Impaired oxidative capacity due to decreased CPT1b levels as a contributing factor to fat accumulation in obesity

Cecilia Ratner,1,2 Andreas Nygaard Madsen,1,2 Line Vildbrad Kristensen,1,2 Louise Julie Skov,1,2 Katrine Seide Pedersen,3 Ole Hartvig Mortensen,3 Gitte Moos Knudsen,4 Kirsten Raun,5 and Birgitte Holst1,2

1Laboratory for Molecular Pharmacology, Department of Neuroscience and Pharmacology, University of Copenhagen, Copenhagen, Denmark; 2Section for Metabolic Receptology and Enterodocrinology, The Novo Nordisk Foundation Center for basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark; 3Department of Biomedical Sciences, Cellular and Metabolic Research Section, Symbion, Copenhagen, Denmark; 4Neurobiology Research Unit and Center for Integrated Molecular Brain Imaging, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark; and 5Novo Nordisk Diabetes Research Unit, Novo Nordisk A/S, Maaloev, Denmark

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To study human obesity, animal models are highly important to understand the pathophysiology behind obesity and to pursue novel treatment strategies. These include rodent models fed with high-fat diet [diet-induced obesity (DIO) rodents] and rodent models with a monogenetic cause of obesity such as leptin deficiency (35, 40). Whereas these animal models may be useful in modeling certain aspects of obesity, they do not mirror human obesity that is caused by a complex interplay of environmental, social, and genetic factors. One animal model, the selectively bred obesity-prone (OP) and obesity-resistant (OR) rat employed in the present study, was developed by Barry Levin and colleagues (31), in an attempt to mimic both the polygenetic and environmental (diet) aspects of obesity. In outbred Sprague-Dawley rats, some are genetically predisposed to obesity when placed on a Western diet with relatively high fat and sucrose compared with chow (31.8% fat, 25.7% sucrose), whereas others are obesity resistant gaining no more weight than chow-fed controls (34). Selective breeding of high and low weight gainers has produced the OP and OR substrains (31). When placed on a Western diet, the OP rats are described to have higher energy intake, accumulate more fat, be glucose intolerant, hyperinsulinemic, and hyperleptinemic, and defend their body weight once obesity is established (31, 36).

Excessive fat accumulation is a hallmark of obesity and can be attributed to an imbalance between energy consumed and energy expended or alterations in substrate utilization and trafficking. The underlying molecular mechanisms giving rise to excessive adiposity in both humans and rodent models including the OP rats are incompletely understood. Centrally, the OP rats have higher hypothalamic neuropeptide Y (NPY) mRNA expression (29), as well as more inhibitory synapses onto the orexigenic proopiomelanocortin neurons than OR rats (18) before high-fat diet feeding and thus the establishment of obesity. This could contribute to their higher food intake and body weight, but it does not explain the selective fat accumulation.

Ghrelin is a peptide primarily secreted from the endocrine cells of the stomach (28), which stimulates growth hormone release, appetite, and fat accumulation (38, 50, 52, 53, 57). Ghrelin is most well described for its orexigenic effects in the arcuate nucleus (ARC) of the hypothalamus, where it increases the appetite-stimulating neuropeptides agouti-related peptide (AgRP) and NPY (9, 27, 47). Importantly, ghrelin also pro-
motes adiposity independently of its appetite-stimulating effects by upregulating lipogenic genes and downregulating fat oxidative genes in white adipose tissue (42, 52). Accordingly, it is interesting to study the effect of exogenously administrated ghrelin in this obesity-prone animal model. Furthermore, several studies have demonstrated that obese animal models such as DIO rodents and db/db mice are resistant or have a blunted response to the orexigenic effects of ghrelin, although results are discrepant as to whether it is caused by the high-fat diet per se or the resultant obesity (5, 14, 23, 42, 43). Interestingly, the ghrelin resistance seems to affect only the orexigenic and not lipogenic actions of ghrelin (42).

