The *ob* Gene and Insulin

A Relationship Leading to Clues to the Understanding of Obesity

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Obesity and non-insulin-dependent diabetes are estimated to affect millions of people in the world. This pathology is multifactorial, comprising complex interactions of genetic and environmental factors and lacking a specific therapy. Great interest arose from the recent discovery of the *ob* gene expressed only in adipose tissue and coding for a protein that appears to regulate adiposity, potentially by acting as a satiety factor. We report here that in normal rats, *ob* mRNA is respectively up- or downregulated by a rise in insulinemia (induced by 2-day insulin infusion while maintaining euglycemia) or a decrease in insulinemia (induced by a 3-day fast). Our results also show that in genetically obese *fa/fa* rats studied longitudinally, white adipose tissue *ob* mRNA levels increase in parallel with early occurring and steadily increasing hyperinsulinemia. This results in adult obese animals having markedly higher *ob* mRNA levels than age-matched normoinsulinemic lean rats. Furthermore, in adult obese rats, *ob* mRNA escapes downregulation as normalization of hyperinsulinemia due to fasting fails to reduce the high *ob* mRNA levels. *Diabetes* 44:1467–1470, 1995

Genetically obese rodents, such as the *ob/ob* mice, *db/db* mice, and *fa/fa* rats, the pathology of which is due to the presence of a double recessive gene, share several abnormalities. The most prominent of these abnormalities are hyperinsulinemia, hyperlipidemia, insulin resistance, glucose intolerance, and non-insulin-dependent diabetes (1). Their pathogenesis is largely unknown, illustrating the complexity of analogous syndromes in other species with polygenic alterations, including humans. Mutation of the *ob* gene, recently cloned from genetically obese *ob/ob* mice (2), prevents the *ob* gene product from exerting its putative satiety effect within the hypothalamus, resulting in hyperphagia, increased fat storage, and obesity (2,3). Based on parabiosis experiments (4,5), another obese but more diabetic animal model, the *db/db* mouse, has been suspected to have a functional *ob* gene product that was unable to act at its receptor because such receptor, presumably located in the hypothalamic area, appeared to be defective (3). The discovery that the defective *db* gene was homologous to the *fa* gene of the genetically obese *fa/fa* rat (6) raised the possibility that *fa/fa* rats may also have a defective hypothalamic *ob* receptor, whereas the *ob* gene is normal (7). The aim of the present work was to investigate the regulation of white adipose tissue *ob* mRNA first in lean, then in genetically obese *fa/fa* rats in relation to plasma insulin concentrations. Because the *ob* gene is highly conserved among various species (2,7), we studied *ob* mRNA levels using a mouse *ob* cDNA.

RESEARCH DESIGN AND METHODS

Preweaning 19-day-old, weaned 28-day-old, and adult lean *FA/fa* and obese *fa/fa* rats of the Zucker strain were used throughout the study. They had free access to water and standard laboratory diet (Provimi Lacta SA, Cossonay, Switzerland). To study the effect of hyperinsulinemia on lean animals, freely moving adult lean rats were infused with 2 U/day human insulin (Novo Nordisk) for 2 days while euglycemia was maintained with a superimposed glucose infusion (200 g/l), as previously described (8). Control rats were infused with saline. For fasting experiments, adult lean and obese *fa/fa* rats were prevented from eating for 3 days. Blood samples were taken from the tail vein during the experimental periods to measure plasma glucose and insulin levels (8). Total RNA was extracted from inguinal white adipose tissue collected at the end of the experiments (9). Aliquots of 10 μg were size-fractionated on 1.5% agarose gels (10), and Northern blots were hybridized (Quikhyb, Stratagene) to random primed labeled cDNAs for *ob* (provided by Dr. J.M. Friedman) and β-actin (Clontech Laboratories). The *ob* and β-actin transcripts were estimated to be ~4.3 kb and 2.4 kb in size, respectively (RNA molecular weight markers, Boehringer Mannheim, Germany). Autoradiographs (X-Omat-AR, Kodak) were quantified by densitometry with Image Quant software. Abundance of *ob* mRNA relative to that of β-actin was expressed as a percentage of corresponding controls. Comparisons between experimental groups were made by Student's *t* test for unpaired data.

