Isoproterenol decreases leptin release from rat and human adipose tissue through posttranscriptional mechanisms

Matthew R. Ricci,1,4* Mi-Jeong Lee,1,4* Colleen D. Russell,1 Yanxin Wang,1 Sean Sullivan,1 Stephen H. Schneider,2 Robert E. Brolin,3 and Susan K. Fried1,4
1Department of Nutritional Sciences, Rutgers University; 2Division of Endocrinology and 3Department of Surgery, University of Medicine and Dentistry of New Jersey, New Brunswick, New Jersey; 4Division of Gerontology, Department of Medicine, University of Maryland School of Medicine and Baltimore Veterans Affairs Medical Center, Baltimore, Maryland

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Ricci, Matthew R., Mi-Jeong Lee, Colleen D. Russell, Yanxin Wang, Sean Sullivan, Stephen H. Schneider, Robert E. Brolin, and Susan K. Fried. Isoproterenol decreases leptin release from rat and human adipose tissue through posttranscriptional mechanisms. Am J Physiol Endocrinol Metab 288: E798–E804, 2005. First published December 7, 2004; doi:10.1152/ajpendo.00446.2004.—In vivo and in vitro studies indicate that β-adrenergic receptor agonists decrease leptin release from fat cells in as little as 30 min. Our objective was to determine whether alterations in leptin biosynthesis or secretion were involved in the short-term adrenergic regulation of leptin in human and rat adipose tissue. Isoproterenol (Iso) decreased leptin release from incubated adipose tissue of both nonobese and obese subjects to similar extent (–28 vs. –21% after 3 h). Inhibition of protein synthesis with cycloheximide did not block the effect of Iso on leptin release from human adipose tissue, suggesting that the Iso effect is independent of leptin synthesis. Iso also tended to increase tissue leptin content at the end of the 3-h incubation, as expected from the observed inhibition of release. Consistent with a posttranslational mechanism, Iso treatment did not affect leptin mRNA levels or relative rate of leptin biosynthesis as directly assessed by [35S]methionine incorporation into immunoprecipitable leptin. In contrast to these results in human adipose tissues, Iso did not decrease basal leptin release from rat adipose tissue. However, Iso did decrease insulin-stimulated leptin release by inhibiting the ability of insulin to increase leptin biosynthesis without detectably affecting leptin mRNA levels. Thus, in both human and rat, adrenergic regulation of posttranscriptional events (secretion in humans, translation in rats) may contribute to the rapid decline in circulating leptin that occurs when the sympathetic nervous system is activated, such as during fasting and cold exposure. Furthermore, the rat does not provide an ideal model to study mechanisms of cellular leptin regulation in humans.

Although serum leptin levels in humans are strongly correlated with percent body fat, circulating leptin levels also undergo short-term changes independently of alterations in adiposity (7, 15, 28). Consistent with prior studies using rodent models (29), recent studies in humans suggest that activation of the sympathetic nervous system or stimulation of β-adrenergic receptors (β-AR) decreases plasma leptin over a relatively short time frame (hours) (8, 9, 22, 23). Cold exposure (23) or infusion of adrenergic agonists (8, 9, 21) decreases plasma leptin and subcutaneous adipose tissue interstitial leptin concentration in humans after ~3 h (20). With fasting, decreases in leptin levels are thought be due to an activation of β-AR in combination with the decrease in insulin levels (11).

We (22) have previously shown that isoproterenol (Iso) decreases leptin release from human adipose tissue fragments in vitro within the same time frame and by the same magnitude as has been reported in vivo, i.e., in 30–60 min. Thus the in vivo decreases in leptin may be due, at least in part, to direct stimulation of adipose tissue β-AR rather than a secondary effect, e.g., an alteration of blood flow to adipose tissue. Because we previously studied adipose tissue of only obese subjects, one goal of this study was to compare the ability of Iso to decrease leptin release in adipose tissue between obese and nonobese subjects.

