Effect of leptin deficiency on metabolic rate in ob/ob mice

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Breslow, Michael J., Kyung Min-Lee, Daniel R. Brown, V. P. Chacko, David Palmer, and Dan E. Berkowitz. Effect of leptin deficiency on metabolic rate in ob/ob mice. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E443–E449, 1999.—Reduced metabolic rate may contribute to weight gain in leptin-deficient (ob/ob) mice; however, available studies have been criticized for referencing O2 consumption (VO2) to estimated rather than true lean body mass. To evaluate whether leptin deficiency reduces energy expenditure, four separate experiments were performed: 1) NMR spectroscopy was used to measure fat and nonfat mass, permitting VO2 to be referenced to true nonfat mass; 2) dietary manipulation was used in an attempt to eliminate differences in body weight and composition between ob/ob and C57BL/6j mice; 3) short-term effects of exogenous leptin (0.3 mg·kg⁻¹·day⁻¹) on VO2 were examined; and 4) body weight and composition were compared in leptin-repleted and pair-fed ob/ob animals. ob/ob animals had greater mass, less lean body mass, and a 10% higher metabolic rate when VO2 was referenced to lean mass. Dietary manipulation achieved identical body weight in ob/ob and C57BL/6j animals; however, despite weight gain in C57BL/6j animals, percent fat mass remained higher in ob/ob animals (55 vs. 30%). Exogenous leptin increased VO2 in ob/ob but not control animals. Weight loss in leptin-repleted ob/ob mice was greater than in pair-fed animals (45 vs. 17%). We conclude, on the basis of the observed increase in VO2 and accelerated weight loss seen with leptin repletion, that leptin deficiency causes a reduction in metabolic rate in ob/ob mice. In contrast, these physiological studies suggest that comparison of VO2 in obese and lean animals does not produce useful information on the contribution of leptin to metabolism.

Leptin is a fat cell protein that plays a critical role in the control of body weight (9). Initial studies in congenitally obese mice suggested that the major action of leptin was to modulate appetite via specific receptors located in the central nervous system (5, 10, 18). However, recent data suggest that leptin also influences energy balance through additional mechanisms, thus confirming the central role of leptin in defending body mass. According to this hypothesis, plasma leptin concentrations signal the adequacy of nutritional reserves and thus regulate a variety of physiological functions (1). Studies in leptin-deficient mice suggest a possible role for leptin in modulating metabolic activity.

Leptin-deficient mice (ob/ob) have a lower core temperature than normal mice (18), a reduced level of sympathetic nervous system activity (17), and decreased expression of β-adrenergic receptors (4, 7). Direct measurements of oxygen consumption, however, have yielded data that are difficult to interpret (14). Traditionally, oxygen consumption data are referenced to estimates of lean body mass, because fat is considered to be metabolically inert. In normal animals, lean body mass is roughly proportional to body surface area, which, in turn, is proportional to body weight raised to the 0.7 power (3). When this normalization technique is used to compare oxygen consumption in ob/ob and control animals, ob/ob animals appear to have markedly decreased energy expenditure (18). However, these calculations assume a constant ratio of fat mass to lean body mass. This is clearly not the case for ob/ob animals, which have massively increased fat stores. When calculations are made that assume identical lean body mass in age-matched ob/ob and control mice, leptin-deficient animals actually have similar or even increased energy expenditure (14). The goal of the present study was to determine whether leptin deficiency alters oxygen consumption. Four different methodologies were employed: NMR spectroscopy was used in an attempt to accurately and noninvasively measure lean body mass and allow proper indexing of oxygen consumption to nonfat mass; dietary manipulation was utilized to achieve equivalent body weights in age-matched ob/ob and normal mice in an attempt to eliminate differences in body composition; the short-term effects of leptin administration on metabolic rate were examined; and weight loss was compared in leptin-repleted and pair-fed ob/ob animals.

