Alterations in lipid metabolism and thermogenesis with emergence of brown adipocytes in white adipose tissue in diet-induced obesity-resistant Lou/C rats

Christelle Veyrat-Durebex,1 Anne-Laure Poher,1 Aurélie Caillon,1 Xavier Montet,2 and François Rohner-Jeanrenaud1

1Laboratory of Metabolism, Department of Internal Medicine, 2Department of Radiology, Faculty of Medicine, University of Geneva, Geneva, Switzerland

Submitted 5 October 2010; accepted in final form 10 March 2011

Alterations in lipid metabolism and thermogenesis with emergence of brown adipocytes in white adipose tissue in diet-induced obesity-resistant Lou/C rats. Am J Physiol Endocrinol Metab 300: E1146–E1157, 2011. First published March 15, 2011; doi:10.1152/ajpendo.00575.2010.—Recent studies describe the Lou/C rat as a model of resistance to age- and diet-induced obesity and suggest a preferential channeling of nutrients toward utilization rather than storage under standard feeding conditions. The purpose of the present study was to evaluate lipid metabolism of Lou/C and Wistar rats under a high-fat (HF) diet. Four-month-old male Lou/C and Wistar animals were submitted to a 40% HF diet for 5–9 wk. Evolution of food intake, body weight, and body composition, hormonal parameters, and expression of key transcription factors and enzymes involved in lipid metabolism were determined. Wistar rats developed obesity after 5 wk of HF diet, as previously described. Among the various parameters measured, accumulation of intraperitoneal fat was particularly evident in HF-fed Wistar rats. In these animals, thermogenesis was, however, stimulated as a likely compensatory mechanism against the development of obesity. On the contrary, Lou/C animals failed to develop obesity under such a diet, and intraperitoneal fat, not including epididymal and retroperitoneal fat deposits, was virtually absent. Enzyme measurements confirmed lipid utilization rather than storage, which was accompanied by the striking emergence of uncoupling protein-1, characteristic of brown adipocytes, in white adipose tissue, particularly in the subcutaneous depot.

IN HUMANS, GENETIC PREDISPOSITIONS can modulate the susceptibility of individuals to develop obesity and/or the metabolic syndrome, which is characterized by glucose intolerance, insulin resistance, and dyslipidemia. Both obesity and the metabolic syndrome are well known to increase the risk of developing type 2 diabetes.

In rodents, different susceptibilities to the development of such pathologies have also been described. Thus, in Sprague-Dawley rats fed a high-fat diet for a few weeks, it is possible to distinguish dietary resistant (DR) from diet-induced obese animals (21). The inbred Lou/C rat, originating from the Wistar strain (3), has been described as a model of resistance to the development of obesity as a function of age (14, 35, 36). Furthermore, under a standard laboratory diet, the Lou/C rat ingests fewer calories than Wistar animals and was thus proposed as a model of spontaneous caloric restriction (1, 35, 36). Recently, we demonstrated that, under standard feeding conditions, Lou/C rats exhibit improved insulin sensitivity and a preferential channeling of nutrients, particularly lipids, toward utilization rather than storage (37). Interestingly, when submitted to a self-selection regimen, Lou/C rats spontaneously select a high proportion of fat (70% of daily caloric intake), without any modification of daily caloric intake and of body weight gain (35), whereas Wistar rats rapidly develop obesity when submitted to such a high-fat diet (6, 20). This suggests that Lou/C rats are resistant to the development of diet-induced obesity, a phenomenon whose mechanisms have not been elucidated so far.

An almost exhaustive study of metabolism in Lou/C and Wistar rats has been previously done in our laboratory (37). The aim of this one was therefore to characterize lipid metabolism in Lou/C rats under a high-fat diet. For the first time, this strain was subjected for 5–9 wk to a diet containing 40% of calories as lipids, a regimen usually used to induce obesity in Wistar or Sprague-Dawley animals (6, 21, 33). After assessment of the main metabolic parameters, such as feeding patterns, body weight gain and body composition, and plasma metabolite and hormone levels, expression of several transcription factors and enzymes involved in thermogenesis and lipid metabolism was evaluated in the liver, skeletal muscles, and brown and white adipose tissue. Thermogenic capacities were studied using indirect calorimetry, and particular attention was placed on the possible emergence of brown adipocytes in white adipose tissue.

MATERIALS AND METHODS

Animals and diets. Male Lou/C (n = 68) and Wistar rats (n = 64) were purchased from Harlan UK (Oxon, UK) and Charles River (L’Arbresle, France), respectively. They were housed under controlled temperature (22°C) and lighting (light on: 0700–1900), with free access to water and to standard (Std) laboratory diet (2.61 kcal/g; RM1, SDS, Essex, UK) (Table 1). Animals were handled regularly, and body weight and food intake were recorded twice daily (0830 and 1730). Mature rats (3.5 mo old) were randomly distributed into two groups, fed either the Std or a 40% high-fat (HF) diet (4.31 kcal/g; 2154 KLBA NAFAG High Fat Purified Diet; Provimi Klifa, Kaiseraugst, Switzerland) (Table 1) for 6–9 wk. A first cohort was followed for 6 wk and tested with a glucose tolerance test (week 5) and euglycemic hyperinsulimic clamps (week 6); a second cohort, also followed for 6 wk, was tested with microcomputerized tomography (micro-CT) (week 6) before being euthanized for tissue analyses; a third cohort was tested with indirect calorimetry (week 7) and cold exposure (week 8) and euthanized at 9 wk of Std or HF diet for tissue analyses.

