Different effects of IGF-I on insulin-stimulated glucose uptake in adipose tissue and skeletal muscle

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Frick, Fredrik, Jan Oscarsson, Kerstin Vikman-Adolfsson, Malin Ottosson, Noriko Yoshida, and Staffan Edén. Different effects of IGF-I on insulin-stimulated glucose uptake in adipose tissue and skeletal muscle. Am J Physiol Endocrinol Metab 278: E729–E737, 2000.—The effect of insulin-like growth factor I (IGF-I) on insulin-stimulated glucose uptake was studied in adipose and muscle tissues of hypophysectomized female rats. IGF-I was given as a subcutaneous infusion via osmotic minipumps for 6 or 20 days. All hypophysectomized rats received L-thyroxine and cortisol replacement therapy. IGF-I treatment increased body weight gain but had no effect on serum glucose or free fatty acid levels. Serum insulin and C-peptide concentrations decreased. Basal and insulin-stimulated glucose incorporation into lipids was reduced in adipose tissue segments and isolated adipocytes from the IGF-I-treated rats. In contrast, insulin treatment of hypophysectomized rats for 7 days increased basal and insulin-stimulated glucose incorporation into lipids in isolated adipocytes. Pretreatment of isolated adipocytes in vitro with IGF-I increased basal and insulin-stimulated glucose incorporation into lipids. These results indicate that the effect of IGF-I on lipogenesis in adipose tissue is not direct but via decreased serum insulin levels, which reduce the capacity of adipocytes to metabolize glucose. Isoproterenol-stimulated lipolysis, but not basal lipolysis, was enhanced in adipocytes from IGF-I-treated animals. In the soleus muscle, the glycogen content and insulin-stimulated glucose incorporation into glycogen were increased in IGF-I-treated rats. In summary, IGF-I has opposite effects on glucose uptake in adipose tissue and skeletal muscle, findings which at least partly explain previous reports of reduced body fat mass, increased body cell mass, and increased insulin responsiveness after IGF-I treatment.

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To further understand the role of IGF-I for glucose uptake in skeletal muscle and adipose tissue, we have studied the effects of IGF-I treatment of hypophysectomized rats on basal and insulin-stimulated glucose incorporation into lipids and glycogen in adipose tissue and skeletal muscle tissue, respectively.

**MATERIALS AND METHODS**

Animals. Female Sprague-Dawley rats (Mollegaard Breeding Center, Ejby, Denmark) were used in these experiments. Hypophysectomy (Hx) was performed at Mollegaard Breeding Center when animals were 50 days old. The animals were maintained under standardized conditions of temperature (24–26°C) and humidity (50–60%), with a light period between 0500 and 1900. The rats were given free access to standard rat chow (rat/mouse standard diet, B&K Universal, Sollentuna, Sweden) and tap water. A body weight gain of >0.5 g/day in Hx rats during a 7-day observation period was regarded as a sign of remaining pituitary tissue and used as an exclusion criterion (23).

After a period of hormonal treatment (see next section), the rats were killed by decapitation. All animals were killed in the morning between 0900 and 1100. Blood was collected from the trunk, and serum was separated and stored at 20°C. Parametrial adipose tissue and soleus muscles were dissected and directly used for ex vivo experiments, which we will describe. The spleen was weighed in initial experiments (cf. Table 1) as an indication of IGF-I effect.

In one experiment, during the evening before the day animals were killed, blood samples (150 µl) were collected from the tip of the tail into heparinized capillary tubes for measurements of serum C-peptide concentrations.

**Hormonal treatment.** After the 7-day observation period, all hormone therapy commenced on the same day. All Hx rats were given replacement therapy with l-thyroxine (10 µg·kg⁻¹·day⁻¹; Nycocem, Oslo, Norway) and cortisol phosphate (400 µg·kg⁻¹·day⁻¹; Solu-Cortef, Upjohn, Puurs, Belgium) diluted in saline. Injections were given daily subcutaneously at 0800 (31–33). Human recombinant IGF-I was generously provided by Genentech (San Francisco, CA). IGF-I was diluted in saline (0.9% NaCl) and given as a continuous infusion via an osmotic minipump (model 2004, Alza) diluted in saline. Injections were given daily subcutaneously into the flank, and insulin was injected subcutaneously under anesthesia (xylazine, 10 mg/kg, Rompun; Bayer, Lever-Kusen, Germany, and ketamine-hydrochloride 77 mg/kg, Ketalar; Parke-Davis, Detroit, MI) (33, 40). When the rats were treated with IGF-I for 6 days (1.25 mg·kg⁻¹·day⁻¹), osmotic minipumps (model 2001, Alza, Palo Alto, CA) were used (33, 40). IGF-I was also given as a continuous infusion for 20 days (0.85 mg·kg⁻¹·day⁻¹) with osmotic minipumps (model 2004, Alza).