The mechanism underlying Iso to decrease leptin release is unknown. A decrease in leptin release could result from an inhibition of leptin synthesis or a direct effect on leptin secretion. Our previous work (25) demonstrated that obese human adipose tissue possessed substantial tissue leptin stores and that inhibition of protein synthesis with cycloheximide (CHX) affected leptin secretion only after 3 h. However, inhibition of protein synthesis did decrease tissue leptin content after a 3-h incubation, implying that ongoing protein synthesis is necessary for maintenance of tissue leptin stores. Thus, in the present study, we assessed whether Iso affected tissue leptin content in human adipose tissue. We reasoned that, if Iso decreased leptin synthesis, it would decrease tissue leptin content too and that a further effect would not be observed when protein synthesis was inhibited with CHX. To confirm these results, we measured the effect of short-term Iso on leptin mRNA levels and directly assessed the rates of leptin biosynthesis with metabolic labeling and immunoprecipitation in human adipose tissue. Because of the difficulties inherent in studying human adipose tissue, we also determined whether rat adipose tissue provides a suitable model for more detailed mechanistic studies of the β-adrenergic regulation of leptin. Insulin appears to be a more important short-term regulator of leptin release in rats compared with humans. In rats, increases in serum insulin in vivo or in vitro produce increases in serum leptin within 1 h (27). In contrast, insulin increases secreted leptin in humans over the longer term (5–8 h after meals or after ≥24 h of adipose tissue culture) (25, 26, 28). Whereas we find no short-term (<3 h) effects of insulin on leptin release from human adipose tissue (unpublished observation), insulin acutely increases leptin release in rat adipose tissue (13). Thus

* These two authors contributed equally to this work.

Address for reprint requests and other correspondence: S. K. Fried, Dept. of Medicine, Division of Gerontology, Univ. of Maryland School of Medicine, Baltimore Veterans Affairs Medical Center, GRECC (BT/GR/18), 10 North Greene St., Baltimore, MD 21201 (E-mail: sfried@grecc.umaryland.edu).

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we determined whether Iso decreases leptin biosynthesis in rat adipose tissue incubated in the presence or absence of insulin.

**MATERIALS AND METHODS**

**Human subjects and adipose tissue collection.** The protocols were approved by the institutional review boards of the University of Medicine and Dentistry of New Jersey, St. Peter’s Hospital, Rutgers University, and the University of Maryland at Baltimore. All subjects gave informed written consent. Subjects did not have cancer or diabetes by medical history, were not taking glucocorticoids or β-blockers, and were by self-report weight stable for at least 1 mo before surgery. Human abdominal subcutaneous adipose tissue samples used in these studies were obtained from two sources: needle aspirations and during surgery for morbid obesity. To obtain hypogastric subcutaneous adipose tissue, the skin was anesthetized with 1% lidocaine, and 1-2 g of adipose tissue were aspirated using a 3-mm Sprirotri cannula (Unitech Instruments, Fountain Valley, CA) connected to a 50-cm³ syringe. Samples of epigastric subcutaneous adipose tissue were obtained at the time of bariatric surgery and immediately placed in serum-free medium 199 (M199; GIBCO-BRL) and 5 μg/ml gentamicin for transportation to the laboratory. Upon arrival, tissue was finely minced (5- to 10-μg fragments), approximately the size of the fragments of adipose tissue obtained from the needle aspirations. The data in Figs. 1 and 2 were obtained from the study of abdominal (epigastric) subcutaneous (SC) adipose tissue sampled from 14 severely obese individuals undergoing elective surgery to treat obesity at Robert Wood Johnson University Hospital and St. Peter’s University Hospital in New Brunswick, NJ plus seven samples of abdominal SC fat (hypogastric, at the level of the umbilicus) obtained by needle aspiration from seven healthy, nonobese subjects [3 females: age 37 ± 3 yr, body mass index (BMI) 27 ± 1 kg/m², fat cell size 0.5 ± 0.2 μg lipid/cell; and 4 males: age 31 ± 5 yr, BMI 26 ± 5 kg/m², fat cell size (n = 3) 0.5 ± 0.05 μg lipid/cell]. The 14 surgical subjects included 12 females (age 41 ± 2 yr, BMI 47 ± 2 kg/m², fat cell size 0.8 ± 0.1 μg lipid/cell) and 2 males (age 30 yr, BMI 57 ± 10 kg/m², fat cell size 0.7 ± 0.08 μg lipid/cell). We also measured the effect of 10⁻⁶ M Iso in 16 additional subjects plus 5 subjects who were also included in the studies with 10⁻³ M Iso presented in Figs. 1 and 2. Thus these data sets included a total of 11 fat aspiration samples from nonobese subjects (BMI <30 kg/m², mean 24.6 ± 0.7 mg/kg²; 4 F, 7 M) and 10 surgical samples from severely obese subjects (mean BMI 40.4 ± 3.3 mg/kg²; 3 M, 7 F).

