British Journal of Nutrition (2002), **87**, 427–434

DOI: 10.1079/BJN2002570

Dietary fat source regulates *ob* gene expression in white adipose tissue of rats under hyperphagic feeding

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(Received 6 August 2001 – Revised 2 January 2002 – Accepted 19 January 2002)

This work was designed to investigate the effect of different lipid sources on *ob* gene expression and serum leptin levels in rats with two different feeding protocols: (1) free access to food; or (2) energy-controlled feeding. Male Wistar rats were fed diets containing 40 % energy as fat (olive oil, sunflower oil or beef tallow), for 4 weeks. In Expt 1 rats had free access to food, and in Expt 2 rats were fed a controlled amount of food (16 g/d, equivalent to 300 kJ/d). Insulin and leptin were determined by ELISA and *ob* mRNA by Northern blot. When rats had free access to food, *ob* mRNA levels were higher in animals fed either olive oil or sunflower oil than in those fed beef tallow. In marked contrast with feeding *ad libitum*, no differences were found among dietary fat groups in rats fed energy-controlled diets. When both feeding protocols were compared, free access to food induced an increased expression of *ob* mRNA in perirenal and/or epididymal adipose tissues from rats fed either olive oil or sunflower oil, but not from rats fed beef tallow. Dietary lipid type did not induce modifications in serum leptin concentrations. A tendency to higher serum leptin levels was observed more in rats with free access to food than in rats fed energy-controlled feeding. No differences were found in insulin levels. Dietary fat type importantly affects *ob* mRNA expression in rat white adipose tissue under hyperphagic conditions. Further study is needed in order to elucidate the mechanism underlying this effect.

***ob* gene: Leptin: Dietary fat: Adipose tissue**

Leptin, the product of the *ob* gene, is a hormone produced mainly by white adipose tissue, secreted into blood and transported into the brain via a saturable system, where it releases or inhibits factors that ultimately reduce energy intake and increase energy expenditure (Ahima & Flier, 2000; Palou *et al.* 2000).

Many studies have shown a positive correlation between leptin levels and body fat both in human subjects (Considine *et al.* 1996; Havel *et al.* 1996) and rodents (Frederich *et al.* 1995; Maffei *et al.* 1995). Thus, this protein has been suggested as an ‘adipostat’ to signal the state of body triacylglycerol stores to the brain, an important feed-back which is necessary for precise regulation of long-term energy balance (Ahima & Flier, 2000). A role for leptin in the short-term control of energy balance has also been suggested (Bado *et al.* 1998; Cinti *et al.* 2000).

Dietary manipulations appear to influence leptin secretion through mechanisms other than just by altering total fat mass. It has been demonstrated that factors such as food restriction (Cha & Jones, 1998), carbohydrate intake (Jenkins *et al.* 1997) and fasting (Trayhurn *et al.* 1995*b*) induce changes in circulating level concentrations.

It has been shown that dietary fatty acid composition can influence different integrating processes of lipid metabolism, such as adipose tissue lipolysis (Awad & Chattopahyay 1986*a*,*b*; Raclot *et al.* 1997; Portillo *et al.* 1999*b*), lipogenesis (Perdereau *et al.* 1992; Camara *et al.* 1996; Portillo *et al.* 2001), lipoprotein lipase activity (Murphy *et al.* 1993; Raclot *et al.* 1997), thermogenic capacity (Takeuchi *et al.* 1995; Kawada *et al.* 1998; Takahashi & Ide, 2000; Rodríguez *et al.* 2002) and fatty acid oxidation (Leyton *et al.* 1987). The potential influence of dietary fatty acids on expression of *ob* gene and secretion of leptin remains to be elucidated.

The aim of the present study was to investigate the effect of different lipid sources, olive oil (a vegetal oil rich in monounsaturated fatty acids), sunflower oil (a vegetal oil rich in polyunsaturated fatty acids) and beef tallow (an

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animal fat rich in saturated fatty acids) on *ob* gene expression and serum leptin levels in rats on two different feeding protocols: (1) free access to food: or (2) energy-controlled feeding. Due to the fact that differences in *ob* gene expression among adipose tissues from different anatomical locations have been demonstrated (Trayhurn *et al.* 1995*b*; Oliver *et al.* 2001), the possibility of a different pattern of response to dietary fat in different fat depots should not be discarded. In order to test this hypothesis analysis of perirenal and epididymal depots was carried out, depots, which have a relatively high expression of this gene.