Insulin (100 U/ml, Insulatard; Novo Nordisk, Copenhagen, Denmark) was diluted in saline and given as a daily subcutaneous injection at 1600. The insulin dose was gradually increased by 10.220.33.1 on October 21, 2017 http://ajpendo.physiology.org/ Downloaded from
mixed with 100 μl of dissolved glycogen. Absorbance at 610 nm was then measured 50 min after the addition of the enzyme solution. Background absorbance was subtracted, and the obtained value was plotted against a glycogen standard for determination of glycogen concentration.

**RESULTS**

Six days of IGF-I treatment (1.25 mg·kg⁻¹·day⁻¹) of HX rats resulted in a significant increase in body weight gain, spleen weight, and IGF-I concentrations. However, no effects on serum glucose or FFA concentrations were observed (Table 1). Analysis of IGFBPs in serum from Hx rats with Western ligand blotting showed a reduction in the 45-, 40-, and 30-kDa IGFBP bands after Hx compared with those in normal rats (Fig. 1). IGF-I treatment of Hx rats restored the reduced levels of the 45- and 40-kDa bands to the levels even higher than in normal rats.

In vivo effects of IGF-I on glucose incorporation into lipids in adipose tissue. The effect of 6 days of IGF-I treatment of Hx rats on insulin-stimulated [²⁴C]glucose incorporation into lipids in adipose tissue segments is shown in Fig. 2. IGF-I treatment resulted in a decrease in basal and insulin-stimulated glucose incorporation into lipids (P < 0.05, controls vs. IGF-I treatment, 2-way ANOVA). Insulin had a statistically significant effect (P < 0.05, vs. different insulin concentrations, 2-way ANOVA). However, the magnitude of the insulin-stimulated increase in glucose incorporation was similar in adipose tissue segments from the Hx control rats (53%) and IGF-I-treated rats (46%). Thus the difference in glucose incorporation found in basal incubations remained and could not be overcome by increasing insulin concentrations. To test the possibility that this effect was retained in isolated adipocytes, adipocytes were isolated from parametrial adipose tissue after 6 days of IGF-I treatment of Hx rats. The basal as well as insulin-stimulated glucose incorporation into lipids was reduced in a similar manner to the adipose tissue segments (data not shown).

The effect of prolonged treatment (20 days) of Hx rats with IGF-I on basal and insulin-stimulated glucose incorporation into lipids in isolated adipocytes was also investigated (Fig. 3). The weight gain was 0.2 ± 0.1 (±SE) g/day among the Hx controls and 0.7 ± 0.2 g/day in the IGF-I-treated rats. In this experiment, basal and insulin-stimulated glucose incorporation into lipids was reduced in manner similar to that after 6 days of IGF-I treatment (P < 0.05, controls vs. IGF-I treatment, and P < 0.05, no insulin vs. 6 nM insulin, 2-way ANOVA).
The magnitude of the insulin-stimulated increase in glucose incorporation was 32% in isolated adipocytes from Hx rats and 41% in adipocytes from rats treated with IGF-I (Fig. 3). Retroperitoneal (RP) fat pad weight from Hx rats and 41% in adipocytes from rats treated with IGF-I (1.25 mg·kg⁻¹·day⁻¹) was given as a continuous sc infusion via osmotic minipumps for 6 days. Values are means ± SE of 4 observations. Each observation is parametrial adipose tissue from 1 rat. There was an overall effect of IGF-I treatment that decreased [¹⁴C]glucose incorporation (F = 14; P < 0.001, 2-way ANOVA). Addition of insulin increased glucose incorporation (F = 33; P < 0.001, 2-way ANOVA). The interaction between IGF-I treatment and addition of insulin was not significant (F = 0.23, 2-way ANOVA). *P < 0.05, significant effect of IGF-I treatment vs. control at indicated concentrations of insulin (1-way ANOVA followed by Student-Newman-Keuls multiple range test). P < 0.05, significant effect of indicated insulin concentration vs. values obtained in tissue segments incubated without insulin for each treatment group (1-way ANOVA followed by Student-Newman-Keuls multiple range test).

