The Lou/C rat: a model of spontaneous food restriction associated with improved insulin sensitivity and decreased lipid storage in adipose tissue

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Veyrat-Durebex C, Montet X, Vinciguerra M, Gjinovci A, Meda P, Foti M, Rohner-Jeanrenaud F. The Lou/C rat: a model of spontaneous food restriction associated with improved insulin sensitivity and decreased lipid storage in adipose tissue. Am J Physiol Endocrinol Metab 296: E1120–E1132, 2009. First published February 10, 2009; doi:10.1152/ajpendo.90592.2008.—The inbred Lou/C rat, originating from the Wistar strain, has been described as a model of resistance to diet-induced obesity, but little is known about its metabolism. Since this knowledge could provide some clues about the etiology of obesity/insulin resistance, this study aimed at characterizing glucose and lipid metabolism in Lou/C vs. Wistar rats. This was achieved by performing glucose and insulin tolerance tests, euglycemic hyperinsulinemic clamps, and characterization of intracellular insulin signaling in skeletal muscle. Substrate-induced insulin secretion was evaluated using perfused pancreas and isolated islets. Finally, body fat composition and the expression of various factors involved in lipid metabolism were determined. Body weight and caloric intake were lower in Lou/C than in Wistar rats, whereas food efficiency was similar. Improved glucose tolerance of Lou/C rats was not related to increased insulin output but was related to improved insulin sensitivity/responsiveness in the liver and in skeletal muscles. In the latter tissue, this was accompanied by improved insulin signaling, as suggested by higher activation of the insulin receptor and of the Akt/protein kinase B pathway. Fat deposition was markedly lower in Lou/C than in Wistar rats, especially in visceral adipose tissue. In the inguinal adipose depot, expression of uncoupling protein-1 was detected in Lou/C but not in Wistar rats, in keeping with a higher expression of peroxisome proliferator-activated receptor-γ coactivator-1 in these animals. The Lou/C rat is a valuable model of spontaneous food restriction with associated improved insulin sensitivity. Independently from its reduced caloric intake, it also exhibits a preferential channelling of nutrients toward utilization rather than storage.

metabolism; obesity; animal model

THE CURRENT EPIDEMIC OF OBESITY is linked to the development of the metabolic syndrome, which is characterized by glucose intolerance, insulin resistance, and dyslipidemia. The changes in nutrition, in particular the consumption of high-fat (HF) diets, as well as the concomitant decrease in physical activity can drastically modify insulin sensitivity (16). In addition, genetic predispositions also can modulate the susceptibility of individuals to develop obesity and type 2 diabetes after a HF diet (9).

Animal models that are prone or resistant to obesity are of valuable help for improving our knowledge on the etiology of obesity/insulin resistance. In this respect, the inbred Lou/C rat, originating from the Wistar strain (5), is of interest, since it is a model of resistance to the development of obesity as a function of age (13, 47, 48). When fed a normal diet, the Lou/C rat ingests fewer calories per day than the Wistar rat and was therefore proposed as a model of spontaneous caloric restriction (1, 47, 48). Moreover, 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 body weight gain over age (47). On the contrary, the Wistar rat usually becomes obese with age (31) and rapidly develops obesity and insulin resistance when submitted to a HF diet (9).

At present, only a few data about the regulation of glucose and lipid metabolism, as well as insulin secretion, have been reported in the Lou/C rat. It was shown that these rats exhibit an improved capacity to maintain euglycemia during a 60-min period of swimming while preserving hepatic glycogen stores (12). These results are in keeping with those of in vitro studies showing that the capacity for gluconeogenesis from glyceral is higher in hepatocytes from Lou/C than in those from Wistar rats (34). Euglycemic hyperinsulinemic clamps performed in 22-mo-old animals have shown that the glucose infusion rate required to maintain euglycemia during hyperinsulinaemia is higher in these old Lou/C than in age-matched Wistar rats, suggesting a better insulin sensitivity in the former than in the latter group of animals (34). A higher lipid utilization rate in Lou/C than in Wistar animals was suggested by the observation that Lou/C rats store less fat than Wistar rats under conditions of similar food intake (i.e., pair-feeding Wistar rats the same amount of food as consumed by Lou/C animals) (13).

The aim of this study was to further characterize glucose and lipid metabolism in Lou/C vs. Wistar rats by studying young adult animals fed a standard diet. After assessment of the main metabolic parameters, such as feeding and body weight gain patterns, glucose tolerance was assessed using a glucose tolerance test. Because we observed improved glucose tolerance in Lou/C rats, we then tried to discriminate between the potential role of either improved insulin secretion or insulin sensitivity. For this purpose, insulin secretion was studied using the perfused rat pancreas and isolated islets, and insulin sensitivity was determined using insulin tolerance tests (ITT) and euglycemic hyperinsulinemic clamps associated with the labeled 2-deoxy-D-glucose technique. Insulin signaling was thereafter studied in skeletal muscles using Western blot analyses. Expression of various enzymes involved in lipid metabolism was evaluated both in the liver and in adipose tissue. Finally, some of the changes observed in Lou/C rats were studied in a
pair-fed group of Wistar animals to determine whether they are strain specific or secondary to the low food intake.

