Diet- and diabetes-induced changes of ob gene expression in rat adipose tissue

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Abstract ob gene regulation is as yet unknown. We first examined whether the ob gene is under physiological control by the nutritional state. Fasting produced a sharp (95%) decrease of ob mRNA in epididymal and inguinal fat pads from 24 h onward. Refeeding rapidly (3-6 h) re-induced ob gene expression and corrected it within 24 h. Similar changes in fatty acid synthase (FAS) and GLUT4 mRNAs were observed, whereas phosphoenolpyruvate carboxykinase (PEPCK) mRNA showed an opposite evolution. We next examined the potential role of insulin. In adipose tissue of streptozotocin-diabetic rats, ob mRNA levels were decreased by 80%. Insulin treatment (4 days) only marginally increased ob mRNA, but restored euglycemia and overcorrected FAS, GLUT4 and PEPCK expression. In conclusion, we provide evidence for a physiological regulation of ob gene by variations in the nutritional state. We also show that ob expression is impaired in streptozotocin-diabetic rats and only slightly restored by insulin treatment, which suggests that ob gene is not or only minimally regulated by the hormone.

Key words. ob gene; Obesity; Diabetes; Adipose tissue; Fatty acid synthase; Glucose transporter

1. Introduction

The precision of body-fat regulation (within ± 1% over many years) is thought to require a slow and powerful feedback control of energy balance. The lipostat theory suggests that a factor specifically secreted by fat cells acts on the hypothalamus to exert a long-term control of appetite, thereby regulating the size of body-fat depot [1,2]. An important step towards the identification of this factor has recently been made. Thus, the murine obese (ob) gene has been cloned and its expression found to be restricted to adipose tissue [3,4]. Moreover, it seems that morbidly obese mice, homozygous for the ob mutation, fail to produce the normal protein product – making the ob protein a putative ‘fat-derived satiety factor’ [3]. Hyperinsulinemia is a major characteristic of obesity in general [5], and ob/ob mice in particular [6]. Moreover, continuous infusion of insulin to normal rodents has been shown to mimic some features of the syndrome (i.e. increased adipose tissue metabolic activity and body weight gain) [7].

How expression of the ob gene might be acutely or chronically regulated is totally unknown. The aim of the present work was two-fold. We first investigated whether the ob gene is under physiological control by the nutritional state, and chose the fasting-refeeding model to address this question. We next examined the potential role of insulin by measuring ob expression in insulin-deficient diabetic rats treated with the hormone.

2. Materials and methods

2.1. Animals and research design

Male Wistar/CPB rats were purchased from IFFA Credo (Brussels, Belgium). The animals were housed in individual cages at a constant temperature (22°C) with a fixed 12-h light-dark cycle (lights on 07:00-19:00 h). Unless otherwise stated, rats received ad libitum a standard laboratory chow (A04, UAR, Villemoisson-sur-Orge, France). For experiment I, the rats were starved at 08:00 h for up to 48 h, then re-fed ad libitum for an additional period of up to 48 h. They had free access to tap water during the whole experiment. For experiment II, diabetes was induced by an i.v. injection of streptozotocin (STZ; 50 mg/kg body weight) (Zanosar; Upjohn, Kalalmazor, MI). Control animals received buffer only. Twelve days after STZ injection, the diabetic animals (glycemia > 25 mmol/l in the fed state) were treated with 2 s.c. injections of insulin/day (combination of Actrapid HM and Monotard HM: Novo Nordisk, Copenhagen, Denmark). The treatment started at 08:00 h for up to 96 h. Daily insulin doses were adjusted according to the results of frequent tail vein blood samplings (5 glycemia/day) and averaged 3-40 Unit.

At various times after starvation or refeeding (Exp. I) and after insulin treatment (Exp. II), the animals were killed by decapitation and larger blood samples were saved. Pairs of epididymal and inguinal fat pads were immediately removed, weighed, frozen in liquid nitrogen and stored at −70°C for subsequent RNA extraction.

