Korean J Physiol Pharmacol
Vol 9: 125-130, April, 2005

Insulin Resistance of Skeletal Muscle was Recovered by Leptin Injection *in vivo*, but not *in vitro*, in High-fat Diet Fed Rats

Kyung-Oh Doh*, Jeong-Oak Park*, Jeong-Ryae Jeon, Jong-Yeon Kim

Department of Physiology, Yeungnam University College of Medicine, Daegu 705-717, Korea

We examined the effect of leptin on the insulin resistance in skeletal muscles by measuring the glucose transport. Male Wistar rats were fed with chow or high-fat diets for 30 days. Three days before sacrifice, high-fat fed rats were subcutaneously injected with leptin (1 mg/kg body weight) for 3 days. The glucose transports in the epitrochlearis and soleus muscle were not different among the experimental groups under basal state, however these were decreased significantly in the high fat-diet rats under insulin-stimulation ($p<0.01$). Leptin treatment recovered the decreased glucose transport in the epitrochlearis ($p<0.05$) and soleus ($p=0.08$). Triglyceride concentration in the soleus muscle was increased significantly in the high fat-fed rats, compared to chow diet rats ($p<0.01$), and it was decreased significantly by leptin treatment ($p<0.01$). The glucose transport was measured under basal and 60 μU/ml of insulin with or without 50 ng/ml of leptin. Leptin had no direct stimulatory effect on glucose transport under both basal and insulin-stimulated conditions *in vitro*. These results demonstrate that leptin injection to high fat diet fed rats recovered impaired insulin responsiveness of the skeletal muscles and muscle triglyceride concentration. However, there was no direct stimulatory effect of leptin on insulin sensitivity of the skeletal muscle *in vitro*.

Key Words: Leptin, Glucose transport, Insulin, Skeletal muscle

INTRODUCTION

Fat is a physiologically important energy store in the body, however its excessive accumulation results in various metabolic disorders such as diabetes, hyperlipidemia, and coronary heart disease. As accumulation of fat increases, the production of leptin, the protein product of the ob gene, increases in fat cells. Most significantly, leptin affects the feeding regulatory center in the hypothalamus, where it binds to the leptin receptor in order to prevent obesity by reducing food intake through the inhibition of secretion of neuropeptide Y and by increasing lipid oxidation through the sympathetic nerve system (Schwartz et al, 1996).

Since leptin receptors have been shown to be expressed in skeletal muscles, fat cells, and pancreatic β-cells as well as the hypothalamus, studies on their physiological significance were vigorously pursued (Tartaglia et al, 1995; Girard, 1997; Siegrist-Kaiser et al, 1997; Houseknecht, 1998; Hickey & Calsbeek, 2001). Although leptin decreases the secretion of insulin in pancreatic β-cells (Timothy et al, 1997; Seufert et al, 1999), it can also increase the insulin sensitivity in the peripheral tissues, when treated for a long period of time (Rouru et al, 1999; Yaspelkis et al, 1999; Malendowicz et al, 2004). It has, therefore, been assumed that such leptin function can possibly cure hyperinsulinemia caused by obesity and insulin resistance. Recently, adipose tissues were reported to be an active endocrine organ that secretes leptin, plasminogen activator inhibitor-1, angiotensinogen, and cytokines, such as tumor necrosis factor-α, interleukin-6 and transforming growth factor-β. (Mohamed-Ali et al, 1998). Therefore, fat cells seem to control the peripheral tissues and various metabolic processes through these mediators. It was reported that the principal target tissues of those mediators are mostly skeletal muscles (Tartaglia, 1997), therefore, the metabolic link between adipocytes and skeletal muscles has attracted a great deal of attention. Taking up about 40% of the body weight, skeletal muscles are the nearest to intramuscular adipocyte, so that the mediators secreted from fat cells can act in paracrine form. It is also known that skeletal muscles are the most important organ for metabolic insulin action, since insulin resistance is expressed in high-fat diets fed animals (Rifkin & Porte, 1991). Moreover, since leptin receptors are expressed in skeletal muscles, leptin's function as a mediator for delivering signals from adipose tissues to skeletal muscles is being noticed. In addition to such direct action of leptin on skeletal muscles, it indirectly affects glucose metabolism through the central nervous system or by altering fat metabolism. However, its effectiveness still remains debated. Some reported that the glucose metabolism increases after leptin was administrated to a living body (Kamohara et al,

