GH but not IGF-I or insulin increases lipoprotein lipase activity in muscle tissues of hypophysectomised rats

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Abstract

Changes in GH secretion are associated with changes in serum lipoproteins, utilisation of fuels and body composition. Since lipoprotein lipase (LPL) is a key enzyme in the regulation of lipid and lipoprotein metabolism, changes in LPL activity may contribute to these effects of GH. The present study was undertaken to investigate the role of GH and the GH-dependent growth factor, IGF-I, in the regulation of LPL in heart, skeletal muscle and adipose tissue. Female rats were hypophysectomised at 50 days of age. One week later, hormonal therapy was commenced. All hypophysectomised rats received L-thyroxine and cortisol. Adipose tissue, the heart, soleus and gastrocnemius muscles were excised after 1 week of hormonal therapy. The effect of insulin injections on adipose tissue and heart LPL activity was also studied. In separate experiments, LPL activity in post-heparin plasma was measured. Hypophysectomy had no effect on adipose tissue LPL activity, whereas activity was reduced in heart, soleus and gastrocnemius muscle tissues. GH treatment had no significant effect on LPL activity in adipose tissue or soleus muscle, but increased the LPL activity in heart and gastrocnemius muscle. GH treatment increased post-heparin plasma LPL activity. Recombinant human IGF-I treatment (1·25 mg/kg per day) markedly reduced LPL activity in adipose tissue, but had no effect in muscle tissues. The effect of IGF-I treatment on adipose tissue LPL was not reflected by a decrease in post-heparin plasma LPL activity. Daily injections of insulin for 7 days increased LPL activity in adipose tissue but had no effect on heart LPL activity. In adipose tissue, LPL mRNA levels tended to decrease as a result of IGF-I treatment. In the muscle tissues, no significant effects of hypophysectomy, GH or IGF-I treatment on LPL mRNA levels were observed.

It is concluded that GH increases heart and skeletal muscle tissue LPL activity, which probably contributes to an increased post-heparin plasma LPL activity. The effect of GH on muscle LPL activity is probably not mediated by IGF-I or insulin. Insulin and IGF-I have opposite effects on LPL activity in adipose tissue.

Journal of Endocrinology (1999) **160,** 247–255

Introduction

Lipoprotein lipase (LPL) is a key enzyme in the regulation of the flux of fatty acids. LPL is localised, when active, to the endothelium of the vessel wall, where it hydrolyses triglyceride-rich lipoproteins, e.g. chylomicrons and very low density lipoproteins. Released fatty acids are either re-esterified or metabolised as an energy source. LPL activity is expressed in most tissues of adult animals, except the liver (Cryer 1981, Bensadoun 1991). LPL activity affects levels of circulating lipoproteins, especially serum triglycerides, and is rate limiting for the supply of triglyceride-derived fatty acids to skeletal muscle tissue (Levak-Frank *et al.* 1995).

The role of growth hormone (GH) in the regulation of LPL activity is not clear. Stimulation and inhibition by GH have been observed, as well as no effects. Post-heparin plasma LPL activity was reduced in acromegaly (Murase *et al.* 1980) and in GH-treated GH-deficient children (Asayama *et al.* 1984). However, post-heparin plasma LPL activity was not affected by GH treatment of GH-deficient adults (Oscarsson *et al.* 1996) or healthy obese men (Oscarsson *et al.* 1994). In hypophysectomised female rats, post-heparin plasma LPL activity was reduced and GH treatment markedly increased the activity (Vikman-Adolfsson *et al.* 1994).

Skeletal muscles and adipose tissue are the major tissues expressing LPL activity, and are therefore the main contributors to post-heparin plasma LPL activity (Tan *et al.* 1977, Cryer 1981, Bensadoun 1991). In adipose tissue, GH has been shown to decrease LPL activity in humans (Ottosson *et al.* 1995, Oscarsson *et al.* 1996) and

  Printed in Great Britain
0022–0795/99/0160–0247
Online version via http://www.endocrinology.org

rats (Murase *et al.* 1981, Barber *et al.* 1992). Comparatively less attention has been paid to the hormonal regulation of muscle LPL activity.

Insulin-like growth factor-I (IGF-I) expression and secretion are mainly regulated by GH and nutritional factors (Thissen *et al.* 1994). GH regulates the expression and secretion of IGF-I in many tissues (Humbel 1990), including adipose (Vikman *et al.* 1991) and muscle tissues (Isgaard *et al.* 1989). Circulating IGF-I is mainly derived from the liver and is believed to mediate some of the effects of GH via endocrine mechanisms (Guler *et al.* 1988, Humbel 1990).

In order to understand further the effects of GH and the role of IGF-I in the effects of GH on the regulation of LPL, we studied the effects of GH and IGF-I treatment of hypophysectomised rats on LPL activity in adipose and muscle tissues, as well as LPL mRNA expression.

