Increase in Plasma Leptin and *Lep* mRNA Concentrations by Food Intake Is Dependent on Insulin

B.K. Patel, J.I. Koenig, L.M. Kaplan, and S.C. Hooi

Obese (*Lep*) gene expression and leptin secretion are regulated by changes in food intake. However, the mechanism by which leptin concentrations are altered by fasting and feeding is unclear. Since these changes occur in parallel with changes in plasma insulin, it is possible that the changes observed are mediated by insulin. To test this hypothesis, we studied the role of insulin in the regulation of *Lep* gene expression in epididymal fat and leptin secretion during feeding. As shown previously, fasted animals showed significant reductions in *Lep* mRNA, plasma leptin, and plasma insulin concentrations. Conversely, feeding increased plasma insulin, *Lep* mRNA, and plasma leptin. In streptozotocin (STZ)-treated animals, plasma insulin concentrations were low. This was associated with low *Lep* mRNA and plasma leptin concentrations. Changes in food intake, whether fasting or feeding, did not significantly alter plasma insulin levels in STZ-treated animals. Under these circumstances, *Lep* mRNA and plasma leptin concentrations also remained low. Our results demonstrate that the decrease in *Lep* mRNA and plasma leptin during fasting and the increase with feeding are dependent on changes in the plasma insulin concentration.

THE RECENT DISCOVERY of the obese (*Lep*) gene[1] has provided new insights into the regulation of energy metabolism in the body. The *Lep* gene is specifically expressed in adipocytes[1,2] and encodes a 167–amino acid secreted protein called leptin. Leptin has potent effects on body weight and appetite. Peripheral and central injections of leptin significantly reduce body weight and food intake in animals.[3-5] Conversely, food intake has effects on both the plasma leptin concentration and *Lep* gene expression. Fasting decreases and refeeding increases *Lep* gene expression[6-8] and the plasma leptin concentration.[9] Changes in *Lep* gene expression have been shown to be associated with parallel changes in plasma insulin concentration.[9-11] However, the role of insulin in mediating changes in *Lep* gene expression and leptin secretion during feeding is unclear. Since insulin itself has a stimulatory effect on *Lep* gene expression and leptin secretion,[12-14] it is possible that the feeding-induced changes in leptin concentrations are dependent on insulin. To test this hypothesis, we studied the effect of streptozotocin (STZ) treatment on *Lep* gene expression and plasma leptin concentrations during fasting and refeeding, and demonstrate that the fasting- and feeding-induced changes in *Lep* gene expression and leptin secretion are dependent on insulin.

MATERIALS AND METHODS

Animals and Treatments

Thirty-five male Sprague-Dawley rats (230 to 270 g) were used for the studies. All of the animals were kept on a 12-hour light-dark cycle (lights on at 8 AM) and given free access to standard rat chow and water, except for rats that were fasted. The rats were randomly divided into seven groups (n = 5 per group) as follows: control (CTR), fasted (FAST), fasted and then refed (FR), STZ-treated, STZ-treated and fasted (SF), STZ-treated, fasted, and refed (SFR), and STZ-treated, fasted, and insulin-treated (SFI).

All animals received an intraperitoneal injection of STZ (Sigma, St Louis, MO; 125 mg/kg body weight) or vehicle (citrate buffer, pH 4.5) at 3 PM on day 1. Tail blood glucose concentrations were determined in all animals 24 hours after the injection using an Accutrend glucometer (Boehringer Mannheim, Indianapolis, IN). Blood glucose levels were also measured at the time of death, 48 hours after treatment, using the Accutrend glucometer. All animals were killed at 3 PM on day 3.

Animals in the FAST, FR, SFR, and SFI groups were fasted by removing the food from the cages at 9 PM on day 2. Food was returned to the cages of the refed groups (FR and SFR) at 9 AM on day 3, and ad libitum feeding was permitted until death at 3 PM. The insulin-treated group (SFI) received a single subcutaneous injection of 2 U human insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) at 9 AM and were killed 6 hours later. The duration of action of the insulin preparation is 8 hours.

