

This is the peer reviewed version of the following article:

Regulation of ob gene expression: evidence for epinephrine-induced suppression in human obesity / Carulli, Lucia; Ferrari, S; Bertolini, M; Tagliafico, Enrico; DEL RIO, Graziano. - In: THE JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM. - ISSN 0021-972X. - ELETTRONICO. - 84:(1999), pp. 3309-3312. [10.1210/jcem.84.9.6007]

Terms of use:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

10/04/2024 10:29

Regulation of *ob* Gene Expression: Evidence for Epinephrine-Induced Suppression in Human Obesity

L. CARULLI, S. FERRARI, M. BERTOLINI, E. TAGLIAFICO, AND G. DEL RIO

Dipartimento di Medicina Interna (Centro di Nutrizione Clinica e Malattie Metaboliche) (L.C., M.B., G.D.R.) and Dipartimento di Scienze Biomediche (S.F., E.T.), Università di Modena, 41100 Modena, Italy

ABSTRACT

Leptin acts as satiety factor and increases energy expenditure. Studies conducted on animals and *in vitro* on adipocytes culture have shown that infusion of catecholamines leads to a significant reduction of *ob* gene expression; it appears of interest to evaluate the *in vivo* effects of adrenergic activation on the expression of the *ob* gene in humans.

We studied *ob* gene expression in adipose tissue samples from 13 obese subjects before and after epinephrine (25 ng/min·kg ideal body weight for 3 h) and 6 obese patients during saline infusion. Hormonal infusion led to a significant increase in epinephrine plasma levels (from 27 ± 4 to 339 ± 75 pg/mL; $P < 0.001$), plasma free fatty acids

(from 0.73 ± 0.05 to 0.98 ± 0.07 ; $P < 0.05$), heart rate (13.5 ± 3.1 beats/min; $F = 2.9$; $P < 0.03$), and systolic blood pressure ($F = 2.7$; $P < 0.05$), whereas diastolic blood pressure did not show significant variation. Plasma leptin levels decreased by the end of the infusion (from 63 ± 13 to 49 ± 11 ng/mL; $P < 0.05$), and *ob* messenger ribonucleic acid levels were significantly reduced (decrease amounting to $47 \pm 5\%$ of basal values). Our study shows that adrenergic activation contributes to regulate *ob* messenger ribonucleic acid levels in humans. The interaction between epinephrine and leptin may operate during metabolic and psychological stress to regulate energy expenditure and food intake. (*J Clin Endocrinol Metab* 84: 3309–3312, 1999)

OBESITY is defined as increased mass of adipose tissue resulting from a systemic imbalance between caloric intake and energy expenditure. This condition increases the risk for a variety of cardiovascular and metabolic disorders, including noninsulin-dependent diabetes mellitus. The molecular factors regulating food intake and body weight are poorly understood. Recently, a major advance in our understanding has been achieved by Zhang and colleagues, who, using positional cloning, have isolated the *obese* (*ob*) gene, which causes severe hereditary obesity and noninsulin-dependent diabetes mellitus in mice when mutated (1). This gene is expressed in both sc and visceral white adipose tissue and codes for a 18,000 molecular mass protein with a signal sequence. It is suggested that the protein, termed leptin, is secreted from white adipocytes as a 16,000 molecular mass product that signals the size of the white adipose tissue depots (1, 2). Circulating leptin levels change in parallel with changes in adipose tissue messenger ribonucleic acid (mRNA) levels (1, 2). Human obesity does not appear to be caused by mutations in the *ob* gene, but it might be due to resistance to leptin (3, 4). However, about 10% of obese patients have relatively low leptin levels; therefore, it has been suggested that obesity could be secondary to an abnormal regulation of the leptin gene that leads to a relative decrease in the synthesis of leptin mRNA (5). The expression of the *ob* gene is subject to nutritional regulation; the level of *ob* mRNA falls during fasting and rises with refeeding (6, 7). Hormonal factors, such as glucocorticoids and insulin, are involved in regulation of the *ob* gene in rats, but controversy

