

The role of growth hormone and glucocorticoid in glucose handling *in vivo*

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Abstract

Growth hormone (GH) can oppose the catabolic effects of glucocorticoids. However, both hormones have adverse effects on carbohydrate metabolism. Here we examined the interactive effects of GH and the glucocorticoid methylprednisolone (MP) on glucose tolerance, insulin resistance and [³H]2,6-deoxyglucose uptake of peripheral tissues in rats.

Female Wistar rats received either saline, GH (2.7 mg/kg), MP (5.0 mg/kg) or GH+MP. After 7 days treatment, animals were subjected to an i.v. glucose tolerance test. In a second experiment, animals treated as above were anesthetized and injected with human insulin (0.5 U/kg), [³H]2,6-deoxyglucose (500 µCi/kg), and [¹⁴C]mannitol (25 µCi/kg), to estimate insulin resistance and [³H]2,6-deoxyglucose uptake in fat and muscle.

Weight gain in controls was 7.6 ± 1.7 g, while GH treatment increased the mean body weight by 18.7 ± 2.2 g ($P < 0.0002$) and MP inhibited weight gain down to 0.0 ± 1.0 g ($P < 0.004$). This drop in weight gain was reversed back to normal when GH was given in

combination with MP. After a glucose tolerance test no significant differences in glucose area under the curve were detected when comparing individual groups with the control group, but samples taken just before this test revealed that basal insulin was significantly elevated in the group treated with GH (174 ± 27 pM, $P < 0.008$), or GH+MP (209 ± 21 pM, $P < 0.004$), when compared with controls (107 ± 17 pM). MP alone had no effect (122 ± 19, $P < 0.3$). After an i.v. bolus of insulin the group receiving GH+MP had a significantly ($P < 0.007$) higher level of circulating glucose compared with controls (6.5 ± 0.3 mM vs 4.4 ± 0.7 mM). Despite this, there were no differences in peripheral glucose uptake between the two groups.

In conclusion this study shows that a combined administration of GH and MP decreases the potency by which insulin decreases circulating glucose levels, but that peripheral tissues are not primarily involved in this insulin resistance.

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Introduction

Growth hormone (GH) and glucocorticoids are potent regulators of metabolism (Davidson 1987, Dallman *et al.* 1993, Yarasheski 1994, Haymond & Mauras 1996). Glucocorticoids induce proteolysis and inhibit protein synthesis, resulting in decreased nitrogen balance and weight loss (Ortoft *et al.* 1992, Strack *et al.* 1995, Weinstein *et al.* 1995). Thus, extensive therapy with high doses of glucocorticoids induces a risk for serious protein wasting. Furthermore, glucocorticoids cause insulin resistance and impair glucose utilisation resulting in hyperglycemia and hyperinsulinemia (Marfaing *et al.* 1991).

Recent studies show that the protein anabolic action of GH has a potential role in the treatment of catabolism (Haymond & Mauras 1996). Accordingly, GH has been shown to increase net balance of amino acids in the human forearm (Fryberg *et al.* 1991, 1992) and in the hind limbs

of pigs (Malmjöf *et al.* 1994a), improve whole body leucine balance in humans (Horber *et al.* 1991) and increase body weight (Ortoft *et al.* 1992). However, a major drawback of GH administration is the diabetogenic actions of the hormone (Davidson 1987), thus complicating its use in conditions with glucocorticoid excess. The combined effect of GH and glucocorticoids regarding glucose and insulin levels appear more dramatic than when using the hormones alone (Haymond & Mauras 1996) and require further studies.

This study was performed to investigate the ability of GH to counteract the growth retarding effect of the glucocorticoid methylprednisolone (MP), and to elucidate further the effects of GH and MP on glucose handling. MP was employed to induce a controlled state of catabolism (Horber *et al.* 1991, Ortoft *et al.* 1992). GH and MP were administered singly or in combination. The actions of the hormones on carbohydrate metabolism were

investigated with an intravenous glucose tolerance test, insulin challenge and with a glucose tracer technique, measuring intracellular glucose accumulation in muscle and fat.

