Cell Metabolism
Short Article

UCP1 Ablation Induces Obesity and Abolishes Diet-Induced Thermogenesis in Mice Exempt from Thermal Stress by Living at Thermoneutrality

Helena M. Feldmann,[1] Valeria Golozoubova,[1,2] Barbara Cannon,[1] and Jan Nedergaard[1,*]
[1]The Wenner-Gren Institute, The Arrhenius Laboratories F3, Stockholm University, SE-106 91 Stockholm, Sweden
[2]Present address: LAB Research, Copenhagen, Denmark
*Correspondence: jan@metabol.su.se
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SUMMARY

As original studies of UCP1-ablated mice failed to demonstrate an obesogenic effect, alternative mechanisms for adaptive adrenergic thermogenesis have been sought. However, we demonstrate here that in C57Bl6 mice exempt from thermal stress (i.e., kept at thermoneutrality), UCP1 ablation in itself induced obesity, even in mice fed control diet, and vastly augmented diet-induced obesity (high-fat diet); i.e., the mice exhibited increased metabolic efficiency. In wild-type mice, high-fat diet increased norepinephrine-induced thermogenesis; i.e., diet-induced thermogenesis was observed, but no such effect was observed in UCP1-ablated mice, demonstrating that diet-induced thermogenesis fully emanates from UCP1 activity. We conclude that ambient temperature is qualitatively determinative for the outcome of metabolic studies, that no other protein and no other mechanism can substitute for UCP1 in mediating diet-induced adrenergic thermogenesis, and that UCP1 activity can be determinative for obesity development in mice and possibly in humans.

INTRODUCTION

To combat obesity, essentially two alternatives exist: to decrease energy intake or to increase energy expenditure. Chronic intake of certain diets in itself augments the capacity for increased energy expenditure—the phenomenon referred to as diet-induced thermogenesis. The understanding of the nature of this response is a prerequisite for developing methods for its augmentation as a possible remedy against obesity.

When it was realized that diet-induced thermogenesis was associated with recruitment of brown adipose tissue and enhanced adrenergic thermogenesis (Rothwell and Stock, 1979)—and that genetic obesity was associated with diminished brown adipose tissue capacity and activity (Trayhurn, 1979)—it was thought that diet-induced thermogenesis could be fully explained by adrenergic stimulation of brown adipose tissue activity and was thus fully mediated via the brown-fat-specific uncoupling protein UCP1. Obesity should thus be counteracted by recruitment of diet-induced thermogenesis, and the prediction would then be that in the absence of UCP1, obesity would develop spontaneously.

The expected phenotype of UCP1-ablated mice would therefore not only be that they should be more susceptible to cold (Enerbäck et al., 1997), have to rely on shivering for thermoregulatory thermogenesis (Golozoubova et al., 2001), and lack the ability to recruit adrenergic thermogenesis during acclimation to cold (Golozoubova et al., 2006), all of which have been demonstrated. It was additionally expected that the UCP1-ablated mice should become obese. Thus, it was unexpected—and perhaps even disappointing—that the UCP1-ablated mouse failed to demonstrate an obese phenotype (Enerbäck et al., 1997), an observation later confirmed by Liu et al. (2003); by E.C. Backlund, B.C., and J.N. (unpublished data); and—except under special conditions—by Kontani et al. (2005). The absence of an obesogenic effect of UCP1 ablation also meant that interest in developing a means to recruit brown adipose tissue to combat obesity has successively waned.

Nonetheless, adaptive adrenergic thermogenesis is considered to be closely related to metabolic control in general, especially to questions relating to metabolic efficiency and thus, ultimately, to obesity (Snitker et al., 2000). Indeed, most obesities are associated with low sympathetic activity (Bray and York, 1998), obesity in both rodents (Trayhurn, 1979) and man (Jung et al., 1979) may be associated with a decreased metabolic (thermogenic) response to norepinephrine, and the thermogenic effect of leptin in genetically obese mice is associated with sympathetic stimulation (Collins et al., 1996). The effect of some drugs used to treat obesity seems to be associated with a stimulation of sympathetic mechanisms (Hirsch et al., 2000). Considering the occurrence of these responses to adrenergic stimulation and the reported nonobese phenotype of the UCP1-ablated mouse, questions can be raised as to whether a UCP1-independent alternative adaptive adrenergic thermogenic response to diet could exist.