Animals were euthanized using isoflurane anesthesia (Halocarbon Laboratories, River Edge, NJ), and rapid decapitation between 0900 and 1300. Blood was sampled, and tissues were rapidly removed,
freeze-clamped, and stored at −80°C. All procedures were approved by the ethics committee of our university and were in accordance with the Swiss guidelines for animal experimentation.

Glucose tolerance test and euglycemic hyperinsulinemic clamps. Rats were first fasted for 4 h (0830–1230). For glucose tolerance test (GTT) (week 5 of HF diet), a glucose load of 1.5 g/kg ip was administered, and glycemia was recorded over 120 min using Glucojet Active strips (Roche Diagnostics, Basel, Switzerland). Euglycemic hyperinsulinemic clamps were realized as previously described in detail (34, 37).

Indirect calorimetry (LabMaster). Analyses for indirect calorimetry, spontaneous activity, and food and drinking behavior were performed using the 12-cage LabMaster system (TSE Systems, Berlin, Germany) of the Small Animal Phenotyping Core facility (CMU, University of Geneva, Geneva), under controlled temperature (22 ± 1°C) and lighting (12:12-h light-dark cycle). The calorimetry system is an open-circuit determining O2 consumption (ml·kg−1·h−1), CO2 production (ml·kg−1·h−1), respiratory exchange rate (RER = VCO2/VO2, where V is volume), and heat (H) (kcal·h−1·kg−0.75) produced by the animal. Detection of animal location and movements was delineated regions correspond to retroperitoneal fat in blue, subcutaneous in turquoise and interscapular in pink. For the abdominal part, delineated regions correspond to retroperitoneal fat in blue, subcutaneous fat in turquoise, epididymal fat in yellow, and intraperitoneal fat in red (not including epididymal and retroperitoneal depots).

Intestinal lipid absorption and muscle and liver triglyceride content. Intestinal lipid absorption was evaluated indirectly by measuring total lipid content in feces. Weighed quantities of frozen feces (=200 mg) were powdered under liquid N2 and extracted overnight at 4°C in 5 ml of chloroform-methanol (2:1, vol/vol). After filtration, extracts were added with 1 ml of sterile water and centrifuged (3,000 g, 10 min). They were washed twice by adding 1.5 ml of upper-phase washing buffer (3.1% chloroform, 49% methanol, CaCl2·2H2O 1.77 mM) followed by centrifugation (3,000 g, 10 min) and removal of the aqueous phase. A known volume was then transferred into preweighed glass tubes and evaporated to dryness with N2. Lipid content was determined by weighing the tubes.

For the determination of TG content in skeletal muscles and the liver, weighed quantities of frozen tissue (=100 mg) were powdered under liquid N2 and extracted overnight at 4°C in chloroform-methanol as previously described (29, 37). The TG content was determined by colorimetric enzymatic analysis as in plasma samples.

Tissue processing and RT-PCR. Total RNAs were extracted from frozen tissue by using a single-step extraction with TRizol reagent (Sigma-Aldrich, Buchs, Switzerland). RNA integrity was assessed by electrophoresis on a 1% agarose gel, and concentration was determined by spectrophotometry. A quantity of 2.5 µg of total RNA was used for RT using random hexamers (Microsynth, Geneva, Switzerland), dNTPs (Promega, Madison, WI), Rnasin as a RNase inhibitor (Promega), and the M-MLV-RT enzyme kit (Invitrogen, Basel, Switzerland). For quantitative PCR (qPCR), amplification of genes was performed from 6.25 or 12.5 ng of cDNA using the SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK), and an ABI 7500 machine (Applied Biosystems, Foster City, CA). For classical PCR, amplification was performed using Taq DNA polymerase (Qiagen, Hilden, Germany), 10 mM dNTP and 3 mM MgCl2. All primers were designed with the PrimerExpress software and used at 200 to 300 nM for qPCR (Suppl. Table S1), and at 20 µM for classical PCR (Suppl. Table S2). Results were normalized to the expression levels of housekeeping genes, such as the ribosomal protein S29 (RPS29), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and cyclophilin.

Western blots. Frozen white (WAT) and brown (BAT) adipose tissues were mechanically homogenized in ice-cold RIPA buffer (100 mM Tris, 2% NaPO4, 0.2% SDS, 0.3 M NaCl, and 1% sodium deoxycholate, pH 7.5), supplemented with protease inhibitors (Complete Mini, Roche Diagnostics). Proteins (25 and 50 µg for BAT and WAT, respectively) were separated by SDS-10% polyacrylamide electrophoresis gel. After a blocking period (milk 5%, 1 h), blots obtained after transfer on nitrocellulose membrane were incubated overnight at 4°C with purified anti-UCP1 (1:1,000) and anti-actin (1:100,000) (Chemicon International, Billerica, MA) antibodies. Detection was performed using horseradish peroxidase-conjugated secondary antibodies (1:5,000) and an enhanced chemiluminescence (ECL) detection system (Amersham Biosciences). Quantifications were then performed using the ChemiDoc XRS from Bio-Rad Laboratories (Hercules, CA) and the Quantity One software.