All of the animals were killed by decapitation. Trunk blood was collected in chilled tubes containing 200 μL 10% tetrasodium EDTA. The plasma was separated by centrifugation and stored at −70°C for insulin and leptin assays. Epididymal fat was dissected, immediately frozen on dry ice, and stored at −70°C for RNA extraction.

Plasma Insulin and Leptin Assays

Plasma insulin concentrations were determined using a human radioimmunoassay (ICN, Costa Mesa, CA) that cross-reacts 100% with rat insulin; 200 μL plasma was used for the assay. The intraassay coefficient of variation was 2.6%, and the sensitivity was 2.5 μIU/mL. Plasma leptin was determined using a mouse radioimmunoassay (Linco Research, St Charles, MO); 100 μL plasma was used for the assay. The intraassay coefficient of variation was 4%, and the sensitivity was 0.2 ng/mL.

RNA Extraction and Northern Analysis

Total RNA was extracted using guanidinium-thiocyanate as previously described.[15] Fifteen micrograms of RNA was denatured and size-fractionated by 1% formaldehyde agarose gel electrophoresis. The RNA was transferred to a nylon membrane (Qiagen, Chatsworth, CA) and cross-linked by UV irradiation using a Stratalinker (Stratagene, La Jolla, CA).

The rat *Lep* cDNA (pOb22) was provided by Dr L.M. Kaplan (Massachusetts General Hospital and Harvard Medical School, Boston, MA). The pOb22 plasmid contains the entire coding region of the rat *Lep* gene. To make a cDNA probe, the rat *Lep* cDNA was amplified

From the Department of Physiology, Faculty of Medicine, National University of Singapore, Singapore; Maryland Psychiatric Research Center, University of Maryland School of Medicine, Baltimore, MD; and the Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA.

Submitted June 23, 1997; accepted October 26, 1997.

Supported by National University of Singapore Grant No. RP950371.

Address reprint requests to S.C. Hooi, MBBS, PhD, Department of Physiology, Faculty of Medicine, National University of Singapore, 10, Kent Ridge Cresent, Singapore 119260.

0026-0495/98/4705-0019$03.00/0

using the following primers: 5′-CCAGCGAGGAAAATGTGCTG-3′ and 5′-GGAATCGTGCGGATAACTTT-3′. The amplified fragment contains the entire coding region for rat leptin. After amplification, the polymerase chain reaction product was separated by agarose gel electrophoresis, and the rat *Lep* fragment was cut out of the gel and recovered by Qiaquick gel extraction (Qiagen). The fragment was labeled with ^{32}P-dCTP using the Rediprime labeling system (Amersham, Arlington Heights, IL). The 18S rRNA primer was synthesized using a Beckman 1000M oligonucleotide synthesis machine (Beckman, Buckinghamshire, UK). The sequence of the 18S primer is as follows: 5′-GACAAGCATATGCTACTGGC-3′.[16] The 18S oligonucleotide was end-labeled with ^{32}P-γATP using T4 polynucleotide kinase (Promega, Madison, WI). The probe was purified using a Qiaquick nucleotide removal kit (Qiagen).

For hybridization to the *Lep* probe, the Northern blots were prehybridized in 6× SSC (0.9 mol/L NaCl, 0.09 mol/L Na_3citrate · $2H_2O$), 5× Denhardt solution, 0.5% sodium dodecyl sulfate (SDS), and 100 μg/mL denatured, fragmented salmon sperm DNA for 8 hours at 50°C. Denatured *Lep* probe was then added to the prehybridization mix to a concentration of 0.5 × 10^6 cpm/mL and hybridized overnight at the same temperature. The blots were then washed twice in 2× SSC/0.1% SDS at room temperature for 15 minutes each and then another two times in 0.2× SSC/0.5% SDS at 50°C for 15 minutes each.

After hybridization to the *Lep* probe, the blots were stripped by pouring boiling 0.5% SDS solution over the blots and shaking for 10 minutes, followed by a room-temperature wash with 2× SSC for another 10 minutes. The blots were then hybridized to the 18S oligonucleotide to correct for minor variations in loading.

