Insulin action in growth hormone-deficient and age-matched control rats: effect of growth hormone treatment

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Abstract

The isolated effect of growth hormone on carbohydrate metabolism in rat skeletal muscle was studied in growth hormone-deficient dwarf rats (dw/dw) treated with either recombinant human growth hormone or saline for 10 days. In addition, age-matched heterozygous (DW/dw) (normal weight and plasma IGF-I) control rats were treated with saline. Growth hormone increased weight gain from 0.1 ± 0.1 (S.E.M.) to 3.6 ± 0.1 g/day and plasma IGF-I concentration from 364 ± 23 to 451 ± 32 ng/ml. Glucose metabolism in skeletal muscle perfused with basal, sub-maximal and maximal concentrations (0, 600 and 60 000 pmol/l respectively) of insulin was not changed by growth hormone. No change could be detected in the total number of glucose transporters (GLUT1 and GLUT4) in the skeletal muscles, except from a lower amount of GLUT4 in the soleus muscle in the hetero-

zygous control group. However, at submaximal insulin concentrations, skeletal muscle glucose uptake and transport were significantly lower in the heterozygous control group compared with the growth hormone-deficient group. This could indicate either a direct long-term effect of growth hormone or more likely a secondary effect attributable to the difference in body weight (205.2 ± 3.1 vs 361.6 ± 5.9 g for dwarf rats and heterozygous controls respectively), and thereby muscle fibre size, between the groups probably resulting in lower average interstitial insulin and glucose concentrations at a given plasma concentration in the heterozygous rats. It is concluded that restoration of subnormal growth hormone concentrations for 10 days has no effect on insulin-stimulated glucose metabolism in skeletal muscle *in vitro*.

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Introduction

Growth hormone has been used for treatment of growth hormone-deficient patients for several decades. Whereas the growth-promoting effects of treatment with growth hormone are unequivocal, the effects on metabolism of fat and carbohydrate in skeletal muscle are not known in any detail. In normal man, sustained increased levels of growth hormone have been found to result in impairment of peripheral insulin sensitivity (Bratusch-Marrain *et al.* 1982, Møller *et al.* 1989). Interestingly, when studying insulin action in growth hormone-deficient patients, where the effect of restoring subnormal growth hormone levels to normal can be studied, the picture is less clear. In these patients, who are prone to hypoglycaemia during childhood (Goodman *et al.* 1968, Hopwood *et al.* 1975) but insulin resistant during adulthood (Johansson *et al.* 1995, Hew *et al.* 1996), long-term replacement treatment with recombinant human growth hormone (rhGH) has revealed conflicting results (Fowelin *et al.* 1993, Hussain *et al.* 1994, Jørgensen *et al.* 1994, O'Neal *et al.* 1994, Weaver *et al.* 1995).

The mechanism behind the growth hormone-induced changes in insulin action is not known, but since the primary site of insulin-mediated glucose uptake is skeletal muscle the insulin resistance following treatment with growth hormone has been studied in this tissue. Both relatively old (Bratusch-Marrain *et al.* 1982, Rizza *et al.* 1982, Rosenfeld *et al.* 1982) and newer studies (Møller *et al.* 1989, Bak *et al.* 1991) suggest that the decrease in insulin sensitivity in skeletal muscle following treatment with growth hormone on top of normal levels can, at least in part, be explained by a defect distal to the insulin receptor.

In rats, the effect of growth hormone on insulin action in skeletal muscle has been studied both *in vivo* and *in vitro* adding extra growth hormone to normal (Ng *et al.* 1990, Dimitriadis *et al.* 1994, Cartee & Bohn 1995, Hittiarachchi *et al.* 1996, Napoli *et al.* 1996) and to hypophysectomised rats (Manchester *et al.* 1959, Riddick *et al.* 1962). The studies have revealed conflicting results, some showing a decrease in insulin sensitivity in skeletal muscle following treatment with growth hormone (Manchester *et al.* 1959, Riddick *et al.* 1962, Ng *et al.*

1990, Dimitriadis *et al.* 1994, Cartee & Bohn 1995, Hittiarachchi *et al.* 1996) and others showing no such effect (Napoli *et al.* 1996). Since in skeletal muscle the glucose transporters GLUT1 and GLUT4 are thought to be responsible for the basal and insulin-stimulated glucose transport respectively (Richter 1996) these transport proteins have been studied in some detail. The studies seem to agree that in skeletal muscle, growth hormone has no effect on the total number of GLUT4 (Cartee & Bohn 1995, Napoli *et al.* 1996) whereas no clear-cut picture exists for GLUT1, with one study showing a small decrease in the total number of GLUT1 following treatment with rhGH (Napoli *et al.* 1996) and another showing no such effect (Cartee & Bohn 1995).

