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Circulating asymmetric dimethylarginine and lipid profile in pre-pubertal children with growth hormone deficiency: Effect of 12-month growth hormone replacement therapy

S. De Marco [a], M.L. Marcovecchio [a,b], D. Caniglia [a], C. De Leonibus [a], F. Chiarelli [a,b], A. Mohn [a,b,*]

[a] Department of Pediatrics, University of Chieti, Chieti, Italy
[b] Center of Excellence on Aging, "G. D'Annunzio" University Foundation, University of Chieti, Italy

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ABSTRACT

Objective: Growth hormone deficiency (GHD) in adults is associated with cardiovascular complications, which lead to reduced life expectancy. At present, data on cardiovascular risk factors in GHD children are limited. The aim of this study was to evaluate whether pre-pubertal GHD children have increased cardiovascular risk factors, and whether 12-month growth hormone (GH) treatment can reverse them.
Design: Twenty pre-pubertal GHD children (6 boys, mean (±SD) age: 9.5 ± 1.8 years) were matched for sex and age with 20 healthy controls (6 boys, mean (±SD) age: 8.8 ± 1.5 years). Asymmetric dimethylarginine (ADMA), lipid profile, glucose metabolism parameters, IGF-1, blood pressure and anthropometric parameters were assessed at baseline and after 12 months of GH treatment.
Results: At baseline, GHD patients showed significantly higher ADMA levels (median [interquartile range]: 78.5 [69.6–123.5] vs 54.0 [38.3–60.8] ng/ml, $p < 0.001$), total cholesterol (mean ± SD: 177.5 ± 30.4 vs 150.1 ± 21.4 mg/dl; $p = 0.004$) and LDL-cholesterol (mean ± SD: 111.2 ± 22.2 vs 84.9 ± 15.9 mg/dl; $p < 0.001$) than controls. After 12-month GH treatment, ADMA (median [interquartile range]: 55.4 [51.2–73.8] ng/ml), total cholesterol (mean ± SD: 155.6 ± 43.2 mg/dl), and LDL-cholesterol (mean ± SD: 95.4 ± 32.1 mg/dl) significantly decreased in GHD children, reaching values comparable to those in controls.
Conclusions: This study showed that, as in adults, pre-pubertal GHD children manifest increased cardiovascular risk markers and that 12-month GH treatment can improve them.

1. Introduction

Adults with growth hormone deficiency (GHD) are known to have reduced life expectancy because of increased cardiovascular and cerebrovascular events [1].

Previous studies have reported that adults as well as adolescents with severe GHD have a cluster of cardiovascular risk factors, including abnormal lipid profile, abdominal obesity, insulin resistance, hypertension, reduced exercise capacity, increased carotid intima-media thickness, and impaired cardiac morphology and function [2–4].

Impaired vascular reactivity, reduced bioavailability of nitric oxide (NO) and impaired oxidant-antioxidant status are all alterations detected in subjects with GHD, where they contribute to endothelial dysfunction, which in turn is one of the first events leading to cardiovascular disease [5–7].

During the last years there has been an increasing interest in a circulating marker of endothelial dysfunction, asymmetric dimethylarginine (ADMA), which is a naturally occurring L-arginine analogue found in plasma and various tissues [8]. ADMA is an endogenous inhibitor of endothelial NO synthase and in this way it contributes to the pathogenesis of endothelial dysfunction [9]. ADMA is considered as an emerging cardiovascular marker and its levels have been found to be increased in patients with hypercholesterolemia, hypertension, coronary artery disease, diabetes mellitus, chronic renal failure and hypopituitarism [10]. Interestingly, adult studies have shown that growth hormone (GH) replacement can decrease ADMA levels [11].

Increased levels of ADMA have also been reported in children and adolescents with pathological conditions, where they have been associated with early markers of atherosclerosis, such as increased carotid artery intima-media thickness [12]. However, up to now there are no studies on ADMA in GHD children. Limited are also pediatric studies [13–15] assessing other cardiovascular risk factors in children with GHD, and the potential beneficial effect of GH treatment on the cardiovascular profile of young people with this condition.

* Corresponding author at: Department of Pediatrics, University of Chieti, Ospedale Policlinico, Via dei Vestini 5, 66100 Chieti, Italy. Tel.: +39 0871 358015; fax: +39 0871 574538.
E-mail address: amohn@unich.it (A. Mohn).

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The aim of the present study was to evaluate ADMA levels along with other cardiovascular risk factors in pre-pubertal children with idiopathic GHD and to test whether 12-month GH replacement therapy could improve their cardiovascular profile.

2. Material and methods

2.1. Study population

We recruited 20 Caucasian pre-pubertal children (6 boys and 14 girls, mean age (±SD): 9.5 ± 1.8 years), who had been referred to the Endocrine Clinic of the Department of Pediatrics, University of Chieti, Italy, between May 2010 and July 2012, for short stature and in whom a diagnosis of GHD was made. These children were a subgroup of patients with a diagnosis of GHD made during that time period and who consecutively agreed to take part in the study.

These patients were matched for sex and age with 20 healthy controls (6 boys and 14 girls, mean age (±SD): 8.8 ± 1.5 years). Control children were recruited from those who had been referred to the pediatric outpatient clinics of our hospital for minor health problems not affecting pubertal development and growth, in particular for the assessment of routine child health and development. Potential causes of growth impairment in these children were excluded through clinical and biochemical assessments.