Materials and Methods

Animals

Intact and hypophysectomised female Sprague–Dawley rats, 50–65 days old (Møllegaard Breeding Centre Ltd, Ejby, Denmark), were used. They were kept at constant temperature with a 14 h:10 h light:darkness cycle. Tap water and pelleted food (Type R36, Ewos, Södertälje, Sweden) were freely available. Body weight was measured daily and rats gaining more than 0·5 g/day in body weight during the observation period of 1 week after hypophysectomy were excluded. The hypophysectomised rats were given hormone therapy for 7 days. In order to determine post-heparin plasma LPL activity, the rats were anaesthetised with a combination of ketamine hydrochloride (77 mg/kg, Ketalar, Parke-Davis, Detroit, MI, USA) and xylazine (9 mg/kg, Rompun, Bayer, Lever-Kusen, Germany). An i.v. injection of heparin (250 IU/kg, Lövens, Ballerup, Denmark) was given into the tail vein. The rats were exsanguinated by open heart puncture 10 min after the administration of heparin (Krauss *et al.* 1973, Vikman-Adolfsson *et al.* 1994). In the other experiments, the rats were killed by decapitation and parametrial and retroperitoneal adipose tissue, heart, soleus and gastrocnemius muscles were taken out, blotted, weighed and stored at −70 °C until analysis. If not otherwise stated, the rats were killed between 1000 and 1200 h. The study was approved by the local Ethics Committee.

Hormonal treatment

L-Thyroxine (Nycomed Ltd, Oslo, Norway; 10 μg/kg per day) and hydrocortisone phosphate (Solu-Cortef, Upjohn, Puurs, Belgium; 400 μg/kg per day) diluted in saline were given as a daily s.c. injection to all the hypophysectomised rats at 0800 h. Recombinant bovine GH (bGH; potency not specified) was generously provided by American Cyanamid Co. (Princeton, NJ, USA). bGH (0·1, 1·5 or 5 mg/kg per day according to the design of the experiment) was diluted in 0·05 mM phosphate buffer, pH 8·6, with 1·6% glycerol and 0·02% sodium azide and given as a continuous infusion via an osmotic mini-pump (Model 2001, Alzet Corporation, Palo Alto, CA, USA) or as two daily injections for 7 days (Oscarsson *et al.* 1991, Vikman-Adolfsson *et al.* 1994). Human recombinant IGF-I, generously provided by Genentech Inc. (San Francisco, CA, USA), was diluted in saline and infused by osmotic mini-pumps at a dose of 1·25 mg/kg per day (Sjöberg *et al.* 1994). Insulin (Insulatard, 100 IU/ml, Novo Nordisk A/S, Denmark) was diluted in saline and given as a daily s.c. injection at 1600 h. The insulin dose was gradually increased (Gause *et al.* 1985): day 1–4, 1·0 U/day; day 5–7, 2·0 U/day. The treatments continued for 7 days.

Serum analysis

Serum glucose concentrations were measured by the glucose 6-phosphate dehydrogenase method (Merck, Darmstadt, Germany). Serum insulin concentration was determined by a RIA (Phedebas, Pharmacia, Uppsala, Sweden). Serum IGF-I concentrations were determined by a hydrochloride acid–ethanol extraction RIA, using human IGF-I for labelling (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) (Sjöberg *et al.* 1994).

LPL

LPL activity in the heart, soleus and gastrocnemius muscle tissues was determined according to Lithell & Boberg (1978). Briefly, frozen tissue was incubated in a reaction medium at 37 °C. The reaction medium contained 2·1 M glycine buffer, pH 8·4, and heparin (240 IU/ml). The triolein emulsion used was according to Nilsson-Ehle & Schotz (1976). The intra-assay coefficient of variation (CV) calculated from duplicates was 12·5%. Activity was expressed as milliunits (mU) per gram tissue (1 mU=1 nmol free fatty acid (FFA) released per minute).

LPL activity was measured in adipose tissue as well as in muscle tissues according to the method of Peterson *et al.* (1985). In brief, frozen tissue was homogenised in a detergent-containing buffer. To each gram of tissue, 9 ml buffer was added. The homogenate was centrifuged for 5 min at 15 000 ***g*** and the clear solution between the sediment and the floating fat layer was used for the assay of LPL. Muscle and heart tissues were diluted 10 times in buffer before assay. The substrate emulsion used was Intralipid (10%, Kabi Pharmacia, Stockholm, Sweden) labelled with [^{3}H]triolein (Dr Krabisch, Lund University, Lund, Sweden). The samples were incubated in triplicate at 25 °C. Liberated fatty acids were extracted (Spooner *et al.* 1977) and quantified in a liquid scintillation counter. The intra-assay CV for determinations in adipose tissue

Table 1 Effects of recombinant bGH (1 mg/kg per day) or recombinant human IGF-I (1·25 mg/kg per day) treatment of hypophysectomised (Hx) female rats on body weight gain, adipocyte diameter, serum IGF-1, glucose and insulin. The values are compared with those of normal age-matched females rats (N). Values are means ± S.E.M. of 10–12 observations, except adipocyte diameter, which was analysed in four rats of each group