Corresponding to: Jong-Yeon Kim, Department of Physiology, Yeungnam University College of Medicine, 317-1 Daemyeong-dong, Nam-gu, Daegu 705-717, Korea. (Tel) 82-53-620-4332, (Fax) 82-53-651-3651, (E-mail) jykim@med.yu.ac.kr
*These authors contributed equally to the study.

ABBREVIATIONS: 2-DG, 2-[1, 2-^{3}H]-deoxy-D-glucose; HF, high-fat diet group; HF-L, leptin treated high-fat diet group; TG, triglyceride.

1997; Burcellin et al, 1999; Yaspelkis et al, 2004) or to cells in an incubation medium (Bates et al, 2002), whereas others observed no effect or inhibition in animals (Rossetti et al, 1997; Widdowson et al, 1998) and cells (Sweeney et al, 2001; Tajmir et al, 2003). When treated for a long period of time, however, insulin sensitivity was increased (Rouru et al, 1999; Yaspelkis et al, 1999; Yaspelkis et al, 2004), and glucose metabolism also increased, when injected directly into the ventromedial nucleus of hypothalamus (Minokoshi et al, 1999). However, the direct effects of leptin in skeletal muscles have variously been reported and remains controversial; it increases the glucose metabolism in C2C12 myotubes and skeletal muscles (Berti et al, 1997; Ceddia et al, 1998), and it dose not affect the glucose metabolism of skeletal muscles directly (Furnsinn et al, 1998; Ranganathan et al, 1998; Zierath et al, 1998), or it rather inhibits the glucose metabolism of skeletal muscles (Liu et al, 1997). Skeletal muscles use stored glycogen and triglycerides, and glucose comes from oral ingestion as the main source of energy. High-fat diets increase the triglycerides concentration in skeletal muscles (Corbalan et al, 1999), and its excessive accumulation inhibits the glucose metabolism, which in turn causes insulin resistance (Koyama et al, 1997). Leptin stimulates the expression of uncoupling protein-2 in adipocytes (Zhou et al, 1997), decreases the storage of triglycerides in fat cells and liver (Lopez-Soriano et al, 1998), and inhibits the incorporation of free fatty acids into triglycerides in skeletal muscles (Muoio et al, 1999). Such results suggest the possibility of improving the insulin sensitivity by reducing the amount of triglycerides in skeletal muscles.

In this study, the glucose transport of skeletal muscles was examined in order to elucidate leptin's effect on the glucose transport in skeletal muscles. After induction of insulin resistance by high-fat diets, leptin was injected into the peritoneum for 3 days (*in vivo* effect), and also leptin was added to incubation media of skeletal muscles *in vitro* in order to evaluate leptin's direct effect (*in vitro* action). The amount of abdominal fat and the concentration of triglycerides in skeletal muscles were measured in order to examine the mechanism of insulin resistance of skeletal muscles and leptin's effects on them.

METHODS

Materials

2-[1,2-^{3}H]-deoxy-D-glucose (2-DG) was obtained from American Radiolabeled Chemicals (St. Louis, MO, USA), and D-[1-^{14}C]mannitol was from NEN Life Science Products (Boston, MA, USA). Insulin was purchased from Novo Nordisk. All other reagents were obtained from Sigma Chemical (St. Louis, MO, USA).

Animals

Wistar male rats (~50 g) were used for the study of indirect effect of leptin in high-fat diet groups, and were given rat chow or high-fat diet for 4 weeks (Table 1). Male (~100 g) rats were used for the study of direct effect of leptin.

Leptin administration

Rats fed the high-fat diet were separated into two groups matched for body weight, after 24 days on the diet. The animals in one group were given daily subcutaneous injection of rat leptin (Research Diagnostics, NJ, USA; 1 mg/kg body weigh) between 0900 h and 1,000 h for 3 days before the experiment. The control animals were given daily injections of phosphate buffer.

