Short-term effects of leptin on lipid metabolism in the rat

Joaquín López-Soriano, Neus Carbó, Francisco J. López-Soriano, Josep M. Argilés*

Departament de Bioquímica i Biologia Molecular, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, 08071-Barcelona, Spain

Received 19 June 1998

Abstract In this study, we have examined the short-term effects of leptin on lipid metabolism in the rat. Acute leptin administration induced hypertriglyceridaemia (31% increase in plasma triacylglycerols) which was not associated with changes in lipoprotein lipase activity in white adipose tissue. Surprisingly, leptin administration did not induce any changes in the lipogenic rate in either white adipose tissue or liver. Leptin administration caused a decreased tissue uptake of exogenous ^{14}C-triacylglycerols. These data suggest that leptin induces important changes in lipid uptake in adipose tissue and skeletal muscle which could be responsible for the observed hypertriglyceridaemia.

Key words: Leptin; Lipogenesis; Lipoprotein lipase; Triacylglycerol; Adipose tissue; Skeletal muscle

1. Introduction

The *ob* gene protein, also known as leptin, is a 16-kDa protein synthesized and secreted by adipose tissue in proportion to fat stores. It has been proposed to have a role as a ponderostat signal informing the brain of the adipose tissue mass and therefore to be involved in the regulation of energy balance [1–4]. Administration of recombinant leptin reduces food intake and produces weight loss [2,3,5], increases energy expenditure [2,6] and increases plasma insulin and glucose [7] in *ob/ob* mice. Leptin also decreases food intake and body weight in non-obese mice [2,3], therefore a failure to produce adequate amounts of leptin or resistance to its central actions would result in the development of obesity.

Leptin is thought to exert its actions on energy homeostasis through the long form of the leptin receptor which is present in the hypothalamus (although at least five other receptor forms have been cloned) and in certain peripheral organs, including adipose tissue. In the hypothalamus, leptin may modulate the activity of neuropeptide Y (NPY), glucagon-like peptide-1 (GLP-1), and other peptides that influence feeding behaviour [8–11]. Indeed, Fan et al. [12] and Huszar et al. [13] have shown that the melanocortin-4 receptor and its peptide ligand, melanocyte-stimulating hormone (MSH), are important in the pathogenesis of obesity in mice with the yellow Agouti mutation. Very recently, Kristensen et al. [14] have shown that CART (cocaine- and amphetamine-regulated transcript) is a satiety factor that may mediate hypothalamic leptin action. Many studies have indicated that leptin and obesity are clearly associated. Thus, *ob/ob* mice are obese and manifest great similarities to animals with lesions in the ventromedial hypothalamus, leading to the prediction that leptin acts on the central nervous system to suppress appetite. This hypothesis is supported by the fact that intraventricular infusion of leptin is more effective than intraperitoneal administration in causing weight loss in mice [5]. However, pair-feeding studies in both obese and lean mice have demonstrated that leptin effects cannot be accounted for entirely by reduced food intake [15]. Thus it is not clear whether leptin acts solely through its central effects or peripherally via functional receptors detected in non-neuronal tissues, such as lung, liver, kidney, gonads and fat [16–19]. In addition other molecules may also be involved. Thus, tumour necrosis factor-α (TNF) contributes to obesity-related hyperleptinemia by regulating leptin release from adipocytes [20]. Indeed, the elevated expression of the cytokine in adipose tissue during obesity [21] may be responsible for the increased leptin production [20]. Leptin also increases the expression of uncoupling proteins [22], UCP1 (present only in brown adipose tissue) and UCP2 (widely distributed), and this may certainly be related to the effects of the ob protein on energy expenditure. In fact, the effects on UCP1 could be mediated by β_3-adrenergic receptors [22].

In spite of the plethora of investigations that have appeared since its recent discovery, very few studies have concentrated on leptin metabolic effects, particularly in relation to lipid metabolism. Therefore it was the aim of the present investigation to see if the acute administration of leptin to rats could have any effects on lipid metabolism.

