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Metabolic alterations in paediatric GH deficiency

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Growth hormone (GH) has a large number of metabolic effects, involving lipid and glucose homoeostasis, lean and fat mass. Growth hormone deficiency (GHD) is associated with a metabolic profile similar to the Metabolic Syndrome which is characterized by dyslipidemia, insulin resistance, haemostatic alterations, oxidative stress, and chronic inflammation. GH replacement treatment in GHD children improves these cardiovascular risk factors, while cessation of GH is associated with a deterioration of most of these risk factors. However, it is unclear whether the changes of these risk factors are associated with an increased risk of cardiovascular diseases especially after discontinuing GH treatment. GH treatment itself can lead to insulin resistance, which probably also influences the cardiovascular health status. Therefore, longitudinal studies with the primary outcome cardiovascular diseases are needed in GHD children. Furthermore, new approaches such as metabolomic studies might be helpful to understand the relationship between GHD, GH treatment, and cardiovascular diseases.

Introduction

The main role of growth hormone (GH) replacement therapy in children with GH deficiency (GHD) is to promote linear growth. However, GH brings also about a large number of metabolic effects, involving lipid and glucose homoeostasis, lean and fat mass, since GH has both insulin-like (insulin-like growth factor-1 [IGF-1] dependent) and anti-insulin-like effects on cells and tissues [1–6]. GH

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decreases lipogenesis and stimulates lipolysis as well as lipid oxidation in order to switch metabolism from glucose and protein to lipid utilization [7].

Therefore, there is an ongoing discussion that GHD in childhood is associated with decreased lean mass and increased body fat, unfavourable lipid and glucose profile, and insulin resistance. All these factors may accelerate atherosclerosis and promote higher cardiovascular morbidity. Existing evidence suggests that the development of the atheromatous plaque begins early in childhood [8]. The effect of dyslipidemia and insulin resistance on cardiovascular diseases (CVD) is well known from the Metabolic Syndrome (MetS). MetS is defined by the clustering of obesity, dyslipidemia, glucose intolerance, and hypertension [9]. Of interest, the clinical feature of MetS is similar to the metabolic effect of untreated GHD in adults characterised by obesity, dyslipidemia and increased diabetes risk [1,2,10]. It is widely accepted that untreated GHD leads to CVD in adults [1,2,10] whereas GH treatment in adults produces beneficial metabolic effects [1,2,11]. In contrast, the situation in childhood is less clear, since relatively few studies have investigated the effect of GHD and GH replacement therapy on the metabolic abnormalities and the associated CVD.

In this review the metabolic alterations in paediatric GHD are discussed focussing not only on classical cardiovascular risk factors but also on new risk factors of CVD such as chronic inflammation, adipokines, and disturbances in the clotting system [12–14]. Epigenetic and metabolomics studies, which have shown specific fingerprints that might be associated with an increased risk of CVD [8], are also included.

Glucose metabolism

Patients with GH overproduction (acromegaly) are insulin resistant and glucose intolerant whereas children with GHD can develop severe hypoglycaemia especially in the neonatal period (see below) [15]. These clinical features demonstrate that GH is an anti-insulin hormone increasing insulin resistance and blood glucose levels.

GH administration inhibits insulin-stimulated glucose uptake especially in muscles [16–18]. Additionally, GH is also able to reduce hepatic insulin sensitivity in healthy humans as proven in clamp studies [7,17]. Several studies in adults demonstrate that GH therapy deteriorates insulin sensitivity resulting in hyperglycaemia with an improvement in the long term in the majority of the studies [11,17,19]. However, GH therapy increases the risk of type 2 diabetes mellitus especially in GHD adults with pre-existing metabolic risk factors such as obesity and/or MetS [20]. Insulin sensitivity increases after cessation of GH treatment [5,7,21]. Euglycaemic hyperinsulinaemic clamp studies show a normalisation of insulin resistance parallel to decreased rates of lipid oxidation in a short GH withdrawal in GHD treated young adults [5,7]. On the other hand, hypopituitary adults with unsubstituted GHD also tend to be insulin resistant with a decreased peripheral insulin sensitivity, a modest fasting hyperinsulinaemia, and abnormal glucose tolerance [10,15].

