CLINICAL STUDY

Decreased insulin sensitivity in small for gestational age males treated with GH and preterm untreated males: a study in young adults

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Abstract

Background: Low birth weight and preterm birth are associated with growth delay as well as the development of insulin resistance. Insulin resistance is especially seen in subjects with catch-up growth. GH therapy induces growth in short subjects with low birth weight at term, but little is known about the long-term effects on insulin sensitivity. GH therapy is now also proposed for preterms that remain short.

Methods: We investigated insulin sensitivity using the gold standard hyperinsulinemic-euglycemic clamp technique in 10 young adult males born small for gestational age (SGA) who had been treated with GH during childhood (GH) in comparison with 15 males born preterm AGA (premAGA), 13 males born preterm SGA (premSGA), and 15 males born at term with normal birth weight (CON). Furthermore, we investigated the presence of the metabolic syndrome.

Results: Insulin sensitivity was decreased in premAGA, premSGA, and GH subjects compared with CON males. The metabolic syndrome was not present in any of the groups.

Conclusion: Insulin sensitivity is decreased in GH-treated SGA born males as well as in preterm born males. With respect to the SGA subjects, whether the difference results from perinatal-, postnatal-, or GH therapy-related factors are not known. With respect to the preterm born subjects, close surveillance is needed when commencing GH therapy.

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Introduction

Low birth weight is considered as a risk factor for the development of the insulin resistance. The development of insulin resistance has been found in subjects born small for gestational age (SGA) at term (1) as well as in subjects born preterm (2, 3). In 10% of the term born SGA subjects, the low birth weight is associated with growth delay that can effectively be treated with GH (4). Comparable growth patterns have been shown to be present in subjects born preterm (5) and the use of GH therapy in subgroups of preterm born subjects is now under debate (6). Although preterms may follow growth trajectories comparable with that of term SGA subjects, it is not known whether the long-term metabolic consequences of term SGA and premature birth are similar.

There have been concerns about the long-term effects of GH therapy on insulin sensitivity. During therapy, GH therapy causes insulin resistance (7, 8) that does not lead to the development of type 2 diabetes during treatment (9).

After cessation of GH therapy insulin sensitivity has been reported to be normal. Van Dijk *et al.* (10) measured insulin sensitivity in the fasting state and found no differences in SGA subjects who had been

treated with GH and controls. The hyperinsulinemic-euglycemic clamp design (11) has never been used for evaluation of SGA subjects after treatment with GH. In one study (12), using the hyperglycemic clamp that is not designed for the measurement of insulin sensitivity (13), a decrease in insulin sensitivity was observed in subjects treated with GH compared with controls.

In the present study, we investigated insulin sensitivity as well as the metabolic syndrome in GH-treated males born SGA (GH). They were compared with male controls born at term with normal birth weight (CON). We also compared the GH and CON males with males born prematurely appropriate for gestational age (premAGA) and males born premSGA.

Methods

Subjects

The study consisted of four groups of young adults.

Previously GH-treated males (GH) The study group consisted of 10 male subjects who previously

participated in a Dutch multicenter, double blind, randomized, dose–response GH trial that originally involved 79 children. The dose–response GH trial started in 1991 and investigated the effect of two doses GH, 1 and 2 mg/m² day respectively. Inclusion criteria for the study were previously described (14). Briefly, children included were prepubertal, with a birth length and height SDS below –1.88, without any or with incomplete catch-up growth in height, and without growth failure caused by other diseases. Biosynthetic GH was administered s.c. once daily and GH treatment was stopped after reaching adult height.

At the time of the present study, inclusion criteria were male subjects who participated in the above-described trial and had been treated with GH in our center. Five out of the fifteen to be recruited participants who were originally treated according to the GH project could not be included due to loss of follow-up. The ten male subjects who could be reached all agreed to take part in the present study. The GH treatment in these subjects was initiated at a mean age ($\pm$ s.d.) of 8.7 ($\pm$ 2.1) years. The dose was 6 U/m² (2 mg/m²). The mean duration of the therapy was 8.1 ($\pm$ 1.4) years. During GH treatment, the mean height SDS ($\pm$ s.d.) increased from –3.3 (0.8) to –1.6 (0.7) ($P<0.0001$). The mean body mass index (BMI) SDS ($\pm$ s.d.) tended to increase from –0.95 (1.4) to –0.17 (1.0) ($P=0.075$). GH treatment was stopped for at least 3 years or more at the start of the present study. Two subjects were born prematurely (gestational age 29 weeks and 30 2/7 weeks). Two subjects were twin brothers. One subject was diagnosed to have Silver–Russell syndrome on clinical grounds.