The above mentioned studies in rats suffer from some methodological problems. For instance, when adding exogenous growth hormone to rats with normal growth hormone levels one is studying the effect of supraphysiological, not physiological, concentrations of growth hormone. Furthermore, the hypophysectomised rat is not a model of isolated growth hormone deficiency because all pituitary hormones are lacking. To circumvent these problems and to be able to elucidate the isolated effects of long-term replacement treatment (10 days) with growth hormone on skeletal muscle insulin action, we have chosen the growth hormone-deficient rat (dw/dw) (Charlton *et al.* 1988) as our model. The dw/dw rat arose from a spontaneous mutation and it is characterised by having a very low growth hormone secretion (5–10% of normal) whereas all other known pituitary hormones are found in normal levels (Charlton *et al.* 1988). As our control animal a group of heterozygous age-matched control rats (normal weight and plasma insulin-like growth factor-I (IGF-I)) was chosen, since age is known to influence insulin action in both rat (Goodman *et al.* 1983) and human (Fink *et al.* 1983) skeletal muscle.

Materials and Methods

Forty-eight adult (21–23 weeks old) male growth hormone-deficient rats (DW-4/OLA/HSD/NN) homozygous for the dwarf (dw/dw) trait, were obtained from Novo Nordisk, Bagsvard, Denmark, and 21 adult (21–23 weeks old) male rats (DW-4/OLA/HSD) heterozygous for the dwarf (Dw/dw) trait were obtained from Harlan UK Ltd, Bicester, UK. Upon arrival the rats were housed individually in plastic cages with a controlled temperature (20 °C) and a 12 h:12 h light–darkness cycle. Lights were on at 0800 h. After 4 weeks the growth hormone-deficient rats were randomly assigned to placebo (PLA) or rhGH-treated (GH) groups. The heterozygous rats were age-matched controls (AGE). The rats were weighed in the morning and received s.c. injections twice daily at 0900 and 1700 h, for 10 consecutive days. The GH group was injected with rhGH (Norditropin, Novo Nordisk)

(0.25 mg/kg body weight) dissolved in sterile saline containing 0.5% bovine serum albumin (BSA) (Cohn fraction V, Sigma Chemical Co., St Louis, MO, USA), which had been passed through a sterile filter, and the PLA group was injected with an equivalent volume of vehicle. BSA was added to minimise rhGH binding to plastic. The AGE group was treated as the PLA group. Rats had free access to standard rat chow (Altromin nr. 1324, Chr. Petersen, Ringsted, Denmark) and tap water until killing. The amount of rat chow remaining in the cage was weighed every morning. The morning after the last evening rhGH injection, animals were again weighed and thereafter anaesthetised with pentobarbital sodium (50 mg/kg body weight). Since the rats were fed the night before the perfusion and the food was removed in the morning 3–7 h before the experiment they must be considered to be under post-prandial conditions.

The rats were surgically prepared for hindlimb perfusion according to Ruderman *et al.* (1971). Before inserting the catheters the rats were heparinised with 500 IU heparin in the inferior vena cava. Blood for analysis of glucose, insulin and IGF-I was sampled from the catheter in the inferior vena cava. Then the rats were killed and the hindlimbs were subsequently perfused.