RESULTS

As shown in Fig. 1, imposition of hyperinsulinemia on lean animals for 2 days while maintaining euglycemia, i.e., insulinization (8), produced an 88% increase in white adipose tissue *ob* mRNA levels relative to control values. It was further observed in separate experiments that decreasing basal insulinemia of lean rats by means of a 3-day fast markedly reduced the levels of *ob* mRNA compared with those in fed controls (Fig. 1). Genetically obese *fa/fa* rats were then compared with lean animals before weaning (19 days old) and after weaning (28 days old), as well as in 12-week-old adult rats. As depicted in Fig. 2, autoradiographs did not reveal any difference between lean and preobese pups before weaning with respect to *ob* mRNA levels in white

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NPY, neuropeptide Y.

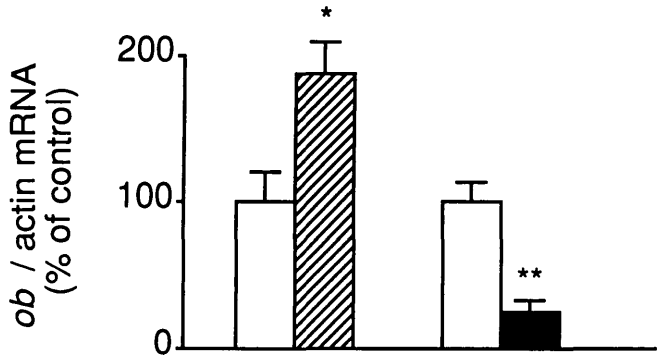

FIG. 1. Effect of 2 days of insulinization and 3 days of fasting on *ob* mRNA levels in white adipose tissue of normal rats. Insulinemia was 351 ± 26 pmol/l for control rats and 904 ± 131 pmol/l for insulinized rats (n = 7 or 8, $P < 0.005$). Glycemia was 7.0 ± 0.3 mmol/l for control rats and 6.3 ± 0.3 mmol/l for insulinized rats (NS). Insulinemia of control fed rats was 146 ± 11 vs. 96 ± 8 pmol/l for rats fasted for 3 days (n = 4 or 5, $P < 0.01$). Their glycemia was 7.4 ± 0.1 and 5.8 ± 0.2 mmol/l, respectively ($P < 0.001$). Abundance of *ob* mRNA relative to that of β-actin was expressed as a percentage of corresponding controls. Values are means ± SE of 4–8 rats. *$P < 0.05$ and **$P < 0.005$. □, control; ▨, insulinized; ■, fasted.

adipose tissue. In marked contrast, adult obese rats had greatly elevated *ob* mRNA levels relative to age-matched lean animals (Fig. 2). Quantification of the results of this longitudinal study revealed, as illustrated by Fig. 3, that *ob* mRNA levels were increased in the 28-day-old obese group after weaning (75% increase) to reach a 725% increase over lean values in adult obese animals. It should be noted that at 28 days of age, when *ob* mRNA levels had begun to increase, insulinemia was approximately threefold higher in obese *fa/fa* rats compared with in lean rats and a similar threefold increase in insulinemia in insulinized lean rats led to an elevation of adipose tissue *ob* mRNA levels of comparable magnitude (Figs. 1 and 3, legends).

Because fasting had a pronounced effect on the levels of *ob* mRNA in lean rats (Fig. 1), the impact of such a fast on the adult obese *fa/fa* rats was examined. Although the fasting period normalized the marked hyperinsulinemia of obese rats, it completely failed to lower the high adipose tissue *ob* mRNA levels (Fig. 4). Note that values of insulinemia for all experimental groups are provided in the figure legends.

DISCUSSION

These data show for the first time that in lean rats, adipose tissue *ob* mRNA levels are positively regulated by chronic hyperinsulinemia. Furthermore, when basal insulinemia is decreased by fasting in lean animals, *ob* mRNA levels are markedly decreased. This study further shows that a defect in white adipose tissue *ob* mRNA levels may not be a primary alteration of the genetically obese *fa/fa* rat. This is substantiated by the observation that *ob* mRNA levels are similar in lean and preobese pups before weaning, albeit preobese pups already have several metabolic alterations at that time (11). In genetically obese *fa/fa* rats, basal insulinemia and *ob* mRNA levels increase in parallel, suggesting that hyperinsulinemia may be a driving force for upregulating *ob* mRNA toward higher than normal levels from the postweaning period onward. These data are in keeping with studies showing that in the dynamic phase of obesity, adipose tissue metabolic activity is also upregulated by insulin to a higher degree than in lean rats (12). However, as shown by this study, once obesity is fully established in the adult rat, *ob* mRNA levels are no longer downregulated by a short-term normalization of hyperinsulinemia produced by fasting.

