Growth Hormone Deficiency in Prepubertal Children: Predictive Markers of Cardiovascular Disease

Chiara De Leonibus\textsuperscript{a}  Stefania De Marco\textsuperscript{a}  Adam Stevens\textsuperscript{c}  Peter Clayton\textsuperscript{c}
Francesco Chiarelli\textsuperscript{a, b}  Angelika Mohn\textsuperscript{a, b}

\textsuperscript{a}Department of Paediatrics, and \textsuperscript{b}Center of Excellence on Aging, ‘G. D’Annunzio’ University Foundation, University of Chieti, Chieti, Italy; \textsuperscript{c}Institute of Human Development, Faculty of Medical and Human Sciences, University of Manchester and Manchester Academic Health Science Centre, Royal Manchester Children’s Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK

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Abstract
Background: Cardiovascular (CV) risk factors have been identified in adults with untreated growth hormone deficiency (GHD). Existing evidence suggests that the development of the atheromatous plaque begins early in childhood. Previous reports have shown that GHD children are prone to increased CV risks including impaired cardiac function, dyslipidemia and abnormalities in body composition. Recent studies in epigenetics and metabolomics have defined specific fingerprints that might be associated with an increased risk of CV disease. Aim: The aim of this review is to point out the most significant biochemical and clinical predictive markers of CV disease in prepubertal children and to evaluate the effect of recombinant human growth hormone therapy on most of these alterations. The novel findings in epigenetics and metabolomics are also reviewed, with a particular focus on translating them into clinical practice.

Introduction
Growth hormone deficiency (GHD) in adulthood is associated with an increased risk of developing cardiovascular (CV) events and reduced life expectancy [1]. A number of CV risk factors have been identified in adults with untreated GHD, including metabolic alterations, visceral obesity and altered cardiac morphology [2]. However, the existing evidence in normal children and young adults suggests that the development of the atheromatous plaque begins early in childhood during prepubertal years [3].

Prepubertal children with untreated GHD have been found to have increased carotid intima-media thickness (cIMT) compared to a control population [4] and have a higher risk of developing cardiovascular disease (CVD) at an early age [5, 6]. Therefore, the identification of early predictive markers of atherosclerotic CVD and primary prevention should begin in childhood. Previous reports have identified a cluster of CV risk factors in children with GHD, including reduced cardiac size and impaired cardiac function, along with dyslipidemia, abnormalities in body composition and in peripheral inflammatory, fibrinolysis and oxidative stress markers [2, 7, 8].
More recently, epigenetics and metabolomics have been increasingly developed as a tool to detect distinctive fingerprints which could predict an increased risk of CVD [9, 10] (fig. 1).

The aim of this review was to examine biochemical and clinical predictive markers of CVD identifiable early in childhood, with a particular focus on the prepubertal age, and to evaluate the effect of recombinant human growth hormone (r-hGH) therapy on these markers. The second part of the review reports on the most recent findings in epigenetics and metabolomics and the possibility to translate these results into clinical practice.

Biochemical Alterations and the Effect of r-hGH Therapy

Children with GHD have been found to have biochemical alterations already in the prepubertal years, including lipid profile abnormalities, the elevation of inflammatory and oxidative stress markers, along with impaired fibrinolysis.

Lipid Profile

An adverse lipid profile has been shown in prepubertal children with GHD throughout their lifespan, presenting with elevation in triglyceride, total cholesterol and low-density lipoprotein cholesterol levels, along with lower high-density lipoprotein cholesterol levels compared with healthy controls [4, 11, 12]. An adverse lipid profile has also been documented with an increased atherogenic index, that is the ratio between total and high-density lipoprotein cholesterol [4, 11]. r-hGH therapy has been demonstrated to induce favorable changes in lipid profiles [13], as 2 years of growth hormone (GH) replacement was associated with reduced levels of all components of the lipid profiles [4, 11, 14].

