundings is linearly related to decreasing temperature (Fig. 3). This is because the slope is a measure of the insulation of the animal (i.e. the thermal conductance of the animal), and insulation is a measure of how much heat is lost per degree difference between the internal and ambient temperatures. This line can be extrapolated to apparent zero metabolism. It follows from the definition of insulation that this point on the *x*-axis constitutes the internal temperature of the animal, i.e. the defended body temperature.

A further consequence of this fully physical phenomenon is that the extent of the thermoneutral zone is also dependent upon the insulation of the animal. As the insulation line has to extrapolate to the defended body temperature, the better insulated the animal, the lower the lower limit of the thermoneutral zone (Fig. 4). The magnitude of the thermoneutral zone can vary in different animals from a few degrees to as much as 70°C. The slope of increasing metabolism with decreasing temperature is steep for a poorly insulated animal (humans, mice) and very shallow for a very well-insulated animal (primarily arctic animals).

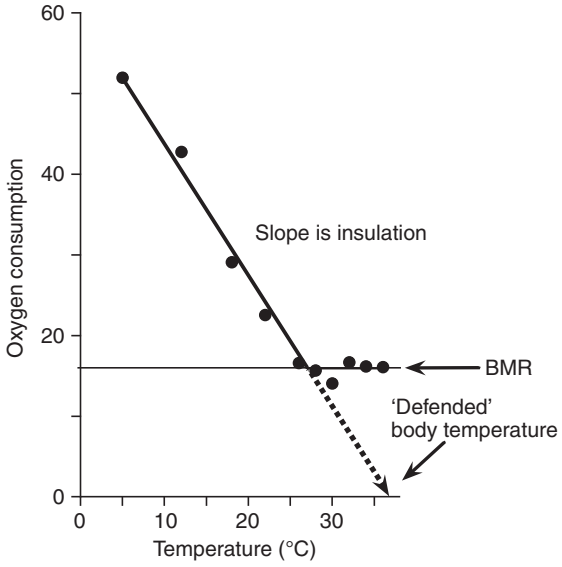

Fig. 3. The relationship between metabolism and body temperature. The increase in extra heat needed to defend the body temperature is, as seen, directly proportional to the decrease in body temperature. The slope of the increase in metabolism as an effect of decreased temperature is a measure of the insulation of the animal [formally, it is the thermal conductance: watts per degree difference between external and internal (i.e. body) temperatures]. This line may be extrapolated (stippled line) to zero. Thus, when the difference between the ambient and body temperatures is zero, heat loss is zero. This is therefore the controlled (defended) temperature (i.e. the body temperature).

Curves such as those shown in Fig. 1 are obtained from studies in metabolic chambers. It may therefore be questioned whether the animals under normal conditions (experimentally, this simply means in their home cages) increase their metabolism to the same extent. This can be deduced from food intake, because animals must replenish all of the energy that is lost as heat. Thus, at 5°C, a mouse will have a food intake approximately 3–4 times that at 30°C. Even at normal animal house temperatures, the food intake is approximately 60% above that at thermoneutrality (Cannon and Nedergaard, 2009), not a negligible alteration from basal levels.

Thermoneutrality is operationally defined

Although the thermoneutral zone is operationally defined as the temperature zone at which the lowest metabolic rate is observed, a nominal value for a given species cannot be tabulated. Normally, the thermoneutral zone for mice is said to be 29–31°C (Fig. 1). However, for newborn and young animals, the zone moves to higher temperatures, approaching body temperature. By contrast, gestational and lactating mammals produce vast amounts of heat due to foetal metabolism and as a by-product of milk production (Roberts and Coward, 1985; Quek and Trayhurn, 1990). This means that the thermoneutral zone moves markedly down the temperature scale, perhaps to approximately 15°C, in lactating mice.

It also follows that any genetic manipulation may alter the thermoneutral zone, notably due to manipulation-induced alterations in insulation (as will be discussed below). Thus, nominal temperatures that are thermoneutral for wild-type mice may not be so for manipulated mice. Consequently, mice of the two genotypes may have to be placed at different nominal temperatures to obtain an identical functional temperature – conditions that will probably not be readily accepted by most reviewers but that are in reality the correct way to perform the experiments. Thus, the thermoneutral zone should optimally be established in independent studies for any mouse modification before further experiments are undertaken. Although this seems a harsh demand, reality has demonstrated that it may be well worth the effort.

In extension of this, the fact that mice are very small – compared with rats – may qualitatively affect the outcome of metabolic experiments. Because of their size, rats are not as cold sensitive as mice. Adult rats probably have a lower critical temperature that approaches 24°C and it may even be slightly lower under normal housing conditions. This means that, under normal housing conditions, adult (male) rats may be experiencing thermoneutrality whereas this is never the case for mice. Apparent physiological differences between rats and mice may therefore be due to the rats being studied under thermoneutral conditions whereas the mice are cold-stressed, leading to qualitatively different outcomes. Similarly, differences between younger and older rats – or between female and male rats – may be secondary to their different thermoneutral zones rather than being true manifestations of age or sexual dimorphisms. Considering the significance of the thermal responses for metabolic studies, it is remarkable that differences in thermal responses are very poorly documented with regard to age, sex, strains, etc.

Even within humans, females are generally smaller than males and thus probably have a different thermoneutral zone. Clearly, they have zones of comfort some degree higher than those of men (Rohles, 1971). Thus, again, metabolic differences between men and women, examined under conditions with identical nominal temperatures, may in reality be secondary to differences in thermal responses rather than to more basic metabolic modalities.

Classical nonshivering thermogenesis

All mammals exposed to cold will initially shiver in order to elevate heat production (Griggio, 1982). Thus, extensive periods of life in the cold would seem difficult and stressful. However, during the Second World War, cold-room food stores were invaded with mice

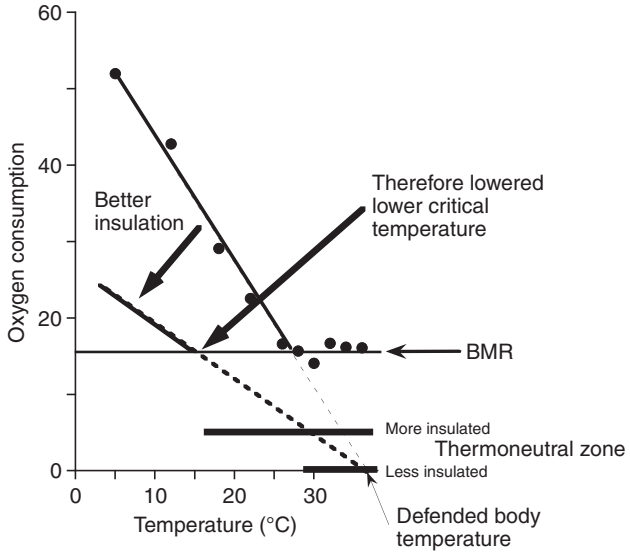

Fig. 4. The effect of increased insulation on the thermoneutral zone. As seen, animals with a better insulation (a smaller slope of the line) must necessarily also obtain a broader thermoneutral zone, because the line must extrapolate to the same defended body temperature.

that apparently lived there all their life, even had their young there and seemed to thrive well under conditions that would seem very unpleasant for all small mammals (–10°C) (Barnett, 1965). That all of this would happen under conditions of constant shivering seemed unlikely.

