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Carotid intima-media thickness in children treated with growth hormone

Abstract

Background: There is an ongoing discussion whether high doses of growth hormone (GH) may lead to cardiovascular diseases. Therefore, we studied the relationships between GH treatment and carotid intima-media thickness (cIMT), which is predictive of the development of atherosclerosis.
Methods: We measured cIMT in 38 children with supraphysiological doses of GH (mean age 10.9±2.2 years; 47% male; GH indication: small for gestational age, n=31; Turner syndrome, n=5; *SHOX* deficiency, n=2) and in 38 age- and gender-matched healthy children without GH treatment. Furthermore, we examined cIMT in 61 children with physiological doses of GH (mean age 12.0±3.1 years; 64% male; GH indication: GH deficiency) and in 61 age- and gender-matched healthy children without GH treatment. Moreover, we analyzed blood pressure, lipids, HbA_{1c}, IGF-1, and IGFBP-3 in children treated with GH.
Results: The cIMT levels did not differ significantly between children with and without GH treatment either in high-dose GH treatment or in physiological GH doses. In backwards linear regression analyses, cIMT was significantly related to HbA_{1c}, but not to age, gender, BMI, pubertal stage, indication of GH treatment, duration or doses of GH treatment, IGF-1, IGFBP-3, or to any cardiovascular risk factor.
Conclusions: We found no evidence that GH treatment is associated with changes in cIMT.

Keywords: growth hormone; intima media thickness; SHOX deficiency; small for gestational age; Turner syndrome.

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Introduction

The cardiovascular risk for patients receiving treatment with growth hormone (GH) has, to date, hardly been investigated. From studies with adults, it is known that GH deficiency may lead to dyslipidemia with consequential vascular changes (1). Relative GH deficiency has been reported as a significant contributor to increased markers of cardiovascular risk in obese adolescents (2). GH concentrations predicted various markers of cardiovascular risk (3). Substitution of deficient endogenous GH has been demonstrated to produce positive effects on the cardiovascular alterations associated with GH deficiency (4–6).

In contrast, it is also conceivable that administration of supraphysiological doses for indications of small for gestational age (SGA), stature homeobox-containing gene (*SHOX*) deficiency or Turner's syndrome could have negative effects on the cardiovascular system (7). It is well known that overproduction of GH in acromegaly is associated with increased mortality due to cardiovascular diseases besides other medical conditions (8). GH is considered to be a mediator of inflammatory mechanisms underlying atherogenesis through direct effects (7). Initial reports from France of the long-term mortality and morbidity after GH treatment in childhood (SAGhE study) showed an increased mortality due to cardiovascular diseases in children especially when high doses of GH were used (9). However, the same study reported no increased mortality in children treated with GH in Belgium, the Netherlands, and Sweden (10). Therefore, studies on early cardiovascular changes in children treated with GH are necessary but scarce.

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Very early vascular modifications can be identified by the method of intima media-thickness measurement (IMT), which is predictive of myocardial infarction and stroke (11, 12). Previous investigations by our research group have shown that increased carotid IMT (cIMT) can be measured in obese children with metabolic syndrome, a disease also associated with early cardiovascular changes

(13–15). Furthermore, increased cIMT has been measured in adults with acromegaly (16, 17). In contrast, untreated relative growth hormone deficiency has been reported to be associated with increased cIMT in adults (5, 6, 18). Studies on GH treatment in GH-deficient adults showed a decrease in cIMT (4, 5). However, no studies analyzing cIMT in children with GH deficiency or high-dose GH treatment have been published to date.

Therefore, we performed the following study to compare the cIMT between children receiving GH and healthy age- and gender-matched children without GH treatment. We hypothesized that children treated with supraphysiological GH doses have an increased cIMT compared to healthy children, while children with GH deficiency treated with physiological GH doses do not differ in their cIMT compared to healthy children. Furthermore, we analyzed whether doses of GH treatment, indication of GH treatment, duration of GH treatment, or other cardiovascular risk factors such as hypertension, dyslipidemia, or overweight had an impact on cIMT in children treated with GH.

Methods

The local Ethics Committee of the Vestische Children's Hospital, University of Witten/Herdecke approved this study. Written informed consent was obtained from all subjects and their parents.