The same findings have been reported in both adolescents and adults with GHD, with the identification of elevated fasting and postprandial triglyceride levels. There is considerable evidence suggesting a positive correlation between the postprandial triglyceride response to an oral lipid load and atherosclerosis of the carotid and coronary arteries [15, 16].
Inflammatory and Fibrinolysis Markers

A number of serum circulating markers, including C-reactive protein (CRP), fibrinogen, active plasminogen activator inhibitor type-1 and homocysteine have been proposed as potential risk factors for atherothrombotic vascular disease [17, 18].

A chronic low-grade inflammation has been considered as necessary for the initiation and development of the atherosclerotic plaque [19, 20]. Serum markers of inflammation and in particular CRP may predict the risk for acute CV events in patients with GHD [7]. Increasing evidence suggests that inflammatory status as expressed by CRP is a strong and independent predictor of the severity of coronary artery disease [20].

Furthermore, changes in markers linked to coagulation and fibrinolysis have been found in untreated adults with GHD. The most common example resides in the elevation of pregnancy-associated plasma protein A (PAPP-A) levels in GHD adults [21]. PAPP-A is a member of the matrix metalloproteinase family and it has been included among markers of CV risk being associated both with the presence of carotid atherosclerosis and acute coronary syndrome [7].

In the pediatric population, a few studies have been reported, some in adolescents with GHD, finding elevated CRP and fibrinogen levels [16, 22–24]; however, less studies were conducted in prepubertal children with the identification of high levels of fibrinogen, CRP and homocysteine [11, 16, 17, 25]. Of note, in all these studies, the biochemical parameters were reversed by short-term r-hGH therapy.

Oxidative Stress Markers

In the prepubertal years, children with GHD have been found to have an impaired oxidant-antioxidant status with reduced nitric oxide (NO) bioavailability and vascular reactivity, which in turn led to endothelial dysfunction and CVD [26] (fig. 2).

Asymmetric dimethylarginine (ADMA) is an endogenous plasmatic inhibitor of endothelial NO synthase and is considered as an emerging CV marker as its levels have been found to be increased in prepubertal patients with GHD in some reports [26], but not in others [27]. Elevated ADMA levels are supposed to be associated with increased inhibition of the endothelial NO synthase and vasoconstriction, which represents the first phenomenon leading to endothelial dysfunction. ADMA levels have

Fig. 2. The impaired oxidant-antioxidant status in children with GHD is associated with reduced NO bioavailability. This, in turn, induces increased vascular inflammation and reactivity, leading to endothelial dysfunction and CVD in later life.
been found to be elevated in other conditions with increased oxidative stress, including type 2 diabetes mellitus [28] and obesity [29], and correlate with cIMT, being considered strong predictors of CVD and/or mortality. r-hGH therapy has been found to decrease ADMA levels, reaching values comparable to those in control children [26].

Moreover, in addition to the antioxidant system impairment, untreated prepubertal children with GHD have been shown to have modifications within the morphology and oxygen-transporting properties of erythrocytes. Specifically, the functional abilities of the erythrocytes to transport and release oxygen were altered and the saturation of hemoglobin with oxygen was modified. A 23% higher binding ability, and increased levels of oxyhemoglobin (by 54%) and NO-hemoglobin (by 50%) were found [30]. After r-hGH treatment, the morphological and functional parameters of erythrocytes have been found to be reduced to normal [30].

**Body Composition Abnormalities and the Effect of r-hGH Therapy**

Body composition abnormalities represent an important marker of visceral adiposity [31]. The measurement of waist circumference has been recommended as a clinical tool to identify the level of excess of visceral adiposity and to predict potential patients at higher risk of developing CVD [31].

Prepubertal children with GHD have been found to have a higher waist circumference and a higher waist-to-height and waist-to-hip ratio (WHR) than controls, thus suggesting increased visceral adiposity. Two years of r-hGH therapy were associated with significantly reduced levels of WHR, therefore improving body composition [11].