Data analyses. Results are expressed as means ± SE. Comparison between the four groups was performed using the parametric two-way analysis of variance followed by the Bonferroni a posteriori test (SPSS, Chicago, IL). The effect of HF diet within one strain was analyzed by Student’s t-test or the Kruskall-Wallis nonparametric test when normality and equal variance tests failed. Statistical significance was established at P < 0.05.

Table 1. Composition of Std and HF diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Std</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude oil</td>
<td>2.60</td>
<td>2.50</td>
</tr>
<tr>
<td>Pork fat</td>
<td>17.50</td>
<td>17.50</td>
</tr>
<tr>
<td>Corn starch</td>
<td>61.50</td>
<td>15.00</td>
</tr>
<tr>
<td>Sucrose</td>
<td>26.00</td>
<td>26.00</td>
</tr>
<tr>
<td>Sugar, ND</td>
<td>6.50</td>
<td>6.50</td>
</tr>
<tr>
<td>Crude protein</td>
<td>14.70</td>
<td>20.00</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>NA</td>
<td>10.00</td>
</tr>
<tr>
<td>Cellulose</td>
<td>4.32</td>
<td>5.00</td>
</tr>
<tr>
<td>Trace element mix</td>
<td>3.60</td>
<td>3.70</td>
</tr>
</tbody>
</table>

Values are given in percentage of grams. Std, standard diet; HF, high-fat diet. ND, nondefined; NA, not available.
RESULTS

The follow-up measurement of feeding patterns showed that, although only 3 days was required in the Lou/C group to decrease food intake to maintain the daily caloric intake, Wistar animals needed 1 wk (Fig. 1A). However, on week 4, both HF-fed Wistar and Lou/C rats still had a higher daily caloric intake than Std-fed animals (Fig. 1A: 198.5 ± 3.9 vs. 145.4 ± 2.1 kcal/kg body wt in HF- and Std-fed Wistar rats, respectively, \( P < 0.001 \); 202 ± 5.1 vs. 163.8 ± 4.2 kcal/kg body wt in HF- and Std-fed Lou/C rats, respectively, \( P < 0.001 \)). After 4 wk, the HF diet promoted a higher body weight gain in both Wistar and Lou/C rats (Fig. 1B), but when food efficiency was calculated, it was almost doubled by HF feeding in the Wistar group, whereas it was unaltered in Lou/C animals (Fig. 1C). Finally on week 9, an identical caloric intake between Std- and HF-fed animals was observed in both the Wistar and the Lou/C group (Fig. 1A). Of note, measurement of lipid content in feces did not reveal any difference in intestinal lipid absorption between the two strains (data not shown).

To validate the deleterious effect of the HF diet in Wistar animals (6, 20), glucose tolerance and insulin sensitivity were evaluated using a GTT and euglycemic hyperinsulinemic clamps on weeks 5 and 6. First, fasting plasma glucose and insulin levels were increased by the HF diet in Wistar rats only (Table 2), in keeping with previous data in the literature (30). Second, significant glucose intolerance was detected in HF-fed Wistar rats compared with controls, whereas this was not the

---

Fig. 1. Effect of a high-fat (HF) diet on food intake, body weight, and insulin sensitivity. Std, standard diet. A: evolution of food intake during the 1st wk of HF diet presentation. Daily food intake expressed on week 4 and week 9. B: overall body weight gain during the 1st 4 wk of HF diet. C: food efficiency (g body wt gained/100 g food intake) over 4 wk of HF diet. D: glucose tolerance test. Glycemia after acute glucose injection (1.5 g/kg ip) and areas under curves (AUC) over 120 min. E: glucose infusion rate (GIR) obtained during euglycemic hyperinsulinemic clamps. Values are means ± SE of 6 animals per group. * \( P < 0.05 \) vs. Std rats; # \( P < 0.05 \) vs. diet-matched Wistar animals (ANOVA).
case in Lou/C animals (Fig. 1D). Concerning insulin sensitivity, the Lou/C strain was described as being more insulin sensitive than Wistar rats (37). When euglycemic hyperinsulenic clamps were performed, the glucose infusion rate (GIR) needed to maintain euglycemia was lower in HF- than in Std-fed Wistar rats, indicating a state of diet-induced insulin resistance, which did not occur in Lou/C rats (Fig. 1E).

To explain the decreased food efficiency in Lou/C rats, analyses of metabolic parameters were conducted using indirect calorimetry. Results showed a higher energy consumption ($V_O2$) (Fig. 2A) and heat (H) production (Fig. 2B) during both nocturnal and diurnal periods in HF-fed Lou/C than in HF-fed Wistar rats. This was not accompanied by any increase in food intake (Fig. 2C) or locomotor activity (Fig. 2D). Of note, HF-fed Wistar rats were less active than Std-fed ones, especially during the nocturnal period, which could potentially contribute to the obesity development. As expected, RER was decreased in rats under HF diet in the two strains (Fig. 2E).