In preliminary studies, we found a similar magnitude of Iso effect on leptin in hypogastric fat aspirations as in epigastric surgical samples. Thus the lack of lean-obese differences observed is not likely to be confounded by possible site differences within the SC depot or related to the method of sampling.

**Acute tissue incubations.** Minced tissue fragments (~100 mg total) were incubated in 1.5 ml M199 +1% BSA at 37°C with shaking (55 cycles/min) for 3 h with or without Iso (10⁻³ or 10⁻⁶ M). We performed preliminary dose-response experiments to compare the effect of 10⁻⁶ and 10⁻⁵ M Iso on leptin release in five subjects (3 nonobese, 2 obese). There was an apparent dose-dependent effect on leptin release (7.5 ± 8 and 25 ± 7% (P < 0.05 vs. controls) for 10⁻⁶ and 10⁻⁵ M Iso, respectively). Because 10⁻⁵ M Iso consistently decreased leptin release, whereas the effect of 10⁻⁶ M Iso was more variable, we chose to pursue our mechanistic studies with the former dose.

When indicated, incubations were carried out in the presence or absence of the general protein synthesis inhibitor cycloheximide (CHX, 10 μg/ml). In preliminary experiments, CHX inhibited protein synthesis by 87% during a 3-h incubation (C. D. Russell and S. K. Fried, unpublished observation). Before and after the incubation, tissue fragments and medium were frozen at −80°C for subsequent determination of leptin content.

**Determination of tissue leptin content.** Tissue fragments were homogenized in a ground-glass homogenizer in 4 volumes of cold TES (10 mM Tris, 1 mM EDTA, 250 mM sucrose) buffer, pH 7.4, containing proteolytic inhibitors (2.5 mg/ml leupeptin, 3.5 mg/ml aprotinin, 1 mM PMSF), with 1% Triton X-100. Homogenates were centrifugated at 14,000 rpm (15 min, 4°C), and the internant below the fat cake was removed. Infranant and media samples were assayed for leptin with a commercially available radioimmunoassay (RIA) kit from Linco (St. Charles, MO). For the human RIA, we included a blank (incubation medium and tissue lysis buffer) and an additional standard at 0.25 ng/ml in each assay, and the assay was done in duplicates. Leptin values are expressed as nanograms per gram of tissue or as nanograms per 10⁶ cells over stated time intervals.

**Biological synthesis.** Minced adipose tissue (1 g) from four obese subjects was preincubated without or with 1 or 10 μM Iso in 10 ml of methionine and cysteine-free minimal essential medium containing 4% BSA, pH 7.4, under an atmosphere of 95% O₂-5% CO₂ with shaking at 70 oscillations/min in a 37°C water bath. After 2.5 h of incubation, tissue (1 g) was placed in 1 ml of the same medium with 1 mM [³⁵S]methionine (Easy Tag Express; PerkinElmer, Boston, MA) under the same conditions (with or without Iso), and pulse labeled during a 30-min incubation under 95% O₂-5% CO₂ with shaking at 70 oscillations/min in a 37°C water bath. Tissue was then homogenized in lysis buffer (50 mM Tris base, 300 mM NaCl, 5 mM EDTA, 0.1% Triton X-100, pH 7.4), containing protease inhibitors (Protease Inhibitor Cocktail Set III; Calbiochem, La Jolla, CA). After centrifugation at 14,000 rpm for 15 min at 4°C, the internant below the fat cake was removed. Volumes of homogenates with equal counts of [³⁵S]methionine incorporation into total protein (determined after TCA precipitation) from +Iso and -Iso conditions were immunoprecipitated with 5 μg of rabbit anti-human leptin antibody (Biovendor, Brno, Czech Republic) to bound to protein A-Sepharose. Pellets were washed three times with 0.15 M NaCl, 0.2 M Tris, 0.1% N-lauryl-sarcosine, and 3% Triton X-100, pH 7.4, containing protease inhibitors (10). Immunoprecipitated proteins were separated by SDS-PAGE and visualized by fluorography (PhosphorImager, Storm; Molecular Dynamics, Sunnyvale, CA). After exposure to radiography, bands were cut out of the gel and digested with H₂O₂, and radioactivity was determined by liquid scintillation counting. Relative rates of leptin biosynthesis in each experiment were calculated as percent TCA-precipitable protein counts and presented as means ± SE of the indicated numbers of experiment. Control experiments showed that the immunoprecipitated leptin migrated in the gel at the same molecular mass as [¹²⁵I]-labeled leptin standard (~16 kDa). Control immunoprecipitations with nonimmune rabbit serum did not show any band at the position of leptin (unpublished observation). Furthermore, competition with excess unlabeled leptin during the immunoprecipititation abolished the radiolabeled leptin band. Labeling of leptin was linear with time for 60 min, and no [³⁵S]methionine-labeled leptin appeared in the medium during the 30-min pulse; thus our pulse labeling reflects initial rates of leptin synthesis.