To clarify the fate of incorporated glucose into lipids, glucose incorporation into lipids of the adipocyte suspension was further analyzed by TLC. The radioactivity recovered in the total lipid fraction after TLC was 73 ± (SE) 2.6% (n = 8). Most of the radioactivity was found in the triglyceride (68 ± 0.6%, n = 8) and fatty acid fractions (27 ± 0.7%, n = 8). There was no effect of IGF-I treatment on the proportion of the radioactivity present in these fractions, either in the absence or the presence of various concentrations of insulin (data not shown).

The results of these experiments suggested a decreased capacity to metabolize glucose into fatty acids and triglycerides after 6 or 20 days of IGF-I treatment, an effect which was similar in adipose tissue segments and isolated adipocytes. We hypothesized that these effects were due to a decreased secretion of insulin as a result of IGF-I treatment. Serum insulin and C-peptide levels were therefore measured (Table 2).

Table 2. Effects of IGF-I treatment in Hx rats on serum insulin and serum C-peptide concentrations

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Insulin, mU/l</th>
<th>Serum C-Peptide, pM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morning sample</td>
<td>Evening sample</td>
</tr>
<tr>
<td>Hx</td>
<td>13.7 ± 2.5</td>
<td>1,269 ± 520</td>
</tr>
<tr>
<td>IGF-I</td>
<td>6.1 ± 0.8*</td>
<td>204 ± 35*</td>
</tr>
</tbody>
</table>

See Table 1 for procedures. Blood samples were taken from the tip of the tail into heparinized capillary tubes in the evening of the day before animals were killed (2000), and trunk blood was obtained on the day animals were killed (0900–1100). Serum concentrations were analyzed as described in MATERIALS AND METHODS. Values are means ± SE from 1 experiment, with 4–6 observations in each group. *P < 0.05 vs. Hx controls (unpaired Student’s t-test).
compared with Hx control rats and normal female rats (Fig. 4). At the time animals were killed, serum insulin concentration was eight- to ninefold higher in the Hx rats treated with insulin than in the Hx controls (3). Insulin treatment increased both basal and insulin-stimulated glucose incorporation into lipids in isolated adipocytes from parametrial adipose tissue (P < 0.05, normal rats vs. Hx controls vs. insulin treatment, and P < 0.05 no insulin vs. 0.6 nM insulin, 2-way ANOVA). The magnitude of the insulin response was similar in adipocytes from Hx controls (35%) and insulin-treated rats (45%) but higher in adipocytes from normal female rats (91%).

In vitro effects of IGF-I on glucose incorporation into lipids in adipocytes. These experiments were conducted to test the possibility that preincubation of isolated adipocytes with IGF-I in vitro affects basal and insulin-stimulated glucose incorporation into lipids in another way from in vivo. The effects of IGF-I and insulin in vitro were studied in isolated adipocytes from normal female rats.

In initial experiments, the effects of different concentrations of IGF-I and insulin on glucose incorporation into lipids in isolated adipocytes were compared. Both hormones increased glucose incorporation into lipids in a concentration-dependent manner. However, the potency of insulin to stimulate glucose incorporation into lipids was ~100-fold higher than that of IGF-I (Fig. 5A). In subsequent experiments, isolated adipocytes were preincubated for 2 h with 3 nM IGF-I (a dose which gives a submaximal effect on glucose uptake) and then exposed to different concentrations of insulin or IGF-I, and glucose incorporation was measured. Values are means ± SE of triplicates. B: effect of preincubation (preinc.) with IGF-I (3 nM) for 2 h on basal and insulin-stimulated glucose incorporation in isolated adipocytes. Adipocytes were isolated from parametrial adipose tissue from 10 normal female rats. Cells were pooled and then preincubated in the presence or absence of insulin (0.6 nM), and glucose incorporation was measured as described in MATERIALS AND METHODS. Values are means ± SE of quadruplicates.

