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Metabolic activity of brown, “beige,” and white adipose tissues in response to chronic adrenergic stimulation in male mice

Sébastien M. Labbé,1 Alexandre Caron,1 Kanta Chechi,1 Mathieu Laplante,1 Roger Lecomte,2 and Denis Richard1

1Centre de recherche de l’Institut universitaire de cardiologie et de pneumologie de Québec, Université Laval, Québec, Canada; and 2Departments of Nuclear Medicine and Radiobiology, Centre d’imagerie moléculaire de Sherbrooke, Université de Sherbrooke, Sherbrooke, Canada

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Labbé SM, Caron A, Chechi K, Laplante M, Lecomte R, Richard D. Metabolic activity of brown, “beige,” and white adipose tissues in response to chronic adrenergic stimulation in male mice. Am J Physiol Endocrinol Metab 311: E260–E268, 2016. —Classical brown adipocytes such as those found in interscapular brown adipose tissue (iBAT) represent energy-burning cells, which have been postulated to play a pivotal role in energy metabolism. Brown adipocytes can also be found in white adipose tissue (WAT) depots [e.g., inguinal WAT (iWAT)] following adrenergic stimulation, and they have been referred to as “beige” adipocytes. Whether the presence of these adipocytes, which gives iWAT a beige appearance, can confer a white depot with some thermogenic activity remains to be seen. In consequence, we designed the present study to investigate the metabolic activity of iBAT, iWAT, and epididymal white depots in mice. Mice were either 1) kept at thermoneutrality (30°C), 2) kept at 30°C and treated daily for 14 days with an adrenergic agonist [CL-316,243 (CL)], or 3) housed at 10°C for 14 days. Metabolic activity was assessed using positron emission tomography imaging with fluoro-[18F]deoxyglucose (glucose uptake), fluoro-[18F]-6-thiaheptadecanoic acid (fatty acid uptake), and [14C]acetate (oxidative activity). In each group, substrate uptakes and oxidative activity were measured in anesthetized mice in response to acute CL. Our results revealed iBAT as a major site of metabolic activity, which exhibited enhanced glucose and nonesterified fatty acid uptakes and oxidative activity in response to chronic cold and CL. On the other hand, beige adipose tissue failed to exhibit appreciable increase in oxidative activity in response to chronic cold and CL. Altogether, our results suggest that the contribution of beige fat to acute-CL-induced metabolic activity is low compared with that of iBAT, even after sustained adrenergic stimulation.

IN RECENT YEARS, BROWN ADIPOCYTE thermogenesis has gained renewed attention. Brown adipocytes are thermogenic cells predominantly found in classical brown adipose tissue (BAT) depots such as interscapular BAT (iBAT). They are responsible for cold adaptation and survival during cold challenge (6). The thermogenic potential of classical BAT is indeed impres-
PET/computed tomography (CT) experiments were initiated immediately after the insertion of a cannula in the tail vein for injections of PET tracers. All imaging experiments were performed on the Avana-
cache photodiode-based small animal PET scanner (LabPET/Tri-
umph; Gamma Medica, Northridge, CA) of the Sherbrooke Molecular Imaging Center, having a 7.5-cm axial field-of-view. The animals were anesthetized with isoflurane (1.75%) delivered through a nose cone and were placed in the prone position on the scanner bed with the heart centered within the field-of-view of the scanner to include the interscapular region and also the inguinal fat. Just before the injection of the first PET tracer (<1 min), an intravenous injection of CL (2 mg/kg, no. C5976; Sigma) was performed. Acute CL allows for appreciating the effects of chronic cold and CL on both substrate uptakes and oxidative activity, which are otherwise minimal under anesthesia, especially in mice kept at thermoneutrality. Boluses of each radiopharmaceutical compound (10 MBq, in 0.2 ml of 0.9% NaCl) were injected via the caudal vein over 30 s after starting PET data acquisition ([11C]Clacetate) and over 60 s for [18]FDG and [18]FTHA.

