Regulation of Lipolysis and Leptin Biosynthesis in Rodent Adipose Tissue by Growth Hormone

John N. Fain and Suleiman W. Bahouth

The present study examined the effects of growth hormone (GH) on lipolysis and leptin release by cultured adipose tissue from rats and mice incubated for 24 hours in primary culture. A stimulation of leptin release by GH in rat adipose tissue was found in the presence of 25 nmol/L dexamethasone, and this was accompanied by a 28% increase in leptin mRNA content. GH stimulated lipolysis in rat adipose tissue in the presence of 0.1 nmol/L CL 316,243. In contrast, basal lipolysis in mouse adipose tissue was stimulated by GH, but this was not accompanied by an increase in leptin release. However, in the presence of insulin plus triiodothyronine (T_3), the stimulation of lipolysis by GH was abolished and GH increased leptin release. These results indicate that GH can stimulate leptin release by both mouse and rat adipose tissue in the absence of a stimulation of lipolysis. In contrast, under conditions in which lipolysis is stimulated by GH, there is no effect on leptin release.

LEPTIN is a polypeptide produced by white adipocytes that is involved in the central regulation of appetite and in the release of pituitary hormones.[1,2] The plasma concentration of leptin correlates with total body fat in both rodents and humans.[3,4] In growth hormone (GH)-deficient humans, administration of GH increased plasma leptin 30% after only 2 days of treatment.[5] Wolthers et al[6] reported that plasma leptin was elevated by 53% after treatment of normal males for 4 days with GH plus glucocorticoid, as compared with a 25% increase in men given only glucocorticoid. There was no effect of GH alone in their experiments. The short-term effect of GH in humans is to increase lipid mobilization and elevate plasma leptin,[5,6] while the longer-term effect is to reduce body fat content, which decreases plasma leptin.[7,8] The increase in plasma leptin due to GH might have a role in the reduction in fat content that occurs after long-term GH treatment.[8,9]

The present studies were designed to determine whether direct effects of GH could be found on leptin release by adipose tissue. Most in vitro studies on the release of leptin or leptin mRNA have used adipocytes,[10-14] but Reul et al[15] and Lonnqvist et al[16] used cut pieces of adipose tissue. The present study used cut pieces of adipose tissue, since in studies using rat adipocytes, we found a large loss of leptin mRNA over a 24-hour incubation.[17] We therefore turned our attention to pieces of rat or mouse adipose tissue incubated in primary culture for 24 hours.

MATERIALS AND METHODS

Epididymal adipose tissue for each experiment was obtained by pooling the tissue from 2 to 3 fed male euthyroid Sprague-Dawley rats (335 to 345 g) or 2 to 8 mice (30 to 40 g). The average amount of epididymal adipose tissue used in each experiment was 6.5 g for studies with rat tissue and 3.6 g for studies with mouse adipose tissue. The mice were either C57/B6, C57/B6 × FVN, or C57/B6 × SGL strain fed on mouse breeder chow containing 10% fat. The pooled adipose tissue was cut into pieces of 5 to 20 mg and incubated in 50-mL polypropylene tubes in an upright position in a gyratory water bath shaker (100 rpm). The buffer for incubation of adipose tissue was Dulbecco's modified Eagle's medium/Ham's F12 (1:1, Sigma no. 2906; Sigma, St Louis, MO) containing 17.5 mmol/L glucose, 121 mmol/L NaCl, 4 mmol/L KCl, 1 mmol/L $CaCl_2$, 25 mmol/L HEPES, 2.4 mmol/L sodium bicarbonate, 40 mg/mL bovine serum albumin (Bovuminar L59410; Intergen, Purchase, NY), 5 μg/mL ethanolamine, 0.1 ng/mL sodium selenite, 90 μg/mL penicillin G, 150 μg/mL streptomycin sulfate, 50 μg/mL gentamicin, 55 μmol/L ascorbic acid, 1 μg/mL leupeptin, and 1 μg/mL aprotinin. The pH was adjusted to 7.4 and the buffer was then filtered through a 0.2-μm filter. All agents were added at the start of the incubation.

