Ectopic UCP1 Overexpression in White Adipose Tissue Improves Insulin Sensitivity in Lou/C Rats, a Model of Obesity Resistance

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Abstract

Brown adipose tissue (BAT), characterized by the presence of uncoupling protein 1 (UCP1), has been described as metabolically active in humans. Lou/C rats, originating from the Wistar strain, are resistant to obesity. We previously demonstrated that Lou/C animals express UCP1 in beige adipocytes in inguinal white adipose tissue (iWAT), suggesting a role of this protein in processes such as the control of body weight and the observed improved insulin sensitivity. A β3 adrenergic agonist was administered for 2 weeks in Wistar and Lou/C rats to activate UCP1 and delineate its metabolic impact. The treatment brought about decreases in fat mass and improvements in insulin sensitivity in both groups. In BAT, UCP1 expression increased similarly in response to the treatment in the two groups. However, the intervention induced the appearance of beige cells in iWAT, associated with a marked increase in UCP1 expression, in Lou/C rats only. This increase was correlated with a markedly enhanced glucose uptake measured during euglycemic-hyperinsulinemic clamps, suggesting a role of beige cells in this process. Activation of UCP1 in [...]
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Brown adipose tissue (BAT), characterized by the presence of uncoupling protein 1 (UCP1), has been described as metabolically active in humans. Lou/C rats, originating from the Wistar strain, are resistant to obesity. We previously demonstrated that Lou/C animals express UCP1 in beige adipocytes in inguinal white adipose tissue (iWAT), suggesting a role of this protein in processes such as the control of body weight and the observed improved insulin sensitivity. A β3 adrenergic agonist was administered for 2 weeks in Wistar and Lou/C rats to activate UCP1 and delineate its metabolic impact. The treatment brought about decreases in fat mass and improvements in insulin sensitivity in both groups. In BAT, UCP1 expression increased similarly in response to the treatment in the two groups. However, the intervention induced the appearance of beige cells in iWAT, associated with a marked increase in UCP1 expression, in Lou/C rats only. This increase was correlated with a markedly enhanced glucose uptake measured during euglycemic-hyperinsulinemic clamps, suggesting a role of beige cells in this process. Activation of UCP1 in ectopic tissues, such as beige cells in iWAT, may be an interesting therapeutic approach to prevent body weight gain, decrease fat mass, and improve insulin sensitivity.

Obesity has reached epidemic proportions worldwide and has become a major global public health problem in recent decades (1). It represents a considerable risk factor for the development of several comorbidities, among which is type 2 diabetes (1). Globally, the key component of the obesity epidemic is long-term dysregulation of energy balance. In view of the relative lack of drugs suppressing appetite, approaches to increase energy expenditure are viewed as potential new therapeutic options to treat obesity and metabolic complications. Along this line, brown adipose tissue (BAT), now known to be present in adult humans (2–4), is the focus of major interest, due to its role in inducing thermogenesis (5). The mitochondria of brown adipocytes are characterized by the presence of uncoupling protein 1 (UCP1), which uncouples oxidative phosphorylation from ATP synthesis, resulting in heat production (6). This process consumes substantial amounts of free fatty acids (FFAs) and glucose (7).

In rodents, brown adipocytes are found in discrete areas, such as interscapular, cervical, peri-renal, and intercostal depots (8), which are referred to as “classical” BAT depots. In white adipose tissue (WAT), brown-like cells, called beige or brite cells, express UCP1 (9). The existence of a specific precursor, different from classical white or brown adipocytes, arising from smooth muscle cells that would differentiate into beige adipocytes in WAT has been proposed (10). Moreover, some studies suggest that, under specific conditions, most or all of white adipocytes transdifferentiate into beige adipocytes (11).

Interestingly, whatever their developmental origin, white, beige, and brown adipocytes seem to greatly differ in their function. As mentioned above, BAT is the effector organ of nonshivering thermogenesis that, by utilizing large quantities of glucose and lipids from the circulation, can promote negative energy balance. Moreover, as recently reviewed by Peirce and Vidal-Puig (12), the role of BAT...
activation might be broader than solely promoting negative energy balance. Indeed, such activation was described to exert anti–type 2 diabetic effects (13,14), associated with improvements of dyslipidemia (rev. in 15). These effects are partly interrelated but can also be dissociated and exerted by different UCP1-expressing adipocyte types.