Table 2. Effect of 5-wk HF diet on body weight and metabolic and hormonal parameters

<table>
<thead>
<tr>
<th></th>
<th>Wistar Std</th>
<th>Wistar HF</th>
<th>Lou/C Std</th>
<th>Lou/C HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body wt, g</td>
<td>403.7 ± 7.0</td>
<td>410.0 ± 4.4</td>
<td>226.7 ± 7.1†</td>
<td>228.5 ± 4.8†</td>
</tr>
<tr>
<td>Final body wt, g</td>
<td>444.3 ± 7.0*</td>
<td>471.0 ± 7.4*</td>
<td>232.1 ± 5.5†</td>
<td>241.8 ± 3.6†</td>
</tr>
<tr>
<td>Fasting glucose, mM</td>
<td>5.1 ± 0.1</td>
<td>5.7 ± 0.3*</td>
<td>4.5 ± 0.1†</td>
<td>4.7 ± 0.1†</td>
</tr>
<tr>
<td>Fasting insulin, ng/ml</td>
<td>2.02 ± 0.26</td>
<td>3.65 ± 0.38*</td>
<td>1.78 ± 0.20</td>
<td>1.21 ± 0.15†</td>
</tr>
<tr>
<td>NEFA, mM</td>
<td>1.66 ± 0.31</td>
<td>5.16 ± 0.47*</td>
<td>0.94 ± 0.14†</td>
<td>1.96 ± 0.30†</td>
</tr>
<tr>
<td>TG, mM</td>
<td>1.36 ± 0.20</td>
<td>2.67 ± 0.34*</td>
<td>0.79 ± 0.07†</td>
<td>0.77 ± 0.06†</td>
</tr>
<tr>
<td>Ghrelin, ng/ml</td>
<td>1.3 ± 0.1</td>
<td>1.0 ± 0.1*</td>
<td>2.1 ± 0.2†</td>
<td>1.7 ± 0.11*</td>
</tr>
<tr>
<td>$T_s$, mM</td>
<td>1.08 ± 0.10</td>
<td>1.41 ± 0.10*</td>
<td>1.37 ± 0.05†</td>
<td>1.24 ± 0.04†</td>
</tr>
<tr>
<td>Free $T_s$, pg/ml</td>
<td>1.44 ± 0.21</td>
<td>1.58 ± 0.32</td>
<td>1.93 ± 0.26†</td>
<td>1.58 ± 0.32</td>
</tr>
<tr>
<td>$T_4$, mM</td>
<td>61.8 ± 3.1</td>
<td>70.8 ± 1.6*</td>
<td>53.8 ± 2.3†</td>
<td>44.7 ± 2.1†</td>
</tr>
<tr>
<td>Free $T_4$, ng/dl</td>
<td>0.95 ± 0.09</td>
<td>1.10 ± 0.08</td>
<td>1.26 ± 0.05†</td>
<td>1.16 ± 0.06</td>
</tr>
</tbody>
</table>

Values represent means ± SE of 8–10 animals per group. NEFA, nonesterified fatty acid; $T_s$, triiodothyronine; $T_4$, thyroxine; TG, triglyceride. *$P < 0.05$ vs. Std rats; †$P < 0.05$ vs. diet-matched Wistar animals (2-way ANOVA).

Fig. 2. Effect of HF diet on energy metabolism. Production of $O_2$ ($V_O2; A$), heat production (H; $B$), food intake ($C$), ambulatory and fine locomotor activities ($D$), and respiratory quotient (RER; $E$), measured using the LabMaster system. Values are means ± SE of 3 animals per group. *$P < 0.05$ vs. Std rats; †$P < 0.05$ vs. diet-matched Wistar animals (ANOVA).
strains, especially when the animals were fed the HF diet. Indeed under standard conditions, we observed significantly higher diameter of adipocytes in inguinal WAT (WATi; Fig. 3B) and in epididymal WAT (WATe) (37) of Wistar than in Lou/C rats. Under the HF diet, the diameter of epididymal adipocytes was almost doubled in the Wistar group (63 ± 2 μm for Std diet and 109 ± 3 μm for HF diet, P < 0.05), whereas it was not modifed in Lou/C animals (54 ± 2 μm for Std diet and 63 ± 2 μm for HF diet, not significant). To quantify these observations, body composition was determined using micro-CT, and, after 3-D reconstruction of the data (Fig. 3C), fat deposition was manually segmented in different anatomic compartments. Table 3 shows that, except for subcutaneous (thorax and abdomen) and axilar fat, all fat depots were significantly heavier in Wistar than in Lou/C rats under Std diet, the difference being particularly marked for retroperitoneal fat, as well as for the intraperitoneal (not including epididymal and retroperitoneal depots) fat depot, which was...
Table 3. Effect of 5-wk HF diet on the mass of various adipose tissue depots, as assessed by microcomputerized tomography

