Modulation of *obese* gene expression in rat brown and white adipose tissues

Madelaine Moinat[a], Chengjun Deng[a], Patrick Muzzin[a], Françoise Assimacopoulos-Jeannet[a], Josiane Seydoux[b], Abdul G. Dulloo[b], Jean-Paul Giacobino[a,*]

[a]*Département de Biochimie Médicale, Centre Médical Universitaire, 1 rue Michel-Servet, 1211 Genève 4, Switzerland*
[b]*Département de Physiologie, Centre Médical Universitaire, 1 rue Michel-Servet, 1211 Genève 4, Switzerland*

Received 29 July 1995

Abstract The *ob* gene mRNA expression in rat brown adipose tissue (BAT) and epididymal white adipose tissue (WAT) was measured on Northern blots hybridized with a rat *ob* gene probe. The level of *ob* gene mRNA in BAT was about 40% of that in WAT. Fasting (36 h) or semi-starvation (10 days) decreased the *ob* gene mRNA level in both tissues by 62–68%, and cold exposure at 6°C (24 h) decreased it in BAT (−84%) but not in WAT. Acute administration of the β_3-adrenergic agonist Ro 16-8714 decreased the *ob* gene mRNA level in BAT (−51%) and WAT (−28%) of lean Zucker rats and only in BAT (−74%) of obese *falfa* rats. This study demonstrates that, in the rat, the *ob* gene is not only expressed in WAT but also in BAT, and suggests that in these two tissues, the modulation of the *ob* gene expression might be more closely associated with known alterations in cell lipid content than with changes in sympathetic activity.

Key words: *ob* Gene; Fast; Cold; Rat brown and white adipose tissue

1. Introduction

In 1994, Zhang et al. [1] cloned a gene referred to as *obese* (*ob*) gene, whose mutation is believed to be responsible for the phenotype of the hereditary obese (*ob/ob*) mouse. The *ob* gene codes for a protein which presumably controls the size of the body fat mass by acting on the ventromedial nucleus of the hypothalamus (VMH) to inhibit food intake and/or to stimulate energy expenditure via activation of the sympathetic nervous system (SNS) [1].

Mutation of the *ob* gene in the C57 BL/6J *ob/ob* mice results in overeating and in a large increase in the white adipose tissue (WAT) *ob* gene mRNA level [1]. In recent studies performed in the obese Zucker *fa/fa* rat and in *db/db* mice, no change was observed in the *ob* gene structure, but a marked increase in the *ob* gene mRNA in WAT could be detected, suggesting that the defect might take place at the *ob* gene product receptor [2,3]. Taken together, these results have led to the notion of a feedback loop between the VMH and the WAT controlling *ob* gene expression. This loop would be switched off in the obese C57 BL/6J *ob/ob* mouse, in the obese Zucker *fa/fa* rat and in the *db/db* mouse and that would account for the up-regulation of the *ob* gene expression in the WAT of these animals.

To date, the *ob* gene expression has been demonstrated in WAT [1,2] and also detected in brown adipose tissue (BAT) [3]. Since BAT is known to play an important role in the regulation of energy balance in rodents [4], the expression of the *ob* gene was examined in this tissue. The aims of this study therefore were: (i) to quantify the *ob* gene expression in rat interscapular BAT and to compare its level to that measured in epididymal WAT; and (ii) to investigate if the *ob* gene level is modulated by conditions which are known to reduce or increase BAT sympathetic and thermogenic activity, namely caloric restriction [5,6], cold exposure [6,7], or the administration of a β_3-adrenoceptor agonist [8]. Under each of these conditions, possible changes in the *ob* gene expression in epididymal WAT were also studied in parallel.

