

CLINICAL STUDY

Short-term low-dose growth hormone administration in subjects with impaired glucose tolerance and the metabolic syndrome: effects on β -cell function and post-load glucose tolerance

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Abstract

Objective: Modest elevations in circulating IGF-I levels have been suggested to protect against the development of glucose intolerance in insulin-resistant subjects. To further understand the interactions of GH and IGF-I on β -cell function and post-load glucose tolerance in glucose-intolerant subjects predisposed to diabetes, we performed a pilot study in 12 subjects with impaired glucose tolerance and the metabolic syndrome using a low GH dose (1.7 μ g/kg per day) known to increase endogenous IGF-I production.

Design: Fourteen daily GH or placebo injections in a double-blind cross-over study.

Methods: Baseline and post-treatment oral glucose tolerance tests were performed. The homeostasis model assessment and the insulinogenic index was used to estimate fasting insulin sensitivity (S_I) and β -cell function respectively, whereas changes in the incremental area under the curve were used to estimate post-load glucose tolerance (ΔAUC_{glu}) and post-load insulin levels (ΔAUC_{ins}).

Results: GH increased total IGF-I ($P < 0.02$), free IGF-I ($P < 0.04$) and fasting insulin ($P < 0.04$) levels, but did not modify plasma IGF-binding proteins (IGFBPs)-1 and -3, fasting glucose, non-esterified fatty acid and C-peptide levels, and fasting S_I . After oral glucose intake, glucose tolerance improved ($P < 0.03$), but post-load insulin levels and β -cell function remained unchanged.

Conclusion: Short-term low-dose GH administration induced fasting hyperinsulinaemia possibly by reducing insulin clearance but improved post-load glucose tolerance, suggesting that increased bioavailable IGF-I enhanced post-load S_I without altering β -cell function. Longer-term studies are required to ascertain whether these positive effects on post-load glucose tolerance and the preservation of β -cell function can be sustained by this GH dose in these high-risk subjects.

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Introduction

Previous studies examining the effects of growth hormone (GH) on glucose metabolism have yielded contrasting results, with some investigators demonstrating acute insulin-like effects (1, 2), whereas others demonstrated diabetogenic effects at the insulin receptor and post-receptor sites (3, 4). These conflicting results raise the question whether variable GH doses have distinct, independent effects on glucose homeostasis. We recently demonstrated that low-dose GH administration (1.7 μ g/kg per day); a dose that mimics the daily physiological GH production rate in adults (5); increased insulin-like growth factor-I (IGF-I) levels and improved β -cell function in healthy adults (6).

With the advent of recombinant human insulin-like growth factor-I (rhIGF-I), it has become apparent that

IGF-I enhances insulin sensitivity (S_I) in conditions such as Type 2 diabetes mellitus (7, 8) and insulin resistance syndromes (9, 10). It has also been proposed that low basal IGF-I levels predict worsening of insulin-mediated glucose uptake in older adults (11). The importance of IGF-I in glucose metabolism was further substantiated by the results of our recently reported observational study demonstrating a possible protective role of IGF-I against the development of glucose intolerance in normoglycaemic adults (12). Taken together, these studies suggest that the interrelated actions of insulin and IGF-I are important determinants of glucose homeostasis in both normal and insulin-resistant states.

To broaden our understanding of the interactions of GH and IGF-I on glucose metabolism in glucose-intolerant subjects predisposed to develop Type 2 diabetes,

we performed this pilot study in subjects with impaired glucose tolerance (IGT) that fulfilled the criteria of the metabolic syndrome by World Health Organization (WHO) standards (13). Using an identical low GH dose (1.7 µg/kg per day) to that used in our previous study involving young healthy adults (6), we aimed to examine the effects of GH and IGF-I on β-cell function and glucose tolerance during a standard oral glucose tolerance test (OGTT).

