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Mitochondrial proton leak in obesity-resistant and obesity-prone mice

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Mitochondrial proton leak in obesity-resistant and obesity-prone mice. Am J Physiol Regul Integr Comp Physiol 293: R1773–R1780, 2007. First published August 29, 2007; doi:10.1152/ajpregu.00478.2007.—We quantified uncoupling proteins (UCPs) in molar amounts and assessed proton conductance in mitochondria isolated from interscapular brown adipose tissue (IBAT) and hindlimb muscle [known from prior work to contain ectopic brown adipose tissue (BAT) interspersed between muscle fibers] of obesity-resistant 129S6/SvEvTac (129) and obesity-prone C57BL/6 (B6) mice under conditions of low (LF) and high-fat (HF) feeding. With usual feeding, IBAT mitochondrial UCP1 content and proton conductance were greater in 129 mice than B6. However, with HF feeding, UCP1 and proton conductance increased more in B6 mice. Moreover, with HF feeding GDP-inhibitable proton conductance, specific for UCP1, equaled that seen in the 129 strain. UCP1 expression was substantial in mitochondria from hindlimb muscle tissue (ectopic BAT) of 129 mice as opposed to B6 but did not increase with HF feeding in either strain. As expected, muscle UCP3 expression increased with HF feeding in both strains but did not differ by strain. Moreover, the proton conductance of mitochondria isolated from hindlimb muscle tissue did not differ by strain or diet. Our data uncover a response to weight gain in obesity-prone (compared to resistant) mice unrecognized in prior studies that examined only UCP1 mRNA. Obesity-prone mice have the capacity to increase both IBAT UCP1 protein and mitochondrial proton conductance as much or more than obesity-resistant mice. But, this is only achieved only at a higher body mass and, therefore, may be adaptive rather than preventative. Neither obesity-prone nor resistant mice respond to HF feeding by expressing more UCP1 in ectopic BAT within muscle tissue.

uncoupling protein; mitochondria; obesity

**UNCOUPLING PROTEIN 1 (UCP1) IS THE MAJOR thermogenic protein driving heat production in mitochondria mainly within interscapular brown adipose tissue (IBAT).** The activity of this protein manifests as proton conductance from the outside of the inner mitochondrial membrane back to the matrix, thereby discharging potential and generating heat. Obesity-resistant strains A/J (40) and SWR/J (33) express more uncoupling protein 1 (UCP1) mRNA in IBAT compared with obesity-prone strains (33, 40, 41). Upon high-fat (HF) feeding IBAT UCP1 mRNA expression increases more in these obesity-resistant mice than obesity-prone strains (33, 40, 41). This has led to the concept that obesity-resistant mice are protected from weight gain induced by HF feeding through increased IBAT UCP1 expression.

However, important issues remain unresolved. UCP mRNA data may not parallel uncoupling protein content or actual mitochondrial uncoupling (31, 38). Hence, it is not clear whether obesity-resistant mice fed HF actually manifest a greater increase in IBAT UCP1 protein content than obesity-prone mice. Most important, nothing is known of how HF feeding actually affects the UCP1-mediated protein leak in mitochondria of obesity-prone or resistant mice.

It is known that, in addition to IBAT, traditional white fat depots have the capacity to express brown adipocytes and UCP1 and that this capacity is genetically determined (12, 42). Likewise, we recently reported (2) that obesity-resistant 129S6/SvEvTac (129) mice expressed substantial UCP1 in ectopic depots of brown adipose tissue (BAT) located within hindlimb muscle interspersed between muscle fibers. In contrast, BAT and UCP1 were barely, if at all, evident in muscle tissue of obesity-prone C57BL/6 (B6) mice. However, this study did not examine the effect of HF feeding on the extent of muscle (ectopic BAT) UCP1 expression.

In view of the above considerations, the current study was carried out to examine two issues. One goal was to determine how HF feeding affects IBAT mitochondrial UCP1 protein and the actual UCP1-mediated mitochondrial proton leak and how this effect might differ between obesity-resistant and obesity-prone mice. A second goal was to examine the strain-specific degree of UCP1 protein in muscle tissue mitochondria, with and without HF feeding, and to determine whether any strain differences would be associated with measurable differences in the proton leak of mitochondria isolated from muscle tissue.