2. Materials and methods

2.1. Animals

All animals (male Wistar rats weighing 65–85 g) were fed ad libitum on a chow diet consisting (by weight) of 54% carbohydrate, 17% protein and 5% fat (the residue was non-digestible material), with free access to drinking water, and were maintained at an ambient temperature of 22 ± 2°C with a 12-h light/12-h dark cycle (lights on from 08.00 h).

Animals were intravenously injected with murine recombinant leptin (1 mg/kg body weight) dissolved in sterile saline, and sacrificed 3.5 h later under diethyl ether anaesthesia. Samples of arterial blood and tissues (skeletal muscle, heart, adipose tissue and liver) were rapidly collected for further processing.

2.2. Biochemicals

Biochemicals were all reagent grade and obtained either from Boehringer Mannheim S.A. (Barcelona, Spain) or from Sigma Chemical Co. (St. Louis, MO, USA). Radiochemicals were purchased from Amersham Int. (Amersham, Bucks., UK). Recombinant murine leptin was purchased from PeproTech Ltd. (London, UK). The purity of the reagent was greater than 95% and it contained less than 0.1 ng/μg of endotoxin.

2.3. Circulating leptin and insulin

Plasma leptin was determined by means of a rat radioimmunoassay kit (Linco Res. Inc., St. Charles, MO, USA). Plasma insulin was quantified with a rat radioimmunoassay kit from Amersham Int. (Amersham, Bucks., UK).

*Corresponding author. Fax: (34) (93) 4021559.
E-mail: argiles@porthos.bio.ub.es

PII: S0014-5793(98)00784-4

2.4. Circulating triacylglycerols and cholesterol

Plasma triacylglycerols were measured by the method of Eggstein and Kreutz [23]. Plasma cholesterol was measured by means of a kit (Menarini Diagnostics, Italy).

2.5. Lipoprotein lipase activity

Tissue lipoprotein lipase (LPL) activity was measured by a modification of the technique of Nilsson-Ehle and Ekman [24]. Tissue samples (retroperitoneal adipose tissue and tibialis muscle) were homogenized and used in an assay system containing [^{3}H]triolein as substrate; [^{3}H]-fatty acids released after a 30-min incubation period were extracted and determined by the method of Nilsson-Ehle and Schotz [25].

2.6. Lipogenic rate in vivo

Tissue lipogenesis and liver cholesterogenesis were measured by using ^3H_2O as previously described [26]. At 1 h before being sacrificed, the animals were injected with 0.2 ml (2 mCi) of ^3H_2O intraperitoneally. Tissues were saponified and fatty acids extracted by the method of Stansbie et al. [27].

2.7. Lipid oxidation and tissue lipid accumulation

The metabolic fate of an orally administered [^{14}C]-lipid load was examined as described by Oller do Nascimiento and Williamson [28] in another group of animals of the same characteristics indicated above. About 0.5 g (2 μCi) of [^{14}C]triolein per rat were given enterally by gastric intubation, without anaesthetic but with minimal stress to the animal. After 3.5 h, the animals were killed, using diethyl ether as anaesthesia. The gastrointestinal tract (plus contents) was homogenized in 150 ml of 3% (w/v) $HClO_4$. Samples were taken of liver, interscapular brown adipose tissue (IBAT), retroperitoneal white adipose tissue (WAT), hindleg muscle and heart. Samples of tissues were saponified and the lipid was extracted [27]. The extracted fatty acids were dissolved in 8 ml of liquid scintillation fluid for determination of [^{14}C]-lipid formation. Triolein absorption was calculated by subtracting total gastrointestinal radioactivity from that administered.

2.8. Statistical analysis

Statistical analysis of the data was performed by means of Student's *t*-test.