Muscle and plasma preparation

Food was removed after 1,800 h the day before the experiment. Rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg body weight) and blood samples were drawn from the tail vein for measurement of glucose, insulin, triglycerides and leptin concentration, and then the epitrochlearis and soleus muscles were excised. Before incubation, soleus muscle was longitudinally split into strips with an average weight of 20~25 mg.

Weights of fat-pads

After the muscle dissection was completed, the abdominal cavity was opened, and the epididymal, mesenteric and retroperitoneal fat-pads were excised and weighed.

Incubations of muscle (effects of insulin)

To allow recovery from the dissection and splitting procedures, muscles were incubated for 30 min at 35°C in a shaking incubator in 2 ml of oxygenated Krebs-Henseleit buffer (KHB) supplemented with 8 mmol/l glucose, 32 mmol/l mannitol, and 0.1% bovine serum albumin (BSA). After the 30 min recovery period, epitrochlearis muscles and soleus strips were incubated for 60 min at 35°C in 2 ml of oxygenated KHB containing 8 mM glucose, 32 mM mannitol, and 0.1% BSA in the presence or absence of maximally effective concentration of insulin (2 mU/ml) before measurement of 2-DG transport activity.

Measurement of 2-DG transport activity

Glucose transport activity was measured using 2-DG, according to Kim et al. (1999). After incubation with insulin or stimulation of contraction, muscles were incubated for 20 min at 30°C in 2 ml KHB containing 4 mM 2-[1,2-^{3}H]-DG (1.5 μCi/ml), 36 mM [^{14}C]mannitol (0.2 μCi/ml), 0.1% BSA, and insulin if it was present in the previous incubation. Extracellular space and intracellular concentration of 2-DG (ìmol/ml intracellular water/20 min) were determined according to the method of Kim et al (1999).

Analytical procedures

Plasma glucose concentration was determined, using the glucose oxidase method, by YSI Glucose Analyzer. Plasma insulin and leptin were measured by radioimmuno-assay. Serum triglyceride concentration was measured using a kit obtained from Sigma Chemical (St. Louis, MO, USA). Muscle triglyceride concentration was determined by extracting total lipids from clamp-frozen muscle samples with chloroform-methanol (2 : 1, vol/vol) as described by Folch et al. (1957), separating the chloroform and methanol-water

phases, removing phospholipids and further processing the sample using Frayn and Maycock's (1980) modification of the method of Denton and Randle (1967). Triglycerides were then quantified spectrophotometrically as glycerol using a Sigma enzymatic assay kit (Sigma Chemical, St. Louis, MO, USA).

Statistical analysis

Values are expressed as means±SE. The significance of difference between two groups was evaluated using Student's t-test. For multiple comparisons, a one-way analysis of variance (ANOVA) was used. When ANOVA showed significant differences, post-hoc analysis was performed with the Newman-Keuls multiple range test.

RESULTS

In the high-fat diet group, epididymal, mesenteric, and retroperitoneal fat mass increased significantly, compared with fats in the chow diet group ($p<0.01$), although there was no significant difference in body weight. Increased fats of the abdominal cavity in the high-fat diet group were decreased significantly by leptin treatment ($p<0.05$), but they were still significantly higher than those in the chow-diet group ($p<0.01$) (Table 1). Plasma glucose and triglycerides levels had no significant difference among the groups. Plasma insulin and leptin levels were significantly higher in both the high-fat diet group and the high-fat diet-leptin group ($p<0.01$) compared with the chow diet group. There was no difference in these levels between the high-fat diet group and the high-fat diet-leptin group (Table 2). The glucose transport (μmol/20 min) of the epitrochlearis muscle had no significant difference among the groups under the basal state. However, it was significantly lower in the high-fat diet group than in the chow diet group by insulin stimulation (1.42±0.109 vs 1.94±0.163, $p<0.01$), and significantly higher in the high-fat diet-leptin group than in the high-fat diet group (1.85±0.144 vs 1.42±0.109, $p<0.05$) (Fig. 1). Also, the glucose transport of the soleus muscle had no significant difference among the groups under the basal state. It was significantly lower in the high-fat diet group than in the chow diet group by insulin stimulation (3.61±0.278 vs 4.98±0.370, $p<0.01$), but tended to increase in the high-fat diet-leptin group,