For hybridization to the 18S oligonucleotide probe, the blots were prehybridized in 6× SSC, 0.1% SDS, 200 μg/mL denatured salmon sperm DNA, and 5× Denhardt solution for 8 hours at 42°C before addition of probe to a concentration of 0.5 × 10^6 cpm/mL. The membranes were hybridized to the 18S probe overnight at 42°C. The membranes were washed twice in 6× SSC/0.1% SDS at room temperature for 15 minutes each and then another time in 6× SSC/0.1% SDS for 30 minutes at 50°C.

After the final wash, the blots were exposed to Biomax (Eastman Kodak, Rochester, NY) autoradiography film overnight and developed using a Kodak RP X-Omat developer. The intensity of the bands was quantified by densitometric analysis using a digital imaging system (BIO-1D; Vilber Lourmat, France) and accompanying software (Bio-Profil).

Statistical Analysis

The *Lep* mRNA concentrations were expressed against 18S RNA controls. The mean ratio for the controls was normalized arbitrarily to 100%. The mean values of the various treatment groups were compared using one-way ANOVA followed by the Newman-Keuls multiple comparisons test (SPSS for Windows; SPSS, Inc, Chicago, IL). Differences were considered significant at P less than .05.

Differences in mean blood glucose and plasma leptin and insulin concentrations were also compared using ANOVA followed by the Newman-Keuls multiple comparisons test. Differences were considered significant at P less than .05.

The difference in body weight for each of the animals was calculated by subtracting the weight at the start from the weight at the end of the experiment. The mean difference in body weight was calculated and compared among treatment groups using ANOVA followed by the Newman-Keuls multiple comparisons test.

To determine if there is a significant correlation of plasma leptin concentrations and *Lep* mRNA levels with plasma insulin concentrations, regression statistics for each of the correlations were determined using SigmaPlot software (Jandel Scientific, San Rafael, CA) and Microsoft Excel (Redmond, WA) for Windows version 5.

RESULTS

Effect of STZ and Feeding on Blood Glucose and Body Weights

Control animals gained, on average, about 16 g over 2 days. Animals fasted overnight (12 hours, FAST) gained about 7 g at the end of the experiment. Animals refed for 6 hours also gained about 7 g. STZ treatment resulted in a significant reduction in the body weight of the animals. On average, STZ-treated animals lost about 63 g in body weight after 2 days. Despite the weight loss, STZ-treated animals were observed to eat, although food intake was not quantified (Table 1).

Twenty-four hours after injection of STZ, all animals had blood glucose concentrations greater than 250 mg/100 mL. Blood glucose increased to greater than 400 mg/100 mL 48 hours after the injection, except for the insulin-treated group, which had a mean blood glucose concentration of 91.2 ± 10.3 mg/100 mL. A 12-hour fast decreased blood glucose from 100.0 ± 11.1 to 83.1 ± 5.7 mg/100 mL in non–STZ-treated animals. Refeeding the fasted animals for 6 hours restored blood glucose to control levels. However, in STZ-treated animals, fasting did not significantly decrease blood glucose concentrations. On the other hand, insulin treatment resulted in a significant decrease of blood glucose in STZ-treated animals.

Effect of STZ and Feeding on Plasma Insulin, Leptin, and Lep mRNA Concentrations

Changes in plasma leptin concentrations are shown in Fig 1A. An overnight fast decreased plasma leptin by 73%. Refeeding for 6 hours increased plasma leptin by 60% compared with controls. STZ treatment decreased plasma leptin 60% compared with controls. Fasting in STZ-treated animals did not significantly reduce leptin concentrations further, unlike in control animals. Also, refeeding the fasted STZ-treated animals did not significantly increase leptin concentrations. However, injection of 2 U insulin resulted in a dramatic increase in leptin. Insulin treatment increased plasma leptin about sixfold in STZ-treated rats compared with non–insulin-treated STZ animals and 2.5-fold compared with controls after 6 hours.