Material and methods

Animals, diets and experimental design

The experiment was conducted in male Wistar rats (8-week-old) purchased from IFFA-Credo (Barcelona, Spain), that were adapted to the room and cage environment 4 d before the beginning of the protocol. They were housed individually in polycarbonate metabolic cages (Tecniplast Gazzada, Guggugiate, Italy) and maintained in a temperature (23 ± 2°C) and humidity (50 %)-controlled room with 12 h light–dark cycle, lights on at 08.00 hours.

When animals (*n* 60) reached a body weight of 240 (SEM 2) g, they were randomly divided into six groups (*n* 10 per group). Three groups were used for Expt 1, in which rats had free access to diets that provided different lipid sources for 4 weeks. Daily determination of food intake was carried out.

The remaining three groups were used for Expt 2. In order to prevent a spontaneous hyperphagia due to the high content of fat and sucrose of the experimental diets, each rat was given 16 g (300 kJ) of the same diets as those used in Expt 1/d, for 4 weeks. This amount was equivalent to the spontaneous energy intake observed in a pilot study where rats with a similar mean body weight to that of rats in this present study were fed a laboratory chow diet. Potential differences among dietary fat groups in energy intake and possible obesity were avoided. All rats had free access to water.

Table 1. Composition of experimental diets

Diet…	Olive oil	Sunflower oil	Beef tallow
Ingredients (g/kg)			
Casein*	200	200	200
DL-Methionine	4	4	4
Sucrose	244	244	244
Wheat starch	245	245	245
Olive oil	200	–	–
Sunflower oil	–	200	10
Beef tallow	–	–	190
Cellulose	50	50	50
Mineral mix†	45	45	45
Vitamin mix†	10	10	10
Choline chloride salt	2	2	2
Total energy (MJ/kg)	18·8	18·8	18·8
Composition by energy (%)			
Protein	16·4	16·4	16·4
Lipid	40·1	40·1	40·1
Carbohydrate	43·5	43·5	43·5

* 900 g casein/kg.
† Formulated according to AIN-93 recommendations (Reeves *et al.* 1993).

The experimental diets were freshly prepared once per week, gassed with N_2 and stored at 0–4°C to avoid rancidity. The composition of all diets are described in Table 1. The fats used were olive oil, sunflower oil and beef tallow; they represented 40 % total energy. Dietary supply of vitamins, minerals and protein was in accordance with dietary recommended allowances for rats from the American Institute of Nutrition AIN-93 (Reeves *et al.* 1993). The fatty acid composition of the diets was determined by GC and is shown in Table 2. The beef-tallow diet was supplemented with sunflower oil (10 g/kg diet) to maintain an adequate intake of linoleic acid and to avoid growth alterations due to the linoleic acid deficiency (Cunnane & Anderson, 1997). The dietary oils were obtained from local markets, vitamins and minerals were purchased from Dyets (Bethlehem, USA) and casein from Sigma (Barcelona, Spain).

At the end of the feeding period, and after an overnight fast, animals were weighed. After anaesthesia for body-fat measurement, they were decapitated. Blood was collected and centrifuged and adipose tissues from perirenal and epididymal anatomical locations were dissected and weighed. Serum and tissue samples were immediately frozen in liquid N_2 and stored (-80°C) for subsequent analyses.

Fatty acid composition of dietary lipid sources

The oils and the beef tallow were transmethylated with methanol in $H_2 SO_4$. Analysis of fatty acids was conducted using a HP 6890 Series II GC equipped with flame ionization detector and a 30 m × 320 μm HP 19091N-213 PEG capillary column (HP Innowax) (Hewlett-Packard, Avondale, PA, USA). The carrier gas was N_2 at a flow rate of 1 ml/min. The temperatures of the oven and the injection port were maintained at 170°C and 225°C respectively. Peaks were identified using fatty acid methyl esters standards obtained from Sigma. All samples were analysed in quadruplicate. The replicate error (CV) was 5 % or less of the mean for all fatty acids.