The perfusion apparatus was similar to that described by Ruderman *et al.* (1971). To maintain the temperature of the perfusate at 37 °C, a heating coil was inserted into the system. The perfusate consisted of Krebs–Henseleit solution, 1- to 3-day-old washed bovine erythrocytes at a haematocrit of 30%; 4% BSA, 8 mM glucose, 0.15 mM pyruvate, 1 mM mannitol and 4.2 IU/ml heparin. The pH of the perfusate was adjusted using bicarbonate and it was continuously gassed during perfusion with a mixture of 35% O₂, 3% CO₂ and 62% N₂, yielding an arterial pH of 7.39 ± 0.01 ($n=68$) (mean $\pm$ S.E.M.), CO₂-pressure of 35.0 ± 0.5 mmHg ($n=69$) and an O₂-pressure of 225.9 ± 3.1 mmHg ($n=69$). Insulin (Actrapid, Novo Nordisk) in the following concentrations was used: 0 (basal (BAS)), 600 (submaximal (SUB)) and 60 000 (maximal (MAX)) pmol/l cell-free perfusate. Insulin at 60 000 pmol/l is a maximally stimulating insulin concentration in the perfused hindquarter (Richter *et al.* 1984).

The first 25 ml of perfusate that passed through the rat was discarded, after which the perfusate was recirculated at a flow rate of 12.5 ml/min. Perfusate samples were obtained from the arterial inflow and venous outflow tubing. Immediately following perfusion the soleus (SOL) and the extensor digitorum longus (EDL) from both legs were rapidly dissected out, freeze clamped and stored at –80 °C until analysis.

To measure muscle membrane glucose transport, 3-O-[glucose-¹⁴C(U)]methyl-D-glucose (specific activity 315 mCi/mmol) and D-mannitol-[1-³H(N)] (specific activity 22.5 mCi/mmol) were added simultaneously to the perfusate, yielding an activity of 0.075 and 0.10 μ Ci/ml respectively. To avoid significant

efflux of 3-O-[glucose- $^{14}\text{C}(\text{U})$]methyl-D-glucose from the intracellular space, the duration of exposure to isotopically labelled compounds was varied depending on the expected glucose transport during the different insulin concentrations. In BAS, SUB and MAX the hindlimb was exposed to the isotopically labelled compounds for 31, 12 and 7 min respectively, always beginning 20 min after starting the perfusion. At the time when the isotopically labelled compounds reached the muscles, recirculation of the perfusate was stopped and one-way perfusion was commenced to secure a constant specific activity of glucose in the arterial perfusate during the period of glucose transport measurement.

Plasma glucose was measured with a Yellow Springs Glucose Analyzer (model 2700 select, Yellow Springs, OH, USA). Insulin in plasma was determined by a commercially available RIA kit especially designed for measurement of rat insulin (Amersham International, Amersham, Bucks, UK). The coefficient of variation of the assay was 5%. IGF-I in plasma was also determined using an RIA. The primary antibody was raised against human IGF-I and the secondary antibody was a sheep anti-rabbit (UCB, Brussels, Belgium). As tracer a moniodinated isomer, $^{125}\text{I}(\text{Tyr})\text{-IGF-I}$ was used (Schäffer *et al.* 1992). The detection limit of the IGF-I assay was 0.3 ng/ml; the coefficient of variation was 10%.

Muscle samples were freeze dried and dissected free of blood and connective tissue before analysis. Glycogen was measured after acid hydrolysis by the hexokinase method (Lowry & Passonneau 1972). Glucose uptake was calculated by multiplying arteriovenous differences by the flow rate and dividing by the perfused muscle mass. Muscle mass of the hindquarter was estimated as one-sixth of body weight (Ruderman *et al.* 1971, Richter *et al.* 1982).

Uptake of 3-O-[glucose- $^{14}\text{C}(\text{U})$]methyl-D-glucose in muscles ($\mu\text{mol/g}$ per h) was detected in perchloric acid extracts and corrected for radiolabel in the extracellular space as determined by the ^3H counts for mannitol. Radioactivity was measured in a liquid scintillation counter (Model 2000 Tri-carb, Packard Instruments, Downers Grove, IL, USA). From the intracellular accumulation of 3-O-[glucose- $^{14}\text{C}(\text{U})$]methyl-D-glucose, the rate of glucose transport was calculated using a 'specific activity of glucose' determined by the glucose concentration and 3-O-[glucose- $^{14}\text{C}(\text{U})$]methyl-D-glucose counts in the perfusate.