The Lou/C rat is a model of age- and diet-induced obesity resistance, which also exhibits a lower body fat mass, increased leptin sensitivity, and improved insulin sensitivity compared with Wistar animals (16–18). We also demonstrated that the most striking differences between Lou/C and Wistar rats were the presence, in inguinal WAT (iWAT) of the Lou/C group, of Ucp1 (16,17) and of a marked Adrb3 overexpression (17).

In the current study, we used the Lou/C rat as a model to investigate the impact of UCP1 activation on glucose metabolism. To this end, various groups of Wistar and Lou/C rats were subcutaneously infused for 2 weeks with a β3 adrenoreceptor agonist (CL-316243), and UCP1 in BAT and WAT depots, as well as the insulin-stimulated glucose utilization rate, of different tissues was determined.

RESEARCH DESIGN AND METHODS

Animals and Diets

Two month-old male Lou/C and Wistar rats were purchased from Harlan UK Limited (Oxon, U.K.) and Charles River (L’Arbresle, France), respectively. They were housed in pairs under controlled conditions (22°C; light on 7:00 A.M. to 1:00 P.M.) and were allowed free access to water and diet (RMI; Hersteller, Essex, U.K.). Osmotic minipumps (Alzet, Cupertino, CA) delivering a β3 agonist (CL-316243), and UCP1 in BAT and WAT depots, as well as the insulin-stimulated glucose utilization rate, of different tissues was determined.

GTT

Wistar and Lou/C rats were food deprived for 4 h (from 9:00 to 1:00 P.M.). A glucose load of 1.5 g/kg i.p. was administered. Blood samples were collected by tail nicking and were used for further analyses of plasma glucose and insulin levels.

EHC

Rats were overnight fasted and anesthetized with pentobarbital (50 mg/kg i.p.; Abbott Laboratories, Chicago, IL). The glucose infusion rate (GIR) required to maintain euglycemia under insulin-stimulated conditions (18 mU/kg/min; Actrapid HM, Novo Nordisk, Bagsvaerd, Denmark) was determined as previously described (19,20). At the end of the EHC, the insulin-stimulated glucose utilization index of individual tissues was measured, using 2-deoxy-[1,2-3H]glucose (30 μCi/rat, NET 328A; PerkinElmer, Schwarzenbach, Switzerland) (20). Rats were killed by decapitation and tissues stored at −80°C. The 2-deoxy-[1,2-3H]glucose–specific activity was measured in deproteinized blood samples. Determination of tissue concentration of [1,2-3H]glucose was performed using the SYBR green PCR Master Mix (Applied Biosystems). Results were normalized to the expression of the housekeeping gene, ribosomal protein S29 (Rps29).

Plasma Measurements

Plasma glucose levels were measured by the glucose oxidase method (Glu; Roche Diagnostics GmbH, Rotkreuz, Switzerland). FFA and triglyceride (TG) levels were determined using a Wako Chemicals GmbH (Neuss, Germany) and a Biomérieux (Marcy l’Etoile, France) kit, respectively. Plasma leptin (Linco Research, Inc., St Charles, MO) levels were measured using a double antibody radioimmunoassay kit. Plasma insulin levels were determined using an ELISA commercial kit (10-1250; Merckodia, Uppsala, Sweden).

Tissue Processing and RT-PCR

RNA was reverse transcribed (M-MLV-RT; Invitrogen, Basel, Switzerland) and quantitative PCR (qPCR) was performed using the SYBR green PCR Master Mix (Applied Biosystems, Warrington, U.K.) on a Stepone Plus machine (Applied Biosystems). Primers (Supplementary Table 1) were designed with the PrimerExpress software (Applied Biosystems). Results were normalized to the expression levels of the housekeeping gene, ribosomal protein S29 (Rps29).

Mitochondrial DNA Copy Number

Quantification of mitochondrial DNA (mtDNA) copy number was achieved by qPCR. Briefly, DNA was extracted from iWAT using the DNeasy Blood and Tissue kit (Qiagen, Düsseldorf, Germany). Nuclear and mtDNA copy numbers were assessed by real-time PCR using primers targeted toward the Cox1 gene (for mtDNA) and nuclear RNaseP (for nuclear DNA).