Premature males born premAGA This group comprised 15 young adult male subjects born prematurely with normal birth weight for gestational age (GA) who had previously participated in a study to investigate the

metabolic profile of young adults born prematurely. Subjects were recruited from the Project on Preterm and Small for gestational age infants (POPS) cohort (15). The POPS cohort comprises of 94% of all Dutch neonates ($n=1338$) who were born alive in 1983 with a GA of below 32 weeks and/or a birth weight below <1500 g. Perinatal (birth weight, birth length, GA, Apgar score, and congenital anomalies) and obstetric parameters were known since birth.

Premature males born premSGA

Subjects were also recruited from the POPS cohort (15). This group comprised 15 young adult male subjects born prematurely with low birth weight for GA.

Healthy controls (CON) This group comprises 15 males born at term with normal birth weight who had participated as controls in a study on the metabolic profile of young adults after premature birth.

Patient characteristics Patient characteristics at birth and at the time of the study are shown in Table 1. Auxology and metabolic characteristics of the subject with Silver–Russell syndrome and of the two preterm born subjects of the GH group were not statistically different from the rest of the GH group.

In GH subjects, height of the mother ($P=0.004$) and target height ($P=0.008$) were significantly lower than CON subjects. Also in GH subjects, height of the mother ($P<0.0001$) and target height ($P=0.016$) were significantly lower than premAGA subjects. There were no differences in height of the father between any of the groups.

GH subjects were slightly older than CON subjects ($P=0.002$), premAGA subjects ($P=0.02$), and premSGA subjects (0.05). GH subjects were shorter than CON subjects ($P<0.0001$) and premAGA subjects

Table 1 Patient characteristics at birth and at the time of the study of the four groups: growth hormone-treated subjects (GH), premature appropriate for gestational age (AGA) subjects (premAGA), premature small for gestational age subjects (premSGA), and healthy untreated control subjects (CON).

N	CON 15	premAGA 15	premSGA 13	GH 10	P
Characteristics at birth					
GA (weeks)	Term	28.9 (1.7)	30.8 (0.9)	36.9 (4.0)	<0.0001
BW (g)	3726 (444)	1566 (364)	990 (108)	1994 (635)	<0.0001
Height mother (cm)	167.8 (5.2)	169.7 (5.3)	165.8 (5.9)	159.2 (7.0)	<0.0001
Height father (cm)	183.1 (6.0)	180.2 (7.9)	180.1 (7.0)	176.6 (7.7)	0.19
Target height (cm)	186.5 (4.6)	186.0 (5.6)	183.9 (5.5)	178.9 (6.3)	0.007
Characteristics at the time of the study					
Age (years)	21.9 (0.3)	22.1 (0.4)	22.2 (0.3)	23.5 (2.5)	0.003
Height (cm)	185.9 (4.8)	183.1 (8.9)	178.9 (6.7)	172.8 (5.3)	<0.0001
Weight (kg)	76.2 (8.2)	77.1 (16.8)	70.3 (8.5)	66.6 (7.3)	0.08
Fat-free mass %	77.8 (4.0)	78.2 (7.1)	78.8 (5.0)	80.7 (5.0)	0.59

Results expressed as mean $\pm$ s.d. GA, gestational age; BW, birth weight; fat-free mass%, percentage of fat-free mass measured with bio impedance analysis (BIA).

($P=0.003$). Weight and fat-free mass percentage were not significantly different between the groups.

Study protocol

Subjects were assessed after a 9-h overnight fast. Measurement of the subject's weight and height were performed using an electronic scale and stadiometer (SECA, Hanover, MD, USA). A tape measure was used to measure the circumference of the non-dominant arm, as well as hip and waist circumferences.

Biometrical impedance analysis was used to calculate lean body mass (Akern BIA 101/S RJL systems, Detroit, MI, USA). Systolic and diastolic blood pressure (BP) were measured after a 15-min rest in the recumbent position and measured by a Dynamap Critikon (Southern Medical Corp. Baton Rouge, LA, USA) using an appropriately sized cuff.

The various components of the metabolic syndrome were assessed in all groups. According to criteria formulated by the ATP III (16), the metabolic syndrome is diagnosed if three or more of the following symptoms are present: central obesity (waist circumference ≥ 102 cm for males), raised triglycerides (TG) levels (TG ≥ 1.7 mmol/l), reduced high-density lipoproteins (HDL) levels (HDL < 1 mmol/l for males), high BP (systolic BP ≥ 130 and/or diastolic BP ≥ 85 mmHg), and increased fasting glucose levels (glucose ≥ 6.1 mmol/l).