Aliquots (20 to 50 μL) of the medium were used to measure the leptin content using radioimmunoassay kits with antibody raised against rat or mouse leptin and with rat or mouse leptin standards from Linco Research (St. Charles, MO). Lipolysis was measured by analysis of glycerol release into the medium (10- to 50-μL aliquots) by the procedure of Boobis and Maughan.[17] The GH preparation was human recombinant GH (lot PS9033AX) produced by Genentech (San Francisco, CA). The insulin was bovine insulin obtained from Sigma, as were the other hormones and reagents. Total cellular RNA was extracted by the procedure of Chomczynski and Sacchi,[18] and the analysis of leptin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs was performed by Northern blot analysis as previously described.[19,20]

The effects of added agents were calculated as the percent change from the incubation control in each experiment, since this resulted in a more normal distribution of the data. Statistical comparisons were made using Student's *t* test on the paired differences.

RESULTS

Stimulation of Leptin Biosynthesis by GH in Rat Adipose Tissue in the Presence of Dexamethasone

The addition of 10 nmol/L GH to pieces of rat adipose tissue incubated in primary culture for 48 hours had no effect on leptin release in the absence of dexamethasone. Dexamethasone stimulated leptin release 2-fold over basal by 24 hours and 48 hours in culture, but not significantly at 6 hours. In the presence of dexamethasone, GH stimulated leptin release at 24 hours of incubation and the increase was sustained over the next 24 hours. Insulin-stimulated leptin release was found at 24 and 48 hours and differed from that due to GH by its independence from dexamethasone (Fig 1).

Another difference between insulin and GH was found in their effect on leptin mRNA accumulation. GH enhanced leptin

From the Departments of Biochemistry and Pharmacology, College of Medicine, University of Tennessee, Memphis, Memphis, TN.

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Address reprint requests to John N. Fain, PhD, Department of Biochemistry, College of Medicine, University of Tennessee, Memphis, Memphis, TN 38163.

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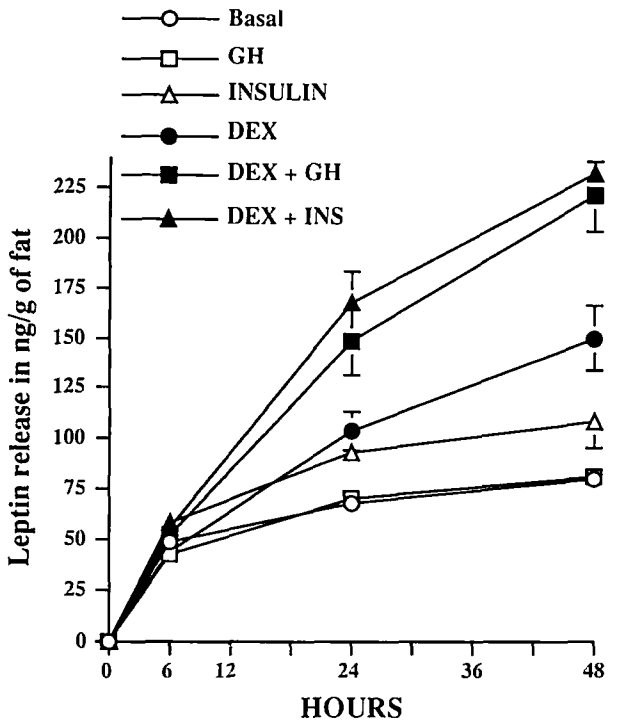


Fig 1. GH stimulates leptin release by adipose tissue after a lag period of 6 hours. Pieces of adipose tissue from euthyroid rats (250 mg) were incubated for 48 hours in 5 mL basal medium (○), with 25 nmol/L dexamethasone (●), 10 nmol/L insulin (△), insulin + dexamethasone (▲), 10 nmol/L GH (□), or GH + dexamethasone (■). Values are the mean ± SEM of 6 paired replications from 3 separate experiments, and aliquots of the medium were removed at 6 and 24 hours.

mRNA by 28% within 24 hours, while insulin reduced leptin mRNA content by 32%. The effect of insulin also differed from that of GH in that insulin stimulated the accumulation of GAPDH mRNA and 18S RNA content. GH had no effect on 18S RNA and inhibited GAPDH mRNA accumulation (Table 1). The insulin-induced increase of GAPDH mRNA confirms similar findings in 3T3 adipocytes[21] and indicates that this so-called "housekeeping" mRNA cannot be used as an index for RNA loading. These data indicate that while GH mimics the ability of insulin to stimulate leptin release in the presence of dexamethasone, its effects on GAPDH mRNA and 18S RNA content are unlike those of insulin.