What was unexpectedly observed in rodents in the 1950s was that after a prolonged period in the cold, the animals ceased to shiver but retained an equally high metabolic rate (Sellers et al., 1954; Cottle and Carlson, 1956; Hart et al., 1956). This would allow for a more comfortable life in the cold. As this elevated metabolism was observed to occur in the absence of measurable shivering, it was appropriately termed nonshivering thermogenesis. The mediator and site of this nonshivering thermogenesis were initially unknown. Starting from experiments originally directed at thyroid hormone effects (Ring, 1942), it turned out that the disappearance of shivering was accompanied by an increase in the thermogenic response to adrenergic stimulation, i.e. the mediator was norepinephrine (noradrenaline), released from the sympathetic nervous system (Hsieh and Carlson, 1957; Depocas, 1960). Consequently, it was concluded that it was possible to estimate the capacity for nonshivering thermogenesis in an animal by injecting norepinephrine into the animal when it was at its thermoneutral temperature. Although this technique indeed activates nonshivering thermogenesis by mimicking the release of norepinephrine from specific regions of the sympathetic nervous system, it also unavoidably activates other adrenergic receptors in the body. This leads to some elevation of metabolism and thus to an overestimate of the nonshivering thermogenic capacity, as will be discussed below.

The organ generating nonshivering thermogenesis remained controversial long after the mediator was identified. Many researchers believed that the predominant site was skeletal muscle, mainly because of its large size and thus potential large capacity for heat production. Based on now classical studies by R. E. Smith in the 1960s (Smith, 1961; Smith and Hock, 1963; Cameron and Smith, 1964; Smith, 1964; Smith and Roberts, 1964), a few scientists believed that brown adipose tissue was the main site of nonshivering thermogenesis. That brown adipose tissue could generate heat was not in question, but the magnitude and thus significance of the heat production were controversial, particularly considering the small size of the organ. The controversy was resolved by the blood flow studies of Foster and colleagues in the late 1970s, which demonstrated massive blood flow increases to brown adipose tissue, both on cold exposure (Foster and Frydman, 1979) and following norepinephrine injection (Foster and Frydman, 1978), with no increases in blood flow to skeletal muscle; the blood leaving brown adipose tissue was also observed to be practically depleted in oxygen. Since then, practically all rodent researchers have agreed that brown adipose tissue is – at least – the main site of nonshivering thermogenesis; some, such as we, would maintain that it is the only site. Concerning humans, the idea that nonshivering thermogenesis (provided it exists) originates from muscle has persisted, not least because it has been the general view that brown adipose tissue did not exist in adults. Very recent observations have altered this view (reviewed in Nedergaard et al., 2007; Nedergaard et al., 2011). We would consider it likely that, in humans too, all nonshivering thermogenesis emanates from brown adipose tissue.

During the 1960s and onwards, studies were also performed that showed that brown adipose tissue went through a process of cell proliferation and increased differentiation when an animal was kept in a cold environment (Cameron and Smith, 1964). Hence, the growth of the tissue could be seen both as the reason and the rate-limiting step for the development of nonshivering thermogenesis. This growth of brown adipose tissue following prolonged cold exposure is termed recruitment. In addition to the increased cell proliferation (Hunt and Hunt, 1967; Bukowiecki et al., 1982; Bukowiecki et al., 1986; Rehnmark and Nedergaard, 1989) and differentiation, there is an extensive mitochondriogenesis and a large increase in the total amount of uncoupling protein 1 (UCP1) (earlier referred to as thermogenin) in the animal (Jacobsson et al., 1994; Cannon and Nedergaard, 2004).

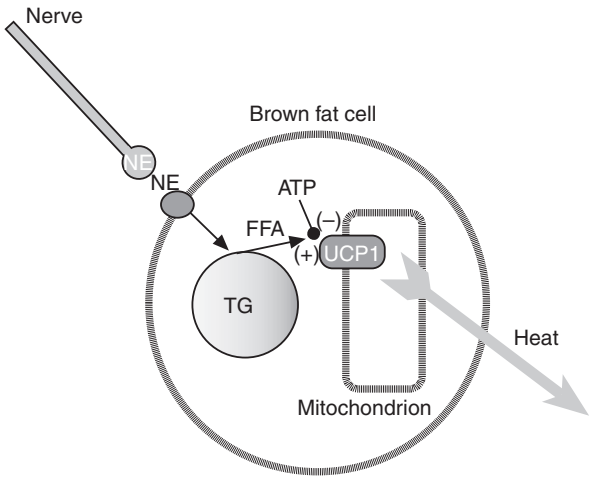

Fig. 5. The basic principles for heat production in brown adipose tissue. The brown-fat cells are stimulated by norepinephrine (NE) released from the sympathetic nervous system. The norepinephrine binds, as indicated, to its receptor in the plasma membrane, and through intracellular signalling processes, this leads to degradation of the triglycerides (TG) in the lipid droplets, and the released free fatty acids (FFA) interact with uncoupling protein-1 (UCP1) and, through this, overcome the inhibition of UCP1 caused by cytosolic purine nucleotides such as ATP (and ADP, GTP and GDP). This leads to respiration in the mitochondria that is uncoupled from ATP synthesis. All energy from the combustion of substrate (food) is therefore directly released as heat.

Thus, classical nonshivering thermogenesis is a facultative (meaning that it can be turned on and off within minutes), adaptive (meaning that it needs weeks to develop) form of thermogenesis that can be acutely induced by norepinephrine injection (i.e. an adrenergic thermogenesis).

The mechanism of heat production in brown adipose tissue

In the 1970s, the biochemical mechanism for heat production in brown adipose tissue was extensively investigated. It became apparent that the heat production occurred in the mitochondria as a consequence of a regulated uncoupling process mediated by a unique protein (Nicholls, 1976). The protein was isolated in 1980 (Lin and Klingenberg, 1980) and is now known as UCP1. In Fig. 5, the action of UCP1 is described. In resting cells, the activity of UCP1 is inhibited by bound purine nucleotides. When the cell is activated by norepinephrine, a lipolytic cascade is initiated that results in UCP1 activation. The exact mechanism of this activation is still not fully resolved (Nedergaard et al., 2001). During early mammalian evolution, UCP1 developed rapidly (Saito et al., 2008) from a probably nonthermogenic protoUCP1 that is still found in fish (Jastroch et al., 2005; Jastroch et al., 2007). UCP1 is principally found in all mammals – with the pig family being the only exception (Berg et al., 2006). Pigs have secondarily lost the ability to express UCP1 and are thus incapable of nonshivering thermogenesis (Mount, 1968). In our opinion, UCP1 is the only true thermogenic uncoupling

protein, and the other proteins with similar names (UCP2–5) have received their names based on homology in amino acid sequence, not on homology in function.