Such an alternative adaptive adrenergic thermogenesis has been suggested in relation to metabolic control in general and in particular in relation to obesity (Lowell and Spiegelman, 2000; Spiegelman and Flier, 2001; Lowell and Bachman, 2003). The localization of this alternative thermogenesis has been discussed to be, for example, muscle (Block, 1994; Jansky, 1995; Lowell and Spiegelman, 2000; Lowell and Bachman, 2003), particularly in adult man, who until recently (Nedergaard et al.,

2007) has generally been supposed to lack significant amounts of brown adipose tissue and thus of UCP1.

An important but often overlooked issue in metabolic research is the fact that "normal" animal house conditions (i.e., 18–22°C) are a chronic thermal stress to mice. To defend their body temperature, the mice have to increase their metabolism—and thus their food intake—to 50%–60% above basal, day and night (Golozoubova et al., 2004). Indeed, defense of body temperature has high priority, and this demand for extra energy determines the total metabolism of the mice. Thus, the constant thermal stress of normal animal house conditions may have severely influenced the outcome of earlier metabolic studies. We therefore undertook to examine the effect of UCP1 ablation under conditions where thermal stress was eliminated (i.e., at thermoneutrality, ~30°C for mice). This had remarkable qualitative effects on the result: UCP1 ablation in itself became obesogenic even with normal diets, and the obesogenic effect of high-fat diets was much augmented, all presumably due to an inability to recruit diet-induced thermogenesis. These results are important not only for evaluating the significance of UCP1, but in general for the analysis of genotypes in metabolic research. They may also be directly relevant for the understanding of human obesity, given that modern man lives a thermoneutral life and is now acknowledged to possess metabolically active brown adipose tissue.

RESULTS

UCP1 Ablation Induces Obesity

The elimination of thermal stress resulted in remarkable effects on energy metabolism of the mice, particularly with respect to effects of UCP1 ablation. In contrast to what was originally reported (Enerbäck et al., 1997), as well as E.C. Backlund, B.C., and J.N. (unpublished data), the ablation of UCP1 was sufficient in itself under these conditions to cause obesity, even in mice exposed to a control diet (chow) (Figure 1A). The body weight of the UCP1-ablated mice was increased at a rate more than 50% higher than in wild-type ($p < 0.05$). (In independent experiments, UCP1-ablated mice kept for 4 months at thermoneutral temperatures showed a 55% increase in body weight, compared to 30% in wild-type [data not shown]).

Even more dramatic was the effect of UCP1 ablation in mice exposed to an obesogenic diet. In earlier investigations performed at 20°C, UCP1-ablated mice exposed to a high-fat diet were paradoxically protected against its obesity-inducing effect (Liu et al., 2003). However, as seen in Figure 1B, while the obesogenic diet had its expected, pronounced effect on body weight in wild-type mice, the effect in UCP1-ablated mice was much higher, showing a further 50% increase in the rate of weight gain ($p < 0.05$).

The effect of UCP1 ablation was not only manifest as an increase in body weight: in both control-diet- and high-fat-fed mice, body fat depots were significantly higher in the UCP1-ablated mice than in wild-type (Figure 1C and 1D). In an independent experiment, we followed lean mass, fat mass, and adiposity index (fat mass/body mass) in similar mice under similar conditions. As seen in Figure S1, UCP1 gene ablation had no effect on lean mass; i.e., the body weight increase was entirely due to an increase in fat mass, and the adiposity index was accordingly increased in the UCP1-ablated mice on both control and high-fat diets.

The increase in body weight and fat depot size in the control-diet-fed mice could not be explained by an increase in food intake (Figure 1E). Wild-type mice exposed to the high-fat diet did not have a markedly greater energy intake than those exposed to chow but, remarkably, still increased in weight; in mice on this diet, UCP1 ablation led to a small increase in food intake (Figure 1F).

Metabolic efficiency is the hallmark of metabolic control of obesity. It represents the fraction of ingested energy that is saved as extra body energy stores (in contrast to the majority of the energy intake that is metabolized to heat). Metabolic efficiency was substantially higher in the UCP1-ablated mice on both diets (Figures 1G and 1H), fully in agreement with increased metabolic efficiency being the main cause of obesity. Thus, even in the high-fat-fed mice, the increase in energy accumulation was not only a result of an increase in food intake but also due to a large increase in metabolic efficiency.