The GLUT1 and GLUT4 content was measured in total crude muscle (TCM) membranes prepared essentially as described by Ploug *et al.* (1993). Twenty to thirty mg muscle were homogenised, TCM were recovered after centrifugation for 90 min at 100 000 *g* and stored in aliquots at -80°C . Protein concentrations were determined and TCM were subjected to SDS-PAGE on a gradient gel followed by transfer to a membrane. The

membrane was blocked, followed by incubation with primary antibody. The GLUT4 antibody is a mouse monoclonal antibody produced against a synthetic peptide corresponding to the 13 carboxy-terminal amino acids of GLUT4 coupled with haemocyanin (Ploug *et al.* 1993). The GLUT1 antibody was a mouse monoclonal antibody produced against the human erythrocyte glucose transporter (Ploug *et al.* 1993). After incubation with primary antibody, membranes were washed and incubated with horseradish peroxidase-labelled secondary antibody. Membranes were again washed and antibody-antigen complexes were visualised by enhanced chemiluminescence. Exposures were obtained and densitometric scanning was performed.

The maximal enzymatic activities of lactate dehydrogenase (LDH), citrate synthase (CS) and β -3-hydroxyacyl-CoA dehydrogenase (HAD) were determined using NAD-NADH enzymatic fluorometric assays according to Lowry & Passonneau (1972). Muscle water content was calculated as: (muscle wet weight) – (muscle freeze-dried weight).

Statistics

All results are expressed as means $\pm$ S.E.M. Significant differences between groups were identified using a one-way ANOVA. When a significant F ratio was obtained the Newman-Keul post-hoc test was utilised to identify differences between mean values. The level of significance during all tests was set at $P < 0.05$.

Results

The mean weight gain in the PLA rats was 0.1 ± 0.1 g/day (Table 1). In GH the mean weight gain was significantly higher than in PLA at 3.6 ± 0.1 g/day (Table 1). The mean weight gain (-0.3 ± 0.1 g/day) in AGE was not different from that in PLA but significantly lower than in GH (Table 1). The growth curve demonstrates that the weight of both the PLA and the AGE group hardly changed during the 10 days of treatment, whereas the weight of the GH animals increased dramatically (Fig. 1). The food intake in the AGE group was significantly greater than that in GH and PLA (Table 1). The food intake in the GH group was significantly greater than that in PLA (Table 1). When the mean food intake per day over the 10 day treatment period was expressed per g mean body weight over the same 10 day period the values were 0.07, 0.07 and 0.05 g food/g body weight per day in PLA, GH and AGE respectively. The food intake per g mean body weight in AGE was significantly lower than in both PLA and GH and no difference between PLA and GH could be found. Thus when expressed per kg body weight the AGE rats ate less than both GH and PLA. The mean wet weights of the SOL and EDL were significantly

Table 1 Body weight, food intake, muscle weight and water content (means ± S.E.M.) in growth hormone-deficient rats (GH and PLA) and heterozygous age-matched control rats (AGE)

	PLA	GH	AGE
Body weight (g)			
Before treatment (n=24)	204.5 ± 2.8	201.1 ± 2.5	364.3 ± 6.0 ⁺
After treatment (n=24)	205.2 ± 3.1	237.5 ± 2.3 [#]	361.6 ± 5.9 ⁺
Food intake (g/day) (n=24)	14.2 ± 0.1	15.4 ± 0.3 [#]	19.0 ± 0.3 ⁺
Muscle weight (mg)			
SOL (n=10)	64.9 ± 1.6	76.3 ± 2.8 [#]	122.4 ± 4.2 ⁺
EDL (n=10)	106.2 ± 2.3	121.0 ± 2.0 [#]	159.7 ± 4.6 ⁺
Water content (%)			
SOL (n=24)	75.4 ± 0.5	75.5 ± 0.5	76.3 ± 0.4
EDL (n=24)	74.4 ± 0.1	74.5 ± 0.2	75.3 ± 0.2 ⁺

[#]Significantly different from PLA, ⁺significantly different from PLA and GH.