<table>
<thead>
<tr>
<th>Region</th>
<th>Wistar Std</th>
<th>Wistar HF</th>
<th>Lou/C Std</th>
<th>Lou/C HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thorax</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>0.38 ± 0.05</td>
<td>1.15 ± 0.36*</td>
<td>0.73 ± 0.04</td>
<td>0.87 ± 0.08</td>
</tr>
<tr>
<td>Axil</td>
<td>3.49 ± 0.16</td>
<td>6.52 ± 0.51*</td>
<td>3.34 ± 0.09</td>
<td>5.83 ± 0.54*</td>
</tr>
<tr>
<td>Interscapular</td>
<td>3.29 ± 0.43</td>
<td>5.37 ± 0.45*</td>
<td>1.84 ± 0.13†</td>
<td>3.06 ± 0.12‡†</td>
</tr>
<tr>
<td>Abdomen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>16.08 ± 6.34</td>
<td>29.45 ± 8.51</td>
<td>13.73 ± 1.63</td>
<td>23.37 ± 2.90*</td>
</tr>
<tr>
<td>Retroperitoneal</td>
<td>28.15 ± 5.29</td>
<td>58.02 ± 13.27*</td>
<td>5.65 ± 0.81†</td>
<td>10.79 ± 3.35‡†</td>
</tr>
<tr>
<td>Epididymal</td>
<td>16.21 ± 2.55</td>
<td>25.22 ± 4.59*</td>
<td>10.09 ± 1.41†</td>
<td>13.11 ± 1.74‡†</td>
</tr>
<tr>
<td>Intrapitoneal</td>
<td>21.35 ± 6.73</td>
<td>51.15 ± 13.01</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values represent means ± SE, in g/kg body wt, of 6 animals per group. *P < 0.05 vs. Std rats; †P < 0.05 vs. diet-matched Wistar animals (2-way ANOVA).

Concerning adipose tissue, the HF diet markedly increased fat storage-promoting enzymes in WATe of Wistar rats, such as LPL, ACCα, FAS, and SCD-1 (Fig. 4C). In Lou/C rats, the mRNA expression of these enzymes was not modified.

In contrast to WATe, no significant effect of the HF diet was observed for LPL, ACC, FAS, or SCD-1 expression in WATi of Wistar rats (Fig. 4D). However, the expressions of CPT Iα and UCP2 were significantly upregulated in Lou/C rats, suggesting that the increased oxidation in this specific fat depot.

The next step was to attempt understanding the mechanisms responsible for the higher thermogenesis observed in HF-fed Lou/C compared with HF-fed Wistar rats. Due to the fact that thyroid hormones are among the main regulators of thermogenesis, plasma T3, free T3, T4 and free T4 levels were determined (Table 2). Under the Std diet, Lou/C rats exhibited higher plasma T3, free T3, and free T4 levels. However, the HF diet induced a significant increase in T3 and T4 in Wistar rats only. The expression levels of UCPs were then investigated in interscapular BAT. No differences in UCPs, type II deiodinase (D2), and thyroid hormone receptor-α (TRα) expression levels between Wistar and Lou/C rats fed the Std diet were detected (data not shown). It was observed that the HF diet increased UCP3, D2, and TRα mRNA levels (Fig. 5A), as well as UCPI protein levels (Fig. 5B) in Wistar rats. All together, these data suggest elevated BAT energy expenditure in response to the HF diet in Wistar animals only. However, as none of these results could explain the increased thermogenesis in HF-fed Lou/C animals, we turned to our recent observation about the emergence of brown adipocytes in subcutaneous adipose tissue in Lou/C rats (37) and measured the mRNA expression of specific brown adipocyte markers in inguinal WAT of HF-fed animals. Markedly higher mRNA levels of peroxisome proliferator-activated receptor (PPARγ)2 and its coactivator PGC-1α were observed in Lou/C than in Wistar rats (Fig. 5C). Since PPARγ2 and PGC-1α expression in WAT was reported to be linked to the emergence of brown adipocytes within this tissue (13), the expression of UCP1, as well as that of PRDM16 and CIDEB, which are the most specific brown adipocyte markers (28, 38) was determined. As illustrated by a representative blot shown in Fig. 5D, the mRNA coding for UCP1 was clearly detected by using standard RT-PCR in HF-fed Lou/C rats, whereas this was not the case in Wistar animals. Using qPCR, a threefold increase of UCP1 was observed in Lou/C vs. Wistar rats (Fig. 5D). PRDM16 expression was similar in the two groups of Lou/C rats, whereas CIDEB expression was significantly decreased in HF- compared with Std-fed Lou/C rats (Fig. 5E).

Concerning adipose tissue, the HF diet induced the development of axilar and interscapular depots in the two strains, whereas it increased the retroperitoneal, the epididymal and the intraperitoneal depots in the Wistar group only. Of special interest is the fact that the HF diet induced the development of subcutaneous abdominal fat specifically in Lou/C rats. Plasma NEFA and leptin levels were at least doubled by HF feeding in both Wistar and Lou/C rats, although the increase was of higher amplitude in the former than in the latter group (Table 2 and Fig. 3E). On the other hand, plasma ghrelin levels were decreased by HF feeding in both groups, being higher in Lou/C than in Wistar rats whatever the diet consumed (Table 2). Finally, while plasma TG levels of Wistar rats were also almost doubled by HF feeding, they were unaltered by the diet in the Lou/C strain (Table 2). Increased plasma leptin levels were partly corroborated by the measurement of the mRNA expression of the ob gene in WATe. Thus, as can be seen in Fig. 3F, the ob gene expression was more than doubled in response to HF feeding in Wistar animals, whereas it was unchanged by the diet in Lou/C rats. The same tendency was observed in WATi and WATm, without reaching statistical significance. No fat deposition was observed in skeletal muscles of Wistar and Lou/C rats, as determined by the measurement of the tissue TG content. This was in line with the lack of changes in the mRNA expression levels of lipoprotein lipase (LPL), fatty acid-binding protein (FABP)3, and uncoupling protein (UCP)3 (data not shown).