METHODS

NMR determination of body composition. All NMR experiments were carried out on a GE Omega NMR spectrometer equipped with a 4.7-T 40-cm Oxford magnet containing actively shielded gradients. The mice were anesthetized with ketamine-xylazine and placed inside a home-built cylindrical radio frequency probe consisting of a single turn copper foil that was tuned to 200 MHz and had nonmagnetic variable capacitors. The dimensions of the radio frequency probe were such that the entire mouse body could be contained inside, even for the most obese mouse in this study. The homogeneity of the radio frequency probe was checked by imaging a large tube of water almost filling the entire probe and was found to be excellent. After the probe containing the mouse was placed inside the magnet, the magnetic field homogeneity was optimized with the automatic shimming procedure available on the spectrometer. The final water line width from the entire mouse body was typically <150 Hz. After the field...
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Homogeneity was adjusted, a free induction decay consisting of eight averages was collected with a nominal flip angle of <5 degrees and a recycle delay of 2 s. The free induction decays were analyzed off-line on a Sun workstation with standard Fourier transform techniques to obtain intensity information. In all cases, the NMR signal from water was well separated from that of the lipids (fat). Fat and nonfat body mass were calculated by multiplying corrected body weight by the fractional volume represented by the water and lipid peak. Corrected body weight excludes gastrointestinal tract contents (estimated at 1 g; Ref. 2) and bone and other tissue not detected by NMR (estimated at 5 and 10% for ob/ob and control animals, respectively; Ref. 2).

Measurement of oxygen consumption by indirect calorimetry. Metabolic rate was measured with indirect calorimetry with a four-chamber Oxymax system (Columbus Instruments, Columbus, OH). The instrument was housed in a laboratory in a thermonuclear environment (22–24°C). The Oxymax system is an indirect, open-circuit calorimeter. It monitors oxygen and carbon dioxide gas concentrations at the inlet and outlet of sealed chambers, through which a known flow of ambient air is forcibly ventilated. The product of gas concentration difference and airflow yields oxygen consumption (V˙O2). Data are represented as milliliter per hour and milliliter per kilogram nonfat mass. V˙O2 was measured every 4 min for 2 h. Data from the initial 30 min were excluded to allow animals time to settle after handling stress. Metabolic rate measurements were averaged over the subsequent 90 min. Points greater than two standard deviations from the mean were excluded from the analysis. Core body temperature was measured in all animals before each determination of V˙O2 with a rectal probe.

Administration of exogenous leptin. Fourteen-day, 100-µl capacity, osmotic minipumps (Alza, Palo Alto, CA) were filled with mouse recombinant leptin (R & D) and were used for continuous administration of leptin. Pumps were filled with leptin (0.3 mg·kg−1·day−1 × 14 days) and implanted subcutaneously in the interscapular region with a brief halothane anesthetic. A single skin clip was used to close the skin. Animals were inspected daily to confirm satisfactory wound healing and general health status. This method for leptin administration has been used by us and others previously.

Animal protocols. All procedures and protocols were approved by the Institutional Animal Care and Use Committee and are accepted by the Association for Assessment and Accreditation of Laboratory Animal Care International. In most protocols, five animals were used in each group. Animals were housed at 22–24°C, five to a cage, with free access to water.

In experiment 1, 6-wk-old C57BL/6j control and ob/ob mice were followed over a 10-wk period. Animals had ad libitum access to rodent chow. Weight and food intake were determined daily, and V˙O2 was measured weekly. NMR spectra were used to quantify fat and nonfat mass; these were obtained at four time intervals during the 10-wk protocol.

In experiment 2, dietary manipulation was used to obtain age-matched ob/ob and control animals of the same body weight. C57BL/6j animals were fed a diet of specially formulated rodent chow (48% fat; 19% protein; 19% carbohydrate; 14% fiber-ash-water), whereas ob/ob mice were diet restricted by pair feeding to normal control mice. Animals were weighed daily. When body weights were similar in both groups, total body V˙O2 was measured and NMR spectra were obtained to determine fat and nonfat mass.

In experiment 3, ob/ob and control animals (n = 8/group) received exogenous leptin via osmotic minipumps at 0.3 mg·kg−1·day−1 and had V˙O2 measured on days 0, 2, 7, and 10. Animals were allowed free access to rodent chow during the study period. Food intake and body weight were measured daily. At the completion of the study, animals were euthanized and blood was obtained for measurement of plasma leptin concentration. Leptin concentrations were measured by radioimmunoassay at the time the animals were euthanized (Linco Research, St. Charles, MO). The sensitivity of the assay is 0.5 ng/ml with an interassay coefficient of variation of ~5%. All leptin assays for this experiment were performed in a single batch.