higher in GH than in PLA (Table 1). In AGE the mean wet weights of SOL and EDL were significantly higher than in both PLA and GH (Table 1). To evaluate whether the muscle growth was proportional to the total weight the percentage increase was calculated. When comparing the GH group and the PLA group after treatment for 10 days, body weight was increased by 13.6%, SOL weight by 14.9% and EDL weight by 12.2%. Thus the muscle weight and the body weight seem to express proportional growth. In SOL, no difference in muscle water content was observed when the three experimental groups were

compared (Table 1). In EDL, no difference in muscle water content was observed when PLA and GH were compared, while the muscle water content in AGE was significantly higher than in both GH and PLA (Table 1). Glucose uptake increased significantly in all groups with increasing concentrations of insulin (Fig. 2). Glucose uptake in PLA was identical to glucose uptake in GH at all insulin concentrations (Fig. 2). In AGE, glucose uptake was not significantly different from that in PLA and GH at BAS, whereas glucose uptake was significantly lower at SUB and MAX (Fig. 2).

Figure 1 Growth curves of growth hormone-deficient rats (GH and PLA) and heterozygous age-matched control rats (AGE) during the 10 day treatment period (n=24 in GH and PLA and n=21 in AGE). #P<0.05 compared with PLA. +P<0.05 compared with PLA and GH. Values are means ± S.E.M.

Figure 2 Glucose uptake was measured as arteriovenous difference multiplied by the perfusate flow rate in growth hormone-deficient rats (GH and PLA) and heterozygous age-matched control rats (AGE). Hindlimbs were perfused with insulin concentrations at 0 (BAS), 600 (SUB) and 60 000 pmol/l (MAX) (n=8 in each of the nine groups). #P<0.05 compared with PLA and GH. Values are means ± S.E.M.

Table 2 Glucose transport in individual skeletal muscles in growth hormone-deficient rats (GH and PLA) and heterozygous age-matched control rats (AGE). Values are means $\pm$ S.E.M.

	PLA	GH	AGE
SOL ($\mu\text{mol/g per h}$)			
BAS	2.0 $\pm$ 0.4	3.0 $\pm$ 0.6	1.2 $\pm$ 0.1 ^S
SUB	11.6 $\pm$ 1.2*	12.5 $\pm$ 1.1*	4.6 $\pm$ 0.8* ⁺
MAX	27.8 $\pm$ 3.6 [^]	23.4 $\pm$ 2.1 [^]	22.7 $\pm$ 1.4 [^]
EDL ($\mu\text{mol/g per h}$)			
BAS	1.9 $\pm$ 0.3	1.8 $\pm$ 0.3	0.9 $\pm$ 0.1 ⁺
SUB	8.6 $\pm$ 0.9*	11.3 $\pm$ 0.9* ^{##}	3.8 $\pm$ 0.7* ⁺
MAX	19.2 $\pm$ 1.6 [^]	19.9 $\pm$ 0.8 [^]	15.3 $\pm$ 2.3 [^]

Glucose transport was measured as accumulation of ^{14}C -3-O-methyl-glucose in individual muscles ($n=8$ in each of the nine groups). Hindquarters were perfused with insulin concentrations at 0 (BAS), 600 (SUB) and 60 000 pmol/l (MAX). ^{##} $P<0.05$ compared with PLA, ^S $P<0.05$ compared with GH, ⁺ $P<0.05$ compared with PLA and GH, ^{*} $P<0.05$ compared with BASAL, [^] $P<0.05$ compared with BASAL and SUB.

Glucose transport, measured as accumulation of 3-O-[glucose- ^{14}C (U)]methyl-D-glucose in SOL and EDL, increased significantly in all groups with increasing concentrations of insulin (Table 2). In both SOL and EDL there was no difference in glucose transport when PLA was compared with GH at BAS and at MAX. However, at SUB in EDL, glucose transport was slightly but significantly lower in PLA compared with GH, whereas no difference could be detected in SOL (Table 2). In AGE,

glucose transport was significantly different from that in GH and PLA at SUB in SOL and at BAS and SUB in EDL (Table 2).