The observation of elevated *ob* expression in adult obese rats is concordant with results obtained from parabiotic linkage of obese *fa/fa* with lean rats: parabiosis decreased the fat mass of lean rats, not of obese rats (13). This suggested the production by *fa/fa* rats of a normal satiety factor that would act on lean but not on obese rats because of the putative existence of a hypothalamic receptor defect for such a satiety factor. This view is in keeping with recent data showing that the administration of the *ob* protein produces a marked decrease in food intake and body weight in obese mice with presumably normal hypothalamic *ob* receptors, while such is not the case in *db/db* mice with hypothetically defective *ob* receptors (14–16). Because of the homology between the *db* and *fa* genes (6), a hypothalamic

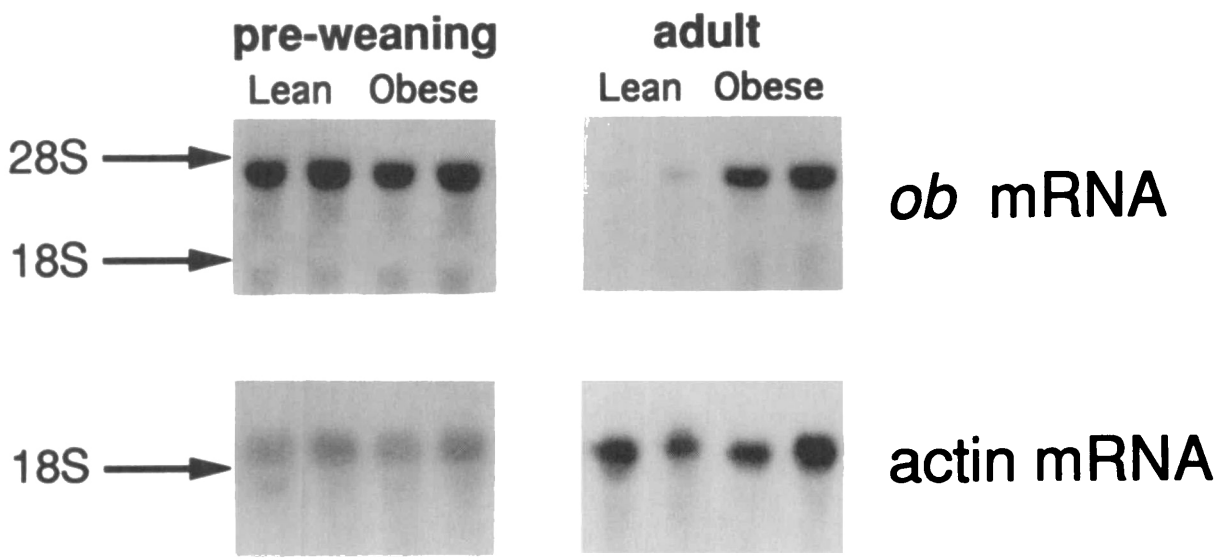

FIG. 2. Autoradiographs of *ob* and β-actin mRNAs in white adipose tissue of 19-day-old preweaning and adult lean and obese *fa/fa* rats of the Zucker strain. Note that the signal intensity on autoradiographs from preweaning pups was stronger than on those from adult rats because of a mandatorily longer exposition time; hence, all results were expressed as percent increase over corresponding control values (see Fig. 3). It should be noted that no signal was detected for *ob* mRNA in brain extracts of lean and obese *fa/fa* rats (data not shown).