Western Blotting

Frozen tissues were homogenized in ice-cold RIPA buffer. Protein levels were quantified using a BCA protein assay (Pierce,
Lausanne, Switzerland). Ten and 50 μg protein, respectively, was used for BAT and WAT SDS-PAGE. UCP1 antibody (Abcam, Cambridge, U.K.) was used at a concentration of 1/10,000 for BAT and 1/1,000 for WAT before the secondary antibody (anti-rabbit 1/5,000) was added. Housekeeping proteins ERM (Santa Cruz Biotechnology, Santa Cruz, CA) and actin (Millipore, Billerica, MA) were used at 1/2,000 and 1/100,000, with anti-goat (1/10,000) or anti-mouse (1/5,000) antibody, respectively. Detection was performed with an enhanced chemiluminescence detection system (Amersham Biosciences, Amersham, U.K.). Signals were quantified using the PXi and the Genetools software from Syngene UK.

Body Composition
An EchoMRI-700 quantitative nuclear magnetic resonance analyzer (Echo Medical Systems, Houston, TX) was used to measure body composition (total fat and lean body mass). Rats were also scanned on a multidetector CT scanner (Discovery 750 HD; GE Healthcare, Milwaukee, WI) to determine the volume of the various fat depots.

Light Microscopy
Tissues were fixed in formalin (10%) after dissection. They were embedded in paraffin blocks, which were cut at 7 μm and then stained with hematoxylin-eosin (Sigma-Aldrich, Buchs, Switzerland). For immunohistochemistry, the same UCP1 antibody, respectively. Detection was performed with an enhanced chemiluminescence detection system (Amersham Biosciences, Amersham, U.K.). Signals were quantified using the PXi and the Genetools software from Syngene UK.

[18F]-fluorodeoxyglucose Glucose Uptake Measurement in Tissues
Rats were overnight fasted and anesthetized using isoflurane. Glycemia was measured before the experiment. Animals received a 3.5 mg/kg i.p. injection of furosemide (Lasix; Sanofi, Paris, France) to empty their bladder. [18F]-fluorodeoxyglucose ([18F]-FDG) was injected via the pudendal vein (20 MBq/rat). Glucose uptake was measured with a microPET/CT (Triumph; TriFoil, Chatsworth, CA). A micro-CT analysis was simultaneously performed to localize the [18F]-FDG emission in different structures. Data were analyzed by the Osirix software (Pixmeo, Geneva, Switzerland).

Data Analyses
Results are expressed as mean ± SEM. Comparison between the four groups was performed by GraphPad Prism (San Diego, CA), using the two-way ANOVA analysis followed by a Tukey post hoc test. Correlation between the Ucp1 mRNA expression and the glucose uptake index in WAT was calculated using the Pearson correlation coefficient. Statistical significance was established at P < 0.05.

RESULTS
As previously reported (16,17), Lou/C had a lower body weight than Wistar rats. The 2-week CL-316243 treatment had no impact on body weight gain in Wistar and Lou/C animals (Fig. 1A). Total food intake of Lou/C was lower than that of Wistar rats, but it was unaffected by the treatment in both groups (Fig. 1A). Interestingly, food efficiency was similar in Wistar and Lou/C rats whether treated or not (Fig. 1A). Analysis of body composition by MRI (EchoMRI) further showed that the β3 agonist treatment decreased the total fat mass and consequently increased the lean body mass in both Wistar and Lou/C animals (Fig. 1B). In addition, CT scan analysis showed a significant treatment-induced decrease in the mass of all fat depots (interscapular BAT, subcutaneous WAT, and intra-abdominal WAT) in the two animal groups, except for subcutaneous WAT of Lou/C rats (Fig. 1C). This was in keeping with a significant or with a trend toward a significant decrease in leptinemia in β3 agonist–treated Wistar and Lou/C rats, respectively (Table 1). Qualitatively similar results were obtained for plasma glucose, insulin, and TG levels, while plasma FFA concentrations were unaffected by the treatment in both groups (Table 1).