Dose-Response and Time-Course Relationships of GH and Leptin Biosynthesis in Rat Adipose Tissue

The addition of 1 nmol/L GH to adipose tissue incubated in the presence of 25 nmol/L dexamethasone resulted in a maximal stimulation of leptin release and leptin mRNA accumulation at 24 hours (Fig 2). The effects of GH on leptin mRNA were the same whether the concentration was 1 or 10 nmol/L. However, while no increase was found for leptin release at 6 hours due to GH, the stimulation of leptin mRNA accumulation at 6 hours was comparable to that found at 24 hours (Fig 2). Therefore, enhanced leptin release by GH at 24 hours is preceded by stimulation of leptin mRNA accumulation, which is maximal by 6 hours. These data are reminiscent of data we obtained previously with dexamethasone, where it had little effect on leptin release in isolated adipocytes at 6 hours despite a marked effect on leptin mRNA.[20]

Effects of GH and the β_3-Adrenoceptor Agonist CL 316,243 on Lipolysis in Rat Adipose Tissue

A delayed lipolytic effect of GH has been described in isolated rat adipocytes incubated for 4 hours in the presence of dexamethasone.[22] However, in intact pieces of rat adipose tissue incubated for 24 hours in the presence of dexamethasone, there was no stimulation of lipolysis by GH in the absence of the β_3-adrenergic receptor agonist CL 316,243 (Fig 3). In contrast, in the presence of as little as 0.1 nmol/L CL 316,243, there was greater than 100% stimulation of lipolysis by GH (Fig 3). The potentiation of the lipolytic action of GH was also found with 0.33 and 1 nmol/L CL 316,243. In a separate experiment, the rate of lipolysis in the presence of 10 nmol/L CL 316,243 was approximately 85 μmol/g and no further increase was found in tissue incubated with GH, while at 0.1 and 0.33 nmol/L CL 316,243, lipolysis was enhanced by 40% in the presence of GH. The stimulation of leptin release by GH was markedly reduced in the presence of 0.1, 0.33, or 1 nmol/L CL 316,243 (Fig 3), which was expected, since CL 316,243 reduces leptin biosynthesis in rat adipose tissue.[20] These data suggest that under conditions in which lipolysis is stimulated by GH, the increase in leptin release is inhibited just as with other lipolytic agents.

GH Stimulates Lipolysis by Mouse Adipose Tissue in the Absence of Insulin and Triiodothyronine But Stimulates Leptin Release in Their Presence

In mouse adipose tissue incubated in primary culture for 24 hours in the presence of 25 nmol/L dexamethasone, there was no stimulation of leptin release by GH in the absence of insulin plus triiodothyronine (T_3). In contrast, there was a marked stimulation of lipolysis by GH that was abolished in the presence of T_3 plus insulin. In the presence of 10 nmol/L T_3, there was actually an inhibition of leptin release by GH, and in

Table 1. Effect of Insulin and GH on Leptin Release and Leptin mRNA, GAPDH mRNA, and 18S RNA Content in Intact Rat Adipose Tissue

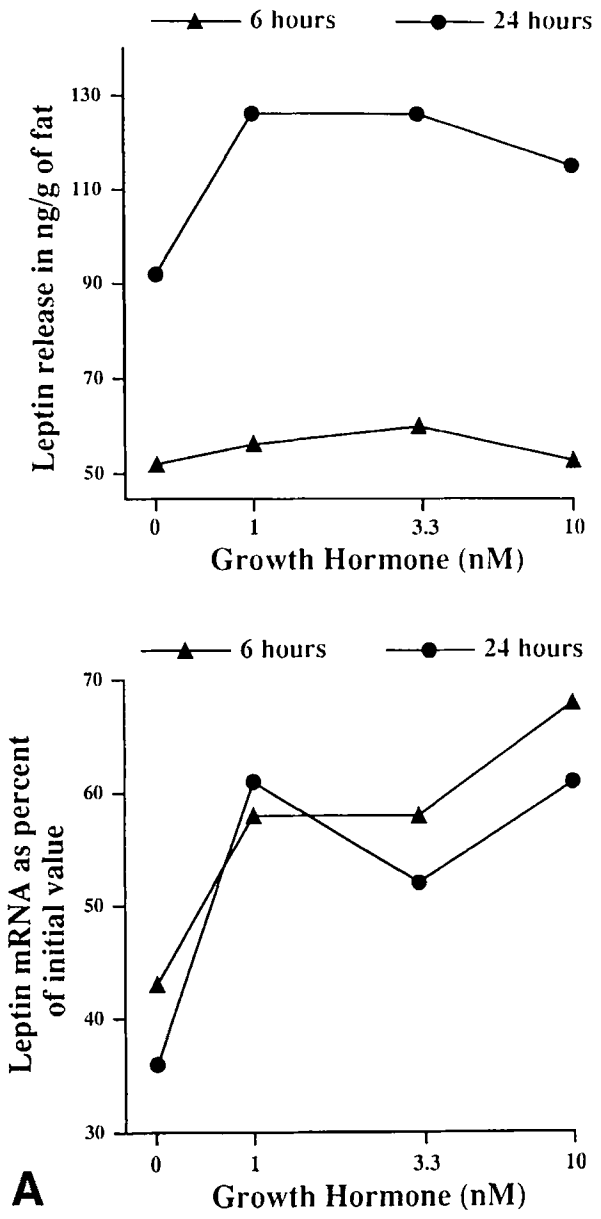
Parameter	Basal Value	% Change Due to Insulin 10 nmol/L	% Change Due to GH 10 nmol/L
Leptin release (ng/g)	156 ± 12	+55 ± 13†	+50 ± 5‡
Leptin mRNA (% of initial)	98 ± 7	−32 ± 5‡	+28 ± 11*
GAPDH mRNA (% of initial)	195 ± 38	+91 ± 21†	−24 ± 6†
Ratio of leptin mRNA/ GAPDH mRNA	.50	−64 ± 4‡	+68 ± 10‡
18S RNA (% of initial)	81 ± 14	+65 ± 25*	−5 ± 25