Table 2. Fatty acid composition of dietary fats (g/100 g total fatty acids)*

Fatty acids	Olive oil	Sunflower oil	Beef tallow
12 : 0	< 0·10	< 0·10	0·20
14 : 0	< 0·10	0·10	4·89
14 : 1*n*-5	< 0·10	< 0·10	0·60
16 : 0	11·87	6·13	29·33
16 : 1*n*-7	0·94	0·11	2·69
18 : 0	2·92	4·60	24·21
18 : 1*n*-9	72·80	24·76	33·77
18 : 1*n*-7	4·46	1·51	1·75
18 : 2*n*-6	5·69	61·74	1·91
18 : 3*n*-3	0·50	0·10	0·30
20 : 0	0·39	0·29	< 0·10
Σ Saturates	15·18	11·12	58·63
Σ Monounsaturates	78·20	26·38	38·81
Σ Polyunsaturates	6·19	61·84	2·21

* Only fatty acids detected at levels > 0·10 g/100 g total fatty acids are listed.

Total body fat

Body composition was assessed in anaesthetized rats before killing by using an EM-SCAN TOBEC SA-3000 (EM-SCAN Inc., Springfield, IL, USA). Measures were based on energy absorption in the presence of a radio-frequency electromagnetic field.

Northern blot analysis of ob mRNA

Adipose tissue RNA were extracted with Tripure Isolation reagent. Total RNA (20 μg) was size fractionated in denaturing formaldehyde (180 ml/l) agarose (12 g/l) gel. RNA was transferred onto nylon membranes with 20 × SSC (saline sodium citrate) and u.v. cross-linked.

Membranes were prehybridizated at 42°C with DYG Easy Hyb solution and hybridizated overnight at 42°C in the same solution containing the 33-mer antisense oligonucleotide *ob* probes $5'$-CGTCTGAGGCAGGGAGCAGCTCTTGG-AGAAGGC-$3'$ (34 ng/ml), synthesized commercially and labelled with a single digoxigenin ligand (Trayhurn *et al.* 1995*a*). They were then washed twice with 2 × SSC SDS (1 g/l) at room temperature and after that twice with 0·1 × SSC SDS (1 g/l) at 48°C. Membranes were then blocked with blocking reagent solution (10 ml/l maleic acid buffer) and then incubated with phosphatase alkaline labelled anti-digoxigenin. CDP-Star substrate was used to produce a chemiluminescence signal, and the membranes were exposed to hyperfilm ECL films (Amersham International; Amersham, Bucks, UK). Signals obtained were analysed by scanner photodensitometry and quantified using the Bio Image software (Millipore; Belford, MA, USA).

Finally, blots were stripped by exposure to boiling SDS (1 g/l) and re-probed for 18S rRNA to check the loading and transfer of RNA during blotting. For 18S rRNA detection, the 31-mer digoxigenin-labelled antisense oligonucleotide $5'$-CGCCTGCTGCCTTCCTTGGATGTGGTAGCCG-$3'$ at a concentration of 70 pg/mL was used (Trayhurn *et al.* 1995*a*).

The RNA isolation reagent, nylon membranes, and reagents for Northern blotting, if not indicated, were purchased from Roche Applied Science (Mannheim, Germany).

Serum analyses

Insulin and leptin were determined using commercial kits by ELISA Rat Insulin ELISA, DRG Instruments GmbH, Marburg, Germany, and Quantikine M Murine kit, R&D Systems, Minneapolis, MN, USA respectively.

Statistical analysis

Statistical analysis was performed using SPSS 8.0 (SPSS Inc., Chicago, IL, USA). ANOVA was used to analyse the data. A *post hoc* test, using the Newman–Keuls method was performed when appropriate. Differences between means were considered to be significant at $P<0·05$ The results are expressed as means values with their standard errors.

Results

Body weight, weight gain, food consumption, adipose tissue weights and total body fat

Final body weight, weight gain and food intake values are shown in Table 3. Adipose tissue weights from perirenal and epididymal anatomical locations and total body fat are shown in Table 4.