2. Materials and methods

All organic and inorganic chemicals were of analytical or molecular biology grade. They were purchased from Merck (Darmstadt, Germany), Boehringer (Mannheim, Germany), Sigma (St. Louis, MO, USA) and Fluka (Buchs, Switzerland). Hybond N^+ membranes, [α-^{32}P]dCTP (3000 Ci/mmol) and [^{35}S]dATP αS (1000 Ci/mmol) were from Amersham (Bucks, UK). The TA Cloning Kit was from Invitrogen Corp. (San Diego, CA, USA), the Micro RNA Isolation Kit and Taq DNA polymerase were from Stratagene (La Jolla, CA, USA). The T7 Sequencing Kit and prepacked oligo(dT) cellulose columns were from Pharmacia (NJ, USA). Ro 16-8714 was a generous gift from Hoffman-La Roche (Basle, Switzerland).

2.1. Animals

Male Sprague–Dawley rats as well as lean (*Fa/Fa*) and obese (*fa/fa*) Zucker rats (7–10 weeks old) were kept at room temperature (about 21°C) with 12 h of illumination per day and fed ad libitum with Provimi Lacta chow (Cossonay, Switzerland). One group of Sprague–Dawley rats was fasted for 36 h, with free access to water containing NaCl 0.45 g/l. In the chronic food restriction experiment, Sprague–Dawley rats received for 10 days a fixed ration (15 g) of the diet, representing ~ 50% of the daily food intake of their ad libitum fed controls. In the cold-exposure experiments, the rats were kept at 6 ± 1°C for either 24 h or 3 weeks with free access to food and water. In the experiment designed to test the effect of Ro 16-8714 treatment, lean and obese Zucker rats were dosed orally with the drug (2 μmol = 0.87 mg/kg) in 5% (w/v) gum arabic at time 0 and 24 h and sacrificed at 32 h, i.e. 8 h after the last administration. Control animals were dosed with the vehicle alone. The rats were killed by decapitation, after which epididymal WAT and interscapular BAT (carefully freed from surrounding WAT) were dissected and quickly dipped into liquid nitrogen.

2.2. Northern blots

Epididymal WAT and interscapular BAT RNAs were isolated using the Micro RNA Isolation Kit. 20 μg of total RNA were electrophoresed in a 1% agarose gel containing formaldehyde as described by Lehrach et al. [9] and transferred to Hybond N^+ membranes by capillary blot-

*Corresponding author. Fax: (41) (22) 702-5502.
E-mail: giacobin@cmu.unige.ch

Abbreviations: BAT, brown adipose tissue; WAT, white adipose tissue; SNS, sympathetic nervous system; VMH, ventromedial hypothalamus; RT-PCR, reverse transcription-polymerase chain reaction.

SSDI 0014-5793(95)01030-0

ting. An *ob* gene probe was obtained by reverse transcription-polymerase chain reaction (RT-PCR) of rat WAT RNA. First strand cDNA synthesis was carried out using 1 μg of total RNA and the PCR 3′-primer according to the instructions of the First Strand Synthesis Kit. Amplification of the *ob* gene cDNA was performed using a 5′-primer matched with the mouse *ob* gene sequence [1] from position 213 to 235 (5′-CCCTCATCAAGACCATTGTCACC-3′) and a 3′-primer from position 547 to 566 (5′-GCAGCCTGCTCAAAGCCACC-3′). PCR amplification was performed as described previously [10], except that formamide was omitted from the medium, 10 μg/ml of nuclease-free bovine serum albumin was added and Taq DNA polymerase was used. The PCR amplification was performed in a thermal cycler programmed to cycle at 95°C for 1 min, 59°C for 1 min, and 72°C for 1 min for 30 cycles. A RT-PCR probe of the theoretical length of 354 bp was obtained. The PCR fragment was cloned and sequenced using the TA Cloning and T7 Sequencing Kits, respectively. It revealed the expected portion of the *ob* gene sequence. The probe was labelled by random priming with [α-^{32}P]dCTP to a specific radioactivity of approximately 1×10^9 dpm/μg DNA. RNA blots were hybridized for 2 h at 68°C in Quik-Hyb, then washed in a solution of 0.1 × SSC (1 × SSC is 150 mM NaCl, 15 mM sodium citrate, pH 7.0)/0.1% sodium dodecyl sulphate at 56°C for 15 min and exposed to Kodak X-AR film at −70°C. Size estimates for the RNA species were established by comparison with a RNA ladder. The amount of RNA in the signals on the autoradiograms was quantified by scanning photodensitometry. Student's unpaired *t*-test was used to determine statistical significance.

