Leptin Levels in Humans Are Acutely Suppressed by Isoproterenol Despite Acipimox-Induced Inhibition of Lipolysis, But Not by Free Fatty Acids

Michael Stumvoll, Andreas Fritsche, Otto Tschritter, Rainer Lehmann, Hans Günther Wahl, Walter Renn, and Hans Häring

Leptin secretion is complexly regulated in humans. Insulin has been shown to stimulate leptin secretion, whereas in vitro data suggest that catecholamines and free fatty acids (FFAs) inhibit leptin secretion. To dissect differential effects on leptin secretion, we performed two experimental protocols in 11 lean healthy subjects in addition to a saline infusion plus oral acipimox to suppress lipolysis (SAL + ACX) as a control experiment: (1) isoproterenol (~30 ng/kg · min, to increase the heart rate by ~50 bpm) plus oral acipimox (ISO + ACX, 240 minutes) and (2) Intralipid (Pharmacia & Upjohn, Erlangen, Germany) plus heparin (LIP, 420 minutes). During SAL + ACX, FFAs decreased from 0.44 ± 0.04 to 0.06 ± 0.02 mmol/L ($P = .001$), while serum insulin and leptin remained unchanged. During ISO + ACX, FFAs decreased similarly from 0.41 ± 0.13 to 0.09 ± 0.02 mmol/L ($P = .001$), while insulin increased from 47 ± 8 to a maximum of 116 ± 15 pmol/L ($P = .001$) and serum leptin decreased acutely from 6.4 ± 2.1 to a minimum of 5.4 ± 1.8 ng/mL after 90 minutes ($P = .003$ v SAL + ACX). After 150 minutes, leptin returned to control levels. During LIP, the elevation of FFAs from 0.34 ± 0.04 to 1.71 ± 0.19 mmol/L ($P = .001$) had no effect on serum insulin or leptin concentrations (both P = nonsignificant). In conclusion, our results show that in humans, isoproterenol acutely suppresses leptin levels independently of increased FFAs, and elevated FFAs have no acute effect on leptin levels. The fact that an inhibition of leptin secretion occurred despite conditions that are known to suppress intracellular cyclic adenosine monophosphate (cAMP) levels, as demonstrated by suppressed lipolysis, suggests that signaling mechanisms other than those mediated by cAMP must be involved in modulating leptin secretion.

LEPTIN, the *ob* gene product, is thought to be an adipocyte-derived signal contributing to the regulation of body weight.[1-3] The observation that genetically defective leptin secretion is characterized by extreme obesity in children emphasizes its relevance for human energy homeostasis.[4]

In addition to the long-term regulation by body fat mass, there is increasing evidence that leptin secretion is also under acute hormonal control. Humoral factors stimulating leptin secretion include glucocorticoids[5] and insulin.[6,7] In contrast, catecholamines have been shown to inhibit leptin secretion in animals,[8,9] and infusion of isoproterenol in healthy subjects resulted in an acute decrease in leptin levels.[10,11] However, in these studies, lipolysis was markedly stimulated and plasma free fatty acids (FFAs) increased about 3-fold. Since FFAs have been shown to inhibit *ob* gene expression in cultured adipocytes,[12,13] the β-adrenergic effect may have been mediated by elevated FFA levels. But in a single in vivo study in humans, experimental elevation of circulating FFAs has not been shown to affect leptin levels.[14]

In vitro data have suggested that the acute regulation of leptin secretion and/or *ob* gene expression is mediated through the second messenger cyclic adenosine monophosphate (cAMP). Increases in cAMP (di-bu-cAMP, forskolin) have been shown to decrease leptin secretion,[13,15] while decreases in intracellular cAMP (insulin) have been shown to increase leptin secretion.[15]

In the present study, in addition to a saline control, we performed two sets of experiments in 11 healthy subjects to differentiate the effects of β-adrenergic stimulation, suppression of lipolysis, a condition known to involve a decrease in intracellular cAMP, and elevation of circulating FFAs on leptin secretion: (1) isoproterenol, a selective β-agonist, together with oral acipimox to suppress lipolysis and FFAs and (2) Intralipid plus heparin to increase circulating FFAs.