	Weight gain (g/day)	**Adipocyte diameter** (μm)	**IGF-I** (μg/l)	**Glucose** (mmol/l)	**Insulin** (mU/l)
Group					
N	2·5 ± 0·1[a]	66·3 ± 1·8	1148 ± 68[a]	7·4 ± 0·3	11·5 ± 0·8[a]
Hx	− 0·7 ± 0·3[b]	54·2 ± 5·0	183 ± 33[b]	7·1 ± 0·2	7·5 ± 0·2[b]
Hx+GH	3·4 ± 0·3[c]	57·3 ± 1·6	712 ± 48[c]	7·4 ± 0·3	10·3 ± 0·9[a]
Hx+IGF-I	1·8 ± 0·2[d]	58·1 ± 1·5	302 ± 60[d]	7·7 ± 0·2	6·5 ± 0·4[b]

Rats were hypophysectomised at 50 days of age. After 1 week of observation, the rats were given hormones for 7 days. All hypophysectomised rats were given L-thyroxine (10 μg/kg per day) and cortisol phosphate (400 μg/kg per day). GH and IGF-I were given as a continuous infusion by means of osmotic mini-pumps. IGF-I, insulin, glucose concentrations and adipocyte diameter in parametrial fat were analysed as described in Materials and Methods.
In all columns, values with different superscripts are significantly different from each other ($P<0·05$, ANOVA followed by Student–Newman–Keul's test).

was 6%, and in heart and gastrocnemius muscle tissues, 5%. Activity was expressed as mU per gram tissue (1 mU=1 nmol FFA released per minute).

The diameter of adipocytes in parametrial fat was determined as previously described (Smith *et al.* 1972). Each observation is the mean of 100 cell diameters.

Post-heparin plasma LPL activity was determined according to Nilsson-Ehle & Ekman (1977), as previously described (Vikman-Adolfsson *et al.* 1994). The activity was expressed as micromoles fatty acids released per minute per millilitre plasma. The intra-assay CV was 6%. All samples were run in one single assay.

LPL probe

[^{32}P]UTP- or [^{35}S]UTP-labelled antisense LPL RNA was synthesised using an EcoR1 linearised plasmid pGem 4Z (Samuelsson *et al.* 1991) as template. The plasmid contains a 550 bp fragment of guinea pig cDNA which has 82% homology with rat cDNA (Enerbäck *et al.* 1987, Brault *et al.* 1992). This probe was found to give a single transcript of 3·6 kb using Northern blot (data not shown), which is the expected size of LPL mRNA in the rat (Brault *et al.* 1992). Sense LPL RNA was synthesised using an SST linearised plasmid.

RNase protection solution hybridisation assay

Total nucleic acids (TNA) were prepared according to the method of Durnam & Palmiter (1983). In brief, the tissue was homogenised in a buffer containing 1% SDS, 20 mM Tris–HCl (pH 7·5) and 4 mM EDTA and digested with proteinase-K (20 μg/ml) overnight. TNA were then extracted with phenol–chloroform. The hybridisation was performed with TNA samples at 70 °C for 24 h in 0·6 M NaCl, 20 mM Tris–HCl (pH 7·5), 4 mM EDTA, 0·1% SDS, 0·75 mM dithiothreitol, 3·3 μg tRNA, 25% formamide with a [^{35}S]UTP-labelled LPL cRNA probe in a volume of 40 μl. The samples were then treated with 40 μg RNase A and 2 μg RNase T_1 in the presence of 100 μg herring sperm DNA for 45 min at 37 °C in a volume of 1 ml. Protected probe was precipitated with 100 μl trichloroacetic acid (6 M). The precipitate was collected on glass-fibre filters (GF/C, Whatman International Ltd, Maidstone, UK) and radioactivity counted in a liquid scintillation counter. The signal was compared with a standard curve which was obtained by hybridisation of *in vitro*-transcribed LPL mRNA (Samuelsson *et al.* 1991). The DNA content in the samples was analysed according to Labarca & Paigen (1980) and 3–20 μg DNA were assayed. Within this range, the hybridisation signal paralleled the standard curve. The intra-assay CV calculated from duplicates was 6%. The results are expressed as amount of LPL mRNA/DNA (amol/μg).

Statistics

Values are given as means ± S.E.M. Comparisons between groups were performed with ANOVA followed by Student–Newman–Keul's multiple range test. *P* values less than 0·05 were considered significant. Values were transformed to logarithms when appropriate.