In one set of experiments, a 20-min dynamic data acquisition with [11C]Clacetate was done to determine tissue blood flow and oxidative metabolism, followed 10 min later by a 30-min dynamic data acquisition with either [18]FDG or [18]FTHA to also determine glucose or NEFA utilization, respectively, as previously described (29). List-

mode dynamic data acquisition allowed for flexible time framing of the data for kinetic modeling of all tracers. Low-dose CT scan imaging was performed using a Gamma Medica Triumph X-O small-

animal CT scanner composed of a 40 W X-ray tube with a 75-μm focal spot diameter and a 2,240 × 2,368 Csi flat panel X-ray detector. The detector pixel size was 50 μm, and a 2 × 2 pixel binning scheme was used. Scans were performed at 60 kVp and 230 μA using 512 projections in fly mode to reduce exposure. Blood samples were taken at the end of experiments by heart punctures.

**Imaging data analysis.** For [11C]Clacetate images, dynamic series of 28 frames (1 × 30, 12 × 10, 8 × 30, 6 × 90, and 1 × 300 s) were sorted out, whereas 30 frames (1 × 30, 12 × 10, 8 × 30, 6 × 90, and 3 × 300 s) were used for [18]FDG and [18]FTHA imaging, and three-
dimensional images were reconstructed using 15 iterations of a maximum-likelihood expectation-maximization algorithm incorporating physical description of the detector response function. Regions of interest (ROIs) were drawn on short-axis images and confirmed with the μCT scan (19). Time-activity curves were quantified in matching ROIs of [11C]Clacetate with either [18]FDG or [18]FTHA. Input curves were extracted by means of a ROI drawn on the left ventricular cavity blood pool in summed last-frame images to seek better contrast. The sizes of these almost-circular ROIs were compared with images of eight cylinders of different diameters from which a recovery factor was extracted and applied to the ROIs for partial volume correction (29). For [11C]Clacetate, we used a three-compartment kinetic model that estimates the generation of CO2 from the tricarboxylic acid cycle using the k2 constant (50) and the tissue blood flow through the K1 constant (12, 49). The dynamic glucose and NEFA uptake was determined using the Patlak graphical analysis (36). Whole tissue glucose and NEFA uptake was corrected for the total mass of adipose tissue determined at the end of PET experiments.

**Measurement of mitochondrial DNA content.** Tissues for assessing mitochondrial DNA (mtDNA) content were prepared as described previously (10, 41). Briefly, total DNA was isolated by proteinase K digestion followed by phenol/chloroform extraction and ethanol precipi-
tation. Ten (10) nanograms of total DNA were then used to amplify mtDNA-encoded NADH dehydrogenase I (ND1) and nuclear DNA-encoded β-actin using real-time PCR in a 10-μl reaction mixture. mtDNA content was calculated from the ratio of ND1 to genomic β-actin quantity.

**Statistical analyses.** Results are expressed as means ± SE. Comparisons were done on normally distributed data using ANOVA followed by Bonferroni post hoc tests to assess the differences between the various treatments with Graph Pad Prism Software version 6.0h for Mac (San Diego, CA). Differences of P < 0.05 were considered statistically significant.
RESULTS

After 14 days, cold-exposed (10°C) mice tended to exhibit lower weight than mice kept at thermoneutrality (30°C) or kept at 30°C and chronically injected with CL (Table 1). The trend for lower weight gain was accompanied by a significant increase in total food intake and by a tendency for a decrease in feed efficiency (Table 1). This cold-induced decrease in food/energetic efficiency supports previous observations (6, 11, 19).

Chronic adrenergic stimulation effects on adipose tissues. As shown in Fig. 1A, cold exposure (10°C) induced a 12-fold increase (over 30°C) in Ucp1 expression in iBAT, whereas chronic CL (30°C-chronic CL) had no effect. We also observed significant increases (over 30°C) in Ucp1 expression in iWAT and eWAT following chronic CL (13- and 124-fold) and chronic cold (168- and 72-fold).