exists on their role in humans (8–10). Trayhurn and colleagues (11) have shown that acute exposure of mice to cold led to suppression of the *ob* gene, and the same effect could be mimicked by the administration of norepinephrine and β_3 -adrenoceptor agonist; the effect of cold on the *ob* gene was therefore mediated primarily by the sympathetic nervous system in animals. On the other hand, it is well known that the sympathoadrenal system plays an important role in energy expenditure (EE) by affecting its different components, such as the basal metabolic rate and food-induced and exercise-induced EE (12). All of these data taken together suggest that the sympathoadrenal system plays a role in the maintenance of body weight affecting both EE and food intake and in regulating the expression of the *ob* gene, in the pathogenesis of obesity. The relationship between the leptin axis and the adrenergic system in human obesity has not been investigated. The aim of this study was to evaluate the effect of sympathoadrenal system activation, obtained by epinephrine administration, on leptin gene expression in human obese subjects.

Subjects and Methods

Nineteen obese patients were recruited for the study. The clinical characteristics of the patients are shown in Table 1. We studied 13 obese subjects before and during epinephrine infusion (9 women and 4 men), whereas 6 obese patients (4 women and 2 men) were recruited as controls and studied during saline infusion without epinephrine. The subjects had been weight stable from at least 6 months before the time of presentation and were free from clinical and laboratory signs of cardiovascular or metabolic disease other than obesity; none of them was taking any medication. The protocol was approved by the local ethics committee, and all the subjects gave informed consent to enter the study.

Protocol

Epinephrine infusion. On arrival, a 19-gauge iv catheter, for blood sampling, was inserted into a vein of the forearm and kept patent with a slow

Received February 24, 1999. Revision received June 1, 1999. Accepted June 7, 1999.

Address all correspondence and requests for reprints to: Dr. Graziano Del Rio, Dipartimento di Medicina Interna, Policlinico di Modena, Via Del Pozzo 71, 41100 Modena, Italy. E-mail: delrio@unimo.it.

running infusion of saline. A second catheter was placed in a contralateral antecubital vein for the infusion of epinephrine at the dose of 25 ng/min/kg ideal body weight (IBW) (13) for 3 h. The epinephrine infusates (Laboratorio Chimico Biologico SRL, Venice, Italy) were prepared as 1 µg/mL solutions in saline, with 1 mg/mL ascorbic acid added as preservative. Heart rate and systolic and diastolic blood pressure were monitored by using an automated cuff device during the test (Lifestat, Physiocal, Richmond, VA). Blood samples were taken at 5 and 0 min before the infusion and at 60, 120, 150, and 180 min during the epinephrine and saline infusions to evaluate concentrations of leptin and epinephrine and free fatty acids (FFA). Leptin was determined by RIA (Human Leptin RIA Kit, Linco Research, Inc., St. Charles, MO); catecholamines were determined by reverse phase high performance liquid chromatography with electrochemical detection (14). Plasma FFA were measured by enzymatic colorimetric assay (Roche Molecular Biochemicals, Lewes, UK). Samples of sc abdominal adipose tissue were obtained, before and after the epinephrine infusion, by means of a new liposuction technique (15), using a 20-cm long, 4-mm wide liposuction cannula connected to a 10-mL syringe to obtain a 5- to 7-mL sample. The specimens were taken from a triangle-shaped area, with the tip at the edge of the umbilicus and the base 5–7 cm lateral and below. The procedure, carried out under local anesthesia, was well tolerated.

RNA extraction and preparation of human *ob* complementary DNA (cDNA)

Total cellular RNA was extracted using a modification of the guanidium-cesium chloride centrifugation technique. Briefly, the adipose tissue biopsy was lysed in 4 mol/L guanidium thiocyanate, and RNA was pelleted by ultracentrifugation through a cesium chloride cushion (5.7 mol/L CsCl and 0.1 mol/L ethylenediamine tetraacetate, pH 7.5). Total RNA was recovered, extracted with phenol-chloroform, precipitated with ethanol, and resuspended in sterile, double distilled water supplemented with ribonuclease inhibitor (RNasin; 0.2 U/L; Roche Molecular Biochemicals, Mannheim, Germany). Ethidium bromide staining of a 1.1% agarose/formaldehyde minigel was used to check the integrity of isolated RNA and to confirm the presence of equivalent amounts of RNA in each lane. RT-PCR was carried out using 3 µg total RNA extracted from an adipose tissue biopsy, reverse transcribed using 200 U SuperScript II Reverse Transcriptase (Life Technologies, Inc., Gaithersburg, MD) and 0.5 µg oligo(deoxythymidine)₁₅ primer (Roche Molecular Biochemicals).