Expression levels of key factors involved in lipid metabolism were also investigated in the liver, WATe, and WATi. We have previously observed that the expression of enzymes implicated in lipogenesis was lower in Lou/C than in Wistar rats fed a Std diet (37). During HF diet, and with regard to the liver, a significant increase in TG content and in the expression of stearoyl-coenzyme A desaturase-1 (SCD-1) was observed in Wistar rats only (Fig. 4, A and B). No effect of the HF diet was detected for the expression of hormone-sensitive lipase (HSL), acetyl-CoA carboxylase (ACC)α, fatty acid synthase (FAS), sterol response element-binding protein (SREBP)-1c, and the insulin-induced gene (INSIG)1 and INSIG2 in the liver (data not shown). However, as a likely compensatory mechanism against the development of obesity, expression of the fat oxidation-promoting enzyme, carnitine palmitoyltransferase (CPT) Iα, was significantly increased in both HF-fed Wistar and Lou/C rats (Fig. 4B).

The next step was to attempt understanding the mechanisms responsible for the higher expression of key factors involved in lipid metabolism in high-fat-fed Lou/C rats.
PRDM16 was present in WATi of Wistar rats but at levels twice below those observed in Lou/C animals (ratio of PRDM16/RPS29 concentration in ng: 0.44 ± 0.12 and 0.90 ± 0.25 in Std-fed Wistar and Lou/C rats, respectively). Concerning CIDEA, only marginal levels were detected in WATi of Wistar rats (ratio of CIDEA/RPS29 concentration in ng: 0.13 ± 0.06 and 1.72 ± 0.43 in Std-fed Wistar and Lou/C rats, respectively). Finally, when the epididymal fat depot was considered, the following was observed: low levels of PRDM16 in both groups (ratio of PRDM16/RPS29 concentration in ng: 0.22 ± 0.08 and 0.33 ± 0.19 in Std-fed Wistar and Lou/C rats, respectively); higher levels of CIDEA without intergroup difference (ratio of CIDEA/RPS29 concentration in ng: 9.0 ± 2.0 and 7.0 ± 1.5 in Std-fed Wistar and Lou/C rats, respectively); and no detection of UCP1 in either Wistar or Lou/C rats (Fig. 5F).

Interestingly, emergence of brown adipocytes in WAT was reported to occur in response to cold exposure or to treatment

Fig. 4. Effect of HF diet on hepatic and WAT metabolism. A: hepatic triglyceride (TG) content after 5 wk of experimental diet. B: expression levels of stearoyl-CoA desaturase-1 (SCD-1) and carnitine palmitoyltransferase Iα (CPT1α). Results are expressed as %relative density measured in animals fed a Std diet, which, after normalization with cyclophilin expression, is set at 100% for each group. C and D: expression levels of lipoprotein lipase (LPL), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), SCD-1, hormone-sensitive lipase (HSL), CPT1α and uncoupling protein (UCP)2 in epididymal (WATe) and inguinal (WATi) WAT. Results are expressed as %relative density measured in animals fed a Std diet, which, after normalization with RPS29 expression, is set at 100% for each group. Values are means ± SE of 8–10 animals per group. *P < 0.05 vs. Std rats (ANOVA).
Fig. 5. Effect of HF diet on brown adipose tissue (BAT) metabolism and on markers of BAT emergence in WAT. A: expression levels of UCP1, UCP3, type II deiodinase (D2), and thyroid hormone receptor (TRα) in BAT. Results are expressed as %relative density measured in animals fed a Std diet, which, after normalization with GAPDH expression, is set at 100% for each group. B: representative Western blots of UCP1 in BAT of 3 rats per group revealed using horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence detection. Quantification using ChemiDoc XRS and the Quantity One software. C: expression levels of peroxisome proliferator-activated receptor (PPARγ)2, and its coactivator PGC-1α in WATi. Results are expressed as %relative density measured in Wistar rats fed a Std diet, set at 100% after normalization with RPS29 expression. D: migration on a 2% agarose gel of UCP1 and RPS29 RT-PCR products from WATi. UCP1 RT-PCR product from BAT was used as a positive control and water as a negative control. L, Lou/C; W, Wistar. mRNA expression of UCP1 in WATi of Std- and HF-fed Wistar and Lou/C rats. Results are expressed as %relative density measured in Wistar rats fed a Std diet, set at 100% after normalization with RPS29 expression. E: migration on a 2% agarose gel of UCP1 and RPS29 RT-PCR products from WATi and WATe of HF-fed Lou/C rats. UCP1 RT-PCR product from BAT was used as a positive control and water as a negative control. Some lanes (in D and F, indicated in solid lines) have been rearranged from the resultant image of the autoradiograph, since nonessential samples were run on the same gel and removed for final presentation. Values are means ± SE of 8–10 animals per group. *P < 0.05 vs. Std rats; #P < 0.05 vs. diet-matched Wistar animals (ANOVA).
with specific β3-adrenoreceptor agonists (8, 9, 16). Lou/C and Wistar rats were therefore submitted to cold exposure for 2 h. At standard temperature of housing (22°C), no significant strain differences were observed for rectal temperature either under Std (36.2 ± 0.3 and 36.3 ± 0.2°C for Lou/C and Wistar rats, respectively) or HF diet (36.0 ± 0.3 and 36.2 ± 0.3°C for Lou/C and Wistar rats, respectively). However, when rats were placed at 6°C for 2 h, Lou/C rats showed higher potency to generate heat, increasing (Std, Fig. 6A, top) or maintaining (HF, Fig. 6A, bottom) body temperature. As cold-induced temperature of Wistar rats was either maintained (Std, Fig. 6A, top) or even decreased (HF, Fig. 6A, bottom), this parameter was higher in the Lou/C than in the Wistar group, regardless of the food consumed.