The changes in *Lep* mRNA levels in epididymal fat resulting from the various treatments are shown in Fig 1B. Essentially, the changes are qualitatively similar to the changes in plasma

Table 1. Effect of Treatments on Body Weight and Blood Glucose

Group	Weight Gain (g)	Blood Glucose (mg/100 mL)	
		24 h After Injection	At Decapitation
CTR	16.12 ± 5.0	124.6 ± 11.1	117.8 ± 14.0
FAST	6.83 ± 4.0	100.0 ± 11.1	83.1 ± 5.7*
FR	6.78 ± 5.1	108.2 ± 5.3	106.8 ± 7.0
STZ	−62.7 ± 11.1*	295.2 ± 18.2*	449.0 ± 40.2*
SF	−48.20 ± 11.6*	316.8 ± 24.7*	429.0 ± 35.0*
SFR	−51.00 ± 4.11*	305.6 ± 31.3*	444.6 ± 24.6*
SFI	−58.48 ± 5.17*	384.4 ± 40.0*	91.2 ± 10.3*

NOTE. The animals were weighed at the start of the experiment and just before decapitation. Blood glucose was determined using an Accutrend glucometer 24 hours after intraperitoneal injection of STZ or vehicle and again just before decapitation. Results are the mean ± SEM. The treatments are detailed in the Methods and Fig 1.

*$P < .05$ v CTR.