Fasting blood samples were taken for the determination of HDL (HDL-C plus, Roche) and TG (glycerol phosphate oxidase-p-aminophenazone method (GPO)-PAP, Roche). Blood glucose was measured immediately by the glucose oxidase method using a Yellow Springs Instrument Co. Glucose analyzer (YSI Inc., Yellow Springs, OH, USA). Insulin levels (immunometric assay, Bayer Diagnostics) were measured after fasting and during the subsequent clamp. After centrifugation, all samples were frozen (-70°C) until assayed.

Hyperinsulinemic-glucose clamp

The hyperinsulinemic-euglycemic clamp was performed to determine insulin sensitivity by peripheral glucose uptake as described by De Fronzo *et al.* (11). Insulin (Velosulin, Novo Nordisk, Bagsvaerd, Denmark) was infused at a rate of 60 mU/kg per h after a priming dose of 6 mU/kg. Hepatic glucose production is known to be suppressed in non-diabetic subjects by this infusion rate. The blood glucose level was measured every 5 min (2300 STATplus C, Yellow Springs Incorporated). Blood glucose levels were clamped to a level of 5 mmol/l. During the last hour, every 15 min, blood was drawn to determine plasma insulin concentrations. Euglycemia (5 mmol/l) was maintained with 20% D-glucose infusion. Under steady-state conditions of euglycemia, the rate of exogenous glucose infusion is equal to the rate of insulin-stimulated glucose disposal. Insulin sensitivity was calculated from the glucose infusion rate

(milligrams per minute) between 60 and 120 min of the euglycemic clamp, divided by body weight and expressed as M -value (mg/kg per min).

To correct for blood glucose levels during the steady state, the Mc -value was obtained as follows: (M -value $- 1$) $\times$ (100/mean plasma glucose (mg/dl) $+ 1$). To correct for fat-free mass, the M_{ffm} -value was obtained as follows: M -value $\times$ percentage fat-free mass.

To correct for both blood glucose levels in fat-free mass, the Mc_{ffm} -value was obtained as follows: Mc -value $\times$ percentage fat-free mass.

Statistical analysis

Analyses were carried out using the computer statistical package SPSS (SPSS Inc., Chicago, IL, USA) for Windows version 11.5. Results in Tables 1–3 are expressed as mean $\pm$ s.d. Differences between the groups were evaluated using ANOVA and regression analysis. Statistical significance was defined as $P \leq 0.05$.

Ethical considerations

The study was approved by the local ethical committee. Informed consent was obtained.

Results

Insulin sensitivity

Results on insulin sensitivity are displayed in Fig. 1. Differences between the groups are shown in Table 2.

Insulin sensitivity expressed as M -value (mg/kg per min), M -value corrected for steady-state blood glucose levels (Mc), M -value corrected for fat-free mass (M_{ffm}), and Mc -value corrected for fat-free mass (Mc_{ffm}) were all significantly lower in premAGA, premSGA, and GH subjects than in CON males (Table 2).

There were no differences in M , Mc , M_{ffm} , and Mc_{ffm} -value between premAGA and premSGA subjects in comparison with GH subjects (Table 2).

Metabolic syndrome components

The components of the metabolic syndrome (increased waist circumference, raised TG levels, decreased HDL levels, high BP, and raised fasting glucose) are shown in Table 3. Mean values were within the normal range. No significant differences in waist circumference, triglyceride levels, HDL levels, and fasting glucose levels were observed between any of the groups. Systolic BP was higher in premAGA subjects than in GH ($P < 0.0001$) and CON ($P < 0.0001$) subjects. In premSGA subjects, systolic BP was higher than in GH subjects (0.03).

Diastolic BP was higher in premAGA subjects than in CON subjects.

Table 2 Regression analysis of differences in insulin sensitivity. (a) Controls in comparison with premature appropriate for gestational age (premAGA), prem small for gestational age (premSGA), and growth hormone (GH) subjects. (b) GH subjects in comparison with premAGA and premSGA subjects.

	PremAGA versus CON		PremSGA versus CON		GH versus CON	
	B (95% CI)	P	B (95% CI)	P	B (95% CI)	P
M-value	-3.7 (-6.2 to -1.2)	0.004	-3.6 (-6.3 to -1.0)	0.007	-4.4 (-7.2 to -1.6)	0.003
Mc	-4.1 (-7.0 to -1.2)	0.007	-3.5 (-6.5 to -0.5)	0.022	-4.6 (-7.8 to -1.3)	0.007
Mffm	-2.8 (-5.0 to -0.6)	0.015	-2.7 (-5.0 to -0.3)	0.025	-3.1 (-5.6 to -0.6)	0.016
Mcffm	-3.1 (-5.6 to -0.5)	0.019	-2.6 (-5.2 to 0.1)	0.05	-3.3 (-6.1 to -0.5)	0.024