3. Results and discussion

In spite of the plethora of investigations devoted to leptin, very few have considered the effects of leptin on intermediate metabolism. The results presented here constitute the first study of the short-term effects of leptin administration on lipid metabolism in the rat. Previous investigations have shown that in addition to being produced by adipocytes [1] and having a role in the control of food intake [29], leptin has important effects on thermogenesis and metabolism. Leptin stimulates glucose metabolism increasing glucose turnover and decreasing liver glycogen [30]. Leptin also seems to act on muscle metabolism where it increases glycogen [30] and induces fatty acid oxidation [31]. This last effect is observed in incubating muscle, therefore demonstrating that leptin effects are direct. In spite of these studies, very few have concentrated on the metabolism of the adipocyte, the leptin-producing cell. Very recent studies suggest that leptin can directly modulate adipose tissue lipolysis, both in normal rats [32] and obese (*ob*/*ob*) mice [33], but not in obese *fa*/*fa* rats or *db*/*db* mice, where there is a lack of leptin receptor function. Leptin produced by the fat cells could constitute a mechanism to modulate its own lipid metabolism. The main objective of this investigation was therefore to analyze the short-term effects of exogenous leptin administration on lipid metabolism, particularly in adipose tissue.

Table 1
Plasma leptin and insulin levels following leptin administration

	Experimental group	
	Control	Leptin-treated
Leptin (ng/ml)	0.74 ± 0.13	5.78 ± 1.63***
Insulin (ng/ml)	0.60 ± 0.03	0.63 ± 0.07

For more details, see Section 2. The values are mean ± S.E.M. for five animals. Statistical significance of the results (Student's *t*-test) (leptin vs. control): ***$P < 0.001$.

Following leptin administration, the levels of the protein increased 7.8-fold in relation with the non-treated controls (Table 1). Interestingly, leptin administration did not cause any changes in circulating insulin (Table 1). It has been previously reported that leptin impairs metabolic actions of insulin in adipose tissue [34] and muscle [31]. In addition, the circulating levels of leptin seem to be correlated with those of circulating insulin [35]. However, non-insulin-dependent diabetics show leptin levels which do not differ from those of non-diabetic humans of the same body mass index [36]. Although *ob* gene expression in rats is up-regulated by insulin under euglycaemic conditions, interestingly short-term insulin administration does not increase leptin secretion in humans [37]. Our data seem to indicate that the contrary is also true, at least in experimental animals.

Leptin administration resulted in an increase in plasma triacylglycerols (31%) which was not associated with changes in total plasma cholesterol (Table 2). We therefore decided to investigate the mechanism responsible for the leptin-induced hypertriglyceridaemia. One of the factors that could be involved is a decreased clearance of circulating triacylglycerols in the form of either chylomicra or very low density lipoproteins (VLDL). In fact, the uptake of this type of triacylglycerols is mediated through the activity of lipoprotein lipase (LPL), an enzyme present in the endothelium of many tissues that cleaves triacylglycerols into fatty acids and glycerol and allows the cell internalization of these metabolites. Surprisingly, leptin administration did not have any effects on LPL

Table 2
Tissue lipoprotein lipase activity and circulating triacylglycerols and cholesterol in leptin-treated rats

Tissue	Experimental group	
	Control	Leptin-treated
Plasma triacylglycerols (mg/100 ml)	72.2 ± 1.7	94.8 ± 7.4*
Plasma cholesterol (mg/100 ml)	83.5 ± 2.6	80.6 ± 4.1
LPL activity		
White adipose tissue (retroperitoneal fat pads)	0.830 ± 0.067	0.687 ± 0.069
Skeletal muscle (tibialis)	0.158 ± 0.006	0.141 ± 0.006

For further details, see Section 2. Lipoprotein lipase (LPL) activity is expressed as nmol of fatty acid released/min per mg of tissue. The results are mean values ± S.E.M. for five animals. Statistical significance of the differences (leptin vs. control) (Student's *t*-test): *$P < 0.05$.