Finally, the mRNA expression of β-adrenergic receptors was quantified in WATi. Interestingly, the expression levels of the β3-receptors were tripled in Lou/C compared with Wistar rats fed either diet (Fig. 6B). Moreover, under the HF diet, the mRNA levels of the β1-receptor were significantly increased in Lou/C rats (Fig. 6B).

DISCUSSION

Previous studies have reported that, when submitted to a self-selection diet, Lou/C rats spontaneously select up to 70% of their calories as lipids but succeed in maintaining normal daily caloric intake and body weight gain (35, 36). On the other hand, it is well known that Wistar rats placed on HF diet rapidly develop diet-induced obesity with increased caloric intake (6). A rapid adaptation to the HF diet was observed in Lou/C rats (within 3 days) with decreased food intake to maintain caloric intake, while a similar behavior was observed after 7 days in Wistar rats; however, the caloric intake of Wistar rats fed the Std or the HF diet was similar only after 7 wk. In terms of body weight gain, Wistar rats took ~15% of their initial body weight during 5 wk of HF diet, whereas body weight gain of Lou/C rats accounted for only 4%. Consequently and very interestingly, whereas food efficiency was doubled by the HF diet in Wistar rats, it was unchanged in Lou/C animals. From results obtained with body composition or lipid metabolism it could be hypothesized that body weight gain of Wistar rats is essentially associated with changes in fat mass. This was particularly striking for intraperitoneal fat, which increased more than threefold in the Wistar group in response to HF feeding, whereas it remained undetectable in the Lou/C group. This observation is of importance, as intraperitoneal fat is known to be particularly deleterious for metabolic homeostasis, being tightly correlated with quantitative and qualitative changes in plasma lipids and lipoproteins as
well as with the development of insulin resistance (24) and metabolic disorders in humans (12). Of note, it cannot be argued that lack of response to HF in Lou/C rats could be due to extreme low adiposity under standard conditions. Percentage of fat mass determined by EchoMRI at 10 wk of age was in the normal range (11.7 ± 0.8 and 10.7 ± 0.5% for Wistar and Lou/C rats, respectively).

With regard to hormonal and metabolic parameters, we confirmed that Lou/C rats displayed optimized metabolic and hormonal values, such as lower plasma glucose, insulin, leptin, NEFA, and TG levels than Wistar rats, that were not (or only modestly) modified by the HF diet. This is in contrast to the effect of the HF diet in Wistar rats. Concerning leptin, it is known that rats fed a HF diet develop central leptin resistance (23). In this respect, it would be of great interest to test whether, in contrast to Wistar rats, adequate central leptin sensitivity is maintained in HF-fed Lou/C animals, which would partially explain their decreased daily caloric intake in response to the high-calorie diet. Along this line, upon following the dynamic changes of plasma leptin levels during the first 2 wk of HF diet, distinct patterns were interestingly observed in Lou/C and Wistar rats. Indeed, while plasma leptin levels doubled after 1 day of HF feeding in both groups, these levels subsequently decreased in the Lou/C but not in the Wistar group. It therefore appears that HF feeding promotes an initial increased leptinemia in all animals, likely representing a general adaptive response to counteract the effect of overfeeding. It can be hypothesized that the sensitivity to this change may be different in obesity-prone and -resistant rodents, with increased sensitivity in the latter group. This is currently under investigation in different age groups by injecting leptin either peripherally or centrally.

When considering the dynamic changes of plasma insulin levels during the initial phase of HF feeding, we observed that these remained unchanged in Lou/C rats, whereas they started to rise after 19 days of HF diet in Wistar rats (data not shown). Of additional interest is the fact that total plasma ghrelin levels, a gastric hormone stimulating food intake (31), were higher in Lou/C than in Wistar rats, even in the face of a HF diet, which usually decreases plasma ghrelin levels (22). Concerning this issue, a clear conclusion about ghrelin in Lou/C vs. Wistar rats cannot be drawn before measurement of the active octanoylated form of ghrelin. In preliminary experiments, we observed that the ratio between total and active ghrelin was higher in Wistar than in Lou/C rats (P = 0.0205) fed the Std diet, a difference that was no longer present under HF diet. These data might indicate that the ghrelin system is more “efficient” in Wistar than in Lou/C rats. However, they need to be considered with caution, given that the validity of active ghrelin measurements using commercial kits is a matter of debate.