	PremAGA versus GH		PremSGA versus GH	
	B (95% CI)	P	B (95% CI)	P
M-value	0.6 (-2.1 to 3.4)	0.66	0.7 (-2.2 to 3.4)	0.62
Mc	0.5 (-2.8 to 3.7)	0.76	1.0 (-2.3 to 4.4)	0.54
Mffm	0.3 (-2.2 to 2.8)	0.80	0.5 (-2.1 to 3.0)	0.73
Mcffm	0.2 (-2.6 to 3.1)	0.87	0.7 (-2.2 to 3.7)	0.62

M-value=glucose disposal mg/kg pre min. Mc=(M-value-1)×(100/mean glucose during clamp)+1. Mffm=M-value×percentage fat-free mass. Mcffm=Mc-value×percentage fat-free mass.

Table 3 Components of the metabolic syndrome (ATP-III) of the growth hormone (GH)-treated subjects GH and healthy untreated control subjects (CON).

N	CON 15	premAGA 15	premSGA 13	GH 10	P
Waist circumference (cm)	77.3 (5.6)	79.3 (9.6)	77.7 (6.7)	76.5 (3.6)	0.78
Triglycerides (mmol/l)	0.88 (0.4)	1.0 (0.5)	1.14 (0.5)	1.27 (0.84)	0.29
HDL (mmol/l)	1.41 (0.26)	1.35 (0.20)	1.39 (0.30)	1.30 (0.18)	0.56
Systolic blood pressure (mmHg)	120 (10)	139 (15)	130 (11)	116 (6)	<0.001
Diastolic blood pressure (mmHg)	64 (5)	73 (8)	68 (8)	65 (8)	0.015
Fasting glucose (mmol/l)	4.3 (0.6)	4.6 (0.4)	4.5 (0.4)	4.5 (0.3)	0.21

Results GA and BW expressed as mean±s.d. HDL, high-density lipoproteins.

M-value= glucose disposal mg/kg per min
 Mc= (M-value - 1) × (100 / mean glucose level during steady state) + 1
 Mffm = M-value × percentage fat-free mass
 Mcffm= Mc-value × percentage fat-free mass

Figure 1 Insulin sensitivity expressed as M, Mc, Mffm, and Mcffm-value for CON, premAGA, premSGA, and GH subjects.

Discussion

In the present study, the hyperinsulinemic-euglycemic clamp design was used to study insulin sensitivity in subjects born SGA who were treated with GH during childhood as well as subjects born preterm who were never treated with GH. Our study shows that young adult males born SGA who had been treated with GH have a decreased insulin sensitivity compared with controls. The reduction of insulin sensitivity in the GH group is larger than in some other studies conducted in young adults with a low birth weight (1, 17) and comparable with the insulin sensitivity that we found in the preterm born males.

The original study in which the GH subjects participated did not include control subjects born SGA not treated with GH, which is a limitation of this study. Therefore, our study cannot distinguish between prenatal, postnatal, and GH therapy-related factors that caused the insulin resistance of GH subjects compared with controls. Within the SGA population, the GH subjects represent a subgroup without spontaneous catch-up growth and it is possible that short SGA subjects may have different insulin sensitivity than tall SGA subjects due to differences in catch-up growth. It is conceivable that the GH subjects were already partly insulin resistant before the start of the GH treatment, although there have been reports that in prepubertal children born SGA the highest insulin sensitivity is found in children without catch-up growth while those with spontaneous catch-up growth are insulin resistant (18). It has not been studied how insulin sensitivity changes with age in term born SGA subjects. Also the effect of duration of GH therapy on insulin sensitivity is not known.

The effect of spontaneous catch-up growth can be studied in the premAGA and premSGA subjects. PremAGA subjects were taller than the GH subjects but had a comparable decrease in insulin sensitivity. This may indicate that spontaneous catch-up growth is unfavorable for metabolic health. Differences in body composition, especially the balance between fat mass and lean mass are often proposed as explanations for differences in insulin sensitivity (19). In our subjects, the fat-free mass percentage was comparable between the groups, suggesting that body composition is not the only factor that is important in the relation between growth and insulin sensitivity.

Except for BP, we did not find differences in the components of the metabolic syndrome between the groups. It is already known that the preterm born have increased BP (3).

To answer the questions on the relation between growth, GH therapy and insulin sensitivity, it is necessary to perform long-term studies that measure insulin sensitivity and body composition before, during and after GH therapy. This is also important now that

subgroups of the preterm born that remain short are considered for GH therapy.

In conclusion, GH-treated subjects born SGA and untreated subjects born preterm have a low insulin sensitivity compared with controls. Whether the difference in the GH-treated subject results from perinatal-, postnatal-, or GH therapy-related factors is not known. In children born preterm, careful follow-up is important when they are treated with GH.

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