In Expt 1, animals fed the different lipid sources gained comparable amounts of weight during the experimental period. No differences in adipose tissue weights and total body fat were found between dietary fat groups. In Expt 2, despite the isoenergetic feeding, rats fed the beef-tallow diet gained significantly less weight than rats fed the other two diets ($P<0·05$). In contrast, no significant differences were found between rats fed the olive-oil and

Table 3. Effects of dietary fat source on body weight, food intake and on serum insulin and leptin levels in rats fed either *ad libitum* or energy-controlled diets†

(Mean values with their standard errors for ten rats per group)

Diet...	Olive oil		Sunflower oil		Beef tallow	
	Mean	SEM	Mean	SEM	Mean	SEM
Rats fed *ad libitum*						
Final body weight (g)	374	5	370	4	371	5
Body weight gain (g)	135	6	133	5	139	8
Food intake (g/d)	21·1^{b}	0·5	20·7^{b}	0·4	23·3^{a}	0·5
Insulin (pmol/l)	265	23	220	12	242	13
Leptin (ng/ml)	9·94	1·00	10·89	1·18	9·25	2·31
Rats fed energy-controlled diet						
Final body weight (g)	331^{a*}	4	340^{a*}	2	315^{b*}	4
Body weight gain (g)	88^{a*}	4	96^{a*}	3	72^{b*}	4
Food intake (g/d)	16·0*	0·0	16·0*	0·0	16·0*	0·0
Insulin (pmol/l)	208	7	202	7	246	16
Leptin (ng/ml)	6·12	1·49	6·95	1·61	5·46	1·87

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).

Mean values were significantly different from those of the groups fed *ad libitum* for each dietary fat source: *$P<0·05$.

† For details of diets and procedures see Tables 1 and 2 and p. 428.

Table 4. Effects of dietary fat source on adipose tissue weights and on total body fat in rats fed either *ad libitum* or energy-controlled diet†

(Mean values with their standard errors for ten rats per group)

Diet…	Olive oil		Sunflower oil		Beef tallow	
	Mean	SEM	Mean	SEM	Mean	SEM
Rats fed *ad libitum*						
Perirenal adipose tissue (g)	7·95	0·60	7·80	0·43	7·89	0·41
Epididymal adipose tissue (g)	8·11	0·55	6·86	0·65	8·42	0·66
Total body fat (g)	72·70	2·41	68·03	3·00	68·46	2·63
Rats fed energy-controlled diet						
Perirenal adipose tissue (g)	5·22^{ab*}	0·33	6·29^{a}	0·63	4·33^{b*}	0·53
Epididymal adipose tissue (g)	5·40^{a*}	0·27	5·48^{a}	0·42	4·29^{b*}	0·38
Total body fat (g)	38·43^{ab*}	1·48	42·80^{a*}	1·54	35·79^{b*}	1·29

^{a,b}Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).

Mean values were significantly different from those of the groups fed *ad libitum* for each dietary fat source: *$P<0·05$.

† For details of diets and procedures, see Tables 1 and 2 and p. 428.

the sunflower-oil diets. Beef tallow-fed rats showed the lowest values for perirenal and epididymal adipose tissues and total body fat.

When rats had free access to food there was a main effect of the type of fat on food intake, with those rats eating the saturated fat having a higher food intake than those rats eating unsaturated fats ($P<0·05$). Under this feeding protocol, a significant increase in food consumption was induced for all the dietary fat sources as compared with rats fed controlled amounts of diets ($P<0·001$).

Northern blot analysis of ob mRNA in white adipose tissues

When rats had free access to food, *ob* mRNA levels were higher in animals fed either olive oil or sunflower oil than in those fed beef tallow in both perirenal and epididymal adipose tissues ($P<0·05$) (Fig. 1). In contrast to feeding *ad libitum*, no differences were found among dietary fat groups in rats fed energy-controlled diets (Fig. 2).

Serum variables

Serum levels of leptin and insulin are shown in Table 3. Dietary lipid type did not induce modifications in these variables. By comparing feeding *ad libitum* with energy-controlled feeding for each dietary fat type, a tendency to higher leptin levels was observed in rats with free access to food, but this increase did not reach statistical significance. In contrast, when the three dietary fat type groups were pooled for each feeding protocol, serum leptin levels were significantly higher in rats fed *ad libitum* than in rats fed energy-controlled diets ($P<0·01$).