Table 3
Tissue lipogenic and cholesterogenic rates in leptin-treated rats

Tissue		Experimental group	
		Control	Leptin-treated
Liver	a	9.61 ± 1.22	9.49 ± 1.83
	b	41.1 ± 5.41	41.7 ± 8.92
	c	1.73 ± 0.14	1.88 ± 0.26
	d	7.42 ± 0.48	8.26 ± 1.26
White adipose tissue	a	5.38 ± 1.10	7.36 ± 1.75
	b	0.85 ± 0.20	0.88 ± 0.19
Brown adipose tissue	a	29.0 ± 5.26	23.6 ± 4.00
	b	7.33 ± 0.54	8.47 ± 0.78

For further details, see Section 2. Tissue lipogenesis is expressed both as: (a) μmol of ^3H_2O/h/g of tissue and (b) μmol of ^3H_2O/h/total tissue (in the case of adipose tissue, only retroperitoneal fat pads were considered). Cholesterogenic rate was calculated as (c) μmol of ^3H_2O/h (non-saponified lipids)/g of tissue or (d) μmol of ^3H_2O/h (non-saponified lipids)/total tissue. The results are mean values ± S.E.M. for five animals.

activity in white adipose tissue. It has been previously reported that chronic leptin administration (20 mg/kg/day) in mice resulted in decreased circulating triacylglycerols with an increase in LPL gene expression [22]. Similarly Chen et al. [38] reported that sustained hyperleptinemia of 8 ng/ml induced for 28 days by infusing a recombinant adenovirus containing the rat leptin cDNA (AdCMV-leptin) in rats resulted in hypotriglyceridaemia. It has to be pointed out, however, than these chronic leptin administration models involve an important reduction in food intake which partly accounts for the reduced triacylglycerol concentration. To further investigate the cause of the hypertriglyceridaemia, we decided to analyze the lipogenic rate in vivo as estimated by the incorporation of ^3H_2O into tissue lipids. Interestingly, the lipogenic rate was unchanged in the liver, suggesting that an increased hepatic fatty acid synthesis and ulterior esterification and VLDL release were not involved in the increase in circulating triacylglycerols that followed leptin administration (Table 3). Both WAT and IBAT lipogenic rates were also unaltered by leptin administration.

From the results obtained it seems quite clear that an enhanced liver lipogenic rate could not account for the leptin-induced hypertriglyceridaemia. In spite of the fact that administration of the ob protein resulted in no significant changes in tissue LPL activity, a tendency for the activity values to be lower is observed in both adipose tissue and skeletal muscle. Since adipose tissue and skeletal muscle represent about 50% of body weight in the rat [39], a small decrease in LPL activity could be responsible for the observed increase in circulating triacylglycerols. To further investigate this possibility, we decided to examine the tissue uptake of an exogenous triacylglycerol load (Table 4). The results clearly show that the total uptake of [^{14}C]triolein was decreased both in skeletal (34%) and cardiac (30%) muscles and white (34%) and brown (57%) adipose tissues, while no changes in uptake were observed in liver (Table 4). Interestingly, leptin administration resulted in no changes in the intestinal absorption of the exogenous lipid load (Table 4). The results presented here are in agreement with those of Chen et al. [38] demonstrating that in vivo hyperleptinemia (induced in normal rats by adenovirus gene transfer) completely ablated fat tissue suggesting a specific lipoatrophic activity for leptin. The same research group [40] has shown that leptin depletes triacylglycerol content in the liver, skeletal muscle and pancreas without increasing fatty acid oxidation, thus suggesting intracellular oxidation. Our results are also in full agreement with those of Muoio et al. [31], clearly demonstrating that leptin alters lipid partitioning in skeletal muscle and suggesting that, in some cases, the skeletal muscle mass can exert important effects on triglyceridaemia.

Acknowledgements: This work was supported by grants from the Fondo de Investigaciones Sanitarias de la Seguridad Social (F.I.S.) (97/2059) of the Spanish Health Ministry, from the DGICYT (PB94-0938) of the Spanish Ministry of Education and Science, and from the Fundació Pi i Sunyer (E00667).