To evaluate the impact of the β3 agonist treatment on glucose metabolism, a GTT was performed at day 9 of the study (Fig. 2A). The treatment had no impact on basal glycemia in both groups (data not shown). As indicated by the changes in glycemia from baseline values, as well as the areas under the curve (AUCs) during the whole tests, glucose tolerance of Wistar and Lou/C rats was improved by the treatment (Fig. 2A). With regard to insulin, the treatment effect on the AUC during the GTT almost reached statistical significance (P = 0.057), this effect being clearly more marked in the Lou/C than in the Wistar group (Fig. 2B).

Peripheral insulin sensitivity was then evaluated by performing EHC. Basal and steady-state (clamp) values of glycemia and insulinemia are provided in Supplementary Table 2. As previously reported (16,17), the GIR of Lou/C rats was higher than that of Wistar animals, indicating improved insulin sensitivity (Fig. 2C). In both the Wistar and the Lou/C group, the β3 agonist treatment increased the GIR (Fig. 2C). As clearly depicted by the dynamic GIR changes, the values reached in Wistar-treated rats were similar to those of the untreated Lou/C group, while the β3 agonist treatment further increased the GIR in Lou/C animals (Fig. 2C). Glucose uptake by individual insulin-sensitive tissues was then assessed, using the 2-deoxyglucose technique (20–22). The treatment had no impact on skeletal muscle glucose uptake in both groups (Fig. 2D). It resulted in similar two- to threefold increases in glucose uptake in BAT of Wistar and Lou/C rats (Fig. 2D). Interestingly, glucose uptake by different WAT depots was not significantly modified in Wistar but was markedly increased in Lou/C animals. This was observed in abdominal WAT (epididymal WAT [eWAT] and retroperitoneal WAT) and in the iWAT fat depot (Fig. 2D).

To try understanding the mechanisms underlying the β3 agonist treatment–induced increases in BAT and WAT glucose uptake, and in view of the well-known effect of CL-316243 on UCP1 (23), we measured the expression of this protein in these tissues. Considering BAT, hematoxylin-eosin
staining revealed less lipid inclusions and therefore activated tissue in the Wistar and the Lou/C treated groups (Fig. 3A). Accordingly, Ucp1 mRNA expression was increased by fourfold by the treatment in the two groups (Fig. 3B). These results were confirmed by Western blot analysis (Fig. 3C). The treatment also had similar effects in Wistar and Lou/C rats regarding the expression of the Adrb3, which was markedly inhibited (Fig. 3B).

Subsequently, BAT activity was evaluated by PET scan analysis, using [18F]-FDG. Figure 3D shows micro-CT, PET scan, and merged micro-CT and PET scan images of one representative animal per group. PET scan allowed for the

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Results are means ± SEM of 8 experiments per group. Plasma was collected from rats receiving NaCl or CL-316243 treatment for 14 days starting at the age of 12 weeks. Statistical analyses were performed using two-way ANOVA (Tukey posttest) with ††<0.01, †††<0.001, Wistar NaCl vs. Wistar CL; ‡<0.05 Lou/C NaCl vs. Lou/C CL.
Figure 2—Improvement in insulin sensitivity in Lou/C rats is due to an increase in glucose uptake by WAT. A: ΔGlycemia during a GTT (1.5 g/kg i.p.) after 4 h of fasting; glucose AUC at 120 min. B: Plasma insulin levels during the GTT; insulin AUC after 60 min. C: GIR during (right panel) and at the end (left panel) of EHC in Wistar NaCl, Wistar treated with CL-316243 (CL), Lou/C NaCl, and Lou/C treated with CL-316243 rats. D: Tissue-specific insulin-stimulated glucose uptake. eWAT, epididymal WAT; rpWAT, retroperitoneal WAT; Gr, quadriceps red; Qw, quadriceps white. Results are means ± SEM of 6–9 experiments. Statistical significance assessed by two-way ANOVA: #strain effect; @treatment effect; $interaction between strain and treatment. *Significant difference by post hoc pairwise comparison between different conditions. Single, double, and triple symbols indicate $P < 0.05, P < 0.01, and $P < 0.001, respectively. For the dynamic changes of Δglucose and insulinemia during the GTT (A and B, left panels), as well as of GIR as a function of time (C, right panel): *Wistar NaCl vs. Lou/C NaCl; †Wistar NaCl vs. Wistar CL-316243; ‡Lou/C NaCl vs. Lou/C CL-316243; §Wistar CL vs. Lou/C CL-316243. gluc, glucose.
detection of three main BAT depots: at the basis of the brain (cervical BAT), in the interscapular region, and along the vertebral column. As clearly revealed after quantification of the signals in all the animals studied, the β3 agonist treatment increased glucose uptake to a similar extent in each of these three depots in Wistar and Lou/C rats, whereas the signals were absent under standard conditions (Fig. 3D).