As a partial adaptive response to the HF diet, the expression of CPT 1α, an enzyme that is involved in the transport of fatty acids into the mitochondria for β-oxidation, was augmented in the liver of both Lou/C and Wistar rats. Similarly, the RER was significantly decreased in the two groups on a HF diet, confirming lipid oxidation.

Furthermore, higher VO₂ and heat production, without any modification of fine or ambulatory locomotor activities was observed in HF-fed Lou/C rats compared with Wistar. This led us to investigate the thyroid function and expression of uncoupling proteins in BAT and WAT. No data arguing toward a specific stimulation of the thyroid axis in the liver were observed. Interestingly, the BAT mRNA or protein expression of UCP1, UCP3, D2, TRα, and the circulating levels of total, but not free, thyroid hormones were significantly increased in response to the HF diet in Wistar rats only. Such changes in BAT UCP expression are the likely consequence of the marked increase in plasma NEFA levels observed in this group, given that NEFA are well known activators of these two UCPs (2, 17). In Lou/C rats, no significant stimulation of UCP1 expression was observed in BAT, skeletal muscles, or epididymal WAT (data not shown). In contrast, a very specific expression profile was observed in the inguinal fat pad of this strain, the expressions of PPARγ2 and PGC-1α being markedly stimulated. Due to the facts that 1) PGC-1α coactivates PPARγ on the UCP1 promoter (27), 2) forced expression of PGC-1α in the 3T3-L1 cell line or in human white adipocytes induces the expression of UCP1 (27, 32), 3) increased expression of these two factors in WAT was reported to be linked with the emergence of brown adipocytes (13) and that we previously observed UCP1 expression in WAT of Std-fed Lou/C rats (37), the WATi expression of UCP1 and of two specific brown adipocyte markers, namely CIDEA and PRDM16, was measured in the two groups. There was a clear-cut expression of UCP1 in HF-fed Lou/C rats of similar amplitude to that seen in Std-fed animals. The expression of PRDM16 and of CIDEA also indicates the presence of brown adipocytes in WATi. However, expression levels of UCP1 in subcutaneous WAT are still lower than in BAT. Whether it would imply a relevant physiological function will need further investigations.

All together, these results imply that the stimulation of thermogenic activity of UCP1 in BAT is likely an adaptive mechanism to counteract the development of HF-induced obesity. However, as demonstrated in Wistar rats, such an adaptive mechanism is apparently not sufficient. In contrast, the Lou/C rats exhibit a specific stimulation of lipolysis and probably thermogenesis in subcutaneous WAT, correlating with their resistance to the development of diet-induced obesity. These results are in keeping with the observation that low susceptibility to develop obesity and diabetes is observed in specific strains of mice exhibiting an elevated number of brown adipocytes in their WAT (11). They also fit with the data showing that transgenic mice expressing UCP1 in WAT are resistant to diet- and genetic-induced obesity (18, 19).

Finally, our study demonstrates the presence of higher expression levels of β₁- and β₂-adrenergic receptors in inguinal WAT of HF fed Lou/C vs. Wistar rats. This is in keeping with the high density of noradrenergic fibers in WAT, leading to high sensitivity to sympathetic activation, such as observed in epididymal and retroperitoneal WAT after fasting (10) or in subcutaneous WAT after cold exposure (25). It is obviously of particular interest, since β₃-adrenergic receptors seem to play a pivotal role in transdifferentiation of white into brown adipocytes (7). In keeping with the importance of the sympathetic nervous system for transdifferentiation phenomena (7, 25), it should be mentioned that previous studies reported elevated norepinephrine content in retroperitoneal and epididymal WAT of Lou/C compared with Wistar rats (26). No data have been reported so far for this parameter in subcutaneous depots, in which a precise assessment of the sympathetic activity would be of a great interest. The functional importance of the presence of brown adipocytes in WATi of Lou/C rats shown in the
present study is likely demonstrated by their improved response to maintaining body temperature in response to cold exposure. Further work, under current investigation, will help strengthen the link between body weight regulation and expression of brown adipocytes in WAT in Lou/C rats.

In conclusion, fat storage is greatly stimulated in response to a HF diet in the Wistar strain, with a site-specific distribution pattern. Stimulation of BAT thermogenesis was insufficient to protect animals from developing obesity and did not occur in Lou/C animals. In these animals, optimal adaptation of food pattern. Stimulation of BAT thermogenesis was insufficient to explain their resistance to the development of diet-induced obesity. A clear understanding of the mechanisms underlying these phenomena represents important issues, as they could have a critical impact on the development of new therapeutic tools to treat human obesity and associated disorders.

ACKNOWLEDGMENTS

We acknowledge the platform of “Phénotype du petit animal” (http://www.medecine.unige.ch/lafaculte/services/phenotypage) for micro-CT utilization, and of “Histology” for histochemistry. We also thank Dr. T. J. Visser for the quantification of plasma thyroid hormone levels. We are grateful to Prof. L. Orci for providing excellent expertise on adipose tissue morphology and histology, to Prof. J. P. Giacobino for helpful discussion, and to Prof. B. M. Spiegelman for providing help in the gene probe design.

GRANTS

This work was supported by the Swiss National Science Foundation Grant no. 310030-120147.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