Another study has evaluated body composition in untreated prepubertal GHD children using dual-energy X-ray absorptiometry to assess the percentage of body fat (BF%), lean body mass (LBM%) and bone mineral content [32]. The dual-energy X-ray absorptiometry analysis identified comparable BF% values to controls, but with significantly lower LBM% and bone mineral density levels. r-hGH therapy for 1 year has been proven to show a beneficial effect on body composition, i.e. a significant reduction in BF% by 15% and an increase in LBM% and bone mineral content by 40 and 44%, respectively [32].

**Cardiac and Carotid Artery Ultrasound Abnormalities and the Effect of r-hGH Therapy**

Ultrasound abnormalities in the GHD population include alterations in both the cardiac and carotid echography, which will be considered in detail below.

**Cardiac Morphology**

In children with GHD, echocardiographic studies of systolic function have yielded contrasting results on the effect of both GHD and r-hGH therapy on cardiac performance [6].

However, the majority of the studies in this field point out the importance of GH for the maintenance of normal cardiac function. The results indicate that GH, directly or indirectly through the effect of the insulin-like growth factor I (IGF-I), is not only involved in the regulation of the somatic growth in children but also in the cardiac growth, probably through the modulation of the size of cardiomyocytes [4, 33].

In a number of studies, a substantially reduced cardiac mass has been identified in prepubertal children with GHD. A reduction of the left ventricular mass (LVM) and left ventricular mass index (LVMi) was found, resulting in impairment of cardiac function. One year of r-hGH replacement has been shown to significantly increase both LVM and LVMi at echocardiography compared to pretreatment values [4, 34].

Other cardiac morphological abnormalities have been identified in prepubertal children with GHD, including decreased left ventricular posterior wall thickness and left ventricular end-diastolic diameter, which significantly increased after 12 months of GH replacement [35]. Increased epicardial adipose tissue has also been shown in GHD adolescents, which has been thought to be a good indicator of abdominal/visceral fat and to contribute to an increased CV risk in the long-term period [36].

**Carotid Echography – cIMT and Endothelial Dysfunction**

cIMT represents the area of tissue from the luminal edge of the artery to the boundary between the media and the adventitia. cIMT increases at a rate of 0.005–0.010 mm/year and its values are age-dependent [37]. In young individuals, a cIMT of >1.00 mm is considered as abnormal [38]. cIMT is increasingly used as a surrogate marker for atherosclerosis and provides an effective and noninvasive method to predict future CV events in young adults [39].

Only a few studies have been conducted in prepubertal children [32], adolescents [22, 36] and young adults [40].
Overall, the results in this field indicate a relationship between GHD and increased cIMT and that even 1 year of GH replacement is able to reduce cIMT values [32], thus reducing the risk of atherosclerotic events.

Other reports show endothelial dysfunction in adults and adolescents with GHD manifested by decreased flow-mediated dilatation of the brachial artery and increased large-artery stiffness assessed by pulse wave analysis of the radial artery. GH replacement resulted in an improvement in endothelial function and a reduction in arterial stiffness [36, 41].

**Future Perspectives**

Recent experimental studies in animal models and reports in humans have yielded interesting findings in the field of epigenetics and metabolomics.

Overall these novel findings, although preliminary, may help in the prediction and identification of patients at risk of developing a CV event later in life. Moreover, understanding the epigenetic mechanisms around GH action and metabolomic markers associated with GHD may help us in identifying new therapeutic possibilities.

**What Is Epigenetics?**

The term ‘epigenetics’ refers to the complex interaction between the genome (DNA) and the environment that is involved in the regulation of gene expression [42]. The DNA accessibility and chromatin structure are changed by DNA methylation and histone modifications [42].