Materials and Methods

Animals and treatment

Study 1 Twenty-five three-month-old female Wistar rats, initially weighing an average of 207 g, were kept at constant temperature and humidity and under a 12 h/12 h light/darkness cycle. The animals were randomly assigned to four treatment groups. One received saline alone (control, $n=5$), another received recombinant rat GH (Geneworks, Rundle Mall, Adelaide, Australia) and saline (GH, $n=6$), a third received MP (Solu Medrol, Pharmacia & Upjohn, Rijksweg 12, Puurs, Belgium) and saline (MP, $n=6$) and a fourth was treated with GH and MP simultaneously (GH+MP, $n=8$). GH was given in two equal doses morning and afternoon in a total daily dose of 2.7 mg/kg, and MP was given once each morning in a daily dose of 5.0 mg/kg. All solutions were given subcutaneously in volumes of 1.0 ml/kg. Treatment continued for 7 days and animals were weighed in the morning every day.

Surgical procedures After one week in the animal facility animals were fitted with permanent indwelling catheters in the left carotid artery and the right jugular vein (Tygon Microbore Tubing, Norton Performance Plastic, Corby, Northamptonshire, UK). A mixture of halothan, nitrogendioxide and oxygen was used for anaesthesia. The catheters were led subcutaneously to the back of the neck. They were filled with polyvinylpyrrolidone (PVP K30, Fluka Chemicals, Buchs, Switzerland) (300 mg/ml) and heparin (500 U/ml) in 0.9% saline, or glucose (500 mg/ml) and heparin (500 U/ml) in 0.9% saline and closed with fishing line. During the procedure the animals received antibiotic (Borgal Mite Vet., Hoechst Veterinär GmbH, Unterschleissheim, Germany) and analgesic (Finadyne Vet., Scanvet, Fredensborg, Denmark).

Experimental procedures During the week following surgery animals were accustomed to the test facilities after which treatments were initiated. On the morning of the last treatment day food was removed, and solutions in the catheters were replaced with heparin containing 20 U/ml saline (0.9%). Three to four hours later blood was sampled for glucose, rat insulin, insulin-like growth factor-I (IGF-I) and rat GH. The intravenous glucose tolerance test was then performed. The animals were given 1.0 g/kg glucose in 0.9% saline solution as a bolus in volumes of 2.0 ml/kg through the jugular vein catheter. Blood was sampled for plasma analysis of glucose and insulin at 0, 5, 15, 30, 60 and 120 min. All samples were kept on ice until

centrifugation (3 min, 10 000 r.p.m.), and were then refrigerated (5 °C, GH samples) or frozen (−20 °C, all others). Immediately after this the animals were given an intravenous overdose of pentobarbital.

Study 2 Thirty-five female Wistar rats, on average weighing 211 g were treated as in study 1. In this experiment there were 6 animals in the control group, 11 in the GH group, 7 in the MP group, and 11 in the group receiving both hormones (GH+MP). The animals were kept in metabolism cages during treatment.

Surgical procedures After 7 days of treatment animals were fitted with indwelling catheters in the left carotid artery (polyethylene) during pentobarbital anaesthesia (60 mg/kg). Catheters were filled with heparin in 0.9% saline (20 U/ml) to prevent occlusion.

Experimental procedures Food was removed on the morning of the last treatment day. During anaesthesia blood samples were withdrawn for analysis of glucose, prior to insulin challenge and glucose uptake measurement. This consisted of injection of a 0.2% albumin solution (Bovine Serum Albumin, Sigma, St Louis, MO, USA) containing human insulin (Actrapid, Novo Nordisk, Gentofte, Denmark), 0.5 U/kg, [³H]2,6-deoxyglucose, 500 µCi/kg, and [U-¹⁴C]mannitol, 25 µCi/kg (Amersham International, Amersham, Bucks, UK), through the catheter over 30 s. The catheter was rinsed by filling it with blood three times before closing it. Twenty-five minutes after insulin injection the blood was sampled for analysis of glucose, IGF-I and isotope content. The rats were then decapitated and the entire soleus muscle, the long digital extensor muscle (soleus and EDL respectively), and 50–100 mg inguinal subcutaneous and intra-abdominal fat were removed. Tissue samples were frozen immediately on solid nitrogen and stored at −80 °C. The muscles were chosen because of their fibre type composition. The soleus muscle consists of 89% slow twitch oxidative fibres, and EDL muscle consists of 79% fast twitch glycolytic fibres (Armstrong & Phelps 1984).