Subjects and methods

Subjects

All of the subjects in this study were participants of the ongoing Ely Study (14), a prospective population-based study of Type 2 diabetes in Ely, a town in the county of Cambridgeshire, UK. The Ely Study has been previously described (14); in brief, during 1990–1991 (Phase 1), 1122 Caucasian adults (aged 40–65 years) free of diabetes were randomly selected from a population-based sampling frame to undergo a 75 g OGTT. Subjects who did not have diabetes at baseline were followed up 5-yearly with further OGTTs. During Phase 3 of the study in 2001, 22 subjects with IGT that satisfied the WHO criteria (13) of the metabolic syndrome were identified and were invited to participate in the present pilot study. Twelve subjects (six males, six females; age range 40–65 years) agreed, whereas ten subjects declined to participate in the study. Baseline characteristics of the subjects that participated in the study did not differ from those that declined to participate. The Cambridge Local Research Ethics Committee granted ethical approval for this study and informed consent was obtained from each subject.

Study design

In a double-blind cross-over study, the subjects were randomised to receive 14 daily GH or placebo injections, exchanging their treatment for a further 14 days after a 7-day washout period. The subjects self-injected GH (Genotropin; Pharmacia Ltd, Milton Keynes, UK) or placebo subcutaneously at 2200 h with a Genotropin pen device. Following an overnight fast, a 75 g OGTT was performed at baseline and post-GH treatment, and at baseline and post-placebo treatment. Fasting blood glucose levels were analysed immediately, whereas haemoglobin A1c (HbA1c) samples were sent to the laboratory for analysis. The remaining samples were centrifuged in a cooled centrifuge at 2500 r.p.m. Aliquoted samples were then transferred to the laboratory and stored at –70 °C within 4 h until assayed for insulin, C-peptide, non-esterified fatty acid (NEFA), total IGF-I, free IGF-I, IGF-binding protein (IGFBP)-1 and IGFBP-3 concentrations.

Anthropometric assessment

Height and weight were measured while the subjects stood in light clothing. The same investigator measured waist circumference using a standard soft tape measure midway between the lowest rib margin and the iliac crest in the standing position. Body fat mass and percentage of body fat were estimated using a bioelectrical impedance monitor (Bodystat 1500, Bodystat Ltd, Douglas, Isle of Man, UK).

Blood samples and assays

Blood glucose concentrations were measured using whole blood samples with a YSI (Yellow Springs Instrument) model 2300 stat plus analyser (Farnborough, Hants, UK). The intra-assay coefficient of variation (CV) was 1.5% at 4.1 mmol/l, and inter-assay CVs were 2.8 and 1.7% at 4.1 and 14.1 mmol/l respectively. Plasma insulin, C-peptide, total IGF-I, IGFBP-3 and IGFBP-1 concentrations were measured using Diagnostic Systems Laboratories, Inc., ELISAs (Oxford Bio-innovations, Upper Heyford, Oxon, UK) according to the manufacturer's instructions. For insulin concentrations, intra-assay CV was 4.4% at 62 pmol/l and 5.1% at 215 pmol/l, and equivalent inter-assay CVs were 4.3 and 2.9% respectively. For C-peptide concentrations, intra-assay CV was 4.8% at 0.7 ng/ml and 3.2% at 2.2 ng/ml, and equivalent inter-assay CVs were 15.8 and 8.1% respectively. For total IGF-I concentrations, sensitivity was 0.03 ng/ml, intra-assay CVs were 8.8 and 9.4% at 107 and 262 ng/ml, and equivalent inter-assay CVs were 6.1 and 8.0% respectively. For IGFBP-3 concentrations, sensitivity was 0.04 ng/ml, intra-assay CVs were 4.9 and 2.8% at 5.2 and 34.7 ng/ml, and equivalent inter-assay CVs were 9.7 and 1.9% respectively. For IGFBP-1 concentrations, sensitivity was 0.25 ng/ml, intra-assay CVs were 6.1% at 7.0 ng/ml and 5.3% at 48.4 ng/ml, and equivalent inter-assay CVs were 10.4 and 5.1% respectively. Serum free IGF-I concentrations were determined using ultrafiltration by centrifugation at conditions approaching those *in vivo* (15). All samples were analysed in triplicate and within the same run, with an intra-assay CV of 13.9% and inter-assay CV of 16.5%. NEFA concentrations were measured using a peroxidase technique with a commercial kit (Half Micro test, Boehringer Mannheim).