SUBJECTS AND METHODS

Subjects

After approval of the protocol by the local ethics committee and provision of informed written consent, we studied 11 lean healthy subjects (6 males and 5 females aged 24.2 ± 3.1 years; body mass index [BMI], 23.6 ± 2.8 kg/m^2, mean ± SD). Before the study, all subjects provided their medical history and underwent a physical examination, a routine blood test, and an electrocardiogram.

Experimental Protocols

Subjects were admitted to the university's research unit in the morning after an overnight fast, and indwelling catheters were inserted into an antecubital vein for infusions and retrogradely into a dorsal hand vein that was kept in a heated chamber for arterialized blood sampling. At 9 AM (−60 minutes) baseline blood samples were performed, and at 10 AM the experiments started.

Each subject underwent 2 or 3 experiments performed in random order no more than 4 weeks apart. All subjects underwent a control experiment (saline + acipimox [SAL + ACX]) for both the isoproterenol and the Intralipid/heparin protocol. For this purpose, normal saline was infused over 7 hours and acipimox 250 mg was administered orally at −60 minutes and 60 minutes after starting the infusion. Eight subjects underwent protocol 1 and 8 subjects who were not identical to those undergoing protocol 1 underwent protocol 2, so that 5 subjects

From the Medizinische Klinik, Abteilung für Endokrinologie, Stoffwechsel und Pathobiochemie, Eberhard-Karls-Universität, Tübingen, Germany.

Submitted April 8, 1999; accepted August 24, 1999.

M.S. and A.F. contributed equally to this study.

Supported in part by a grant from the Deutsche Forschungsgemeinschaft (DFG Stu 192-2/1 to M.S.).

Address reprint requests to Michael Stumvoll, MD, Medizinische Universitätsklinik, Otfried-Müller-Str. 10, 72076 Tübingen, Germany.

0026-0495/00/4903-0010$10.00/0

underwent all 3 protocols. Thus, subjects served as their own controls and essentially two sets of experiments were performed.

Experimental protocol 1 (isoproterenol + acipimox, n = 8). Subjects (BMI, 22.6 ± 3.0 kg/m^2) received an unprimed infusion of isoproterenol (Sanofi, Brussels, Belgium) over 4 hours at a rate of 20 to 40 ng/kg · min, titrated to achieve an increase in heart rate of at least 40 bpm but no higher than twice the resting heart rate. Acipimox (Olbemox 250 mg; Pharmacia & Upjohn, Erlangen, Germany) was administered orally at −60 minutes and 60 minutes after starting the infusion (ISO + ACX).

Experimental protocol 2 (Intralipid + heparin, n = 8). Subjects (BMI, 24.4 ± 3.2 kg/m^2) received an Intralipid 20% (Pharmacia & Upjohn) infusion at a rate of 0.83 mL/kg · h over a 7-hour period together with sodium heparin at a rate of 800 IE/h (priming dose, 1,000 IE). The longer duration of this protocol (LIP protocol) was chosen in order not to miss any delayed effects of elevated FFAs on leptin levels, in analogy to the effects on insulin-stimulated glucose uptake, where the inhibition does not occur until 3 to 4 hours after the elevation of FFAs.[16]

Sampling and Analytical Procedures

Blood samples for determination of blood glucose and serum insulin, leptin, and FFAs were obtained every 30 minutes during ISO + ACX and every 60 minutes during LIP and SAL + ACX. In addition, during infusions of isoproterenol and saline, blood pressure and heart rate were recorded at regular intervals using a Dynamap monitor (Critikon, Tampa, FL).