A unifying mechanism explaining insulin resistance in both conditions (unsubstituted GHD and GH treatment) could be an increased flux of free fatty acids (FFA) caused by visceral obesity (untreated GHD) or enhanced lipid oxidation (GH substitution), respectively [15] (see Figs. 1 and 2). During fasting, which may be considered the natural domain for the metabolic effects of GH, the induction of insulin resistance by GH is associated with enhanced lipid oxidation and protein conservation. In this particular context, insulin resistance appears to constitute a favourable metabolic adaptation. Of interest, maximal GH peaks physiologically occur at night during the fasting state. Probably, administration of GH not only in the fasting state such as performed by using long-acting GH may be associated with greater consequences of insulin resistance such as higher prevalences of atherosclerosis and type 2 diabetes mellitus.

Studying children demonstrates that untreated GHD children have no deterioration in their glucose metabolism after the neonatal period [22–25], but during the treatment with GH insulin resistance increases compensated by hyperinsulinaemia [22–28]. Accordingly, an increased daily insulin requirement has been reported in patients with type 1 diabetes mellitus treated with GH [29].

However, most studies in children report no hyperglycaemia [22,23,25,26], while few studies show also increased fasting glucose levels after the onset of GH treatment in GHD children [24,27,28]. Clamp studies demonstrate that GHD treated adolescents have the well-known higher insulin resistance at fasting [30] but it is coupled with a compensatory insulin secretion both at fasting and during OGTT

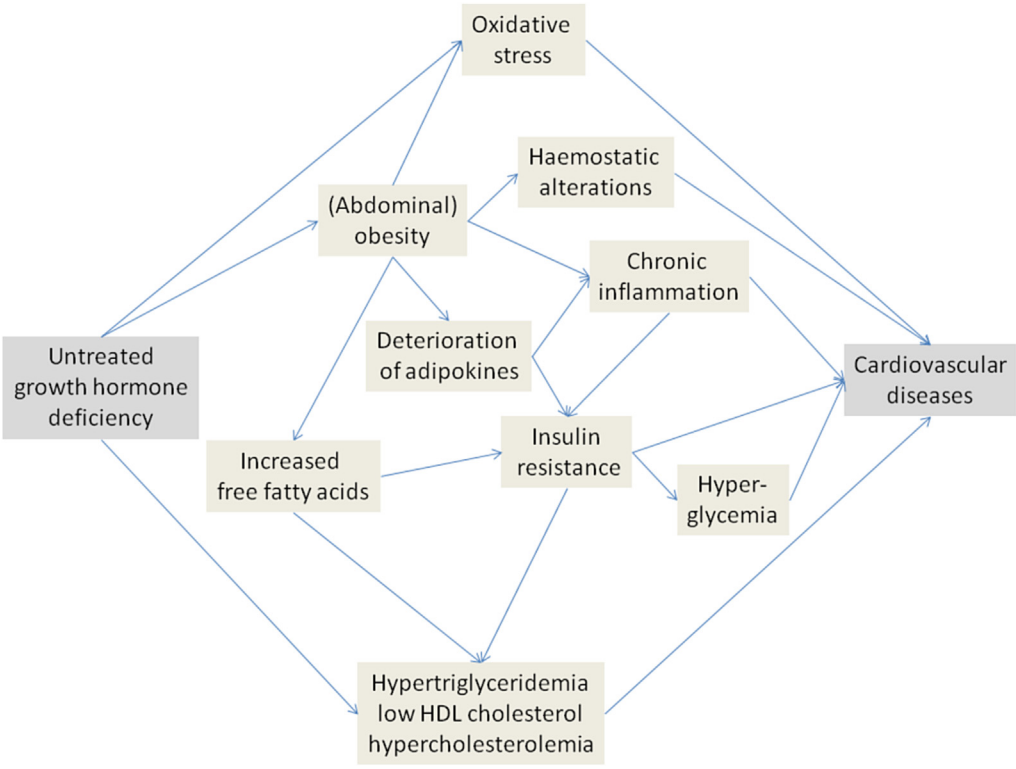

Fig. 1. Effects of untreated growth hormone deficiency on the metabolism and the cardiovascular system.

explaining the normal glucose levels [7]. In youths, the pancreas maintains a beta-cell compensatory capacity. This mechanism probably enables the pancreas to secrete more insulin counterbalancing the GH inhibited glucose uptake in the muscles or liver [7,18].