**Determination of fat cell size.** Mean fat cell weight was determined by Coulter counting of osmium-fixed fragments of adipose tissue, as described by Hirsch and Gallian (16).

**Determination of glycerol.** Media samples were assayed for glycerol by use of a fluorometric enzymatic method.

**RNA extraction and northern blotting.** Total RNA was extracted from human (or rat) adipose tissue (frozen immediately or after incubation with or without insulin or Iso for 2 h), using a modification of the method of Chomczynski and Sacchi (6). Total RNA (12 μg) was separated in 1% agarose gels, transferred to a nylon membrane (Zetabind, Eastman Kodak, Rochester, NY), and baked for 2 h at 80°C for subsequent hybridization with [³²P]dCTP (PerkinElmer)-labeled human leptin insert (EasyGo Labeling; Amersham, Piscataway, NJ) followed by exposure to the PhosphorImager. The intensities of each band were quantified using ImageQuant 5.0 (Storm, Molecular Dynamics). 18S
rRNA was captured on the image, quantified, and used as loading control. Data were presented as leptin mRNA-to-18S rRNA ratios.

**Rat studies.** Male rats (3–4 mo old; 300–400 g, or ~1 yr old; 550–650 g; Wistar Strain; Charles River Laboratories, Wilmington, MA) were housed with free access to laboratory chow and water. A dark-light cycle was maintained as 1800–0600 dark and 0600–1800 light. On the day of an experiment, rats were anesthetized using CO2 at 0900–1000 and killed by decapitation. Adipose tissue (epididymal and retroperitoneal fat pads combined) were collected, minced, and then used for experiments.

**Measurement of leptin release from rat adipose tissue.** Aliquots (~100 mg) of minced tissue were incubated in 1 ml of Krebs-Ringer bicarbonate buffer with 4% BSA for 3 h at 37°C with shaking (70 oscillations/min). Incubation media were collected and used for measuring leptin secretion via leptin RIA (Linco). This buffer was chosen for rat studies because it allowed a more robust response to insulin compared with M199.

**Measurement of relative rates of leptin biosynthesis.** Biosynthetic labeling was performed as described for human adipose tissue, except that the preincubation period was 1 h and pulse labeling was conducted with 0.5 mCi/ml [35S]methionine for 45 min. Iso (1 μM) and/or insulin (6 nM) was included in the medium when indicated. Our preliminary experiments showed that [35S]methionine incorporation into adipocytes is linear up to 90 min, and our preliminary data also showed that [35S]methionine incorporation into leptin is linear up to 60 min in rat adipose tissue.

**Statistical analyses.** All data are means ± SE. The main objective of this work was to assess basic mechanisms by which Iso affects leptin secretion, not to detect possible subtle sex-related differences between the magnitudes of responses to Iso. Thus data from men and women were combined. Before statistical analysis, data were log-transformed to normalize variance. One-way analysis of variance was used to determine treatment effects vs. the appropriate control group at a specific time point. When significant main effects and/or interactions were found, post hoc comparisons between treatments were made with a Bonferroni test. Significance level was set at *P* < 0.05.