Table 1. Body weight and regional fat mass (g) in experimental groups

	Chow	HF	HF-L
Body weight			
Feeding state	254.5±4.71	253.3±3.50	253.5±1.74
Fasting state	235.8±5.12	244.8±4.13	245.5±1.44*
Total fat	7.1±0.84	11.7±0.54**	9.8±0.44**,#
Epididymal fat	2.2±0.31	3.3±0.22**	2.8±0.18**,#
Mesenteric fat	3.1±0.24	4.1±0.23**	3.6±0.13*,#
Retroperitoneal fat	1.9±0.31	4.3±0.30**	3.4±0.24**,#
n	7	7	8

Values are mean±S.E. *n* indicates the number of experimental cases. HF: high- fat diet group, HF-L: leptin treated high -fat diet group. *$p<0.05$, **$p<0.01$, vs chow. #$p<0.05$, vs HF.

Table 2. Plasma concentrations of glucose, insulin, leptin, and triglyceride (TG) in experimental groups

	Chow	HF	HF-L
Glucose (mg/dl)	91.7±2.6	96.2±3.6	98.5±4.6
TG (mg/dl)	59.6±2.9	63.1±2.6	60.5±4.4
Insulin (uU/ml)	9.8±0.9	17.2±2.1**	16.9±3.1**
Leptin (ng/ml)	0.7±0.1	2.0±0.2**	1.6±0.3**
n	7	7	8

Values are mean±S.E. *n* indicates the number of experimental cases. HF: high- fat diet group, HF-L: leptin treated high -fat diet group. *$p<0.05$, **$p<0.01$, vs chow.

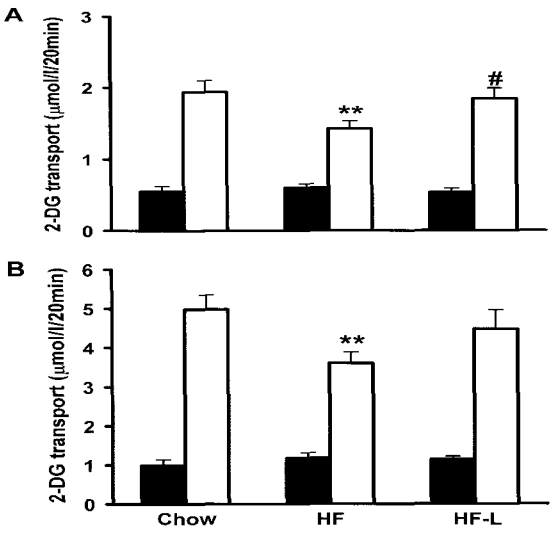

Fig. 1. 2-Deoxy-glucose (2-DG) uptake in epitrochlearis (A) and soleus (B) muscle in chow, high-fat (HF), and leptin treated high-fat (HF-L) diet rats. Open bars indicate basal state and closed bars indicate insulin (2 mU/ml) stimulated state. **$p<0.01$ vs chow, #$p<0.05$ vs HF.

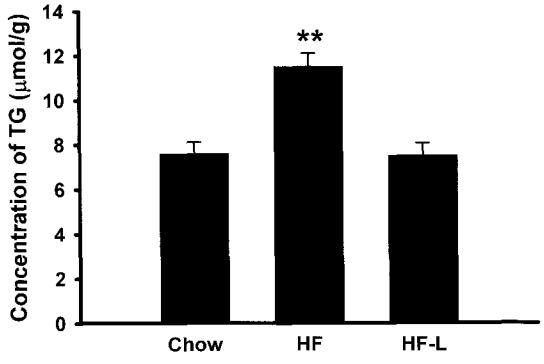

Fig. 2. Concentrations of triglyceride (TG) in soleus muscle in chow, high-fat (HF), and leptin treated high-fat (HF-L) diet fed rats. **$p<0.01$, vs chow or HF-L.