In experiment 4, weight loss and changes in body composition and metabolic rate were compared in two groups of ob/ob animals. Group 1 animals received exogenous leptin (0.3 mg·kg−1·day−1 via osmotic minipump); group 2 were pair-fed to group 1 animals and received saline via minipump. Animals were monitored for 4 wk. Food intake was measured daily. NMR spectra were measured immediately before implantation of the first 14-day minipump, on day 14 after removal of the first pump and before insertion of the second pump, and on day 28 after pump removal. V˙O2 was measured twice weekly.

Data analysis. Independent t-tests were used to compare body weight, fat and lean body mass, V˙O2, food intake, and core temperature between groups at the beginning and end of the protocols. Changes in measured parameters over time and with specific interventions were evaluated with one-way ANOVA techniques; Neuman-Keuls tests were used for pairwise comparisons. Comparisons between groups were analyzed by two-way ANOVA. P values < 0.05 were considered significant. All data are expressed as means ± SE.

RESULTS

Experiment 1: comparison of V˙O2 in ob/ob and control animals. ob/ob animals weighed 50% more than control animals at the start of the protocol and throughout the study (Fig. 1A). Body composition data are shown in Fig. 1, B and C. Fat mass accounted for the difference in body weight between ob/ob and control animals (52 vs. 15%); nonfat mass was actually lower in ob/ob mice than in control animals (15.8 ± 0.5 vs. 19.9 ± 0.7 g, P < 0.05, n = 5). Body weight increased in ad libitum-fed ob/ob and control animals over the 10-wk study period by 58 and 31%, respectively. Percent fat mass remained at 15% in control animals but increased from 52 ± 2 to 59 ± 1% in ob/ob animals. V˙O2 data for ad libitum-fed ob/ob and control animals are shown in Fig 2. When no attempt was made to correct for differences in body weight or composition, V˙O2 data were similar in ob/ob and control animals (Fig. 2A). When V˙O2 was referenced to body weight to the 0.7 power (Fig. 2B), ob/ob animals had lower metabolic rates. However, when V˙O2 was expressed as a function of measured nonfat mass (Fig. 2C), ob/ob animals consumed considerably more oxygen than controls. Core body temperature was ~1°C degree higher in control animals at all times.

Experiment 2: dietary manipulation to achieve identical body weight in ob/ob and control mice. Effects of dietary manipulation on body weight and composition are shown in Fig. 3. Control animals receiving the high-fat diet demonstrated accelerated weight gain compared with normal chow-fed animals in experiment 1. Fat mass increased from 13 to 30%, whereas nonfat
mass increased only minimally (from 25 ± 1.0 to 25.8 ± 1.8 g). Diet-restricted ob/ob animals lost weight and at 7 wk weighed the same as control animals on the high-fat diet. Weight loss in diet-restricted ob/ob animals came from both fat and nonfat body mass. There was little change in body composition; fat mass and nonfat mass decreased 11 and 18%, respectively. Final fat mass was 55% of total body weight in ob/ob animals. At equivalent body weights, VO₂ was higher in diet-restricted ob/ob animals if referenced to nonfat mass and was equivalent if expressed on a per animal basis.

Fig. 1. A: change in body weight over 10-wk study period in ob/ob and C57BL/6J (control) mice. B and C: fat and nonfat mass determined by NMR spectroscopy in ob/ob and control mice at beginning and end of study period. Data are means ± SE of 5 animals/group. *P < 0.05 vs. control animals.

Fig. 2. Oxygen consumption (VO₂) data in ob/ob and control animals at beginning and end of 10-wk study period. VO₂ data are on a per animal basis (A) relative to body surface area (kg body wt⁰.⁷) estimates of lean body mass (lbm, B) and relative to measured nonfat mass (C). Data are means ± SE of 5 animals/group. *P < 0.05 vs. control animals.