3. Results

Autoradiography of the Northern blots of rat epididymal WAT RNA hybridized with the *ob* gene probe revealed a main signal with a mean size of 4 kb. This size of the transcript is smaller than that originally described by Zhang et al. [1] (about 4.5 kb) but similar to that more recently reported by Trayhurn et al. [11]. A much fainter signal was also detected at a size of 2.3 kb. Only the 4 kb transcript level was found to be modulated by changes in experimental conditions studied. An *ob* gene transcript with a mean size of 4 kb was also detected in the interscapular BAT and was found to amount to 39 ± 10% (n = 5) of the *ob* gene transcript level in the WAT (Fig. 1A).

The effects of food restriction or cold exposure on the *ob* gene mRNA level in BAT and WAT were subsequently studied. Under both conditions of food restriction, i.e. acute (36 h) and chronic (10 days), *ob* gene mRNA levels were decreased (−62% to −68%) as compared to those in BAT and WAT of their respective controls (Figs. 1B, and 2A and B).

Acute cold exposure (24 h) markedly decreased (−84%) the *ob* gene mRNA level in BAT while it had no effect in WAT. Chronic cold acclimation (3 weeks) did not change the *ob* mRNA level in both BAT and WAT (Fig. 2A and B).

The effect of an acute β-adrenergic stimulation was also studied in lean and obese Zucker rats. As shown in Fig. 3A and B, the *ob* gene mRNA levels in WAT and BAT of the obese rats were about 2-fold higher than that in their lean controls. Administration of the β_3-adrenergic agonist Ro 16-8714 to the lean Zucker rats decreased the *ob* gene mRNA level in both BAT and WAT by 51% and 28%, respectively, whereas in the obese Zucker *falfa* rats it decreased this level only in the BAT (by 74%).

4. Discussion

A new and consistent finding of this study is that the *ob* gene mRNA is expressed in the BAT of the rat. Direct comparison shows that the average level of the *ob* gene mRNA in interscapular BAT amounts to about 40% of that in epididymal WAT. These data cannot be explained by contamination of the BAT by white adipocytes for several reasons. *First*, the degree of expression of the *ob* gene mRNA in BAT (i.e. 40% of that in WAT) is much higher than what could be accounted for by a simple contamination with white adipocytes. *Second*, it is known that the amount of white adipocytes infiltrating brown adipose tissue, which is practically negligible in very young (3 weeks old) rats, increases with age. However, it was found in this study (results not shown) that the level of *ob* gene mRNA in BAT did not vary in rats aged between 3 and 12 weeks. *Third*, recent unpublished data from our laboratory indicate that the *ob* gene mRNA is highly expressed in cultured brown adipocytes.

A likely explanation for the effects of the various conditions used in this study on the *ob* gene expression in BAT seems to reside in their known effects on the cell lipid content rather than in changes in sympathetic activity. Acute fasting and chronic food restriction, acute cold exposure [12] as well as treatment with Ro 16-8714 [13] are all stimuli that have been shown to reduce the lipids stores in BAT. The same explanation holds true for the effect of acute fasting and chronic food restriction in WAT. The finding of a substantial fall in the *ob* gene mRNA level in this tissue during fasting is in line with the recent report of Trayhurn et al. [11]. Under condition of acute cold exposure