We measured cIMT in 38 children treated with supraphysiological doses of GH (GH indication: SGA, n=31; Turner syndrome, n=5; *SHOX* deficiency, n=2) and in 38 age- and gender-matched healthy children without GH treatment. Furthermore, we analyzed cIMT in 61 children with GH deficiency treated with physiological doses of GH and in 61 age- and gender-matched healthy children without GH treatment. Additionally, we collected the clinical data and cardiovascular risk factors such as hypertension, dyslipidemia, and disturbed glucose metabolism [as measured by glycosylated hemoglobin (HbA_{1c})] in the children treated with GH.

Height was measured to the nearest millimeter using a rigid stadiometer. Weight was measured in underwear to the nearest 0.1 kg using a calibrated balance scale. Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters. The weight status was quantified using Cole's least mean square method, which normalizes the BMI skewed distribution in childhood and expressed BMI as a standard deviation score (BMI-SDS) (19) using German population-based reference data (20).

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Pubertal stage was determined by well-trained physicians in children treated with GH. Pubertal developmental stage was categorized into two groups based on breast and genital stages according to Marshall and Tanner (21, 22) (prepubertal: boys with genital stage I, girls with breast stage I; pubertal: boys with genital stage >I; girls with breast stage >I).

Blood pressure was measured using a validated protocol (23). Blood pressure was measured at the right arm after a 10-min rest in the supine position with an oscillometric device (Omron M6). Two repeated recordings were made with 5 min in between, and the lower value of the two recordings of systolic blood pressure and diastolic blood pressure measurements was recorded. The cuff size was based on the length and circumference of the upper arm and was as large as possible without having the elbow skin crease obstructing the stethoscope (23).

IGF-1, IGFBP-3, HbA_{1c}, HDL-cholesterol, LDL-cholesterol, and total cholesterol concentrations were measured in the non-fasting state using commercially available test kits (HDL-C Plus and LDL-C Plus, Roche Diagnostics, Mannheim, Germany; Vitros analyzer, Ortho Clinical Diagnostics, Neckargemuend, Germany; MEIA, Abbott, Wiesbaden, Germany; Tina-quant Hemoglobin A1c Gen. Cobas Integra 400/800, Roche, Mannheim, Germany; Immulite 2000 IGF-1 and Immulite 2000 IGFBP3, Siemens Healthcare Diagnostics). SD scores (SDS) were calculated as follows: patient's parameter minus the mean of the reference population for patient's age and gender divided by the SD of the reference population.

One investigator, who was blinded to the participants' cardiovascular risk factor status and GH treatment, measured the cIMT by B-mode ultrasound using a 14-MHz linear transducer following a standardized protocol. Four measurements were performed at the common carotid artery near the bifurcation at the far wall after a 10-min rest. We took the maximum value and mean value for statistical purposes. The strongest association between the different measurements of cIMT and coronary risk factors has been to shown to be the maximum and not the mean value of cIMT (12, 15). The patients were examined in the supine position with the head turned slightly to the side. The intra-observer coefficients of variability of cIMT measurements were 6% for a cIMT of 0.4 mm and 4% for a cIMT of 0.7 mm (15, 24).

Statistical analyses

Statistical analyses were performed using the Winstat software package (R. Fitch Software, Bad Krozingen, Germany). Normal distribution was tested by the Kolmogorov-Smirnov test. Except mean cIMT and maximal cIMT, all variables were normally distributed. To compare the variables between children with and without GH treatment, the χ^2 test, Student's t-test for unpaired observations, and Mann-Whitney U-test were used as appropriate. Additionally, cIMT was compared between children with and without GH treatment adjusted to BMI-SDS. Furthermore, the cIMT measurements in the children with different indications of GH treatment were compared by analysis of variance (ANOVA). The cIMT measurements in children treated with GH were correlated to age, gender, BMI-SDS, dose of GH, duration of treatment with GH, IGF-1, IGFBP-3, HDL-cholesterol, LDL-cholesterol, total cholesterol, triglycerides, HbA_{1c}, and blood pressure by Spearman rank correlation. For this purpose, the characteristics of children with physiological and supraphysiological GH doses were summarized. Furthermore, in this summarized collective, multiple linear regression analyses were conducted for the dependent variable log cIMT measurement (max or mean), including weight status (BMI), age, gender, pubertal stage, IGF-1, blood pressure, lipids, HbA_{1c}, dose of GH, duration of treatment with GH, and indication for GH as independent variables. In these analyses, gender, pubertal stage, and indication of GH treatment were used as categorical variables. A p-value <0.05 was considered as significant. Data were presented as mean and standard deviation or as median and interquartile range (IQR) if the variables were not normally distributed.