Linear regression analyses were performed for circulating leptin levels *v.* adipose tissue weights, total body fat, body weight and serum insulin levels. Significant positive

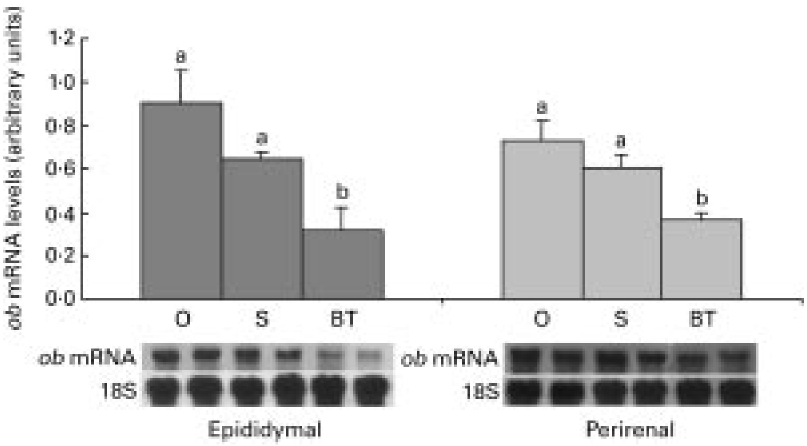

Fig. 1. *ob* mRNA expression in epididymal and perirenal adipose tissues from rats fed different lipid sources *ad libitum*. *ob* mRNA was measured by Northern blot and expressed relative to 18S rRNA in arbitrary units. O, olive oil; S, sunflower oil; BT, beef tallow. For details of diets and procedures, see Tables 1 and 2 and p. 428. Values are means for ten rats per group with standard errors shown by vertical bars. ^{a,b}Mean values with unlilke superscript letters were significantly different ($P<0·05$).

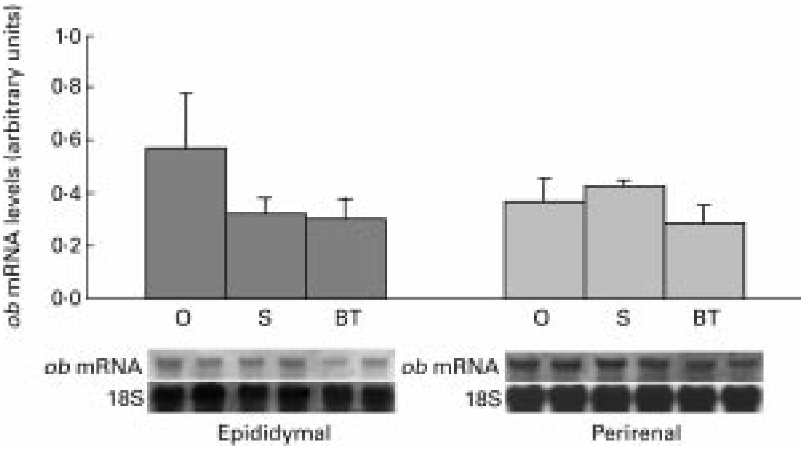

Fig. 2. *ob* mRNA expression in epididymal and perirenal adipose tissues from rats fed energy-controlled diets that provided different lipid sources. *ob* mRNA was measured by Northern blot and expressed relative to 18S rRNA in arbitrary units. O, olive oil; S, sunflower oil; BT, beef tallow. For details of diets and procedures, see Tables 1 and 2 and p. 428. Values are means for ten rats per group with standard errors shown by vertical bars. There were no significant differences between the dietary groups.

correlations were found when the six experimental groups were pooled (*n* 6 per group) for body weight (*r* 0·470, $P=0·008$), epididymal adipose tissue (*r* 0·398, $P=0·027$) and total body fat (*r* 0·443, $P=0·012$).

Discussion

When rats had free access to food, dietary lipid source did not influence body-weight gain, adipose tissue weights or total fat accumulation. Nevertheless, significant differences in food intake were found; rats eating the saturated fat showed higher food intake than those rats eating unsaturated fats ($P<0·05$). This suggests different utilization of dietary fats. In a previous study we found that unsaturated fats were better absorbed than saturated fats; absorbability coefficients were 98·2, 97·4 and 93·7 % for olive oil, sunflower oil and beef tallow respectively (Portillo *et al.* 2001). This situation can help to explain the similarity in final body weights and adipose depot sizes among the three groups, despite some differences in food intake.