The aim of the present study was to examine the molecular and physiological backgrounds for increased fat accumulation by performing an integrated and simultaneous analysis of food intake, energy expenditure, substrate utilization, and activity levels under unstressed home-cage conditions in free-fed and fasted male OP and OR rats using an indirect calorimetry system. This was done after feeding a Western diet for 76 days where obesity was fully established, to study the translational value of this model for human obesity. In addition, juvenile OP and OR rats were also examined before severe obesity had developed in the indirect calorimetry system to establish whether the phenotype observed in the adult obese rats were primary or secondary to obesity. Adult OP and OR rats were also given a single dose of peripherally administered ghrelin to assess potential differences in ghrelin sensitivity between OP and OR rats.

MATERIALS AND METHODS

Animals and indirect calorimetry. Two cohorts of selectively bred male OP and OR rats (Taclevin DS and DR rats, Taconic, Hudson, NY) were used in these studies. All rats were single housed from 4 wk of age with ad libitum access to water and a Western diet (31.8% fat, 16.8% protein, and 51.4% carbohydrate (25.7% sucrose), no. 12266B, Research Diets, Brogaarden, Lunge, Denmark) or a chow diet (13% fat, 27% protein, and 60% carbohydrate; altomin no. 1310, Brogaarden) under a 12-h light/dark cycle (lights off at 1800 h). The first cohort of rats (12 OP and 12 OR rats) was placed on the Western diet for 76 days. During this time period, food intake, body weight, and activity levels were monitored every 3–4 days, and body composition was determined every 2 wk by quantitative magnetic resonance imaging (MRI) using EchoMRI (Echo Medical Systems, Houston, TX) in unanesthetized rats. At 15 or 17 wk of age (balanced between OP and OR rats), rats were housed singley in an indirect calorimetry system (Phenomaster; TSE systems, Bad Homburg, Germany), where food intake, energy expenditure, respiratory exchange ratio (RER: VCO₂/VO₂), and activity levels were recorded. Rats were acclimatized to the system for 3 days followed by 3 days of baseline measurements. Next, rats were fasted for 24 h followed by 24 h of refeeding and a recovery period, after which ghrelin (2 ng/kg in 2.5 ml/kg sc, Polypeptide, Linham, Sweden) or saline was administered just before the onset of the dark phase in a crossover design. The ghrelin dose was chosen based on previous publications (1, 5) and further optimized in our laboratory; in lean rats, this dose increases the active ghrelin concentration about fivefold from 903 ± 82 pg/ml to 4,393 ± 162 pg/ml in plasma after 40 min (Ratner C, Skov LJ, Madsen AN, and Holst B; unpublished observations).

The second cohort of rats (8 OP and 8 OR) was immediately placed in the indirect calorimetry system upon arrival with access to standard chow. The rats were acclimatized for 1 wk before measurements were started at an age of 5 wk. Data were collected for 1 wk and then the rats were switched to the Western diet and data collected for another week. Body composition was determined before and after measurements. The indirect calorimetry system was calibrated before each study and for each study period the indirect calorimetry system measured each cage every 20 min for 1 min and 10 s and the reference air every 20 min.

DIO mice and lean controls (C57BL/6) were purchased from Taconic after they had been fed a high-fat diet (60% fat, D12492, Research diets) or control diet for 10 wk from 6 wk of age (17). DIO rats and lean controls (Sprague-Dawley, Taconic, Ry, Denmark) were started on a high-fat/high-sucrose diet (45% fat, 12451, Research diets) or a low-fat control diet (10% fat, D12450B, Research diets) at a body weight of 200 g and maintained on the diets for 15 wk. DIO mice were euthanized at 17 wk of age and DIO rats at 22 wk of age. Tissue was collected as described for OP and OR rats. At euthanasia, DIO mice had a body weight of 38.2 ± 0.6 g versus chow-fed animals 27.5 ± 0.6 g (17). DIO rats had a body weight of 665.2 ± 39.3 g versus control rats of 470.6 ± 18.0 g. All animal experiments were approved by the Animal Experiments Inspectorate (Copenhagen, Denmark) and conducted in accordance with institutional guidelines (license no. 2012-15-2934-0005).