Power analyses

Assuming an increase in cIMT of 0.05 mm in children treated with GH compared to healthy untreated children, which is 50% of that reported for the impact of metabolic syndrome on cIMT in children (25), this study has a power of 0.93 to detect differences in cIMT in a two-sided comparison with an α error of 0.05 and a standard deviation of 0.07 mm in cIMT based on a study sample of 38 children treated with supraphysiological GH doses and 38 age- and gender-matched healthy children without GH treatment. The power was 0.99 to detect differences in cIMT in a two-sided comparison with an α error of 0.05 and a standard deviation of 0.07 mm based on a study sample of 61 children treated with physiological GH doses and 61 age- and gender-matched healthy children without GH treatment.

Results

The patients' characteristics are shown in Table 1. The mean duration of GH treatment was 4.4±2.0 years in the 38 children treated with supraphysiological GH doses and 4.4±2.4 years in the 61 children treated with physiological GH doses. The mean GH dose per day was 1.1±0.1 mg/m^2 in the 38 children treated with supraphysiological GH doses and 0.8±0.1 mg/m^2 in the 61 children treated with physiological GH doses.

The mean and maximum cIMT levels did not differ significantly between children with and without GH treatment either in the children treated with supraphysiological GH doses or in the children with physiological doses of GH (see Figure 1). The mean and maximum cIMT levels did not differ significantly between healthy controls and children treated either with supraphysiological or with physiological doses of GH even after adjustment for BMI-SDS (supraphysiological GH doses: mean cIMT, p=0.246; max cIMT, p=0.184; physiological GH doses: mean cIMT, p=0.789; max cIMT, p=0.689).

The cIMT values grouped according to indications of GH treatment are shown in Figure 2. There were no significant differences either in the maximal cIMT (p=0.883) or in the mean cIMT (p=0.625) between the different GH indication groups (p-values derived from ANOVA). Comparing the children with physiological GH doses (indication: GH deficiency) and the children with supraphysiological GH doses (summarizing the indications SGA, Turner syndrome, and *SHOX* deficiency) demonstrated no significant differences in mean cIMT [median 0.40 (IQR 0.40–0.50) mm vs. median 0.40 (IQR 0.40–0.50) mm; p=0.514] or maximal cIMT [median 0.50 (IQR 0.40–0.50) mm vs. median 0.45 (IQR 0.40–0.50) mm; p=0.959].

The mean IGF-1 SDS was 1.3±1.3 in the 38 children treated with supraphysiological GH and 0.6±1.2 mg/m^2 in the 61 children treated with physiological GH doses. The mean IGFBP-3 SDS was 0.3±0.7 in the children treated with supraphysiological GH doses and 0.3±1.0 mg/m^2 in the 61 children treated with physiological GH doses. IGF-1 levels above the normal range were measured in 42% of 38 children treated with supraphysiological GH doses and in 12% of the 61 children treated with physiological GH doses. IGFBP-3 levels above the normal range were observed in 5% of 38 children treated with supraphysiological GH doses and in 12% of the 61 children treated with physiological GH doses.

Table 1 Clinical and anthropometric characteristics of the study population.

	Supraphysiological GH doses	Healthy controls without GH treatment	p-Value	Physiological GH doses	Healthy controls without GH treatment	p-Value
Number	38	38	–	61	61	–
Age, years	10.9±2.2	10.9±2.3	0.890	12.0±3.1	11.8±3.7	0.745
Gender	47% male	47% male	0.999[a]	64% male	64% male	0.999[a]
Height, cm	137.5±11.5	145.4±15.1	0.015	142.9±17.6	154.0±16.9	<0.001
Weight, kg	32.9±8.1	40.2±12.5	0.005	38.7±14.6	46.1±14.4	0.007
BMI, kg/m^2	17.2±2.3	18.1±2.6	0.110	18.2±3.3	18.9±2.8	0.202
BMI-SDS	−0.33±1.06	0.15±1.10	0.056	−0.20±1.03	0.11±1.20	0.167
Pubertal stage	47% prepubertal, 52% pubertal	Not determined	–	48% prepubertal, 52% pubertal	Not determined	–
Systolic blood pressure, mm Hg	109±12	Not determined	–	114±12	Not determined	–
Diastolic blood pressure, mm Hg	64±9	Not determined	–	63±8	Not determined	–
HbA_{1c} (%)	5.3±0.2	Not determined	–	5.4±0.3	Not determined	–
Total cholesterol, mg/dL	159±21	Not determined	–	161±26	Not determined	–
LDL cholesterol, mg/dL	80±20	Not determined	–	82±24	Not determined	–
HDL cholesterol, mg/dL	59±10	Not determined	–	59±12	Not determined	–