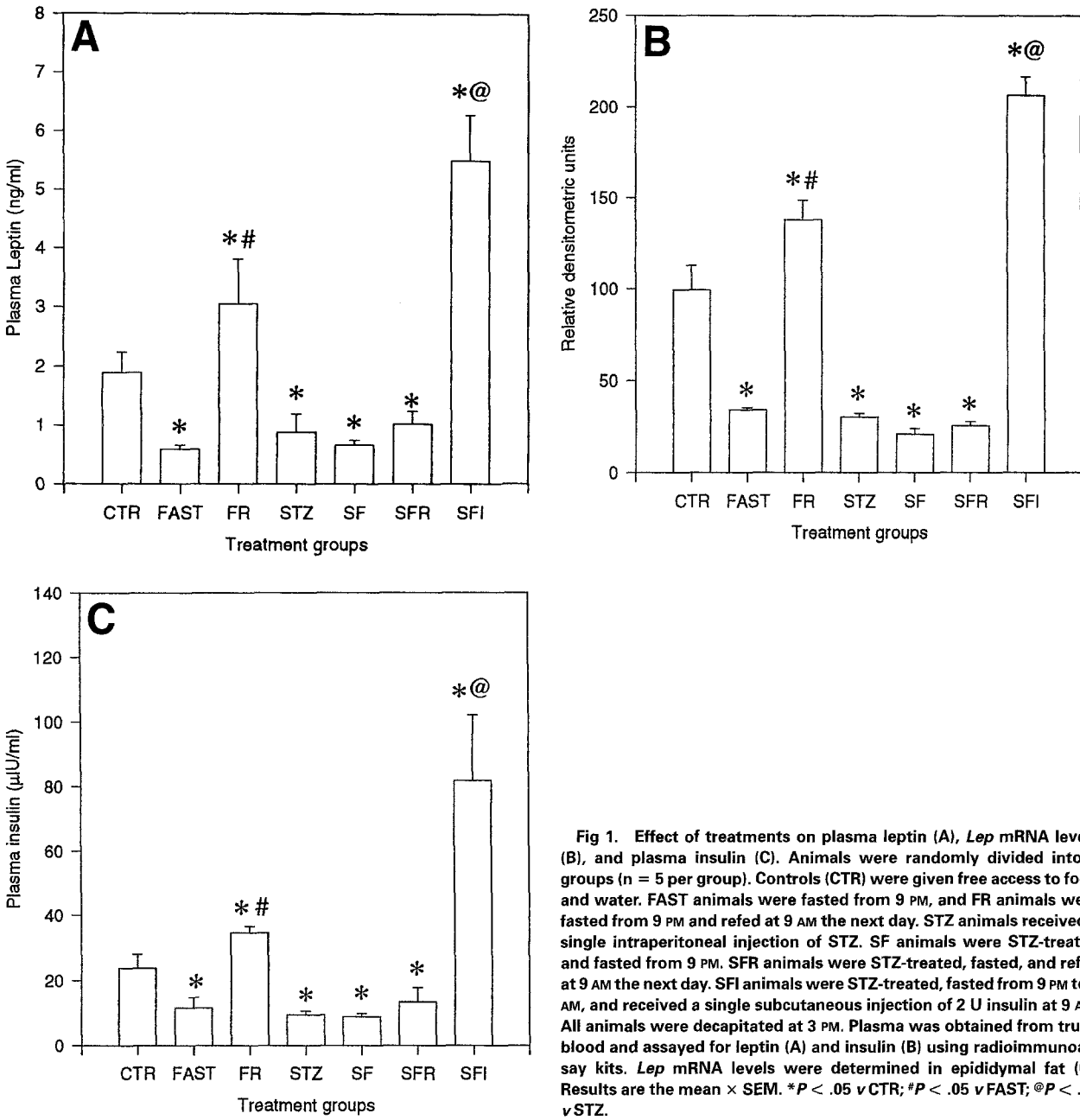

Fig 1. Effect of treatments on plasma leptin (A), *Lep* mRNA levels (B), and plasma insulin (C). Animals were randomly divided into 7 groups (n = 5 per group). Controls (CTR) were given free access to food and water. FAST animals were fasted from 9 PM, and FR animals were fasted from 9 PM and refed at 9 AM the next day. STZ animals received a single intraperitoneal injection of STZ. SF animals were STZ-treated and fasted from 9 PM. SFR animals were STZ-treated, fasted, and refed at 9 AM the next day. SFI animals were STZ-treated, fasted from 9 PM to 9 AM, and received a single subcutaneous injection of 2 U insulin at 9 AM. All animals were decapitated at 3 PM. Plasma was obtained from trunk blood and assayed for leptin (A) and insulin (B) using radioimmunoassay kits. *Lep* mRNA levels were determined in epididymal fat (C). Results are the mean × SEM. *$P < .05$ *v* CTR; #$P < .05$ *v* FAST; @$P < .05$ *v* STZ.

leptin concentrations. Fasting resulted in a 62% reduction in *Lep* gene expression, which was normalized by refeeding. Compared with the controls, STZ treatment resulted in a reduction of *Lep* mRNA to the fasting levels. There was no further reduction in *Lep* mRNA levels when the animals were fasted. Refeeding the STZ-treated and fasted animals did not increase *Lep* mRNA levels. However, insulin injection rapidly increased *Lep* mRNA approximately sevenfold compared with the levels in STZ animals and twofold compared with control levels.

Plasma insulin concentrations paralleled the changes in plasma leptin and *Lep* mRNA levels (Fig 1C). Fasting significantly decreased plasma insulin by 50% compared with controls. Refeeding increased insulin concentrations by 50% compared with controls. STZ treatment decreased insulin levels by greater than 50% compared with controls. Refeeding fasted STZ-treated animals did not significantly increase the insulin levels compared with those in non-refed STZ-treated animals. A single subcutaneous injection of 2 U soluble insulin resulted in a ninefold increase of insulin in STZ-treated animals.

Correlations Between Plasma Insulin, Leptin, and Lep mRNA Levels

Individual plasma leptin concentrations (Fig 2A) and *Lep* mRNA levels (Fig 2B) were plotted against the plasma insulin concentrations. There was a highly significant correlation between plasma insulin and leptin concentrations ($R^2 = .8$, $P < .001$). Similarly, the correlation between plasma insulin

