Effects of fasting and refeeding on *ob* gene expression in white adipose tissue of lean and obese (*ob/ob*) mice

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Abstract A 33-mer antisense oligonucleotide has been utilized as a probe for the rapid chemiluminescence-based detection of *ob* (obese) mRNA. Expression of the *ob* gene was evident in several white adipose tissue depots of mice (epididymal, highest; subcutaneous and omental, lowest), but not in other organs. Fasting (24 h) induced a substantial fall in *ob* mRNA in the epididymal fat of lean mice, which was rapidly reversed on refeeding, responses consistent with the putative role of *ob* in energy balance. Fasting had no effect, however, on *ob* mRNA levels in obese (*ob/ob*) mice.

Key words: *ob* (obese) gene; *ob* mRNA; *ob/ob* mouse; White adipose tissue; Fasting; Digoxigenin-labelled oligonucleotide

1. Introduction

The genetically obese (*ob/ob*) mouse has been widely used as an animal model in obesity research [1,2]. Despite the characterization of a number of abnormalities in these mutants, following the application of conventional physiological and biochemical approaches, the fundamental defect has remained unresolved. Positional cloning has, however, recently led to the identification and sequencing of the *ob* (obese) gene [3]. The gene appears to be expressed in adipose tissue, and codes for a previously unknown protein, M_r = 18,000 [3]. The protein contains a putative signal sequence, and is considered to be secreted from adipocytes as an M_r = 16,000 product [3].

The target organ and function of *ob* protein are unknown, but it is evident from the profound obesity that occurs in mice with the *ob/ob* mutation that the protein must play a critical role in the regulation of energy balance. One possibility is that it acts as a satiety factor [3,4], although this is not consistent with the observations that the early development of the obesity in *ob/ob* mice takes place without an increase in food intake [2,5–7]. Nevertheless, given the importance of *ob* protein in energy balance, it would be predicted that expression of the *ob* gene should be highly sensitive to nutritional status.

In the present study we have examined the effects of fasting, and subsequent refeeding, on the level of the mRNA encoding *ob* in white adipose tissue of mice. The response to fasting has been examined in both lean and obese (*ob/ob*) mutant animals. An antisense oligonucleotide has been designed as a probe for *ob* mRNA, and used with a chemiluminescence-based detection procedure [8] to give a rapid and simplified measurement of the mRNA on Northern blots.

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2. Materials and methods

2.1. Animals and tissues

Ten-week-old male lean and obese (*ob/ob*) mice of the 'Aston' variety were taken from a colony maintained at the Rowett Research Institute. Tissues were removed following cervical dislocation, frozen in liquid N2, and stored at −80°C. White adipose tissue was taken from the epididymal, omental, perirenal and subcutaneous regions; subcutaneous fat was taken separately from near the hind limbs and adjacent to the forelimbs.

To investigate the effects of fasting and fasting-refeeding, mice housed at 22°C were divided into three groups (5 or 6 mice per group) and housed individually in cages with wire-mesh bottoms. Two groups were fasted for 24 h, one of which was subsequently refed for 6 h. The control group had continuous access to food (Biosure; Special Diet Services, Cambridge) containing 18% protein and 2.4% fat (w/w).

2.2. Northern blotting

Total RNA was extracted from tissues [9] and fractionated by agarose gel electrophoresis. The RNA was then transferred to a charged nylon membrane (Boehringer-Mannheim) by capillary blotting, and fixed with UV light. The mRNA for *ob* was detected by a chemiluminescence-based procedure [8] utilizing a 33-mer antisense oligonucleotide probe end-labelled (5′) with a single digoxigenin ligand (Boehringer-Mannheim). The oligonucleotide (5′-GGTCTGAGGCAGGGAGCAGCTCTTGGAGAAGGC-3′), which was synthesized commercially (R&D Systems Europe), was based on a region of the mRNA downstream from the site of the primary mutation in *ob/ob* mice [3]. An alternative antisense oligonucleotide was also tested, based on the region of the *ob* mRNA containing the putative signal sequence; although this detected *ob* mRNA, a weaker signal with higher background was obtained.

Pre-hybridization was performed at 42°C for 1.5 h in 50% formamide, 5 × SSC, 2% blocking reagent (Boehringer-Mannheim), 50 mM sodium phosphate (pH 7.0), 0.1% *N*-lauroylsarcosine and 7% SDS. Hybridization was at 42°C overnight in pre-hybridization buffer, together with the oligonucleotide (25 ng/ml). Post-hybridization washes were performed as previously [8]. The membranes were incubated with an anti-(digoxigenin)serum/alkaline phosphatase conjugate, and processed essentially as in the protocols provided by Boehringer-Mannheim. Both CSPD and CDP-*Star* (Tropix) were used as chemiluminescence substrates [10–12]. Signals were visualized by exposure of membranes to film, and quantified with a molecular imager using phosphor storage screens sensitive to chemiluminescence (Bio-Rad), or by densitometry.