2.2. RNA extraction and Northern blot analysis

Total RNA was isolated with an acid guanidinium–thiocyanate–phenol–chloroform mixture, as previously described [8]. For Northern blot analysis, 30 µg of RNA was denatured in a solution containing 2.2 mM formaldehyde and 50% formamide (v/v) by heating at 95°C for 2 min. RNA was then size-fractionated by 1% agarose gel electrophoresis, transferred to a Hybond-N membrane (Amersham Int., Amersham, Bucks, UK) and cross-linked by ultraviolet irradiation. The integrity and relative amounts of RNA were assessed by methylene blue staining of the blot.

The ob cDNA probe was prepared as follows. Using the described primers [3], a 100 bp product was obtained after RT-PCR on total RNA from adipose tissue of ob/ob mice [6] kindly provided by Dr. C.J. Bailey. This product was then cloned into plasmid pCR-Script SK+ (Stratagene, La Jolla, CA), controlled by sequencing, and amplified by PCR on plasmid DNA. The other cDNA probes were obtained from Drs. R.W. Hanson for phosphoenolpyruvate carboxykinase (PEPCK) [9], A.G. Goodridge for fatty acid synthase (FAS) [10] and D.E. James for the glucose transporter GLUT4 [11]. Probes were labelled with 32P using the Multiprime labelling system kit (Amersham) Hybridizations with PEPCK, FAS, GLUT4 and β-actin probes were performed as previously described [12] Hybridizations with ob cDNA were similar to those with PEPCK [12]. Filters were then exposed to Kodak X-Omat AR films for 1.30-45 h at −70°C with intensifying screens. Intensity of the mRNA bands on the blots was quantified by scanning densitometry (Ultrorcan XL, LKB, Sweden) and expressed as optical density units.

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2.3 Statistical analysis

Results are presented as means ± S.E.M. for the indicated number of rats. Comparisons between groups were carried out by analysis of variance followed by the Dunnet's test for multiple comparisons. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Experiment I

Plasma glucose levels of fed rats averaged $6.2 ± 0.2$ mmol/l. During fasting, they progressively decreased to reach a minimum of $3.3 ± 0.2$ mmol/l at 24 h ($P < 0.01$). During refeeding, they returned to normal values from 6 h, after a transient phase of hyperglycemia (Fig. 1). Plasma insulin levels also declined from fed values of $6.6 ± 0.7$ ng/ml to a nadir of $0.31 ± 0.01$ ng/ml at 24 h of fasting, and were normalized within 3 h of refeeding. The body weight of the rats ($220 ± 1$ g) decreased by 15% after 48 h of fasting, and progressively returned to normal values after 48 h of refeeding. The weight of epididymal and inguinal fat pads showed a roughly similar evolution, without significant change within 24 h of fasting or refeeding (Fig. 1).

Fasting produced a sharp (~95%) decrease in $ob$ mRNA levels (~4.5 kb transcript) in epididymal fat from 24 h onward (Fig. 2). Refeeding re-induced the expression of $ob$ gene significantly after 6 h and completely after 1 day. The expression of a key lipogenic enzyme, FAS and of the glucose transporter, GLUT4 followed a rather similar evolution, although their re-induction was not so precocious (Fig. 2). Since, in line with previous data [13], β-actin mRNA also tended to decrease with fasting (though less markedly: ~50%) and to re-increase during refeeding (not shown), the specificity of the changes in mRNA levels was assessed by hybridizing the blots with a probe coding for a key glyceroneogenic enzyme. In agreement with reports on enzyme activity [14], mRNA levels of PEPCK increased with fasting and decreased with refeeding. This pattern was thus opposite to that of the changes of $ob$, FAS or GLUT4 mRNAs (Fig. 2).

In inguinal fat pads, the evolution of the different mRNA levels was roughly similar to that observed in epididymal tissue. However, the re-induction of $ob$ gene was slower and not so complete, suggesting that $ob$ expression may be characterized by an adipose region-specificity (Fig. 2). This could also be the case for GLUT4, whose re-increase was more sluggish, and for PEPCK, whose amplitude of changes was greater than in epididymal fat.