In line with our previous observation of measurable UCP1 expression in iWAT of Lou/C, but not of Wistar rats (16,17), we looked at the potential presence of UCP1-positive beige adipocytes in this tissue after the β3 agonist treatment. Examination of hematoxylin-eosin staining revealed the presence of multilocular adipocytes in the β3 agonist–treated Lou/C group only (Fig. 4A). Immunofluorescence analysis further showed that these
Figure 4—UCP1 is overexpressed in the subcutaneous iWAT depot in Lou/C rats by CL-316243. A: Representative hematoxylin-eosin staining in iWAT (7 μm). Scale bars = 50 μm. iWAT sections were stained with an anti-UCP1 antibody. Hematoxylin staining was used to recognize structures. The sections were examined by fluorescence microscopy. Red indicates UCP1. B: Ucp1, Adrb3, and Pgc1α mRNA expression in iWAT. C: UCP1 protein expression measured by Western blot with relative quantification in iWAT. D: Representative axial cuts of [18F]-FDG uptake measured by microPET. microCT was performed to localize glucose uptake in the tissues; quantification of glucose uptake in iWAT of control and CL-316243 (CL)-treated Wistar and Lou/C rats. SUV, standard uptake value. E: Mitochondrial DNA copy number in iWAT. DNA copies of mitochondrial complex I (COX1, mtDNA) were normalized to the DNA levels of nuclear RNaseP (nDNA).
multilocular adipocytes expressed UCP1, demonstrating the presence of beige cells in the iWAT of Lou/C treated rats (Fig. 4A). In keeping with these results, Ucp1 mRNA expression was increased a hundred fold in iWAT of Lou/C rats in response to the treatment. It was also induced in iWAT of Wistar treated animals, reaching values measured in the untreated Lou/C group (Fig. 4B). Western blot analysis clearly showed that UCP1 protein was expressed in the iWAT of the Lou/C treated group only (Fig. 4C). In line with the results obtained for Ucp1, the expression of Pgc1α (Fig. 4B), of Tbx1 (Supplementary Fig. 1), and of various markers of mitochondrial biogenesis (Supplementary Fig. 1), as well as mitochondrial DNA (Fig. 4E), were markedly enhanced by the treatment in Lou/C rats (600% increase in the mtDNA copy number). Next, we measured the activity of iWAT by PET scan coupled with micro-CT and observed a higher glucose uptake in treated Lou/C rats compared with the three other groups (Fig. 4D). To substantiate the existence of the previously proposed link between glucose uptake/insulin sensitivity and Ucp1 expression in adipose tissue (24), we looked at the correlation between these two parameters, considering all the animals studied. As shown on Fig. 4F, the data fitted a hyperbolic regression with a positive and significant correlation, suggesting that UCP1-positive beige cells in iWAT may be involved in the improvement in insulin sensitivity in this tissue. With regard to the expression of the Adrb3, it was markedly overexpressed in iWAT of Lou/C compared with Wistar rats, in which it was barely detectable. The β3 agonist treatment downregulated the expression of the Adrb3 in the Lou/C but not in the Wistar group (Fig. 4B).

Given that the insulin-induced glucose uptake was increased by the β3 agonist, not only in iWAT, but also in the eWAT, we examined the potential presence of beige adipocytes in this depot. Multilocularization of adipocytes was observed in Lou/C-treated rats only (Supplementary Fig. 2A), in keeping with the induction of a marked Ucp1 gene expression (by 160-fold) (Supplementary Fig. 2B). The mRNA expression of the Adrb3 was low in both Wistar and Lou/C rats, with no intergroup difference and no impact of the β3 agonist treatment (Supplementary Fig. 2B).

