Expression of the ob (obese) gene during lactation in mice

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The mutant gene responsible for the development of obesity in the ob/ob mouse has recently been localised and sequenced [1]. The identification of the ob (obese) gene provides an opportunity to make rapid progress in unravelling the critical factors involved in the regulation of body weight. The ob gene appears to be expressed exclusively in adipose tissue [1-3], where it codes for an 18,000 M<sub>r</sub> protein which is secreted from the adipocyte as a 16,000 M<sub>r</sub> product [1], termed leptin [4]. Leptin may act as a satiety factor [1,4-6], signalling the size of the white adipose tissue depots, but there is also evidence that it stimulates energy expenditure [4,5]. Increased expression of the ob gene has been observed following the administration of glucocorticoids [7], while fasting leads to a marked fall in gene expression which is reversed on subsequent re-feeding [3]. Cold exposure induces a substantial increase in energy expenditure and substrate flux in an animal, and we have recently found that expression of the ob gene is rapidly suppressed in the cold, a response which appears to be mediated by the sympathetic nervous system [8]. In the present study we have examined the effects of lactation, a physiological state which also induces major changes in nutrient flux and food intake in small animals [9], on the expression of the ob gene.

Female mice of the 'Aston' variety were mated at 6-8 weeks of age. The main studies focused on mid-lactation (10-12 days post-partum), but analyses were also performed at early and late lactation (1-2 days and 18-20 days post-partum, respectively). The effects of abrupt weaning were examined in mid-lactation, tissue being taken 24 h after removal of the pups. Virgin mice, age-matched to the lactating animals, were used as controls. The mice were killed by cervical dislocation and gonadal fat (and other tissues in pilot studies) rapidly removed and frozen in liquid N<sub>2</sub>

Total RNA was extracted and fractionated by agarose gel electrophoresis [3,8]. After vacuum blotting onto a charged nylon membrane (Boehringer Mannheim), the RNA was fixed with UV light. The blots were probed with a 33-mer antisense oligonucleotide (5'-GGTCTGAGGCAGGGAGCAGCTCTTGG-3', where it codes for an 18,000 M<sub>r</sub> protein which is secreted from the adipocyte as a 16,000 M<sub>r</sub> product [1], termed leptin [4]. Leptin may act as a satiety factor [1,4-6], signalling the size of the white adipose tissue depots, but there is also evidence that it stimulates energy expenditure [4,5]. Increased expression of the ob gene has been observed following the administration of glucocorticoids [7], while fasting leads to a marked fall in gene expression which is reversed on subsequent re-feeding [3]. Cold exposure induces a substantial increase in energy expenditure and substrate flux in an animal, and we have recently found that expression of the ob gene is rapidly suppressed in the cold, a response which appears to be mediated by the sympathetic nervous system [8]. In the present study we have examined the effects of lactation, a physiological state which also induces major changes in nutrient flux and food intake in small animals [9], on the expression of the ob gene.

Fig. 1. Representative Northern blot of ob mRNA in white adipose tissue of lactating (mid) and control (virgin) mice. Total RNA (10 µg/lane) from mouse gonadal white adipose tissue was probed with a 33-mer antisense oligonucleotide end-labelled with DIG. Detection was with the chemiluminescence substrate, CDP-Star, with 30 min exposure to light. The blots were probed with a 33-mer antisense oligonucleotide targeted to the mRNA encoding mouse ob mRNA [3,8]. The oligonucleotide was synthesised commercially, and labelled with DIG at the 5' end (R & D Systems Europe).

The present study indicates that ob mRNA levels do not greatly change during lactation in mice. Thus the substantial increases in food intake that occur in lactating mice are not associated with major alterations in the expression of the ob gene. There was little change in the amount of gonadal white adipose tissue in lactating mice in this study, and this may explain the absence of a substantive effect of lactation on the ob system, i.e. the adipocytes may be expressing ob at a level appropriate to their cell size, there being no specific regulatory signals linked to the hormonal alterations of the lactating state.

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Abbreviations used: DIG, digoxigenin; CDP-Star, disodium 2-chloro-5-(4-methoxyphenyl)-1,2-dioxetane-3,2'-[5′-chlooro]tricyclo [3.3.1.1<sup>3.8</sup>]decane-4-yl-1-phenyl phosphate