3.2. Experiment II

High plasma glucose levels of diabetic rats ($27 ± 1$ mmol/l) were decreased by ~25-45% during the first 12 h of insulin treatment, and were normalized from 24 h of therapy onward (mean value at 24, 48, 72 and 96 h: $6.2 ± 0.8$ mmol/l) (Fig. 3). The low plasma insulin levels of diabetic rats ($0.74 ± 0.16$ ng/ml vs. $4.20 ± 0.70$ ng/ml in control rats) were considerably increased during the first 2 days of treatment (reflecting marked insulin resistance), before reaching normal levels at the end of the study ($5.7 ± 2.2$ ng/ml at 96 h). The decrease in body weight

![Fig. 1. Time course of the changes in plasma glucose and insulin levels, and in body weight and fat pad weight during the fasting/refeeding transition. Rats were starved for up to 48 h (□), then refed for an additional period of up to 48 h (●). Values are means ± S.E.M. for 4-8 rats in each group.](image-url)
produced by 12 days of diabetes was about 18% (233 ± 6 g in diabetic rats vs. 285 ± 7 g in control rats). It was progressively attenuated after 1 day of insulin treatment and was almost completely corrected after 3 days. The weight of epididymal fat pads was markedly (70%) reduced by diabetes, but also returned to near normal values after 3 days of insulin therapy (Fig. 3). The inguinal fat was not studied in this experiment because its marked atrophy prevented its reliable isolation in diabetic animals.

*ob* mRNA levels were decreased by 80% in adipose tissue of diabetic rats (Fig. 4). These levels increased slightly after 1.30 h of insulin treatment, and then plateaued at about 43% of control levels (Fig. 4). By contrast, the pronounced reductions in FAS and GLUT4 mRNAs produced by diabetes were over-corrected by insulin, and reached 2.5- to 4-fold higher values than in controls (Fig. 4). PEPCK mRNA levels, which were elevated by diabetes, were reduced to infra normal values after 24 h of treatment (Fig. 4).

4. Discussion

The recently identified *ob* gene is thought to code for a satiety factor, but nothing is known about its regulation [3]. This study shows that *ob* mRNA levels in adipose tissues decrease when fasting extends beyond 12 h and rapidly (3-6 h) re-increase upon refeeding, to be fully corrected within 24 h. This re-induction is likely to be due to an increased gene transcriptional rate. This is the first evidence for a physiological regulation of
ob gene by variations of the nutritional state. The decrease of ob gene expression in fasted, hungry animals and its increase in sated ones are fully compatible with the hypothesis that the ob gene codes for a satiety factor.

This study also shows that ob gene expression is altered in a common pathological state, diabetes. This might in part contribute to the classical diabetic polyphagia. Thus, abnormal ob gene expression is not limited to anabolic states (i.e. ob/ob mice), but can also be extended to catabolic states. In both cases, production of the ob protein may be defective, but the etiology of the defect is different: constitutive abnormality (ob gene mutation) in ob/ob mice [3] vs. functional impairment of ob gene expression in fasted or STZ-diabetic rats.

Fasting and diabetes are characterized by opposite changes in glycemia, but similarly low insulinemia and ob expression. Refeeding also produces parallel increases in insulinemia and ob mRNA levels. This might suggest that insulin plays a role in ob gene regulation. To test this hypothesis, STZ-diabetic rats were treated with the hormone for 4 days. After a rapid but modest increase, ob mRNA levels plateaued at lower than normal levels. Yet, insulin treatment was efficient as shown by the restoration of euglycemia and the overcorrection of other genes. Thus, mRNA levels of FAS and GLUT4, two genes positively regulated by insulin in vivo [13,15] rapidly and markedly surpassed the control. This overshoot is in agreement with that previously reported for GLUT4 mRNA in fat cells [13]. On the other hand, PEPCK expression, which is under negative control of the hormone, was strongly inhibited by insulin in adipose tissue, as in liver [16]. Taken together, these data suggest that the ob gene is not or only minimally specifically regulated by insulin. This appears teleologically meaningful. Thus, if a rise in insulin levels, after a period of severe or long-lasting catabolism, were rapidly enhancing the expression of a gene coding for a satiety factor, the ensuing anorexia would prevent rebuilding of fat free-mass and energy stores (a ‘suicide behaviour’).