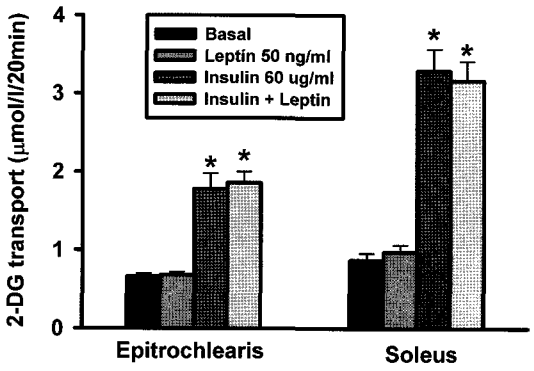

Fig. 3. 2-Deoxy-glucose (2-DG) uptake in epitrochlearis and soleus muscles after incubation with leptin *in vitro* *p<0.01 vs basal or leptin.

compared with the high-fat diet group (4.48±0.484 vs 3.61 ±0.278, p=0.08). The concentration of triglyceride in the soleus muscle was significantly higher (p<0.01) in the high-fat diet group (11.5±0.57 μmol/g) than in the chow diet group (7.6±0.49 μmol/g) (Fig. 2). In case of the high-fat diet- leptin group, it was about the same level as the chow diet group, since it was significantly lower (7.5±0.55, p< 0.01) than in the high-fat diet group. In order to examine the direct effect of leptin on the glucose transport of skeletal muscles, leptin was added to the incubation medium of muscle tissues, and the rate of glucose transport was measured. The result showed that leptin had no effect on the glucose transport of both epitrochlearis and soleus muscles under the basal state. Also, there was no stimulatory effect on the glucose transport by insulin (Fig. 3).

DISCUSSION

Rats fed high-fat diets for 30 days had an increased amount of fat in the abdomen without any significant difference in the body weight, compared with rats on chow diets, and had an increased plasma insulin and leptin levels. Fats accumulated by high-fat diets in the abdominal cavity became significantly lower as a result of leptin treatment for 3 days, but were still significantly high, compared with fats in the chow diet group. This result agrees with the report that leptin was considerably effective in reducing the fats in abdominal cavity (Barzilai et al, 1997).

In the high-fat diet group, the fasting plasma glucose was similar to that in the chow diet group. However, the plasma insulin level in the high-fat diet group was about 1.7 times higher than in the chow diet group. This result apparently differs from the reports that leptin directly acts on pancreatic islets or improves the insulin sensitivity (Timothy et al, 1997; Seufert et al, 1999). The plasma leptin level increased about 2.8 times in the high-fat diet group, compared with the chow diet group, however there was no significant difference due to leptin treatment.

Insulin responsiveness of skeletal muscles was evaluated by the rate of glucose transport. It was evident that insulin resistance developed in the high-fat diet group, since the glucose transport by insulin stimulation decreased significantly in both epitrochlearis muscles and soleus muscles compared with the chow diet group. When leptin was administrated to the high-fat diet group for 3days, the glucose transport of epitrochlearis muscles increased significantly up to the level of the chow-diet group, and it tended to increase also in soleus muscles, thus verifying that insulin responsiveness of skeletal muscles was improved by leptin treatment. This means that leptin can recover insulin resistance of skeletal muscles, in agreement with other reports (Kamohara et al,1997; Burcellin et al, 1999; Minokoshi et al, 1999).

In order to know the direct effect of leptin on the insulin responsiveness of skeletal muscles, leptin was added to the incubation medium of epitrochlearis and soleus muscles. Both muscles did not show any increase of the glucose transport under the basal condition as well as with insulin stimulation. This result agrees with the reports that, although leptin receptors were expressed in skeletal muscles, it did not have direct effects on the glucose transport of skeletal muscles (Furnsinn et al, 1998; Ranganathan et al, 1998; Zierath et al, 1998). In other studies (Berti et al, 1997; Ceddia et al, 1998), it was reported that, although leptin could not magnify the function of insulin, leptin itself to a certain degree could act like insulin. However, as opposed to these reports, our result showed that leptin had no effects at all on the glucose transport under the basal condition. In the above two studies, Berti et al (1997) used NIH3T3 and HepG2 cell strains, while Ceddia et al (1998) used albino rats, although the cultivation time of one hour was same. Therefore, the different results could possibly be explained, based on the condition of skeletal muscles or species difference.