One microliter of cDNA was then amplified by adding 2.5 U Expand High Fidelity Polymerase (Boehringer Mannheim, Mannheim, Germany), 0.5 µg direct primer, and 0.5 µg reverse primer. Oligonucleotide primers were synthesized on a solid phase synthesizer (model 394, Applied Biosystems, Inc., Foster City, CA). The primers enclose the coding sequence corresponding to nucleotides 22–55 and 561–594 of the human *ob* gene (GenBank accession no. U 43653): direct primer, 5'-GTTGCAAGGCCCAAGAAGGGATCCTGGGAAGGAA-3'; and reverse primer, 5'-CGTAGTCTTGCAGGATCCGTGACCTTCAAGGCC-3'.

DNA labeling

Human *ob* cDNA was labeled using the random prime labeling procedure described by Feinberg and Volgelstein (16). The specific activities obtained ranged from 1–3 × 10⁹ cpm/µg DNA.

TABLE 1. Anthropometric features of the subjects included in the study

No. of patients	19
Age (yr)	42.1 ± 2.2
Ht (cm)	164.2 ± 3.2
Wt (kg)	133.8 ± 7.5
BMI	49.6 ± 2.4
W/H	1.00 ± 0.05
FM (kg)	63.9 ± 5.8
FFM (kg)	69.5 ± 4.4

Data are the mean ± SEM.

Northern blot analysis

The total cellular RNA was extracted as described above. Ten micrograms of RNA for each lane were loaded onto a 1.1% agarose/formaldehyde gel in 1 × 4-morpholine propanesulfonic acid buffer. The RNA was transferred to a positively charged nylon membrane (Roche Molecular Biochemicals) using the electroblotting procedure. After UV fixation of the transferred RNA, the membrane was prehybridized in 6 × SSC (standard saline citrate)-5 × Denhardt's solution-0.5% SDS and then hybridized in 6 × SSC-5 × Denhardt's solution-10% dextrane sulphate-0.5% SDS with ³²P-labeled probe and then washed with 0.1 × SSC-0.5% SDS at 65 °C for several hours. Autoradiography was performed using intensifying screen at -80 °C. The films were analyzed using a soft laser densitometer scanner. A further densitometric scanning of ethidium bromide staining of the gel was used to normalize the *ob* mRNA abundance. Data were analyzed using repeated measures ANOVA (17) or paired *t* test when appropriate.

Results

As a result of the epinephrine infusion, the mean plasma epinephrine levels increased from 27 ± 4 to 339 ± 75 pg/mL at the end of the infusion (*P* < 0.001). A small, but significant, increase in systolic blood pressure (*F* = 2.7; *P* < 0.05; Fig. 1) was observed, whereas diastolic blood pressure did not change during epinephrine infusion (*F* = 1.4; *P* = NS; Fig. 1); by the end of the infusion heart rate had risen by 13.5 ± 3.1 beats/min (*F* = 2.9; *P* < 0.03; Fig. 1). As a result of epinephrine infusion, plasma FFA increased from a basal value of 0.73 ± 0.05 to 0.98 ± 0.07 mmol/L at 180 min (*P* < 0.05; Fig. 2). Leptin levels during the infusion decreased from 63 ± 13 to 61 ± 12 ng/mL at 60 min (*P* = NS), 52 ± 9 at 120 min, 46 ±