UCP1 Ablation Abolishes Diet-Induced Thermogenesis

As the alterations in efficiency were not (primarily) due to alterations in energy intake, the effects should be understandable based on alterations in metabolic rates. However, in neither control-diet-fed nor in high-fat-fed mice was there a decrease in resting metabolic rate due to UCP1 ablation (Figures 2A and 2B). Similarly, we did not observe any effect of UCP1 ablation on mean activity (Figures 2A and 2B).

As no differences in these measures were observed that could explain the increased metabolic efficiency, we measured diet-induced thermogenesis. The term "diet-induced thermogenesis" is often used rather vaguely, but we refer here to the increased ability of animals exposed to certain diets to respond to adrenergic stimulation, i.e., "diet-adaptation-recruited norepinephrine-induced thermogenesis" (Cannon and Nedergaard, 2004).

Even in control-fed mice living at thermoneutral temperatures, a thermogenic response to norepinephrine injection was seen (Figure 2C). The response to norepinephrine injection was lower in the UCP1-ablated mice, principally in agreement with earlier observations in mice acclimated to thermoneutral temperature (Golozoubova et al., 2006). A high response to norepinephrine was seen in high-fat-fed wild-type mice (Figure 2D); the response to norepinephrine here was markedly lower in the UCP1-ablated mice (Figure 2D).

In Figure 2E, a comparison of the maximal norepinephrine-induced thermogenic rates from Figures 2C and 2D is made. It is evident that in the wild-type mice, thermogenesis was augmented due to the high-fat diet; the difference—which thus constitutes diet-induced thermogenesis—is depicted in Figure 2F. Thus, even at thermoneutrality, these animals exhibit diet-induced thermogenesis.

The result was dramatically different for the UCP1-ablated mice. For both diets, the norepinephrine-induced thermogenesis was lower than that of wild-type (Figure 2E). Most conclusively, no positive effect of the high-fat diet was seen in these mice (Figure 2F). Thus, no diet-induced thermogenesis could be induced in mice without UCP1.