Numerous studies (Storlien et al, 1991; Koyama et al, 1997; Pan et al, 1997) indicate that the increase in the triglyceride of skeletal muscles induces insulin resistance. Therefore the triglyceride of soleus muscles was measured in this study in order to examine whether the improved insulin resistance of skeletal muscles by leptin was related with the decreased triglyceride. The triglyceride of soleus muscles increased by 50% in the high-fat diet group compared with the chow diet group, in agreement with the report that verified the increase in the triglyceride of skeletal muscles in rats fed high-fat diets (Corbalan et al, 1999). It was reported that the accumulation of triglyceride in skeletal muscles on high-fat diets was due to excess energy intake and inhibition of the expression of uncoupling protein-2 and 3, which caused the decrease of lipid oxidation (Corbalan et al, 1999; Muoio et al, 1999). After leptin treatment, however, the triglyceride accumulated in skeletal muscles by high-fat diets decreased to the level of the chow diet group. These outcomes are most likely due to leptin's specific effect, since leptin has been shown to increase the expression of uncoupling protein-2 (Zhou et al, 1997), decrease the influx of triglyceride into adipose tissues (Lopez-Soriano et al, 1998), and inhibit the accumulation of fatty acid as triglyceride in skeletal muscles (Muoio et al, 1999). Therefore, the mechanism of the improved insulin sensitivity of skeletal muscles by leptin in this study can be attributed to the effect of leptin in reducing the triglyceride in skeletal muscles.

There are two pathways that leptin directly affecst the glucose transport of skeletal muscles. First, it combines with the leptin receptor and participates in the signal transfer process related with the glucose transport. Second, it increases the degree of bonding in the insulin receptor residing in the cell membrane, since leptin causes some

changes in lipid components of the cell membrane (Muoio et al, 1997). The former pathway would shown the effect after one-hour preincubation executed in the present study, but the latter might need a longer period of action time. Therefore, the result in the present study could not exclude the change of fat component of the cell membrane as the mechanism of increasing the glucose transport of skeletal muscles with leptin dosage into a living body for three days. In order to examine whether leptin actually increases the glucose transport by changing the components of the cell membrane of skeletal muscles, further studies should be carried out to measure the insulin-binding capacity of skeletal muscles and to analyze lipid components of the cell membrane of skeletal muscles.

To sum up the above results, although leptin was effective in decreasing the amount of fat in abdominal cavity and triglyceride in skeletal muscle induced by high-fat diet as well, no direct effect on the skeletal muscles was shown.

ACKNOWLEDGEMENT

This study was supported by Research Grant from the Yeungnam University Medical Center.

REFERENCES

Barzilai N, Wang J, Massilon D, Vuguin P, Hawkins M, Rossetti L. Leptin selectively decreases visceral adiposity and enhances insulin action. *J Clin Invest* 100: 3105–3110, 1997

Bates SH, Gardiner JV, Jones RB, Bloom SR, Bailey CJ. Acute stimulation of glucose uptake by leptin in L6 muscle cells. *Horm Metab Res* 34:111–5, 2002

Berti L, Kellerer M, Capp E, Haring HU. Leptin stimulates glucose transport and glycogen synthesis in C2C12 myotubes: Evidence for a PI3-kinase mediated effect. *Diabetologia* 40: 606–609, 1997

Burcellin R, Kamohara S, Li J, Tannenbaum GS, Charron MJ, Friedman JM. Acute intravenous infusion increases glucose turnover but not skeletal muscle glucose uptake in ob/ob mice. *Diabetes* 48: 1264–1269, 1999