MATERIALS AND METHODS

*Animals and diets.* Two-month-old male Wistar and Lou/C rats were purchased from Harlan UK Limited (Oxon, UK) and Charles River (L’Arbresle, France), respectively. They were housed in pairs under controlled conditions (23°C; lights on 0700–1900) and were allowed free access to water and diet (RMI; Herstelser, Essex, UK). At 3.5 mo of age (14 wk), body weight and food intake were recorded twice daily (0830 and 1730) during 6 wk. A first colony (Wistar, n = 10; Lou/C, n = 10) was submitted to a glucose tolerance test (GTT) on week 6 and to a euglycemic hyperinsulinemic clamp on week 7. A second colony (Wistar, n = 8; Lou/C, n = 10) was analyzed for ITT on week 4 and body fat composition by microcomputerized tomography (micro-CT) on week 5. A third colony (Wistar, n = 9; Lou/C, n = 10) was used for analyses of pancreatic islet morphology and insulin secretion using the isolated perfused pancreas and preparation of isolated islets. Finally, a fourth colony was used to determine the effects of the chronic caloric restriction observed in Lou/C rats on various metabolic parameters. Food intake of Lou/C (n = 8) and Wistar (n = 8) rats was recorded daily from 5 to 21 wk of age. A third group of Wistar rats (n = 8) was included that was restricted to the same amount of food as that consumed by the Lou/C group (pair-fed group; PF). These rats were weighed twice a week. At 19 wk of age, all the animals were submitted to a GTT, and body composition was determined at 20 wk using a micro-CT. All rats were killed between 0900 and 1300 using isoflurane (Halocarbon Laboratories, River Edge, NJ) anesthesia and rapid decapitation. Blood was collected to measure the concentration of various hormones and metabolites. Tissues were freeze-clamped and stored at −80°C for determination of gene expression. The procedures were approved by the ethics committee of our university and were in accordance with the Swiss guidelines for animal experimentation.

*Plasma measurements.* Plasma glucose was measured using the glucose oxidase method (Glu; Roche Diagnostics, Rotkreuz, Switzerland). Plasma nonesterified fatty acid and triglyceride levels were determined using kits from Wako Chemicals (Neuss, Germany) and Biomérieux (Marcy l’Etoile, France), respectively. Plasma corticosterone (Immunodiagnostic Systems, Boldon, UK), ghrelin, and leptin (Linco Research, St. Charles, MO) levels were determined using double-antibody radioimmunoassay (RIA) kits. Plasma insulin levels also were measured by RIA, as previously described (20). Finally, plasma adiponectin levels were determined using an ELISA commercial kit (AdipoGen, Seoul, Korea).

*Glucose and insulin tolerance tests.* Wistar, Wistar PF, and Lou/C rats were fasted for 4 h. For GTT, a glucose load of 1.5 g/kg was administered intraperitoneally. Blood samples were collected by tail nicking for further analyses of glucose and insulin concentrations. For ITT, insulin was administered intraperitoneally at a dose of 0.75 UI/kg, and glycemia was recorded using Glucotrend Active (Roche, Basel, Switzerland).

*Immunofluorescence.* For insulin immunostaining, pieces of pancreas were fixed in Bouin’s solution and processed according to standard methods (10). Briefly, 5-μm-thick sections were incubated for 2 h at room temperature with a guinea pig polyclonal serum

### Table 1. Body weight, food intake, and plasma and tissue metabolite and hormone levels in Wistar and Lou/C rats

<table>
<thead>
<tr>
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<th>Wistar</th>
<th>Lou/C</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>444.3±7.0</td>
<td>233.8±5.5*</td>
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<tr>
<td>Relative body weight gain, g/day</td>
<td>1.05±0.35</td>
<td>0.77±0.14</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>9.56±0.77</td>
<td>7.66±0.39*</td>
</tr>
<tr>
<td>NEFA, mM</td>
<td>1.32±0.12</td>
<td>0.99±0.56*</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>147±22</td>
<td>84±5*</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>1.31±0.14</td>
<td>0.79±0.07*</td>
</tr>
<tr>
<td>Corticosterone, ng/ml</td>
<td>64.2±8.5</td>
<td>36.0±7.4*</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>5.51±0.69</td>
<td>1.83±0.11*</td>
</tr>
<tr>
<td>Ghrelin, ng/ml</td>
<td>1.31±0.12</td>
<td>2.13±0.18*</td>
</tr>
<tr>
<td>TG content in liver, mg/g tissue</td>
<td>15.3±2.1</td>
<td>14.5±2.1</td>
</tr>
<tr>
<td>TG content in gastrocnemias, mg/g tissue</td>
<td>5.0±1.2</td>
<td>5.8±0.7</td>
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</table>

Values are means ± SE of 8 Wistar and 10 Lou/C rats. Body weight is given as measured at the end of the experiment (i.e., at 20 wk of age); relative body weight gain was calculated on the 4th week of the experiment (i.e., from 18 to 19 wk of age). NEFA, nonesterified fatty acids; TG, triglyceride. *P < 0.05 using Student’s t-test.
against insulin (1:800) (49). After being rinsed in phosphate-buffered saline (PBS), sections were incubated for 1 h at room temperature with a fluorescein-conjugated goat anti-guinea pig IgG (1:400). After a 4-min staining in 0.03% Evans blue, sections were coverslipped with a drop of 0.02% paraphenylenediamine in glycerol-PBS (2:1) and photographed with an Axiophot fluorescence microscope. Negative controls included different dilutions of the primary antibody, exposure to a nonimmune serum, and incubation with the secondary antibody only. None of these controls resulted in a specific staining of the cells and tissues studied.

**Perfused pancreas and isolated islets.** Rats were processed for pancreas perfusion as previously described (10, 45). Briefly, the pancreas was perfused with a Krebs-Hank’s buffer (KHB) at a constant rate of 5 ml/min via mesenteric and transileac arteries, and the perfusate was collected every minute from the portal vein. After an initial equilibration period with no sample collected, each pancreas was successively perfused for 15-min periods with KHB containing the following glucose concentrations sequence: 2.8 mM (basal), 8.4 mM (postprandial levels), 2.8 mM, 16.8 mM (supraphysiological stimulation), 2.8 mM, 16.8 mM plus 1 nM glucagon-like peptide-1 (GLP-1; PolyPeptide Laboratories, Hillerod, Denmark) (receptor plus supraphysiological stimulation), and 2.8 mM. Aliquots of perfusates were collected on ice and stored at −20°C until RIA. The pancreas was sampled, weighed, and immediately extracted in 5 ml of acid ethanol for evaluation of total insulin content by RIA (Linco Research) (29).