Measurement of [³H]deoxyglucose uptake

The method used for determination of [³H]deoxyglucose uptake was modified from Hom *et al.* (1984). In the present study the extracellular tracer molecule [U-¹⁴C]sucrose was replaced by [U-¹⁴C]mannitol. Because mannitol is a monosaccharide like glucose it would therefore in theory be a better extracellular tracer than the disaccharide sucrose, as it is likely to share the dispersion kinetics of glucose. Frozen tissue samples were weighed directly into 20 ml glass scintillation vials, 1.0 ml BTS-450 toluene solubiliser (Beckmann, Brea, CA, USA) was added, and the vials were left at 55 °C overnight. Next

Table 1 Data from study 1: basal plasma glucose and insulin, peak values after 5 min, and area under the curve (AUC) after 120 min, during an i.v. glucose tolerance test (1.0 g/kg) in rats after 7 days of treatment with saline (Control), rat growth hormone 2.7 mg/kg (GH), methylprednisolone 5.0 mg/kg (MP) or GH and MP in the same doses as in the groups treated with only one hormone (GH+MP). Values are presented as means \pm S.E.M.

	Control	GH	MP	GH+MP
Glucose				
Basal (mM)	8.3 \pm 0.3	8.0 \pm 0.3	8.9 \pm 1.1	9.5 \pm 0.5
Peak (mM)	22.7 \pm 0.9	27.5 \pm 2.4	25.3 \pm 1.0	26.1 \pm 1.5
AUC (mM/min)	1064 \pm 62	1149 \pm 76	1337 \pm 53	1276 \pm 67
Insulin				
Basal (pM)	107 \pm 17	174 \pm 27*	122 \pm 19	209 \pm 21**
Peak (pM)	1182 \pm 263	1406 \pm 294	554 \pm 73	916 \pm 100
AUC (pM/min)	19 933 \pm 3105	32 450 \pm 4574	25 160 \pm 4889	39 260 \pm 3253***

* $P < 0.008$, ** $P < 0.004$, *** $P < 0.003$ compared with controls.

day 10 ml scintillation liquid (Hi Safe, Beckmann) and 80 μ l iced acetic acid were added, and samples were counted using external standards, with a double isotope counter program in a liquid scintillation counter (Beckmann LS6000). Each tissue type was counted separately and the value of the blank samples was subtracted. Calculations of intracellular [3 H]deoxyglucose uptake was carried out as described by Hom *et al.* (1984).

Blood analyses

Blood was drawn into tubes containing 4–8 μ l heparin (1000 U/ml), and all were analyzed with double determinations. Plasma glucose was analyzed on a Beckmann autoanalyzer (Synchron CX-5, Beckmann, kits no. 442640 and 442785). Plasma IGF-I was measured with an RIA procedure (modified from IGF-I 100T kit, Nichols Institute, San Juan Capistrano, CA, USA), which has intra- and interassay coefficients of variation of 5.8% and 10.1% respectively, and a detection limit of 0.3 ng/ml. Plasma GH was analyzed with a double monoclonal ELISA procedure with a detection limit of 1.0 ng/ml, and intra- and interassay coefficients of variation of 5.4% and 5.3% respectively.

ELISA for plasma rat insulin Rat insulin in plasma was analyzed with an ELISA developed at our laboratory using polyclonal antibodies from guinea pig (GP114 and GP116, SDC, Gentofte, Denmark). Immunoplates were coated with diluted anti-insulin antibody and stored at 4 °C for at least 24 h. Samples, diluted to the appropriate concentration and calibrators prepared from rat insulin (no. 735 41118, Novo Nordisk A/S), were applied and incubated overnight at 4 °C. Peroxidase-labeled detecting antibody was applied followed by TMB-enzyme substrate (3,3',5,5'-tetramethyl-benzidine peroxidase substrate, no. 50–76–00, Kierkegaard & Perry Lab. Inc., Gaithersburg, MD, USA) prior to incubation. The reaction was stopped by adding phosphoric acid and absorption was measured at

450 nm with a 620 nm reference (Spectra II SLT, Wallac, Turku, Finland). Cross-reaction with glucagon and IGFs -I and -II were less than 1 p.p.m. and 0.5% respectively. Recovery was between 95% and 107% and linearity was 100%. Intra- and interassay coefficients of variation were less than 5% and 10% respectively. Detection limit was 0.7 pM and the matrix effect of rat plasma was acceptable at a minimum dilution of 1:3. The correlation coefficient was $r = 0.98$ between sandwich ELISA and RIA (Heding 1972).