Table 4
Intestinal absorption and metabolic fate of [^{14}C]triolein in leptin-treated rats

Parameter	Experimental group	
	Control	Leptin-treated
[^{14}C]Triolein absorption (% of administered dose)	85.4 ± 2.7	86.4 ± 1.6
[^{14}C]-Lipid accumulation (% of absorbed dose)		
Liver	0.975 ± 0.063	0.956 ± 0.059
White adipose tissue	2.660 ± 0.330	1.260 ± 0.380*
Brown adipose tissue	1.422 ± 0.298	0.610 ± 0.177*
Skeletal muscle (tibialis)	0.228 ± 0.022	0.151 ± 0.024*
Heart	0.246 ± 0.013	0.173 ± 0.010**

For further details, see Section 2. Intestinal absorption was assessed 3.5 h after an oral [^{14}C]triolein administration. Tissue lipid accumulation values are expressed as % of absorbed dose/g. The results are mean values ± S.E.M. for five animals. Values that are significantly different (leptin vs. control) by Student's *t*-test are indicated by: *$P < 0.05$, **$P < 0.01$.

References

[1] Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L. and Friedman, J.M. (1994) Nature 372, 425–432.
[2] Pelleymounter, M.A., Callen, M.J., Baker, M.B., Hecht, R., Winters, D., Boone, T. and Collins, F. (1995) Science 269, 540–543.
[3] Halaas, J.L., Gajiwala, K.S., Maffei, M., Cohen, S.L., Chait, B.T., Rabinowitz, D., Lallone, R.L., Burley, S.K. and Friedman, J.M. (1995) Science 269, 543–546.
[4] Caro, J.F., Sinha, M.K., Kolaczynski, J.W., Zhang, P.L. and Considine, R.V. (1996) Diabetes 45, 1455–1462.
[5] Campfield, L.A., Smith, F.J., Guisez, Y., Devos, R. and Burn, P. (1995) Science 269, 546–549.
[6] Levin, N., Nelson, C., Gurney, A., Vandlen, R. and De Sauvage, F. (1996) Proc. Natl. Acad. Sci. USA 93, 1726–1730.
[7] Schwartz, M.W., Baskin, D.G., Bukowski, T.R., Kuijper, J.L., Foster, D., Lasser, G., Prunkard, D.E., Porte, D., Woods, S.C., Seeley, R.J. and Weigle, D.S. (1996) Diabetes 45, 531–535.
[8] Schwartz, M.W., Seeley, R.J., Campfield, L.A., Burn, P. and Baskin, D.G. (1996) J. Clin. Invest. 98, 1101–1106.
[9] Goldstone, A.P., Mercer, J.G., Gunn, I., Moar, K.M., Edwards, C.M., Rossi, M., Howard, J.K., Rasheed, S., Turton, M.D., Small, C., Heath, M.M., O'Shea, D., Steere, J., Meeran, K., Ghatei, M.A., Hoggard, N. and Bloom, S.R. (1997) FEBS Lett. 415, 134–138.
[10] Wang, Q., Bing, C., Al-Barazanji, K., Mossakowaska, D.E., Wang, X.M., McBay, D.L., Neville, W.A., Taddayon, M., Pickavance, L., Dryden, S., Thomas, M.E., McHale, M.T., Gloyer, I.S., Wilson, S., Buckingham, R., Arch, J.R., Trayhurn, P. and Williams, G. (1997) Diabetes 46, 335–341.
[11] Sahu, A. (1998) Endocrinology 139, 795–798.
[12] Fan, W., Boston, B.A., Kesterson, R.A., Hruby, V.J. and Cone, R.D. (1997) Nature 385, 165–168.
[13] Huszar, D., Lynch, C.A., Fairchild-Huntress, V., Dunmore, J.H., Fang, Q., Berkemeier, L.R., Gu, W., Kesterson, R.A., Boston, B.A., Cone, R.D., Smith, F.J., Campfield, L.A. and Lee, F. (1997) Cell 88, 131–141.