It is well-known that thermoneutrality in rodents is reached at ~28–30°C (24°C for rats and 30°C for mice) and that, although it is the usual temperature used in animal quarters, 22°C represents a cold stimulus increasing UCP1 expression and activity (25). It was therefore possible that the presence of UCP1-positive beige cells in WAT observed in the Lou/C group without any treatment was a consequence of a better temperature sensing than in Wistar rats. To address this issue, we maintained groups of Wistar and Lou/C rats at 22°C or at 30°C for 4 weeks. At 30°C, both food intake and body weight gain were reduced in Wistar, as well as in Lou/C rats (Fig. 5A). This resulted in similar food efficiencies in response to the treatment under the two experimental conditions, with lower values in the Lou/C than in the Wistar groups (Fig. 5B). Regarding BAT Ucp1 expression, it was lower in Lou/C than in Wistar animals, and it was reduced by the exposure at 30°C in the Wistar group (Fig. 5D). Such conditions also brought about a decrease in BAT Adrb3 expression in Wistar and Lou/C rats (Fig. 5D). In iWAT, Ucp1 expression was doubled in Lou/C compared with Wistar at 22°C, although this did not reach statistical significance, and it remained at the same values at 30°C (Fig. 5E). In this tissue, expression of the Adrb3 was higher in Lou/C than in Wistar rats, with no effect of the temperature (Fig. 5E). Similar observations were made for the GIR measured during EHC (Fig. 5C), indicating that the improved insulin sensitivity of the Lou/C group is maintained at 30°C and is therefore not linked to cold exposure, even of minor magnitude.

Finally, the expression of genes encoding for enzymes known to be involved in lipid metabolism was measured in BAT and iWAT. Acetyl CoA carboxylase represents the rate-limiting step in fatty acid (FA) synthesis. Carnitine palmitoyl transferase-1α is the rate-limiting step of the FA oxidation pathway, mediating FA transport from the cytosol to the mitochondria. Medium- and large-chain acyl-CoA dehydrogenases (MCAD and LCAD) are involved in FA oxidation. PEPCK allows for the in situ production of glycerol-3-phosphate (glyceroneogenesis) and therefore for the formation of TG. Hormone-sensitive lipase (HSL) is one of the main enzymes involved in lipolysis. In iWAT, Acc1 expression was twofold higher in Lou/C than in Wistar rats in the absence of β3 agonist treatment. Furthermore, it was more than doubled in the Lou/C group in response to the treatment, while it remained unchanged in Wistar rats (Fig. 6A). As expected from its inhibitory regulation by malonyl CoA, Cpt1a expression was lower in Lou/C than in Wistar animals, with a trend toward a further decrease in the β3 agonist–treated group (Fig. 6A). On the contrary, the β3 agonist treatment resulted in an enhancement of Hsl, Lcad, and Mcad expression in Lou/C rats, without any change in the Wistar group (Fig. 6A). Similar results were obtained for the expression of Pepck (Fig. 6A).

In BAT, Acc1 expression was similar in Wistar and Lou/C untreated rats and it was reduced by the treatment in the Lou/C group only (Fig. 6B). The expression of Cpt1α was under the detection limit, as a likely consequence of

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_F_ Nonlinear regression with hyperbolic correlation between 2-DG glucose uptake and Ucp1 expression in iWAT. Results are means ± SEM of 8 experiments per group. Statistical significance assessed by two-way ANOVA: #strain effect; @treatment effect; $interaction between strain and treatment. *Significant difference by post hoc pairwise comparison between different conditions. Single and triple symbols indicate P < 0.05 and P < 0.001, respectively. gluc, glucose._
Figure 5—Differences between Wistar and Lou/C rats are not due to a difference in thermoneutrality. 

A: \( \Delta \text{BW gain (g)} \) of Wistar and Lou/C rats measured between days 4 and 30 of 22°C (control) and 30°C exposure. Total food intake (FI) measured between days 4 and 30.

B: Food efficiency calculated from \( \Delta \text{body weight gain/total food intake in %} \).