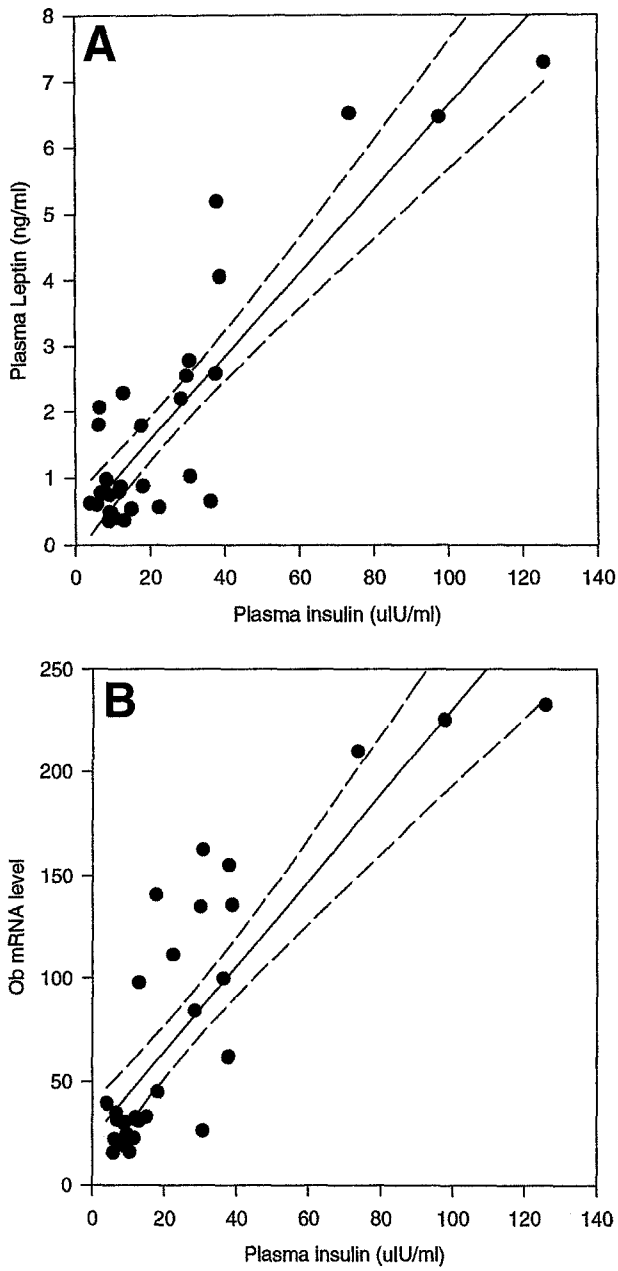

Fig 2. Correlation between plasma insulin, leptin, and *Lep* mRNA levels. Plasma leptin concentrations (A) and *Lep* mRNA levels (B) were plotted against plasma insulin. A regression line (—) and the 95% confidence intervals (-----) were derived using SigmaPlot software.

and *Lep* mRNA levels was also highly significant ($R^2 = .7$, $P < .001$).

DISCUSSION

Leptin has potent inhibitory effects on appetite and weight gain in animals.[3-5,17] It is postulated that leptin serves as an adipose tissue–specific endocrine signal acting on the hypothalamus to effect changes in appetite and metabolism.[18] The presence of leptin receptors in the hypothalamus[19,20] and the efficacy of intracerebral injections of leptin in reducing appetite and body weight are consistent with this hypothesis.[5,21] The changes in plasma leptin concentrations and *Lep* mRNA levels observed in fasting and refeeding are also consistent with the hyopthesis. However, the mechanism by which plasma leptin concentrations and *Lep* mRNA levels in adipose tissue are regulated by fasting and refeeding is unclear.

Plasma leptin and insulin concentrations appear to be regulated in parallel.[22] The data presented in this report suggest a parallel association between plasma insulin, leptin, and *Lep* mRNA levels in adipose tissue. The concentrations of the three parameters (plasma leptin, insulin, and *Lep* mRNA) are regulated in parallel by the various treatments (Fig 1). Figure 2 shows a good correlation between plasma insulin and plasma leptin concentrations (A) and between plasma insulin and *Lep* mRNA levels (B). Given that insulin has a stimulatory effect on *Lep* expression,[12-14] the data support the hypothesis that plasma leptin and *Lep* mRNA levels are regulated by circulating insulin in the body.