Data from 11,686 GH-treated patients in the Genetics and Neuroendocrinology of Short Stature International Study (GeNeSIS), a multinational observational study of children with growth disorders, were analysed for diabetes incidence [31]: During a median 1.8 year of GH treatment, diabetes standardized incidence ratios for U.S. patients were 8.5 (2.8–19.5) for type 2 diabetes mellitus, which was slightly higher compared to the U.S. population with a type 2 diabetes mellitus standardized incidence ratios of 6.5 (3.3–11.7). In contrast, the incidence of type 1 diabetes did not differ. Among the 11 patients with the onset of type 2 diabetes mellitus in the GeNeSIS database, risk factors for diabetes such as obesity or type 2 diabetes in first grade relatives were identified in 10 patients.

A further large international pharmacoepidemiological retrospective survey of children treated with GH (KIGS database) showed that 85 (0.36%) of 23,333 children treated with GH had an abnormal glucose metabolism [32]. After investigation, 43 had confirmed glucose disorders (11 with type 1 diabetes, 18 with type 2 diabetes, and 14 with impaired glucose tolerance). In the children treated with GH, the incidence and the age at the time of diagnosis of type 1 diabetes did not differ from expected values based on population studies [32]. In the KIGS database, the incidence of type 2 diabetes mellitus was 34.4 cases per 100,000 years of GH treatment which was six-fold higher than reported in those children who were not treated with GH [32]. In this database, type 2 diabetes mellitus did not resolve after GH therapy had been stopped [32]. This suggests that GH treatment may accelerate the onset of type 2 diabetes in predisposed individuals. It has to be highlighted, that in both surveys not only children with GHD were included but also children with other growth disorders treated with GH at supraphysiological doses [31,32]. Therefore, the risk of type 2 diabetes is likely to be lower for children with GHD as demonstrated in these two pharmaco-epidemiological surveys.

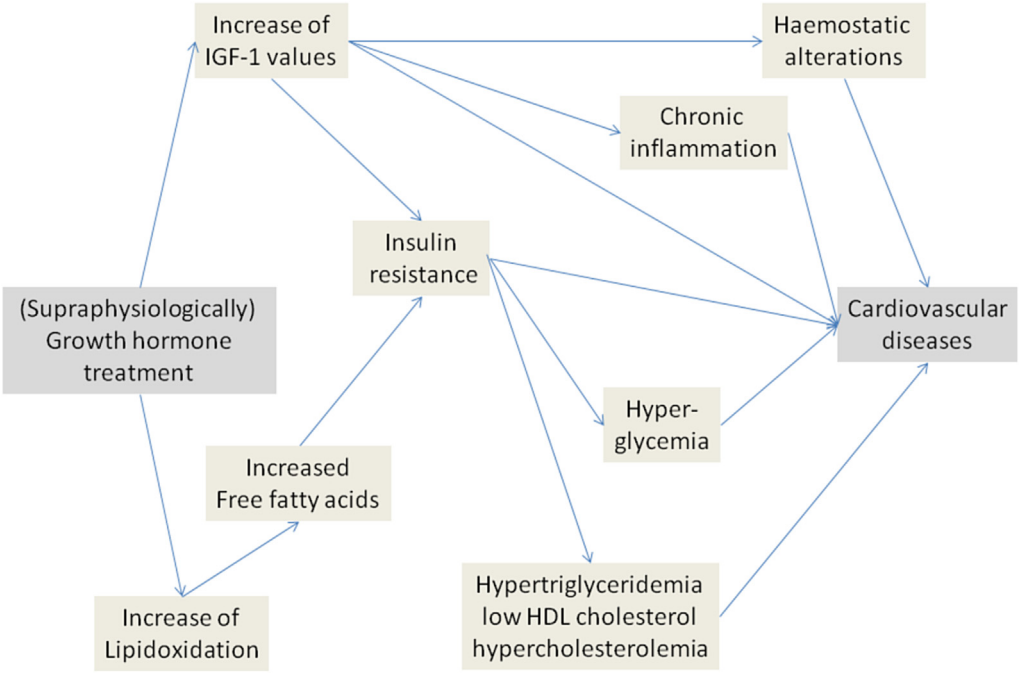

Fig. 2. Impact of (supraphysiological) growth hormone treatment on the metabolism and the cardiovascular system.

Interestingly, the glucose metabolism during GH treatment also depends on the genetic background. The presence of at least one GH receptor (GHR) exon 3 deletion (d3GHR) allele is associated with lower glucose levels after GH withdrawal [7]. One study in children reported lower fasting insulin, HOMA-IR, and lipid profile in the homozygous and heterozygous d3GHR group than in the full-length GHR group [33]. The d3GHR variant consisting of genomic exon 3 deletion has been linked with increased receptor activity due to an enhanced signal transduction [34]. Its biological role on glucose metabolism is still controversial.