Recent data suggest that epigenetic mechanisms play a major role in human development, growth and metabolism. The idea that GHD may be linked to epigenetic modifications has emerged from epigenetic theories defined as ‘gestational programming’ linking maternal environmental factors and epigenetic alterations in the newborn, which result in offspring metabolic abnormalities [43–45]. A link between epigenetics, decreased IGF-1/ prenatal growth and increased risk of CV events has already been described in newborns with intrauterine growth restriction, where specific epigenetic signatures including methylation within the IGF2 gene and genes involved in carbohydrate metabolism have been identified [46]. Other reports indicate the role of IGF2 gene promoter region methylation in characterizing GH response in children with idiopathic short stature [47, 48].

Overall these reports suggest that long-lasting growth and cardiometabolic changes arise, at least in part, from epigenetically mediated alterations in gene expression that occur very early in life [49].

To the best of our knowledge, although there is growing evidence to suggest a causative role of epigenetics in the pathological mechanisms of CVD [9], there are few reports on GHD in humans. Up to now, the majority of the studies use GH-deficient animal models to support the role of epigenetic changes in affecting growth and metabolism, which will be considered in detail below.

**Epigenetic Markers**

The most important epigenetic tag found in DNA is the covalent attachment of a methyl group to cytosine residues in CpG dinucleotide sequences, known as ‘DNA methylation’. CpG methylation occurs usually within gene promoters and can repress transcriptional activation of gene expression. The percentage of CpG methylation is variable, and unmethylated ‘islands’ of CpG in the genome can become methylated during development with consequent silencing of the associated gene [50]. Therefore, the state of CpG methylation probably controls accessibility of the transcription factors to regions of DNA, with methylated CpG restricting transcription and unmethylated CpG allowing the gene to be expressed.

In addition, chromatin remodeling may occur by histone modifications including histone acetylation at the amino group of lysine residues in H3 and H4 tails that is most consistently associated with promotion of transcription [51] (fig. 3).

**GHD and Epigenetic Signatures: The Role of GH Supplementation**

The majority of long-term physiological effects of GH require hormone-mediated changes in gene expression. The transcription factor, Signal Transducer and Activator of Transcription 5b (Stat5b), plays a critical role in the actions of GH on growth and metabolism by regulating a large number of GH-dependent genes. However, most of the functional related mechanisms remain poorly understood.

In recent years, specific epigenetic features have been found in in vivo animal models of GHD, including histone acetylation and DNA methylation with hepatic chromatin changes. Specifically, in the hypophysectomized rat, it has been found that in the absence of GH, there was minimal occupancy of Stat5b at proximal promoter sites and a reduction of transcription of Stat5b-dependent genes involved in growth and metabolism.
These included Socs2, Cish, Igfals, Spi2, Igf1 with relatively closed hepatic chromatin, as evidenced by low levels of core histone acetylation [52]. GH treatment has been found to cause acute changes in chromatin structure and facilitate a rise in transcriptional activity of these 5 genes by enhanced histone acetylation at all promoter sites [52].

Another study has been conducted in diet-induced obese mice to evaluate the effects of GH supplementation on gene expression by analyzing mRNA levels of metabolism-related genes. GH has been demonstrated to directly regulate adipose tissue function by decreasing adipose mass via a decline in leptin gene expression, along with an increase in adiponectin gene expression by preventing its decline in the presence of hydrogen peroxide in cultured adipocytes. GH has also been found to improve oxidative stress and chronic inflammation of visceral fat, inducing enhanced liver gene expression of antioxidant enzymes, interleukin-10 and CD206 [53].

Therefore, r-hGH not only promotes growth, but also influences key metabolic processes at an epigenetic level by directly regulating gene expression (fig. 4).

What Is Metabolomics?

Metabolomics is the untargeted measurement of endogenous and exogenous metabolites and it is a rapidly developing tool to holistically study metabolism in many human conditions and diseases [54]. It specifically defines the ‘metabolome’, which is the pattern of low-molecular weight compounds present in cells, tissues or biofluids. It is performed by using specific techniques including mass spectrometry and nuclear magnetic resonance spectroscopy [55]. Its use is important in defining meta-
bolic profiles as indicators of physiopathological conditions and to provide predictive markers of disease (fig. 5).