STZ treatment results in the ablation of pancreatic islet β cells[23] and a consequent reduction in basal insulin secretion and responsiveness to stimuli that increase or decrease insulin secretion. The dose used in this study causes the animals to become diabetic 24 hours after STZ injection.[24] The significant decrease in plasma insulin 48 hours after STZ injection was associated with a similar decrease in both plasma leptin and *Lep* mRNA levels. This suggests that normal basal levels of insulin are required to maintain basal concentrations of circulating leptin and *Lep* gene expression in adipose tissue. Fasting did not further reduce plasma insulin levels in STZ-treated rats, in contrast to non–STZ-treated rats. Under these circumstances, plasma leptin and *Lep* mRNA levels also were not reduced. It appears that the reduction of plasma leptin concentrations and *Lep* mRNA levels in fasted rats is dependent on the associated reduction in plasma insulin that occurs with fasting. Similarly, the data also show that the refeeding response is dependent on insulin. STZ-treated animals were unable to respond to a stimulus to increase insulin secretion during refeeding. When the increase in insulin is prevented, plasma leptin and *Lep* mRNA levels also are not increased. This shows that the increase in plasma leptin concentrations and *Lep* mRNA levels with food intake is dependent on an increase in plasma insulin. Interestingly, a single injection of insulin increased plasma leptin concentrations and *Lep* mRNA levels, showing that the ability of adipose tissue to respond to insulin is unchanged. The lack of response of adipose tissue to refeeding is not due to a defect in the adipose tissue itself, but results from the inability of the pancreas to secrete insulin. The rapid and marked increase in plasma leptin and *Lep* mRNA levels after 6 hours of insulin treatment is significant in STZ-treated rats compared with both control and STZ-treated animals. The increase in plasma leptin and *Lep* mRNA levels occurred despite no recovery of body weight over this period. Although others have shown stimulatory effects of insulin on *Lep* mRNA[7,25-26] and plasma leptin levels,[27] the observation that insulin rapidly increased plasma leptin and *Lep* mRNA levels in STZ-treated rats is novel. The demonstration of a stimulatory effect of insulin on plasma leptin and *Lep* mRNA levels is important in view of conflicting responses in humans, where acute adminis-

tration of insulin may not have significant effects on plasma leptin concentrations.[28-30] It is likely that there are species differences in the response of leptin to insulin.

The results also suggest that plasma leptin and *Lep* mRNA levels may be more tightly associated with the plasma insulin concentration than with the body weight. Animals refed for 6 hours did not show a significant increase in weight compared with those that were fasted and not refed. Rats fasted for 12 hours showed a mean increase in weight of about 7 g compared with their original body weight. Similarly, rats fasted and refed for 6 hours also showed a 7-g increase in weight compared with the original body weight. However, refeeding caused a significant change in plasma insulin concentrations that also resulted in increased plasma leptin and *Lep* mRNA levels. This increase in leptin secretion and *Lep* mRNA levels without a significant alteration in body weight suggests that plasma insulin levels are more important in the regulation of leptin in the short-term. The acute regulation of leptin levels by food intake suggests that while leptin may be important in the long-term regulation of neuroendocrine responses to fasting,[11] it may also be important in the short-term to regulate meal size. Ingestion of food causes an increase in insulin that rapidly increases plasma leptin, causing satiety and an increase in the metabolic utilization of the ingested food.