Data analysis

The computer program SAS (SAS Institute, Cary, NC, USA) was used for analysis of the data. A two-way ANOVA was performed with the general linear models (GLM) procedure to test for significant effects across all groups. This was considered significant if the P value was less than 0.05. If significant effects were found in the ANOVA the main effects of GH and MP were analyzed, and considered significant when $P < 0.01$. Lastly, each group mean was compared with the control group, using the Estimate option of the GLM procedure, and a P value less than 0.01 was considered significant. The software program Excel (Microsoft Corp., Redmond, WA, USA) was used for calculation of the area under the curve (AUC). Data in the text and tables are presented as means \pm S.E.M.

Results

The results are presented in Tables 1 and 2.

Growth

Both GH and MP had significant main effects on weight gain in both experiments. GH increased weight gain ($P < 0.001$) while MP decreased weight gain ($P < 0.003$). In

Table 2 Data from study 2: weight gain, plasma IGF-I, plasma glucose levels and [³H]-2,6-deoxyglucose uptake in muscle and fat 25 min after an i.v. insulin challenge (0.5 U/kg). Rats were treated for 7 days with saline (Control), rat growth hormone 2.7 mg/kg (GH), methylprednisolone 5.0 mg/kg (MP) or GH and MP in the same doses as in the groups treated with only one hormone. Values are presented as means ± s.e.m.

	Control	GH	MP	GH+MP
Weight gain (g)	8.7 ± 4.5	21.8 ± 2.4*	-4.7 ± 5.3	11.7 ± 2.9
IGF-I (ng/ml)	882 ± 10	999 ± 40**	853 ± 45	1142 ± 53***
After insulin challenge				
Glucose (mM)	4.4 ± 0.7	5.2 ± 0.5	5.0 ± 0.6	6.5 ± 0.3****
Deoxyglucose uptake (d.p.m./mg)				
Muscle				
Soleus	1034 ± 97	907 ± 46	1092 ± 96	1063 ± 73
EDL	790 ± 73	793 ± 70	935 ± 104	802 ± 70
Fat				
Subcutaneous	141 ± 34	162 ± 33	209 ± 59	234 ± 39
Abdominal	73 ± 25	101 ± 18	89 ± 8	85 ± 14

Soleus, the soleus muscle; EDL, the long extensor muscle of the digits; subcutaneous fat, subcutaneous inguinal fat; abdominal fat, intra-abdominal fat. **P*<0.003, ***P*<0.001, ****P*<0.005, *****P*<0.007 compared with controls.

study 1 GH treatment increased the mean body weight by 18.7 ± 2.2 g (*P*<0.0002), whereas MP inhibited weight gain: 0.0 ± 1.0 g (*P*<0.004). Treatment with both hormones resulted in a weight gain of 10.8 ± 1.8 g (*P*<0.02) which is comparable to the control value of 7.6 ± 1.7 g. Growth results from study 2 are shown in Table 2.

Growth hormone and IGF-I

GH treatment increased GH levels approximately 40-fold, from a mean of 7.3 ± 2.3 ng/ml in the controls and MP-treated animals to 302 ± 34 ng/ml in the two groups receiving GH. MP did not affect GH levels significantly (*P*<0.7). When comparing the two studies the IGF-I levels were slightly lower in the study where animals were chronically catheterized (study 1). Here control values were 754 ± 34 ng/ml and after GH alone or in combination with MP the values were 790 ± 60 ng/ml and 799 ± 32 ng/ml respectively. The IGF-I level was 666 ± 60 ng/ml after MP treatment. The IGF-I levels in study 2 were significantly elevated in the groups receiving GH (*P*<0.002) and were not affected by MP (*P*<0.3). Group means and statistics from study 2 are listed in Table 2.