Serum leptin was determined by a radioimmunoassay (Linco, St. Charles, MO) with an intraassay and interassay coefficient of variation less than 6% and a lower detection limit of 0.5 ng/mL.[17] Serum insulin and FFA concentrations were determined by a standard enzyme-linked immunosorbent assay (Enzymun Test; Boehringer, Mannheim, Germany) and an enzymatic method (NEFA C kit; WACO Chemicals, Neuss, Germany), respectively. Blood glucose was determined using a HemoCue analyzer (HemoCue, Mission Viejo, CA).

Statistical Analysis

Unless stated otherwise, data are expressed as the mean ± SEM. For statistics and figures, data from the experimental protocol (ISO + ACX or LIP) are compared with the respective SAL + ACX data from the same subjects only, so that all subjects served as their own control. For clarity, leptin levels in the figures are expressed as the relative change from the mean baseline level (mean of −60, −30, and 0 minutes for ISO + ACX; mean of −60 and 0 minutes for LIP). For statistical comparisons with the SAL + ACX protocol, multivariate ANOVA (MANOVA) with a repeated-measures design was used over the whole infusion period. For between-experiment comparisons at single time points or over a defined time interval, the paired Student's *t* test (2-tailed) was used. For example, for the specific comparison of the maximal effect of isoproterenol, the mean leptin value at 60, 90, and 120 minutes (ISO + ACX) was compared with the mean leptin value at 60 and 120 minutes (SAL + ACX). The statistical software package SPSS/PC+ (SPSS, Chicago, IL) was used.

RESULTS

Experimental Protocol 1 (ISO + ACX versus SAL + ACX)

During SAL + ACX, both the heart rate and blood pressure remained unchanged. During ISO + ACX, the heart rate increased by approximately 50 bpm to a plateau of about 120 bpm ($P < .001$, MANOVA). Systolic blood pressure increased from 117 ± 5 mm Hg to a maximum of 145 ± 4 mm Hg at 30 minutes ($P = .002$) and subsequently decreased progressively to 122 ± 6 mm Hg, which was not different from baseline ($P = .22$) but was higher versus SAL + ACX (108 ± 3 mm Hg, $P = .03$). The slight progressive decrease in diastolic blood pressure was not significantly different between groups ($P = .25$, MANOVA) (Fig 1).

In the SAL + ACX experiment, neither blood glucose nor serum insulin levels changed significantly. During ISO + ACX, glucose remained unchanged ($P = .73$), while serum insulin increased from 47 ± 8 to a peak level of 116 ± 15 pmol/L at 30 minutes ($P < .001$) and subsequently decreased progressively to 34 ± 5 pmol/L at 240 minutes, which was not different versus SAL + ACX ($P = .25$). Following oral administration of acipimox, FFAs decreased from 0.44 ± 0.04 mmol/L to a plateau (average values from 60 to 240 minutes) of 0.07 ± 0.01 mmol/L ($P < .001$) during SAL + ACX and from 0.41 ± 0.03 to a plateau of 0.10 ± 0.02 mmol/L ($P < .001$), which was not significantly different between experiments ($P = .29$, MANOVA) (Fig 2).

In contrast to SAL + ACX, where leptin levels showed a slow progressive decline from 7.3 ± 2.9 ng/mL at baseline to 6.3 ± 2.5 ng/mL at 240 minutes ($P = .04$), leptin showed a biphasic response during ISO + ACX, decreasing sharply from 6.4 ± 2.1 ng/mL to a minimum of 5.4 ± 1.8 ng/mL ($P = .003$ *v* SAL + ACX by *t* test and $P = .033$ by MANOVA) at 90 minutes. Thereafter, leptin began to increase, reaching control levels at 180 minutes, and subsequently were not significantly different versus SAL + ACX until the end of the study ($P = .13$, *t* test at 240 minutes) (Fig 2).