Results

GH and IGF-I treatment of hypophysectomised rats resulted in a significant increase in body weight. Serum IGF-I concentrations were increased by both treatments. However, IGF-I treatment resulted in lower serum IGF-I levels and body weight gain than GH treatment (Table 1). GH treatment resulted in higher serum insulin concentrations compared with hypophysectomised rats, whereas IGF-I treatment had no effect on the insulin concentrations. Neither GH nor IGF-I treatment had any effect on serum glucose concentrations (Table 1). After 2 days of treatment, serum insulin was already increased by GH treatment, but IGF-I treatment had no effect (data not

shown). LPL activity was determined in heart and predominantly white (gastrocnemius) and red (soleus) skeletal muscle by the method of Lithell & Boberg (1978). In heart, gastrocnemius muscle and soleus muscle, LPL activity was reduced in the hypophysectomised rats given thyroxine and cortisol substitution (Fig. 1). GH treatment increased LPL activity in heart (20%) and gastrocnemius muscle (50%). The LPL activity in the gastrocnemius muscle was lower compared with heart and soleus muscle. In soleus muscle, there was no significant effect of GH treatment, although the activity tended to be higher also in this muscle after GH treatment. IGF-I treatment had no effect on LPL activity in the muscle tissues (Fig. 1). LPL activity in the parametrial adipose tissue was measured by the method of Peterson *et al.* (1985). LPL activity in adipose tissue was not affected by hypophysectomy or GH treatment. In contrast, IGF-I treatment markedly reduced LPL activity in the parametrial adipose tissue (Fig. 1). The diameter of adipocytes isolated from parametrial adipose tissue was not affected by the hormonal treatment (Table 1), indicating that the LPL activity per cell was affected.

In order to determine if there was any effect of the hormones on LPL mRNA levels, LPL mRNA was quantified with an RNase protection solution hybridisation assay (Table 2). In adipose tissue from IGF-I-treated animals, LPL mRNA levels were reduced compared with those in adipose tissue from normal and GH-treated hypophysectomised rats, but there was no effect of any of the treatments on LPL mRNA levels in heart or gastrocnemius muscle.

The lack of effect of GH treatment on LPL activity in parametrial adipose tissue was surprising in view of previous findings in the rat on this activity (Murase *et al.* 1981, Barber *et al.* 1992). Although the experimental designs differ in several ways, one obvious difference is the mode of administration of GH. It is possible that two daily injections, as previously used by Barber *et al.* (1992), could result in another response, which could be dependent on the time of killing. Hypophysectomised rats were therefore given two daily injections or a continuous infusion of GH for 7 days. The rats were killed as indicated in Table 3. There was no effect of either two daily injections or a continuous infusion of GH on the parametrial adipose tissue LPL activity compared with control (one-way ANOVA). However, at all time-points LPL activity tended to be more stimulated when the rats were given a continuous infusion of GH (Table 3). When the two modes of administration were compared (two-way ANOVA), it was revealed that a continuous infusion resulted in a higher LPL activity than two daily injections, and that the time of killing had no impact on LPL activity.

LPL activity in heart and skeletal muscle was also analysed in tissue homogenate using the method of Peterson *et al.* (1985). Heart LPL activity in hypophysectomised rats was 54 ± 7% (n=7) of that in normal rats (100 ± 3%, n=6) (P<0·05). This method also detected a significant reduction (P<0·05) in LPL activity in gastrocnemius muscle following hypophysectomy (74 ± 5%, n=7) compared with normal rats (100 ± 6%, n=6). Thus, the methods used gave similar results regarding the effect of hypophysectomy on LPL activity in heart and the gastrocnemius muscle. The effect of different doses of GH in hypophysectomised rats on LPL activity in heart and the gastrocnemius muscle was then determined (Table 4). The highest dose of GH used (5 mg/kg) resulted in a 26% increase in heart LPL activity and a 35% increase in gastrocnemius muscle LPL activity (Table 4).

GH treatment increased serum insulin concentrations (Table 1). In order to investigate if the effect of GH on LPL activities was indirect via the effect on insulin, daily injections of insulin were given to hypophysectomised rats for 7 days. The effect of insulin was compared with that of a continuous infusion of GH (Fig. 2). Insulin treatment had no effect on heart LPL activity, in contrast to GH. In adipose tissue, insulin treatment increased LPL activity by nearly 80%, whereas GH had no significant effect (cf. Table 3). Serum insulin concentrations were increased 2- to 3-fold by the insulin treatment (data not shown), in line with previous results (Gause *et al.* 1985).

Finally, the effect of IGF-I and GH on post-heparin plasma LPL activity was investigated. IGF-I treatment had no effect on heparin-releasable LPL activity (Table 5). However, post-heparin LPL activity was reduced after hypophysectomy and increased after GH treatment.