Glucose tolerance test (study 1)

The basal and peak levels of plasma glucose and insulin, as well as the glucose and insulin AUCs from the glucose tolerance test are shown in Table 1. There were no significant effects of any treatment on basal (*P*<0.4) and peak (*P*<0.3) glucose levels. Glucose AUC was increased by MP treatment (*P*<0.008), while GH did not affect this variable (*P*<0.9). However, no significant disturbances of glucose AUC were detected when comparing the individual groups with the control group (Table 1).

Basal insulin was significantly elevated by GH treatment (*P*<0.002), but was not changed significantly by MP injections (*P*<0.3). The effect of GH on the individual treatment groups can be seen in Table 1. Peak insulin levels were significantly decreased by MP (*P*<0.009), whereas GH treatment did not change this variable (*P*<0.2). However, when comparing the MP-treated groups individually with the controls no significant differences were detected (Table 1). GH elevated insulin AUC significantly (*P*<0.004), while this was not the case after MP treatment (*P*<0.2). However, when comparing each group mean with the controls only the group receiving both hormones had a significantly elevated insulin AUC (*P*<0.003), while the increase seen after GH alone only approached significance (*P*<0.02) (Table 1).

Insulin challenge (study 2)

As in study 1, basal glucose levels were not affected significantly by the hormones (*P*<1.0, data not shown). The post insulin glucose levels were increased by GH treatment to a degree approaching significance (*P*<0.02), whereas MP did not seem to affect this variable (*P*<0.08). Only in the group receiving both hormones did treatment significantly increase post insulin glucose levels when compared with the control group (*P*<0.007) (Table 2).

Peripheral glucose uptake

The ANOVA did not detect any significant treatment effects on the uptake of [³H]deoxyglucose in fat or muscle tissue. The tracer levels in each tissue are shown in Table 2.

No significant interaction between GH and MP was detected in any instance.

Discussion

This study was undertaken to examine some effects of GH on growth and carbohydrate handling in intact growing rats treated with the glucocorticoid, methylprednisolone.

Growth

It is well established that glucocorticoids have a catabolic effect (Horber & Haymond 1990, Horber *et al.* 1991, Malmjöf *et al.* 1994b), which in some situations leads to wasting of nitrogen and growth retardation. With the dose regimen of MP used in the present study a majority of animals responded with an arrest of body weight gain or even a decrease. If GH was given in conjunction with MP these negative effects were prevented and animals grew at more or less the same rate as the control animals. This finding supports previous results (Kovacs *et al.* 1991), although some authors have reported that GH was not able to inhibit the catabolic effects of glucocorticoids (Ortoft *et al.* 1992, 1993, Malmjöf *et al.* 1997).

Probably, the ability of GH to counteract the effect of glucocorticoids on growth may depend on the levels of circulating IGF-I (Ortoft *et al.* 1993). In both of the experiments performed in this study GH appeared to normalize or elevate circulating levels of IGF-I in MP-treated animals. This was particularly noticeable in the second experiment. The glucocorticoid used in this study has a relatively short half life which might have increased the potential of GH to exert its anabolic effects by increasing IGF-I production.

Carbohydrate metabolism

The effects of glucocorticoids on glucose metabolism have been reported to be variable (Stojanovska *et al.* 1990, Haber & Weinstein 1992, Ortoft *et al.* 1992, 1993, Venkatesan *et al.* 1996). This was probably to do with differences in the type of glucocorticoid used as well as dose and mode of administration. In this study animals treated with MP were able to maintain normal basal glucose levels. Even their ability to cope with an i.v. glucose challenge was not dramatically changed although a statistically significant main effect of MP to decrease glucose tolerance could be discerned from the data.