Lipid metabolism

GH is also able to compensate the anti-lipolytic actions of hyperinsulinaemia [17]. GH leads to lipolysis by inhibiting insulin-stimulated glucose uptake predominately in muscles but also in the liver [16–18]. Furthermore, GH plays an important role in the regulation of lipoprotein metabolism [7]. In concordance, GHD in adult life is associated with higher total and LDL-cholesterol levels and a variable prevalence of hypertriglyceridaemia [1,2,10]. GH replacement therapy in adults has beneficial effects on these lipid parameters [1,2,11].

However, the situation in GHD children is not as evident. Some studies report no abnormalities in lipid profiles [23,25,35,36] or only marginal changes of lipids [24,27] in untreated GHD children. As opposed to these findings, other studies demonstrate that total and LDL-cholesterol are higher in untreated GHD children than in controls, whereas HDL-cholesterol and triglycerides are similar [6,22,26,37,38].

GH replacement therapy in GHD children improves the lipid profile with a significant reduction in total cholesterol, LDL-cholesterol, and triglyceride levels and a concomitant slight rise in HDL-cholesterol after 1–2 years GH treatment in most studies [3,22,23,25,26,37]. However, one study reports no changes of lipids after GH treatment in GHD children [4]. Apolipoproteins CII and CIII as well as apolipoprotein A1 increase throughout GH administration, while lipoprotein(a) and apolipoproteins AI and B do not change significantly [4,35,38].

It is interesting to note that in GHD adolescents who discontinue GH treatment at the completion of linear growth, there are unfavourable effects on lipid profiles [21,39]. Differences in apolipoprotein-B, LDL-cholesterol, and smaller lipoparticles (LDL-3 and non-HDL) have been reported between GHD adolescents discontinuing GH treatment and GHD adolescents continuing GH treatment in a prospective trial [21].

The interpretation of the true effect of GH replacement therapy on metabolic status in GHD children is often complicated by differing aetiologies of GHD and by the presence of additional hormone deficiencies. Therefore, studies analysing well-characterised patients are helpful to understand the effect of GH on lipids in GHD children. One study analysed the response of the lipid profile to GH treatment in a cohort of children with the same mutation in the GHRH receptor gene [40]: At baseline, the subjects with GHD had an adverse lipid profile, including elevated triglycerides, elevated LDL-cholesterol and elevated total cholesterol. After starting GH treatment triglycerides normalized within 3 months, LDL-cholesterol within 12 months and total cholesterol within 24 months.

Body composition

It is well known that GH influences body composition in adults and children [41,42]. GHD is associated with a weight gain in children and adults [1,2,42–44], which results in increased proportion of fat tissue in relation to lean body mass (LBM) [41]. Furthermore, GHD predisposes to abdominal fat accumulation as demonstrated by higher waist circumference in adults and children [24,45–47] [8]. Whereas GH replacement in GH-deficient states is associated with a decrease in fat mass [3,4,27,35,48–51] and a reduction of waist circumference [8,37], these effects are not seen with IGF-1 replacement in GH-resistant states (as in Laron's syndrome) suggesting that the effect of GH on body fat is independent from IGF-1 levels [52]. In addition, replacement of GH in children or adults with GHD reverses truncal fat accumulation [49,53]. Some studies also demonstrate a reduction in truncal fat with GH administration in obese adults without GHD [45,47].

This is why administration of GH is suggested not only to treat short stature but also to improve body composition in GHD children [42]. Based on the KIGS database, we have reported (n = 2643 children with GHD) that overweight and obese children with GHD reduced their degree of overweight after initiating GH replacement treatment [54]. This effect was more pronounced until reaching final height if GH treatment started before the age of 8 years [54]. If the children had a normal weight, when GHD was diagnosed, GH treatment was associated with a higher body mass index (BMI) at near final height compared to BMI at the onset of GH treatment in this database [54]. Therefore, one might speculate whether this increase is a negative effect of GH treatment. However, higher GH doses were associated with a decrease of BMI in children with GHD pointing against an adverse effect of GH treatment on weight status [54]. It is likely that the increase in BMI in normal-weight children treated with GH reflects the natural course of BMI in children of industrialized countries [55]. Furthermore, from the present experience, it might be possible that there is a predominate increment in LBM during GH treatment in normal-weight and underweight individuals while the lipolytic effect of GH appears to prevail especially in the omental fat mass in obese children [41].