Calculations

The homeostasis model assessment (HOMA), described by Matthews *et al.* (16) and previously validated against independent measures of S_I (17–19), was used to estimate fasting S_I (HOMA-S, in %) from fasting glucose and insulin levels. These values are expressed in relation to values in a 'standard individual' in which they are each accorded the value 100. The HOMA-CIGMA

computer program (20) calculated HOMA-S values, and with such a method, high HOMA values denote high S_I .

The insulinogenic index, a commonly used index of β -cell function because of the ability of this index to detect anomalies in β -cell function in many circumstances, was calculated from the OGTT data as the ratio of the increment of insulin concentrations to that of glucose concentrations at 30 min after oral glucose loading ($Ins_{30} - Ins_0 / Glu_{30} - Glu_0$) (21).

The suprabasal incremental area under the curve (AUC) using the trapezoidal rule, as previously described (22, 23), was used to estimate post-load glucose tolerance (ΔAUC_{glu}) and post-load insulin levels (ΔAUC_{ins}).

Power calculations

The sample size and power calculations for the present study were based on our previous study (6). In that study, using the HOMA, the low GH dose similar to the dose used in the present study did not alter insulin sensitivity, but led to reductions of 15–20% in fasting blood glucose levels. Therefore, based on that study, 12 subjects were recruited in the present study, and the HOMA assessment of insulin sensitivity should enable the detection of 10% difference with a 90% power at the 1% level.

Statistical analyses

Statistical analyses were performed using SPSS for Windows (version 10.0, SPSS, Chicago, IL, USA). Data are expressed as means \pm s.e., except for data in Table 1 which are presented as means \pm s.d. Carryover effect was tested for by comparing the baseline values prior to each treatment phase. Treatment order effect was analysed by comparing the treatment difference from baseline between the two treatment orders (i.e. GH/placebo and placebo/GH) using unpaired Student's *t*-test. As no carryover or order effect was found, the analysis was performed on combined data. Post- vs pre-treatment comparison was performed by paired Student's *t*-test and Wilcoxon signed-rank test, where appropriate. *P* values < 0.05 defined statistical significance.

Table 1 Baseline characteristics of study subjects.

Age (years)	59.3 \pm 6.1
HbA1c (%) at study entry*	5.5 \pm 0.6
Body weight (kg)	87.3 \pm 18.8
Body mass index (kg/m ²)	30.0 \pm 6.0
Waist (cm)	97.9 \pm 13.6
Fat mass (%)	34.6 \pm 10.5
Lean body mass (kg)	54.3 \pm 13.8

All values are presented as mean \pm s.d.

* Reference range of HbA1c, 4.9–6.3%.

Results

Baseline characteristics and anthropometric changes of study subjects

Baseline characteristics of the study subjects are shown in Table 1. No significant changes in body mass index (BMI), body composition and waist circumference measurements were observed following either GH or placebo injections.

IGF-I and IGFBP levels

The placebo injections did not modify any of the measured biochemical indices (Tables 2 and 3). GH increased total IGF-I ($P < 0.02$) and free IGF-I ($P < 0.04$), whereas IGFBP-I and IGFBP-3 levels were unchanged (Table 2).