Data are shown as percentage or as mean±standard deviation; p-Value derived from the Student's t-test for unpaired observations, unless otherwise indicated. [a]p-Value derived from the χ^2-test.

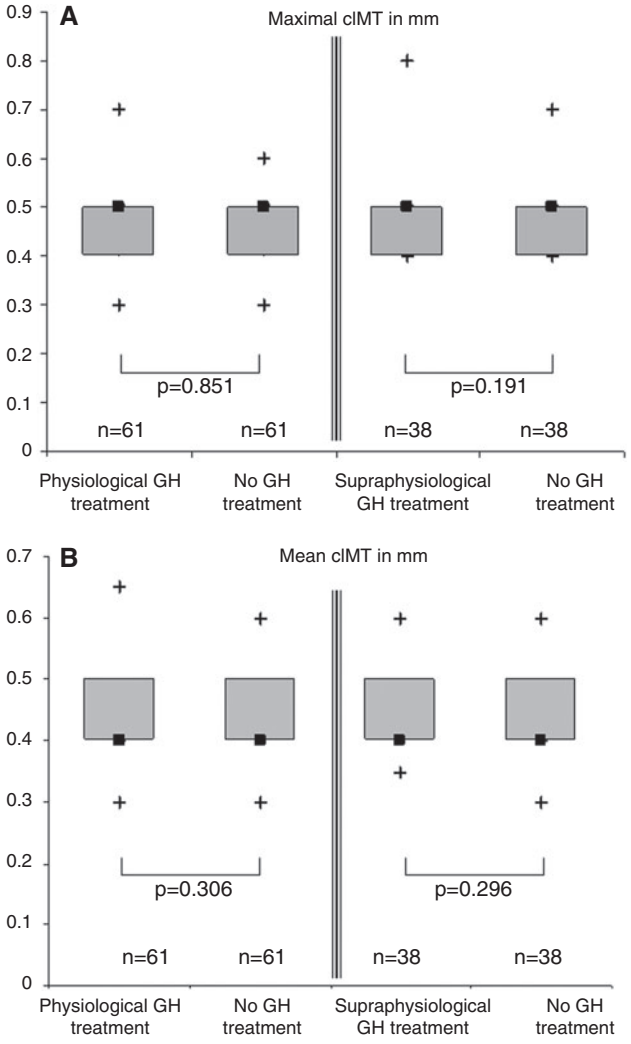

Figure 1 Box plots of carotid IMT levels in 38 children treated with supraphysiological GH doses and in 38 age- and gender-matched healthy children without GH, as well as box plots of cIMT in 61 children treated with physiological GH doses and in 61 age- and gender-matched healthy children without GH (A: maximal cIMT; B: mean cIMT; GH: growth hormone; unadjusted p-values derived by the Mann-Whitney U-test; p-values adjusted for BMI-SDS: supraphysiological GH doses: mean cIMT, p=0.246; max cIMT, p=0.184; physiological GH doses: mean cIMT, p=0.789; max cIMT, p=0.689).