This lipolytic effect of GH has been proven by dual energy X-ray absorptiometry (DEXA) measurements demonstrating a 15% reduction in fat and an increase of 40% in LBM after 1 year of GH treatment [50]. Interestingly, after beginning GH therapy, the body composition improved more in pubertal than in prepubertal children, where only a slight effect could be shown [40]. Stopping GH therapy for 1 year in adolescent GHD patients induces fat accumulation, while continued GH treatment has been reported to have beneficial effects on the body composition by reducing the fat mass and increasing the fat-free mass [5]. Another study in severe GHD adolescents defined by a peak GH < 3 μg/l shows that LBM increases by 2.5 ± 0.5 kg (approximately 6%) over 12 months in those receiving GH but is unchanged after GH discontinuation [21]. Continuation of GH at completion of linear growth results in ongoing accrual of LBM, whereas skeletal muscle mass remains static after GH cessation in these adolescents with GHD [21]. This divergence of gain in LBM is of potential importance because increases in LBM occur as a feature of healthy late adolescent development. However, studies on body composition after cessation of GH in children with not severe GHD are still missing.

Adipokines

The changes in body composition during GH treatment in GHD children are also associated with changes of adipokines. Adiponectin, resistin and leptin are the most famous hormones secreted in adipose tissue all improving insulin sensitivity [12,14]. Therefore, these adipokines may be an important link between GHD and insulin resistance (see Fig. 1).

In most studies, decreased leptin concentrations in a parallel manner to decreased body mass are described under GH therapy [22,51,56]. Conversely, another study reported a significant rise in leptin levels after 12 months of GH treatment in GHD children [27]. Resistin was shown to decrease after the onset of GH replacement therapy in GHD children [27]. Adiponectin did not change after 12 month GH treatment in GHD children [22,51]. Other studies reported higher adiponectin levels in GHD children before GH therapy than in controls [27,51], while a further study reported that adiponectin levels were significantly lower in untreated GHD adolescents than in treated GHD subjects or in control subjects [38]. The reason for these controversial findings may be attributed to the small sample sizes, which was always <40 in these studies. Studies with other adipokines such as fibroblast growth factor-21, apelin, retinol-binding protein, adipocyte fatty acid-binding protein have not been performed yet, but are of interest since all these hormones are involved in the regulation of insulin resistance [14].

Inflammatory system

A chronic low-grade inflammation has been considered necessary for the initiation and development of the atherosclerotic plaque [8]. Serum markers of inflammation and in particular CRP may predict the risk for acute cardiovascular events in patients with GHD [8]. Increasing evidence suggests that inflammatory status as expressed by CRP is a predictor for the severity of coronary artery disease [8].

Increment of visceral fat mass (as described in untreated GHD adults) is associated with rising C-reactive protein (CRP), tumour necrosis factor alpha (TNF-α) and interleukin-6 [57]. These findings fit well to the hypothesis that obesity is a chronic inflammatory disease [13].

Untreated GHD children have higher TNF-α and CRP levels compared to healthy controls and GHD children treated with GH [36,58,59]. Under GH treatment TNF-α decreases [27,37]. On the other side, GH is discussed to be a mediator of inflammatory mechanisms [60]. However, much more research is needed to understand the relationships between inflammatory status, GHD, and CVD.

Haemostatic alterations

Changes in markers linked to coagulation and fibrinolysis have been found in untreated adults with GHD [8,10]. The most common example resides in the elevation of pregnancy-associated plasma protein A (PAPPA) levels in GHD adults [61,62]. PAPPA is a member of the matrix metalloproteinase family and seems to play a role in the pathophysiology of atherosclerotic plaques [61]. In a longitudinal study, we were able to demonstrate that PAPPA serum levels correlated significantly with other cardiovascular risk factors in obese children [63].

Higher fibrinogen levels have also been demonstrated in untreated GHD children [36,64]. Additionally, elevated homocystein levels have been reported in untreated GHD children, which normalized after GH treatment initiation [65]. Furthermore, vascular cell adhesion molecule-1 (VCAM-1) and P-selectin concentrations has been shown to be increased in untreated GHD children compared to healthy controls or treated GHD children, while vascular endothelial cell activation intercellular adhesion molecule-1 (ICAM-1) and E-selectin concentrations did not differ [59]. These findings point towards a prothrombotic state in untreated GHD but further studies are necessary.