**RESULTS**

**Iso decreased leptin in the incubation medium, but not tissue leptin, in human adipose tissue.** Addition of Iso (10^{-5} M) decreased the amount of leptin secreted into the medium from SC adipose tissue of nonobese subjects (−28 ± 6%, *n* = 6, *P* < 0.01; Fig. 1, bottom). The magnitude of the decrease was similar to what we previously reported in obese subjects (22) and confirmed here (−21 ± 5%, *n* = 10, *P* = 0.004; Fig. 1, top). Conclusions were similar when the data were expressed per gram of tissue (Fig. 1) or per 10^6 fat cells: nonobese, 4.5 ± 1.4 (control) vs. 3.2 ± 1.0 (Iso) ng/10^6 cells, *P* = 0.008; obese, 14.6 ± 2.6 (control) vs. 11.3 ± 1.9 (Iso) ng/10^6 cells, *P* = 0.05. Because of the higher absolute rate of leptin secretion in the obese, the absolute decrease (control − Iso) tended to be greater in the obese (−3.3 ± 1.0 vs. −1.2 ± 0.4 ng/10^6 cells, *P* = 0.08).

To reinforce our conclusion that β-adrenergic activation decreases leptin release, we also studied the effect of a lower concentration of Iso. Iso at 10^{-6} M significantly decreased leptin release [−14.1 ± 6.6%, *P* = 0.02 by paired t-test of log-transformed data, *n* = 21 (11 nonobese and 10 obese subjects)]. The magnitude of the response to 10^{-6} M Iso was not related to the BMI of the subjects (data not shown).

To gain insight into the mechanism of the inhibitory effect of Iso on leptin release, we assessed the effect of Iso on tissue leptin content. We hypothesized that if Iso decreased leptin release by decreasing leptin protein synthesis it would also decrease leptin content of the tissue. However, if Iso blocked leptin secretion but not synthesis it would increase tissue leptin content. Consistent with the latter hypothesis, we found that the Iso-induced decline in leptin release was not associated with any decrease in tissue leptin content at the end of the incubation (Fig. 1). Rather, there was a trend (*P* = 0.07) for tissue leptin content to be higher with Iso compared with control in nonobese subjects. These data are consistent with the hypothesis that Iso does not decrease leptin release primarily by decreasing leptin synthesis.

**Inhibition of leptin release by Iso is independent of protein synthesis in human adipose tissue.** To more directly test the hypothesis that the inhibitory effect of Iso on leptin release depended on protein synthesis, abdominal SC tissue fragments were incubated with 10 μM Iso and/or CHX (10 μg/ml), a general inhibitor of protein synthesis. In this subset of subjects (7 obese, 1 nonobese), incubation of SC adipose tissue with Iso decreased media leptin accumulation (−25 ± 6%) compared with control (*P* = 0.005, *n* = 8; Fig. 2). Importantly, addition of Iso in the presence of CHX decreased leptin release into the media by ~35% compared with CHX alone (*P* = 0.02, *n* = 8; Fig. 2). Whereas CHX decreased the amount of leptin remaining in the tissue at the end of the incubation (*P* < 0.01 vs. initial), Iso did not affect tissue leptin content.
We noted some individual variability in the effect of Iso on leptin release and therefore questioned whether this was due to a generalized resistance in some subjects. Thus we compared the Iso effect on leptin and lipolysis (glycerol release). Subjects showing little response to Iso effects on leptin compared the Iso effect on leptin and lipolysis (glycerol release). We have demonstrated that the \( \beta \)-AR agonist Iso decreases leptin release within 3 h from human subcutaneous adipose tissue of both nonobese and obese subjects. The mechanism involved was posttranslational, as shown by several lines of evidence. First, Iso decreased leptin release even when protein synthesis was inhibited with CHX. Additionally, Iso did not