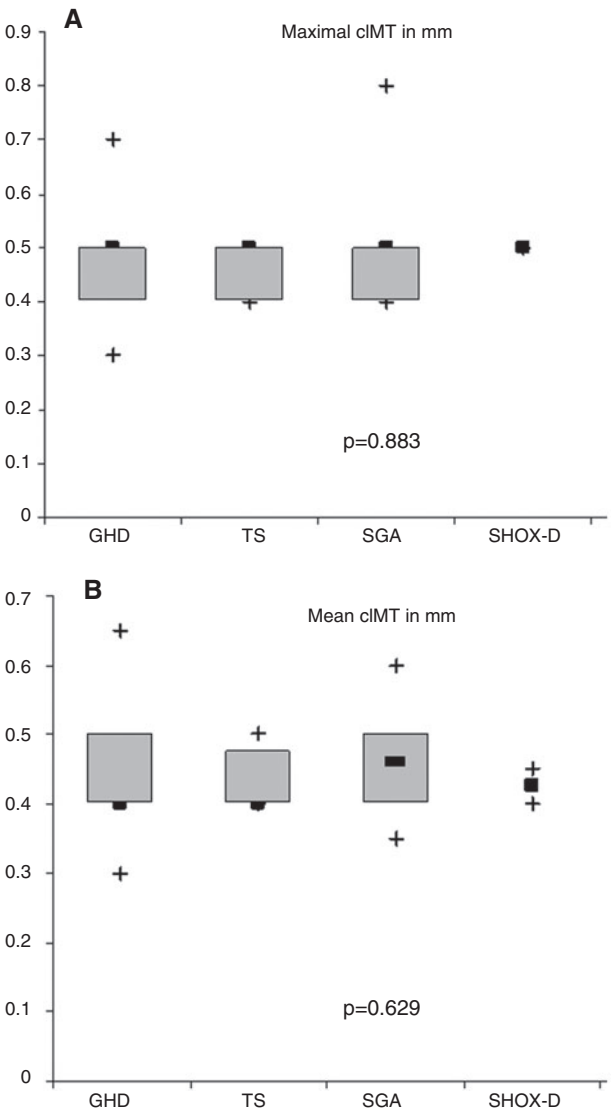

Figure 2 Box plots of carotid IMT measurements (A: maximal cIMT; B: mean cIMT) in 99 children treated with GH grouped according to indications of GH treatment (GHD: growth hormone deficiency; TS: Turner syndrome; SGA: small for gestational age; SHOX-D: *SHOX* deficiency).

The correlations between maximal cIMT or mean cIMT and age, pubertal stage, IGF-1, and cardiovascular risk factors in the children treated with GH are shown in Table 2. HbA_{1c} and BMI-SDS were significantly related to mean cIMT (but not to maximal cIMT), while all other parameters showed no significant relationships to mean or maximal cIMT.

In the backwards linear regression analyses, mean cIMT was significantly related to HbA_{1c} (β-coefficient 0.06, 95% confidence interval ±0.05, p=0.020, r^2=0.05), but not to age, gender, BMI, pubertal stage, duration or doses of GH treatment, indication of GH treatment, IGF-1, IGFBP-3, or to any cardiovascular risk factors in the children treated with GH. Furthermore, maximal cIMT was not related to age, gender, BMI, pubertal stage, duration or doses of GH treatment, indication of GH treatment, IGF-1 levels, or to any cardiovascular risk factor including HbA_{1c}.

Discussion

This is the first study analyzing the impact of GH treatment on cIMT in children. The cIMT measurements did

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Table 2 Spearman rank correlation between maximal or mean carotid IMT (cIMT) and age, pubertal stage, IGF-1, IGFBP-3, and cardiovascular risk factors in 99 children treated with GH.

	Maximal cIMT	Mean cIMT
Age	−0.04	−0.11
BMI	0.05	0.08
BMI-SDS	0.13	*0.19*[a]
Pubertal stage	−0.06	−0.09
Treatment duration of GH	−0.09	−0.04
GH doses	−0.11	−0.06
IGF-1	0.04	0.09
IGF-1 SDS	−0.08	−0.07
IGFBP-3	0.01	0.06
IGFBP-3 SDS	−0.09	0.03
HbA_{1c}	0.09	*0.19*[a]
Total cholesterol	0.06	0.13
LDL cholesterol	0.03	0.09
HDL cholesterol	−0.03	−0.04
Systolic blood pressure	0.01	0.01
Diastolic blood pressure	−0.08	−0.05

[a]$p<0.05$.

not differ between the children with and without GH treatment either in the children with supraphysiological or in the group of children with physiological doses of GH. Accordingly, the doses of GH and the duration of treatment did not correlate to cIMT measurements, suggesting that GH treatment in children is not associated with cardiovascular changes measurable with cIMT. These findings are in line with reports that adult mortality and morbidity are not increased in childhood-onset GH-deficient patients who received pediatric GH treatment (26) and that GH therapy is not associated with increased mortality when IGF-1 levels were targeted within normal age-related reference ranges (27).