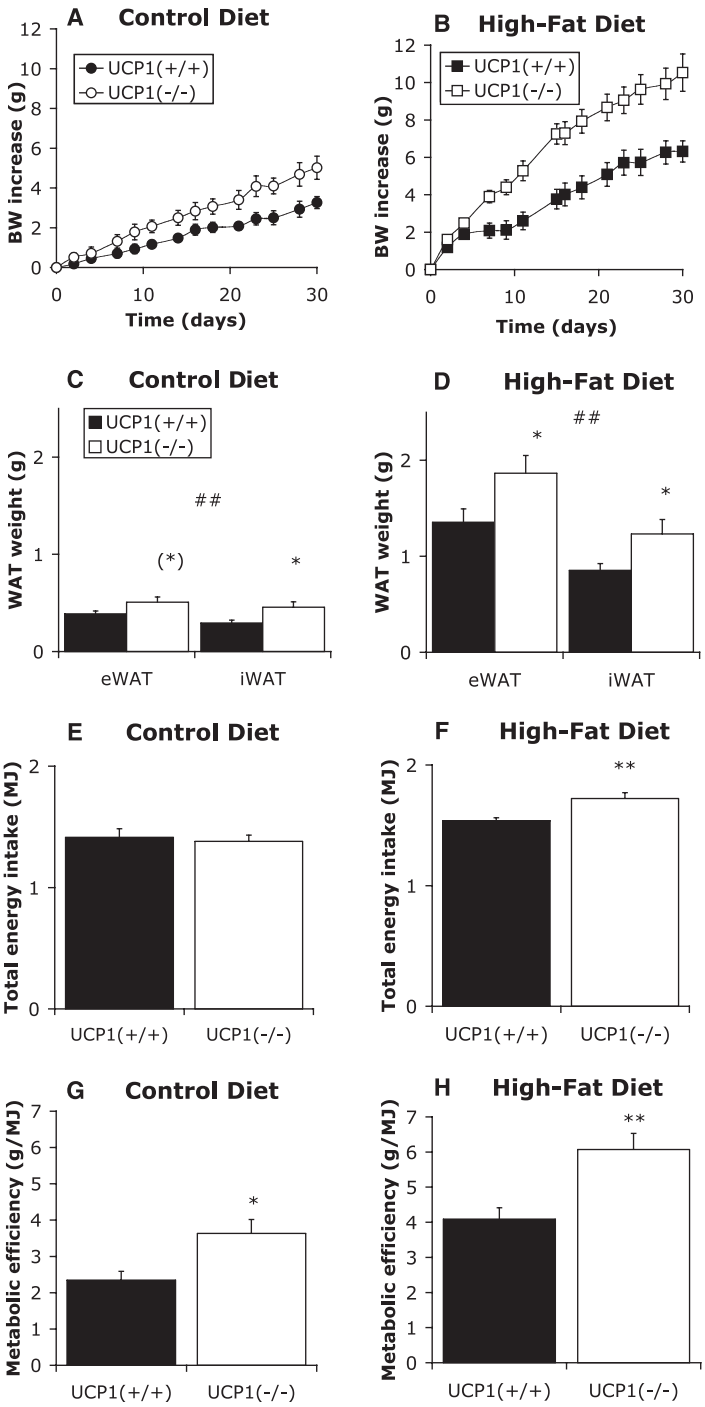

Figure 1. Effect of UCP1 Ablation on Energy Metabolism

(A and B) Body weight (BW) increase of wild-type and UCP1(−/−) mice. Both wild-type and UCP1(−/−) mice on control diet had an initial weight of 23.0 ± 0.3 g (n = 6). Wild-type mice on high-fat diet had an initial weight of 24.6 ± 0.5 g and UCP1(−/−) mice of 24.9 ± 0.1 g (n = 6). Average slope was significantly different ($p < 0.05$) between both wild-type and UCP1-ablated mice for control diet (A) (0.11 ± 0.01 g/day versus 0.16 ± 0.02 g/day) and high-fat diet (B) (0.20 ± 0.02 g/day versus 0.32 ± 0.04 g/day).

(C and D) Epididymal (eWAT) and inguinal (iWAT) white adipose tissue weights. n values for wild-type and UCP1(−/−) are 5 and 5 in (C) and 6 and 5 in (D). (*) and * indicate $p < 0.10$ and ≤ 0.05 between the genotypes. ## indicates $p < 0.01$ between the genotypes when both depot data sets were analyzed with a two-way ANOVA.

(E and F) Total energy intake. Energy intake was recorded for 30 days. n values are as in (A) and (B). ** indicates $p < 0.01$ (Student's t test).

(G and H) Metabolic efficiency. Values are calculated as g body weight gained per MJ food consumed, based on (A), (B), (E), and (F). Values shown in all panels are means ± SEM.

Brown Adipose Tissue Recruitment

We examined how UCP1 ablation and adaptation to the two diets affected brown adipose tissue (Figure 3). UCP1 ablation in itself led to an increase in tissue wet weight (Figure 3A) and total protein content (Figure 3C) in control-fed mice. This is explainable by an increased sympathetic activation of the tissue and has been observed earlier (Fredriksson et al., 2005). There was, of course, no UCP1 in the UCP1-ablated mice, but a 4-fold increase in UCP1 content due to high-fat feeding was observed in wild-type mice (Figures 3G and 3H).

UCP1 and Diet-Induced Thermogenesis

In Figure 4A, we have compiled the relationship between the total UCP1 content of the mice and their basal metabolism, as well as their thermogenic response to norepinephrine injection. As seen, the basal metabolic rate was unaffected by the amount of UCP1, but in the response to norepinephrine, two components could be identified: one independent of UCP1 and one directly proportional to UCP1 content. These components are principally delineated in Figure 4B; the nature of these components will be analyzed in the discussion.

DISCUSSION

The Significance of Thermoneutral Temperature

We find here that simply performing the investigations under conditions where thermal stress is eliminated leads to a drastic alteration in our understanding of the significance of UCP1 for metabolic control. The main reason that UCP1 ablation becomes obesogenic at thermoneutrality is probably that under these conditions there is no longer a requirement for a chronically 50% elevated metabolism. At subthermoneutral environmental temperatures (18–22°C), this extra thermogenesis is needed irrespective of its origin. Thus, if brown-fat-derived heat is available, it will be used; if it is not available (as in the UCP1-ablated mice), other sources (e.g., increased activity or shivering) will be used. Thus, a decrease in the contribution of brown adipose tissue will be compensated by other mechanisms. This should be contrasted to the situation at thermoneutrality, where no heat is needed and thus the brown-fat effect will become evident.

