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Effects of a Combination of rhGH and Metformin on Adiponectin Levels in Patients with Metabolic Syndrome

Abstract

Adiponectin is a recently discovered adipocytokine that correlates negatively with body mass index and body fat. In patients with GH deficiency, treatment with recombinant human growth hormone (rhGH) reduces body fat mass and thus may also have a favorable effect in patients with metabolic syndrome, and would also be expected to increase adiponectin levels. However, due to its diabetogenic effect, rhGH treatment also bears an increased risk for the development of type 2 diabetes mellitus. We conducted a 18-month randomized, double-blind, placebo-controlled study to assess the effect of rhGH in combination with metformin (MGH) in 14 obese men (7 MGH; 7 Metformin+Placebo, 54 ± 2 years, BMI 33.0 ± 1.2 kg/m^2) with mildly elevated fasting plasma glucose (FPG) at screening (6.1–8.0mmol/l). All patients received metformin (850 mg twice daily) for treatment of type 2 diabetes mellitus/impaired glucose tolerance, either alone or in combination with rhGH (daily dose 9.5 µg/kg body weight). Glucose disposal rate (GDR) was measured using the euglycemic hyperinsulinemic clamp technique, and body composition was measured by DEXA at 0 and 18 months. After 18 months, the mean adiponectin concentration increased by 32 ± 11% ($p = 0.018$) in the MGH group and did not change in the MP group (– 10 ± 13%; p = n. s.). The difference in relative changes in adiponectin levels between the two groups after 18 months was statistically significant ($p = 0.026$). Improvement in insulin sensitivity (GDR) correlated positively with adiponectin levels ($r = 0.73$; $p = 0.004$). In conclusion, the additional administration of rhGH increased adiponectin levels in patients with metabolic syndrome, indicating its potential role in adiponectin-associated insulin sensitivity alterations.

Key words
Adiponectin · Growth hormone · Metabolic syndrome

Introduction

Abdominal obesity and visceral fat are central findings in metabolic syndrome and have been linked to the development of insulin resistance and type 2 diabetes mellitus. Striking similarities exist between metabolic syndrome and untreated growth hormone deficiency (GHD) [1–3]. In obese males without evidence of pituitary disease, treatment with rhGH over a period of 9 months resulted in reduced abdominal fat mass and improved glucose metabolism [2]. The use of rhGH in patients with central obesity, however, may be limited by an increase in insulin resistance with a highly increased risk of developing type 2 diabetes, which is known to occur during the early course of rhGH therapy [4]. Metformin, a biguanide antihyperglycemic agent, is a well-established treatment of type 2 diabetes and obesity [5], and its administration has been shown to be effective in the prevention of type 2 diabetes in persons at high risk [6].

Adiponectin is a recently characterized adipocytokine that has gained interest as a novel mediator due to its putative insulin-sensitizing and vascular protective effects [7,8]. Adiponectin levels correlate negatively with percentage body fat, central fat distribu-

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tion, abnormal oral glucose tolerance, but correlate positively with glucose disposal rate during euglycemic insulin clamp [9–12]. Expression of the AdipoR2, one of the two recently identified adiponectin receptors, is regulated by GH, indicating its potential role in adiponectin-associated metabolic insulin sensitivity alterations [13,14]. The aim of the present study was to investigate the additional effect of rhGH on adiponectin levels and body fat in patients with metabolic syndrome treated with metformin.