Experimental Protocol 2 (LIP versus SAL + ACX)

During LIP, blood glucose concentrations did not change over time and were not significantly different versus SAL + ACX ($P = .90$, MANOVA). Insulin decreased progressively both during SAL + ACX (from 87 ± 12 to 37 ± 7 pmol/L at 420 minutes, $P = .003$) and during LIP (from 86 ± 12 to

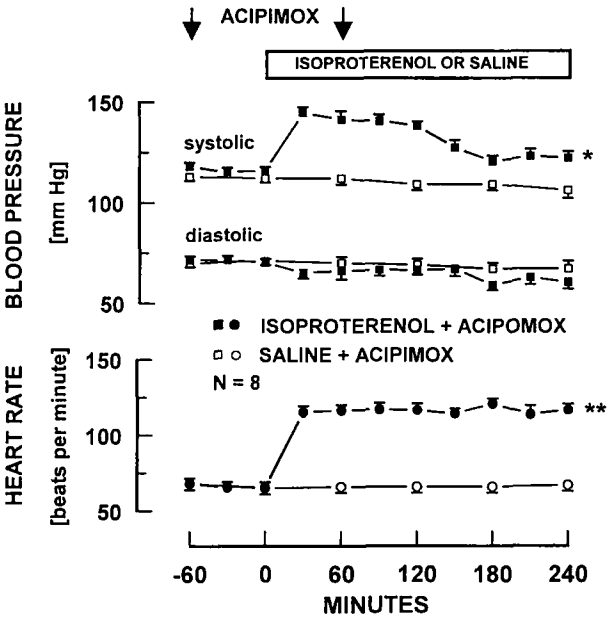

Fig 1. Blood pressure and heart rate before and during infusion of isoproterenol or saline (experimental protocol 1). *P = .002 *v* SAL + ACX, **P < .001 *v* SAL + ACX.

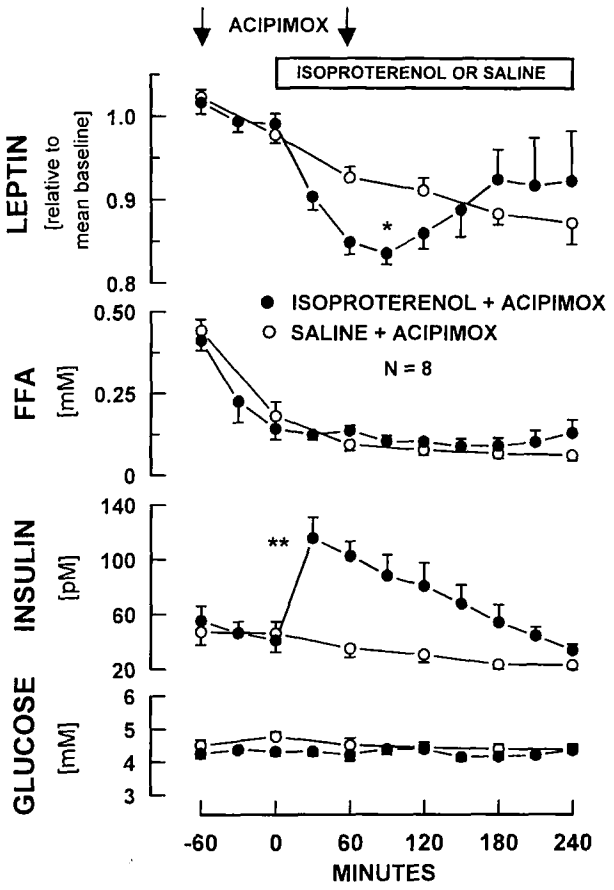

Fig 2. Serum leptin, FFA, insulin, and glucose before and during infusion of isoproterenol or saline (experimental protocol 1). *Mean of 60, 90, and 120 minutes (ISO + ACX) *v* mean of 60 and 120 minutes (SAL + ACX), $P = .003$; **30 minutes (ISO + ACX) *v* mean of 0 and 60 minutes (SAL + ACX), $P < .001$.