To accomplish our objectives, we used quantitative immunoblotting to estimate the molar amounts of UCP1 and UCP3 in IBAT and hindlimb muscle of B6 and 129 mice under conditions of typical low-fat (LF) rodent feeding and HF feeding. In addition, to assess UCP1-mediated proton leak activity in mitochondria of these mice, we measured GDP-inhibitable proton conductance.

Our findings provide novel information regarding the role of IBAT respiratory uncoupling in the differential sensitivity to obesity and reveal a perspective different from that based on...
prior UCP1 mRNA studies. The existing paradigm, based on mRNA data, suggests that obesity protection simply follows from a greater increase in IBAT UCP1 expression by obesity-resistant mice upon HF feeding. Here, we show that this is not actually the case. Rather, we show that UCP1-mediated uncoupling represents a previously unreported adaptive phenomenon that actually comes to play more in obesity-sensitive (to HF feeding) mice than obesity-resistant mice, but at higher body mass.

MATERIALS AND METHODS

Experimental animals. Wild-type B6 and 129 male mice were purchased from Taconic (Germantown, NY). Animals were fed and maintained according to standard National Institutes of Health guidelines and the protocol was approved by our institutional Animal Care Committee. Room temperature was maintained at 25°C. Mice were euthanized by IP injection of 10 mg pentobarbital sodium followed by incision of the left ventricle.

Materials. Affinity purified polyclonal rabbit anti-UCP12-A, directed against a 19 amino acid cytoplasmic, COOH-terminal sequence of mouse and rat UCP-1, and anti-UCP32-A, against a 14 amino acid sequence mapping near the COOH-terminus of human UCP-3, which is 93% homologous (13/14 residues) to rat and mouse UCP-3 were purchased from Alpha Diagnostics International (San Antonio, TX).

Animal studies. Three groups of experiments were performed. Group I experiments examined UCP1 and UCP 3 expression in IBAT and hindlimb muscle mitochondria of B6 compared with 129 mice. All mice were fed standard rodent chow as routinely used by the University of Iowa animal care unit for 1–2 wk after purchase from Taconic. Standard rodent chow diet was obtained from Harlan Teklad (Madison, WI) and consisted of 11.8% fat, 31% protein, and 57.3% carbohydrate (www.tekladcustomdiets.com), which was very similar in fat content to the LF diet described below.

Group II experiments compared UCP1 and UCP 3 expression in IBAT and hindlimb muscle mitochondria of 129 mice fed LF compared with HF. The HF diet consisted of 60% fat, 20% protein, and 20% carbohydrates (Research Diets, New Brunswick, NJ). The LF diet consisted of 10% of calories provided from fat, 20% from protein, and 70% from carbohydrates (Research Diets). Details of these diets are available at www.researchdiets.com.

Group III experiments compared four groups of mice; B6-fed LF, B6-fed HF, 129-fed LF, and 129-fed HF, using the same LF and HF diets as in the group II experiments. In addition to quantifying UCP1 and UCP3 expression, we also measured the proton conductance of mitochondria or 0.25 to 3.0 mM for hindlimb muscle to inhibit succinate dehydrogenase, thereby, decreasing electrons available for the transport system and creating a range of membrane potentials. Proton conductance at any point along the curve of respiration vs. potential is defined by oxygen consumption per unit potential and expressed in units of nmoles O$_2$·min$^{-1}$·mg$^{-1}$ mitochondrial protein/mV or nmoles H$^+$·min$^{-1}$·mg$^{-1}$·mV$^{-1}$ if multiplied by 6 according to the above stoichiometry. To compare curves between conditions, the data for each individual mitochondrial preparation were fit using second-order polynomial curve fitting.