It may particularly be pointed out that in Western society, human life is now lived at thermoneutrality (due to clothes and housing); thus, performing the mouse experiments at thermoneutrality is a way to "humanize" the thermal physiology of the mouse. Indeed, keeping the mice in the thermoneutral zone may humanize their physiology in broader respects. For example, it has classically been considered that mice are poor models of

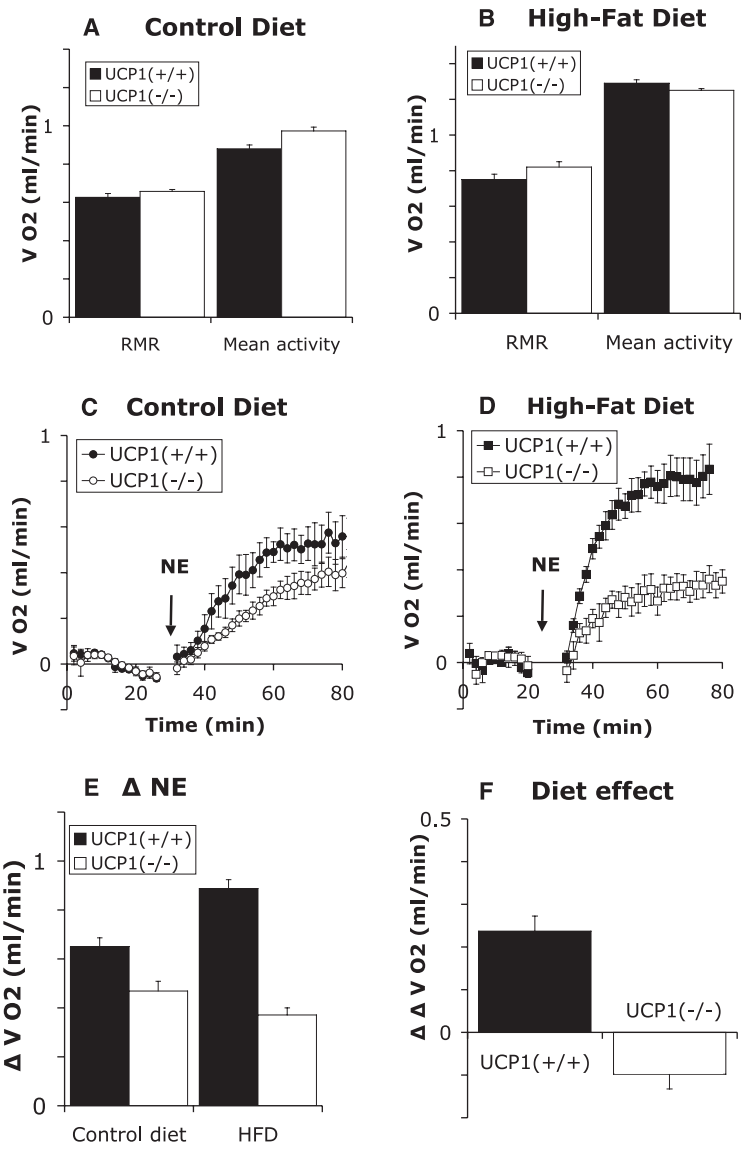

Figure 2. Effects of UCP1 Ablation on Metabolic Rates

(A and B) Resting metabolic rate (RMR) and mean activity. n = 6 and 6 in (A) and 5 and 5 in (B). Values shown are means ± SEM.

(C and D) Norepinephrine-induced oxygen consumption. To facilitate comparisons, responses are expressed as increases over prenorepinephrine (anesthetized) metabolic values. The subtracted value (under anesthesia) is 0.47 ± 0.01 for both wild-type and UCP1(−/−) animals in (C) (n = 5 and 6) and 0.52 ± 0.02 and 0.67 ± 0.02 in (D) (n = 6 and 5). Values shown are means ± SEM.

(E) Mean maximal response to norepinephrine (highest 2 min rate), based on the data in (C) and (D).

(F) Diet-induced effects on response to norepinephrine in wild-type and UCP1-ablated mice. Data are calculated from the compilation in (E); errors given are means of those of the ingoing parameters.

humans with respect to control of heart activity. At rest, human heart rate is under parasympathetic control, but in mice "at rest," the rate is under sympathetic control. The realization that mice are not metabolically resting when they are under the thermal stress of normal animal house conditions has led to a re-evaluation of heart rate control in mice at thermoneutrality and, under these conditions, the heart rate of these mice came under parasympathetic control (Swoap et al., 2008), demonstrating that, in this respect as well, thermoneutrality has a "humanizing" effect.