Life without UCP1

To delineate the significance of brown adipose tissue under different physiological conditions, animals without brown adipose tissue would really be necessary. However, such animals have been difficult to generate, either by attempts to dissect away brown fat (which cannot be done adequately as the tissue depots are found in so many places) or by molecular means. However, because of the significance of UCP1 for the thermogenic mechanism of brown adipose tissue, a mouse with a genetic ablation of UCP1 (Enerbäck et al., 1997) should be a mouse in which the brown adipose tissue is unable to produce heat. Therefore, with such a mouse as an experimental tool, many questions concerning the significance of brown adipose tissue heat production under different physiological conditions have now been stringently addressed.

As anticipated, brown adipocytes isolated from UCP1-ablated mice do not respond to norepinephrine addition with an increase in oxygen consumption, i.e. they do not show adrenergic thermogenesis (Matthias et al., 2000). However, the basal respiration of the cells is identical regardless of whether they possess UCP1. This demonstrates that UCP1 does not show any proton transport activity when non-stimulated, that is to say, it is not 'leaky' (Shabalina et al., 2010b) (i.e. it does not allow for proton flux over the mitochondrial membrane when it is not directly stimulated).

UCP1-ablated mice are viable and fertile. In agreement with the results from the isolated brown adipocytes, there are no differences in basal metabolic rate between mice with and without UCP1 (Golozoubova et al., 2001; Golozoubova et al., 2006). This confirms the tenet that UCP1 is not leaky and does not contribute to basal metabolic rate.

The UCP1-ablated mice were initially observed to be unable to defend body temperature when transferred from normal animal house temperatures of approximately 23 to 5°C (Enerbäck et al., 1997). Although at first sight, this appears to be the expected result if brown adipose tissue were to be ascribed a major role in nonshivering thermogenesis, it seems to be in contradiction to the tenet that mammals initially shiver to maintain body temperature. However, the outcome is understandable within this tenet. A mouse with an ablation in the UCP1 gene that has been living at normal animal house temperatures will have been unable to develop any capacity for thermogenesis in its brown adipose tissue because of the lack of UCP1. Its survival at 23°C has been dependent on the constant use of shivering to increase metabolism. If such an animal is transferred to 5°C, it will – unlike the wild-type animal – have no brown adipose tissue activity, and is therefore forced to rely entirely upon shivering to defend its body temperature. The capacity and endurance of the shivering prove to be inadequate, and gradually the body temperature of the UCP1-ablated animal therefore decreases.

If a UCP1-ablated mouse is housed at an intermediate, cooler temperature, such as 18°C, it can then be transferred to 5°C and survive for prolonged periods (Golozoubova et al., 2001). Similarly, if the ambient temperature is successively decreased (2°C day^{-1}), the UCP1-ablated mice survive in the cold (Ukropec et al., 2006). It seemed initially possible that such cold-acclimated mice had developed an alternative means of nonshivering thermogenesis. However, measurements of electrical activity in muscle (i.e. shivering) showed that, in contrast to the case in wild-type mice, these UCP1-ablated mice shiver with the same intensity after several weeks in the cold as they do on the initiation of exposure to cold (Golozoubova et al., 2001). They have thus not developed any alternative nonshivering thermogenesis. Simple visual inspection of the mice in the cold also makes it clear that whereas the cold-acclimated wild-type mice are comfortable in the cold and move around normally, principally similarly to behaviour at normal temperatures, the cold-acclimated UCP1-ablated mice remain in one position, in the nest if possible, curled up and visibly shivering. Thus, there is no evidence that an alternative mechanism for nonshivering thermogenesis has developed. Rather it would seem that the endurance capacity of the mouse for shivering has increased, and its muscles therefore do not become exhausted, which allows for the uninterrupted maintenance of shivering and, therefore, defence of body temperature. Alterations in muscle capacity are indeed observable in cold-acclimated UCP1-ablated mice, measurable as alterations in muscle mitochondria ATP-synthase capacity (Shabalina et al., 2010a). Thus, no other adaptive adrenergic mechanism of thermogenesis exists or is induced in these mice. We would therefore maintain that all classical nonshivering thermogenesis is located in brown adipose tissue.

Until recently, it has been the general contention that there must be an alternative mechanism for heat production in muscle because it was believed that so-called thyroid hormone thermogenesis took place in muscle. However, it now seems likely that even thyroid hormone thermogenesis emanates from brown adipose tissue, due to thyroid hormone stimulation of the areas in the brain that control brown adipose tissue activity (Sjögren et al., 2007; Lopez et al., 2010; Cannon and Nedergaard, 2010).

Acute cold exposure does not test nonshivering thermogenic capacity

The metabolism of mice is often examined using a cold tolerance test. In this test, the mice are acutely moved from their normal thermal environment (often ~20°C) into the cold (4°C) (Fig. 6). The mice may be able to cope with this challenge, or they may

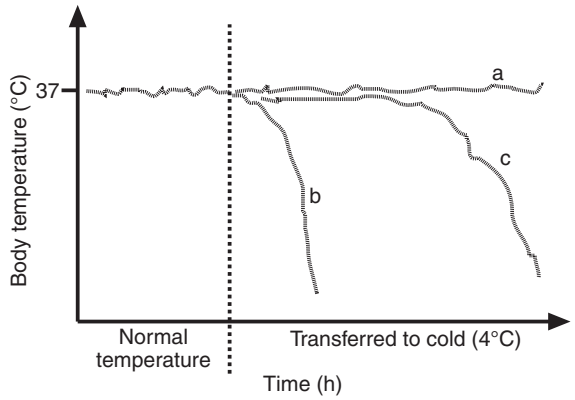

Fig. 6. Typical results of a cold tolerance test. At the indicated time, animals are transferred from normal ambient temperatures (often ~20°C) to cold temperatures (4°C). The animals may either be able to defend their body temperature indefinitely (a) or they may immediately (b) or after some time (c) succumb to the cold. In an acute situation such as this, the extra heat needed comes mainly from shivering, so this experiment mainly tests shivering endurance; however, factors such as animal insulation, heart and lung performance and delivery of substrate (e.g. fatty acids) from the white adipose tissue to the muscle may also be limiting for the cold tolerance. Therefore, this test does not explicitly examine the capacity for adaptive nonshivering thermogenesis, a process that takes weeks in the cold to develop.

immediately or successively succumb to the cold (Fig. 6). This test indeed tests the cold tolerance of the mice, but does not examine nonshivering thermogenesis capacity, despite many implications of this in the literature.