In Expt 2 rats were fed a fixed amount of the experimental diets (16 g/d, 300 kJ/d), providing adequate energy to ensure a normal growth, as explained on p. 428. This protocol removed the differences in food consumption produced in Expt 1 and avoids the energy hyperphagia induced by high-fat feeding. This hyperphagic effect has been described well in the literature (Lim *et al.* 1991; Astrup *et al.* 1994; Portillo *et al.* 1998, 1999*a*). In the present study, energy intake in rats with free access to food was 29·4–45·6 % higher than that of rats fed in a controlled way. Although in the second experiment energy intake was the same in the four groups, the reduced amount of fat absorbed fat by the beef tallow-fed group ($P<0·05$) resulted in an energy deficiency that can explain the lower weight gain and fat accumulation observed in this group (Portillo *et al.* 2001). As expected, and due to higher energy intake, adipose tissue weights and total body fat were higher in rats with free access to food than in rats fed energy-controlled diets.

Information concerning the effects of dietary lipid sources or fatty acids on *ob* mRNA expression is scarce. Raclot *et al.* (1997) observed that a diet rich in oleic acid elicited a higher *ob* mRNA expression in rat adipose tissue than a fish-oil diet, rich in *n*-3 fatty acids. Murata *et al.* (2000) analysed the effects of different doses of eicosapentaenoic acid on cultured 3T3-L1 adipocytes *in vitro*; they demonstrated that this fatty acid caused a time and dose-dependent increase in *ob* mRNA.

The results of the present study demonstrate that only under hyperphagic conditions was leptin gene expression in rat white adipose tissue affected by dietary fat source. It could be hypothesized that, in the absence of energy excess, energy intake was more significant in the control of leptin than the quality of dietary fat. However, when feeding *ad libitum*, fatty acid composition of the diet does affect *ob* mRNA expression: unsaturated fatty acid-diet feeding led to higher *ob* mRNA expression than saturated fatty acid-diet feeding. The existence of changes in *ob* mRNA induced by dietary fat type in *ad libitum*-fed rats without changes in adipose tissue size suggests that dietary fat acts in a way independent of this variable. Cha & Jones (1998) also observed the influence of energy intake on dietary fat effects. They found that dietary fat consumed by rats for 10 weeks modified serum leptin levels when rats were fed *ad libitum*, but not when animals were fed a diet restricted to 70 % of *ad libitum* energy intake.

The mechanisms responsible for the effect of dietary fat type on *ob* mRNA gene expression are not clear. In a previous work (Perona *et al.* 2000) where the same experimental design was used, we observed significant differences in the fatty acid profiles of triacylglycerol stored in adipose tissue depending on dietary fat source. In this context, it could be speculated that the saturated fatty acids deposited in adipose tissue from rats fed beef tallow (35·4 g/100 g of total fat for perirenal adipose tissue), the monounsaturated fatty acids deposited in adipose tissue from olive oil-fed rats (68·0 g/100 g of total fat for perirenal adipose tissue) or the *n*-6 polyunsaturated fatty acids deposited in adipose tissue from sunflower fed rats (46·4 g/100 g total fat for perirenal adipose tissue) may each have an effect that either inhibits or increases leptin gene expression. Nevertheless if this were the case, differences among groups fed energy-controlled diets would have been observed.

Recent reports suggest that the hexosamine biosynthetic pathway contributes to the fatty acid regulation of leptin expression (Wang *et al.* 1998; Murata *et al.* 2000; Perona *et al.* 2000). In the present study, experiments designed to clarify this hypothesis have not been carried out. Changes in fatty acid availability that inhibit lipolysis and promote increased flux of fructose 6-phosphate into the glucosamine pathway appear to be important in the regulation of leptin production (Wang *et al.* 1998). However, the precise role of specific fatty acids or the particular effects of the diets studied as regulators of the glycolysis pathway is not well known.