GH given alone or in combination with MP appeared to have no specific effects on basal glucose levels or the ability to cope with a glucose challenge. However, basal insulin levels were significantly increased in both groups receiving GH, which is in agreement with previous studies of GH administration in rats (Hettiarachchi *et al.* 1996, Thirone *et al.* 1997). In the group receiving GH together with MP basal insulin levels were approximately doubled. Since basal glucose was not decreased, but rather elevated, this indicates that a certain degree of insulin resistance was present. This impression is further

supported by the fact that animals in this group exhibited a significant increase in insulin AUC after an i.v. glucose challenge. Similar findings have been reported in humans (Horber *et al.* 1991). We are not aware of previous reports regarding glucose and insulin dynamics in rats treated with GH and glucocorticoid. Further confirmation of the fact that the combined treatment with GH and MP reduces insulin sensitivity was obtained when an acute insulin challenge was carried out.

Now, the interesting question arises as to which tissues are involved in the relative insulin resistance seen after combined treatment with GH and MP. One possible explanation would be a decrease in insulin-stimulated glucose transport in peripheral tissues such as fat and muscle. To our knowledge no studies dealing with this specific issue have been reported. Therefore, animals were challenged with an acute insulin injection in combination with radiolabeled glucose. The relative [³H]deoxyglucose uptake values of these individual tissues were in the same order of magnitude as described in earlier reports (Hom *et al.* 1984). Animals treated with GH and MP showed no difference in [³H]deoxyglucose uptake in fat or muscle but the potential of insulin to depress circulating glucose levels was significantly decreased. Although some support for these results could be found in the literature (Marfaing *et al.* 1991) they are surprising because there is a large body of evidence published which suggests that the elevated circulating glucose and insulin levels following treatment with GH or glucocorticoids is a consequence of peripheral insulin resistance (Pagano *et al.* 1983, Jorgensen *et al.* 1994, Fowelin *et al.* 1995, Hettiarachchi *et al.* 1996, Venkatesan *et al.* 1996). The reason for these conflicting results is at present not clear but the dose of insulin used might be of importance. In this study and in studies reporting similar results (Marfaing *et al.* 1991) a relatively high dose of insulin has been employed.

It might be asked what influence the dose of insulin has when testing the effect of GH and MP on insulin-stimulated peripheral glucose uptake. One explanation for this could be that the two hormones downregulate the insulin receptors. This would shift the dose-response curve to the right, but not alter the maximal effect of insulin, as not all receptors are needed to attain the maximum response (DeFronzo 1982). Such a reasoning could perhaps explain why neither GH (Dimitriadis *et al.* 1994, Cartee & Bohn 1995, Napoli *et al.* 1996) nor glucocorticoid (Dimitriadis *et al.* 1997) reduce *in vitro* glucose transport activity in muscle during maximal insulin stimulation. However, to elucidate this issue further *in vivo* dose-response curves of insulin-stimulated glucose uptake in rats treated with GH and/or glucocorticoid are required. Until this type of data are presented we have to conclude that the combined administration of GH and MP does not generally decrease peripheral insulin-stimulated glucose disposal. Such a conclusion is further supported by the fact that neither GH (Cartee & Bohn 1995, Napoli

et al. 1996) nor dexamethasone (Haber & Weinstein 1992, Venkatesan *et al.* 1996, Dimitriadis *et al.* 1997) change the abundance of the glucose transporter molecule GLUT-4 in muscle cell membranes.

If the combination of GH and glucocorticoids does not decrease insulin-stimulated glucose transport peripherally then how could the decrease in insulin sensitivity be explained? One explanation could be that the hormones cause hepatic insulin resistance and that the liver consequently is principally involved in disturbing normal insulin function. This possibility is supported by the results from experiments concerning hepatic function after GH or glucocorticoid therapy in several species (Rizza *et al.* 1982, Pagano *et al.* 1983, Horber *et al.* 1991, Goldstein *et al.* 1993, Okuda *et al.* 1994, Hettiarachchi *et al.* 1996).

Conclusions

The growth retarding effect of glucocorticoid treatment can be overcome by GH administration. However, this effect is probably dependent on periods when the glucocorticoid level is relatively low and the GH level is high. In this setting GH elevated insulin levels, but development of outright insulin resistance required treatment with both hormones. Combination of the two hormones did not decrease peripheral glucose uptake in a situation with impaired glucose handling. This suggests that hepatic insulin resistance is important in this condition, probably with increased hepatic glucose mobilisation as a consequence.

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