What it really tests is dependent on the previous thermal history of the animal, which determines the contribution of brown fat thermogenesis to total thermogenesis, and on the ability of an animal to elevate and maintain its total metabolism at the level needed to survive at the exposure temperature, through shivering. Regardless of whether an animal has brown adipose tissue, it must nonetheless elevate its total metabolism to the same extent in order to defend its body temperature. There are statements in the literature that imply that warming an animal through shivering thermogenesis should in some way be more energetically costly than heating it by nonshivering thermogenesis. This suggestion is difficult to reconcile with thermodynamics, and we are unaware of any experimental demonstration of this phenomenon. Indeed the metabolic rates of mice that produce their heat through shivering or nonshivering thermogenesis are identical (Golozoubova et al., 2001; Meyer et al., 2010).

In a cold tolerance experiment, a fraction of the metabolic increase may be from brown fat and the remainder from shivering, or it may all derive from shivering. If the animal fails to maintain body temperature, it can be for any of a variety of reasons. The failure could indeed indicate an inadequacy in brown fat, but equally well an inadequacy in the ability to maintain shivering (i.e. that there is a muscle problem), or that the heart or lungs are unable to meet the challenge of such a high elevation of metabolic rate.

A further confounding issue with such a test, if it is used to investigate the significance of a particular gene in genetically modified mice, is that the gene of interest may alter the insulation of the mouse. As shown in Fig. 3, insulation determines the magnitude of heat loss at a given temperature. If the gene of interest has actually made the fur more sparse, the mouse will, in practice, be exposed to a greater cold challenge and may cool more quickly than the wild-type mice. This could have been interpreted to mean that the gene of interest impairs brown fat thermogenesis but, as will be understood, this is clearly an inadequate interpretation.

The significance of thermal prehistory for cold tolerance

The outcome of a cold tolerance test is much influenced by the thermal prehistory of the mice. We can compare two such prehistories. If a (wild-type) mouse is first maintained at its thermoneutral temperature, approximately 30°C, and is then acutely transferred to 5°C, it will have to increase its metabolism immediately three- to fourfold (see Fig. 1). Some time later its body temperature (and thus its metabolism) may decrease (Fig. 2). Thus, the cold challenge is too great for the mouse to cope with: its ability to maintain a level of shivering that can counteract the cold for a prolonged period is insufficient. This can be interpreted in the way that at thermoneutral temperatures, the animal develops little or no brown adipose tissue. Consequently, on exposure to temperatures below thermoneutral, it will be entirely dependent on shivering thermogenesis for heat production. Constant shivering requires muscles with a capacity for constant endurance activity. Should this endurance ability fail, the animal has no other means to defend body temperature and its body temperature will decrease (principally as illustrated in Fig. 2).

However (Fig. 7), if an animal is maintained at temperatures below thermoneutrality, e.g. normal animal house temperatures, for a prolonged period, it will recruit brown adipose tissue and UCP1 to the extent required to compensate for the temperature challenge represented by these temperatures. Therefore, at 23°C, a mouse will have a metabolic rate constantly ~60% above its basal metabolic rate but will not shiver, because the capacity of its brown adipose tissue has become equivalent to this demand. When such an animal is transferred to 5°C, it will keep full activity in its brown adipose tissue. However, this capacity will be insufficient, as it is adequate only for 23°C. Therefore, the animal will also shiver at a level necessary to compensate for the remainder of the cold demand (Jansky et al., 1967). It thus has available the limited brown adipose tissue capacity plus its total shivering capacity. This means that it only needs to use a fraction of its shivering capacity and does not overtax this system. It can therefore cope with this sudden cold challenge. This illustrates one ecological advantage of developing brown fat thermogenic capacity: the ability to be prepared for successively decreasing temperatures.

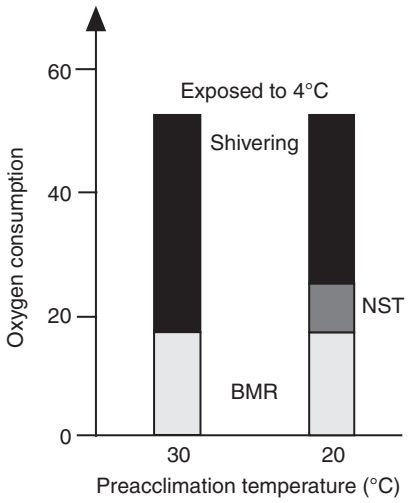

Fig. 7. Effect of thermal prehistory on shivering demand. Animals pre-exposed to temperatures below thermoneutrality will develop a capacity for nonshivering thermogenesis (NST) adequate for that temperature. When the animals are acutely exposed to 4°C, the demand for shivering to compensate for the rest of the heat loss at 4°C is therefore reduced. Such animals will therefore manage better in a cold tolerance test (Fig. 6). Theoretical figure based on the data shown in Fig. 1.

Norepinephrine injection as a test to determine nonshivering thermogenic capacity: certain limitations

Because it is norepinephrine that activates nonshivering thermogenesis, one means to evaluate the nonshivering thermogenic capacity of an animal is to treat it acutely with norepinephrine to mimic activation of the sympathetic nervous system (Fig. 8). Depending on the previous history of the animal, the magnitude of the response will vary.

Under physiological circumstances, when an animal is exposed to cold, it will attempt to activate whatever brown fat it possesses. This is mediated by activation of the sympathetic nerves that directly innervate the brown adipose tissue depots (Foster et al., 1982). This is thus not a generalized sympathetic activation but a highly localized one (Cannon and Nedergaard, 2004). However, when norepinephrine is injected into an animal, a concentration must be given that is sufficiently high to mimic the local synaptic concentration (Depocas et al., 1980). This results in all the cells of the animal being bathed in a high amount of norepinephrine. As essentially all cells possess adrenergic receptors that are coupled to