As shown in Fig. 1C, UCP1-positive multilobular cells were indisputably detected in iWAT, following both chronic CL (30°C-chronic CL) and cold (10°C), with cold being the strongest inducer. As shown in Fig. 1C, brown adipocytes were heterogeneously grouped in multiple clusters across iWAT. At 10°C, multilobular UCP1-immunostained adipocytes of iBAT appeared morphologically similar to the brown adipocytes in iBAT at 30°C. However, following chronic CL and cold, brown adipocytes in iBAT looked more lipid depleted and more densely immunostained for UCP1 than brown adipocytes in iWAT. In eWAT, we also observed sparse UCP1-positive multilobular cells following chronic CL and cold.

As shown in Fig. 1B, mtDNA content was significantly increased in iBAT after chronic CL (2.5-fold, P < 0.01) and even more after chronic cold exposure (4.4-fold, P < 0.001). In iWAT, chronic CL increased mtDNA levels by 23-fold, and chronic cold exposure increased these levels by 7.3-fold. However, the ratio of mtDNA to nuclear DNA in iBAT following chronic CL was lower than that of iBAT at 30°C (6.06 vs. 7.98). In eWAT, this ratio was even lower than iWAT both after chronic CL (1.41) and chronic cold (0.35).

Glucose uptake in response to acute CL following chronic CL at 30°C and cold. Figure 2 shows the effects of chronic CL and cold on glucose uptake in iBAT, iWAT, and eWAT. Table 2 presents the blood glucose levels, which were similar under each condition (average value around 9.0 mmol/l). Figure 2A presents the glucose fractional uptake rate, whereas Fig. 2B shows the rate of glucose uptake by the whole tissue. We first measured basal glucose uptake at 30°C without any stimulation. As shown in Fig. 2, A and B, there was no statistical difference in uptake in either iBAT or WAT depots. However, uptake was statistically different in iBAT and iWAT when animals were kept at 30°C and acutely injected with CL. Although there was no difference between the 30°C and 30°C-chronic CL groups in their glucose uptake, chronic cold exposure significantly increased CL-induced glucose uptake in iBAT. This increase was accounted for by elevations in both the ability of iBAT to extract glucose from blood (fractional uptake rate, 4-fold increase) and its mass (1.4-fold).

NEFA uptake in response to acute CL following chronic CL at 30°C and cold. Figure 3 shows the effects of chronic CL and cold exposure on NEFA uptake in the adipose depots. As shown in Table 2, we observed that NEFA levels were lower in chronic CL and cold mice than in animals kept at 30°C. At baseline, NEFA fractional uptake was larger in iBAT than in iWAT and eWAT (Fig. 3A). However, the net uptake contribution was similar in all depots (Fig. 3B). Similar to glucose, acute injection of CL following 30°C adaptation was associated with a significant increase in NEFA uptake. Both chronic CL and cold were associated with an elevation (2.5-fold above thermonutrality) of NEFA fractional uptake rate in iBAT (Fig. 3A). However, NEFA fractional uptake rate in iWAT and eWAT was not significantly increased (above 30°C) following either chronic CL or cold, despite being significantly lower than that of iBAT (Fig. 3A).