In SOL and EDL, no significant difference in total GLUT1 content was observed when the three experimental groups were compared (Fig. 3). In SOL, no difference in GLUT4 content could be observed when PLA was compared with GH (Fig. 3). In AGE, GLUT4 content was significantly lower than in both GH and PLA (Fig. 3). In EDL, no difference in total GLUT4 content was observed when the three experimental groups were compared (Fig. 3).

In both SOL and EDL, no difference in the maximal activity of CS and LDH was observed when the three experimental groups were compared (Table 3). In SOL, the maximal activity of HAD in GH and AGE was significantly higher than the maximal activity of HAD in PLA (Table 3). There was no difference in the maximal activity of HAD when GH and AGE were compared (Table 3). In the EDL, no difference was observed when the maximal activity of HAD was compared in the three groups (Table 3). In both SOL and EDL, no difference in basal glycogen content was observed when the three experimental groups were compared (Table 3). However, muscle glycogen concentrations generally were higher after perfusion at SUB and MAX compared with BAS (data not shown).

There was no difference in blood glucose concentration when PLA, GH and AGE were compared (Table 4).

Figure 3 GLUT1 (A) and GLUT4 (B) content was determined by Western blot analysis in SOL and EDL in growth hormone-deficient rats (GH and PLA) and heterozygous age-matched control rats (AGE). Values are expressed relative to a rat heart standard ($n=8$ in each group). [#] $P<0.05$ compared with PLA and GH. Values are means $\pm$ S.E.M.

Table 3 Activities of lactate dehydrogenase (LDH), citrate synthase (CS) and β-3-hydroxyacyl-CoA dehydrogenase (HAD) and glycogen concentration in growth hormone-deficient rats (GH and PLA) and heterozygous age-matched control rats (AGE). Values are means ± S.E.M. (n=9–10)

	PLA	GH	AGE
LDH (μmol/g per min)			
SOL	627 ± 40	637 ± 42	560 ± 15
EDL	1984 ± 93	1963 ± 114	1944 ± 92
CS (μmol/g per min)			
SOL	42.3 ± 1.5	46.0 ± 1.2	46.2 ± 1.9
EDL	43.0 ± 1.7	43.3 ± 2.1	41.1 ± 2.6
HAD (μmol/g per min)			
SOL	33.4 ± 1.6	40.8 ± 2.4 [#]	41.5 ± 2.5 [#]
EDL	15.8 ± 1.5	17.6 ± 1.4	20.5 ± 1.8
Glycogen (μmol glycosyl/g wet weight)			
SOL	34.3 ± 1.9	30.9 ± 1.0	35.2 ± 1.7
EDL	36.3 ± 2.1	37.2 ± 2.2	42.2 ± 2.7

[#]Significantly different from PLA.

Plasma insulin concentration was similar in PLA and GH (Table 4). In AGE, the plasma insulin concentration was significantly higher than in both PLA and GH (Table 4). In GH the plasma IGF-I concentration was 23% higher than in PLA, and in AGE the plasma IGF-I concentration was 38% higher than in PLA (Table 4). There was no difference in plasma IGF-I concentration when GH and AGE were compared (Table 4). For comparison, IGF-I concentration in normal 15-week-old Lewis rats without the dwarf gene was 448 ± 37 ng/ml (n=6), which was not significantly different from that in GH and AGE.

Discussion

Our results demonstrate that in growth hormone-deficient rats treatment with growth hormone for 10 days, restoring IGF-I plasma levels to normal, does not change *in vitro* insulin action in skeletal muscle or the total amount of muscle GLUT1 and GLUT4. On the other hand, in heterozygous non-growth hormone-deficient rats insulin action is decreased compared with the growth hormone-

Table 4 Concentrations of blood glucose, plasma insulin and plasma IGF-I in growth hormone-deficient rats (GH and PLA) and heterozygous age-matched control rats (AGE). Values are means ± S.E.M.