Tissue collection. Adult obese rats were returned to baseline maintenance conditions after calorimetry studies for at least 1 wk before tissue collection at an age of 20 wk. Rats were food deprived from the early light phase and euthanized 3 to 5 h into the light phase by decapitation after brief CO₂ anesthesia. The hypothalamus was dissected by making a cut posterior to the optic chiasm and anterior to the mammillary body, and then it was scooped out along its lateral margins in an approximate depth of 2 mm and snap frozen in liquid nitrogen. Epididymal and subcutaneous white adipose tissue and soleus and extensor digitorum longus (EDL) muscle were removed and snap frozen in liquid nitrogen. Trunk blood was collected and plasma separated. All samples were stored at −80°C until further processed.

Quantitative real-time PCR. RNA (hypothalamus and adipose tissue) was extracted and DNase treated using the RNaseasy lipid tissue mini kit (Qiagen, Hilden, Germany) or TRizol (muscle) (Invitrogen, Carlsbad, CA). cDNA was synthesized by reverse transcription using ImProm-ITM reverse transcriptase (Promega, Madison, WI). Relative mRNA levels were determined in duplicates using a Lightcycler 480 II (Roche Applied Science, Penzberg, Germany) and SYBRPremix Ex Taq (Takara, Otsu, Japan). The relative gene expression level was determined using the ΔΔC₆₇ method normalizing to TATA-binding protein (TBP). All primers used (Table 1) were initially tested for specificity and efficiency by running standard curves.

Western blot. Western blot analysis was performed as previously described (44). The antibodies used were the following: total acetyl-CoA carboxylase no. 3662 and phosphorylated acetyl-CoA carboxylase no. 3661 (Cell Signaling, Boston, MA), diluted 1:1,000.

Plasma assays. Insulin and leptin were analyzed using the mouse/rat insulin kit and the rat leptin kit (Mesoscale Discovery, Rockville, MD).

Statistical analyses. Statistical analyses were performed using Prism version 6.0a (Graphpad Software, San Diego, CA). Data were analyzed using two-way ANOVA with Sidak posttest, two-way ANOVA repeated measures with Sidak posttest, or two-tailed t-tests. Energy expenditure data were analyzed by one-way or two-way ANCOVA as recommended (54) with lean mass as covariate using SAS version 9.2 (SAS software, Cary, NC). All data represent means ± SE. Values of P ≤ 0.05 were considered significant.

RESULTS

Food intake, body weight, and body composition. The body weight of OP and OR rats was similar for the initial part of the feeding period, but during the final 20 days a difference in body weight developed between OP and OR rats (541 ± 9 g vs. 500 ± 12 g, P < 0.01) (Fig. 1A). No difference in food intake among the different groups was found (Fig. 1B). A comparison of the lean mass between the different groups revealed that the OR rats had significantly more lean mass than the OP rats (Fig. 1C). The higher body weight of the OR rats was in part attributable to a significantly higher body fat content, whereas the body water content was not different between the groups (Fig. 1D). A comparison of the body fat content between the different groups revealed that the OR rats had a significantly higher body fat content than the OP rats (Fig. 1E). The lower body fat content of the OP rats was in part attributable to a significantly lower body fat mass, whereas the body water mass was not different between the groups (Fig. 1F).
was detected between OR and OP rats assessed by weighing of remaining food pellets (Fig. 1B). OR and OP rats initially had similar body composition but already after 2 wk on the Western diet, OP rats had significantly higher fat percentage and fat mass (Fig. 1, C and E) and lower lean percentage compared with OR rats, whereas the absolute lean mass was similar between OP and OR rats (Fig. 1, D and F). The difference in body composition between OP and OR rats continued to increase throughout the study and at 76 days the difference in fat percentage was 19.7 ± 0.6% in OP rats versus 14.2 ± 0.4% in OR rats (P < 0.0001).

Indirect calorimetry. During the 72 h of baseline measurements, OP rats had higher RER values (Fig. 2, A and B) and lower activity levels (Fig. 2, C and D) compared with OR rats, whereas OP and OR rats had similar cumulative food intake (Fig. 2H) and energy expenditure (Fig. 2, E–G). To determine whether the differences observed in the adult obese rats were primary and contributing or secondary to the obese phenotype, we examined juvenile rats in the indirect calorimetry system before and during the introduction of the Western diet. Juvenile OP rats on chow showed increased RER values during the dark phase (Fig. 2J) and during the light phase after the diet was switched to the Western diet (Fig. 2L). No differences in activity levels were observed between juvenile OP and OR rats (Fig. 2, K and M). The juvenile OP and OR rats had similar body weight before the calorimetry study, whereas OP rats were heavier afterwards (Fig. 2, I and N). This was likely caused by the higher food intake observed in juvenile OP rats (data not shown).