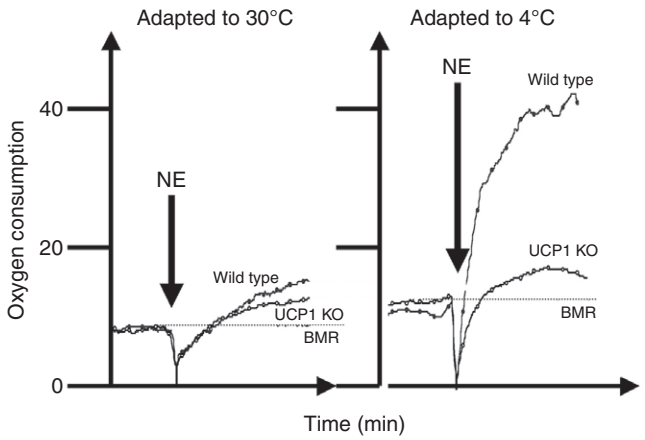

Fig. 8. Effect of cold acclimation on the thermogenic response to norepinephrine (NE). NE was injected into wild-type mice (which can produce heat in their brown adipose tissue) and into UCP1-ablated mice (UCP1 KO) (which are unable to do this); the mice were acclimated to 30 or 4°C for at least 1 mo. There was no effect of the presence or absence of UCP1 with regard to the basal metabolic rate before norepinephrine injection. Acclimation to cold led to some increase in basal respiration (probably related to the effects of the several-times larger food intake in these mice). Cold acclimation had no effect on the response to NE in the UCP1 KO mice. This means that the heat production elicited by NE injection in these animals is not adaptive; it constitutes what we refer to as the 'pharmacological' effect of injected NE on all cells in the body. Only in the mice that possess UCP1 does acclimation to cold result in an increased response to NE. Only the respiration/heat production in excess of that seen in the UCP1-ablated mice constitutes adaptive adrenergic thermogenesis. It corresponds to the development of adaptive nonshivering thermogenesis, and the increase due to cold acclimation represents the recruitment of brown adipose tissue (i.e. the mice get more brown-fat cells, with more mitochondria and more UCP1) [V. Golozoubova, B.C. and J.N., unpublished observations, performed principally as described in Golozoubova et al. (Golozoubova et al., 2006)].

metabolic responses of some type, an elevation of metabolism will ensue that is independent of brown fat and that does not occur under physiological circumstances. This response can therefore be seen as a purely pharmacological response and does not demonstrate any adaptive responsiveness. It leads to an overestimation of nonshivering thermogenic capacity because its magnitude can only be accurately evaluated in mice with a genetic ablation of UCP1. The magnitude of the adrenergic response in animals that have been housed at their thermoneutral temperature is a fairly close approximation (Fig. 8) (Golozoubova et al., 2006) but nonetheless some UCP1 activity is present even at thermoneutral temperatures, the amount being dependent on, for example, diet (Feldmann et al., 2009).

It is important that the norepinephrine injection experiments are performed at thermoneutrality (~30°C for mice) for two reasons. One is that at any temperature below thermoneutrality, nonshivering thermogenesis and/or brown adipose tissue activity is already (partially) activated, and the true capacity for norepinephrine-induced thermogenesis will therefore be underestimated. The other is that at lower temperatures, animals often show what has been called 'paradoxical' effects of norepinephrine injection: their body temperature and metabolism decrease (Zylan and Carlisle, 1991; Zylan and Carlisle, 1992), apparently due to alterations in peripheral blood flow (Carlisle and Stock, 1993). (In animals with an extremely high capacity for nonshivering thermogenesis and with a good insulation, such a high heat production may be induced by norepinephrine that the animal becomes hyperthermic, as it cannot dissipate heat, and then this type of experiment cannot be undertaken.)

Nonshivering thermogenic capacity can be determined in awake, non-anaesthetized animals (Jansky et al., 1967), but such experiments tend to show higher inter-animal variability and, therefore, require more animals to achieve statistical significance (Golozoubova et al., 2006). Principally, an acute stress response is induced by the injection itself, in addition to the direct norepinephrine-induced thermogenesis.

To improve the reproducibility of the measurements and decrease the number of animals required, anaesthetized animals can be studied. It is not possible to use inhalation anaesthetics as these inhibit brown fat activity (Ohlson et al., 1994); agents of the barbiturate type are normally used. The anaesthetized animal is placed in a small-volume measuring chamber at a temperature a few degrees higher than thermoneutral (33°C is needed for a mouse), in order to maintain its body temperature (Golozoubova et al., 2001). After an adequate period of measurement to estimate the basal metabolic rate, the animal is removed and injected with norepinephrine and returned to the chamber. The metabolic rate will rise and plateau (Fig. 8). The increase over basal is the nonshivering thermogenic capacity plus the pharmacological response to norepinephrine. Basically, norepinephrine tests can therefore only be used to compare the difference in magnitude of the response between different conditions (e.g warm- and cold-acclimated animals); the absolute magnitude of nonshivering thermogenesis cannot be obtained by this method in itself.

Adrenergically induced thermogenesis is not (necessarily) nonshivering thermogenesis

It is important to distinguish between adrenergic thermogenesis and nonshivering thermogenesis. There are many investigations in which the effect of injected/infused norepinephrine (or other adrenergic agents or norepinephrine stimulation of isolated organs) is presented as a thermogenic effect in a way implicating that this is a mechanism used by the animal to defend its body temperature, i.e. is a thermoregulatory thermogenesis. In general, this is probably not the case.

It is no surprise that different organs display increased oxygen consumption (thermogenesis) when stimulated with norepinephrine. In these organs, the cognate metabolic processes are stimulated, and any such stimulation leads to thermogenesis. Thus, norepinephrine stimulation of the salivary gland leads to increased oxygen consumption (Terzic and Stoji, 1990), as does stimulation of the liver (Binet and Claret, 1983). These reactions have never been discussed to represent thermoregulatory thermogenesis; the heat is simply a by-product of the increased metabolism related to increased secretion, etc. Only because muscle is traditionally discussed as being a thermogenic organ are similar adrenergically induced responses in muscle discussed as representing a form of thermoregulatory thermogenesis. There is presently no reason to think that the increase in oxygen consumption observed during adrenergic stimulation of muscle is principally of another nature than that observed after stimulation of the salivary gland or liver, and just as norepinephrine stimulation of oxygen consumption in the salivary gland or liver does not require a specific 'thermogenic' mechanism (because it is only thermogenic as a metabolic consequence, not as a purpose), neither does stimulation in muscle.

Importantly, these brown-fat-independent types of adrenergic thermogenesis have never been shown to be adaptive. This means

that they are not recruited during acclimation to cold or adaptation to diet, and they are therefore not part of a thermoregulatory process. Particularly in humans, there are many results from studies using infusions of adrenergic agents and measurements of oxygen consumption (Blaak et al., 1993) to discuss 'adrenergic thermogenesis'. These studies are, for the reasons stated above, probably not relevant for the type of thermogenesis discussed here, i.e. thermoregulatory nonshivering thermogenesis or diet-induced thermogenesis. To our knowledge, there are no indications that this thermogenesis is adaptive. Additionally, there is the problem that the adrenergic concentrations achieved during infusion, particularly in humans, may be so low that only a hormonal action of adrenergic agonists is induced; i.e. the levels may not be high enough to reach the postsynaptic areas in a sufficiently high concentration. In that case, brown adipose tissue may not be stimulated at all.