Discussion

In the present study, we show for the first time that GH increases LPL activity in skeletal muscles and heart. The most prominent effect of GH was observed in predominantly white skeletal muscle (gastrocnemius). The effects of GH did not seem to be mediated via IGF-I, since IGF-I in contrast to GH had no effect on LPL activity in heart or skeletal muscles. Moreover, the effect of GH on muscle LPL activity was probably not mediated by insulin, since insulin treatment, in contrast to GH treatment, had no effect on heart LPL activity. IGF-I treatment and insulin treatment had opposite effects on adipose tissue LPL activity. Although GH enhances the production and secretion of IGF-I in the adipose tissue of the rat (Vikman *et al.* 1991), GH treatment had no effect on LPL activity in adipose tissue. It is therefore possible that GH, via mechanisms not involving IGF-I production, has an effect opposite to that of IGF-I on LPL activity. We did not observe any significant effect of GH or IGF-I on LPL mRNA levels in adipose tissue, although IGF-I treatment tended to decrease LPL mRNA levels. Other studies in the rat have indicated that GH decreases LPL activity in adipose tissue (Murase *et al.* 1981, Barber *et al.* 1992). The discrepant results may be explained by different

experimental designs and differences in the duration of treatment. For example, the higher LPL activity in parametrial adipose tissue following a continuous infusion of GH indicates that the mode of GH administration could be of importance.

Although IGF-I markedly decreased LPL activity in adipose tissue, IGF-I treatment did not affect heparin-releasable LPL activity. However, the increased LPL activity in muscle tissues after GH treatment may explain the increased post-heparin plasma LPL activity as found previously (Vikman-Adolfsson *et al.* 1994) because of the much larger proportion of muscle tissue compared with adipose tissue in the rat (Roberts *et al.* 1992).

In line with previous studies, the highest LPL activity was measured in heart and adipose tissue, followed by red skeletal muscle (soleus), and the lowest activity in predominantly white skeletal muscle (gastrocnemius) (Linder *et al.* 1976, Tan *et al.* 1977). Similar differences between the tissues were also noticed on the LPL mRNA levels. Two different methods were used to measure muscle LPL activity. The effects of hypophysectomy and hormones were similar using these methods, indicating that the

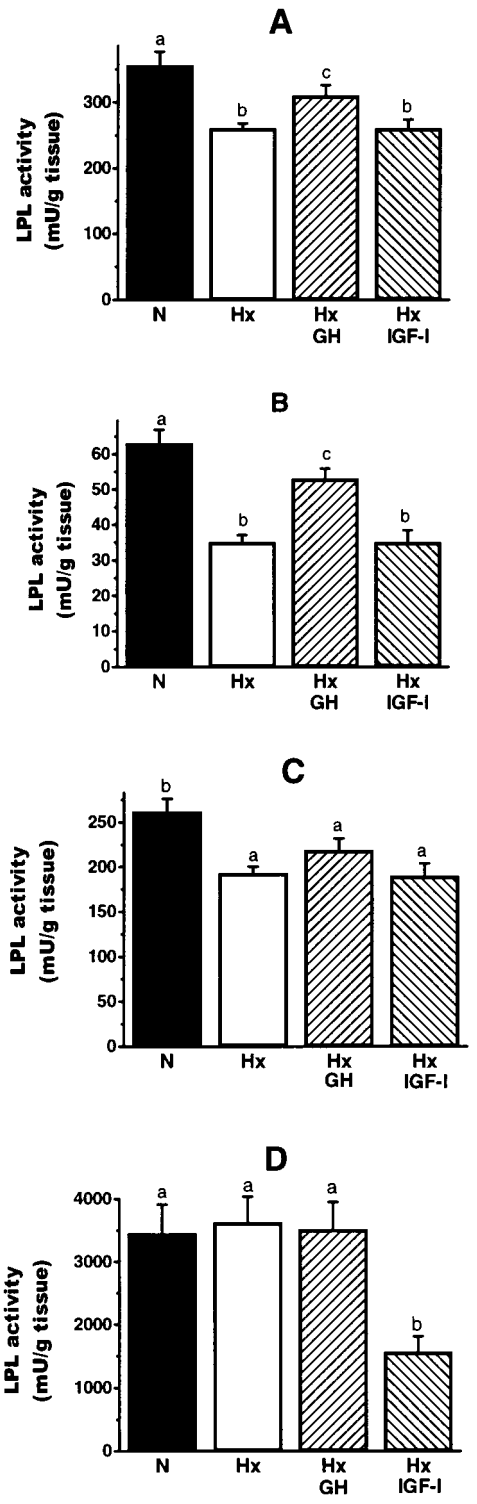

Figure 1 Effect of GH (1 mg/kg per day) or IGF-I (1·25 mg/kg per day) treatment of hypophysectomised (Hx) rats on LPL activity in the heart (A), gastrocnemius muscle (B), soleus muscle (C) and parametrial adipose tissue (D). Rats were hypophysectomised at 50 days of age. After 1 week of observation the rats were given hormones for 7 days. All hypophysectomised rats were given L-thyroxine (10 µg/kg per day) and cortisol phosphate (400 µg/kg per day). LPL activity in heart and skeletal muscle tissues was analysed according to the method of Lithell & Boberg (1978) and LPL activity in adipose tissue analysed according to the method of Peterson *et al.* (1985). The values are compared with those of normal age-matched female rats (N). Values are means ± S.E.M. of eight observations in each group. Values with different superscripts are significantly different from each other ($P<0·05$, ANOVA followed by Student–Newman–Keul's test).