	PLA	GH	AGE
Blood glucose (mmol/l)	7.7 ± 0.4 (n=24)	7.9 ± 0.4 (n=24)	8.9 ± 0.4 (n=21)
Plasma insulin (pmol/l)	416 ± 55 (n=20)	339 ± 34 (n=20)	751 ± 88 ⁺ (n=14)
Plasma IGF-I (ng/ml)	364 ± 23 (n=14)	451 ± 32 [#] (n=14)	503 ± 46 [#] (n=10)

[#]Significantly different from PLA, ⁺significantly different from PLA and GH.

deficient rats. Thus, chronic growth hormone deficiency is associated with retarded growth and increased insulin action in skeletal muscle. Nevertheless, although weight gain is restored by growth hormone treatment for 10 days, insulin action is not changed.

Several groups have examined the effect of growth hormone on insulin action in rat skeletal muscle. However, most of the information was obtained in hypophysectomised animals (Manchester *et al.* 1959, Riddick *et al.* 1962) and in animals bearing a growth hormone-producing tumour (Imamura *et al.* 1993) or by adding exogenous growth hormone to already normal levels of hormone (Ng *et al.* 1990, Cartee & Bohn 1995, Hittiarachchi *et al.* 1996, Napoli *et al.* 1996). Our model has clear advantages compared with hypophysectomy, because in the dwarf rat the only deficient anterior pituitary hormone is growth hormone (Charlton *et al.* 1988). Furthermore, it is more physiologically sound to replace a deficient hormone than to add exogenous hormone on top of endogenous levels.

In the present study, the effect of growth hormone treatment on insulin action was studied *in vitro*. Consistent with our results Napoli *et al.* (1996) showed that growth hormone treatment for 4 weeks in normal rats did not change insulin action on skeletal muscle glucose transport. However, other studies in which exogenous growth hormone was added to endogenous secretion for 10 days in normal rats showed a significant but rather small decrease in insulin-stimulated and basal glucose transport in incubated muscles (Dimitriadis *et al.* 1994, Cartee & Bohn 1995). *In vivo* studies on the other hand clearly show that elevation of the growth hormone level for 2–3 days induces peripheral insulin resistance in normal rats (Ng *et al.* 1990, Hittiarachchi *et al.* 1996).

In agreement with studies in rats, studies in normal humans consistently report that short-term infusion of growth hormone decreases peripheral insulin sensitivity (Bratusch-Marrain *et al.* 1982, Rizza *et al.* 1982, Rosenfeld *et al.* 1982, Möller *et al.* 1989, Bak *et al.* 1991). Since both insulin binding to its receptor and insulin receptor kinase activity have been shown to be unaffected by growth hormone, the decrease in insulin-mediated glucose uptake seems to be mediated through a post-receptor effect (Bratusch-Marrain *et al.* 1982, Rosenfeld *et al.* 1982, Rizza *et al.* 1982). Thus, most of the available data suggest that the outcome of adding excess growth hormone on top of already normal levels is a decrease in *in vivo* peripheral insulin sensitivity of glucose metabolism in both rats and humans. However, when insulin action is evaluated *in vitro* the effects of growth hormone treatment, when added in excess of normal levels or after restoration of subnormal levels, are less consistent.

In line with the view that growth hormone is a diabetogenic hormone, is that it has been shown that growth hormone-deficient children are prone to hypoglycaemia (Goodman *et al.* 1968, Hopwood *et al.* 1975) due