NOTE. Cut pieces of epididymal adipose tissue from euthyroid rats (250 mg in 5 mL medium) were incubated for 24 hours in the presence of 25 nmol/L dexamethasone, and all agents were added at the start of the incubation. Basal values are the mean ± SEM of 8 paired replications. Leptin and GAPDH mRNA values are corrected for recovery of 18S RNA. Effects of insulin and GH are shown as the % change ± SEM.

*$P < .05$.

†$P < .01$.

‡$P < .001$.



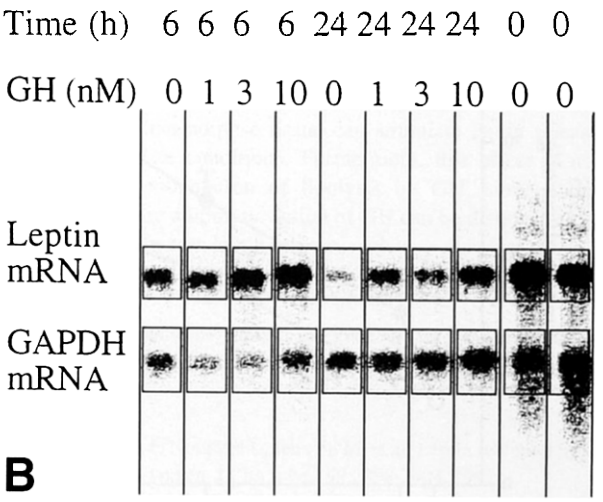


Fig 2. Effect of varying GH concentration on leptin release and leptin mRNA content. (A) Pieces of epididymal adipose tissue from euthyroid rats (500 mg) were incubated for 6 or 24 hours in 10 mL medium containing 10 nmol/L T_3 in the presence of 25 nmol/L dexamethasone with the indicated concentrations of GH. Leptin mRNA content is expressed as a % of the initial value at the start of the incubation corrected for recovery of 18S RNA. Values are the mean of 2 separate paired experiments. (B) Digital image of the Northern blot analysis of leptin and GAPDH mRNAs in 1 of the 2 experiments summarized in A. Time 0 values are in duplicate and are values at the start of incubation, while the other 8 lanes are values at 6 and 24 hours with the indicated concentrations of GH.

the presence of 10 nmol/L insulin, a variable increase in leptin release. Similarly, in the presence of either hormone alone, the lipolytic response to GH was quite variable (Table 2).

DISCUSSION

The present results add GH to the list of agents that can stimulate leptin release in the presence of the appropriate hormonal milieu in adipose tissue from rodents. Hardie et al[12] reported that rat GH did not affect leptin release by rat adipocytes over a 24-hour incubation. We found similar results, since human GH increased leptin release in the presence of dexamethasone in adipocytes by only 11% ± 4% (mean ± SEM of 4 paired replications). These results suggest that adipocytes are less suitable for studies on GH action than incubated pieces of adipose tissue. In contrast, we found a 50% increase in leptin release over 24 hours in incubated pieces of rat adipose tissue in the presence of dexamethasone (Table 1).