Materials and Methods

Subjects

The study was designed as a 18-month, double-blind, placebo-controlled trial. Informed consent was obtained from each patient before enrolment. All patients included had three or more criteria fulfilling the definition for metabolic syndrome according to the Third Report of the National Cholesterol Education Program [ATP III] [15] – 1. Abdominal obesity: waist circumference > 102 cm in men; 2. Hypertriglyceridemia ≥ 1.695mmol/l and/or HDL < 1.036mmol/l in men; 3. high blood pressure ≥ 130/80 mm Hg; and 4. high fasting glucose ≥ 6.1mmol/l) based on the definition of the WHO in 1998 [16]. Inclusion criteria were age between 35–70 years and body mass index between 30–40 kg/m^2, fasting plasma glucose (FPG) between 6.1 and 8.0 mmol/l, and HbA_{1c} < 7.5%. The patients were randomly assigned to one of two treatment groups (metformin and rhGH (MGH) or metformin and placebo (MP)). Treatment assignments were stratified according to BMI and HbA_{1c}. Twelve patients were included in the MGH group and 13 patients in the MP group. Side effects were observed in 7 of 12 patients in the MGH group and 8 of 13 patients of the MP group as mentioned in the previous paper [17]. The data presented here include those patients (7 in each group) available for 18-month follow-up.

In both treatment groups, patients received metformin 850 mg twice daily (Glucophage®, Merck, Darmstadt, Germany) during the whole study period. In the MGH group, rhGH (Genotropin®, Pfizer, Karlsruhe, Germany) was administered sc before bedtime at a daily dose of 9.5 μg/kg/body weight (0.20 IU/kg body weight/week) after an initial 4-week dose-adjustment period.

Methods

Body weight was measured in the morning to the nearest 0.1 kg wearing indoor clothing, and body height was measured barefoot to the nearest 0.01m. Waist circumference was measured in the standing position with a flexible plastic tape midway between the lower rib margin and the iliac crest. Insulin-mediated glucose disposal was determined using the euglycemic insulin clamp technique based on the protocol of De Fronzo et al. [18,19]. In-house reference values for glucose disposal rate (GDR) have been evaluated in healthy young men (30-males, mean age 25 ± 2 years, BMI 23.0 ± 1.6 kg/m^2, GDR 10.2 ± 1.9 mg/kg/min (range 6.4–14.0)). Total body fat mass and lean body mass were assessed by dual energy X-ray absorptiometry (DEXA) (model DPX-L, Lunar Corporation, Madison, WI, USA).

Assays

Adiponectin concentrations were measured on diluted (1 : 500) fasting serum samples using a human adiponectin radioimmunoassay (RIA) kit (Linco Research, Missouri, USA). The sensitivity of the assay was 1 μg/ml, and the limit of linearity was 200 μg/ml. All samples were run in duplicate in the same assay. The mean intraassay and interassay coefficient of variation was 6% and 9%, respectively.

Serum GH levels were determined by chemiluminescence immunometric assay (Nichols Institute Diagnostics GmbH, Bad Nauheim, Germany) and plasma IGF-I concentrations were measured by immunoradiometric assay (Nichols Institute Diagnostics GmbH, Bad Nauheim, Germany). Plasma glucose, HbA_{1c}, total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides were determined by routine methods.

Statistics

Quantitative data are expressed as the mean±SEM from replicate determinations. Absolute differences between time-points were analyzed per group using the paired Wilcoxon signed-rank test (WSR). Comparisons between baseline values in the two groups were performed using the unpaired Wilcoxon/Mann/Whitney test (WMW). Correlation of adiponectin levels to various variables were analyzed using Pearson's test. Statistical analyses were performed using GraphPad InStat version 3.02 (GraphPad Software, San Diego, California USA). Differences were considered statistically significant at $p < 0.05$.

Results

Seven patients in two groups each receiving either metformin and GH (MGH) or metformin and placebo (MP) were followed up for 18 months. The mean IGF-I levels at baseline were in the lower age-adjusted normal range in both groups. In the MGH group, IGF-I increased from 173 ± 22 to 408 ± 50 μg/l ($p < 0.001$) after 18 months, whereas IGF-I in the MP group did not change significantly (from 144 ± 15 to 160 ± 19 μg/l).

The mean baseline adiponectin level was slightly higher in the MGH group (range 3.6–30.8 μg/ml *vs.* 11.3–44.9 μg/ml in the MP group), but the difference was not significant ($p = 0.07$). After 18 months, the mean adiponectin concentration (13.8 ± 3.2 to 20.3 ± 4.4 μg/ml) increased by 32 ± 11% ($p = 0.018$) in the MGH group and did not change in the MP group (25.3 ± 4.7 at baseline to 21.6 ± 2.8 μg/ml; -10 ± 13%; p = n.s.; Fig. **1**).