In vivo effect of IGF-I on lipolysis. The effect of IGF-I treatment on basal and isoproterenol-stimulated lipolysis in isolated adipocytes was subsequently studied. Adipocytes were isolated from Hx rats that had been
treated with IGF-I for 6 days and compared with adipocytes isolated from Hx control rats (Fig. 6). Basal lipolysis was not affected by IGF-I treatment. However, isoproterenol-stimulated lipolysis was slightly increased in cells from IGF-I-treated rats, indicating that IGF-I treatment increases the responsiveness of adipocytes to catecholamines.

In vivo effect of IGF-I on glucose incorporation into glycogen in soleus muscle. The effect of IGF-I treatment on glycogen content and glucose incorporation into glycogen of isolated soleus muscle was studied. The glycogen content of the muscle was higher in IGF-I-treated rats compared with Hx control rats (1.57 ± 0.2 vs. 0.63 ± 0.2 mg/g tissue; P < 0.05). There was an overall significant increase in glucose incorporation into glycogen in soleus muscle of IGF-I-treated rats (P < 0.05, 2-way ANOVA). Basal glucose incorporation into glycogen was significantly increased (Fig. 7). At a submaximal concentration of insulin, glucose incorporation was increased in soleus muscles of IGF-I-treated rats but not in Hx controls, indicating an increased sensitivity to insulin. At the highest insulin concentration, glucose incorporation was similar in the two treatment groups, indicating that IGF-I treatment did not increase the maximal capacity of the soleus muscle to respond to insulin (Fig. 7).

DISCUSSION

Treatment of Hx rats with IGF-I for 6 or 20 days reduced basal and insulin-stimulated lipogenesis in adipose tissue but increased insulin-stimulated glycogen synthesis in skeletal muscle. It is likely that this inhibitory effect of IGF-I on lipogenesis in adipocytes was caused by decreased insulin secretion (18, 27, 29, 36). This conclusion is based on the following observations. First, C-peptide and insulin levels were decreased in IGF-I-treated Hx rats. Second, insulin treatment of Hx rats increased basal and insulin-stimulated glucose incorporation into lipids in adipocytes. Third, preincubation with IGF-I in vitro did not interfere with the insulin response on glucose incorporation into lipids. However, we cannot rule out the possibility that prolonged exposure of adipocytes to IGF-I interferes directly with the insulin response, because only short-term experiments were possible to perform in vitro.

IGF-I is capable of reproducing most of the effects of insulin in adipocytes at 100-fold higher concentrations (2). Accordingly, the type I IGF receptor seems to be very scarce or absent from rat adipocytes (48). These results indicate that IGF-I can have a direct effect on adipocytes only via the insulin receptor (24, 48). The type I IGF receptor seems to be present in high density in skeletal muscle (34, 47). Moreover, in skeletal muscle, IGF-I is 5–10 times less potent than insulin on glucose transport (8). Therefore, it is likely that the physiological effects of IGF-I in skeletal muscle are via IGF receptors and not insulin receptors. However, the possibility that IGF-I can act partly through the insulin receptor in skeletal muscle cannot be completely ruled out. In conclusion, our observations and those of others are in line with the assumption that the direct metabolic effects of IGF-I are mediated through the insulin...
We cannot exclude the possibility that other metabolic changes induced by IGF-I therapy can change the insulin responsiveness of adipocytes. One such possibility is a changed expression of IGFBPs. Our results show that Hx decreases and IGF-I treatment for 7 days increases 45-kDa and 40-kDa IGFBPs in Hx rats. These results are in agreement with previous findings (4). Both 30-kDa and 25-kDa IGFBPs seem to be negatively regulated by insulin (2). In our model, IGF-I treatment reduces both serum insulin and C-peptide concentrations, which could explain an increase in 30-kDa and 25-kDa binding proteins (2). As IGF actions are modified by IGFBPs, the induction of binding proteins by IGF-I may act as a regulator of IGF-I effects in target tissues. However, it is unclear whether these effects of IGF-I on circulating IGFBPs affect glucose uptake in adipose tissue or skeletal muscle.