Glucose, insulin, C-peptide and NEFA levels

GH increased fasting insulin ($P < 0.04$) levels, but did not modify fasting glucose, C-peptide and NEFA levels, and fasting S_I (Table 2). Following oral glucose intake, post-load glucose tolerance ($P < 0.03$) improved. Blood glucose levels decreased at 120 min compared with pre-treatment values ($P < 0.02$), with a corresponding 14% reduction in percentage change from baseline ($P < 0.04$) (Table 3, Fig. 1), whereas post-load insulin levels and β -cell function, as estimated by the insulinogenic index, were unchanged (Table 3).

Discussion

This pilot study demonstrated that short-term low-dose GH administration increased fasting insulin levels and improved post-load glucose tolerance. We hypothesise that the low GH dose reduced insulin clearance, given that β -cell function was unchanged, and increased bioavailable IGF-I, which in turn may have enhanced post-load S_I in subjects with IGT and the metabolic syndrome.

High doses of GH therapy impair both hepatic and peripheral S_I in healthy humans (24), whereas we have recently demonstrated that lower GH doses exerted insulin-like effects (6). In contrast to our previous findings of reductions in fasting blood glucose levels in healthy adults (6), fasting blood glucose levels in subjects with IGT and the metabolic syndrome were unchanged following low-dose GH administration. However, fasting insulin levels increased suggesting an induction of GH-induced insulin resistance, although this was not reflected in the HOMA probably due to the small subject numbers in this study. The unchanged fasting C-peptide levels could also indicate a reduction in insulin clearance, as C-peptide and insulin are released in equimolar amounts from the β -cells. This hypothesis is supported

Table 2 Effect of 14-day placebo and GH treatment on GH pharmacokinetics and fasting biochemical parameters in subjects with IGT and the metabolic syndrome.

	Placebo		GH	
	Day 1 (pre-treatment)	Day 15 (post-treatment)	Day 1 (pre-treatment)	Day 15 (post-treatment)
Total IGF-I (ng/ml)	152±26	125±17	111±18	161±18*
Free IGF-I (ng/ml)	0.64±0.08	0.55±0.10	0.56±0.10	0.69±0.09†
IGFBP-1 (ng/ml)	17.5±4.4	16.3±3.8	18.0±4.1	17.7±4.8
IGFBP-3 (ng/ml)	3396±211	3533±329	3424±230	3615±235
Fasting glucose (mmol/l)	4.9±0.2	5.0±0.3	5.1±0.3	4.9±0.2
Fasting insulin (pmol/l)	71.7±13.7	67.2±12.3	60.7±11.8	73.6±15.3†
Fasting C-peptide (pmol/l)	777±94	746±93	726±81	787±98
Fasting NEFA (μmol/l)	542±53	518±59	528±38	560±35
Fasting S _i (HOMA-S, %)	110.4±21.3	111.1±18.6	122.9±19.5	116.3±24.1

All values are presented as means ± s.e.