Preliminary data have identified metabolomic fingerprints in adolescents and adults with GHD. In the present time, no studies have been performed in prepubertal children with GHD. The only available data on the use of metabolomics in the pediatric population include a selected group of children with other growth disorders, including newborns with intrauterine growth retardation [56] or in children born small for gestational age [57].

In children born small for gestational age, metabolomics has been used as a tool to understand and compare different metabolic profiles in children with catch-up growth who have a greater risk of cardiometabolic diseases than children with no catch-up growth. Metabolic profiling demonstrated a fourfold decrease in urine myoinositol in children with catch-up compared with children with no catch-up growth, and this was associated with an increase in growth factor and IGF-I signaling, along with increased insulin levels in children with catch-up growth, thus implying a possible role of specific metabolic profiles that may relate to cardiometabolic risk [57].

**Metabolomic Markers in Patients with GHD**

At present, only two studies have identified specific metabolic profiles in patients with GHD: one performed as a single-case study in a female adolescent with severe GHD and the second report in adults with GHD compared to healthy controls [58, 59].

The first study has shown that the urine metabolic profile was different in the patient as compared with healthy controls and it changed during r-hGH treatment and after discontinuation of r-hGH replacement. Several metabolite changes were identified and related to modifications in protein turnover (urinary creatine and creatinine) and lipid metabolism (urinary citrate, free fat acids and ketone bodies) [58].

The second pilot study has evaluated 10 adult patients with GHD and compared them with 10 healthy age- and gender-matched controls. The metabolome analysis has identified clear differences between the groups, mostly within the lipid class. During r-hGH replacement, major changes in amino and fatty acids occurred [59].

Specifically, among 285 measured metabolites, 13 were identified as the most important in differentiating GHD patients from controls. Eleven of them were classified as lipids, including phospholipids, and 2 were identified as cysteine and glyceric acid. r-hGH replacement in-

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**Fig. 5.** The ‘metabolome’ is the group of low-molecular weight compounds present in cells, tissues or biofluids. These include lipids, nucleotides, amino acids and sugars. The metabolome derives from the RNA transcription (transcriptome) of the DNA information (genome).

In addition, the use of human leukocyte proteomic profiling has been used to identify protein changes in young individuals on short-term GH treatment. A number of peptides/proteins in the 3–22-kDa range have been found to be either up- or downregulated by GH, including calcium-binding and proinflammatory proteins [60]. The importance of a reduction of the proinflammatory proteins, such as calgranulins, may provide an explanation of the anti-inflammatory effect of GH and the reduction of the inflammatory CV risk markers in GH-deficient subjects [61].

Other reports have identified the metabolic response to GH in normal and GH-deficient patients in the regulation of protein, glucose and lipid metabolism [62, 63].

Overall, these results highlight the importance of metabolomics and of proteomics in the identification of serum biomarkers of GH action in children with GHD and in defining the response to r-hGH treatment [59].

**Conclusions**

Children with GHD already have detectable biochemical and clinical markers of CVD in the prepubertal years; it is important that this is recognized. Most of these alterations are reversible after r-hGH supplementation, and, therefore, effective treatment of GHD children through childhood will lower the burden of CV risk that
these young people could carry into adulthood. However, further studies are needed to evaluate the long-term effect of r-hGH treatment on CV risk.

Novel epigenetic and metabolomic markers are now emerging that could be utilized as clinical tools to detect distinctive fingerprints to help in predicting those children with GHD and other growth disorders who are at an increased risk of CVD. The novelty of these studies resides in a better understanding of the epigenetic and metabolomic changes related to GHD, which are not permanent and could be reversed by r-hGH therapy.

 Disclosure Statement

The authors have no conflicts of interest to disclose.

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De Leonibus/De Marco/Stevens/Clayton/Chiarelli/Mohn


Novel Predictive Markers of Cardiovascular Disease in GHD Children