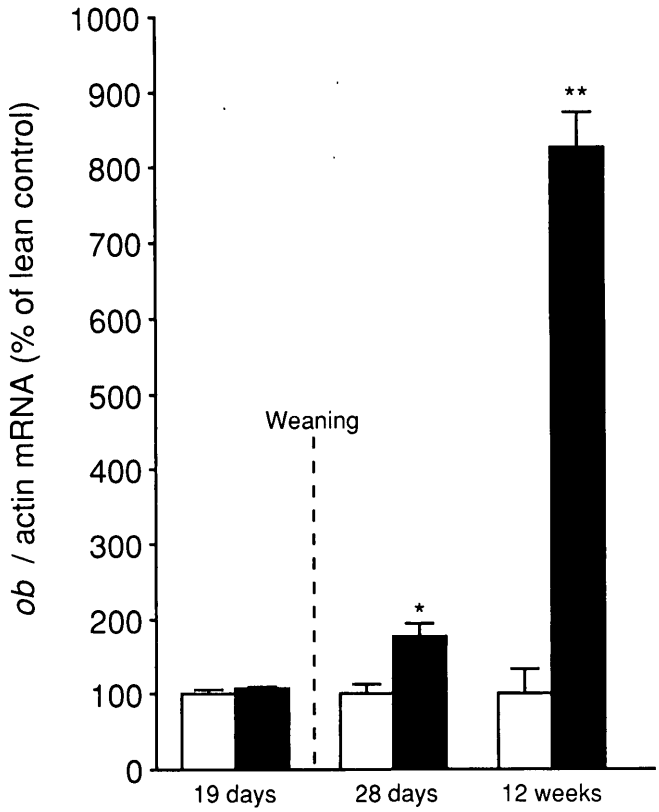

FIG. 3. Levels of *ob* mRNA in white adipose tissue of unweaned 19-day-old, 28-day-old postweaned, and adult lean and obese *fa/fa* rats of the Zucker strain. Insulinemia (from preweaning to adulthood) was, respectively, 87 ± 7, 90 ± 8, and 300 ± 67 pmol/l for lean controls (n = 3–5) compared with 192 ± 16, 307 ± 15, and 3,146 ± 384 pmol/l for obese *fa/fa* rats (n = 3–5). $P < 0.001$ for intergroup differences between lean and obese rats at each age. Abundance of *ob* mRNA relative to that of β-actin was expressed as a percentage of corresponding lean controls (set at 100%). Values are means ± SE of 3–5 rats. *$P < 0.01$ and **$P < 0.0001$. □, lean control; ■, obese.

ob receptor defect is likely to be present in the *fa/fa* rat. Such a defect would prevent the satiety effect from occurring and could result in a downstream increase in those factors that normally stimulate feeding. Among the candidates that could potentially increase, the potent orexigenic agent neuropeptide Y (NPY) may play an important role. Indeed, NPY levels are elevated in the hypothalamus of genetically obese

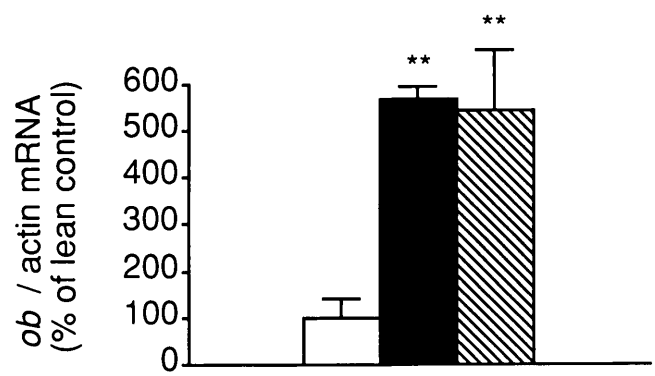

FIG. 4. Effect of 3 days of fasting on *ob* mRNA levels in white adipose tissue of obese *fa/fa* rats compared with that of fed obese *fa/fa* rats and fed lean rats. Insulinemia of obese fed *fa/fa* rats was higher (1,627 ± 330 pmol/l, $P < 0.005$) than that of fasted *fa/fa* rats, which decreased to values not significantly different from that of fed lean controls (292 ± 63 vs. 300 ± 67 pmol/l, NS). Abundance of *ob* mRNA relative to that of β-actin was expressed as a percentage of corresponding lean controls. All values are means ± SE of 4–6 rats. **$P < 0.0005$ relative to lean controls. □, lean control; ■, obese; ▨, obese fasted.

fa/fa rats, in which NPY also escapes normal regulation by either insulin or fasting (17–19). In the present study, it is proposed that in normal rats, the *ob* gene product acting as a satiety signal (possibly by interacting with a neuropeptide such as NPY) is under the regulation of insulin. Furthermore, the expression of *ob* is augmented during the development of the obesity of the *fa/fa* rat because of the progressive occurrence of hyperinsulinemia. Finally, in adult obese rats, for reasons yet to be determined, adipose tissue mRNA levels fail to be decreased when hyperinsulinemia in the animals is normalized by fasting. It is therefore concluded that insulin can be viewed as an up- and downregulator of *ob* expression in lean rats, whereas only the upregulation is present and functional in obese animals. The lack of downregulation of *ob* mRNA levels in the obese group, although of unknown cause so far, could possibly be related to the presence of intracellular factors or signals that would parallel the amount of triglyceride stores, as suggested elsewhere (20).

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