We also fasted the adult obese OP and OR rats for 24 h and monitored their ability to shift from a mixed oxidation of carbohydrate and fat to an almost complete fat oxidation (Table 2). During the first 3 h of fasting the OP rats had significantly higher RER, but during more extreme situations such as longer-lasting fasting, the OP rats obtained the same capacity for fat oxidation as observed for the OR rats. During refeeding OP rats likewise had similar RER values to OR rats. Furthermore, OR rats had higher energy expenditure during dark phase refeeding.

**Ghrelin administration.** Subcutaneous administration of ghrelin in the adult obese rats (2 mg/kg) induced a trend toward increased cumulative food intake (P = 0.07) in OR rats and (P = 0.12) in OP rats with a significant main effect of ghrelin treatment (P = 0.006) (Fig. 3A). Ghrelin decreased activity levels in OR rats (P = 0.002) and energy expenditure in both OR (P < 0.0001) and OP (P = 0.0025) rats at 40 min, whereas no difference was observed after 120 min (Fig. 3, B and C). Ghrelin induced a robust increase in RER values at both time points representative of a shift away from fat oxidation toward carbohydrate metabolism in both the OP and OR rats (Fig. 3D).

**Adipose tissue and muscle gene expression.** To examine possible molecular backgrounds of the observed higher fat accumulation and higher RER values in OP rats, the expression of genes involved in lipogenesis and fatty acid metabolism were examined in the adult obese rats. In the intra-abdominal fat compartment, epididymal fat, OP rats had lower mRNA levels of carnitine palmitoyl transferase 1b (CPT1b) and both isoforms of acetyl-CoA carboxylase (ACC) (Fig. 4A). CPT1b mediates the transport of fatty acids across the mitochondrial membrane, which may be responsible for the impaired capacity of fat oxidation within this adipose compartment for OP rats. ACC mediates the synthesis of two pools of malonyl CoA, of which malonyl CoA produced by ACC1 is used in the synthesis of triglycerides, whereas malonyl CoA produced by ACC2 inhibits CPT1b and thus the β-oxidation pathway (55). Because ACC is active only in the nonphosphorylated state, Western blotting was used to determine the degree of phosphorylation. No difference was observed in total or phosphorylated ACC levels between OP and OR rats (Fig. 4B) and thus the activity of ACC did not differ between OP and OR rats. To examine if low CPT1b levels in intra-abdominal fat is generally observed in obese animals, the CPT1b expression levels were also examined in DIO mice and rats, obtained by simple high-fat diet feeding, hence not predisposed to obesity. In contrast to the OP rats, DIO mice had higher CPT1b levels compared with chow-fed controls (Fig. 4C), whereas DIO rats had similar CPT1b levels to lean control rats (Fig. 4D). High