It has been proposed that the ‘fat-derived satiety factor’ (i.e. possibly the ob protein) is secreted in proportion to adipocyte size or number [2], and would thus be produced in proportion to total body-fat mass (lipostat model) [1,2]. However, we only observed a marginal re-induction of ob expression in insulin-treated diabetic rats despite nearly complete normalization of epididymal fat pad weight. In addition, the largest variations in ob mRNA levels during fasting/refeeding occurred when the weight of fat pads hardly varied (this was particularly striking at the 24 h time point) and vice versa. Thus, no close relationship appears to exist between ob expression and fat pad weight during acute metabolic changes. However, our results do not necessarily question the lipostat theory for at least two reasons. First, the weight of adipose tissue at one site may not be exactly representative of total body-fat stores. Second, in spite of lipid accumulation and increase in size, the metabolic activity of adipocytes from diabetic rats injected with insulin may not be.

Fig. 3. Time course of the changes in plasma glucose and insulin levels, and in body weight and epididymal fat pad weight during insulin treatment of diabetic rats. Rats were made diabetic by streptozotocin injection 12 days earlier. Control rats (open columns) had received buffer only. A group of diabetic rats were untreated (○), while the others were treated with insulin (●) for various periods of up to 96 h. Values are means ± S.E.M. for 4-9 rats in each group.
from Northern-bLOTS, like those shown in the inserts (CPEPCK) mRNA in epididymal adipose tissue from diabetic rats during 328 GLUT4 transporter and phosphoenolpyruvate carboxykinase days earlier. Control rats (open columns) had received buffer only. A indicated periods (hours)). The results are expressed as percentages of group of diabetic rats was untreated means ± S.E.M. for 4-9 rats in each group. mRNA levels were quanti­

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References


Fig. 4. Time course of the changes in ob, fatty acid synthase (FAS), GLUT4 transporter and phosphoenolpyruvate carboxykinase (PEPCK) mRNA in epididymal adipose tissue from diabetic rats during insulin treatment. Diabetic rats were injected with streptozotocin 12 days earlier. Control rats (open columns) had received buffer only. A group of diabetic rats was untreated (○), while the others were treated with insulin (●) for various periods of up to 96 h. Values are means ± S.E.M. for 4–9 rats in each group. mRNA levels were quanti­

fied by scanning densitometry of autoradiographic signals obtained from Northern-bLOTS, like those shown in the inserts (C = control; D = untreated diabetic; 3–96 = diabetic rats treated with insulin for the indicated periods (hours)). The results are expressed as percentages of values in control rats.

completely corrected within the first days of treatment. This is supported by the 2-fold increase in intracellular water and protein content [13], and by the marked overshoot of both FAS and GLUT4 mRNAs. Thus, the concept of adipose tissue mass should not be considered on too restrictive histological grounds, but rather be viewed as a functional and metabolic entity.

Normalization or restoration of a currently unidentified intracellular event in adipose tissue might be a prerequisite for ob re-induction. In this respect, the delay of ob re-induction in insulin-treated diabetic rats, as compared with fasted-refed animals, could be due to the longer period of catabolism (12 vs. 2 days) and the larger involution of adipose tissue (by 68 vs. 29%), with subsequent slower restoration of this putative sensor. The nature of this sensor is unknown. Yet, studies on the expression of recently cloned genes such those coding for peroxisome proliferator-activated receptors, which could play a role in control of lipid metabolism and coordinate the expression of adipocyte-specific genes, might be helpful [17,18].

In conclusion, we provide evidence for a physiological regulation of ob gene by variations of the nutritional state. We also show that ob expression is impaired in STZ-diabetic rats, and only slightly restored by insulin treatment, which suggests that the ob gene is not or only minimally regulated by insulin.