In addition, the possible role of dietary fatty acids as signalling molecules that bind and activate a new class of nuclear receptors, the peroxisome proliferator-activated receptors could be considered (Nisoli *et al.* 2000). It has been demonstrated that different ligands for peroxisome proliferator-activated receptors such as clofibrate, troglitazone or thiazolidinediones repress *ob* mRNA expression (De Vos *et al.* 1996; Nolan *et al.* 1996). However, no direct effect for the more abundant fatty acids in the cells has been described as acting on peroxisome proliferator-activated receptors.

Divergences in the pattern of response of *ob* mRNA levels and circulating leptin concentrations were found. Dietary fat source did not affect serum leptin levels for either feeding *ad libitum* or in energy-controlled feeding. This discrepancy can be found in the literature. Lin *et al.* (1998) observed significant differences in epididymal adipose tissue *ob* mRNA in rats fed either a high-fat diet or a low-fat diet for 5 weeks but no changes in serum leptin concentrations. Several facts could explain these discrepancies. Leptin secreted from several organs addition to white adipose tissue, including the stomach (Cinti *et al.* 2000, 2001), brown fat, placenta and fetal tissues (such as heart and bone cartilage) (Trayhurn *et al.* 1999) as well as subcutaneous adipose tissue and the greater fat pad, accounts for serum leptin levels. Villafuerte *et al.* (2000) reported that, although the expression of *ob* mRNA in subcutaneous tissue is relatively low, the contribution to total circulating leptin may be proportionally higher because of the absolute mass of the tissue. In the present study, information concerning *ob* mRNA regulation in tissues mentioned earlier and organs is not available. On the other hand, differences in the clearance of leptin from circulation due to binding to its soluble receptor should not be discarded. Secretion and/or turnover of leptin may be regulated independently of synthesis.

It has been demonstrated that leptin levels increase as the size of adipose tissue increases (Frederich *et al.* 1995; Maffei *et al.* 1995; Considine *et al.* 1996). An increase in energy intake also results in a sharp increase in serum leptin levels, even in the absence of body weight changes (Kolaczynski *et al.* 1996*a*; Sinha *et al.* 1996). In the present study, rats fed *ad libitum* showed higher values of serum leptin than those showed by rats fed energy-controlled diets, although when considering each dietary fat individually differences did not reach statistical significance. On the other hand, under energy-controlled feeding, rats fed the beef-tallow diet showed significantly lower weights of adipose tissues than rats fed the other two diets, as well as a tendency towards lower serum leptin levels (10·8 % lower than olive oil-fed rats and 21·4 % lower than sunflower oil-fed rats). It is important to point out that serum analyses were carried out after an overnight fast. It has been widely proved that fasting results in a rapid and drastic fall in circulating leptin (Trayhurn *et al.* 1995*b*; Hardie *et al.* 1996; Kolaczynski *et al.* 1996*b*). Thus, this fasting-induced effect might partially mask the effects of increased energy intake and adiposity in rats fed *ad libitum*. A positive and significant correlation was found between leptin levels and body weight, adipose tissue weights and total body fat in concordance with other studies (Frederich *et al.* 1995; Maffei *et al.* 1995).

The role of hormones in the regulation of leptin expression has been intensively explored. A prime candidate for such regulation is insulin. In rats, this hormone stimulates *ob* gene expression and leptin production (Cusin *et al.* 1995; Saladin *et al.* 1995). In order to test whether insulin was involved in the dietary fat type effects found in this study, serum concentrations were measured. However, differences in *ob* mRNA levels in adipose tissue cannot be explained by changes in insulin status because no significant differences were found among rats fed the three different lipid sources.

In summary, under high-fat *ad libitum* feeding conditions, the expression of *ob* mRNA in white adipose tissue from rats is enhanced by unsaturated dietary fats (olive oil and sunflower oil) compared with a saturated dietary fat (beef tallow) by a mechanism which is independent of adipose tissue size. The mechanisms underlying this effect remain unclear, so further study is required in order to shed light on this issue.

Acknowledgements

This study was supported by the Government of the País Vasco (grant PI 96/22 to M.P.P.), DGICYT, Ministerio de Educación y Ciencia, Spain (grant PM97-0094 to A.P.) and by the European Commission DGXII (COST 918 to A.P.). V.M.R. is a recipient of a fellowship from the Spanish Government.

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