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**Table 1. Primers used in the study**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer 5′→3′</th>
<th>Reverse Primer 5′→3′</th>
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<tr>
<td>Acaca</td>
<td>AGTGGCAGTGGTTCCTGAG</td>
<td>GGCCCTTGACTCATCACTGGA</td>
</tr>
<tr>
<td>Acacb</td>
<td>TCCCTGATAGTGGAGGCAC</td>
<td>CCGCCCTCAAGGTCGCTTAA</td>
</tr>
<tr>
<td>Agrp</td>
<td>GATGGCAGTGGTTCCTGAG</td>
<td>GGCCCTTGACTCATCACTGGA</td>
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<tr>
<td>Car1</td>
<td>TGCCCTTGACTCATCACTGGA</td>
<td>GGCCCTTGACTCATCACTGGA</td>
</tr>
<tr>
<td>Cpt1b (rat)</td>
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<td>GGCCCTTGACTCATCACTGGA</td>
</tr>
<tr>
<td>Cpt1b (mouse)</td>
<td>GGCCCTTGACTCATCACTGGA</td>
<td>GGCCCTTGACTCATCACTGGA</td>
</tr>
<tr>
<td>Fasn</td>
<td>ACCTTGCAAGGAGACTAGCTA</td>
<td>CCGCCCTCAAGGTCGCTTAA</td>
</tr>
<tr>
<td>Ghsl</td>
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<td>CCGCCCTCAAGGTCGCTTAA</td>
</tr>
<tr>
<td>Lipe</td>
<td>GCCTTGCTACTGACTCATGGA</td>
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<tr>
<td>Mc4r</td>
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<td>CCGCCCTCAAGGTCGCTTAA</td>
</tr>
<tr>
<td>Npy</td>
<td>GACATAGCAGGAGAGGCTTGGA</td>
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</tr>
<tr>
<td>Pomc</td>
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<td>TGCCCTTGACTCATCACTGGA</td>
</tr>
<tr>
<td>Pparg</td>
<td>CATAGCAGGAGAGGCTTGGA</td>
<td>TGCCCTTGACTCATCACTGGA</td>
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<tr>
<td>Ppargc1a</td>
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<tr>
<td>Scd1</td>
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<td>ACACCCATTTCCCTCAAATGAGGAG</td>
</tr>
<tr>
<td>Sirt1</td>
<td>TGGCTCTGGCTGGGGGGGCTGA</td>
<td>ACACCCATTTCCCTCAAATGAGGAG</td>
</tr>
<tr>
<td>Tbp</td>
<td>TCACAGCTGAGAAGGCTTGCA</td>
<td>TGCCCTTGACTCATCACTGGA</td>
</tr>
<tr>
<td>Ucp2</td>
<td>CTCAGAAGACACCATTGCCAGA</td>
<td>TGCCCTTGACTCATCACTGGA</td>
</tr>
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</table>
levels of CPT1b correspond well with earlier described low RER levels in DIO rodents (15, 49).

In subcutaneous fat, OP rats had higher mRNA levels of stearoyl-CoA desaturase 1 (SCD1), the rate-limiting enzyme in unsaturated fatty acid synthesis (Fig. 4E). No differences in mRNA levels in muscle were observed between OP and OR rats, except a trend toward higher expression of SCD1 in both soleus (oxidative) and extensor digitorum longus (EDL) (mixed oxidative and glycolytic) in OP rats (Fig. 4, F and G).

Hypothalamic gene expression. In the hypothalamus, the adult obese OP rats had lower expression of AgRP, uncoupling protein 2 (UCP2), and the ghrelin receptor (Fig. 4H). As mentioned above, low expression of the ghrelin receptor is associated with obesity (5), which is consistent with these results. AgRP mRNA in ARC is upregulated when ghrelin activates the NPY/AgRP neurons, and this is also the neuronal population described to be responsible for the ghrelin resistance associated with obesity. Furthermore, hypothalamic UCP2 mRNA is also upregulated by ghrelin, and UCP2 is required for a complete food intake response of ghrelin, as it mediates ghrelin’s activation of NPY/AgRP neurons by lowering free radicals generated by hypothalamic fatty acid oxidation (2). Thus low mRNA levels of the ghrelin receptor in OP rats could decrease ghrelin-mediated gene expression of AgRP and UCP2, which in turn likely contributes to the observed attenuated ghrelin sensitivity in OP rats.

Plasma hormones. Plasma levels of leptin and insulin were assessed to determine basal differences in metabolic blood markers in adult obese rats. OP rats had higher leptin and insulin levels than OR rats (Table 3). High leptin and insulin levels are often observed in obese animals as leptin mirrors adiposity, whereas high insulin could be indicative of insulin resistance in OP rats.