Islets were isolated by collagenase digestion from groups of two Lou/C and two Wistar rats in three independent experiments. After an overnight culture in RPMI 140 medium supplemented with 10% fetal calf serum and 11 mM glucose, series of 20 islets (3 islet

![Fig. 2](http://ajpendo.physiology.org/)

Glucose and insulin tolerance tests and tissue-specific glucose utilization index during euglycemic hyperinsulinemic clamps in Wistar and Lou/C rats. **A**: evolution of glycemia at 0, 15, 30, 60, and 120 min after acute glucose injection (1.5 g/kg ip) (left) and areas under curves (AUC) over 120 min after the injection (right). **B**: change in (Delta) glycemia 0, 15, 30, and 60 min after acute insulin injection (0.75 UI/kg ip) expressed as a percentage of basal glycemia. **C**: uptake of 2-deoxy-D-[1-3H]glucose expressed in tissue. RQ, red quadriceps; WQ, white quadriceps; RG, red gastrocnemius; WG, white gastrocnemius; BAT, brown adipose tissue. Values are means ± SE of 10 rats per group. *P < 0.05 using Student’s t-test.
aliquots per condition) were preincubated for 30 min at 37°C in 1 ml of Krebs-Ringer-bicarbonate buffer containing 2.8 mM glucose. The medium was then discarded and replaced for 30 min at 37°C by 1 ml of the test medium supplemented with stimuli as indicated previously. At the end of these experiments, the medium was sampled and rapidly frozen until assayed, while the islets were extracted in 0.5 ml of acid ethanol (10).

Euglycemic hyperinsulinemic clamps. Rats were fasted overnight and anesthetized with Nembutal (50 mg/kg ip; Abbott Laboratories, Chicago, IL). Hepatic glucose production and glucose infusion rate to

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**Fig. 3.** Immunofluorescence and insulin secretion in perfused pancreas and isolated islets of Wistar and Lou/C rats. A: immunofluorescence of insulin immunostaining (green, fluorescein-conjugated antibody) in a representative islet of Wistar (a) and Lou/C rats (b). Scale bar, 25 μm. B: pancreatic weight (left) and insulin content (right). C: profile of glucose- and glucagon-like peptide-1 (GLP-1)-induced insulin secretion. D: cumulative insulin secretion over 15 min expressed as AUC in perfused pancreas. Values are means ± SE of 4 rats per group. E: insulin secretion of isolated islets expressed as a percentage of insulin content. Values are means ± SE of 10–12 islet aliquots from 3 independent experiments. *P < 0.05 using 1-way ANOVA (pancreas weight, insulin content and secretion in isolated islets) and Mann-Whitney and Kolmogorov-Smirnov tests (AUC in perfused pancreas).
maintain euglycemia were measured under basal and insulin-stimulated (200 mU/mL; Actrapid HM; Novo Nordisk, Bagsvaerd, Denmark) conditions, as previously described (44, 46). At the end of the euglycemic hyperinsulinemic clamps, the in vivo insulin-stimulated glucose utilization index of individual tissues was determined using 2-deoxy-o-[1-3H]glucose (30 μCi/rat; Amersham Biosciences UK, Little Chalfont, UK) (44, 46). Rats were killed by decapitation, and tissues were rapidly removed and stored at −80°C. The 2-deoxy-o-[1-3H]glucose and o-[U-14C]glucose specific activities were determined in deproteinized blood samples (44, 46). Measurement of tissue concentration of 2-deoxy-o-[1-3H]glucose-6-phosphate allowed calculation of the in vivo glucose utilization index of individual tissues and was expressed in nanograms per minute per milligram of tissue.

Western blot for insulin signaling in skeletal muscles, white adipose tissue, and the liver. Frozen tissues (red gastrocnemius and the liver) were mechanically homogenized in ice-cold RIPA buffer (15). Lysates were processed for Western blot analyses, and protein levels were quantified (15). Antibodies against insulin receptor (IR), phospho-IR, insulin receptor substrate-1 (IRS-1), phospho-IRS-1 (Tyr632), peroxisome proliferator-activated receptor-γ (PPAR-γ), and PPAR coactivator-1α (PGC-1α) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-Akt, phospho-Akt (Thr308 and Ser473), phospho-IRS-1 (Ser636/639), 44/42 MAP kinase (ERK1/2), and phospho-mTOR (Ser2448), S6 kinase, and phospho-S6 kinase (Thr389) were obtained from Cell Signaling Technology (Danvers, MA). Antibodies against fatty acid synthase and actin were obtained from BD Transduction Laboratories (Lexington, KY) and Chemicon International (Billerica, MA), respectively. Detection was performed using horseradish peroxidase-conjugated secondary antibodies and an enhanced chemiluminescence (ECL) detection system (Amersham Biosciences). Quantifications were then performed using the ChemiDoc XRS from Bio-Rad Laboratories (Hercules, CA) and Quantity One software.

Determination of body fat composition by micro-CT. In vivo micro-CT images of anesthetized rats (Nembutal, 50 mg/kg ip) were acquired on a Skyscan-1076 micro-CT (Skyscan, Aartselaar, Belgium) using an X-ray charge-coupled device camera with a cooled 1,024 × 1,024-pixel 12-bit sensor (http://www.medecine.unige.ch/lafaculte/services/phenotypage). The camera was rotated through 185°, and images were made every 1.4°, achieving an in vivo resolution of 35 μm. The acquisition parameters permitted a clear delineation of fat, muscles, and bone in each of the scans obtained during two 40-min scanning periods for thoracic and abdominal parts. Reconstruction of acquired data was made through a cone-beam reconstruction method to account for the conical geometry of the X-ray source (8). Delineation of fat was straightforward, since fat has a negative Hounsfield unit compared with positive values for muscle and bone (41). After reconstruction, fat deposition was manually segmented with Osirix in the following anatomical compartments: for the thorax: 1) subcutaneous, 2) axilar, and 3) interscapular; and for the abdomen: 1) subcutaneous, 2) visceral, 3) retroperitoneal, and 4) epididymal. Volume of fat was automatically generated from the segmented region. Before the in vivo experiment, known masses of fat ranging from 13 to 500 mg were scanned and their volumes calculated. The correlation between volumes of reconstructed fat with the actual fat weight was linear (correlation coefficient r = 0.999), allowing us to convert the CT results into grams of tissue.