45 ± 13 pmol/L at 420 minutes, $P = .02$), which were not significantly different ($P = .31$, MANOVA). FFA levels decreased progressively from 0.44 ± 0.04 to 0.06 ± 0.02 mmol/L ($P < .001$) in the saline experiment as a consequence of the antilipolytic effect of acipimox. Infusion of Intralipid and heparin resulted in a progressive increase of FFA levels from 0.34 ± 0.04 mmol/L at baseline to a plateau of approximately 1.6 mmol/L between 180 and 420 minutes ($P < .001$) (Fig 3).

Leptin levels decreased progressively during infusion of both saline (from 9.5 ± 2.7 to 8.0 ± 2.4 ng/mL) and Intralipid/heparin (from 8.3 ± 2.7 to 7.2 ± 2.6 ng/mL) until the end of the study (both $P < .05$ *v* baseline, *t* test). The decrease in the Intralipid/heparin experiment was not different versus that found during infusion of saline ($P = .47$) (Fig 3).

DISCUSSION

During infusion of isoproterenol, we found a decrease in leptin, reaching a trough of about 85% of the baseline value after 90 minutes. Although this decrease was less marked, it is in general agreement with two previous reports showing a similarly rapid maximal decline in leptin levels of 20% and 27% after 120 and 180 minutes, respectively, of isoproterenol infusion.[10,11] Our data and others thus substantiate earlier reports presenting indirect evidence for an inverse relationship between plasma epinephrine and leptin levels. During hypoglycemia, a reciprocal relationship between endogenous catecholamine levels and insulin-stimulated leptin secretion has recently been shown in normal and type 1 diabetic subjects.[18] In another study, a negative correlation between leptin and resting epinephrine levels independent of the BMI was found in women but not in men.[19] The decrease in our study was observed despite a lack of increase in FFAs, which have been shown to inhibit leptin secretion.[12,13] This indicates that β-adrenergic inhibition of leptin secretion is due to a direct effect on β-adrenergic receptors and is not indirectly mediated by FFAs.

In addition to eliminating the indirect effects of FFAs, the use of acipimox in this study possibly allows insight into the mechanism of leptin secretion in humans. Intracellular cAMP levels, above all, are thought to control leptin secretion and *ob* gene expression.[13,15,20] In the adipocyte, therefore, not only stimulation of lipolysis[21] but also inhibition of leptin secretion by β-agonists[13,15] have been attributed exclusively to increased cAMP concentrations. However, in the present study, stimulation of lipolysis was effectively prevented by acipimox, a potent inhibitor of adipocyte adenylate cyclase that decreases intracellular cAMP levels.[22,23] It is therefore intriguing that an infusion of isoproterenol designed to nearly double the heart rate should acutely decrease leptin levels while having no effect on lipolysis.

Provided that changes in leptin levels reflect changes in leptin production rather than leptin clearance, and if one accepts that suppression of circulating FFAs qualitatively reflects decreased, or at least not increased, intracellular cAMP, there is but one logical explanation for this discrepancy: mechanisms in addi-