For IBAT mitochondria, proton leak kinetics were determined in the presence and absence of GDP (1.0 mM). The nucleotide, GDP, is a well-known potent inhibitor of UCP1 activity (29). Nucleotide inhibition of UCP homologs has been reported but requires the presence of superoxide and fatty acids (14). For this reason and because UCP1 is much more abundant than any other uncoupling protein in IBAT, GDP inhibition, as studied herein, should be specific for UCP1. This is important not just for specificity between UCP subtypes but also to exclude a role of any less defined uncoupling potentially due to other mitochondrial carriers or possible artificial uncoupling.

Immunoblotting and mitochondrial content of mitochondrial UCP1. Mitochondrial protein was separated on 12.5% polyacrylamide gels, and immunoblotting was carried out as we previously described (15, 21). We documented (15, 21) the specificity of the antibodies to UCP1 and UCP3 with competition by specific (but, not by nonspecific) peptide to which these antibodies were raised, by demonstration of the appropriate tissue distribution of UCP1 or UCP3 immunoreactivity based on the reported mRNA distribution and by demonstration of enhanced immunoreactivity in mitochondrial compared with whole cell extracts. UCP1 was quantified in units of micrograms per milligram mitochondrial protein, as we previously described (16), using standards consisting of purified rat UCP1 protein expressed in Escherichia coli inclusion bodies. Using analogous methodology, we cloned an insert coding the full-length rat UCP3 into pQE-60 (Qiagen) for bacterial expression in M15 E coli and purification. Plasmids (pQE-60) with inserts were verified by fluorescent DNA sequencing by the College of Medicine DNA core facility at the University of Iowa. Rat and mouse UCP1 and UCP3 are homologous over the epitope recognized by the antibodies used. The purity of the inclusion body preparations was determined, as we described in the past (15, 16), by silver staining compared with known amounts of the protein GAPDH (Sigma, St. Louis, MO), which migrates at a similar rate. The UCP3 inclusion body preparation used in these studies was 8.7% pure, and the UCP1 preparation was 2.9% pure.

Potential cross-reactivity between antibodies to UCP1 and UCP3 was evaluated by assessing the immunoreactivity of both antibodies to UCP1 and UCP3 protein expressed by adenoviral infection of insulinoma cells, as we described previously (15, 21).

Plasma leptin and insulin. Mouse leptin and insulin were assayed by RIA using kits obtained from Linco Research.
Statistics. Differences between groups were compared using the unpaired two-tailed t-test, one-way, or two-way ANOVA as described in the figure legends.

RESULTS

Quantitative immunoblotting. We used quantitative immunoblotting to assess both UCP1 and UCP3 expression in mitochondria isolated from IBAT and hindlimb muscle. Figure 1 illustrates representative immunoblots, which demonstrate the use of inclusion body (ib) standards to quantify UCP expression in absolute amounts (µg/mg mitochondrial protein). As shown in Fig. 1D, there was no cross-reactivity between the antibodies to the UCP1 and UCP3 proteins. Specificity was also demonstrated by abolishing the immunoreactive signals by competing peptide (to which the antibodies were raised) but not by noncompeting peptides (data not shown). From the data in Fig. 1, we calculate that, using 10–15 µg mitochondrial protein loaded per lane, that immunoblotting for UCP1 is roughly sensitive down to levels of 0.2 to 0.3 µg/mg mitochondrial protein and for UCP3 roughly 0.03 to 0.04 µg/mg.

Differences in mitochondrial UCP1 and UCP3 protein expression by strain (group I experiments). Weights of the B6 and 129 mice (n = 10 per group) at death were 24.4 ± 0.9 g and 26.1 ± 0.6 g (P = not significant), respectively. B6 mice were slightly older than 129 at death (74 ± 2 compared with 67 ± 2 days, P < 0.01).