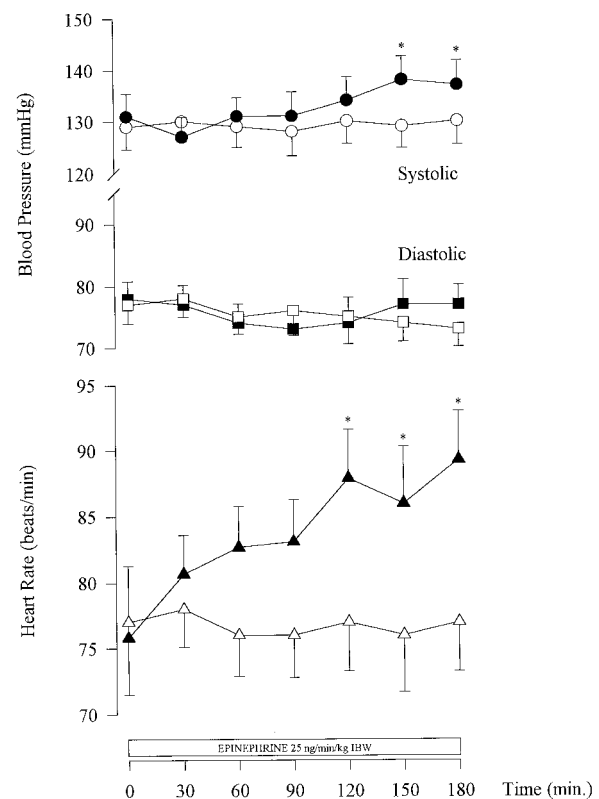


FIG. 1. Systolic and diastolic blood pressure and heart rate during epinephrine infusion (25 ng/min/kg IBW; filled circles) and during saline infusion (open circles). Results are the mean ± SEM. *, *P* < 0.05 vs. basal values.

11 at 150 min, and 49 ± 11 ng/mL at 180 min ($P < 0.05$ for all *vs.* basal value); in parallel, the expression of leptin mRNA in adipose tissue fell markedly in all patients studied (Fig. 3); the decrease was $47 \pm 5\%$ of the basal values ($P < 0.001$; Fig. 4). No significant correlation was found between plasma FFA and plasma leptin during epinephrine infusion ($r = -0.19$; $P = \text{NS}$), whereas the percent decrease in leptin *ob* mRNA was inversely correlated with basal plasma leptin ($r = -0.64$; $P < 0.05$). In the control group no changes were observed in pulse rate or blood pressure (Fig. 1). Plasma epinephrine did not change (from 30 ± 3 to 28 ± 4 pg/mL; $P = \text{NS}$), nor did plasma FFA (from 0.65 ± 0.07 to 0.67 ± 0.08 mmol/L, $P = \text{NS}$; Fig. 2), plasma leptin (from 56 ± 15 to 52 ± 16 ng/mL; $P = \text{NS}$), and leptin mRNA (mean change from basal value, $5 \pm 4\%$; $P = \text{NS}$; Fig. 4).

Discussion

In this study we provide evidence for the first time that increased plasma epinephrine levels are able to acutely regulate *ob* gene expression in white adipose tissue of obese subjects. The decrease in *ob* gene expression was detected in all of the patients studied; these data together with previous findings support the concept that *ob* gene expression is under hormonal control (8–10). Adipose tissue is highly innervated

in humans, and both sympathetic and parasympathetic activities regulate its function (18, 19). Several studies in animals have demonstrated a strict relationship between adrenergic activation and leptin (11, 20, 21). In addition, a study conducted *in vitro* on adipocyte coculture has shown that administration of norepinephrine and isoproterenol reduced the level of leptin mRNA, and this effect was reversed partially by the administration of propranolol (22). The administration of a β_3 -adrenergic receptor agonist in mice produced acute suppression of *ob* gene expression in white adipose tissue (20). The absence of this effect in β_3 -receptor knockout mice supports the view that the suppression of the *ob* gene in adipocytes is mediated by a β_3 -receptor through a G_s protein-coupled pathway (21).

Observations in humans are lacking. It has been shown that circulating leptin is rapidly (within 2 h) and reversibly suppressed by isoprenaline infusion in humans (23). Our data agree with this finding, as we observed a similar decrease in plasma leptin levels ($\sim 20\%$ of basal values) in obese patients during epinephrine infusion. Assessment of arteriovenous differences of leptin release from adipose tissue seems to be a more sensitive technique for detecting changes in secretion than measurement of the systemic concentration, and it is possible that our data indeed underestimate the effect of adrenergic activation on leptin secretion *in vivo* (24). Knowledge of the mechanisms that modulate leptin gene expression is therefore important to understand the interindividual variability in plasma leptin levels.