The problem with the pharmacological response to norepinephrine can to some extent be overcome by using a specific β_3-adrenergic agonist, notably CL-316,243. As β_3-adrenergic receptors are only found in high density in adipose tissues, and as white adipose tissue is nearly inert with respect to oxygen consumption, the response seen would mainly emanate from brown adipose tissue, i.e. there is practically no 'pharmacological' response. However, the response may not represent the true capacity of brown adipose tissue because β_3- and β_1-adrenoreceptors may be needed to elicit the total β-adrenergic response, and there may be an α-adrenergic component (Mohell et al., 1983) that works through augmentation of the β-adrenergic component (Zhao et al., 1997). Thus, only with norepinephrine is it certain that the entire thermogenic response is induced.

The difficult point of the divisor

Metabolic chambers measure the rate of oxygen consumption, and the outcome is thus in litres of oxygen per unit time. This is an approximation of the total heat production but, because the thermal equivalent of an oxygen molecule is different when carbohydrate or fat is combusted, conversion factors depending on the respiratory quotient should be used to convert the oxygen consumption values to energy (W). This is particularly important if the food composition is changed (from carbohydrate to fat) or during day-and-night measurements when the animals change from burning a mixed diet (active phase) to burning stored fat (inactive phase).

Total metabolism	90	100	120
Weight	80	100	200
Metabolism/weight	1.13	1.00	0.6

Fig. 9. The problems occurring by expressing metabolism per kg body weight. The animals symbolized have similar amounts of normal tissue (black) but different amounts of white adipose tissue (grey). Compared with the 'normal' animal in the middle, the lean animal to the left has a slightly lower total metabolism and the obese animal to the right has a slightly higher metabolism. However, when these values for whole-animal metabolism are divided by the respective body weights, exactly the opposite impressions are obtained – the lean animal appears to have an increased metabolism and the obese animal a decreased metabolism – and the final erroneous conclusion becomes that the animals have become lean/obese due to these altered metabolic rates. Thus, to express metabolic rates per body weight (or to any power of body weight) leads to misleading conclusions.

In studies of all types of metabolism, there is one major difficulty in interpretation and representation of the results: the denominator or the divisor, i.e. how the results should be expressed. If the animals are of the same size and body composition, there is no problem, but very often this is not the case. It may initially seem natural to express metabolism per gram body weight; however, in reality, animals are often studied that have become obese, e.g. due to a diet intervention or a genetic alteration. Such animals may have identical amounts of active (lean) tissue but are carrying expanded amounts of lipid around in their white adipose tissue (Fig. 9). Lipid as a chemical is totally metabolically inert, and in no way contributes to metabolism. However, if the metabolic rate is expressed per gram body weight, and one animal carries extra weight in the form of lipid, the metabolic rate expressed in this way will appear smaller in the obese animal. Thus, we have an 'explanation' for the obesity: 'it is due to a decreased metabolism'. This is evidently not an adequate description. By contrast, if a treatment leads to leanness, the lipid carried around is less, the divisor is smaller and thus we have an explanation for leanness: enhanced thermogenesis (Fig. 9). Although these considerations would seem banal, the literature overflows with results calculated this way and conclusions based on these results. The problem has been repeatedly addressed, but still seems to persist (Himms-Hagen, 1997; Butler and Kozak, 2010).

In an apparently more advanced way, metabolic rates and thermogenic capacities can be expressed per gram body weight raised to some power. Most often the conversion is to grams raised to the power 0.75. Firstly, evidently this in no way eliminates the problem discussed above; lipid is still inert even if raised to any power. Secondly, the power 0.75 has not been established for a single species, but is an allometric factor used when metabolic rates are compared between animals whose body weights are orders of magnitude different (e.g. mice and elephants). It turns out that the metabolism increases in proportion to the body weight to the power 0.75 (the reason for this is still unknown). For mathematical reasons, the power raising makes nearly no difference if, for example, mice with only somewhat different body weights are compared, and it should therefore only be used for comparisons between species.

Occasionally, the power 0.66 is used instead. This is the geometrical relationship between the surface area and the volume (weight) of a sphere or cube. The power relationship is of significance if thermal balance is discussed – but to use it to express rates of metabolism implies that all metabolism is due to heat loss to the surroundings, which is of course not the case. (The difference between the powers 0.75 and 0.66 is the reason why animals that are small or large experience the same ambient temperatures differently: 23°C is cold for a mouse but is hot for an elephant.)

What, then, is the solution to the dilemma of the divisor? The easiest – and in most circumstances most correct – solution is simply to give the results as per animal. A more sophisticated, and on occasion advantageous, solution is to express the rate per gram lean body mass. Lean body mass is obtainable with dual-energy X-ray absorptiometry (DEXA) or magnetic resonance imaging (MRI), but in most obese/lean comparisons, it is only the lipid amount that is changed, and in this case the results are almost identical to expressing the rates per animal. (Even to express the metabolic rates per gram lean mass assumes that all lean mass in the body has an equal metabolic rate. This is not the case; therefore, although lean mass is a better approximation, it is not without its own problems.) An even better solution is to examine the animals before any marked difference in body weight between the wild-type/control group and

the experimental group has occurred. After all, if the modification studied should be causative of the development of obesity (or protection against obesity), the altered metabolic rate should be present before the new phenotype becomes evident.

Diet-induced thermogenesis

Brown adipose tissue is an admirable defence mechanism against cold. It has an impressively high oxidative capacity and thermogenic activity per gram of tissue and provides chronically cold-exposed mammals with a comfortable means of defending body temperature. As pointed out above, in its absence, shivering will function but shivering is notably less comfortable than nonshivering thermogenesis and will impose restrictions on the animal's freedom of movement.

Some 30 years ago, it was observed that a nonshivering thermogenic capacity could also be recruited by exposing rodents to so-called cafeteria diets or, later, to high-fat diets (Rothwell and Stock, 1979). The mechanism of recruitment of brown adipose tissue under these conditions has not been clarified but it presumably involves activation of the sympathetic nervous system either directly by components in the diet (as has been the general view) or secondarily to the developing obesity as such. It was proposed that animals that could develop brown adipose tissue in this way could use its thermogenic capacity to combust excess energy in the diet and thus not become as obese as otherwise expected. Extensive studies by many groups have supported this view (Cannon and Nedergaard, 2004) (but see Maxwell et al., 1987; Kozak, 2010). The magnitude of the increase in metabolic rate induced by injected norepinephrine is enhanced following dietary treatment, in a manner similar to that following cold acclimation (i.e. classical nonshivering thermogenesis) (Rothwell and Stock, 1980; Feldmann et al., 2009; Feldmann et al., 2010). The increases seen are smaller, but it would seem to be an adaptive process, as is classical nonshivering thermogenesis. It must be emphasized that this phenomenon ('metaboloregulatory thermogenesis') is only clearly observable in animals living at thermoneutrality (Feldmann et al., 2009), because at lower ambient temperatures, thermoregulatory thermogenesis will dominate metabolism. (Also note that this metabolic increase is in addition to that caused by the direct metabolic costs of digesting the food.)