UCP1 was substantially more abundant in IBAT mitochondria isolated from the 129 mice compared with B6 (Fig. 2A). IBAT UCP3 content was also greater in 129 mitochondria than B6 (Fig. 2B). On a molar basis, the amount of UCP3 in IBAT mitochondria was very small compared with UCP1 (compare Fig. 2, A and B). The UCP1 content of mitochondria isolated from hindlimb muscle tissue of 129 mice was considerably greater than that of B6 mice (Fig. 2C). Actually, the UCP1 content of the B6 muscle mitochondria was close to the lower

Fig. 1. Representative immunoblots used to quantify uncoupling protein 1 (UCP1) and UCP3 in absolute units. A: UCP1 immunoreactivity of BAT mitochondrial extracts from LF-fed B6 and LF-fed 129 mice compared with UCP1 inclusion body (ib) preparations of known purity (2.9%). UCP1 and UCP3 ib preparations migrate slightly slower due to NH2-terminal sequence additions, including histidine tags and restriction sites used in cloning. B: UCP1 immunoreactivity of hindlimb muscle mitochondrial extracts from LF-fed B6 and LF-fed 129 mice compared with UCP1 ib preparations. C: UCP3 immunoreactivity of hindlimb muscle mitochondrial extracts from LF- and HF-fed 129 mice compared with UCP3 ib preparations of known purity (8.7%). D: UCP1 and UCP3 immunoreactivity of identical immunoblots demonstrating absence of antibody crossreactivity. Lane 1: 4-µg extract of mitochondria isolated from rat insulinoma (INS-1) cells infected with adenoviral UCP3. Lane 2: 4-µg extract of mitochondria isolated from INS-1 cells infected with adenoviral UCP1.

Fig. 2. Quantitative UCP1 and UCP3 protein expression in mitochondria isolated from IBAT or hindlimb muscle of B6 compared with 129 mice (group I experiments). All mice were fed normal fat (NF) diets in the form of the typical rodent chow (see METHODS). A: UCP1 in interscapular brown adipose tissue (IBAT) mitochondria; B: UCP3 in IBAT mitochondria; C: UCP1 in muscle mitochondria; D: UCP3 in muscle mitochondria. Data are expressed as means ± SE, n = 10 mice per group. P values determined by unpaired, 2 tailed t-test.
limit of sensitivity. The UCP3 content of mitochondria isolated from muscle tissue of 129 mice did not differ from B6 mice (Fig. 2D).

Effect of HF feeding on mitochondrial UCP1 and UCP3 protein content of obesity-resistant 129 mice (group II experiments). Given the group I results showing that the 129 mice expressed markedly more UCP1 in muscle tissue than the B6 strain and more UCP1 in IBAT, we examined how these parameters might change in these obesity-resistant mice with HF feeding. Duration of diets of the LF and HF mice at death were 74 ± 4 days in both groups, and ages of the mice were 106 ± 4 days in both groups. Weights of the LF and HF mice (n = 14 per group) at death were 29.2 ± 0.7 g and 32.1 ± 0.8 g, respectively, (P = 0.01). Weight gain during feeding period in the LF and HF mice was 13.4 ± 0.8 g and 16.4 ± 0.7 g, respectively, (P < 0.01).

We observed no significant differences between the UCP1 content of mitochondria isolated from either IBAT or hindlimb muscle tissue of 129 mice fed HF compared with 129 mice fed LF (Fig. 3, A and C). In contrast, the UCP3 content of IBAT and muscle tissue mitochondria was greater in mice fed HF (Fig. 3, B and D), although for IBAT, the significance was borderline (P = 0.049).

Mitochondrial UCP protein content and proton leak activity by strain and diet (group III experiments). In these experiments, we examined the kinetics of the proton leak in mitochondria isolated from IBAT and muscle tissue from four groups of mice (B6 LF, B6 HF, 129 LF, and 129 HF). We also determined UCP content and other characteristics of the mice (Table 1). By design, mice in the four groups were of the same age and exposed to the same duration of dietary perturbation. As expected, the B6 mice gained more weight than 129 when exposed to HF feeding.

Mitochondrial UCP content, as well as leptin and insulin concentrations, are depicted in Table 1. IBAT UCP1 content differed by strain (lower in B6 mice) and diet (greater with HF feeding). Moreover, the difference (HF vs. LF) was as much or greater in the obesity-prone B6 mice vs. 129. Although hindlimb muscle tissue UCP1 content was greater in the 129 mice, we observed no increase with HF feeding in either strain. IBAT UCP3 differed only by strain, whereas hindlimb muscle tissue UCP3 differed only by diet. Leptin increased with HF feeding but only to a significant extent in the B6 mice. Insulin concentrations differed by strain, greater in the B6 mice.