Indeed, plasma leptin levels in obese subjects may be high or normal; however, a small fraction of patients shows relatively low leptin levels. Recently, it has been demonstrated that alterations in the regulatory elements of the *ob* gene may lead to an abnormal response to cold in mice, and the researchers asserted that in some cases abnormal regulation of the leptin gene may be an etiological factor in the pathogenesis of obesity in humans (5).

The sympathetic nervous system and the adrenal medulla play an important role in the control of EE (12). Indeed, in humans, the plasma epinephrine threshold for metabolic effects, including lipolysis and stimulation of EE, lies within the physiological range (25). The epinephrine infusion rate used in our study has proved to be effective in stimulating lipolysis and metabolic rate in both lean and obese subjects (13), and the epinephrine concentration reached at the end of the study is similar to that seen during hypoglycemia and strenuous exercise (25). Therefore, it would be interesting to study the leptin variation *in vivo* during different metabolic

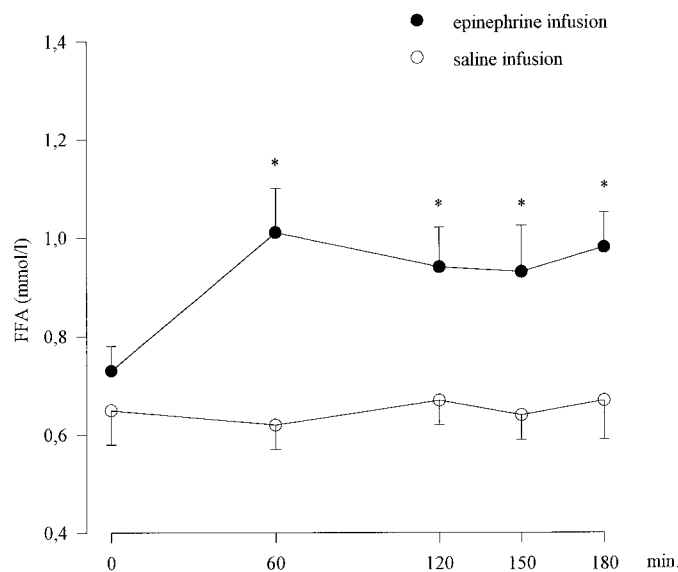


FIG. 2. Plasma FFA levels during epinephrine infusion (25 ng/min·kg IBW; filled circles) and during saline infusion (open circles). Results are the mean \pm SEM. *, $P < 0.05$ *vs.* basal values.

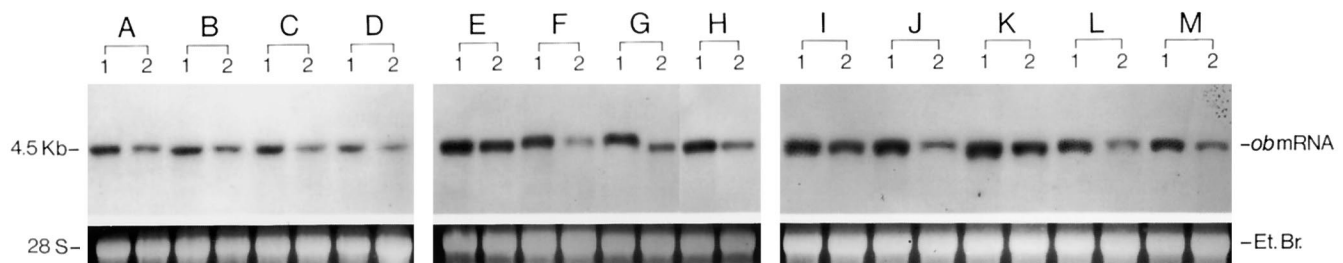


FIG. 3. Northern blot analysis of human *ob* mRNA 1) before and 2) after epinephrine infusion in 13 obese subjects. Total RNA (10 μ g/lane) was used.