Whereas the purpose of classical nonshivering thermogenesis is clear, that of diet-induced thermogenesis is not equally evident. Diet-induced thermogenesis would seem to be an irrational process because animals 'should' not have developed a system to wastefully expend such a rare commodity as energy. There are indications that the magnitude of diet-induced thermogenesis is related to the protein content of the diet. An adequate explanation for the development of diet-induced thermogenesis was proposed by Stock: if diets with inadequate protein (or another essential nutrient) content – i.e. unbalanced diets – were eaten to the extent that sufficient protein was ingested, a system had to exist to remove the excess of energy that this extra ingestion had incurred (Stock, 1999). This system would thus be brown adipose tissue. Good experimental evidence for this hypothesis is still lacking.

Influence of UCP1 ablation on obesity development

If an animal does use brown fat thermogenesis to regulate its amount of stored body fat, it would be reasonable to assume that in the absence of active brown fat (such as in an animal lacking UCP1), the animal would become obese, provided it maintained the same energy intake. It was therefore initially surprising (perhaps even disappointing) that the UCP1-ablated mice did not develop obesity (Enerbäck et al., 1997). However, later studies performed in mice housed at their thermoneutral temperature showed a development of obesity even on a regular chow diet, and to a greater extent on a high-fat diet (Feldmann et al., 2009). This indicates that even the very small amount of UCP1 present in the wild-type mice at thermoneutrality is actually effective in modulating body fat content, its absence is not compensated by other means, and the absence is sufficient for obesity to occur.

Animals living at their thermoneutral temperatures are not under any cold stress and, therefore, clearly do not have UCP1 and brown fat for this reason. Brown fat is classically recruited in parallel with decreasing ambient temperatures. The presence of some active brown fat even at thermoneutrality can be taken to indicate that it indeed has a physiological function. Surprisingly, mice without UCP1 are protected against diet-obesity when studied under normal animal house conditions. The reason for this is still not clarified but this is not a unique outcome for UCP1-ablated mice. Even mice with UCP1 (i.e. wild-type mice) are protected against obesity if they are placed in a cold environment; however, the degree of cold needed for this protection is higher for wild-type than for UCP1-ablated mice (Cannon and Nedergaard, 2009).

The risk of false positives

Perhaps the most important reason to acquire a thermal understanding when approaching studies of metabolism is to not be

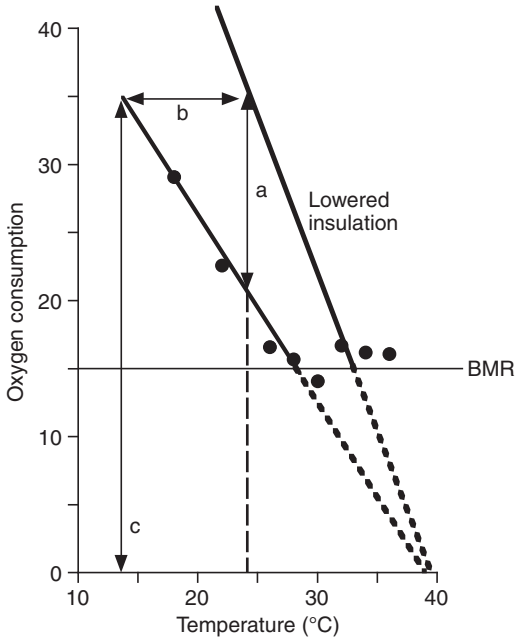

Fig. 10. The risk of false positives. If, for example, a mutant mouse has fur with a decreased insulation, the slope of the thermal control curve becomes higher. If this mouse is only maintained and examined at normal temperature (here 24°C), it will display a higher metabolism (a) than the control. The mutant thus appears to be hypermetabolic. In reality, it feels much colder than the wild-type mouse, i.e. it feels like the wild-type mouse would feel if it were shifted to a lower temperature where the same metabolism would be needed (b); that is it feels as if it were at 14°C (c). It will therefore display all the features expected of mice at 14°C, e.g. recruited brown adipose tissue, protection against diet-induced obesity and the 'britening' of white adipose tissue (UCP1 expression in white adipose tissue). All of these effects are, however, secondary to the animal feeling colder and will disappear if the mouse is kept and examined at thermoneutrality. Such examinations are rarely performed.

misled by false positive observations and thus to invest scientific time and effort in metabolic phenomena that are secondary to thermal regulation rather than to truly altered metabolism.

If an animal of interest (a genetically modified or drug-treated animal) is housed at 'normal' temperature, a situation as that indicated by 'a' in Fig. 10 may be encountered. As seen, the mouse of interest for thermoregulatory reasons necessarily shows an increased metabolism (a) (thermogenesis), which (wrongly) suggests that it has an enhanced metabolism due to some metabolic pathway being modified. Indeed, such a mouse is likely to show decreased metabolic efficiency (it eats more to gain the same weight), be protected against diet-induced obesity, have activated brown adipose tissue and show activated 'brite' adipose tissue [i.e. the appearance of UCP1-containing cells in white adipose tissue depots (Xue et al., 2005; Petrovic et al., 2010; Waldén, 2010)]. All of these observations would undoubtedly be formally correct, but when the thermoregulatory responses of the mouse are examined, these results become trivial in the sense that they are all consequences of decreased insulation. Thus, as indicated in Fig. 10, as the thermoregulatory slope is higher, the mouse would feel thermoregulatorily (b) as if it were exposed to 14°C (c), although it is at 24°C. In this example, all differences would thus be ascribable to the expected effects of feeling colder.

The fact that this is more than a theoretical situation has been demonstrated several times recently. For example, a mouse without the fatty acid elongase Elovl3 demonstrated the above characteristics and was experimentally shown to have decreased insulation (Westerberg et al., 2004). Even simple inspection of its visual appearance indicated skin/fur problems, and the increased metabolism disappeared at thermoneutrality. Similarly, the global absence of stearoyl CoA dehydrogenase 1 (SCD1) leads to this type of apparently hypermetabolic phenotype (Ntambi et al., 2002); however, it turns out that if the knock-out of SCD1 is restricted to the skin, exactly the same phenotype is found as in the global knockout (Sampath et al., 2009), and both the global and the skin-restricted phenotypes show visually observable skin phenotypes. Thus, the metabolic changes can be explained by the altered skin phenotype, the resultant increased heat loss and the effects of this resultant increased metabolism. This is thus not an 'intrinsic' metabolic phenotype, it is a simple metabolic consequence of loss of insulation.