To assess UCP1 activity, we examined the kinetics of the proton leak in mitochondria isolated from IBAT and muscle of the group III mice. As shown in Fig. 4A, the proton conductance of mitochondria isolated from IBAT of B6 mice fed LF was lower compared with 129 mice. However, with HF feed-

Table 1. Characteristics and mitochondrial UCP1 and UCP3 concentrations in IBAT and hindlimb muscle tissue of mice used in studies of mitochondrial proton conductance (group III experiments)

<table>
<thead>
<tr>
<th>B6 (LF)</th>
<th>B6 (HF)</th>
<th>129 (LF)</th>
<th>129 (HF)</th>
<th>P Values by Two-Way ANOVA (diet/strain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at death, days</td>
<td>83.8 ± 1.1</td>
<td>83.8 ± 1.1</td>
<td>83.8 ± 1.1</td>
<td>83.8 ± 1.1</td>
</tr>
<tr>
<td>Weight at death, g</td>
<td>25.4 ± 0.5</td>
<td>37.6 ± 1.5**</td>
<td>27.4 ± 0.9</td>
<td>29.1 ± 1.1</td>
</tr>
<tr>
<td>Number of days on diet</td>
<td>51.8 ± 1.1</td>
<td>51.8 ± 1.1</td>
<td>51.8 ± 1.1</td>
<td>51.8 ± 1.1</td>
</tr>
<tr>
<td>Weight gain on diet, g</td>
<td>10.2 ± 0.5</td>
<td>21.0 ± 1.2**</td>
<td>7.9 ± 0.4</td>
<td>9.9 ± 0.9</td>
</tr>
<tr>
<td>IBAT UCP1, µg/mg protein</td>
<td>44 ± 4</td>
<td>80 ± 5*</td>
<td>87 ± 5</td>
<td>113 ± 11**</td>
</tr>
<tr>
<td>HL UCP1, µg/mg protein</td>
<td>0.43 ± 0.04</td>
<td>0.31 ± 0.06</td>
<td>1.12 ± 0.44</td>
<td>0.99 ± 0.21</td>
</tr>
<tr>
<td>IBAT UCP3, µg/mg protein</td>
<td>0.16 ± 0.02*</td>
<td>0.19 ± 0.02**</td>
<td>0.29 ± 0.05</td>
<td>0.37 ± 0.03</td>
</tr>
<tr>
<td>HL UCP3, µg/mg protein</td>
<td>0.21 ± 0.02</td>
<td>0.37 ± 0.03**</td>
<td>0.20 ± 0.02</td>
<td>0.32 ± 0.03*</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>7.1 ± 1.2</td>
<td>29.3 ± 5.3**</td>
<td>13.2 ± 2.2</td>
<td>19.0 ± 2.4</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>2.46 ± 0.76</td>
<td>3.63 ± 1.05*</td>
<td>0.34 ± 0.07</td>
<td>0.73 ± 0.20</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE; n = 6 for all groups. Mitochondrial uncoupling protein 1 (UCP1) and UCP3 concentrations are given in micrograms per milligram mitochondrial protein. HL, hindlimb tissue; HF, high fat; LF, low fat; IBAT, interscapular brown adipose tissue; NS, not significant. *Significant interaction. **P < 0.05, *P < 0.01, **P < 0.001 compared to LF of same strain by two-way ANOVA and Bonferroni posttest or by one-way ANOVA (when interaction is significant). *P < 0.05, **P < 0.01, ***P < 0.001 compared to 129 strain on same diet by two-way ANOVA and Bonferroni post-test or by one-way ANOVA (when interaction is significant).
ing, the proton conductance of B6 IBAT mitochondria was comparable to that of the 129 mitochondria. For quantification and statistical comparison, we fit curves for individual mitochondrial preparations and determined the potential needed to drive respiration at a midrange (although arbitrarily chosen) oxygen consumption rate of 300 nmoles O$_2$/min$^{-1}$/mg mitochondrial protein$^{-1}$ according to diet and mouse strain. Data points are expressed as the means ± SE; n = 6 mice per group, P < 0.005 for both diet and strain by two-way ANOVA but with significant interaction, *P < 0.001 compared with all other groups by one-way ANOVA with Bonferroni’s post hoc tests.