for further studies of leptin release. Additionally, we assessed the Iso effect on leptin biosynthesis and mRNA at 2 h, the approximate time point at which leptin released at 3 h would have been synthesized. Similar to the preliminary results in Fig. 4, Iso did not consistently affect basal but decreased insulin-stimulated leptin release. Consistent with previous studies of rat adipocytes and our preliminary results using adipose tissue from older rats (Fig. 4), insulin increased leptin secretion from fragments by 26% (insulin 35.2 ± 2.15 vs. basal 27.9 ± 1.84 ng leptin/g tissue \( -1 \cdot 3 \cdot h^{-1} \), \( n = 5 \), \( P < 0.01 \); Fig. 5A), and Iso significantly decreased insulin-stimulated leptin release (Ins + Iso 26.34 ± 1.75 vs. Ins alone 35.2 ± 2.15 ng leptin/g tissue \( -1 \cdot 3 \cdot h^{-1} \), \( n = 5 \), \( P < 0.001 \)). Insulin increased relative rates of leptin biosynthesis by 62 ± 11% (vs. basal, \( n = 3 \), \( P < 0.05 \); Fig. 5B). Although it had no effect on leptin biosynthesis in the absence of insulin, Iso completely blocked insulin-stimulated leptin biosynthesis (Fig. 5B). Incubation with insulin and Iso had no effect on total protein synthesis (TCA-precipitatable counts: control 40.9 ± 3.6, Ins 38.2 ± 3.2, Ins 41.5 ± 1.6, Ins + Iso 41.8 ± 5.5 \times 10^6 \) cpm/g tissue, \( n = 3 \), \( P = NS \)). Leptin mRNA levels did not change compared with initial values over the 2-h incubation and were not affected by either insulin or Iso (leptin mRNA/18S rRNA: initial 8.7 ± 2.1; 2-h control 8.6 ± 2.2; 2-h Iso 8.4 ± 1.9; 2-h Ins 8.9 ± 2.3; 2-h Ins + Iso 9.6 ± 1.9, ratio of densitometry arbitrary units, \( n = 3 \), \( P = NS \)). Taken together, these data indicate that, in rat adipose tissue, Iso blocks the ability of insulin to increase leptin translation and thus release.

**DISCUSSION**

We have demonstrated that the \( \beta \)-AR agonist Iso decreases leptin release within 3 h from human subcutaneous adipose tissue of both nonobese and obese subjects. The mechanism involved was posttranslational, as shown by several lines of evidence. First, Iso decreased leptin release even when protein synthesis was inhibited with CHX. Additionally, Iso did not

![Figure 3](http://ajpendo.physiology.org/)
Fig. 4. Time course of Iso effect on leptin release from rat adipose tissue. Adipose tissue fragments from 420- to 680-g rats (~0.5–1 yr old) were incubated for ≤5 h under basal conditions (B, no additions), with 1 μM Iso (A), or with insulin (6 nM) or Iso + insulin (B), as described in MATERIALS AND METHODS. All 4 incubation conditions were run in parallel using adipose tissue from individual rat (n = 4). Data are means ± SE of 4 independent experiments.

% Significant effect of Iso at given time point by paired t-test of log-transformed data. Insulin effect on leptin release was also significant at 3 and 5 h (comparing data in A and B, P < 0.05 by paired t-test).

affect levels of leptin mRNA or initial rates of leptin biosynthesis as determined by incorporation of [35S]methionine into immunoprecipitateable leptin. Our data suggest that the adrenergic modulation of the secretion of preformed leptin over a relatively short time frame may play a role in the decline in serum leptin levels that occurs during fasting (2, 14, 17) or acute cold exposure (23). In contrast to its effect in human adipose tissue, Iso did not consistently affect basal leptin release or synthesis in adipose tissue from either relatively young (3- to 4-mo-old) or older (~1-yr-old) rats. In contrast to human adipose tissue, new protein synthesis is required for leptin release during incubations of over 1 h (Ref. 18 and unpublished data (M.-J. Lee and S. K. Fried)) from rat adipose tissue, suggesting that preformed leptin stores are smaller in rats than in humans. Iso did, however, antagonize the ability of insulin to increase leptin biosynthesis and secretion in rat adipose tissue. Thus, in the rat, counterregulation of leptin synthesis by insulin and adrenergic effectors appears to modulate leptin biosynthesis and thereby influence the quantity of leptin that is secreted into the circulation. In contrast, in humans, we do not find that insulin affects leptin release or biosynthesis during 3 h of incubation (unpublished observation) and therefore suggest that the major short-term mechanism regulating leptin secretion is adrenergic inhibition of the release of preformed leptin stores. Thus our data suggest that there may be important species differences in the mechanisms that regulate leptin secretion and that caution should be exercised in extrapolating data from the rat model to humans.