Skeletal muscle and hepatic triglyceride content. For the determination of total lipid in skeletal muscles (white gastrocnemius) and in the liver, weighed quantities of frozen tissue (~100 mg) were powdered under liquid N2 and extracted overnight at 4°C in chloroform-methanol (2:1) (42). Phase separation was obtained after addition of H2SO4 (1 M) and centrifugation. The upper phase was discarded, and the lower phase was washed with H2SO4. Phospholipids were removed by adding silicic acid previously activated by heating, and by centrifugation. Two milliliters of the lower organic phase were removed and evaporated under N2. The resulting pellet was suspended in assay buffer (0.4 M Tris, 0.2 M NaCl, 10 mM EDTA, 10 mg/ml bovine serum albumin, and 1% Triton X-100, pH 8.4), and triglyceride content was determined by colorimetric enzymatic analysis, as mentioned above.

Tissue processing and RT-PCR. Total RNAs were extracted from frozen tissues by using a single-step extraction procedure with Trizol reagent (Sigma-Aldrich, Buchs, Switzerland). Total RNA (2.5 μg) was used for reverse transcription with random hexamers (Mircosynth, 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 12.5 ng of cDNA using the SYBR green PCR master mix (Applied Biosystems, Warrington, UK) on an AB7500 machine (Applied Biosystems). For PCR, amplification was performed using TaqDNA polymerase (Qiagen, Hilden, Germany), 10 mM dNTPs, and 3 mM MgCl2. Primers were designed with the PrimerExpress software and used in qPCR at 200–300 nM and in classic PCR at 20 μM (Supplemental Tables 1 and 2, respectively). (Supplemental data for this article is available online at the American Journal of Physiology-Endocrinology and Metabolism website.) Results were normalized to expression levels of housekeeping genes, namely, cyclophilin for the liver, ribosomal protein S29 for adipose tissue, and glyceraldehyde-3-phosphate dehydrogenase for skeletal muscles.

Data analyses. Results are means ± SE. Comparison between two groups was realized using Student’s t-test or the Kruskal-Wallis nonparametric test when normality and equal variance tests failed (SPSS, Chicago, IL). Comparison among three groups was realized using one-way analysis of variance (ANOVA). Correlation between different gene expression and between body and pancreas weight was calculated using the Pearson correlation coefficient. For results obtained during the pancreas perfusion experiment, comparisons were made using one-way ANOVA. Comparisons of pancreas perfusion curves were realized using the Mann-Whitney and Kolmogorov-Smirnov tests. Statistical significance was established at P < 0.05.

RESULTS

From 15 to 18 wk of age, Lou/C rats had a markedly lighter body weight (P < 0.0001) and a lower daily food intake (P < 0.0001) than age-matched Wistar rats (Fig. 1, A and B). Nevertheless, the daily relative body weight gain and food efficiency calculated over 3 wk (i.e., from week 15 to week 17) were similar in the two strains (Table 1 and Fig. 1C). Analyses of feeding pattern provided a similar percentage of food consumed during the dark (81.6 ± 0.9 and 86.8 ± 0.8% for Lou/C and Wistar rats, respectively) and light periods (18.4 ± 0.9 and 13.2 ± 0.8% for Lou/C and Wistar rats, respectively).

Table 2. In vivo glucose metabolism using euglycemic hyperinsulinemic clamps in Wistar and Lou/C rats

<table>
<thead>
<tr>
<th>Wistar</th>
<th>Lou/C</th>
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<tbody>
<tr>
<td><strong>Basal insulinemia, ng/ml</strong></td>
<td>1.34 ± 0.16</td>
</tr>
<tr>
<td><strong>Basal glycemia, mM</strong></td>
<td>3.63 ± 0.17</td>
</tr>
<tr>
<td><strong>Final glycemia, mM</strong></td>
<td>16.4 ± 1.2</td>
</tr>
<tr>
<td><strong>GIR, mg·kg⁻¹·min⁻¹</strong></td>
<td>5.81 ± 0.30</td>
</tr>
<tr>
<td><strong>HGP, mg·kg⁻¹·min⁻¹</strong></td>
<td>15.6 ± 0.8</td>
</tr>
</tbody>
</table>
| **HGP, mg·kg⁻¹·min⁻¹** | 5.2 ± 1.9 | 0.2 ± 1.2

Values are means ± SE of 8 Wistar and 8 Lou/C rats. Experiments were performed after an overnight fast. GIR, glucose infusion rate; HGP, hepatic glucose production. *P < 0.001 using Student’s t-test; †P < 0.05 using the Kruskal-Wallis nonparametric test.
In the fed state, Lou/C rats exhibited lower plasma glucose ($P = 0.034$), nonesterified fatty acid (NEFA) ($P = 0.025$), and triglyceride (TG) ($P = 0.01$) levels compared with Wistar rats (Table 1). They also displayed lower plasma insulin ($P = 0.02$), corticosterone ($P = 0.026$), and leptin levels than Wistar rats ($P = 0.022$), whereas plasma ghrelin concentrations were higher ($P = 0.003$) (Table 1).

Compared with Wistar rats, glucose tolerance of Lou/C animals was improved (Fig. 2A). This could be linked to either higher insulin secretion or improved insulin sensitivity. Measurement of plasma insulin levels at 15, 30, and 60 min after glucose injection did not reveal any difference between Lou/C and Wistar rats [area under curve (AUC) of plasma insulin levels 60 min after glucose injection: $69.8 \pm 7.8$ and $76.4 \pm 9.9$ ng·ml$^{-1}$·min for Lou/C and Wistar rats, respectively]. However, since an increase in plasma insulin levels occurring before 15 min could have affected the glycemic profile, as previously reported (2), we directly investigated pancreas function.