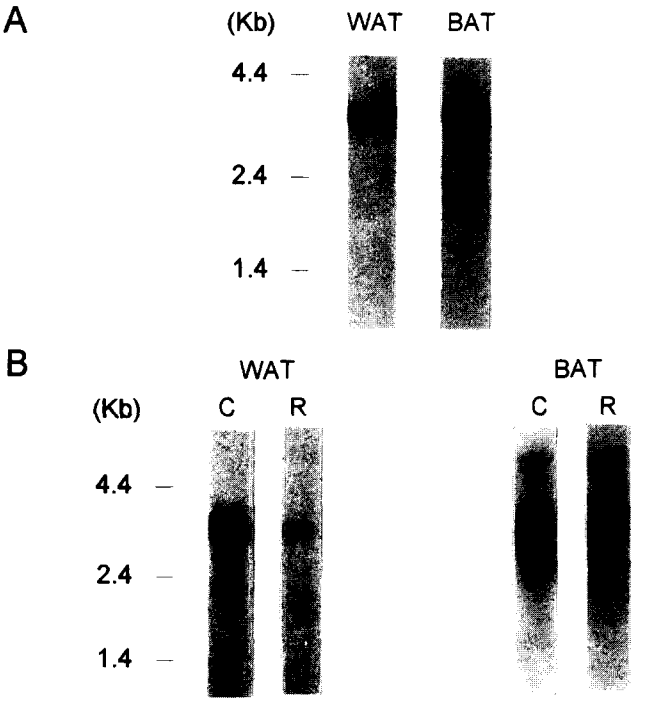

Fig. 1. (A) *ob* gene mRNA signals in the epipidymal white adipose tissue (WAT) and in the interscapular brown adipose tissue (BAT) of Sprague–Dawley rats. Total RNA, 20 μg, was electrophoresed, transferred to membrane filters and hybridized with the rat *ob* gene probe as described in section 2. The representative signals shown were obtained on the same Northern blot. Specific activity of the probe: 10^9 dpm/μg DNA; time of exposure of the autoradiogram: 12 h. (B) Representative *ob* gene mRNA signals in WAT and BAT of Sprague–Dawley rats which were food restricted (semi-starvation) for 10 days (R) as compared to that of their respective controls (C). The positions of marker RNAs are shown in kilobases (kb).

the transient fall in the *ob* gene mRNA in BAT can be associated with a transient lipid depletion in this tissue during the first day, i.e. before the rat increases its food intake sufficiently to meet its energy requirements for both thermoregulation and energy balance. This short delay before the compensatory hyperphagia induced by cold acclimation would have little or no effect on the size of WAT and this would explain why, in this tissue, the *ob* gene mRNA level did not change even transiently in the cold. During chronic cold acclimation the lipid content of BAT would be restored secondary to hyperphagia. This, together with hormonal adaptative changes that are known to occur after a few days in the cold would explain why, during chronic cold acclimation, the *ob* gene mRNA level is not modified in BAT.

In the case of β_3-adrenoceptor activation by Ro 16-8714, the decrease in the *ob* gene mRNA in the WAT of the lean but not of the obese *falfa* rat can also be explained by differences in the degree of lipid depletion in this tissue. Indeed, an acute administration of the thermogenic drug is likely to result in a significant reduction in lipid content of WAT of the lean, but not in the much larger WAT of the obese rat.