As a more specific indicator of UCP1-mediated proton conductance in IBAT, we determined these kinetics in the presence and absence of GDP (Fig. 5). Inhibition by this nucleotide, evident as the right shift in respiration vs. potential, was least in mitochondria isolated from B6 mice fed LF but greatest in mitochondria isolated from B6 mice fed HF. To quantify this shift, we used the curve fits for each mitochondrial preparation to calculate proton conductance at the above oxygen consumption rate of 300 in the presence and absence of the nucleotide and determined the difference by subtraction (Fig. 5E). Proton conductance was expressed in nmoles H$^+$/min$^{-1}$/mg mitochondrial protein$^{-1}$/mV$^{-1}$ and calculated as 6 times the oxygen consumption rate of 300 divided by potential (see MATERIALS AND METHODS). The GDP-inhibitable proton conductance differed by strain, but only in the B6 mice by diet, being substantially greater with HF feeding. The difference in GDP-inhibitable proton conductance in B6 mice (HF over LF) exceeded that expected on the basis of expression of the UCP1 protein alone (Table 1), suggesting that HF feeding of B6 mice (compared to LF) is associated with greater activity per mol-

cule UCP1. This is depicted in Fig. 5F, in which the data are expressed per microgram UCP1. Interestingly, the pattern seen (by diet and strain) resembles that of leptin (Table 1). Moreover, leptin correlated strongly with UCP1 content ($r = 0.71$, $P < 0.01$) and the UCP1 mediated proton leak ($r = 0.91$, $P < 0.0001$) over all (LF fed plus HF fed) B6 mice but not the 129 mice.

Proton conductance did not differ between mitochondria isolated from B6 and B6 mice by strain or diet (Fig. 6).

**DISCUSSION**

There is well-recognized genetic variability in the propensity to develop obesity among both humans (5, 26, 28) and animals, including rodents (1, 23, 24). B6 mice develop obesity, hyperglycemia, and marked insulin resistance in response to diet (4, 36) or engineered genetic defects (24). In contrast, 129 mice respond much less to these manipulations.

It has been assumed on the basis of mRNA studies (33, 40, 41) that obesity-resistant mice, when fed HF diets increase UCP1 more than obesity-prone mice and are thereby protected from weight gain. However, these studies did not assess either mitochondrial UCP1 protein content or mitochondrial function. Therefore, these results need to be interpreted with caution since UCP mRNA levels may not parallel UCP content or activity of the proton leak (31, 38).

In fact, our current results do not support the above simple paradigm of a greater increase in UCP1 with HF feeding and, therefore, more obesity protection in obesity-resistant mice. Rather, our data offer a different and more comprehensive view. Here, we rigorously quantified both UCP1 protein content and the UCP1-mediated proton leak in IBAT mitochondria. Our data show that the increase in UCP1 protein content and the UCP1-mediated proton leak with HF feeding was greater in the obesity-prone mice (Table 1, Fig. 5). Thus, we report the novel finding that obesity-prone mice do have the capability of upregulating the proton leak to a greater extent than obesity-resistant mice at least when a high enough body mass is achieved. In fact, for the obesity-resistant 129 mice, there was actually only a relatively small (group 3 mice, Table 1) or an insignificant (group 2 mice, Fig. 3A) increase in IBAT UCP1 expression and no increase in proton leak activity (Fig. 5) with HF feeding over LF feeding, although these parameters were already high (vs. the B6) on LF intake (Figs. 2A and 5, Table 1). A limitation to our study, as well as to the above-referenced prior studies of UCP1 mRNA (33, 40, 41), is that HF feeding and increased body mass go hand-in-hand, so it is difficult to be sure that changes in UCP expression or in the activity of the UCP1-mediated proton leak result only from one or other of these factors.