DISCUSSION

In the present study we demonstrated that the selectively bred OP rats fed a moderate high-fat diet have an impaired capacity to use fat as an energy source demonstrated by increased RER value during normal physiological baseline conditions. We suggest that this could be one of the causative factors responsible for the severe increase in fat accumulation
in the OP compared with OR rats, since the increased RER level was observed both before and after severe obesity developed in the OP rats. For the first time we also dissect an important molecular pathway that may explain the decreased capacity to oxidize fat in the intra-abdominal fat compartment. Here we observed decreased expression of CPT1b, which is responsible for the transfer of free fatty acids into the mitochondrial matrix suggesting lower fat oxidation. In the subcutaneous fat compartment, the rate-limiting enzyme in unsaturated fatty acid synthesis, SCD1, was upregulated. Also, in the skeletal muscles a trend toward upregulation of this enzyme was observed. The regulation of these markers could contribute to the impaired fat oxidation and explain the increased propensity for fat accumulation observed in OP rats. Interestingly, increased fat accumulation is observed before the increase in body weight, and this redistribution likely contributes to the following increase in body weight. Finally, we have shown that this strain of obese rats does not develop full ghrelin resistance with respect to food intake. However, the ghrelin-induced decrease in activity and energy expenditure were attenuated in the OP rats compared with the OR rats, whereas OR and OP rats responded similarly to ghrelin-induced increase in the RER values.

Valid animal models that reflect the complicated interplay between environmental and genetic factors are required in the attempts to develop novel and efficient pharmaceutical treatments of obesity. In addition, such animal models may also be important for the molecular understanding of the pathophysiology responsible for the development of obesity. The OP rat model that we have studied may constitute such a model system. The OP rats in our study developed a strong degree of fat accumulation, which is a corner stone in the pathophysiology of obesity and important for the accompanied diseases (10). Thus it is important to characterize the metabolic parameters under unstressed “home-cage” conditions for longer periods of times to determine mechanisms of this severe fat accumulation, which the indirect calorimetry system accommodates. In the ad libitum fed state, OP rats had higher RER values indicating a preferential usage of carbohydrate over fat as their energy substrate, despite the higher fat mass of OP rats. This difference was not caused by higher food intake in the OP rats since this study, in contrast to previous studies (12, 25, 26,
and obesity-resistant rats established. While it is well established that low activity levels only observed in adult rats after the obese phenotype was strain. On the contrary, lower activity levels in OP rats were primary and causative and not secondary to obesity in this higher RER values suggesting that impaired fat oxidation is OP rats compared with OR rats. Juvenile OP rats likewise had Table 2. RER, energy expenditure, activity, and food intake during 24 h fasting followed by 24 h refeeding in obesity-prone and obesity-resistant rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dark Phase</th>
<th>Light Phase</th>
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<tr>
<td></td>
<td>OP</td>
<td>OR</td>
</tr>
<tr>
<td><strong>Fasting</strong></td>
<td></td>
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</tr>
<tr>
<td>RER</td>
<td>0.766 ± 0.003</td>
<td>0.760 ± 0.002</td>
</tr>
<tr>
<td>3 h</td>
<td>0.830 ± 0.009</td>
<td>0.809 ± 0.005</td>
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<tr>
<td><strong>Energy expenditure, kcal/h</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3.12 ± 0.06</td>
<td>3.20 ± 0.06</td>
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<tr>
<td>3 h</td>
<td>3.86 ± 0.09</td>
<td>3.83 ± 0.09</td>
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<tr>
<td><strong>Activity (beam breaks)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>225 ± 13</td>
<td>245 ± 17</td>
</tr>
<tr>
<td>3 h</td>
<td>467 ± 29</td>
<td>482 ± 43</td>
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<tr>
<td><strong>Refeeding</strong></td>
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<tr>
<td>RER</td>
<td>0.887 ± 0.003</td>
<td>0.886 ± 0.003</td>
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<tr>
<td><strong>Energy expenditure, kcal/h</strong></td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>3.47 ± 0.06</td>
<td>3.64 ± 0.06</td>
</tr>
<tr>
<td>3 h</td>
<td>296 ± 31</td>
<td>354 ± 22</td>
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<tr>
<td><strong>Activity (beam breaks)</strong></td>
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<tr>
<td>Cumulative food intake, g</td>
<td>17.9 ± 0.9</td>
<td>19.2 ± 0.7</td>
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<tr>
<td><strong>Cumulative food intake, g</strong></td>
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Data are means ± SE; n = 12 per genotype. OP, obesity prone; OR, obesity resistant. RER: respiratory exchange ratio. Energy expenditure represents average values adjusted for lean mass using ANCOVA. All other data were tested with two-tailed t-test.