Experiment 3: effect of short-term leptin administration on VO₂ in control and ob/ob animals. Exogenous leptin administration resulted in equivalent plasma leptin concentrations (8.3 ± 1.8 and 6.2 ± 0.7 ng/ml) in C57BL/6J and ob/ob mice, respectively. Effects of leptin on VO₂ are shown in Fig. 4. Control animals had no change in food intake or body weight during the 10 days of leptin administration. A transient (and not statistically significant) 31% increase in VO₂ was observed on day 3; however, VO₂ on day 10 was identical to baseline levels. In contrast, ob/ob animals demonstrated an 80% reduction in food intake, a 24% decrease in body weight, and a 50% increase in VO₂. Body temperature was lower in ob/ob animals at baseline and did not change in either group with leptin administration.
Experiment 4: comparison of weight loss, body composition, and metabolism in leptin-repleted and pair-fed ob/ob mice. Food intake in the two groups was similar, although somewhat higher in leptin-repleted animals. The difference was due to increased food intake in leptin-treated animals in week 4. Leptin-repleted animals lost weight more rapidly than pair-fed controls (45 vs. 17% over the 28 days; Fig. 5). NMR data (Fig. 6) demonstrated large differences in loss of fat mass in the two groups (74 vs. 18%). As in experiment 3, \( \dot{V}O_2 \) increased in leptin-treated mice; no change in \( \dot{V}O_2 \) was observed in pair-fed controls. Body temperature increased on day 8 of leptin repletion and remained elevated thereafter.

**DISCUSSION**

The present study was undertaken to evaluate whether leptin deficiency alters metabolic activity. The sustained increase in metabolic activity seen with leptin repletion and the accelerated weight loss seen in leptin-treated animals, compared with that seen in pair-fed ob/ob mice, strongly suggest that the answer to the question is yes. A second important finding of the study is that variations in body weight and composition have important effects on metabolic activity that preclude comparing metabolic activity in animals having differences in body composition. NMR spectroscopy demonstrated lower lean body mass in ob/ob mice, and dietary restriction caused loss of nonfat mass in these animals. In addition, leptin repletion accelerated fat loss. These data suggest that leptin-deficient mice are unable to utilize fat efficiently as a fuel source.

Several distinct lines of evidence suggested to prior investigators that ob/ob mice have a lower metabolic rate than control animals. First, excess weight gain in juvenile ob/ob mice occurs without greater food intake (6). Second, preweaned ob/ob mice have lower measured oxygen consumption than wild-type littermates (16). Finally, leptin-deficient animals are hypothermic (18), and there is a well-established relationship between body temperature and metabolic activity. Thurby
and Trayhurn (22) demonstrated that environmental
temperature plays a major role in determining the
excess weight gain of ob/ob mice receiving the same
amount of food as lean controls and that the low energy
expenditure is due primarily to reduced thermoregula-
tory thermogenesis. However, direct measurements of
oxygen consumption, rather than confirming a reduced
metabolic rate in ob/ob animals, have generated consid-
erable controversy (14). At the core of this debate lies
the attempt of some investigators to normalize oxygen
consumption to calculated estimates of lean body mass.
In normal animals, lean body mass is proportional to
body surface area, which, in turn, is proportional to
body weight raised to the 0.7 power (3). When this
approximation for lean body mass is applied to ob/ob
mice, it generates a calculated oxygen consumption
that is less than that found in control animals. How-
ever, fat mass is dramatically increased in ob/ob
animals, and thus lean body mass is no longer propor-
tional to body surface area or weight raised to the 0.7
power.

In the present study, we used proton NMR spectro-
copy to serially and noninvasively measure fat and
nonfat mass. This methodology generates discrete peaks
for fat and water. Absolute fat mass was estimated by
multiplying corrected body weight by the volume ratio
of the fat mass peak. Corrected body weight was
obtained by subtracting the estimated weight of both
intraluminal food and bone, teeth, and other nonfat,
nonwater tissues not included in the NMR spectra.
Estimated values for these were obtained from composi-
tional analysis studies performed four decades ago (2).
This study observed similar intraintestinal and re-
sidual weights in congenitally obese and control ani-
mals. Although the nonfat, nonprotein, nonwater resi-
dues in compositional analysis studies may not exactly
duplicate the tissue mass not detected in our NMR
spectra, any error should be quite small. It should be
noted that the water peak (nonfat mass) includes lean
body mass, blood, and other nonstructural water-based
material. In the absence of water deprivation, however,
changes in nonfat mass reflect alterations in lean body
mass. Thus we believe that NMR spectroscopy provides
valuable information about body composition in living
animals.