Ceddia RB, William WN, Curi R. Leptin increases glucose transport and utilization in skeletal muscle *in vitro*. *Gen Pharmacol* 31: 799–801, 1998

Corbalan MS, Margareto J, Martinez JA, Marti A. High-fat feeding reduced muscle uncoupling protein 3 expression in rats. *J Physiol Biochem* 55: 67–72, 1999

Denton RM, Randle PJ. Concentrations of glycerides and phospholipids in rat heart and gastrocnemius muscle: effects of alloxan-diabetes and perfusion. *Biochem J* 104: 416–422, 1967

Folch J, Sloane M, Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226: 497–509, 1957

Frayn KN, Maycock PF. Skeletal muscle triacylglycerol in the rat: methods for sampling and measurement, and studies of biological variability. *J Lipid Res* 21: 139–144, 1980

Furnsinn C, Brunmair B, Furtmuller R, Roden M, Englisch R, Waldhausl W. Failure of leptin to affect basal and insulin-stimulated glucose metabolism of rat skeletal muscle *in vitro*. *Diabetologia* 41: 524–529, 1998

Girard J. Is leptin the link between obesity and insulin resistance? *Diabetes Metab* 23 (Suppl 3): 16–24, 1997

Hickey MS, Calsbeek DJ. Plasma leptin and exercise: recent findings. *Sports Med* 31: 583–9, 2001

Houseknecht KL, Baile CA, Matteri RL, Spurlock ME. The biology of leptin: a review. *J Anim Sci* 76: 1405–1420, 1998

Kamohara S, Burcelin R, Halaas JL, Friedman JM, Charron MJ. Acute stimulation of glucose metabolism in mice by leptin treatment. *Nature* 389: 373–377, 1997

Kim JY, Nolte LA, Hansen PA, Han DH, Kawanaka K, Holloszy JO. Insulin resistance of muscle glucose transport in male and female rats fed a high-sucrose diet. *Am J Physiol* 276 (3 Pt 2): R665–R672, 1999

Koyama K, Chen G, Lee Y, Unger RH. Tissue triglycerides, insulin resistance, and insulin production: Implications for hyperinsulinemia of obesity. *Am J Physiol* 273 (4 Pt 1): E708–E713, 1997

Liu YL, Emilsson V, Cawthorne MA. Leptin inhibits glycogen synthesis in the isolated soleus muscle of obese (*ob/ob*) mice. *FEBS Lett* 411: 351–355, 1997

Lopez-Soriano J, Carbo N, Lopez-Soriano FJ, Argiles JM. Short-term effects of leptin on lipid metabolism in the rat. *FEBS Lett* 431: 371–374, 1998

Malendowicz LK, Gorska T, Tortorella C, Nowak M, Majchrzak M, Spinazzi R, Nussdorfer GG, Nowak KW. Acute *in vivo* effects of leptin and leptin fragments on corticosteroid hormone secretion and entero-insular axis in the rat. *Int J Mol Med* 13: 829–834, 2004

Minokoshi Y, Haque MS, Shimazu T. Microinjection of leptin into the ventromedial hypothalamus increases glucose uptake in peripheral tissues in rats. *Diabetes* 48: 287–291, 1999

Mohamed-Ali V, pinkney JH, Coppack SW. Adipocyte tissue as an endocrine and paracrine organ. *Int J Obes* 22: 1145–1158, 1998

Muoio DM, Dohm GL, Fiedorek FT, Tapscott EB, Coleman RA. Leptin directly alters lipid partitioning in skeletal muscle. *Diabetes* 45: 1360–1363, 1997

Muoio DM, Dohm GL, Tapscott EB, Coleman RA. Leptin opposes insulin's effects on fatty acid partitioning in muscles isolated from obese ob/ob mice. *Am J Physiol* 276 (5 Pt 1): E913–E921, 1999

Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, Jenkins AB, Storlien LH. Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes* 46: 983–988, 1997

Ranganathan S, Ciaraldi TP, Henry RR, Mudaliar S, Kern PA. Lack of effect of leptin on glucose transport, lipoprotein lipase, and insulin action in adipose and muscle cells. *Endocrinology* 139: 2509–2513, 1998

Rifkin H, Porte D. *Diabetes Mellitus*. 4th ed. Elsevier Science Publishing Co, New York, p 457–459, 1991.