31, 33, 36, 45), did not demonstrate increased food intake in OP rats compared with OR rats. Juvenile OP rats likewise had higher RER values suggesting that impaired fat oxidation is primary and causative and not secondary to obesity in this strain. On the contrary, lower activity levels in OP rats were only observed in adult rats after the obese phenotype was established. While it is well established that low activity levels can maintain and exacerbate an obese phenotype (4), the contribution of low activity levels as the primary underlying factor for obesity is more controversial. Rodent studies show conflicting results as some find activity levels predictive of future weight gain (4, 51), while others do not (7, 30, 41). In this particular strain of OP rats most studies found no preexisting differences in activity between OP and OR rats (25, 39),

Fig. 3. Sensitivity of OP and OR rats to a single dose of ghrelin (2 mg/kg sc). Ghrelin was administered just before the onset of the dark phase and data were measured 40 and 120 min after administration. A: cumulative food intake (40 min: treatment main effect \( P = 0.0062 \); B: energy expenditure (40 min: treatment main effect \( P < 0.0001 \)); C: ambulatory activity represented as average number of beam breaks during 20-min intervals (40 min: treatment main effect \( P = 0.0011 \)), and D: average RER values (40 min: treatment main effect \( P < 0.0001 \), genotype main effect \( P = 0.018 \), 120 min: treatment main effect \( P < 0.0001 \), genotype main effect \( P = 0.0018 \)). Data are means ± SE. Each time point tested with two-way ANOVA with Sidak posttest (two comparisons made) except where two-way ANCOVA was used (B). N = 12 per genotype. ** \( P < 0.01 \), *** \( P < 0.001 \), **** \( P < 0.0001 \).
whereas one study found activity differences before the establishment of obesity (51). Thus the causal relationship between activity and obesity is complex and incompletely understood. This study suggests that low activity is secondary to excessive fat accumulation and not one of the underlying factors contributing to the OP and OR phenotypes of this strain.

A previous study (25) working on the same strain of rats found that selectively bred OP rats preferentially oxidize carbohydrate over fat, but the study by Jackman et al. (25) only measured the energy expenditure and RER value just before and after short (5 days) exposure to high-fat diet, before obesity was fully established. Impaired fat oxidative capacities of the OP rats is also supported by tracer studies after intra-gastric administration of [14C]palmitate, where OP rats oxidized less dietary fat and had higher disposal of tracer to the adipose tissue than OR rats (24, 26). However, it has never previously been shown that OP rats oxidize less fat than the OR rats after long-term, high-fat feeding, where severe fat accumulation in the OP rats has been obtained.

The observation that OP rats display decreased fat oxidation both under lean and fat conditions indicate that this may be one of the primary causative factor for the excessive fat accumulation. Furthermore, our study suggests that the molecular responsible factor is a lower level of CPT1b in the fat tissue. This is in contrast to other obese animal models such as DIO rodents where diet alone is responsible for the obesity, as these have lower RER value compared with chow-fed controls (15, 49). The DIO mice and rats examined in the present study had higher or similar CPT1b levels compared with low-fat fed controls, which is probably an adaptive mechanism to their higher fat intake and the consequent high-fat accumulation. This indicates that obesity or high adiposity per se is not associated with high RER values and low CPT1b levels, substantiating that the high RER and low CPT1b levels observed in the OP rats could be one of the genetic underlying and molecular factors responsible for the high level of fat accumulation in this obese phenotype. Interestingly, human studies suggest that impaired fat oxidative capacities may be an important contributor to obesity because obese individuals oxidize less fat than lean individuals (3) and fat oxidation capacities have been shown to predict body weight (13, 16, 37, 46, 58). Increased desaturase activities and SCD1 expression have been described in skeletal muscle of obese humans (20, 56), whereas decreased CPT1 expression has been observed (48). This is in line with our results from the OP and OR rats and emphasizes the high translational value of this animal model when studying obesity.