Other genetically modified mice have also been shown to exhibit changes in fur and skin properties together with resistance to diet-induced obesity; these include the global knock-out of acyl coenzyme A:diacylglycerol acyltransferase 1 (DGAT1) (Smith et al., 2000; Chen et al., 2002), the global knock-out of the vitamin D receptor (Xie et al., 2002; Narvaez et al., 2009) and mice overexpressing the human apolipoprotein C1 gene (Jong et al., 1998; Jong et al., 2001). Again, it is unlikely that these modifications truly affect intrinsic metabolism; rather, the outcome is due to thermoregulatory thermogenesis. In addition, mice that lack the thyroid hormone receptor α show an increased metabolism, etc. at 22°C but not at thermoneutrality (Marrif et al., 2005; Pelletier et al., 2008). The most probable explanation for this is again an insulation problem (although the authors propose another mechanism). These are just a few examples of mouse models demonstrating increased thermogenesis that, after thermal examination, prove to be likely ascribable to skin/fur alterations. It is likely that a number of recently published metabolic phenotypes where, for example, activation of brite adipose tissue has been demonstrated may in reality be due to alterations in insulation. As these mice have not been examined after housing at thermoneutrality, it cannot be excluded that they demonstrate a thermoregulatory phenotype rather than the metaboloregulatory phenotype advocated. Thus, we would suggest that concerning many of these genetically modified mice, the metabolic effects observed (resistance to diet-induced obesity in combination with higher metabolism at normal animal house temperatures and activation of brown and brite adipose tissues) are the same that would be observed by e.g. simply shaving the mice. The phenotype is thus mainly or fully secondary to the loss of insulation.

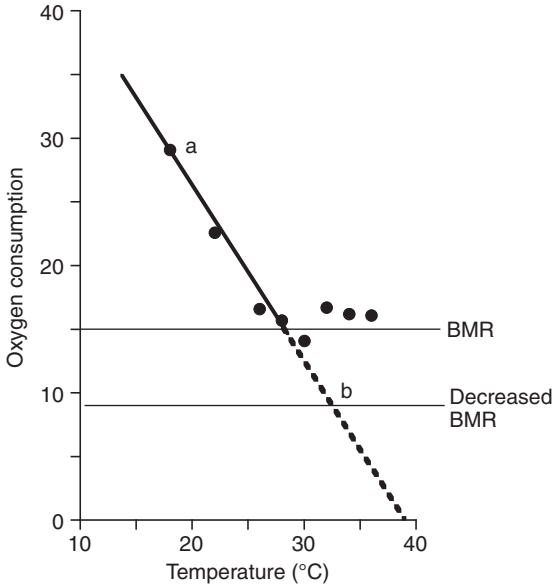

Fig. 11. The risk of false negatives. If a (mutant) animal truly has a decreased intrinsic metabolic rate but unchanged body temperature and insulation, it will not display a metabolic rate different from the wild type if kept and examined at normal temperatures (a). Only if examined so as to establish the thermoneutral zone of the animal will the decreased metabolism become evident (b). This is the case for thyroid receptor null mutants (Golozoubova et al., 2004). It is likely that many mutants (or treatments) with true effects on intrinsic metabolism have been overlooked because they have only been examined under conditions where their metabolic rate is controlled by the ambient temperature.

The risk of false negatives

A scientifically equally disturbing problem – or perhaps an even larger problem – is that housing animals at normal temperatures may mask true metabolic phenotypes. As seen in Fig. 11, a mouse living at normal temperatures may show an identical metabolism to the wild-type (for thermoregulatory reasons) but at thermoneutrality may demonstrate a reduced metabolism. There is also the possibility that although the basal metabolism is not altered, increases in metabolism caused by food or other treatments will not be visible because the extra heat produced is fully 'used' by the animal for thermoregulation and is therefore not metabolically revealed.

One clear example is the UCP1-ablated mouse that does not, as noted above, demonstrate an obesity-prone phenotype when kept at normal temperature (Enerbäck et al., 1997; Liu et al., 2003). However, when thermoregulatory metabolism is switched off when the mice are housed at thermoneutrality, the lack of metaboloregulatory thermogenesis becomes evident and the mice become obese (Feldmann et al., 2009; Feldmann et al., 2010). There are other examples of thermoregulatory thermogenesis resulting from keeping the mice at normal animal house temperatures obscuring

alterations in intrinsic metabolism. Thus, mice that lack the mitochondrial glycerol-3-phosphate dehydrogenase gene (DosSantos et al., 2003) or have a mutated thyroid hormone receptor α1 (Sjögren et al., 2007) show distinctly different phenotypes when housed at normal animal house temperatures and at thermoneutrality.

In a broader sense, mice at thermoneutrality may be said to become more humanized in that, for example, their control of heart activity changes from being sympathetic to being vagal (Swoap et al., 2008), allowing for clear identification of effects of a thyroid hormone receptor mutation (Mittag et al., 2010). Also, effects of pyrogens are only evident at thermoneutrality, not at normal room temperatures (Rudaya et al., 2005). In addition, the serum lipid pattern becomes closer to those of humans (data not shown). As studies on mice are often performed to mimic a human situation, it is clearly most appropriate to house mice at thermoneutrality.

Few genetic modifications and few agents developed for metabolic control have been examined in animals living at thermoneutrality. Therefore, there is a great risk that several important candidate genes and several promising agents have been overlooked and dismissed as a result of the experiments being performed under conditions where metaboloregulatory processes are overshadowed by thermoregulatory processes.

Thermoneutrality is the preferred temperature

Mainly for practical reasons and for the comfort of the animal house personnel, animal house temperatures are normally in the range of 18 to 20°C. To maintain mice at ~30°C appears at first sight to many people to be exposing them to a heat challenge. However, this is not the case. This is most evident if the mouse is allowed to choose its thermal environment. In experiments with temperature gradients, the mice always choose temperatures of approximately 30°C (Gordon et al., 1998). Similarly, if a mouse is given the direct choice between an environment at 20°C or at 30°C, it chooses 30°C [see e.g. wild-type mouse behaviour in supplementary video 1 in Bautista et al. (Bautista et al., 2007)]. Thus, mice, just as humans, prefer to live under conditions of thermoneutrality.

Perspectives

The attempts to humanize mice to gain insights into human metabolism have acquired a much broader significance during the last few years. For decades, it has been assumed that adult humans do not possess active brown adipose tissue, and studies of nonshivering thermogenesis have, therefore, been seen as being only of academic interest. The insight that a significant fraction of adult humans possess brown adipose tissue (Nedergaard et al., 2007; Nedergaard et al., 2011) – and that a significant fraction lack brown adipose tissue – means that the study of nonshivering thermogenesis and its adequate measurement have now become of significance for the understanding of human metabolism; indeed, a new metabolic world is opening (Nedergaard and Cannon, 2010).

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