Table 2 Effects of recombinant bGH (1 mg/kg per day) or recombinant human IGF-I (1·25 mg/kg per day) treatment of hypophysectomised (Hx) female rats on LPL mRNA levels in heart, gastrocnemius muscle and parametrial adipose tissue. The values are compared with those of normal age-matched female rats (N). Values are means ± S.E.M. of 10–12 observations

	LPL mRNA (amol/µg DNA)		
	Heart	Gastrocnemius	Adipose tissue
Group			
N	55 ± 10	14 ± 1	108 ± 9[a]
Hx	45 ± 3	17 ± 2	90 ± 9[ab]
Hx+GH	56 ± 5	14 ± 1	108 ± 5[a]
Hx+IGF-I	50 ± 6	14 ± 2	73 ± 10[b]

Rats were hypophysectomised at 50 days of age. After 1 week of observation, the rats were given hormones for 7 days. All hypophysectomised rats were given L-thyroxine (10 µg/kg per day) and cortisol phosphate (400 µg/kg per day). LPL mRNA levels were analysed as described in Materials and Methods.
In all columns, values with different superscripts are significantly different from each other ($P<0·05$, ANOVA followed by Student–Newman–Keul's test).

Table 3 Effects of two daily injections and continuous infusion of recombinant bGH (1 mg/kg per day) on LPL activity in parametrial adipose tissue at different time points during the day. Values are means ± S.E.M. of six observations in each group

	Hormonal treatment	LPL activity (mU/g tissue)
Time of killing		
1100–1200 h	Controls	3362 ± 260
	GH × 2	3273 ± 127
	GH continuous	4684 ± 670
1600–1700 h	GH × 2	4086 ± 389
	GH continuous	4234 ± 526
1900–2000 h	GH × 2	3487 ± 246
	GH continuous	4801 ± 524

Female rats were hypophysectomised at 50 days of age. After 1 week of observation, the rats were given hormones for 7 days. All rats, including controls, were hypophysectomised and given L-thyroxine (10 µg/kg per day) and cortisol phosphate (400 µg/kg per day). LPL activity in parametrial adipose tissue was analysed according to the method of Peterson *et al.* (1985).
Values were analysed with one-way and two-way tANOVA followed by Student–Newman–Keul's test.

Table 4 Effects of different doses of recombinant bGH in hypophysectomised female rats on LPL activity in the heart and gastrocnemius muscle tissues. Values are means ± S.E.M. of eight observations in each group

	LPL activity (mU/g tissues)	
	Heart	Gastrocnemius muscle
Treatment (mg bGH/kg per day)		
Control	2995 ± 49^{b}	265 ± 25^{b}
0·1	2919 ± 72^{b}	317 ± 28^{ab}
1	3309 ± 168^{ab}	382 ± 28^{a}
5	3722 ± 279^{a}	358 ± 19^{a}

Rats were hypophysectomised at 50 days of age. After 1 week of observation, the rats were given hormones for 7 days. All rats, including controls, were hypophysectomised and given L-thyroxine (10 µg/kg per day) and cortisol phosphate (400 µg/kg per day). LPL activity in heart and gastrocnemius muscle was analysed according to the method of Peterson *et al.* (1985).
Values with different superscripts are significantly different from each other ($P<0·05$, ANOVA followed by Student–Newman–Keul's test).