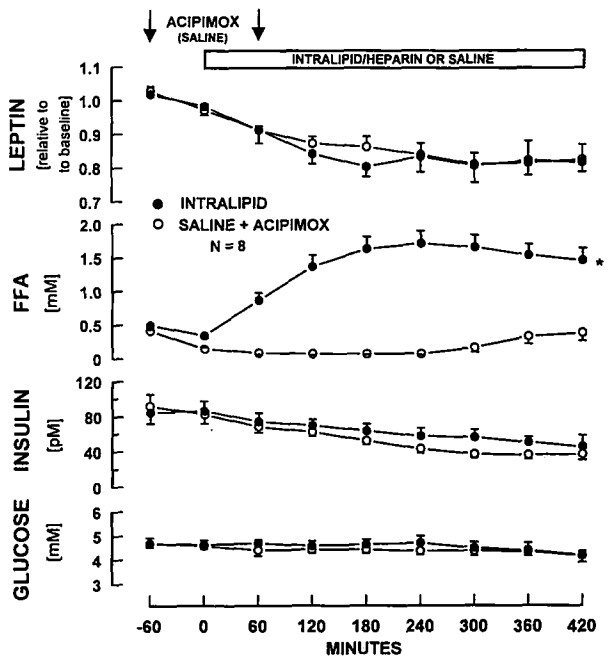

Fig 3. Serum leptin, FFA, insulin, and glucose before and during infusion of Intralipid plus heparin or saline (experimental protocol 2). *$P < .001$ *v* SAL + ACX.

tion to those governed by the adenylate cyclase/phosphodiesterase system must be involved in leptin secretion in humans.

The concept of such a signaling pathway existing in the adipocyte is supported by the recent observation that isoproterenol activates protein kinase B in isolated rat adipocytes independently of cAMP.[24] The postulated existence of an alternative signaling pathway might also explain our finding of a less pronounced effect of isoproterenol versus the two previous studies.[10,11] In those studies, where intracellular cAMP was not suppressed by acipimox, both cAMP-dependent and cAMP-independent pathways should have been operative, resulting in a more pronounced decrease in leptin secretion. Additional evidence that leptin secretion is at least partially independent of cAMP levels is reported in a recent study showing that administration of acipimox did not result in any significant difference in leptin levels over a 420-minute period as compared with placebo.[14] This indicates that a decrease in cAMP alone does not stimulate leptin secretion. However, it should be clearly noted that our conclusion is somewhat limited by the fact that we did not actually measure intracellular cAMP levels but inferred them from the suppression of lipolysis, a condition known to involve reduced intracellular cAMP levels.

Furthermore, in contrast to the two previous reports,[10,11] the decrease in leptin during infusion of isoproterenol was transient in our study. One reason for this discrepancy may simply be the fact that we infused isoproterenol over a longer period, making the increase in leptin visible. However, a different explanation for our observation that leptin started to increase after 90 minutes and reached control levels by about 180 minutes would be the stimulation of leptin secretion by insulin which increased to concentrations observed after a meal challenge. With similar increases in insulin (from 50 to 140 pmol/L) achieved during a euglycemic clamp, a significant increase in leptin has been shown in humans.[7] The presence of such a causal relationship is supported by the significant correlation between the mean increase in serum insulin during infusion of isoproterenol and the increase in leptin after its maximal decrease at 90 minutes ($r = .79$, $P = .02$) (Fig 4). Simultaneous infusion of somatostatin to eliminate any confounding insulin effects would have led to inconclusive results, since somatostatin itself has been shown to decrease leptin levels in humans.[11]

It is noteworthy that we did not find significant changes in blood glucose during the 4-hour isoproterenol infusion. Possibly, offsetting mechanisms may have been operative in maintaining blood glucose constant, ie, stimulation of hepatic glycogenolysis and gluconeogenesis (direct effect of isoproterenol) and stimulation of glucose disposal (indirect effect via the increase in insulin).

Although during LIP, FFAs increased almost 5-fold compared with baseline and 13-fold compared with SAL + ACX, over a 7-hour period, no significant differences in leptin levels were observed. This observation strongly suggests that circulating FFAs have no acute effect on leptin secretion in humans and confirms an earlier report also demonstrating no effect of a 3-hour elevation of FFAs in normal subjects.[14] These findings are at variance with in vitro data showing inhibition of *ob* gene expression by bromo-palmitate, a nonmetabolizable fatty acid, in an embryonic murine adipocyte cell line (3T3-L1).[12] In