In spite of the capacity to increase UCP1-mediated proton conductance with HF-induced weight gain, the B6 mice are prone to greater obesity than 129. This may simply be because, relative to the 129 mice, the capacity to increase the UCP1-mediated proton leak may come on too late. Nonetheless, it seems reasonable to suggest that this UCP1 response does represent an adaptive response mitigating against an even further rise in adiposity. In any case, whether adaptive or not, this capacity to increase UCP1 represents a novel concept not evident from prior studies examining UCP1 mRNA (33, 40, 41). Our data do not imply that UCP1 has no role in obesity
prevention (as opposed to adaptation). In the absence of HF feeding, the 129 mice express more UCP1 and proton leak activity, so they would be relatively protected from weight gain at the onset of HF feeding.

In this regard, it is of note that our proton conductance data by strain and diet (Fig. 5, E and F) resemble the pattern manifest by leptin (Table 1), a protein well known to increase with fat mass preventing even further accumulation of fat. Leptin enhances sympathetic nerve traffic and upregulates IBAT UCP1 expression (11, 18, 37). Thus, the parallel between leptin and IBAT proton conductance suggests that IBAT UCP1 and its activity may occur in response to leptin and support the adaptive nature of these changes in IBAT mitochondrial function.

Obesity-resistant A/J mice, compared with B6, have been reported to manifest a greater increase in IBAT UCP1 mRNA in response to exogenous leptin, therefore, maintaining leptin sensitivity in spite of HF feeding (34). The effect on mitochondrial function and UCP1 protein content was not determined in that study. Our current studies were not designed to address this issue per se, but they do show that B6 mice, although leptin resistant, do, in fact, maintain an adaptive IBAT response (UCP1 protein content and proton conductance) in the face of a large increase in plasma leptin (Table 1). The fact that IBAT mitochondria of obesity-resistant 129 mice, at much lower leptin, have similar proton conductance, supports the fact that obesity-resistant mice are indeed more leptin sensitive.

We recently reported that hindlimb muscle of 129 mice expressed ectopic depots of BAT and substantial levels of UCP1 mRNA and protein (3). The much higher levels of hindlimb UCP1 mRNA in the 129 mice compared with the B6 mice were not due to white fat contamination, as we observed similar levels of expression of adiponectin and aP2 in the B6 and 129 muscle samples and a higher level of leptin in B6. Immunohistochemical study revealed that hindlimb UCP1 was expressed within multilocular brown adipocytes intermixed with white adipose tissue located between muscle bundles (3). Therefore, a second goal of the current work was to quantitatively examine the degree of strain-specific UCP1 protein in muscle tissue mitochondria with and without HF feeding.
feeding. We also sought to determine whether any strain differences would be associated with measurable differences in the proton leak of muscle mitochondria.

Our current findings confirm the strain difference (129 vs. B6) in mitochondrial UCP1 protein content under usual feeding conditions. However, we now show that hindlimb muscle (ectopic BAT) UCP1 expression is not increased by HF feeding in either strain (table 1, Fig. 3). Our past results also showed a marginal but insignificant increase in whole muscle tissue mitochondrial proton conductance in B6 mice compared with 129 fed under usual feeding conditions (2). Our current results again show no strain difference in muscle tissue mitochondrial proton conductance but extend these observations to the HF feeding condition.

Of course, with respect to our studies of muscle tissue mitochondrial proton conductance, we must acknowledge the limitation that we are trying to detect a difference in proton conductance between mitochondrial preparations from whole muscle. So, the ectopic BAT mitochondria, wherein the difference in proton conductance would presumably lie, are diluted by UCP3-expressing myocyte mitochondria. In spite of this limitation, we thought it might be possible, because of the large total molar amount of UCP1 in muscle tissue of 129 mice (which is comparable to or actually exceeds UCP3 when expressed per unit total muscle mitochondrial mass, Table 1, Figs. 2 and 3), a strain differences in proton conductance might, in fact, be measurable. However, if any difference exists, we could not detect it and, unfortunately, we are not able to isolate mitochondria specifically from the BAT interspersed between muscle fibers. So, net muscle mitochondrial proton conductance does not appear to be altered in any strain-specific fashion upon HF feeding and does not increase with HF feeding in either strain.