At baseline, GDR did not differ significantly between the MGH and MP groups (3.9 ± 1.8 and 5.7 ± 2.1 mg/kg/min, respectively), and the difference between the two groups was not significant ($p = 0.12$). After 18 months of treatment, the mean GDR of the MGH group increased to 4.6 ± 2.4 mg/kg/min and slightly decreased in the MP group to 4.8 ± 1.4 mg/kg/min. The relative changes of adiponectin concentrations were positively correlated with the relative changes of GDR (Fig. **2**).

Total body fat decreased in both groups without statistical difference between the two groups. BMI ($r = -0.50$, $p = 0.009$) and total body fat ($r = -0.43$, $p = 0.028$) were negatively correlated to adiponectin concentration. Furthermore, a trend toward a negative correlation of waist circumference to adiponectin levels ($r = -0.39$, $p = 0.059$) was found. After 18 months of treatment, fasting plas-

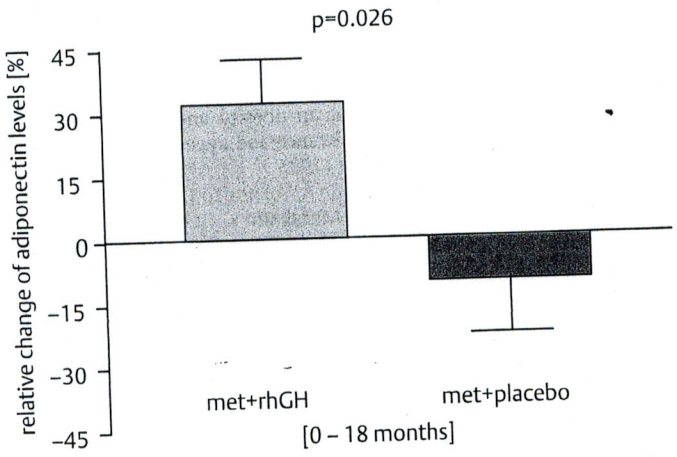

Fig. 1 Changes in adiponectin levels after treatment with metformin+rhGH or metformin+placebo in 14 men with metabolic syndrome.

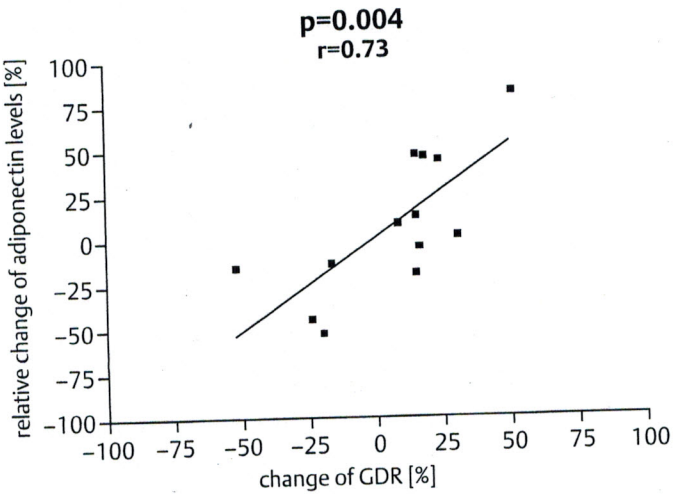

Fig. 2 Correlation of the relative change of adiponectin levels with the changes in GDR (glucose disposal rate) during an euglycemic clamp test in patients with metabolic syndrome.