It is unlikely that the increase in plasma leptin after short-term GH treatment involves insulin-like growth factor-1 (IGF-1) since adipocytes lack functional IGF-1 receptors.[23] However, IGF-1 can interact with insulin receptors in adipocytes, but only at concentrations 33 to 75 times higher than those of insulin.[24] Reul et al[15] reported that 100 nmol/L IGF-1 inhibited leptin mRNA accumulation to about the same extent as 1 nmol/L insulin. Furthermore, administration of IGF-1 to small hypophysectomized male rats for 6 days increased the body weight while decreasing the low leptin mRNA content of adipose tissue to nondetectable levels.[25]

There was a major loss of leptin mRNA and a reduction in plasma leptin within 4 to 5 hours after administration of

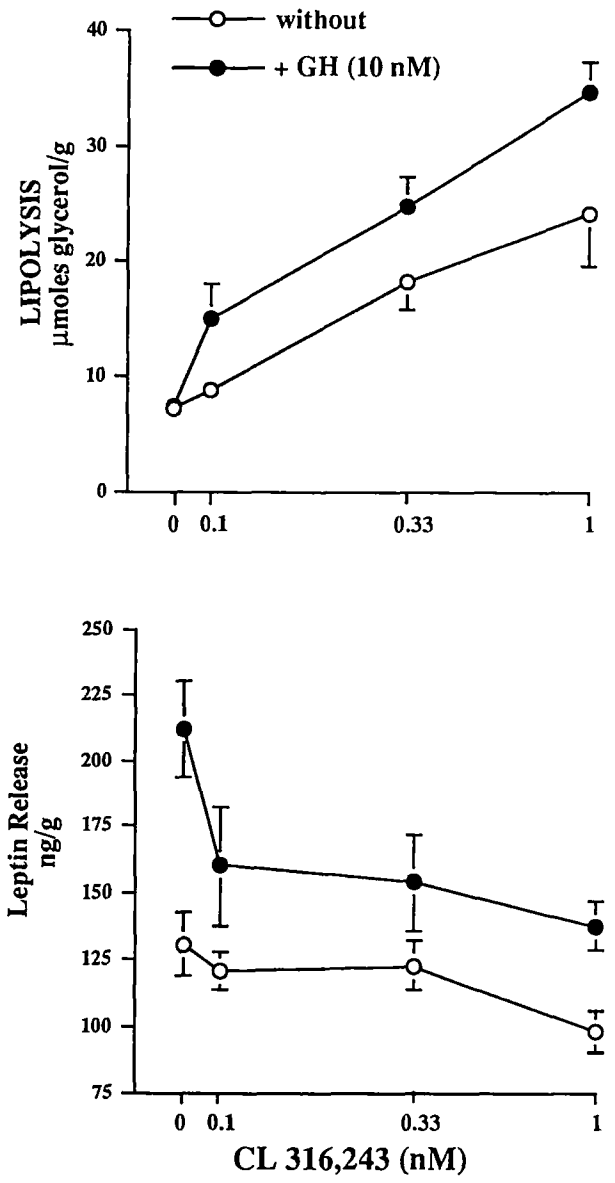


Fig 3. GH stimulates lipolysis in the presence of CL 316,243. Pieces of adipose tissue from euthyroid rats (250 mg) were incubated for 24 hours in 5 mL medium containing 25 nmol/L dexamethasone without or with 10 nmol/L GH in the presence of the indicated concentrations of CL 316,243. Values for leptin release and lipolysis are the mean ± SEM of 4 separate paired experiments. The effects of GH on lipolysis in the presence of 0.1, 0.33, and 1 nmol/L CL 316,243 and on leptin release in both the absence and the presence of 1 nmol/L CL 316,243 were statistically significant based on paired comparisons ($P < .05$).

selective β_3-adrenergic agonists to mice.[26] Similar results were also found after administration of isoproterenol to mice.[27] Gettys et al[11] found that CL 316,243 inhibited leptin release over a 2-hour incubation of rat adipocytes. These results suggest that the inhibition of leptin release by catecholamines is a more rapid process than the stimulation of leptin release and leptin mRNA accumulation by GH and glucocorticoids. We also found little effect of insulin on leptin release by rat adipose tissue at 6 hours (Fig 1), but more rapid stimulatory effects of insulin have been observed by some groups.[11,28,29]

There is little evidence for leptin storage in regulated secretory vesicles in rat adipose tissue.[29] The addition of insulin (700 nmol/L) to incubated pieces of rat adipose tissue doubled the total leptin content at 2 hours of incubation. In mouse adipose tissue, we found that the leptin content was 18 ± 2 ng/g at the start of the 24-hour incubation and 12 ± 6 ng/g tissue at the end of the incubation in 5 experiments. The release of leptin over 24 hours to the medium was 540 ng/g fat in the presence of 25 nmol/L dexamethasone. Therefore, over 99% of the leptin release represented the net synthesis of leptin. These data suggest that the release of leptin in response to GH and other agents represents increased synthesis of leptin.