The significance of this demonstration of the importance of avoiding thermal stress in metabolic studies is not restricted to an understanding of the physiological function of brown adipose tissue and UCP1. Indeed, it can be inferred that phenotypes displaying altered metabolic efficiency may have been overlooked when studying the process at 18–22°C. Only when the thermal drive for heat production to combat heat loss has been eliminated can the true phenotype be revealed with respect to both metabolic and appetite control.

The Three Components of Thermogenesis

Based on the data collected here, we have made a conceptual diagram (Figure 4B) of the thermogenic responses. In this analysis, it is evident that thermogenesis may be separated into three components: basal metabolic rate, norepinephrine-induced UCP1-*in*dependent thermogenesis, and norepinephrine-induced UCP1-dependent thermogenesis. This analysis leads to several important conclusions.

First, it is evident that the presence and amount of UCP1 in the animal has no effect on basal metabolism (values under anesthesia are presented here, but the same conclusion is obtained in awake animals [Figures 2A and 2B]). UCP1 is thus not innately "leaky." The implication is that an increase in the amount of endogenously expressed UCP1, even if induced, for example, pharmacologically, would not in itself alter basal metabolism. This is a conclusion principally in agreement with observations where UCP1 levels have been increased pharmacologically and where the thermogenic effect is observed only after adrenergic stimulation (Sell et al., 2004). These results contrast sharply with those seen in mice where UCP1 is ectopically expressed. In these mice, no adrenergic stimulation is needed to observe an increased metabolism that counteracts obesity (Li et al., 2000). Thus, in ectopic overexpression, UCP1 behaves as an uncontrolled classical uncoupler such as DNP (Caldeira da Silva et al., 2008) and is not under physiological control.

The second component is that of UCP1-*in*dependent norepinephrine-induced thermogenesis. That such a component can be seen experimentally is well known (Enerbäck et al., 1997; Golozoubova et al., 2001, 2006; Granneman et al., 2003). The molecular nature of this response remains enigmatic, but it is understandable as the summation of the effects of pharmacological doses of norepinephrine indiscriminately stimulating all adrenergically responsive cells in the body. What we clearly demonstrate here is that this UCP1-independent response cannot be recruited by diet (Figures 2E and 2F). Thus, it is nonadaptive and cannot be involved in diet-induced thermogenesis. Similarly, it is not recruitable by cold (Golozoubova et al., 2006).