The statistical significance of differences between groups was assessed by Student's unpaired *t*-test.

3. Results

The antisense oligonucleotide employed as a probe for *ob* mRNA detected a single band (~4.1 kb) on Northern blots of total RNA from epididymal white adipose tissue. The mRNA encoding *ob* was detected, using a chemiluminescence-based procedure, within a few minutes of exposure to film. *ob* mRNA was detected in white adipose tissue (Fig. 1A), but not in any of the other tissues examined (liver, brain, heart, skeletal muscle, kidney, gut, spleen, testis), in agreement with Zhang et al. [3].

A weak signal was very occasionally obtained with brown

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adipose tissue (interscapular); it is likely, however, that this reflects some contamination of the tissue with white adipocytes rather than expression of the *ob* gene by brown fat itself. *ob* mRNA was detected in each of the major white adipose tissue depots, including the subcutaneous fat (Fig. 1B). There was, however, a considerable variation between different depots in the level of the mRNA within each individual animal, the highest levels being present in the epididymal tissue and the lowest in the subcutaneous (particularly tissue from near the forelimbs) and omental fat (Fig. 1B).

The effects of fasting and subsequent re-feeding on the expression of the *ob* gene were examined in the epididymal fat. Fasting lean mice for 24 h led to a substantial fall in the level of *ob* mRNA (Fig. 2A), to 28% of that of control animals given continuous access to food (Fig. 3A). Refeeding of fasted animals for 6 h resulted in a rapid rise in the level of the mRNA,

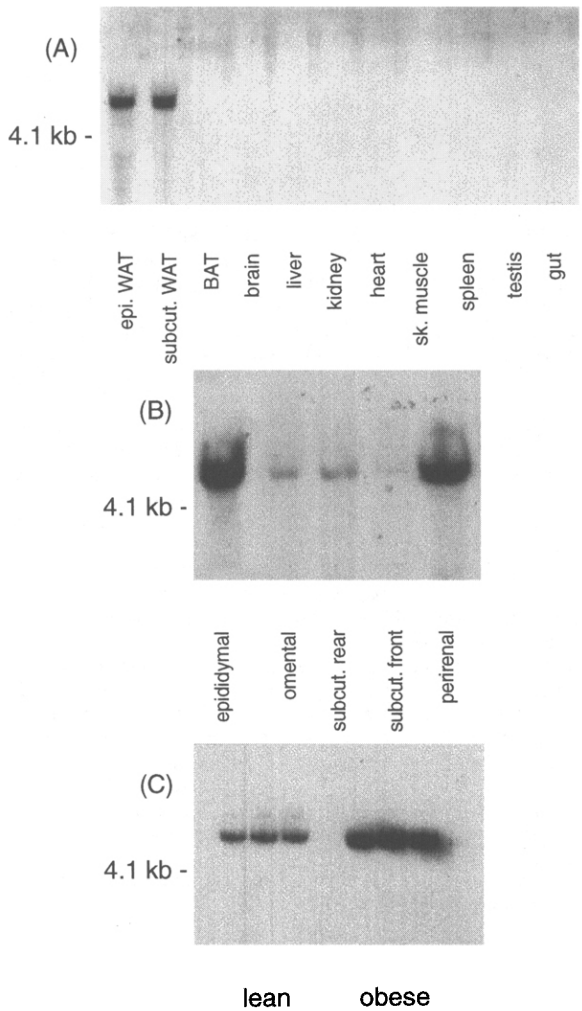

Fig. 1. Representative Northern blots of *ob* mRNA from tissues of mice. In (A), various tissues, both adipose and non-adipose, from lean mice were examined; epididymal and subcutaneous adipose tissue were collected from different animals. In (B), different white adipose tissue depots were examined within the same mouse. In (C), epididymal white adipose tissue was examined in lean and obese (*ob*/*ob*) littermates, aged 10 weeks. 10 μg of total RNA was loaded onto each lane of the gels. A 33-mer antisense digoxigenin-labelled oligonucleotide was used to detect *ob* mRNA using a chemiluminescence-based procedure. Membranes were exposed to film for 60 min.