Table 1 Patient characteristics for 14 men with metabolic syndrome (mean±SEM)

		Metformin + rhGH	*Metformin + Placebo*
BMI (kg/m²)	*a*	33.7±1.4	32.2±0.8
	b	32.5±1.1	30.9±1.0
Waist circumference (cm)	*a*	120±4	112±3
	b	114±10	110±4
Total body fat (kg)	*a*	34.1±2.9	31.1±3.1
	b	29.6±5.5	28.5±3.8
Total muscle mass (kg)	*a*	73.9±3.2	67.0±3.4
	b	74.4±2.4	64.8±4.1
Blood pressure systolic (mmHg)	*a*	135±3	126±4
	b	133±5	133±4
Blood pressure diastolic (mmHg)	*a*	88±10	83±4
	b	83±5	83±4
Fasting plasma glucose (mmol/l)	*a*	6.8±0.2*	6.8±0.2*
	b	6.0±0.2	5.5±0.2
HbA_{1c} (%)	*a*	5.6±0.1	6.0±0.1
	b	5.6±0.2	5.6±0.1
Total cholesterol (mmol/l)	*a*	5.4±0.4	5.0±0.3
	b	5.1±0.2	5.6±0.2
LDL cholesterol (mmol/l)	*a*	3.4±0.3	3.6±0.2
	b	3.1±0.2	3.4±0.2
HDL cholesterol (mmol/l)	*a*	0.9±0.1*	1.0±0.0*
	b	1.2±0.1	1.4±0.1
Triglycerides (mmol/l)	*a*	1.9±0.4	1.8±0.2
	b	2.2±0.4	1.7±0.2

All values are expressed as the means ± SEM
*$p < 0.05$ (differences between 0 and 18 months in each group)
a 0 months (baseline), *b* after 18 months

ma glucose decreased significantly ($p < 0.05$) and HDL cholesterol levels increased significantly ($p < 0.05$) without statistical difference between the two groups (Table 1).

Discussion

In the present placebo-controlled prospective study on a small group of patients with metabolic syndrome, the additional administration of growth hormone to metformin increased adiponectin levels. This increase of adiponectin was closely related to an increase of insulin-sensitivity as determined by euglycemic clamp testing.

Recently, two receptors of this adipose-expressed protein referred to as adiponectin receptor 1 (AdipoR1) and 2 (AdipoR2) have been cloned. AdipoR1 is abundantly expressed in skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver [13]. Fasshauer et al. have shown that GH induced AdipoR2 mRNA by up to 2.4-fold but not AdipoR1 mRNA; its positive effect on AdipoR2 expression could be reversed by withdrawal of GH for 24 h [14]. These *in vitro* experiments with fat cells could be confirmed by the present *in vivo* observation of increased adiponectin levels after treatment with hGH.

The pharmacological effect of adiponectin in reducing insulin resistance is related to a decrease in plasma fatty acid levels and in triglyceride content in muscle and liver in obese mice [20,21]. Moreover, other candidate molecules such as TNF-α may be involved in the pathogenesis of adiponectin-associated insulin resistance. Since TNF-α significantly reduced adiponectin expression and secretion from adipocytes, TNF-α might be partially responsible for the decreased adiponectin production in obesity [21–24].

Over the last few years, several studies have shown that the administration of rhGH can reduce body fat and especially visceral fat in obese patients, which is most likely due to its lipolytic effect [2,25]. Metformin, a biguanide antihyperglycemic agent, is a well-established treatment strategy for type 2 diabetes and obesity [5], and its effectiveness has been demonstrated in the prevention of type 2 diabetes in persons at high risk [6]. Moreover, the aim of the additional administration of metformin was to minimize the insulin antagonistic effect of rhGH in patients with metabolic syndrome and type 2 diabetes mellitus. Therefore, we have compared the effect of combination therapy with

metformin and rhGH to metformin alone [26–28]. While both regimes decreased body fat significantly, increased adiponectin levels were only found in combination therapy, indicating that adiponectin levels seem to precede changes in body fat.

As we have shown in the present study, treatment with metformin alone to reduce blood glucose levels did not change adiponectin levels [29] in obese individuals with type 2 diabetes mellitus, indicating that the increase in adiponectin levels is associated with improved insulin action, but is not directly related to glycemic control.

In conclusion, our data provide evidence for the potential role of rhGH in adiponectin-associated alterations of insulin-sensitivity in patients with metabolic syndrome.

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