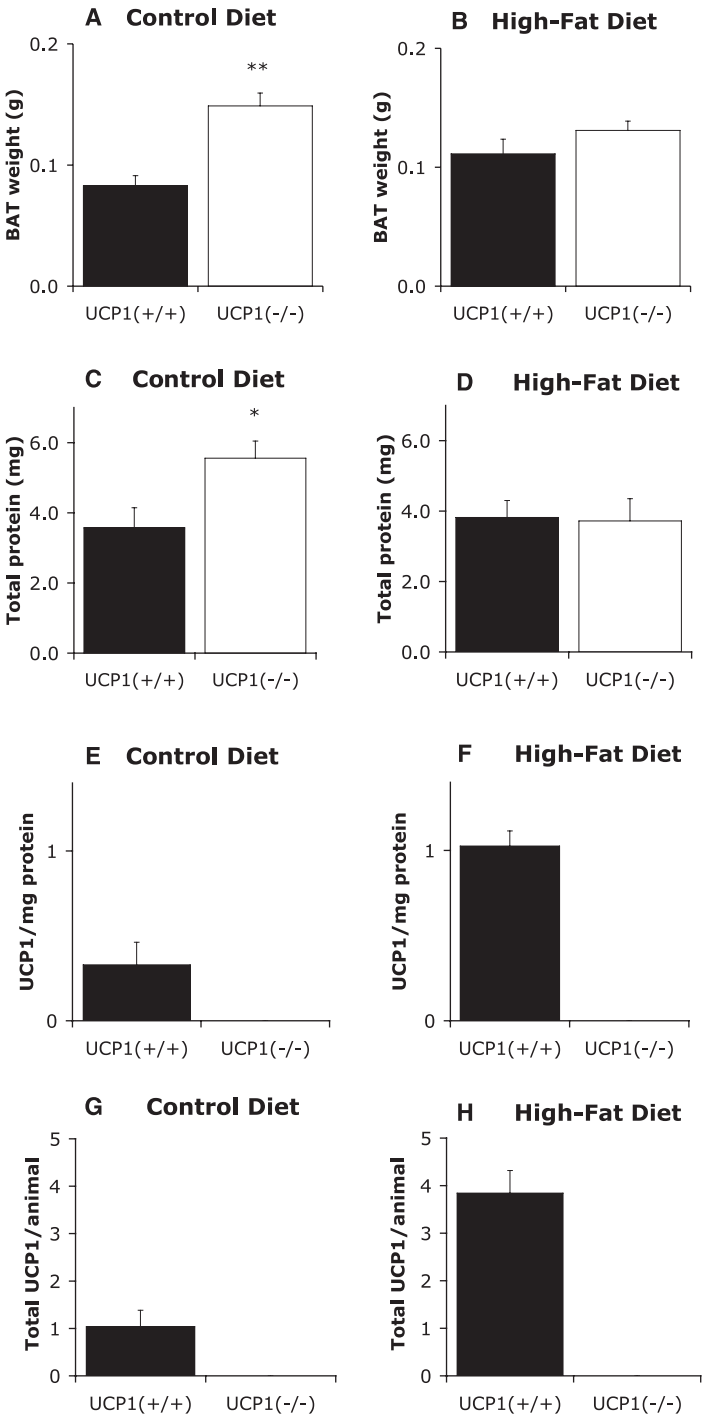

Figure 3. Effect of UCP1 Ablation on Brown Adipose Tissue

(A and B) Wet weight of brown adipose tissue. n = 5 and 5 (A) and 6 and 5 (B).

(C and D) Total protein per brown adipose tissue depot.

(E and F) UCP1 per mg homogenate protein. Values are expressed versus a homogenate standard applied on all gels.

(G and H) Total UCP1 per animal, based on (C) through (F). Values shown in all panels are means ± SEM.

The third component is the UCP1-dependent norepinephrine-induced thermogenesis, i.e., the additional response that is directly proportional to the total amount of UCP1. It can be seen (Figure 4B) that the line obtained from studies of wild-type mice extrapolates exactly to the response in UCP1-ablated mice. Thus, the recruitable adrenergic thermogenesis seen as an effect of different diets, i.e., diet-induced thermogenesis, is fully dependent on the presence of UCP1, and diet-induced thermogenesis is fully mediated via UCP1 activation. There is thus no evidence for any alternative adaptive adrenergic thermogenesis to that mediated by UCP1, even though the existence of such mechanisms has been suggested (Lowell and Spiegelman, 2000; Spiegelman and Flier, 2001; Lowell and Bachman, 2003), and even though there are data on alterations in expression of certain genes in white adipose tissue (Granneman et al., 2003; Ukropec et al., 2006) and muscle (Solinas et al., 2004; de Meis et al., 2005; Martin et al., 2006; Kus et al., 2008) following altered diet. Whereas the gene expression alterations are indisputable, it has been unclear whether these alterations would result in a significant alternative nonshivering thermogenesis. Based on the functional data presented here (Figures 1A, 1B, and 2F), it would seem that the pathways in which such enzymes are found cannot be mobilized as a part of the defense against obesogenic diets nor be part of an adaptive adrenergic thermogenesis. Thus it follows that, at least in the mouse model examined here, no adrenergic diet-induced thermogenesis can emanate from the activity of any protein or mechanism other than that of UCP1 in brown adipose tissue.

Implications for Human Metabolic Control

In addition to the present data being of significance for an understanding of the nature of diet-induced thermogenesis specifically and metabolic control in experimental animals (mice) in general—and thus in the analysis of phenotype manifestations of genotype modifications—there may be specific implications in relation to human metabolic control. Among the important criteria for this is, firstly, that modern humans are practically never exposed to metabolic cold stress, i.e., they are never in situations where their metabolism is dominated by a need to counteract heat loss. In reality, modern humans thus live at thermoneutrality, just as the mice examined here, and for this reason our metabolism should be more similar to that described here than to that of mice normally studied. Second, although it had earlier been believed that active brown adipose tissue is only found in human newborns, recent years have seen an accumulation of unexpected evidence that adult humans also possess active brown adipose tissue (Nedergaard et al., 2007). These depots may vary between individuals, and they may not be very large, but it may still be that in a significant fraction of the population, some degree of obesity/leanness is governed by the amount of UCP1, just as it is in the mice studied here.