To this end, we first compared islets and whole pancreas of Wistar and Lou/C animals. Immunofluorescence revealed that islets of Lou/C rats were normal, with staining of $\beta$-cells similar to that in Wistar animals (Fig. 3A). The average wet weight of fresh pancreas of Lou/C rats, however, represented 70% of that of Wistar rats ($P = 0.020$) (Fig. 3B). This difference was linearly correlated with body weight in the two strains ($P = 0.040$). The total insulin content of Lou/C pancreas also represented 77% of that of Wistar rats ($P = 0.030$) (Fig. 3B), implying similar insulin concentration within the tissue. The profile of insulin secretion was then assessed by in situ perfusion of intact pancreas (Fig. 3C). Evaluation of AUC for each successive stimulation period showed that Wistar rats significantly increased their insulin output over basal value when challenged by 8.4 mM glucose, 16.8 mM glucose, and

Fig. 4. Insulin signaling in the red gastrocnemius muscle after euglycemic hyperinsulinemic clamps in Wistar and Lou/C rats. A: Western blots revealed using horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence detection. p, phosphorylation. IR, insulin receptor; IRS-1, insulin receptor substrate-1; AMPK, AMP-activated protein kinase; mTOR, mammalian target of rapamycin; S6K, S6 kinase. B and C: quantification of total protein expression (B) and the ratio of phosphorylated to total protein (C) in Lou/C rats using the ChemiDoc XRS and Quantity One software. Data are expressed as percentages of values in Wistar rats and normalized to the levels of actin as a housekeeping protein. Values are means ± SE of 5 rats per group. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$ using Student’s $t$-test.
16.8 mM glucose plus 1 nM GLP-1 (Fig. 3D). Compared with Wistar rats, Lou/C animals displayed a significantly lower response when challenged with either 8.4 or 16.8 mM glucose \((P < 0.04)\), as well as a trend toward a lower output in response to 16.8 mM glucose plus 1 nM GLP-1 (no significant difference, NS) (Fig. 3D). However, when the insulin responses were reported to the total insulin content of the pancreas, they were similar in both groups of animals. This is in keeping with additional studies on isolated islets showing similar insulin secretion in Lou/C and Wistar rats, in response to both glucose (Fig. 3E) and to nonmetabolized stimuli, such as GLP-1 (Fig. 3E) or IBMX (data not shown).

An ITT was thereafter performed to determine whether the improved glucose tolerance observed in Lou/C rats was linked to increased insulin sensitivity. As shown in Fig. 2B, a trend toward improved insulin sensitivity was observed in Lou/C compared with Wistar rats (NS). To further unravel a potential difference in insulin sensitivity in the two groups, in vivo glucose metabolism was assessed during euglycemic hyperinsulinemic clamps. Plasma glucose and insulin levels in overnight-fasted animals were similar in the two strains (Table 2). However, the glucose infusion rate required to maintain euglycemia during insulin infusion was higher in Lou/C than in Wistar rats \((P < 0.001)\), indicating a higher overall insulin sensitivity in this strain (Table 2). Moreover, hepatic glucose production was more suppressed by hyperinsulinemia in the Lou/C than in the Wistar group \((P = 0.046)\) (Table 2).

In view of this enhanced insulin sensitivity, the insulin signaling cascade was explored in the gastrocnemius muscle at the end of 2.5 h of euglycemic hyperinsulinemic clamps, the time at which plasma insulin levels were identical in the two groups. Expression levels of IR, IRS-1, Akt, and ERK1/2 were identical in the two strains (Fig. 4, A and B). However, phosphorylation on activating residues of the IR was slightly increased in Lou/C rats \((\sim 20\%) \,(P < 0.01)\) concomitantly with a significant increase in Akt \((\sim 80\%) \,(P < 0.01)\), but not in IRS-1, phosphorylation (Fig. 4, A and C). Surprisingly, activation of the ERK2, but not the ERK1, isoform was specifically reduced in Lou/C rats \((P < 0.001)\) (Fig. 4C).

Activation of AMPK has been shown to be crucial for energy metabolism in muscles (22, 26, 39), and activation of mTOR/S6 kinase can regulate Akt activity (3). Expression and phosphorylation of these signaling molecules were thus examined. There was no intergroup difference when the expression of AMPK or its phosphorylation form was considered.

Measurements of the in vivo insulin-stimulated glucose utilization index of individual tissues using the labeled 2-deoxy-D-glucose technique also indicated enhanced insulin sensitivity in Lou/C compared with Wistar rats, mainly in skeletal muscles and in brown adipose tissue \((P < 0.05)\) (Fig. 2C). A tendency toward a higher (1.8-fold) glucose uptake in epidymal and inguinal white adipose tissue (WAT) of Lou/C compared with Wistar rats also was observed (NS; data not shown).

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increase in the expression of mTOR and S6 kinase (S6K) was observed in Lou/C rats \((P < 0.05)\) (Fig. 4B), but the ratio of phosphorylated to total proteins was similar in the two strains (Fig. 4C).

Of note is the fact that the expression of the proteins whose phosphorylated form was altered in Lou/C rats during hyperinsulinemia (IR, Akt, and ERK2) was unaltered compared with the Wistar group under basal conditions (i.e., normal insulinemia). This was clear for phosho-IR and -Akt, and the trend toward a decrease in phosho-ERK2 was not significant (Supplemental Fig. 1).