REFERENCES

1. Zhang YY, Proenca R, Maffei M, et al: Positional cloning of the mouse obese gene and its human homolog. Nature 372:425-432, 1994
2. Masuzaki H, Ogawa Y, Isse N, et al: Human obese gene expression—Adipocyte-specific expression and regional differences in the adipose tissue. Diabetes 44:855-858, 1995
3. Halaas JL, Gajiwala KS, Maffei M, et al: Weight-reducing effects of the plasma protein encoded by the obese gene. Nat Med 269:543-546, 1995
4. Pelleymounter MA, Cullen MJ, Baker MB, et al: Effects of the obese gene product on body-weight regulation in OB/OB mice. Nat Med 269:540-543, 1995
5. Campfield LA, Smith FJ, Guisez Y, et al: Recombinant mouse OB protein—Evidence for a peripheral signal linking adiposity and central neural networks. Nat Med 269:546-549, 1995
6. Becker DJ, Ongemba LN, Brichard V, et al: Diet-induced and diabetes-induced changes of OB gene expression in rat adipose tissue. FEBS Lett 371:324-328, 1995
7. Saladin R, Devos P, Guerremillo M, et al: Transient increase in obese gene expression after food intake or insulin administration. Nature 377:527-529, 1995
8. Zheng DH, Jones JP, Usala SJ, et al: Differential expression of OB messenger RNA in rat adipose tissues in response to insulin. Biochem Biophys Res Commun 218:434-437, 1996
9. Boden G, Chen X, Mozzoli M, et al: Effect of fasting on serum leptin in normal human subjects. J Clin Endocrinol Metab 81:3419-3423, 1996
10. Havel PJ, Kasimkarakas S, Mueller W, et al: Relationship of plasma leptin to plasma insulin and adiposity in normal-weight and overweight women—Effects of dietary-fat content and sustained weight-loss. J Clin Endocrinol Metab 81:4406-4413, 1996
11. Sivitz WI, Donohoue P, Leibel RL, et al: Plasma leptin levels in diabetic and insulin-treated diabetic rats. J Invest Med 44:A370, 1996 (abstr)
12. Macdougald OA, Hwang CS, Fan HY, et al: Regulated expression of the obese gene product (leptin) in white adipose-tissue and 3T3-L1 adipocytes. Proc Natl Acad Sci USA 92:9034-9037, 1995
13. Wabitsch M, Jensen PB, Blum WF, et al: Insulin and cortisol promote leptin production in cultured human fat cells. Diabetes 45:1435-1438, 1996
14. Leroy P, Dessolin S, Villageois P, et al: Expression of OB gene in adipose cells—Regulation by insulin. J Biol Chem 271:2365-2368, 1996
15. Chirgwin JJ, Przbyla AE, MacDonald RJ, et al: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18:5294-5299, 1979
16. Tiao G, Fagan J, Roegner V, et al: Energy-ubiquitin–dependent muscle proteolysis during sepsis in rats is regulated by glucocorticoids. J Clin Invest 97:339-348, 1996
17. Rentsch J, Levens N, Chiesi M, et al: Recombinant OB gene product reduces food intake in fasted mice. Biochem Biophys Res Commun 214:131-136, 1995
18. Ahima RS, Prabakaran D, Mantzoros C, et al: Role of leptin in the neuroendocrine response to fasting. Nature 382:250-252, 1996
19. Tartaglia LA, Dembski M, Weng X, et al: Identification and expression cloning of a leptin receptor, OB-R. Cell 83:1263-1271, 1995
20. Wang MY, Zhou YT, Newgard CB, et al: A novel leptin receptor isoform in rat. FEBS Lett 392:87-90, 1996
21. Schwartz MW, Seeley RJ, Campfield LA, et al: Identification of targets of leptin action in rat hypothalamus. J Clin Invest 98:1101-1106, 1996
22. Cusin I, Sainsbury A, Doyle P, et al: The OB gene and insulin—A relationship leading to clues to the understanding of obesity. Diabetes 44:1467-1470, 1995
23. Gold G, Manning M, Heldt A, et al: Diabetes induced with multiple subdiabetogenic doses of streptozotocin—Lack of protection by exogenous superperoxide dismutase. Diabetes 30:634-638, 1981
24. Sivitz WI, DeSautel SL, Lee EC, et al: Time-dependent regulation of rat adipose tissue glucose transporter (GLUT4) mRNA and protein by insulin in streptozotocin-diabetic and normal rats. Metabolism 41:1267-1272, 1992
25. Trayhurn P, Thomas MEA, Duncan JS, et al: 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, 1995
26. Mizuno TM, Bergen H, Funabashi T, et al: Obese gene expression—Reduction by fasting and stimulation by insulin and glucose in lean mice, and persistent elevation in acquired (diet-induced) and genetic (yellow agouti) obesity. Proc Natl Acad Sci USA 93:3434-3438, 1996
27. Hardie LJ, Rayner DV, Holmes S, et al: Circulating leptin levels are modulated by fasting, cold exposure and insulin administration in lean but not Zucker (fa/fa) rats as measured by ELISA. Biochem Biophys Res Commun 223:660-665, 1996
28. Kolaczynski JW, Nyce MR, Considine RV, et al: Acute and chronic effect of insulin on leptin production in humans—Studies in-vivo and in-vitro. Diabetes 45:699-701, 1996
29. Muscelli E, Camastra S, Masoni A, et al: Acute insulin administration does not affect plasma leptin levels in lean or obese subjects. Eur J Clin Invest 26:940-943, 1996
30. Clapham JC, Smith SA, Moore GBT, et al: Plasma leptin concentrations and OB gene expression in subcutaneous adipose tissue are not regulated acutely by physiological hyperinsulinaemia in lean and obese humans. Int J Obes 21:179-183, 1997