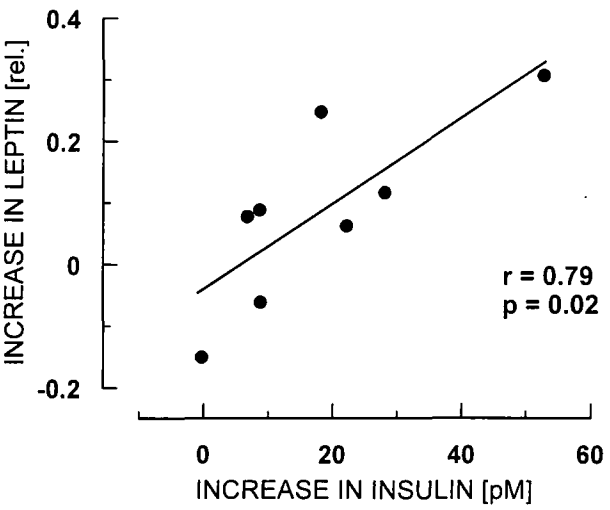

Fig 4. Correlation of the increase in serum leptin after 90 minutes and the increase in serum insulin during infusion of isoproterenol (leptin 240 minutes − leptin 90 minutes) over mean basal leptin plotted against the mean increase in insulin over baseline between 30 and 120 minutes.

adipocytes cultured from young mice, oleic acid had a small but dose-dependent suppressive effect on *ob* gene expression.[13] These discrepancies may be explained by the well-known species differences in the leptin system between mice and humans. Additionally, the lipid infusion periods may have been too short, leaving the possibility that the regulation of leptin secretion by FFAs in man is a chronic rather than an acute mechanism.

Alternatively, it is possible that in vivo, it is not so much the concentration of FFAs delivered to the adipocyte that regulates leptin secretion, but the actual rate of triglyceride synthesis. Therefore, the experimental elevation of FFAs in this study would have downregulated leptin secretion only in the presence of hyperinsulinemia and hyperglycemia, a condition known to maximally accelerate lipogenesis.

It should be pointed out that the baseline leptin and insulin levels of the two protocols differed. This resulted from the fact that not the exact same subjects underwent both protocols. This has potential bearing on the interpretation of the results. For example, in the LIP protocol, two subjects were included who did not participate in ISO + ACX. They were hyperinsulinemic and relatively hyperleptinemic, which certainly affected the baseline levels and probably also later time points. Proportionate changes or differences in high leptin (and insulin) levels, or a lack thereof, would arithmetically more strongly influence the absolute mean than the relatively pronounced changes at low levels. Conversely, increments from higher starting points may appear blunted when expressed as a relative change from baseline. Therefore, comparison of the two protocols is limited by the fact that the degree of obesity varied. Nevertheless, since subjects served as their own controls within a protocol, the main conclusions are not affected.

In conclusion, our results show that in humans, isoproterenol suppresses leptin levels independently of increased FFAs,

indicating a direct effect via β-adrenergic receptors on leptin secretion. Furthermore, the fact that inhibition of leptin secretion occurred despite conditions that are known to suppress intracellular cAMP levels, as demonstrated by suppressed lipolysis, suggests that signaling mechanisms other than those mediated by cAMP must be involved in regulating leptin secretion. Finally, an elevation of FFAs has no acute effect on leptin levels.

ACKNOWLEDGMENT

We are indebted to Anke Wahl, Elisabeth Metzinger, and Sabine Wolff for excellent technical assistance.

REFERENCES

1. Zhang Y, Proenca R, Maffei M, et al: Positional cloning of the mouse obese gene and its human homologue. Nature 372:425-432, 1994

2. Pelleymounter MA, Cullen MJ, Baker MB, et al: Effects of the obese gene product on body weight regulation in ob/ob mice. Science 269:540-543, 1995

3. Considine RV, Sinha MK, Heiman ML, et al: Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 334:292-295, 1996

4. Montague CT, Farooqi IS, Whitehead JP, et al: Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature 387:903-908, 1997