Concerning lipid metabolism, body fat distribution was first determined and quantified after micro-CT reconstruction (Fig. 5A). Strikingly, fat depots were much smaller in Lou/C than in Wistar rats. Thus, in the thorax, total volume of fat was \(1.4 \pm 0.1\) and \(3.2 \pm 0.70\) g for Lou/C and Wistar rats, respectively \((P < 0.05)\). In the abdomen, all adipose tissue depots of Lou/C rats were smaller than those of Wistar animals (Fig. 5B). The largest difference was seen in visceral fat, which had a volume of \(9.5 \pm 3.0\) g in Wistar rats but was not quantifiable in Lou/C animals. Globally, the total fat volume represented \(8.3 \pm 0.5\) and \(40.8 \pm 4.5\) g (i.e., \(3.6\) and \(9.1\) g/100 g tissue) in Lou/C and Wistar rats, respectively \((P < 0.05)\). In tissues such as the liver and skeletal muscles (i.e., white gastrocnemius), TG content was similar in the two strains (Table 1).

Measurements of the mRNA expression of key enzymes involved in lipid metabolism were performed in the liver, skeletal muscles, and adipose tissue depots of Wistar and Lou/C rats. In the gastrocnemius (Table 3), expression levels of enzymes and proteins involved in lipid uptake or utilization, such as lipoprotein lipase (LPL), fatty acid binding protein-3 (FABP3), and uncoupling protein-3 (UCP3), were similar between the two strains. In the liver (Table 3), expression of molecules involved in lipid utilization, such as PPAR\(\alpha\), its coactivator PGC-1\(\alpha\), and sirtuin 1 (SIRT1), was higher in Lou/C than in Wistar rats \((P < 0.05)\). On the contrary, the expression of the sterol response element-binding protein (SREBP)-1c, a transcription factor modulating the expression of enzymes involved in fatty acid synthesis, was lower \((P < 0.05)\), whereas the expression of other enzymes or transcription factors was unaltered.

In epididymal VAT (WATi), the expression of PGC-1\(\alpha\) was increased 3-fold in Lou/C compared with Wistar rats (ratio PGC-1\(\alpha\)/RPS29: 4.27 \pm 1.36 and 1.36 \pm 0.33 for Lou/C and Wistar rats, respectively) \((P < 0.05)\). In inguinal WAT (WATi), higher levels of PGC-1\(\alpha\), PPAR\(\gamma\)2, acetyl-CoA carboxylase (ACC)\(\alpha\), and SIRT1 were observed in Lou/C rats, whereas fatty acid synthase (FAS) and stearoyl coenzyme-A desaturase-1 (SCD-1) expression was lower \((P < 0.05)\) (Fig. 6A). This gene expression profile was confirmed at the protein levels for PGC-1\(\alpha\) and PPAR\(\gamma\)2 (Fig. 6B).

Interestingly, as illustrated by a representative blot using standard RT-PCR (Fig. 6C), the mRNA coding for UCP1 was detected in WATi of Lou/C rats but not in that of Wistar animals. When analyzed by qPCR, the UCP1 expression in inguinal fat of Lou/C rats was similar to the expression of RPS29, used as the housekeeping gene (17.6 \pm 7.1 vs. 16.9 \pm 2.6 ng for UCP1 and RPS29, respectively).

To determine whether the increased insulin sensitivity and the decreased lipid storage observed in Lou/C rats are related to their spontaneous lower caloric intake, we pair-fed a group of Wistar rats (PF Wistar) from the age of 5 to 21 wk the caloric intake of age-matched Lou/C animals. As depicted in Fig. 7A, the body weight of the PF Wistar group was intermediate between that of Lou/C and Wistar rats (280.1 \pm 3.0 vs. 245.8 \pm 7.3 and 354.6 \pm 15.9 g, respectively) at 21 wk \((P < 0.001)\). Because body weight of the PF Wistar group was higher than that of Lou/C rats at 5 wk of age (135.7 \pm 2.2 vs. 77.3 \pm 3.1 g for Wistar PF and Lou/C rats, respectively), body weight gain from 5 to 18 wk was similar in the two groups. However, and very interestingly, food efficiency of the PF Wistar group was more than twice as high as that of Lou/C rats when calculated over 1 wk at 17 wk of age (Fig. 7B).

In the fed state, PF Wistar rats exhibited similar plasma glucose, NEFA, and insulin levels compared with Lou/C rats (Table 4). However, they displayed higher plasma TG levels \((P = 0.020)\) than Lou/C rats and higher corticosterone levels \((P = 0.046)\) than both Lou/C and Wistar animals, whereas plasma leptin levels were significantly lower compared with the other two groups \((P = 0.016)\) (Table 4).

At 19 wk of age, glucose tolerance of PF Wistar rats was not different from that of Lou/C animals, suggesting similar insulin sensitivity in these two groups (Fig. 7C). Interestingly, plasma adiponectin levels were elevated in the PF Wistar group only (Fig. 7D).

Considering lipid metabolism, analyses of body composition using micro-CT showed a similar profile of adipose tissue deposition in PF Wistar and Lou/C rats (Fig. 5B). However, the pattern of expression of different genes expressed in adipose tissue, whose products are involved in lipid metabolism, was very different in Lou/C and PF Wistar rats. Thus, compared with that in Lou/C rats, the expression of ACC\(\alpha\) and FAS in WATe of PF Wistar rats was more than 3-fold higher (Fig. 8A).