As noted above, UCP1 protein expression within ectopic BAT of muscle did not increase with HF feeding. This differed from what occurred in IBAT, wherein UCP1 increased with HF feeding (Table 1). In this regard, we can at least speculate as to why UCP1 would be upregulated by HF feeding in IBAT but not in ectopic BAT in muscle. It is possible that this is related to the parallel between leptin and our UCP1 expression and proton leak data discussed above. Although leptin enhances sympathetic nerve traffic to IBAT (11, 19), we have no reason to think that this is also the case for sympathetic nerve traffic to muscle. So, if this tissue difference in leptin action (presumably based on neuroanatomy) does exist, leptin regulation of UCP1 might be expected in IBAT but not muscle.

The amount of UCP1 per IBAT mitochondria as we report here may seem large. Depending on diet, our data suggest that this amount can range from roughly 3 to 10% of all mitochondrial protein. However, this amount is in line with other reports of UCP1 in IBAT mitochondria of rodents under various circumstances (17, 35, 39). Our data (Table 1, Figs. 2 and 3) also confirm that this molar amount of UCP1 per IBAT mitochondria far exceeds the molar amount of UCP3 per muscle or IBAT mitochondria (17).

Why does the difference in muscle UCP3 (mainly expressed in myocytes) with HF feeding (Fig. 3, Table 1) not manifest as a difference in muscle mitochondrial proton conductance (Fig. 6)? This discordance between muscle UCP3 expression and muscle mitochondrial proton conductance has been noted before. Upregulation of muscle UCP3 expression without concurrent change in the proton conductance of isolated mitochondria has been reported in rodents after fasting and after induction of hyperthyroidism (22, 25). Thus our data, in conjunction with other reports, support the concept that energy dissipation may not actually be the major role for muscle UCP3. Others have suggested that UCP3 may be important in other ways, such as mitigating oxidative stress (6, 7) or exporting fatty acids (20).

Although UCP1 may be important in the differential propensity to obesity between mouse strains, the implications toward human physiology are less clear since BAT in adult humans is not readily identifiable. On the other hand, UCP1 has been detected in 1 of every 100–200 human intraperitoneal adipocytes (30). Moreover, BAT is expressed in newborn humans; adrenergic stimuli can induce UCP1 in adult humans as evident by the expression of BAT in patients with pheochromocytoma (9); and UCP1 can be induced by catecholamine (10) or thiazolidinedione (13) in human preadipocytes. So, given these considerations along with the well-recognized plasticity of white adipose tissue to express characteristics of BAT, understanding the physiology and regulation of UCP1 may prove important. Moreover, in theory, even small amounts of UCP1 might impact energy balance over long time periods.

In conclusion, on the basis of rigid assessment of mitochondrial UCP protein content and mitochondrial proton conductance, we provide a new view of the role of uncoupling in the differential sensitivity toward obesity between mouse strains. Past mRNA studies suggest only those obesity-resistant mice, when fed HF, manifest a greater increase in IBAT UCP1 expression and are thereby prevented from marked weight gain. However, we now show that obesity-prone mice (fed HF) have the capacity to increase both mitochondrial IBAT UCP1 protein and mitochondrial proton conductance as much or more than obesity-resistant mice. But, this is only achieved only at a higher body mass and, therefore, may be adaptive rather than preventative. This seemingly adaptive response appears to parallel changes in circulating leptin. In addition, we show that neither obesity-prone nor resistant mice respond to HF feeding by expressing more UCP1 in ectopic BAT within muscle tissue. The small excess in UCP1 in muscle tissue (ectopic BAT) in 129 vs. B6 mice with usual feeding may add slightly to the preventative effect toward obesity in 129 mice, but the lack of increase with HF feeding suggests that ectopic UCP1 in muscle tissue is not part of an adaptive response to HF intake.
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GRANTS

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