Table 1. Characteristics of mice following 14-day adaptation

<table>
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<th>30°C</th>
<th>30°C-Chronic CL</th>
<th>10°C</th>
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<tbody>
<tr>
<td>Weight gain, g</td>
<td>0.54 ± 0.24</td>
<td>0.52 ± 0.19</td>
<td>0.00 ± 0.28</td>
</tr>
<tr>
<td>Total food intake, g</td>
<td>49 ± 2</td>
<td>45 ± 2</td>
<td>98 ± 3***###</td>
</tr>
<tr>
<td>Energetic efficiency, mg weight gain/g food intake</td>
<td>10 ± 5</td>
<td>11 ± 4</td>
<td>−1 ± 3</td>
</tr>
<tr>
<td>iBAT weight, mg</td>
<td>66.5 ± 2.4</td>
<td>69.8 ± 2.6</td>
<td>92.2 ± 4.0***##</td>
</tr>
<tr>
<td>iWAT weight, mg</td>
<td>162.9 ± 14.5</td>
<td>153.1 ± 13.1</td>
<td>70.8 ± 8.7***###</td>
</tr>
<tr>
<td>eWAT weight, mg</td>
<td>389.6 ± 36.2</td>
<td>141.7 ± 14.2***##</td>
<td>219.7 ± 18.2****##</td>
</tr>
</tbody>
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Data are expressed as means ± SE. CL, CL-316,243; iBAT, inguinal brown adipose tissue; iWAT, inguinal white adipose tissue; eWAT, epididymal white adipose tissue. ***P < 0.001 vs. 30°C and ###P < 0.01 and ####P < 0.001 vs. 30°C-chronic CL, assessed by post hoc Bonferroni test following ANOVA.
shown in Fig. 4E, chronic CL did not affect oxidative activity in iBAT, which contrasted with the 17.2-fold increase (above 30°C) seen following chronic cold. Importantly, we were not able to detect any CL-induced oxidative activity in iWAT (beige) and eWAT (white) following chronic CL and cold.

DISCUSSION

We have investigated the metabolic activity of three adipose depots using 18FDG (glucose uptake), 18FTHA (NEFA uptake), and [11C]acetate (oxidative activity). The use of this triad
of PET tracers has proven to be invaluable to assess brown fat metabolism in laboratory rodents (19) and humans (3, 4, 33). Specifically, our study aimed at comparing the metabolic activity of iBAT, the most investigated classical BAT depot, with that of iWAT and eWAT, which are known to, respectively, undergo or resist browning following adrenergic stimulation (cold exposure or \(\beta\)-adrenergic agonism) (51). We used groups of mice that were kept at either 30°C, 30°C treated chronic CL, or 10°C for 14 days. The metabolic activity was measured acutely after an injection of CL. Our results confirm the ability of iBAT to strongly respond to the chronic adrenergic treatments (CL and cold). Furthermore, our observations emphasize the limited ability of iWAT and eWAT to significantly enhance their metabolic activity in response to chronic CL and cold.

Table 2. Plasma profile of mice following acute CL injection

<table>
<thead>
<tr>
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<th>30°C</th>
<th>30°C-Chronic CL</th>
<th>10°C</th>
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<tr>
<td>Glucose, mmol/l</td>
<td>8.58 ± 0.46</td>
<td>8.89 ± 0.80</td>
<td>9.59 ± 0.52</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>3.812 ± 61</td>
<td>20 ± 9***</td>
<td>27 ± 8***</td>
</tr>
<tr>
<td>NEFA, μmol/l</td>
<td>1.448 ± 114</td>
<td>298 ± 26***</td>
<td>231 ± 32***</td>
</tr>
<tr>
<td>TG, μmol/l</td>
<td>697 ± 35</td>
<td>397 ± 78**</td>
<td>332 ± 54***</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. NEFA, nonesterified fatty acid; TG, triglyceride. **\(p < 0.01\) and ***\(p < 0.001\) vs. 30°C, assessed by post hoc Bonferroni test following 2-way ANOVA.

Classical BAT is a thermogenic tissue (6) that strongly responds to chronic adrenergic stimulation by enhancing its thermogenic capacity and activity. In the present study, chronic...
cold expectedly boosted the stimulating effect of acute CL on the iBAT oxidative activity (measured with $[^{11}C]$acetate) and iBAT glucose uptake (measured with $[^{18}FDG]$). Cold constitutes a potent stimulator of classical BAT (19) via enhancing BAT sympathetic activity (6, 7, 20). In this study, chronic CL at 30°C was less efficient than cold in enhancing the metabolic activity of iBAT. It did not indeed enrich acute CL-induced oxidative activity in iBAT. It is noteworthy that acute CL per se resulted in an increased oxidative activity of iBAT following the three chronic conditions (30°C, chronic CL at 30°C, chronic 10°C). Chronic CL at 30°C nonetheless heightened iBAT NEFA fractional uptake rate following acute CL and increased (above 30°C) iBAT mitochondrial DNA content. It is worth insisting on the fact that chronic CL mice were housed at thermoneutrality (30°C), a condition that per se reduces the thermogenic need and hence likely might have potentially opposed stimulating effects of chronic CL in inducing the thermogenic capacity/activity of iBAT, which is further supported by the lack of iBAT mass expansion (Table 1).