[14] Kristensen, P., Judge, M.E., Thim, L., Ribel, U., Christjansen, K.N., Wulff, B.S., Clausen, J.T., Jensen, P.B., Madsen, O.D., Vrang, N., Larsen, P.J. and Hastrup, S. (1998) Nature 393, 72–76.
[15] Levin, N., Nelson, C., Gurney, A., Vandlen, R. and De Sauvage, F. (1996) Proc. Natl. Acad. Sci. USA 93, 1726–1730.
[16] Hamann, A. and Matthaei, S. (1996) Exp. Clin. Endocrinol. Diabetes 104, 293–300.
[17] Levin, B.E. and Routh, V.H. (1996) Am. J. Physiol. 271, R491–R500.
[18] Schwartz, M.W., Seeley, R.J., Campfield, L.A., Burn, P. and Baskin, D.G. (1996) J. Clin. Invest. 98, 1101–1106.
[19] Trayhurn, P. and Rayner, D.V. (1996) Biochem. Soc. Trans. 24, 565–570.
[20] Kirchgessner, T.G., Uysal, K.T., Wiesbrock, S.M., Marino, M.W. and Hotamisligil, G.S. (1997) J. Clin. Invest. 100, 2777–2782.
[21] Hotamisligil, G.S., Shargill, N.S. and Spiegelman, B.M. (1993) Science 259, 87–90.
[22] Sarmiento, U., Benson, B., Kaufman, S., Ross, L., Qi, M., Scully, S. and DiPalma, C. (1997) Lab. Invest. 77, 243–256.
[23] Eggstein, M. and Kreutz, F.H. (1966) Klin. Wochenschr. 44, 262–267.
[24] Nilsson-Ehle, P. and Ekman, R. (1977) Artery 3, 197–209.
[25] Nilsson-Ehle, P. and Schots, M.C. (1976) J. Lipid Res. 17, 536–541.
[26] Robinson, A.M., Girard, J.R. and Williamson, D.H. (1978) Biochem. J. 176, 343–346.
[27] Stansbie, D., Brownsey, R.W., Crettaz, M. and Denton, R.M. (1986) Biochem. J. 160, 413–416.
[28] Oller do Nascimiento, C.M. and Williamson, D.H. (1986) Biochem. J. 239, 233–236.
[29] Friedman, J.M. (1997) Nature 385, 119–120.
[30] Kamohara, S., Burcelin, R., Halaas, J.L., Friedman, J.M. and Charron, M.J. (1997) Nature 389, 374–377.
[31] Muoio, D.M., Dohn, G.L., Fiedorek, F.T., Tapscott, E.B. and Coleman, R.A. (1997) Diabetes 46, 1360–1363.
[32] Siegrist-Kaiser, C.A., Pauli, V., Juge-Aubry, C.E., Boss, O., Pernin, A., Chin, W.W., Cusin, I., Rohner-Jeanrenaud, F., Burger, A.G., Zapf, J. and Meier, C.A. (1997) J. Clin. Invest. 100, 2858–2864.
[33] Frühbeck, G., Aguado, M. and Martínez, J.A. (1997) Biochem. Biophys. Res. Commun. 240, 590–594.
[34] Muller, G., Ertl, J., Gerl, M. and Preibisch, G. (1997) J. Biol. Chem. 272, 10585–10593.
[35] Zimmet, P.Z. and Collier, G.R. (1996) Med. J. Aust. 164, 393–394.
[36] Haffner, S.M., Stern, M.P., Miettinen, H., Wei, M. and Gingerich, R.L. (1996) Diabetes 45, 822–824.
[37] Kolaczynski, J.W., Nyce, M.R., Considine, B.V., Boden, G., Nolan, J.J., Henry, R., Mudaliar, S.R., Olefsky, J. and Caro, J.F. (1996) Diabetes 45, 699–701.
[38] Chen, G., Koyama, K., Yuann, X., Lee, Y., Zhou, Y.T., O'Doherty, R., Newgard, C.B. and Unger, R.H. (1996) Proc. Natl. Acad. Sci. USA 93, 14795–14799.
[39] Arola, L., Herrera, E. and Alemany, M. (1979) Rev. Esp. Fisiol. 35, 215–218.
[40] Shimabukuro, M., Koyama, K., Cheng, G., Wang, M.Y., Trieu, F., Lee, Y., Newgard, C.B. and Unger, R.H. (1997) Proc. Natl. Acad. Sci. USA 94, 4637–4641.