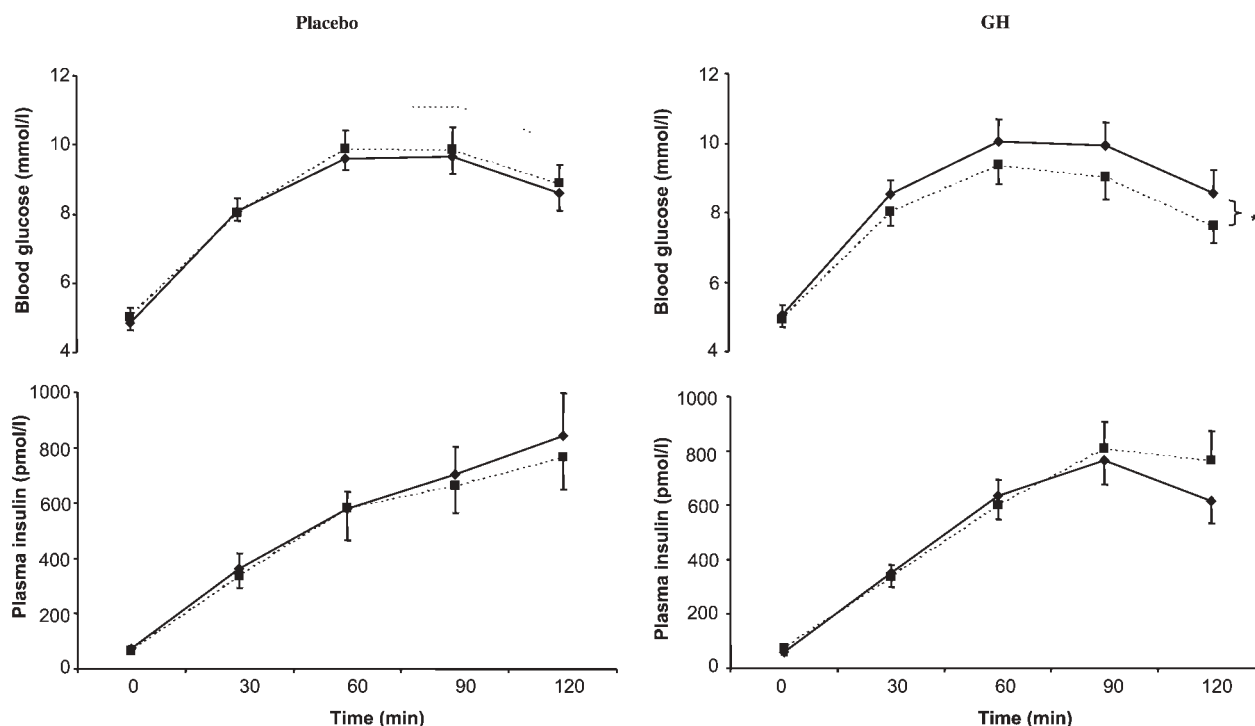
* $P < 0.02$ and † $P < 0.04$ vs day 1 GH pre-treatment.

Table 3 Effect of 14-day placebo and GH treatment on insulin and glucose parameters during the OGTT in subjects with IGT and the metabolic syndrome.

	Placebo		GH	
	Day 1 (pre-treatment)	Day 15 (post-treatment)	Day 1 (pre-treatment)	Day 15 (post-treatment)
Insulinogenic index ($\text{Ins}_{30} - \text{Ins}_0 / \text{Glu}_{30} - \text{Glu}_0$)	87.9±15.1	93.0±13.7	84.6±16.7	87.0±11.1
Post-load insulin levels ($\Delta\text{AUC}_{\text{ins}}$) (pmol/l per hour)	27 256±3104	25 933±3073	27 654±3210	28 046±3538
120-min glucose levels (mmol/l)	8.6±0.5	8.9±0.5	8.5±0.7	7.6±0.5*
Post-load glucose tolerance ($\Delta\text{AUC}_{\text{glu}}$) (mmol/l per hour)	219.8±15.8	219.9±18.9	226.9±20.3	194.9±18.5†

All values are presented as means ± s.e.

* $P < 0.02$; † $P < 0.03$ vs day 1 GH pre-treatment.

**Figure 1** Mean (\pm s.e.) blood glucose and plasma insulin concentrations during OGTT before (day 1: \blacklozenge and continuous line) and after (day 15: \blacksquare and dotted line) placebo and GH injections. * $P < 0.02$ vs day 1 before GH treatment.

by studies demonstrating that factors such as obesity in the presence or absence of Type 2 diabetes (25), glucose intolerance (26) and increased circulating GH levels (27) reduced insulin clearance rates.