Basal lipolysis and the proportion of labeled fatty acids to triglycerides were not changed in cultured adipocytes after IGF-I treatment of Hx rats, indicating that the apparent decreased lipogenesis was not due to an increased lipolysis. Thus it can be concluded from the present results that the decreased lipogenesis after IGF-I treatment is mainly due to a decreased capacity of glucose metabolism in adipocytes. After Hx, when basal and stimulated insulin serum levels are decreased (11, 40), basal and insulin-stimulated lipogenesis and glucose oxidation in adipose tissue have been shown to be decreased (9, 11). When Hx rats were treated with insulin, these changes were partly reversed, as shown in this study and previously (9, 11). These results indicate that insulin is important for the long-term regulation of the lipogenic capacity of adipose tissue. A further decrease in insulin secretion induced by IGF-I, as shown in this study and by others (18, 27, 29, 36), may thus contribute to the low lipogenic capacity of adipose tissue. The decreased lipogenic capacity explains observations from both experimental and clinical studies, which showed that long-term treatment with IGF-I reduces fat deposits (14, 25, 44).

In contrast to lipogenesis in adipose tissue, glycogen content and insulin-stimulated glycogen synthesis in muscle tissue were increased by IGF-I treatment. Our results are in line with those reported by Dimitriadis et al. (5), who showed that prolonged treatment of normal rats with IGF-I resulted in increased glucose utilization and glycogen synthesis in the soleus muscle.

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 (42). The doses used in our experiments increased body weight to the same range as in previous reports (14, 39, 40). IGF-I treatment of Hx rats does not affect food intake (40). Moreover, the doses resulted in serum concentrations of IGF-I similar to those of normal rats (40). Glucose concentrations were not altered by IGF-I treatment, indicating that the treatment did not induce hyperglycemia. This result is in agreement with the observation that more frequent injections of IGF-I had no effect on blood glucose concentrations, whereas few daily doses resulted in hypoglycemia (45). The dose of thyroxine (10 µg·kg⁻¹·day⁻¹) has been shown to be physiologically with respect to plasma concentrations of thyroxine (30) and longitudinal bone growth (43). A dose of 500 µg/kg cortisone per day has been shown to be within the physiological range with respect to body growth and longitudinal bone growth (22) and GH binding (9).

Although these results indicate that the effects of GH on body composition may be partly mediated via increased circulating levels of IGF-I, the effect of IGF-I treatment of Hx rats differs in some aspects from effects of GH. We have shown that GH treatment of Hx rats increased lipoprotein lipase activity in skeletal muscle but did not affect this activity in adipose tissue. In contrast, IGF-I treatment had no effect on muscle lipoprotein lipase activity but decreased lipoprotein lipase activity in adipose tissue (33). Thus it is possible that the inhibition of lipoprotein lipase activity in adipose tissue after IGF-I treatment is also due to the decreased serum insulin levels.

Moreover, GH-treated Hx rats and human GH transgenic mice have unaltered or decreased serum triglyceride levels, whereas IGF-I-treated Hx rats and IGF-I transgenic mice have increased serum triglyceride levels (32, 35, 40). Thus several effects of GH on lipid metabolism are not mediated via IGF-I; rather, IGF-I may modulate the effect of GH both at the level of GH secretion and insulin secretion.

However, IGF-I and GH may also act in concert, e.g., decrease body fat mass. For example, GH treatment of Hx rats increased catecholamine-stimulated lipolysis, an effect that has been shown to be partly due to an increase in β-adrenergic receptor numbers (46). Thus it is possible that the increased lipolytic responsiveness induced by GH may be partly mediated via IGF-I.

Our results suggest that IGF-I reduces body fat mass via inhibition of the lipogenic capacity of adipocytes. IGF-I probably reduces lipogenesis in adipose tissue via inhibition of insulin secretion. Moreover, an increased catecholamine-induced lipolysis may also contribute to reduced body fat mass. 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 insulin in this regulation. The potential of IGF-I for treatment of type II diabetes is further substantiated in this study by the findings of a decreased lipogenesis in adipose tissue and an insulin-like effect in skeletal muscle.

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