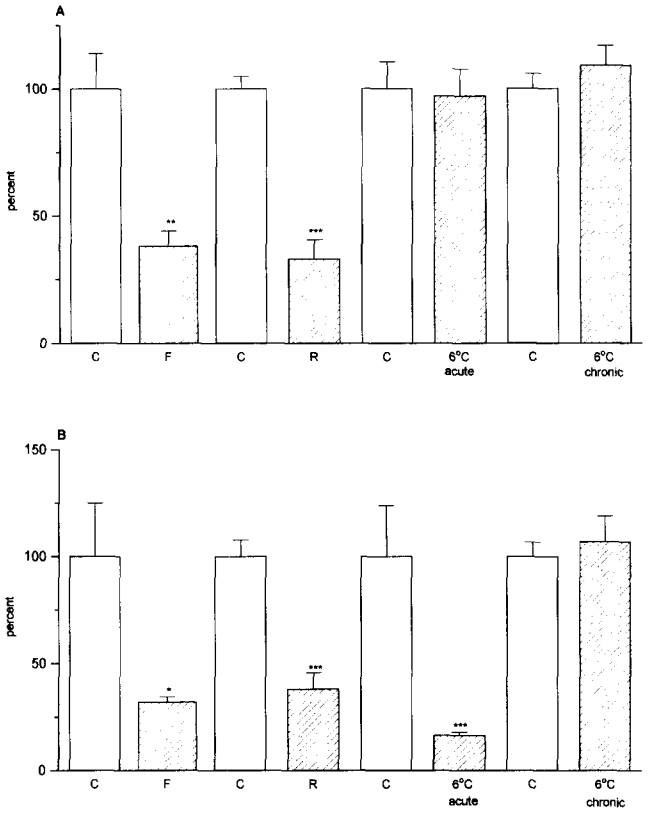

Fig. 2. (A) *ob* gene mRNA level in the epididymal WAT of Sprague–Dawley rats which were fasted for 36 h (F) or food restricted (semi-starvation) for 10 days (R) or exposed at 6°C for 24 h (acute) or for 3 weeks (chronic) as compared to that of their respective controls (C). (B) *ob* gene mRNA level in the interscapular BAT of Sprague–Dawley rats submitted to the same experimental conditions as in (A), as compared to that of their respective controls. Total RNA, 20 μg, was electrophoresed, transferred to membrane filters and hybridized with the rat *ob* gene probe as described in section 2. The *ob* gene mRNA levels are expressed in percent of the mean control values ± S.E.M. The number of experiments varied between 3 and 7. Only signals obtained on a same Northern blot were compared. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ compared to the respective control values.

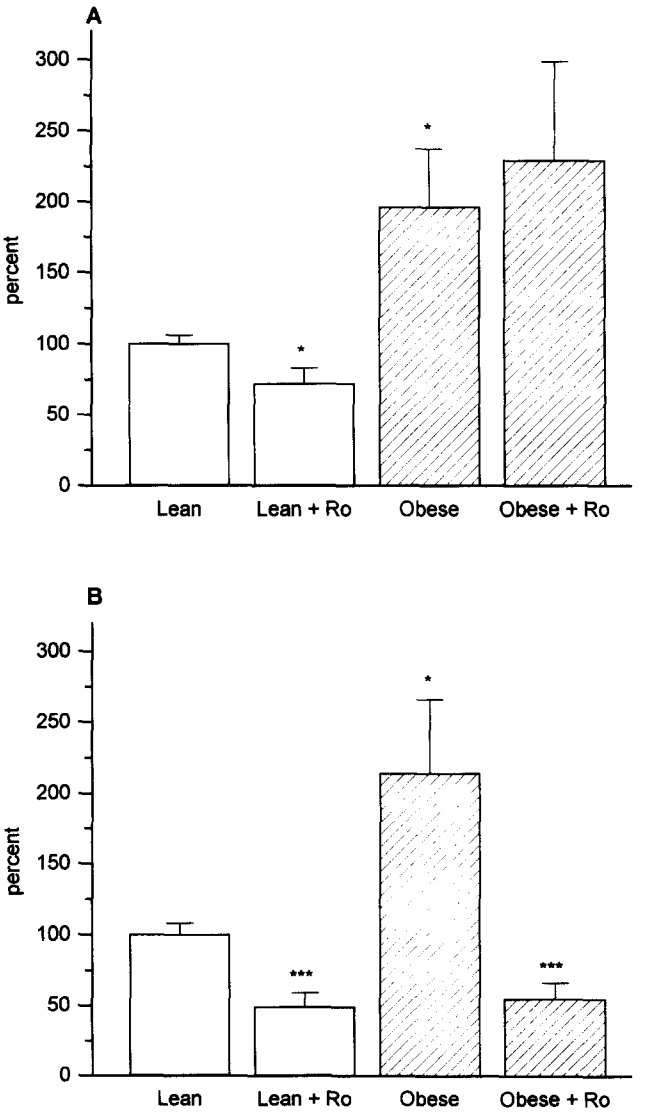

Fig. 3. (A) *ob* gene mRNA level in the epididymal WAT of lean (empty columns) and obese (hatched columns) Zucker rats treated or not with the β_3-adrenergic agonist Ro-168714. (B) *ob* gene mRNA level in the interscapular BAT of lean (empty columns) and obese (hatched columns) Zucker rats treated or not with Ro 16-8714. Total RNA (20 μg) was electrophoresed, transferred to membrane filters and hybridized with the rat *ob* gene probe as described in section 2. The results are expressed as in Fig. 2.