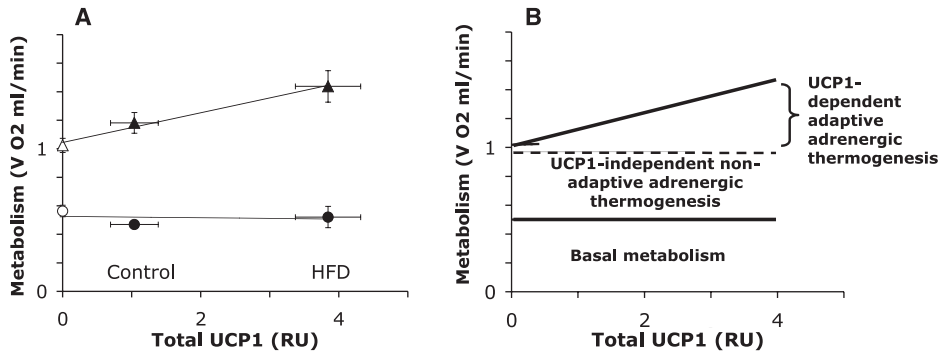

Figure 4. Relationship between UCP1 Amounts and Thermogenesis

(A) Oxygen consumption as a function of UCP1 amount. Values from anesthetized mice before norepinephrine injection are shown at the bottom and the maximum oxygen consumption reached after norepinephrine injection at the top. Values for UCP1(−/−) animals were combined. Filled symbols: wild-type; open symbols: UCP1 (−/−) mice.

(B) Schematic representation of slopes from (A). See discussion for elaboration of concepts. Values shown are means ± SEM.

EXPERIMENTAL PROCEDURES

Animals

UCP1-ablated male mice were derived from those described in (Enerbäck et al., 1997) and were backcrossed to the C57BL/6 strain for ten generations. The mice, as well as the corresponding wild-type mice, were bred at the institute. For each diet, young mice of each genotype were singly caged and housed at 29°C, with a 12/12 hr light-dark cycle, for the duration of the experiment. The control-diet mice had access only to chow (R70, Lactamin), and the high-fat-fed mice had access only to high-fat diet (Research Diets D12451). The experiments were approved by the North Stockholm Animal Ethics Committee. All groups had free access to water and food. Three times per week, body weight and food weight were recorded. The energy content of the food ingested was calculated based on data from the manufacturers of the food.

Indirect Calorimetry

To determine basal/resting metabolic rate (RMR), oxygen consumption was measured by indirect calorimetry (INCA System, Somedic; Hörby, Sweden) weeks 4–6 in conscious animals at 30°C during 3 hr in the light period in the absence of food and water. RMR was defined as the average of the three lowest points (2 min determinations) and "mean activity" as the average oxygen consumption in the last 60 min of the measurement.

To measure norepinephrine-induced thermogenesis, the animals were anesthetized on a separate occasion with pentobarbital (90 mg/kg, i.p.), and indirect calorimetry was performed for 30 min at 33°C to obtain basal values, principally as earlier described (Golozoubova et al., 2006). The individual mice were then briefly removed from the calorimetry chambers, injected with norepinephrine (1 mg norepinephrine bitartrate/kg, subcutaneously), and returned to the chamber, and oxygen consumption was measured for another 60–80 min.

Protein Analysis

Animals were sacrificed on the same day as anesthesia took place, and interscapular brown adipose tissue, inguinal white adipose tissue, and epididymal white adipose tissue were recovered quantitatively and weighed. Samples were stored immediately at −80°C. The brown adipose tissue samples were homogenized in RIPA buffer with protease inhibitor (Complete Mini, Roche), and protein concentration was measured. Immunoblot analysis of UCP1 was performed as described (Petrovic et al., 2008).

Statistics

For each diet group, the differences between genotypes were examined with Student's t test ($p < 0.05$).

SUPPLEMENTAL DATA

Supplemental Data include one figure and can be found online at http://www.cell.com/cellmetabolism/supplemental/S1550-4131(08)00421-X.

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