Our previous studies of the effects of Iso on leptin release examined only adipose tissue from obese subjects. In the present study, we demonstrate that, on average, the absolute Iso-induced decrease in secreted leptin tended to be greater in obese compared with nonobese subjects. However, the relative Iso-induced decrease in media leptin with Iso was similar (nonobese: −28 ± 6%; obese: −21 ± 5%). In vivo, β-AR agonists decrease plasma leptin in nonobese subjects (15, 21) and SC adipose tissue interstitial leptin concentration in obese subjects (5, 9, 21). Couillard et al. (8) found that the absolute decrease (ng/ml) in plasma leptin in response to epinephrine infusion was greater in obese compared with lean women. However, it appears from their data that the relative decrease in plasma leptin was similar between the groups, discordant with our present in vitro findings. The initial decline (% decrease) in leptin with 14–22 h of fasting is blunted in obese compared with lean subjects but similar after 52 h. From our in vitro results, it seems unlikely that resistance to adrenergic effects on leptin contributes to the initial blunted fall in leptin with fasting in the obese. Rather, the higher insulinemia of the obese may prevent a rapid fall in leptin with fasting in vivo.

Fig. 5. Iso dose not affect leptin release from rat adipose tissue, blocks insulin-induced leptin secretion. A: minced adipose tissue (100 mg) from ad libitum-fed rats (300–400 g, 3–4 mo old) were incubated in 1 ml Krebs-Ringer bicarbonate with 4% BSA in basal condition (B, without any hormone), Iso (1 μM), insulin (I, 6 nM), or combination of insulin and Iso (1 μM Iso in MEM-Met-free medium for 1 h and then pulse labeled with [35S]methionine during an acute 45-min incubation in same medium but containing 0.5 mCi/ml [35S]Met/Cys. Tissue was then homogenized, and [35S]leptin was immunoprecipitated with rabbit anti-human leptin antibody. After SDS-PAGE and fluorography, a single specific band of 16 kDa was detected. Data were corrected for differences in total protein synthesis and compared with 1-way ANOVA followed by post hoc Bonferroni t-test (n = 3). *Control vs. Insulin, P < 0.05; #Insulin alone vs. Insulin + Iso, P < 0.05.
The effect of Iso on leptin release from human adipose tissue fragments is likely to be due to an inhibition of leptin secretion, but we cannot exclude an increase in the rate of degradation of leptin within the tissues. The hypothesis that Iso inhibits the rate of leptin secretion is supported by the observation that there tended to be more leptin remaining in the tissue after incubation with Iso compared with controls (in the nonobese). It is likely that this increase is more apparent in the nonobese because it is difficult to determine the change from the initially higher leptin content in the obese. In contrast, as we have previously shown, inhibition of protein synthesis markedly decreased tissue leptin content (25). In addition to a possible effect of Iso on leptin secretion from the adipocyte, it is possible that Iso may directly or indirectly affect release of leptin from extracellular sites within the tissue fragments. Studies with isolated adipocytes would be needed to address this question. However, we find that, similar to results in rat fat cells (1), constitutive leptin release from isolated human fat cells is rapid and not reproducibly regulated by hormones.

Our data do not resolve the question of whether Iso is acting directly on the adipocyte to influence leptin release. The effect of Iso may, in theory, be mediated through effects on nonadipose cells present in adipose tissue. Future studies with a novel adipocyte model will be needed to address this question.

Increasing evidence indicates that leptin secretion from adipocytes is regulated. Recent ultrastructural and immunohistochemical data of adipose tissue indicate that leptin is present in small vesicles near the plasma membrane of adipocytes (1, 3). Insulin stimulates both leptin secretion and production by rat white adipose tissue (1). Studies in rodent adipocytes and cell lines indicate that insulin and TNF-α stimulate the release of leptin from preformed pools. Activation of adrenergic receptors may inhibit leptin secretion by a mechanism similar to that by which it decreases the insulin-stimulated translocation of the glucose transporter GLUT4 to the plasma membrane (24).