Table 3. Plasma levels of leptin and insulin in obesity-prone and obesity-resistant rats

<table>
<thead>
<tr>
<th>Hormone</th>
<th>OP</th>
<th>OR</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin, ng/ml</td>
<td>19.6 ± 1.7</td>
<td>15.5 ± 0.9</td>
<td>0.047</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>4.4 ± 0.4</td>
<td>3.2 ± 0.4</td>
<td>0.049</td>
</tr>
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</table>

Data are means ± SE; n = 12 per genotype. Data were tested with two-tailed t-test.
We also examined the sensitivity of the OP and OR rats to the orexigenic hormone ghrelin. As described in lean animal models, we observed a trend toward increased food intake, a shift in the substrate use toward carbohydrate, and decreased activity and energy expenditure in OR rats, despite their high-fat diet. OP rats also showed a trend toward increased food intake and an attenuated decrease in activity levels and energy expenditure compared with OR rats. Only with respect to increased RER values a comparable ghrelin response was observed between OP and OR rats. Several obese and diabetic rodent models are resistant to the orexigenic effects of ghrelin (5, 14, 23, 43). One study showed that feeding a high-fat diet overnight before central ghrelin administration was sufficient to blunt the orexigenic effects of ghrelin, suggesting that the diet interferes with ghrelin signaling (42). However, others find that longer time periods and increased body weight are necessary before ghrelin resistance occurs (6). Thus the underlying cause, e.g., the diet or the resultant obesity, is not fully understood. A recent study suggest that hyperleptinemia associated with obesity is important for ghrelin resistance (6). Interestingly, obese humans are not ghrelin resistant as associated with obesity is important for ghrelin resistance (6). Thus, the diet interferes with ghrelin signaling (42). However, others find that longer time periods and increased body weight are necessary before ghrelin resistance occurs (6). Thus the underlying cause, e.g., the diet or the resultant obesity, is not fully understood. A recent study suggest that hyperleptinemia associated with obesity is important for ghrelin resistance (6). Interestingly, obese humans are not ghrelin resistant as increased food intake and hunger ratings were observed after infusion of 5 pmol·kg⁻¹·min⁻¹ ghrelin for 75 min in both obese and lean subjects (11). Also, very obese subjects (mean body mass index 51.4) responded similarly to ghrelin infusions with respect to food intake as lean subjects (19). We did not observe full ghrelin resistance in the OR rats despite long-term Western diet feeding, supporting that diet per se does not cause ghrelin resistance. The OP rats also showed a trend toward increased food intake after ghrelin administration but with attenuated response compared with OR rats. Thus the OP and OR rats may also in this respect, constitute a better and more predictable model for human obesity compared with other rodent models. An attenuated ghrelin response of OP rats compared with OR rats was also observed in ghrelin’s suppression of activity levels and energy expenditure, overall suggesting that adiposity or downstream factors affect ghrelin sensitivity. In contrast, OP and OR rats responded similarly to the ghrelin-induced shift in substrate use toward carbohydrate oxidation, in line with previous publications demonstrating that ghrelin’s adipogenic effects are not blunted in the obese state (42). Thus the increased fat accumulation and decreased oxidative capacity observed for the OP rats is not caused by an increased sensitivity to ghrelin.

In ghrelin, other studies have shown that the OR rats are more sensitive to leptin (21, 22, 32, 33), insulin (8, 21), and glp-1 agonists (12) compared with OP rats. Thus it seems that NPY/AgRP neurons lose their ability to correctly integrate and respond to both orexigenic and anorexigenic hormonal signals in obese animals independently of the high-fat diet.

Perspectives and Significance

In conclusion, the present study suggests that increased fat accumulation in the selectivity bred OP rats may be caused by impaired capacity to oxidize fat. Impaired fat oxidative capacities were demonstrated both in adult obese and juvenile OP rats before the development of obesity, indicating that this defect could be causative. The low capacity to metabolize fat could be mediated via decreased expression of CPT1b. This, in combination with the observed high expression of SCD1, may constitute the molecular explanation for the increased fat accumulation in OP rats. The impaired fat oxidative capacity combined with increased fat accumulative capacity has also been shown to characterize human obesity. Furthermore, ghrelin did not seem to contribute to the high fat accumulation of OP rats, although OP rats were less resistant to ghrelin in terms of food intake compared with other rodent models of obesity. This also points to a high translational value for human obesity. Overall, we suggest that the OP and OR rat model constitute a suitable model for future studies on human obesity.