Furthermore, our study demonstrated no relationship between cIMT and IGF-1 or IGFBP-3 under GH treatment. These findings are of interest since endothelial cells have high-affinity binding sites for IGF-1 (1, 28). Moreover, IGF-1 and its IGFBPs participate in inflammation-linked angiogenesis (1, 29). Decreased IGF-1 levels due to GH deficiency and increased IGF-1 levels in acromegaly have been reported to be associated with increased risk of ischemic heart disease and stroke (1, 30, 31). Since IGF-1 levels were in the normal range in the majority of our children, this may explain the lack of association between IGF-1 and cIMT.

In concordance with our previous studies (13–15) and further reports (11, 32), BMI-SDS and HbA_{1c} were related to mean cIMT in univariate analyses. Of interest, the mean and not the maximal cIMT was related to HbA_{1c} and BMI-SDS, while in adults the maximal cIMT was reported to have a stronger association between cIMT and coronary risk factors as compared to mean values of cIMT (12, 15). Future longitudinal studies have to analyze whether there is a difference between children and adults concerning the predictive value of mean and maximal cIMT on cardiovascular risk factors.

However, in multiple linear regression analyses adjusted for multiple confounders, we did not find significant relationships between cIMT measurements and BMI-SDS or cardiovascular risk factors except for HbA_{1c} levels. Interestingly, we have reported previously that disturbed glucose metabolism appears to be the strongest contributory factor for cardiovascular risk parameters leading to increased cIMT in obese children (25). The lack of significant association between cardiovascular risk factors or BMI-SDS and cIMT in our study can be explained, at least in part, by the fact that only a minority of the patients analyzed in this study were overweight and that most patients demonstrated normal blood pressure and lipids values.

Study limitations

First, we analyzed only Caucasian children. Therefore our findings may not be applicable to other populations. Second, we did not measure 24-h blood pressure, which is clinically more relevant than single blood pressure measurements. Third, lipids were not measured in the fasting state, so any conclusion concerning lipids has to be treated very cautiously. Fourth, this is a cross-sectional study, which does not allow drawing final conclusions. For this purpose, longitudinal studies are necessary. Fifth, cIMT is only a surrogate measurement and clinical end points would be preferable. However, longitudinal studies in children over decades are very difficult to perform. Sixth, most reports presume that IMT is related to an initial atherosclerotic process (11, 12), an increased IMT has also been discussed to reflect a non-atherosclerotic adaptive response to changes in shear stress and tensile stress (33). Furthermore, the ultrasound measurement of IMT does not allow to differentiate between intima thickening due to an atherosclerotic process or to medial hypertrophy (smooth muscle growth) caused by the hemodynamic stimulation of a progressive increase in systolic blood pressure, pulse pressure, or arterial diameter over time (33). Seventh, changes in cIMT may not reflect all elements of cardiovascular morbidity, but this limitation holds true for all surrogate outcomes. For example, it is

well known that acromegaly, a disease with increased GH levels, is associated with cardiac muscle hypertrophy, which was not measured in this study (8). Eight, the number of children with Turner syndrome or *SHOX* deficiency was small. Therefore, the impact of high GH doses in special disorders such as Turner syndrome or *SHOX* deficiency and cIMT cannot be ruled out by our study. Finally, the mean duration of GH treatment was 4.4 years. Therefore, we cannot exclude cIMT changes after long-term GH treatment. Furthermore, changes in cIMT may occur later in life in children treated with GH.

In summary, we found no evidence that GH treatment is associated with changes in the cardiovascular system measurable by cIMT either in children with supraphysiological or in children with physiological doses of GH. Future longitudinal studies over decades should ideally confirm our findings.

Author contributions: Thomas Reinehr and Rainer Wunsch developed the study design. Thomas Reinehr, Caroline Knop, Barbara Wolters, and Nina Lass performed the anthropometrical measurements. Rainer Wunsch performed the cIMT measurements. Thomas Reinehr wrote the first draft of the paper and performed the statistical analyses. All authors discussed the findings, contributed, and approved the final version of the manuscript.

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Conflict of interest statement: All authors declare that there is no conflict of interest.

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