One important aspect of the present study was the investigation of the metabolic activity of iWAT after its transformation into beige fat following chronic adrenergic stimulation. It has been known for years that cold exposure (24–26, 54) and $\beta_3$-adrenergic agonism (9, 17, 35, 53) both lead to the browning of iWAT. Our results confirm the ability to iWAT to undergo browning after chronic CL and cold and to exhibit increased mtDNA content. However, our results do not demonstrate that the browning of iWAT was paralleled by a major.

Fig. 3. Nonesterified fatty acid (NEFA) metabolism in various adipose depots. Black bars represent iBAT, orange bars represent iWAT, and open bars represent eWAT ($n = 5$ mice for 30°C at basal state, $n = 4$ mice for 30°C and 30°C-chronic CL, and $n = 6$ mice for 10°C following acute CL). A: fractional NEFA uptake determined using the Patlak graphic approach following a 30-min dynamic scan. B: total dynamic NEFA uptake corrected for tissue weight. C: representative coronal CT images. Brown arrows show iBAT region. D: representative PET-CT coregistration following chronic cold exposure. Brown arrows show positive 14(R,S)-fluoro-$[^{18}F]$-6-thiaheptadecanoic acid ($[^{18}FTHA]$) uptake in iBAT. E: representative coronal CT images. Orange arrows show iWAT region, and pink arrows show eWAT region. F: representative PET-CT coregistration following chronic cold exposure. Orange arrows show positive $[^{18}FTHA]$ uptake in iWAT, and pink arrows show positive $[^{18}FTHA]$ uptake in eWAT. Data are expressed as means ± SE. ***$p < 0.001$ vs. 30°C + acute CL, ###$p < 0.01$ and ###$p < 0.001$ vs. 30°C at basal state, $\&$ $p < 0.001$ vs. iBAT, and $\&\&$ $p < 0.001$ vs. iWAT assessed by post hoc Bonferroni test following 2-way ANOVA.
increase in its metabolic activity as assessed with a triad of PET tracers that have been proven to readily detect any metabolic changes in BAT (3, 4, 19, 33) as well as in other metabolically active tissues such as heart or liver (2, 8, 21, 29, 46). The oxidative activity ([11C]acetate) of iWAT following chronic CL and cold was not only trivial compared with that of iBAT, but it was also not higher than that of eWAT, which resisted browning. This finding was somewhat unexpected considering the simultaneous observations of increased oxidative capacity (mitochondria and multilocular UCP1-positive cells) of iWAT following chronic adrenergic stimulation. At the same time, our findings do not really contradict the existing literature, since there is not much evidence that beige adipose tissue can markedly contribute to energy metabolism when activated, despite the evidence that beige fat mitochondria exhibit enhanced ex vivo oxygen consumption following chronic adrenergic stimulation (43). There is also evidence that its thermogenic potential is much less than that of iBAT (16, 30). Recently, Park et al. (35) reported that browning of iWAT following chronic CL treatment (10 days) was associated with an increase in 18FDG fractional uptake in response to acute CL, a finding that tends to be corroborated by the 18FDG uptake results of the present study. However, one has to consider that 18FDG uptake, in contrast to [11C]acetate, does not represent a direct marker of tissue oxidative/thermogenic activity. Moreover, 18FDG uptake following acute CL can be modulated by insulin, whose levels significantly raised following CL, and served for de novo lipogenesis rather than for oxidative activity (22). It is noteworthy that acute CL led to an increase in NEFA uptake in iWAT and eWAT compared with iBAT in mice kept at 30°C. This was likely due to the increase in insulin levels seen as a result of acute CL injection.