C: GIR measured at the end of EHCs in Wistar and Lou/C rats housed at 22°C and at 30°C.

D: \( \text{Ucp1} \) and \( \text{Adrb3} \) mRNA expression in BAT.

E: \( \text{Ucp1} \) and \( \text{Adrb3} \) mRNA expression in iWAT.

Results are means ± SEM of 8–12 experiments. Statistical significance assessed by two-way ANOVA: #strain effect; @treatment effect; $interaction between strain and treatment. *Significant difference by post hoc pairwise comparison between different conditions. Double and triple symbols indicate \( P < 0.01 \) and \( P < 0.001 \), respectively.
elevated Acc1 expression in this tissue. There was no difference in Hsl expression in Wistar and Lou/C rats whether treated or not (data not shown). In control animals, Pepck was less expressed in the Lou/C compared with the Wistar group but it was increased by the treatment in Lou/C rats to reach the expression level measured in Wistar rats (Fig. 6B).

**DISCUSSION**

This study was undertaken to determine the impact of recruiting UCP1-expressing adipocytes in a model of obesity resistance characterized by the presence of beige cells in iWAT (16,17). For this purpose, Lou/C and Wistar rats were treated for 2 weeks with a β3 agonist (CL-316243). The treatment had no effect on food intake and body weight gain, while it decreased the fat mass as well as the weight of interscapular BAT and of various WAT depots in the two groups.

In terms of its effects on UCP1 expression, different types of results were obtained in BAT and WAT when comparing Wistar and Lou/C rats. In BAT, we and others previously reported that Ucp1 mRNA and/or protein levels were lower in Lou/C than in Wistar rats at 22°C or at 25°C.

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**Figure 6**—The higher glucose uptake in iWAT is explained by an increase in FA synthesis in CL-treated Lou/C rats. A: Acc1, Cpt1α, Hsl, Pepck, Lcad, and Mcad mRNA expression in iWAT. B: Acc1 and Pepck mRNA expression in BAT. Results are means ± SEM of 8 experiments per group. Statistical significance assessed by two-way ANOVA: #strain effect; @treatment effect. *Significant difference by post hoc pairwise comparison between different conditions. Single, double, and triple symbols indicate P < 0.05, P < 0.01, and P < 0.001, respectively. AU, arbitrary units; CL, CL, CL-316243; ctrl, control.
(17,26), whereas, in the current study, this was observed for UCP1 protein at 22°C but not for the Ucp1 gene at 22°C or 30°C. Regarding the effects of the β3 agonist treatment, it increased Ucp1 and decreased Adrb3 expression in BAT of both groups to a similar extent. These results, confirmed by the measurement of UCP1 protein, closely correlated with the observation of similar increases in BAT glucose uptake in Wistar and Lou/C rats, as measured during EHC and by \(^{18}\)F-FDG–PET scan analysis.

UCP1-expressing beige cells are known to be present in various WAT depots (27,28). Chronic cold exposure was shown to recruit beige adipocytes in WAT, resulting in WAT "browning" (29,30). Interestingly, resistance to diet-induced obesity in rats and mice has been suggested to depend on the induction of beige adipocyte recruitment in WAT (16,31). Moreover, such increased recruitment of beige cells has been shown to compensate for decreased BAT thermogenesis, and the specific loss of beige adipocytes due to adipose tissue–specific deletion of a transcriptional factor involved in Ucp1 expression (32), PRDM16, was shown to cause obesity (32,33). Beige adipocytes are also strikingly involved in the regulation of glucose metabolism, as demonstrated by the beneficial effects on glucose tolerance and insulin sensitivity observed in transgenic mice overexpressing PRDM16 in iWAT (32).

In the current study, we showed that the β3 agonist treatment affected the Lou/C group only, markedly increasing UCP1 gene and protein expression, mitochondrial biogenesis, and the insulin-stimulated glucose uptake measured during EHC (subcutaneous and intra-abdominal fat depots) or using \(^{18}\)F-FDG–PET scan analysis (subcutaneous fat depot) as previously reported (34). Furthermore, in iWAT from Lou/C–treated rats, a hyperbolic correlation between glucose uptake and Ucp1 mRNA expression could be observed. Although this does not allow conclusions about cause-effect relationships, these data suggest that the amount of beige adipocytes in WAT depots can determine the overall tissue glucose utilization rate. Such conclusion is supported by the appearance of multilocular beige cells in iWAT of CL-316243–treated Lou/C rats, as determined by histological means. Whether these beige cells result from activation of existing adipocytes or from transdifferentiation of white into brown cells is an important issue that will need to be addressed in future work.