With NMR spectroscopy, we found that fat mass
accounted for >50% of body weight in ob/ob animals, a
figure that is in agreement with compositional analysis
studies performed in the 1950s (2). Of interest, ob/ob
animals had less nonfat mass than control animals. As
a result, when we referenced oxygen consumption to
nonfat mass, ob/ob mice had higher levels of oxygen
consumption than control animals. These data would
argue against a reduced metabolic rate in ob/ob animals; however, it is important to differentiate between energy used for basal metabolism and energy expended on movement and related activities. The indirect calorimetric measurements of oxygen consumption employed in the current study were performed on awake, unrestrained animals. Thus these data include both energy expended on basal metabolism and energy used in movement and other nonbasal activities. One would anticipate that the considerable extra mass of the ob/ob animals increases work related to movement. In support of this explanation, Kaplan (15) found lower oxygen consumption per kilogram of lean body mass when oxygen consumption measurements were performed in a water bath, which reduced or eliminated movement and related activities. The indirect calorimetry also results in a dramatic increase in β3-adrenergic receptor expression in ob/ob mice (4), as well as expression of uncoupling protein in brown adipose tissue (20).

The fourth set of experiments evaluated whether weight loss in leptin-repleted ob/ob animals was due to reduced food intake only. Leptin repletion resulted in dramatic reductions in food intake, to levels considerably below those seen in C57BL/6J mice. However, ob/ob mice fed identical amounts of food as leptin-treated mice lost less weight. Our pair-feeding program actually resulted in leptin-repleted animals eating more than the control group because of a significant increase in food intake in the leptin group in the final week of the study. We speculate that obesity may contribute to appetite suppression in ob/ob animals and that with marked weight loss, food intake rises. The accelerated weight loss seen in leptin-repleted animals suggests that leptin increased metabolic activity. Two alternate explanations are that leptin interferes with food absorption or causes external loss of metabolic substrates via urine or feces. These latter hypotheses seem unlikely. In our study, leptin administration resulted in no significant change in body weight or food intake, with only a transient but not statistically significant change in oxygen consumption. Other investigators have demonstrated a metabolic effect of leptin in lean mice (11) (−300 µg·mouse−1·day−1 by twice daily intraperitoneal injection) and rats (20). In a comprehensive study, Harris et al. (12) demonstrated the dose dependence of the leptin effect in ob/ob and lean mice. They clearly demonstrated the markedly enhanced sensitivity of the ob/ob mice to the metabolic effects of leptin (2 µg·mouse−1·day−1 was sufficient to decrease food intake and reduce body weight). In control mice, the only significant response to leptin was a transient but not sustained reduction in food intake and weight loss with doses of 10 or 42 µg/day. Our mice received 6–9 µg/day; thus it is not unexpected that we did not observe significant changes in food intake or metabolism observed by others. Thus the effect of leptin on lean rodents appears to be dependent on the dose and method of administration.

Two additional findings of the current study were that ob/ob animals have less lean body mass than age-matched control animals and are unable to maintain lean body mass with dietary restriction. Similar observations have been made by other investigators (2). Massive adiposity in ob/ob animals reflects the obligatory storage of unused calories. Reduced levels of lean body mass in ob/ob animals suggest that fat synthesis may occur at the expense of protein synthesis. Thus, despite massive obesity, ob/ob animals may actually suffer from malnutrition. Along similar lines, severe dietary restriction in ob/ob animals results in decreases in lean body mass, raising the possibility that these animals have an impaired ability to utilize fat.
stores for energy production. In support of this hypothesis, Shimabukuro et al. (21) recently demonstrated that leptin acts peripherally to inhibit triglyceride synthesis and stimulate lipolysis. Leptin also induces the expression of uncoupling protein 2 and the enzymes of fatty acid oxidation (23). Another somewhat unexpected finding was that acute leptin administration did not restore core body temperature to normal until 8–10 days. The recognized effects of leptin on SNS activity and expression of β3-adrenoeceptors would be predicted to increase thermogenesis. We speculate that the reduced body temperature seen in ob/ob animals may occur secondary to an impaired ability to utilize fuel substrates and that the increase in temperature seen with long-term leptin repletion may require correction of the fairly severe malnutrition seen in these animals.

In conclusion, leptin repletion in ob/ob mice both increased oxygen consumption and caused weight loss independent of its effect on food intake. We believe that these actions of leptin provide evidence that leptin-deficient mice are hypometabolic. The use of magnetic resonance imaging to define body composition enabled us to differentiate fat and nonfat mass rapidly and noninvasively. The technique helped establish the contribution of body weight and composition to metabolic activity, demonstrating the valuable role of NMR in nutritional research. Finally, an important finding of the present study was that ob/ob mice have reduced lean body mass and lose lean body mass preferentially with dietary restriction. These findings suggest that leptin may be required for efficient utilization of fat as an energy source.

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