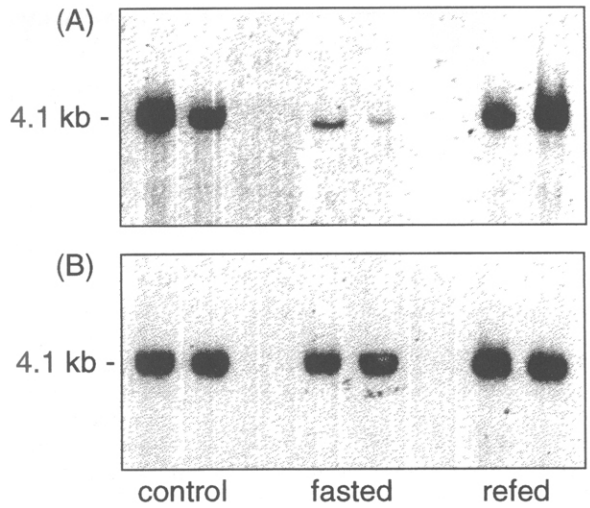

Fig. 2. Representative Northern blots of the effects of fasting and fasting/refeeding on *ob* mRNA levels in epididymal white adipose tissue of lean (A) and obese (B) mice. Mice were fasted for 24 h, or fasted for 24 h and refed for 6 h; control animals received continuous access to food. 10 μg of total RNA was loaded to each lane of the gel. A 33-mer antisense digoxigenin-labelled oligonucleotide was used to detect *ob* mRNA using a chemiluminescence-based procedure. Membranes were exposed to film for 30 min.

to 68% of control values, and there was no significant difference with the control mice (Figs. 2A and 3A).

In marked contrast to lean animals, there was no significant change in the level of *ob* mRNA in the epididymal fat pads of obese (*ob*/*ob*) mice following a 24 h fast (Figs. 2B and 3B). Refeeding fasted animals for 6 h also did not lead to any significant change in *ob* mRNA in the obese mutant. The level of *ob* mRNA was much higher in epididymal white fat of obese animals (*ob* gene on the 'Aston' background) than in lean mice (Fig. 1C).

4. Discussion

ob mRNA was detected using a simplified, chemiluminescence-based procedure for Northern hybridization [8]. This involves employing an antisense oligonucleotide end-labelled with digoxigenin, providing rapid (within a few minutes exposure to film) and sensitive detection. The results demonstrate that the recently identified *ob* gene is expressed in each of the main white adipose tissue depots in mice, both internal and subcutaneous. There are, however, major differences between fat depots in the level of *ob* mRNA. The basis for this is not clear, but it may relate to the size of the fat cells, the level of the mRNA being highest where the adipocytes appear to be largest. Such a view would be consistent with the concept of fat cells signalling the state of energy stores at an individual level, this being integrated into an overall signal for the total body fat of the animal as a whole.

The present study also demonstrates that the level of *ob* mRNA in lean mice is markedly influenced by nutritional status, declining on fasting and rising on refeeding. The responses to fasting and refeeding are consistent with the putative major role of *ob* protein in the regulation of energy balance, and indicate that the *ob* system responds rapidly to changes in

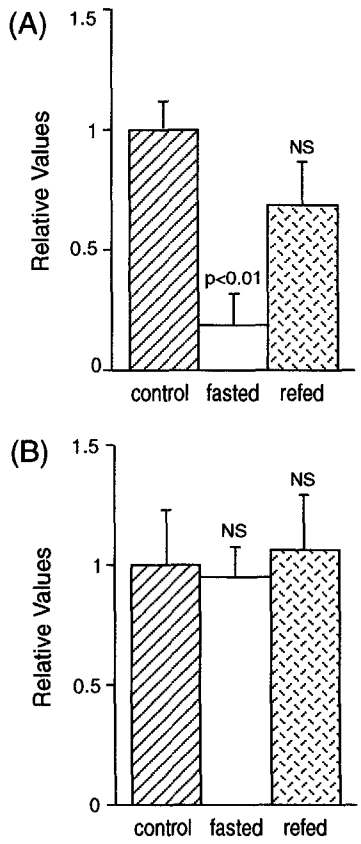

Fig. 3. Quantification of *ob* mRNA levels in epididymal white adipose tissue of lean (A) and obese (B) mice following fasting and fasting/refeeding. Mice were fasted for 24 h, or fasted for 24 h and refed for 6 h; control animals received continuous access to food. Results are mean values+S.E.M. (bars) for 5–6 mice in each group. NS, not significant ($P > 0.05$).

substrate flux, i.e. it is part of a relatively short-term regulatory system rather than being restricted to the monitoring of adipose tissue stores only over the long-term.

The absence of any change in the level of *ob* mRNA in the obese animal in response to fasting is intriguing. It would seem that in the *ob/ob* mutant the signals to white adipose tissue regulating the expression of the *ob* gene may not be directly modified by the removal of food. This raises the possibility that the *ob* protein is not a satiety factor, or immediately linked to satiety as such. The *ob* protein could therefore be involved in the regulation of a component of energy balance other than food intake, relating perhaps to an index of energy expenditure such as thermogenesis or a specific metabolite.

Alternatively, fasting for 24 h may not lead to a sufficient change in the level of the regulatory signals to adipose tissue in *ob/ob* mice. These mutants are normally hyperinsulinaemic and hyperglycaemic [1,2], and consideration of circulating hormone and substrate concentrations in fasting obese animals may provide insight into the nature of the feedback signals regulating the expression of the *ob* gene.

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