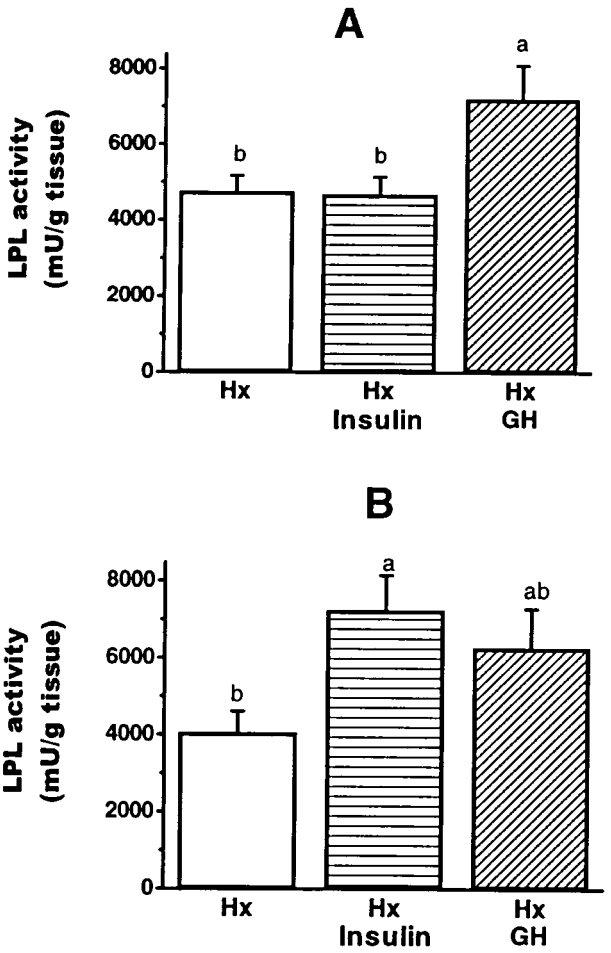

Figure 2 Effect of GH and insulin treatment of hypophysectomised (Hx) rats on LPL activity in the heart (A) and parametrial adipose tissue (B). Rats were hypophysectomised at 50 days of age. After 7–10 days of observation the rats were hormonally treated for 7 days. All hypophysectomised rats were given L-thyroxine (10 µg/kg per day) and cortisol phosphate (400 µg/kg per day) as a daily s.c. injection. bGH (1·5 mg/kg per day) was given as a continuous infusion by means of osmotic mini-pumps. Insulin was given as a daily s.c. injection at 1600 h. The dose was gradually increased: days 1 to 4, 1·0 U/day; days 5 to 7, 2·0 U/day. LPL activity in heart and parametrial adipose tissue was analysed according to the method of Peterson *et al.* (1985). There were four or five rats in each group. Values are means ± S.E.M. Values with different superscripts are significantly different from each other ($P<0·05$, ANOVA followed by Student–Newman–Keul's test).

heparin-extracted activity (Lithell & Boberg 1978) mirrors the total LPL activity in a tissue homogenate (Peterson *et al.* 1985) in these experimental situations.

Apart from the hormonal changes induced by hypophysectomy in the present model, other non-hormonal factors such as food intake may influence the measured LPL activity (Cryer 1981). However, food intake has been shown to be unaffected by GH (Clark *et al.* 1985, Roberts *et al.* 1992) and IGF-I treatment of rats (Sjöberg *et al.* 1994). Hypophysectomised rats were given replacement therapy with L-thyroxine and cortisol in doses previously shown to be within the physiological range (Thorngren & Hansson 1973, Jansson *et al.* 1982). The dose of GH given in these experiments (1 or 1·5 mg/kg per day) is considered to be within the physiological range. This assumption is based on the knowledge of the integrated serum concentration of GH in 50- to 60-day-old female rats (Jansson *et al.* 1985) and the plasma clearance rate of GH (Frohman & Bernardis 1970). Human IGF-I with 3 of 70 amino acids different from rat IGF-I is more potent *in vitro* than rat IGF-I on rat adipocytes (Tamura *et al.* 1989). The dose used in our experiments increased body weight to the

Table 5 Effects of recombinant bGH (1 mg/kg per day) or recombinant human IGF-I (1·25 mg/kg per day) treatment of hypophysectomised (Hx) female rats on post-heparin plasma LPL activity. Values are means ± S.E.M. of five observations in each group

Group of animals	Hormone	Post-heparin LPL activity (μmol/min per ml)
N	—	0·90 ± 0·02[a]
Hx	—	0·64 ± 0·03[b]
Hx	IGF-I	0·74 ± 0·03[b]
Hx	GH	1·11 ± 0·02[c]

Rats were hypophysectomised at 50 days of age. After 1 week of observation, the rats were given hormones for 7 days. All hypophysectomised rats were given L-thyroxine (10 μg/kg per day) and cortisol phosphate (400 μg/kg per day). Post-heparin plasma LPL activity was analysed as described in Materials and Methods. The values are compared with those of normal age-matched female rats (N).
Values with different superscripts are significantly different from each other ($P<0·05$, ANOVA followed by Student–Newman–Keul's test).

same extent as in previous reports (Guler *et al.* 1988) and has been shown to result in nearly normalised serum concentrations of IGF-I (Sjöberg *et al.* 1994). Glucose concentrations were not altered by IGF-I treatment, indicating that the continuous infusion of IGF-I did not induce hypoglycaemia. IGF-I has been reported to acutely inhibit insulin secretion (Leahy & Vandekerkhove 1990). In contrast, longer exposure of rat islets to IGF-I increased insulin secretion (Sieradzki *et al.* 1988). In these and previous experiments (Sjöberg *et al.* 1994), we have not been able to detect any effects of IGF-I treatment on serum insulin concentrations. We therefore consider it unlikely that any change in insulin secretion would have influenced the effect of IGF-I treatment on LPL activity. However, it cannot be excluded that 7 days of IGF-I treatment decreased the insulin responsiveness of adipose tissue.

Hypophysectomy and GH treatment changed serum concentrations of insulin, indicating that the effects of GH could be due to alterations in insulin levels. A stimulatory effect of insulin on LPL activity in adipose tissue due to transcriptional as well as translational and post-translational effects has been described (Eckel *et al.* 1984, Ong *et al.* 1988). In line with these observations, an increase in adipose tissue LPL activity occurred after insulin treatment of hypophysectomised rats.