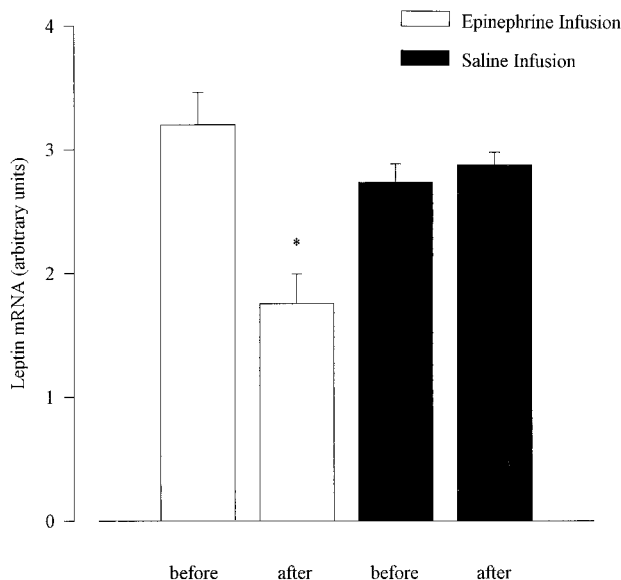


FIG. 4. Quantification of *ob* mRNA levels before and after epinephrine infusion (open columns) and during saline infusion (filled columns). Results are the mean \pm SEM. *, $P < 0.001$ vs. before infusion.

challenges in humans and its relationship to the adrenergic system *in vivo*. Leptin may act as a satiety factor, but there is evidence that it also affects energy expenditure. Studies conducted *in vivo* on mice have shown that daily ip injection of these mice with recombinant OB protein lowered their body weight, percent body fat, food intake, and serum concentration of glucose and insulin (26); in addition, metabolic rate, body temperature, and activity levels were increased, suggesting that the OB protein regulates body weight and fat deposition as well as EE (2, 4). Fasting is another situation in which a relationship between the adrenergic system and leptin could be hypothesized. Actually, the fall in plasma leptin levels during fasting is associated with an increase in epinephrine and a decrease in norepinephrine plasma levels, but does not seem to be linked to changes in insulin or ketones (6, 27). A putative influence of FFA on leptin levels has been suggested; recent papers indicate that plasma leptin concentrations are not correlated with changes occurring in plasma FFA without concomitant adrenergic activation (28, 29). Finally, epinephrine is also part of the physiological response to stress, and recently, leptin has been claimed to be a stress-related hormone, able to modulate the hypothalamic-pituitary-adrenal axis (30). The interaction between epinephrine and leptin may, therefore, operate during metabolic and psychological stress to regulate EE and food intake.