In healthy adults, we postulated that the increased bioavailable IGF-I generated by low-dose GH administration may have increased insulin secretion (6). In contrast, we did not observe any changes in β -cell function in this study following oral glucose intake despite increases in total and free IGF-I concentrations. It has been suggested that the bioactivity of IGF-I is maintained primarily through free IGF-I (28), which constitutes less than 1% of the circulating IGF pool (15). Other studies have demonstrated the importance of IGF-I in enhancing β -cell function (29, 30) and protecting β -cells from apoptosis (31). Indirect evidence implicating IGF-I in enhancing β -cell function has also been reported from studies of knockout mice lacking β -cell IGF-I receptor demonstrating defective glucose-stimulated insulin secretion and glucose intolerance (32, 33). There are several possibilities as to why β -cell function did not improve in our subjects following low-dose GH administration, in contrast to previous observations in healthy (6) and GH-deficient adults (34). In this study, we examined a select group of subjects who, in addition to IGT, had insulin resistance and other cardiovascular risk factors that constituted the metabolic syndrome. It has been proposed that the increasing demand associated with increasing insulin resistance over a prolonged period resulted in β -cell 'exhaustion' (35). It is also possible that the chronically deranged metabolic state of lipid metabolism may contribute to the gradual accumulation of intracellular fat in β -cells (36), thus leading to the inhibition of β -cell mass expansion (29) and the consequent failure to respond to further IGF-I stimulation.

The first-phase insulin secretion has been shown to play a critical role in maintaining postprandial euglycaemia (26,37). In this study, we observed a reduction in 120-min blood glucose levels and improved post-load glucose tolerance suggesting enhanced post-load S_I . As the insulinogenic index, which also reflects first-phase insulin secretion, was unchanged after oral glucose intake, the improvement in post-load glucose tolerance must be largely due to enhanced post-load S_I secondary to the effects of increased free IGF-I levels. The effects of IGF-I on improving S_I and enhancing glucose disposal independent of changes in GH levels have been reported in several short-term studies employing rhIGF-I in healthy subjects (38, 39) and patients with diabetes (7, 8). Subjects with IGT and the metabolic syndrome may be more sensitive to the effects of IGF-I following increased blood glucose levels due to increased hybrid IGF-I/insulin receptors in muscle, which behave more like IGF-I receptors (40), and are increased in subjects with insulin resistance (41, 42). Sakai *et al.* (43) demonstrated that IGF-I enhanced the cellular response to insulin primarily via these hybrid receptors, and

more recently these investigators have provided further evidence that the combination of increased IGF-I and insulin levels further augmented the sensitivity of the hybrid receptors to insulin activation (44).

More recently, several landmark diabetes prevention studies involving subjects with IGT have provided evidence supporting the efficacy of lifestyle intervention, based on diet and exercise, in enhancing S_I and reducing the risk of developing Type 2 diabetes (45, 46). However, these were long-term studies and compliance with lifestyle interventions is often poor in the medium-to-long term (47), thus highlighting the need to define the potential role of physiological increases of circulating IGF-I levels in enhancing glucose disposal. Further to the results of our recent observational study (12), we can only speculate that prolonged physiological increase in IGF-I exposure may be required to fully utilise the effects of IGF-I on β -cell function in these 'high-risk' subjects.

The present study had limitations in that subject numbers were small, and that the OGTT is an inconsistent test because variables such as the rate of glucose absorption, neural activation and incretins are involved. It would have been preferable to utilise more invasive techniques such as the hyperinsulinaemic hyperglycaemic clamp to assess hepatic and peripheral S_I , and β -cell function, but this was not possible as this study was part of a large ongoing epidemiological field study (14). Nevertheless, our data highlight the complex interaction of insulin and the GH-IGF-I axis in insulin-resistant subjects with glucose intolerance.

In conclusion, the effects of increased bioavailable IGF-I generated by the low GH dose in improving post-load glucose tolerance in subjects with IGT and the metabolic syndrome appears to be β -cell independent, thus providing further evidence of a direct role of free IGF-I in facilitating peripheral glucose disposal and enhancing post-load S_I . Additional longer-term studies with larger patient numbers are required to clarify the effects of low-dose GH, rhIGF-I and/or rhIGF-I/IGFBP-3 complex therapy on β -cell function, and to determine whether the effects of IGF-I on post-load S_I are sustained in these high-risk subjects.

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