Rossetti L, Massillon D, Barzilai N, Vuguin P, Chen W, Hawkins M, Wu J, Wang J. Short term effects of leptin on hepatic gluconeogenesis and *in vivo* insulin action. *J Biol Chem* 272: 27758–27763, 1997

Rouru J, Cusin I, Zakrzewska KE, Jeanrenaud B, Rohner-Jeanrenaud F. Effects of intravenously infused leptin on insulin sensitivity and on the expression of uncoupling proteins in brown adipose tissue. *Endocrinology* 140: 3688–3692, 1999

Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG. Identification of targets of leptin action in rat hypothatamus. *J Clin Invest* 98: 1101–1106, 1996

Seufert J, Kieffer TJ, Leech CA, Holz GG, Moritz W, Ricordi C, Habener JF. Leptin suppression of insulin secretion and gene expression in human pancreatic islets: Implications for the development of adipogenic diabetes mellitus. *J Clin Endocrinol Metab* 84: 670–676, 1999

Siegrist-Kaiser CA, Pauli V, Juge-Aubry CE, Boss O, Pernin A, Chin WW, Cusin I, Rohner-Jeanrenaud F, Burger AG, Zapf J, Meier CA. Direct effects of leptin on brown and white adipose tissue. *J Clin Invest* 100: 2858–2864, 1997

Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WS, Khouri S, Kraegen EW. Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and w-3 fatty acids in muscle phopholipid. *Diabetes* 40: 280–289, 1991

Sweeney G, Keen J, Somwar R, Konrad D, Garg R, Klip A. High leptin levels acutely inhibit insulin-stimulated glucose uptake without affecting glucose transporter 4 translocation in l6 rat skeletal muscle cells. *Endocrinology* 142: 4806–4812, 2001

Tajmir P, Kwan JJ, Kessas M, Mozammel S, Sweeney G. Acute

and chronic leptin treatment mediate contrasting effects on signaling, glucose uptake, and GLUT4 translocation in L6-GLUT 4myc myotubes. *J Cell Physiol* 197: 122–130, 2003

Tartaglia LA. The leptin receptor. *J Biol Chem* 272: 6093–6096, 1997

Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Wool EA, Monroe CA, Tepper RI. Identification and expression cloning of a leptin receptor, OB-R. *Cell* 83: 1263–1271, 1995

Timothy JK, Heller RS, Leech CA, Holz GG, Habener JF. Leptin suppression of insulin secretion by the activation of ATP-sensitive K+ channels in pancreatic β-cells. *Diabetes* 46: 1087–1093, 1997

Widdowson PS, Upton R, Pickavance L, Buckingham R, Tadayyon M, Arch J, Williams G. Acute hyperleptinemia does not modify insulin sensitivity in vivo in rats. *Horm Metab Res* 30: 259–262, 1998

Yaspelkis BB 3rd, Ansari L, Ramey EL, Holland GL, Loy SF. Chronic leptin administration increases insulin-stimulated skeletal muscle glucose uptake and transport. *Metabolism* 48: 671–676, 1999

Yaspelkis BB 3rd, Singh MK, Krisan AD, Collins DE, Kwong CC, Bernard JR, Crain AM. Chronic leptin treatment enhances insulin-stimulated glucose disposal in skeletal muscle of high-fat fed rodents. *Life Sci* 74: 1801–1816, 2004

Zhou YT, Shimabukuro M, Koyama K, Lee Y, Wang MY, Trieu F, Newgard CB, Unger RH. Induction by leptin of uncoupling protein-2 and enzymes of fatty acid oxidation. *Proc Natl Acad Sci USA* 94: 6386–6390, 1997

Zierath JR, Frevert EU, Ryder JW, Berggren PO, Kahn BB. Evidence against a direct effect of leptin on glucose transport in skeletal muscle and adipocytes. *Diabetes* 47: 1–4, 1998