In heart and skeletal muscle tissues, insulin has been shown to have a less pronounced effect than in adipose tissue on LPL activity (Deshaise *et al.* 1990). Moreover, fasting/feeding seems to have a less pronounced and less consistent effect on LPL activity in muscle tissues than in adipose tissue, in most studies reflected in no change or increased muscle LPL activity during fasting (Linder *et al.* 1976, Tan *et al.* 1977, Ladu *et al.* 1991). In line with these results, there was no effect of insulin treatment on heart LPL activity, but a marked effect on adipose tissue LPL activity. GH treatment has been shown to decrease basal and insulin-stimulated glucose uptake in muscles of the rat (Goodman & Schwartz 1974, Davidson 1979), indicating decreased insulin responsiveness. Serum insulin levels were increased by GH treatment without any effect on glucose concentrations, indicating that decreased insulin responsiveness was balanced by an increased insulin secretion. Thus, it is unlikely that changes in insulin secretion and insulin responsiveness could explain the effect of GH on muscle tissue LPL activity.

GH has lipolytic and anti-lipogenic effects in adipose tissue, which may result in increased availability of fatty acids in other tissues. It has been proposed that this effect of GH results in a decreased respiratory ratio and a decreased use of glucose as a fuel, especially in muscle tissues (Randle *et al.* 1963, Goodman & Schwartz 1974). An increase in LPL activity in heart and skeletal muscle as a result of GH action could further increase the availability of fatty acids in these tissues, since LPL activity in skeletal muscle has been shown to be rate limiting for the uptake of triglyceride-derived fatty acids in this tissue (Levak-Frank *et al.* 1995). The effect of GH on LPL activity in muscle tissue indicates that the increased use of fatty acids in muscle tissue as a result of GH action is not only a result of increased availability of circulating fatty acids. Moreover, changes in insulin responsiveness in muscle tissues following GH treatment may in part be explained by the effects of GH on LPL activity in these tissues.

Although GH increases the production and secretion of IGF-I from various tissues, the effects of these hormones differ in several aspects, especially regarding effects on lipid and lipoprotein metabolism. GH treatment of hypophysectomised rats reduced serum concentrations of apolipoprotein B and decreased triglyceride concentrations (Oscarsson *et al.* 1991), whereas IGF-I treatment increased apolipoprotein B and triglyceride concentrations (Sjöberg *et al.* 1994). These findings are in line with observations made in GH and IGF-I transgenic mice, in which serum triglyceride levels were increased in IGF-I transgenic mice but not in GH transgenic mice (Quaife *et al.* 1989). The different effects of GH and IGF-I are further substantiated by the present finding of different effects of these hormones on LPL activity. Thus, several effects of GH on lipid and lipoprotein metabolism may actually be balanced by opposing effects of IGF-I.

There are few studies on the effect of IGF-I on LPL activity. In mature 3T3-L1 adipocytes (Spooner *et al.* 1979), as well as in human isolated adipocytes (Kern *et al.* 1988), IGF-I increased LPL activity. The discrepancy between these two studies and our results in adipose tissue could be due to different effects of IGF-I in an acute experiment compared with effects of long-term treatment with IGF-I. The different results could also be due to species differences. Such a difference may be at the level of receptors, since rat adipocytes seem to lack IGF-I receptors

(King & Kahn 1980). Thus, the mechanism for the inhibitory effect of IGF-I on LPL activity in adipose tissue is not clear, but may involve effects of IGF-I on other cells in the vicinity of the adipocytes. In view of the present results and previous reports, it seems appropriate to conclude that IGF-I plays an important role in the regulation of intermediary metabolism, serving as a modulator of the effects of GH and probably also insulin in this regulation.

In the rat, GH treatment has been shown to increase carcass protein without affecting carcass fat or parametrial fat pad weight (Roberts *et al.* 1992). However, IGF-I treatment of hypophysectomised rats (Guler *et al.* 1988) and diabetic rats (Tomas *et al.* 1993) resulted in reduced weight of the epididymal fat pad and decreased carcass fat. Thus, the effects of GH and IGF-I on LPL activity may play a role in metabolic adjustments, resulting in changed utilisation of fuels and body composition.

Acknowledgements

We are grateful to Barbro Basta, Ann-Sofie Lidmer, Birgitta Odén, Carolina Simonsson and Siv Tengblad for excellent technical assistance. This study was supported by the Swedish Medical Research Council (Grant 8269 and 04P-11010), the Nordisk Insulin Foundation, Göteborg Medical Society, Åke Wibergs Foundation, Tore Nilson Foundation, Magnus Bergvalls Foundation, Handlaren Hjalmar Svensson Foundation and The Swedish Medical Society.

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Received 26 September 1997
Revised manuscript received 20 April 1998
Final version received 18 September 1998
Accepted 30 September 1998