The lipolytic action of GH on rat adipose tissue first reported by Fain et al[22] requires the presence of glucocorticoids and is now thought to be secondary to a reduction in the level of $G_{i\alpha 2}$ in adipocyte membranes.[30,31] In adipocytes from rats, there was no lipolytic effect of GH except in the presence of CL 316,243 (Fig 3), which via stimulation of adrenergic β_3 receptors activates $G_{s\alpha}$ and consequently increases cyclic AMP, resulting in activation of protein kinase A. These results suggest that inhibition of $G_{i\alpha 2}$ has little effect on basal lipolysis in rat fat but a pronounced effect on lipolysis when G_s is activated by only 0.1 nmol/L CL 316,243. While a reduction in the level of $G_{i\alpha 2}$ in adipocytes might explain the lipolytic action of GH, the effects of GH on leptin release may not be linked to a reduction in $G_{i\alpha 2}$.

GH administration to animals results in lipid mobilization and a loss of adipose tissue accompanied by an inhibition of insulin action.[32] However, in adipose tissue from hypophysectomized rats, the direct addition of GH results in an inhibition of lipolysis and a stimulation of glucose metabolism.[32] In mouse adipose tissue, GH stimulated lipolysis in the presence of dexamethasone without an effect on leptin release (Table 2). However, in the presence of T_3 and insulin, the lipolytic effect of GH was abolished but now a stimulation was observed in leptin release. These data suggest that in the presence of insulin and T_3, GH is an insulin-like agent in that it no longer stimulates lipolysis but, like insulin, actually enhances leptin formation. The mechanism by which GH stimulates leptin release is different from that of insulin, since GH enhanced leptin mRNA

Table 2. GH Stimulates Leptin Release by Mouse Adipose Tissue in the Presence of Insulin + T_3

	Leptin Release		Lipolysis	
Condition	ng/g Fat	% Change Due to 10 nmol/L GH	μmol/g Fat	% Change Due to GH
Basal	240 ± 45	0 ± 16	22 ± 4	+51 ± 17*
+10 nmol/L T_3	255 ± 45	−21 ± 6*	26 ± 9	+38 ± 22
+10 nmol/L insulin	310 ± 35	+26 ± 37	18 ± 3	+37 ± 27
+T_3 + insulin	245 ± 50	+57 ± 22*	18 ± 3	−10 ± 7

NOTE. Cut pieces of mouse epididymal adipose tissue (150 mg) were incubated for 24 hours in 5 mL medium containing 25 nmol/L dexamethasone. All agents were added at the start of the incubation. Values are the mean ± SEM of 7 paired replications, and adipose tissue was pooled in each experiment from 4 mice.

*Significant stimulatory effects of GH based on the paired % differences ($P < .05$).

accumulation while insulin had the opposite effect. Furthermore, in rat adipose tissue, leptin release was stimulated by GH under conditions where no effect on lipolysis could be found (Fig 3). These data suggest that depending on the species and hormonal milieu, GH will stimulate lipolysis, leptin formation, or both depending on the experimental conditions during primary culture of adipose tissue for 24 hours.

An increase in plasma leptin was found in critically ill patients maintained on normocaloric parenteral and/or enteral feeding within 12 hours after administration of GH secretagogues.[33] Wolthers et al[6] also observed an elevation in plasma leptin after 4 days of GH administration to normal males in the presence of glucocorticoid. Our results indicate that a stimulation of leptin release by a direct action of GH on adipose tissue could contribute to the elevation in plasma leptin after administration of GH or GH secretagogues to humans.

GH can be added to the list of hormones whose direct addition to rodent adipose tissue can stimulate leptin release under appropriate conditions. Furthermore, this effect is not linked to the stimulation of lipolysis by GH, since under conditions where a lipolytic action of GH can be demonstrated, there is little effect on leptin release.

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