Finally, the present study performed on lean and obese Zucker rats shows that, as previously reported for WAT [2], the *ob* gene mRNA level is increased in BAT of obese rats as compared to that of their lean controls. Therefore, the postulated feed-back loop controlling the expression of the *ob* gene in the obese rat WAT would also concern BAT. It cannot, however, be excluded that the increased level of *ob* gene mRNA in the BAT of these obese animals is due to the large amount of white adipocyte-like cells known to be present in their BAT.

Taken together the results of this study show that the modulation of the *ob* gene expression by food restriction, cold exposure and β_3-adrenergic agonist administration in both BAT and WAT seems to be more closely associated with alterations in lipid content of these tissues than with altered sympathetic

activity or β_3-adrenoceptor activation per se. Further studies are warranted to confirm this contention and to determine the intra- or extracellular factor(s) that inhibits *ob* gene expression when the adipocyte lipid content decreases.

Acknowledgements: This work was supported by grants (No. 32-33779.92 and No. 3137616.93) from the Swiss National Science Foundation and by Grants-in-Aid from Ciba-Geigy-Jubiläums-Stiftung and from Ingeborg Naegeli II Foundation. The expert technical assistance of Francine Califano and Françoise Kühne is gratefully acknowledged. We are also indebted to Prof. B. Jeanrenaud for the generous gift of Zucker *fa/fa* and *FA/FA* rats.

References

[1] Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L. and Friedman, J.M. (1994) Nature 372, 425–432.
[2] Murakami, T. and Shima, K. (1995) Biochem. Biophys. Res. Commun. 209, 944–952.
[3] Maffei, M., Fei, H., Lee, G.H., Dani, C., Leroy, P., Zhang, Y., Proenca, R., Negrel, R., Ailhaud, G. and Friedman, J.M. (1995) Proc. Natl. Acad. Sci. USA 92, 6957–6960.
[4] Trayhurn, P. (1986) in: Brown Adipose Tissue (Trayhurn, P. and Nicholls, D.G. eds.) pp. 299–338, Edward Arnold, London.
[5] Rothwell, N.J. and Stock, M.J. (1982) Biosci. Rep. 2, 543–549.
[6] Landsberg, L. and Young, J.N. (1978) N. Engl. J. Med. 298, 1295–1300.
[7] Himms-Hagen, J. (1986) in: Brown Adipose Tissue (Trayhurn, P. and Nichols, D.G. eds.) pp. 214–268, Edward Arnold, London.
[8] Giacobino, J.-P. (1995) Eur. J. Endocrinol. 132, 377–385.
[9] Lehrach, H., Diamond, D., Wozney, J.M. and Boedtker, H. (1977) Biochemistry 16, 4743–4751.
[10] Revelli, J.-P., Muzzin, P., Paoloni, A., Moinat, M. and Giacobino, J.-P. (1993) J. Mol. Endocrinol. 10, 193–197.
[11] Trayhurn, P., Duncan, J.S., Thomas, M.E.A. and Rayner, D.V. (1995) FEBS Lett. 368, 488–490.
[12] Barnard, T. and Skala, J. (1970) in: Brown Adipose Tissue (Lindberg, O. ed.) pp. 33–72, Elsevier, New York.
[13] Muzzin, P., Revelli, J.-P., Ricquier, D., Meier, M.K., Assimacopoulos-Jeannet, F. and Giacobino, J.-P. (1989) Biochem. J. 261, 721–724.