References

- Zhang Y, Proenca R, Maffei M, Barone M, Leopold I, Friedman JM. 1994 Positional cloning of the mouse *ob* gene and its human homologue. *Nature*. 372:425–432.
- Halaas JL, Gajiwalan Maffei M, et al. 1995 Weight-reducing effects of the plasma protein encoded by the obese gene. *Science*. 269:543–546.
- Considine RV, Considine E, Williams CJ, et al. 1995 Evidence against either a premature stop codon or the absence of *ob* gene mRNA in human obesity. *J Clin Invest*. 95:2986–2988.
- Maffei M, Halaas J, Ravussin E, et al. 1995 Leptin levels in human and rodent. Measurement of plasma leptin and *ob* RNA in obese and weight-reduced subjects. *Nat Med*. 1:1155–1161.
- Ioffe E, Moon B, Connolly E, Friedman JM. 1998 Abnormal regulation of the leptin gene in the pathogenesis of obesity. *Proc Natl Acad Sci USA*. 95:11852–11857.
- Kolaczynski JW, Considine RV, Ohannesian J, et al. 1996 Response of leptin to short-term fasting and refeeding in humans. A link with ketogenesis but not ketones themselves. *Diabetes*. 45:1511–1515.
- Trayhurn P, Thomas MEA, Duncan JS, Rayner DV. 1995 Effects of fasting and refeeding on *ob* gene expression in white adipose tissue of lean and obese (*ob/ob*) mice. *FEBS Lett*. 368:488–490.
- De Vos P, Saladin R, Auwerx J, Staels B. 1995 Induction of *ob* gene expression by corticosteroids is accompanied by body weight loss and reduced food intake. *J Biol Chem*. 270:15958–15961.
- MacDougald OA, Hwang CS, Fan H, Lane. 1995 Regulated expression of the obese gene product (leptin) in white adipose tissue and 3T3-L1 adipocytes. *Proc Natl Acad Sci USA*. 92:9034–9037.
- Saad MF, Khan A, Sharma A, et al. 1998 Physiological insulinemia acutely modulates plasma leptin. *Diabetes*. 47:544–549.
- Trayhurn P, Duncan JS, Rayner DV. 1995 Acute cold-induced suppression of *ob* (obese) gene expression in white adipose tissue of mice: mediation by the sympathetic system. *Biochemistry*. 31:729–733.
- Ravussin E, Lillioja S, Knowler WC, et al. 1988 Reduced rate of energy expenditure as a risk factor for body weight gain. *N Engl J Med*. 318:467–472.
- Webber J, Taylor J, Greathead, Dawson J, Buttery PJ, Macdonald IA. 1994 A comparison of thermogenic, metabolic and haemodynamic responses to infused adrenaline in lean and obese subjects. *Int J Obes*. 18:717–724.
- Del Rio G, Velardo A, Zizzo G, Marrama P, Della Casa L. 1993 Sex differences in catecholamine response to clonidine. *Int J Obes*. 17:465–469.
- Kolaczynski JW, Morales LM, Moore JH, et al. 1994 A new technique for biopsy of human abdominal fat under local anaesthesia with lidocaine. *Int J Obes*. 18:161–166.
- Feinberg AP, Vogelstein B. 1983 A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem*. 132:6–13.
- Norusis MJ. 1983 SPSS-X, advanced statistics guide. Chicago: McGraw-Hill.
- Arner P, Kriegholm E, Engfeldt P, Bolinder J. 1990 Adrenergic regulation of lipolysis *in situ* at rest and during exercise. *J Clin Invest*. 85:893–898.
- Andersson K, Arner P. 1995 Cholinergic-mediated effects on glycerol output from human adipose tissue using *in situ* microdialysis. *Br J Pharmacol*. 115:1155–1162.
- Mantzoros CS, Daqing Q, Frederich RC, et al. 1996 Activation of β_3 adrenergic receptors suppresses leptin expression and mediates a leptin independent inhibition of food intake in mice. *Diabetes*. 45:909–914.
- Kosaki A, Yamada K, Kuzuya H. 1996 Reduced expression of the leptin gene (*ob*) by catecholamines through a G(s) protein-coupled pathway in 3T3-L1 adipocytes. *Diabetes*. 45:1744–1749.
- Hardie LJ, Guilhot N, Trayhurn P. 1996 Regulation of leptin production in cultured mature white adipocytes. *Horm Metab Res*. 28:685–689.
- Pinkney JH, Coppack SW, Mohammed-Ali V. 1998 Effect of isoprenaline on plasma leptin and lipolysis in humans. *Clin Endocrinol (Oxf)*. 48:407–411.
- Coppack SW, Stanner S, Rawesh A, Goodrick S, Mohamed-Ali V. 1997 *In vivo* adipose tissue leptin production before and after a high carbohydrate meal. *Int J Obes*. 21:S33.
- Cryer PE. 1980 Physiology and pathophysiology of the human sympathoadrenal neuroendocrine system. *N Engl J Med*. 303:436–444.
- Pelleymounter MA, Cullen MJ, Baker MB, et al. 1995 Effects of the obese gene product on body weight regulation in *ob/ob* mice. *Science*. 269:540–543.
- Young JB, Rosa RM, Landsberg L. 1984 Dissociation of sympathetic nervous system and adrenal medullary responses. *Am J Physiol*. 247:E35–E40.
- Hennes MM, Dua A, Maas DL, Sonnenberg GE, Krakower GR, Kissebah AH. 1997 Relationship of plasma leptin levels to change in plasma free fatty acids in women who are lean and women who are abdominally obese. *Obes Res*. 5:442–446.
- Samra JS, Giles SL, Summers LK, et al. 1998 Peripheral fat metabolism during infusion of an exogenous triacylglycerol emulsion. *Int J Obes*. 22:806–812.
- Bornstein SR. 1997 Is leptin a stress related peptide? *Nat Med*. 3:937.