All study participants were in good health and were not affected by any chronic diseases. Children with multiple pituitary hormone deficiency were excluded in order to avoid any other potential influence on cardiovascular markers and glucose metabolism. Other exclusion criteria were Turner and Prader-Willi syndrome and neurological and psychological disorders. None of the children were taking any medication or vitamin supplementation. None of the patients had ever received GH treatment before entering the study.

Children with GHD and control children underwent a baseline visit. Thereafter, GH treatment at replacement dose was started in GHD children. After 12-month treatment a second study visit was performed in GHD children as well as in control children.

A detailed medical and family history was obtained from all subjects and a complete physical examination was performed, including anthropometric parameters and staging of puberty, on the basis of breast development in girls and genital development in boys according to Tanner's criteria.

Fasting blood samples were collected before the initiation of GH treatment and after 12 months, for the evaluation of ADMA, lipid profile (total cholesterol, triglycerides, high-density lipoprotein (HDL)-cholesterol and low-density lipoprotein (LDL)-cholesterol), glucose metabolism parameters (glucose, insulin, HbA1c) and IGF-1 levels.

Blood pressure was also assessed in all children at baseline and after 12 months of regular therapy.

Patients with GHD received GH at an average dose of 0.035 mg/kg/day. Compliance was monitored by recall in the last month of the study and was estimated at an average of ~90%.

The study was approved by the Ethical Committee of the University of Chieti. Written informed consent was obtained from all parents and oral consent from all children.

2.2. GHD patients

Isolated GHD was diagnosed according to auxological and biochemical parameters as established by the GH Research Society [16]. In particular, height was 2 standard deviations (SD) below the population mean and height velocity over 1 year was more than 1 SD below the mean for chronological age. All cases underwent two GH provocation tests and in both GH peak was below 10 μg/l. IGF-1 levels were below a cut-off less than −2 SD for age and sex. Brain magnetic resonance imaging was normal in all children, establishing the idiopathic nature of GHD.

2.3. Anthropometric measurements

Weight and height were measured with the child in light clothing and without shoes. Body weight was measured to the nearest 0.1 kg with a calibrated scale (Salus, Inc., Italia). Height was measured in triplicate with a Harpenden stadiometer to the nearest 0.1 cm (Holtain, Wales, UK). Each subject stood straight, with feet placed together and flat on the ground, heels, buttock and scapulae against the vertical backboard, arm loose and relaxed with the palms facing medially and the head positioned in the Frankfurt plane. Body mass index (BMI, the weight in kilograms divided by the square of the height in meters) and standard deviation score (SDS) for all anthropometric parameters were based on published normative data for Italian children [17].

2.4. Laboratory procedures

2.4.1. Serum IGF-1

Serum IGF-1 levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Diagnostic System Laboratories, Inc., Webster, TX, USA). The intra-assay coefficients of variation (CVs) were 7.1% (at 26.47 ± 1.87 ng/ml), 4.5% (at 48.36 ± 2.15 ng/ml) and 6.5% (at 169.67 ± 11.01 ng/ml). The inter-assay CVs were 8.8% (at 42.94 ± 3.79 ng/ml), 4.8% (at 132.61 ± 6.35 ng/ml) and 6.4% (at 379.12 ± 24.38 ng/ml).

The results of the measured IGF-1 were converted into SDS according to the formula: SDS = [X − average X] / SD; where X is the observed value, average X is the mean of the normal value at the respective age and SD is the standard deviation from the mean, using normative data, as provided by the manufacturer.

2.4.2. Lipid metabolism

Total cholesterol, HDL-cholesterol and triglycerides were measured with an enzymatic-calorimetric test. LDL-cholesterol was derived according to the Friedewald's equation [18].

2.4.3. Glucose metabolism and insulin resistance

Plasma glucose was determined by using the glucose oxidase method and plasma insulin was measured with two-site immunoenzymometric assay (AIA-PACK IRI; Tosoh, Tokyo, Japan). The limit of detection was 0.5 μU/ml with intra-assay and inter-assay coefficients of variation <7% for quality control.

Insulin resistance was assessed by using the homeostasis model assessment of insulin resistance (HOMA-IR), defined by [fasting insulin (mU/l) × fasting glucose (mmol/l) / 22.5] [19].

2.4.4. HbA1c

HbA1c concentrations were measured using a high-performance liquid chromatography method. The normal range was 4.2–6.0%, with an intra-assay coefficient of variation of 3%.

2.4.5. ADMA

Levels of human serum ADMA were determined by an ELISA kit (Cusabio Biotech Co., LTD., Catalog No. CSB-E09298h, P.R. China), according to the manufacturer's protocols. The minimum detectable dose of human ADMA was less than 2 ng/ml. The intra-assay coefficient of variation was <8%; and the inter-assay coefficient of variation was <10%.

2.4.6. Blood pressure

Blood pressure was measured by the same investigator at all study visits using a validated protocol. Systolic (SBP) and diastolic blood pressures (DBP) were measured three times, at 5-minute intervals, at the non-dominant arm, after 10 min rest using a calibrated sphygmomanometer. The cuff size, which was based on the length and circumference of the upper arm, was chosen to be as large as possible without having the elbow skin crease obstructing the stethoscope. The mean