Oxidative stress markers

Children with GHD were shown to have an impaired oxidant-antioxidant status with a reduced nitric oxide (NO) bioavailability and vascular reactivity, which in turn led to endothelial dysfunction and CVD [8,66]. Asymmetric dimethylarginine (ADMA) is an endogenous plasmatic inhibitor of

endothelial NO synthase and is considered as a cardiovascular marker. Elevated ADMA levels are supposed to be associated with an increased inhibition of the endothelial NO synthase and vasoconstriction, which represents the first phenomenon leading to endothelial dysfunction [8]. ADMA levels were elevated in other conditions with increased oxidative stress, including type 2 diabetes mellitus and obesity, they correlated with carotid IMT [8]. Under GH therapy decreased ADMA levels, reaching values comparable to those in healthy children, were found [8,66].

Cardiovascular alterations

Unpropitious lipid profile, high abdominal fat mass, insulin resistance, chronic inflammation, haemostatic alterations, and oxidative stress increase the cardiovascular risk in children with GHD (see Fig. 1) [8,10,67]. It is known from studies in adults that untreated GHD can lead to premature atherosclerosis and reduced life expectancy [10,67,68]. In concordance, decreased IGF-1 levels due to adult GHD have been reported to be associated with an increased risk of ischaemic heart disease and stroke [67,69]. Proposed mechanisms linking GHD to CVD are dyslipidemia, insulin resistance, hyperglycaemia, and increased IGF-1 levels.

However, GH treatment itself can also induce CVD (see Fig. 2). It is well known that the overproduction of GH in acromegaly is associated with an increased mortality due to CVD [70]. Fasting GH levels in adults are predictive for cardiovascular morbidity and mortality [71]. GH itself is discussed to be a mediator of inflammatory mechanisms underlying atherogenesis through direct effects [60]. Furthermore, endothelial cells have high-affinity binding sites for IGF-1 which are related to GH levels [67]. Moreover, IGF-1 and its IGFBPs are involved in the inflammation-linked angiogenesis [67]. Accordingly, increased IGF-1 levels in acromegaly have been reported to be associated with an increased risk of ischaemic heart disease and stroke [67,69,72]. Therefore, it is also conceivable that at least the administration of supraphysiological doses of GH could have negative effects on the cardiovascular system [8]. First reports from France of the long-term mortality and morbidity after GH treatment in childhood (SAGhE study) showed an increased mortality due to CVD (especially hemorrhagic stroke) especially when high doses of GH were used in children [73]. However, the same study demonstrated no increased mortality in children treated with GH in Belgium, the Netherlands, and Sweden [74,75]. These findings are in line with those of other studies proving that the adult mortality and morbidity is not increased in childhood-onset GHD patients who received paediatric GH treatment [76]. Also, GH therapy was not associated with an increased mortality, when IGF-1 levels were targeted within normal age-related reference ranges [77]. A Swedish study demonstrated that GH treatment in children is not linked to an increased mortality when adjusting mortality for birth weight [75].

The existing evidence in children and young adults suggests that the development of the atheromatous plaque begins early in childhood during prepubertal years [8]. Early vascular modifications can be identified by the measurements of intima media-thickness (IMT), which is predictive for myocardial infarction and stroke [78]. Previous investigations by our research group have shown that increased carotid IMT can be measured in obese children with MetS [79], which has similarities to metabolic alterations in GHD children. Furthermore, increased carotid IMT has been measured in adults with acromegaly [80]. On the other hand, untreated GHD is associated with increased cIMT in adults [81–83]. Studies on GH treatment in adults with GHD show a decrease of carotid IMT [83,84].

However, only a few studies analysing carotid IMT in children with GHD or GH treatment have been published to date. We have recently reported that the carotid IMT measurements did not differ between 99 children with GH treatment and 99 age- and gender matched healthy children, neither in the children with supraphysiological nor in the group of children with physiological doses of GH [85]. Accordingly, the doses of GH and the duration of treatment did not correlate to carotid IMT measurements suggesting that GH treatment in children is not associated with cardiovascular changes measurable by carotid IMT [85]. Furthermore, our study demonstrated no relationships between carotid IMT and IGF-1 or IGFBP-3 under GH treatment [85]. Another smaller study confirmed our finding that GH treatment is not associated with arterial vascular changes [6]. In contrast, two studies reported abnormal carotid IMT in untreated GHD children as compared to controls [26,50]. Post therapy, significant reduction in carotid IMT was seen in one of these studies [50].