The reasons as to why our PET/CT imaging protocol with [11C]acetate did not reveal any enhanced activity in beige adipose certainly deserve attention. One can argue that the PET/CT imaging protocol is not sensitive enough to detect oxidative activity in every circumstance. However, this argument lacks strength, since PET/CT imaging with [11C]acetate has proven to be a very reliable tool for detecting metabolic activity changes in the heart (2, 29), liver (8, 46), skeletal muscle (21), cancer cells (28, 34), and, more recently, in brown fat (3, 4, 19, 33), even in humans, where the density of brown fat adipocytes appears not higher than that of beige fat in laboratory mice (55). One can also argue that either the mouse strain that we used was not a good responder to cold or the cold stimulus that we used did not sufficiently enhance iWAT thermogenic capacity. This reason cannot be excluded, since there are mouse strains that respond more to cold than C57Bl/6 mice (43), and there could be longer and stronger cold stimuli than the one that we used. However, it is clear from our data.

Fig. 4. Effects of chronic adrenergic stimulation on blood flow and total oxidative activity index of various adipose depots. Mean standard uptake value (SUV) time-activity curves for 30°C mice (A), 30°C-chronic CL mice (B), and 10°C mice (C). Red dots show iBAT, orange squares show iWAT, and pink triangles show eWAT. Black bars represent iBAT, gray bars represent iWAT, and open bars represent eWAT (n = 4 mice for 30°C, n = 5 mice for 30°C-chronic CL, and n = 6 mice for 10°C). Total tissue blood flow (D) and total oxidative activity index (E) were calculated based on a 3-compartment kinetic model following a 20-min dynamic scan and corrected for respective tissue weight. Data are expressed as means ± SE. ***P < 0.001 vs. 30°C and ###P < 0.001 vs. iBAT, assessed by post hoc Bonferroni test following 2-way ANOVA.
that we induced a noticeable “beiging” of iWAT and yet no detectable enhanced oxidative activity (with $^{11}$C-acetate), even response in a acute CL injection. It is noteworthy that the amount of UCP1 (protein) following chronic adrenergic activation remains much lower in iWAT than in iBAT (16, 30), whatever the model or species used (43). Further studies need to be conducted to elucidate the beige fat incapacity to demonstrate oxidative activity, and one cannot even exclude at this point the possibility that there could be metabolic particularities of iWAT preventing brown fat cells from increasing their oxidative activity under stimulation.

The inability of beige iWAT to show enhanced $^{11}$C disappearance rate may question the physiological relevance of this depot in contributing to enhanced oxidative metabolism and hence in enhanced energy expenditure. Several investigators have reported strong correlations between the development of beige fat and enhanced energy expenditure with resistance to diet-induced obesity, at least in animal models (5, 14, 38–40, 44, 14a). However, the causality link between beige fat and enhanced energy expenditure remains to be firmly established. It is noteworthy that every beige fat inducer also stimulates classical iBAT, whose oxidative activity exceeds by far that of beige fat, as clearly and further demonstrated here. Finally, one cannot deny a potential indirect role of beige fat in influencing metabolic activity of classical BAT or other metabolic tissues such liver, skeletal muscle, or heart (45) to improve the overall metabolic profile.

In conclusion, our results demonstrate the ability of iBAT to increase its metabolic activity following chronic adrenergic stimulation induced by chronic CL or cold. They also demonstrate the limited ability of beige fat to show a meaningful enhanced substrate uptake and oxidative activity following chronic adrenergic stimulation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.M.L. and D.R. conceived and designed of research; S.M.L. and A.C. performed experiments; S.M.L. analyzed data; S.M.L., K.C., M.L., R.L., and D.R. interpreted results of experiments; S.M.L. prepared figures; S.M.L. performed experiments; S.M.L. analyzed data; S.M.L., K.C., M.L., R.L., and D.R. approved final version of manuscript.

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