As schematized by Fig. 7, the molecular mechanisms underlying the β3 agonist–induced increase in glucose metabolism in iWAT of Lou/C rats seem to involve inductions of increased FA synthesis (de novo lipogenesis [DNL]) and lipolysis (increased Acc1 and Hsl, respectively), as well as of enhanced FA β-oxidation (increased Mcad and Lcad). Additionally, the marked increase in the expression of Pepck in iWAT of Lou/C treated rats suggests the presence of increased glyceroneogenesis. Similar data of gene expression responsible for enzymes involved in lipid metabolism in adipose tissues were reported in normal mice treated with CL-316243 for 7 days (35).

With regard to the overall insulin-stimulated glucose utilization, as reflected by the GIR during EHC (under our experimental conditions of suppressed hepatic glucose production), we observed that it was similarly increased by the treatment in Wistar and Lou/C rats. Given that skeletal muscle glucose utilization was unaltered by the treatment in the two groups, only BAT and WAT glucose uptake could be held responsible for the treatment-induced increase in GIR. By considering the total mass of BAT and WAT, as well as the mean glucose uptake in these tissues, it was possible to approximate the percent contribution of each of these two tissues to the increased GIR. Thus, in Wistar rats, both BAT (≈30%) and WAT (≈60%) contributed to the GIR enhancement in response to the treatment. In contrast, in Lou/C rats, the treatment doubled BAT glucose uptake but decreased the BAT mass by twofold. Therefore, in these animals, the stimulatory effect of the treatment on GIR could only be attributed to increased glucose uptake in WAT depots containing beige cells. It should, however, be mentioned that these calculations may underestimate BAT glucose uptake, as the reduction in tissue weight may reflect loss of lipids due to increased thermogenic activity induced by the β3 agonist treatment. These data are in keeping with previously reported results showing that chronic stimulation of the
sympathetic nervous system stimulates glucose uptake in WAT and BAT without any effect in skeletal muscles (36). The predominant role of beige cells in WAT glucose uptake is further supported by the previous observation that the increased glucose utilization under sympathetic stimulation depends on UCP1 activation (37,38).

Altogether, the results of the current study extend other data showing that chronic cold exposure or β3 agonist treatment exerts beneficial effects on glucose metabolism in mice with high-fat diet–induced obesity (rev. in 12) and normalizes hyperglycemia and hypertriglyceridemia in mouse models of diabetes and dyslipidemia (39–41). They also extend previous data (42), supporting the notion of interdependency between glucose uptake, lipid metabolism, and thermogenesis. As recently further discussed (43), a particularly important role for the futile cycling between FA synthesis and oxidation linked to thermogenesis and glucose disposal is played by DNL. This may be particularly relevant in beige cells of WAT depots, in keeping with the observation that WAT is required for the full thermogenic response to CL-316243 (44). To go a step further in the understanding of the mechanisms involved in the effects of β3 agonists, several data indicate that DNL is required for the synthesis of several signaling lipids with systemic effects (“lipokines”) supporting metabolic homeostasis. The presence of these molecules in our different experimental groups could therefore be of interest and will be investigated in the future.

To conclude, induction of DNL coupled with a simultaneous increase in FA oxidation as occurs during browning of WAT (CL-316243–treated normal mice [35]; CL-316243–treated Lou/C rats [present study]) appears to be important for the regulation of glucose homeostasis and may therefore be of clinical relevance for the treatment of obesity and type 2 diabetes in humans.

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References

7. Matthias A, Ohlson KB, Fredriksen JM, Jacobsen S, Nedergaard J, Cannon B. Thermogenic responses in brown fat cells are fully UCP1-dependent. UCPI or UCPS do not substitute for UCP1 in adrenergically or fatty acid-induced thermogenesis. J Biol Chem 2000;275:25073–25081