to a diminished hepatic glucose output (Bougnères *et al.* 1985). Surprising then is that newer studies in growth hormone-deficient adults show a lower than normal peripheral insulin sensitivity evaluated by the euglycaemic hyperinsulinaemic clamp (Johansson *et al.* 1995, Hew *et al.* 1996). The mechanism behind the decrease in insulin action in growth hormone deficiency has not yet been fully elucidated but it has been proposed that a defect in glucose storage and glycogen synthase activation might be involved (Hew *et al.* 1996). In the present study we were also able to study the effect of chronic growth hormone deficiency on muscle glucose transport by comparing the growth hormone-deficient placebo-treated (PLA) rats with the age-matched heterozygous controls (AGE). We showed that in the SUB state, glucose transport in both SOL and EDL is lower in AGE compared with PLA, whereas no difference could be detected in the BAS and in the MAX states, indicating that insulin sensitivity is decreased in AGE compared with PLA. Since the total level of GLUT1 was the same in PLA and AGE, and GLUT4 was only slightly lower in AGE in SOL, the glucose transporter content does not seem to be able to account for the decrease in insulin sensitivity in AGE. We hypothesise that the difference in body size between PLA and the AGE matched control group is responsible. It is a well known fact that in rats an increase in age is associated with a decrease in insulin sensitivity (Goodman *et al.* 1983). However, this association between ageing and insulin sensitivity is confounded by several factors such as age-related changes in physical activity, body composition and body size. Changes in physical activity are associated with changed activity of enzymes of the citric acid cycle like CS (Saltin & Gollnick 1983). The fact that in the present study CS activity was similar in AGE and PLA indicates that the degree of physical activity in the two groups was similar. Because insulin action was determined in individual muscles the reason for the difference between PLA and AGE should be sought in the muscles rather than in the differences in body composition. A possibility is that in the larger AGE rats the diffusion conditions are less favourable than in PLA rats. We have recently demonstrated that the average muscle fibre size is 29–68% (depending on the muscle fibre type studied) larger in AGE than in PLA (Daugaard *et al.* 1998). Assuming that the average number of capillaries is the same in muscles from PLA and AGE, then the larger fibre area per capillary means that the average diffusion distance from capillary to muscle fibre surface and interior is increased in AGE compared with PLA. This in turn suggests that the average interstitial concentration of insulin may be less in AGE than in PLA at a given perfusate concentration. Since insulin action is closely related to the interstitial concentration of insulin, as reflected by lymph insulin concentration (Castillo *et al.* 1994), this might explain the lesser response to SUB and equal response to MAX insulin concentrations in AGE compared with PLA,

because at the maximal perfusate insulin concentration interstitial insulin concentration will be well above the maximal stimulating concentration in both groups. It could be argued that the inclusion of a heterozygous weight-matched control group could have clarified whether the size of the rats is responsible for the observed differences. However, by including such a control group the age of the rats would have been introduced as a confounding factor.

Treatment with growth hormone did not have any effect on insulin action in the growth hormone-deficient rats. This is in contrast to older studies using hypophysectomised rats displaying increased insulin action, where both Manchester *et al.* (1959) and Riddick *et al.* (1962) observed a normalisation of glucose uptake and 2-deoxyglucose transport respectively, following treatment with growth hormone. In humans, several recent studies have assessed insulin action on glucose metabolism in growth hormone-deficient patients receiving replacement therapy with growth hormone (Fowelin *et al.* 1993, Hussain *et al.* 1994, Jørgensen *et al.* 1994, O'Neal *et al.* 1994, Weaver *et al.* 1995). It has been shown by most but not all (Hussain *et al.* 1994, Weaver *et al.* 1995) that treatment with growth hormone induces a temporary state of insulin resistance which is abolished following long-term treatment (Fowelin *et al.* 1993, Jørgensen *et al.* 1994, O'Neal *et al.* 1994). The mechanisms behind the observed changes are not known, but a change in body composition could be important since adults with growth hormone deficiency are known to have increased body fat mass and decreased lean body mass compared with healthy controls (Binnerts *et al.* 1992). Furthermore, growth hormone treatment of growth hormone-deficient adults seems to increase lean body mass and decrease adipose tissue mass (Salomon *et al.* 1989). In the only study of growth hormone-deficient humans examining skeletal muscle it was found that insulin activation of glycogen synthase was less in growth hormone-deficient adults than in controls, as was insulin stimulation of whole body glucose disposal (Hew *et al.* 1996). This may, however, be an indirect effect of growth hormone deficiency due to differences in body composition, as suggested by Hew *et al.* (1996). In agreement with this interpretation our study suggests that growth hormone does not alter insulin action directly in muscle.

In conclusion, in growth hormone-deficient rats treatment with growth hormone for 10 days restores weight gain and plasma IGF-I concentration but does not affect *in vitro* insulin action on skeletal muscle glucose transport. Compared with the growth hormone-deficient rats, age-matched control rats have a decreased sensitivity to insulin. This could either indicate a direct long-term effect of growth hormone, or more likely be a secondary effect of increased muscle fibre size making the average diffusion distance longer for insulin from the capillaries to the muscle fibre surface in age-matched control rats compared with growth hormone-deficient rats.

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