5. Miell JP, Englaro P, Blum WF: Dexamethasone induces an acute and sustained rise in circulating leptin levels in normal human subjects. Horm Metab Res 28:704-707, 1996

6. Kolaczynski JW, Nyce MR, Considine RV, et al: Acute and chronic effects of insulin on leptin production in humans: Studies in vivo and in vitro. Diabetes 45:699-701, 1996

7. Saad MF, Khan A, Sharma A, et al: Physiological insulinemia acutely modulates plasma leptin. Diabetes 47:544-549, 1998

8. Trayhurn P, Duncan JS, Rayner DV, et al: Rapid inhibition of ob gene expression and circulating leptin levels in lean mice by the beta 3-adrenoceptor agonists BRL 35135A and ZD2079. Biochem Biophys Res Commun 228:605-610, 1996

9. Mantzoros CS, Qu D, Frederich RC, et al: Activation of beta(3) adrenergic receptors suppresses leptin expression and mediates a leptin-independent inhibition of food intake in mice. Diabetes 45:909-914, 1996

10. Pinkney JH, Coppack SW, Mohamed Ali V: Effect of isoprenaline on plasma leptin and lipolysis in humans. Clin Endocrinol (Oxf) 48:407-411, 1998

11. Donahoo WT, Jensen DR, Yost TJ, et al: Isoproterenol and somatostatin decrease plasma leptin in humans: A novel mechanism regulating leptin secretion. J Clin Endocrinol Metab 82:4139-4143, 1997

12. Rentsch J, Chiesi M: Regulation of ob gene mRNA levels in cultured adipocytes. FEBS Lett 379:55-59, 1996

13. Deng C, Moinat M, Curtis L, et al: Effects of beta-adrenoceptor subtype stimulation on obese gene messenger ribonucleic acid and on leptin secretion in mouse brown adipocytes differentiated in culture. Endocrinology 138:548-552, 1997

14. Peino R, Fernandez Alvarez J, Penalva A, et al: Acute changes in free fatty acids (FFA) do not alter serum leptin levels. J Endocrinol Invest 21:526-530, 1998

15. Gettys TW, Harkness PJ, Watson PM: The beta 3-adrenergic receptor inhibits insulin-stimulated leptin secretion from isolated rat adipocytes. Endocrinology 137:4054-4057, 1996

16. Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 46:3-10, 1997

17. Ma Z, Gingerich RL, Santiago JV, et al: Radioimmunoassay of leptin in human plasma. Clin Chem 42:942-946, 1996

18. Fritsche A, Wahl HG, Metzinger E, et al: Evidence for inhibition of leptin secretion by catecholamines in man. Exp Clin Endocrinol Diabetes 106:415-418, 1998

19. Mills PJ, Ziegler MG, Morrison TA: Leptin is related to epinephrine levels but not reproductive hormone levels in cycling African-American and Caucasian women. Life Sci 63:617-623, 1998

20. Slieker LJ, Sloop KW, Surface PL, et al: Regulation of expression of ob mRNA and protein by glucocorticoids and cAMP. J Biol Chem 271:5301-5304, 1996

21. Lafontan M, Berlan M: Fat cell adrenergic receptors and the control of white and brown fat cell function. J Lipid Res 34:1057-1091, 1993

22. Ambrogi V, Cozzi P, Sanjust P, et al: Antilipolytic activity of a series of pyrazine-*N*-oxides. Eur J Med Chem 15:157-163, 1980

23. Fuccella LM, Goldaniga G, Lovisolo P, et al: Inhibition of lipolysis by nicotinic acid and by acipimox. Clin Pharmacol Ther 28:790-795, 1980

24. Moule SK, Welsh GI, Edgell NJ, et al: Regulation of protein kinase B and glycogen synthase kinase-3 by insulin and beta-adrenergic agonists in rat epididymal fat cells. Activation of protein kinase B by wortmannin-sensitive and -insensitive mechanisms. J Biol Chem 272: 7713-7719, 1997