---

**Table 3. mRNA expression of key enzymes involved in lipid metabolism in skeletal muscles and liver of Wistar and Lou/C rats**

<table>
<thead>
<tr>
<th></th>
<th>Wistar</th>
<th>Lou/C</th>
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<tbody>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPL</td>
<td>0.97 \pm 0.28</td>
<td>0.68 \pm 0.13</td>
</tr>
<tr>
<td>FABP3</td>
<td>6.41 \pm 2.39</td>
<td>2.84 \pm 1.18</td>
</tr>
<tr>
<td>UCP3</td>
<td>2.57 \pm 0.65</td>
<td>1.83 \pm 0.24</td>
</tr>
<tr>
<td><strong>PPAR(\alpha)</strong></td>
<td>0.51 \pm 0.07</td>
<td>1.05 \pm 0.31</td>
</tr>
<tr>
<td><strong>PGC-1(\alpha)</strong></td>
<td>0.40 \pm 0.12</td>
<td>1.26 \pm 0.28*</td>
</tr>
<tr>
<td><strong>SIRT1</strong></td>
<td>1.09 \pm 0.08</td>
<td>1.45 \pm 0.14*</td>
</tr>
<tr>
<td><strong>SREBP-1c</strong></td>
<td>2.38 \pm 0.20</td>
<td>2.00 \pm 0.16*</td>
</tr>
<tr>
<td><strong>ACC(\alpha)</strong></td>
<td>0.03 \pm 0.01</td>
<td>0.04 \pm 0.01</td>
</tr>
<tr>
<td><strong>FAS</strong></td>
<td>0.12 \pm 0.02</td>
<td>0.21 \pm 0.07</td>
</tr>
<tr>
<td><strong>SCD1</strong></td>
<td>1.16 \pm 0.25</td>
<td>0.75 \pm 0.21</td>
</tr>
<tr>
<td><strong>INSIG-1</strong></td>
<td>11.84 \pm 2.22</td>
<td>12.73 \pm 1.28</td>
</tr>
<tr>
<td><strong>INSIG-2</strong></td>
<td>18.78 \pm 2.91</td>
<td>18.39 \pm 2.21</td>
</tr>
<tr>
<td><strong>CPT1(\alpha)</strong></td>
<td>1.20 \pm 0.20</td>
<td>1.36 \pm 0.17</td>
</tr>
</tbody>
</table>

Values are means \(\pm\) SE of 8 Wistar and 10 Lou/C rats. Results are expressed as ratios of the quantity (in ng) of the gene of interest to the quantity (in ng) of GAPDH and cyclophilin as the housekeeping gene in muscles and the liver, respectively. \(*P < 0.05\) using Student’s \(t\) test. RG, red gastrocnemius; LPL, lipoprotein lipase; FABP3, fatty acid binding protein-3; UCP3, uncoupling protein-3; PPAR\(\alpha\), peroxisome proliferator-activated receptor-\(\alpha\); PGC-1\(\alpha\), PPAR coactivator-1\(\alpha\); SIRT1, sirtuin 1; SREBP-1c, sterol response element-binding protein-1c; ACC\(\alpha\), acetyl-CoA carboxylase-\(\alpha\); FAS, fatty acid synthase; SCD1, stearoyl coenzyme-A desaturase-1; INSIG, insulin-induced gene; CPT1\(\alpha\), carnitine palmitoyltransferase 1\(\alpha\).
This difference was even more marked for SCD-1, which was 14-fold higher in PF Wistar than in Lou/C rats (Fig. 8A). The profile of gene expression in WATi of PF Wistar was also clearly different from that observed in Lou/C rats (Fig. 8B). Expression levels of ACCα and PPARγ2 were significantly lower (P < 0.05), whereas FAS expression tended to increase. More importantly, the expression of UCP1 was highly decreased in PF Wistar rats. Actually, as illustrated by a blot representing a standard RT-PCR (Fig. 8C), the mRNA coding for UCP1 was almost not detected in WATi of PF Wistar rats, whereas it was clearly present in the Lou/C group.

**DISCUSSION**

As previously reported (1), Lou/C rats of the present study exhibited markedly lighter body weight and lower caloric intake than Wistar animals, without any difference in food efficiency (FE). Lou/C rats also displayed optimized metabolic and hormonal parameters, such as lower plasma glucose, free fatty acid, TG, insulin, leptin, and corticosterone levels. In terms of glucose metabolism, glucose tolerance (by GTT) of Lou/C rats was improved compared with that of Wistar animals. This did not seem to result from insulin oversecretion, because there was no difference in insulinemia during the GTT. When studied in vitro, using the perfused pancreas, the glucose-induced insulin response was similar in Lou/C and Wistar rats, when expressed relative to the total pancreatic insulin content. Such was also the case for the insulin response of isolated islets to glucose or nonmetabolized stimuli, in agreement with the observation that the morphology and arrangement of β-cells were normal in the islets of Lou/C rats.

Considering the other parameter that affects glucose tolerance, namely, insulin sensitivity, a clear difference was ob-
served between Lou/C and Wistar rats. Indeed, hepatic glucose production was more suppressed by hyperinsulinemia in Lou/C than in Wistar rats. In addition, the insulin-stimulated glucose utilization was higher in skeletal muscles of Lou/C than in tissues of Wistar animals. Under hyperinsulinemic conditions, the improved insulin sensitivity/responsiveness of muscles from Lou/C rats was related to a higher activation of the IR and the Akt/protein kinase B pathway, which is responsible for most of the metabolic actions of insulin, with no change in IRS-1 phosphorylation. These results are in keeping with the observation of improved insulin signaling and increased Akt phosphorylation in F344B/N rats submitted to a 60% caloric restriction during 8 wk (28), a model in which no effect on IRS-1 and phosphotyrosine-PI 3-kinase was observed (14). These data, as well as those reported in the present study, are of interest given the fact that muscle insulin resistance occurring in obesity (40) is reportedly characterized by a defect in the insulin signaling cascade, involving IRS and Akt protein kinase (23).

The observation of reduced ERK2 activation in muscle of Lou/C rats deserves to be mentioned. However, little is known about the role of ERK2 in skeletal muscle, except that this isoform is specifically required in muscle mitogenic and differentiation processes (24), its activity being increased by chronic exercise (32). Further analyses are therefore necessary to address the functional significance of this finding.

Many studies in humans (25) and rodents (11) have shown that there is a clear association between insulin sensitivity, particularly at the level of the liver (4, 7), and plasma adiponectin levels. However, plasma adiponectin levels of Lou/C rats were not different from those of the Wistar group. Because of its numerous and well-documented interactions with glucose metabolism and insulin sensitivity, lipid metabolism was then explored in Lou/C and Wistar rats. Despite comparable FE, Lou/C rats had a markedly decreased fat mass. This was investigated using a micro-CT technique, which allows for the precise delimitation and quantification of individual fat deposits.