From these data, it is not possible to assess the effect of obesity on tissue leptin content, because in this experiment there were relatively more males in the nonobese group (nonobese group: 3 males, 3 females vs. obese group: 2 males, 8 females) and males have lower serum leptin. Within this relatively small number of subjects, however, it appeared that, within each sex, obese adipose tissue exhibited higher leptin content (data not shown). Further studies are needed to address the effect of obesity on tissue leptin content in relationship to leptin secretion in obese vs. nonobese men and women.

We (22) previously reported that 24 h of culture with Iso significantly decreased leptin mRNA levels in parallel with the quantity of leptin accumulating in the medium, and others have reported similar results in rat adipose tissue or adipocytes (4, 15, 19). However, no effects of Iso on leptin mRNA levels were found after 3 h of Iso in the current study. Thus we conclude that the activation of β-AR in human adipose tissue decreases leptin release through long-term effects at the pretranslational level as well as short-term effects on the secretion of leptin.

The Iso effect on leptin release during a 3-h incubation is small (≈20%) but reproducible and similar in magnitude to the effect of epinephrine infusion in vivo [10–23% (8, 9)]. Similarly, short-term fasting (17) and acute cold exposure (23) decrease circulating leptin by a similar order of magnitude (e.g., ≈14–39% from 14–22 h of fasting in obese and lean women, respectively). Thus the in vitro effect of β-adrenergic stimulation on leptin that we observed is likely to be relevant to the in vivo situation.

Most of studies we report were conducted using a high concentration of a β-adrenergic agonist (10−3 M Iso), raising a potential concern about physiological relevance. However, 10−6 M also significantly decreased leptin release. Preliminary dose-response studies showed that 10−6 M Iso produced a nearly maximal response in most subjects (3 of 5 in which both concentrations were tested in the same subjects, data not shown). The significant barrier to diffusion into adipose tissue fragments also makes it likely that the effective concentration of the agonist available at the β-adrenergic receptor is probably lower than the added concentration. Furthermore, the release of catecholamines from sympathetic nerves could conceivably result in high local concentrations of catecholamines within adipose tissue. Thus, taking our in vitro data into account, together with available in vivo data (5, 8, 9), we conclude that β-AR activation probably plays a physiological role in the regulation of leptin secretion but that further experiments are needed to establish the in vivo importance of β-adrenergic regulation of leptin release.

Our previous studies of the time course of the Iso effect on leptin release from human adipose tissue showed a significant inhibition after only 90 min of incubation (22). We have not examined earlier time points or nonobese subjects, because the small amounts of accumulated leptin are too close to the limit of detectability of the assay. Thus it is possible that Iso-induced inhibition of leptin occurs within the same time frame as leptin pulsatility, i.e., within 30–60 min. The idea that catecholamines might regulate pulsatility of adipocyte function comes from the recent observation of Bergman’s group (12) that fatty acid release from canine adipose tissue in vivo is pulsatile and is disrupted by β-adrenergic blockade. In vivo, sympathetic input to white adipose tissue is increased by fasting in both rats and humans. Conceivably, variability in the activity of the sympathetic nervous system may influence not only the average rate of release of leptin but also its pulsatile release. Our data suggest that rapid alterations in leptin biochemistry, rather than secretion, is the more important cellular mechanism in rats, but the end result, an adrenergic modulation of leptin release, seems to be similar in both species.

In conclusion, we have provided evidence that stimulation of β-AR within human adipose tissue decreases the release of leptin from human adipose tissue through a posttranslational mechanism, most likely secretion per se. Our data suggest that the adipose tissue leptin response to β-AR stimulation is similar in obese and nonobese humans. We propose that catecholamines may mediate short-term decreases in plasma leptin that occur within hours of fasting and cold exposure. In addition, we hypothesize that sympathetic inhibition of leptin secretion may contribute to its pulsatile secretion. In contrast, in rat adipose tissue, isoproterenol does not affect basal leptin secretion but has a short-term action to antagonize the insulin-stimulated leptin biosynthesis.

Current address of M. R. Ricci: Research Diets, Inc., 20 Jules Ln, New Brunswick, NJ 08901 (E-mail: Ricci@ResearchDiet.com).

GRANTS

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