Table 4. Plasma metabolite and hormone levels in Wistar, Lou/C, and pair-fed Wistar rats

<table>
<thead>
<tr>
<th></th>
<th>Wistar</th>
<th>Lou/C</th>
<th>Pair-fed Wistar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mM</td>
<td>6.29±0.19</td>
<td>5.30±0.16*</td>
<td>5.33±0.21*</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>158±10</td>
<td>97±4*</td>
<td>141±16†</td>
</tr>
<tr>
<td>NEFA, mM</td>
<td>0.15±0.03</td>
<td>0.19±0.03</td>
<td>0.18±0.02</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>1.35±0.17</td>
<td>0.80±0.10</td>
<td>0.79±0.07</td>
</tr>
<tr>
<td>Corticosterone, ng/ml</td>
<td>61.3±4.4</td>
<td>76.6±51.0</td>
<td>148.4±51.2*</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>6.98±3.43</td>
<td>4.75±0.84</td>
<td>2.28±0.84†</td>
</tr>
</tbody>
</table>

Values are means ± SE of 8 Wistar, 8 Lou/C, and 8 pair-fed (PF) Wistar rats per group. *P < 0.05 vs. Wistar rats using 1-way ANOVA. †P < 0.05 vs. Lou/C rats using 1-way ANOVA.
visceral fat contributes to the inhibition of adiponectin insulin resistance (27) and metabolic disorders in humans. Lipids and lipoproteins, as well as with the development of diabetes, are tightly correlated with quantitative and qualitative changes in serum lipids and lipoproteins. The importance of visceral fat is known to be particularly deleterious for metabolic homeostasis, being capable of promoting metabolic disorders. It was observed that all of the fat depots were smaller in Lou/C than in Wistar rats. In addition, and in contrast to the situation in Wistar rats, there was no intra-abdominal fat deposition in the Lou/C group. This is of importance, since visceral fat is known to be particularly deleterious for metabolic homeostasis, being capable of promoting metabolic disorders in humans (18). Moreover, it has been shown in vivo and in vitro that visceral fat contributes to the inhibition of adiponectin secretion from subcutaneous depot (18), likely via the release of TNF-α (25, 27).

Recent studies in PPARγ2−/− ob/ob (POKO) mice have shown that these mice are even more insulin resistant than ob/ob mice on a standard diet (30). Together with other studies, these results strongly suggest that PPARγ2 plays an important role in favoring insulin sensitivity, independently from its effect on adipogenesis (30). Both PPARγ and its coactivator, PGC-1α, are required for the differentiation of brown adipocytes and for the expression of UCP1 in brown adipose tissue (36). These two factors also were shown to favor the biogenesis of mitochondria and the expression of glycerol kinase, resulting in increased fat utilization and energy expenditure (36). Recently, it was proposed that increased expression of PPARγ and PGC-1α in white adipose tissue could be linked with the emergence of brown adipocytes within this tissue (19).

In our study, the expression of PPARγ2 and PGC-1α was increased 3- to 3.5-fold, respectively, in inguinal adipose tissue of Lou/C compared with Wistar rats. Interestingly, UCP1 expression was detected in adipose tissue of Lou/C rats but not in that of the Wistar group. Together, these results further strengthen the existence of a link between the expression of PPARγ2 and PGC-1α and that of UCP1 in white adipose tissue. In this respect, it is worth mentioning that exposure to β-adrenergic agonists was shown to induce the appearance of brown adipocytes in white fat pads of mice (17, 21) and that an enhanced sympathetic innervation was reported in white adipose tissue of Lou/C rats (33).

Finally, the expression of SIRT1 was significantly increased in both adipose tissue and the liver of Lou/C rats. Strong evidence now suggests an important role of this NAD+dependent deacetylase on glucose and lipid metabolism, as well as on insulin signaling, mainly through interaction with PGC-1α (38). In adipose tissue, SIRT1 appears to inhibit fat storage and increase lipolysis (35), whereas in the liver, SIRT1 activates gluconeogenesis and represses glycolysis by deacetylating PGC-1α (6, 37). In WATi of Lou/C rats, the presence of decreased FAS expression, indicative of low adipogenesis, could result, at least partly, from enhanced SIRT1 expression. In contrast, the increase in ACCα expression measured in WATt of Lou/C rats could reflect the tendency toward higher insulin-stimulated glucose utilization. In the liver of Lou/C animals, overexpression of PGC-1α and SIRT1 resembles the situation occurring during caloric restriction (6, 37) and might therefore represent an adaptation to the spontaneous food restriction that occurs in these animals.

One of the important questions that needs to be addressed at that point is whether the metabolic changes observed in Lou/C rats are secondary to their low food intake or whether they are strain specific. Comparing a group of PF Wistar rats with Lou/C rats, it was observed that, in contrast to the situation of Lou/C rats discussed above, food restriction induced adaptive mechanisms to increase the “efficiency” of the food consumed by apparently increasing the capacity of adipose tissue to store lipids. This is in agreement with previously reported results showing that food restriction induces adaptive regional specialization of adipocyte function, favoring the immediate conservation of nutrients by promoting fat storage (43).

In summary, the morphological and molecular data gathered in the present study suggest that the Lou/C rat is a model of spontaneous caloric restriction with improved insulin sensitiv-
ity and preferential channeling of nutrients toward utilization, rather than storage. An interesting phenotype-specific feature of Lou/C rats is the appearance of UCP1 in white adipose tissue, which could result from transdifferentiation of white into brown adipocytes. This, as well as its functional significance during cold exposure and high-fat diet, needs to be examined in detail in future studies. Altogether, the changes in glucose and lipid metabolism reported presently in Lou/C rats fed a standard diet could represent important genetic determinants of resistance to the development of obesity.

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