Other reports showed endothelial dysfunction in adults and adolescents with GHD manifested by a decreased flow-mediated dilatation of the brachial artery and an increased large-artery stiffness assessed by pulse wave analysis of the radial artery [8]. GH replacement resulted in an improvement in endothelial function and a reduction in arterial stiffness [86].

GHD and GH replacement therapy have also an effect on the cardiac mass and size. Young adults with childhood-onset and adulthood-onset GH deficiency have been found to have reduced left ventricular mass and impaired systolic function [26]. The left ventricular mass and left ventricular mass index were significantly lower in 30 GHD children than in 20 controls [26]. GH replacement therapy exerts beneficial effects on the cardiac mass by normalizing the cardiac size after 12 months [26]. Another two-year case-control prospective study in 31 GHD children reported that the left ventricular mass index was significantly lower in GHD children than in controls, whereas left ventricular systolic and diastolic functions were similar [23]. In GHD children left ventricular mass index significantly increased after 1 year of GH replacement and remained stable thereafter [23,26]. These findings suggest that GH, directly or indirectly through the effect of 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 [8,26]. However, another study revealed that interventricular septal thickness, left ventricular posterior wall thickness, and left ventricular mass after correction for body surface were similar in untreated GHD patients and healthy controls [6]. The left ventricular ejection fraction at rest was similar in untreated GHD subjects and controls as were the pulmonary venous flow velocities [6]. Therefore, it is unclear today if GHD has an influence on cardiac mass or function.

Future perspective in predicting cardiovascular risk

Recent studies in epigenetics and metabolomics have defined specific fingerprints that might be associated with an increased risk of CVD [8]. Metabolomics is a new tool to understand the relationship between GHD and CVD [8]. Metabolomics is the untargeted measurement of endogenous and exogenous metabolites. 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. In children born small for gestational age, metabolomics studies have been used as a tool to understand and compare different metabolic profiles in children with catch-up growth who have a greater risk of CVD than those without catch-up growth [8]. 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 of GH and IGF-1. This effect, along with increased insulin levels in children with catch up growth suggests that specific metabolic profiles may relate to a cardiometabolic risk [8]. A single-case study in a female adolescent with severe GHD showed a different urine metabolic profile compared to healthy controls and it changed during GH treatment and after discontinuation of GH replacement [87]. In adults with GHD, the metabolome analysis identified clear differences compared to healthy controls, mostly within the lipid class which normalized during GH treatment [8].

Epigenetic mechanisms play a major role in growth and metabolism. The majority of long-term physiological effects of GH require hormone-mediated changes in gene expression [8]. 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. 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 [88]: In the hypophysectomized rat, there was a reduction of transcription of Stat5b-dependent genes involved in growth and metabolism in the absence of GH. However, in humans it is unclear if GHD is associated with epigenetic mechanisms in the foetus and the neonate. Since all metabolic abnormalities normalized in GH treatment in GHD children (see above), these findings do not support the hypothesis that epigenetic mechanisms are involved in metabolic alterations of GHD in humans. The hypothesis of "fetal programming" suggests that genetic alterations by epigenetic modulations in the first years of life are irreversible. However, GH treatment has been found to cause acute changes in chromatin structure and facilitates a rise in transcriptional activity of these 5 genes by enhanced histone acetylation at all promoter sites in an animal model [8,88].

Further metabolic alterations not associated to the cardiovascular system

Neonatal metabolic alterations

Severe congenital GHD of the newborn is a rare disease, which can cause life-threatening hypoglycaemias beginning in the first week of life [89,90]. Therefore, severe GHD of the newborn needs a fast diagnosis and the substitution with GH. In some cases, the cause of severe GHD is monogenic including mutations of the GH encoding *GH-1* or of transcriptions factors of the pituitary gland such as *Pit1/POU1F1* [90]. The majority of cases are still idiopathic and frequently associated with a significant malformation of the pituitary gland and multiple pituitary hormone deficiencies [89,90].

Neonatal cholestatic hepatitis is another guiding symptom of congenital combined pituitary hormone deficiency including GHD [89]. Infants with combined pituitary hormone deficiency present with early and prolonged jaundice, and failure to thrive [89]. Males with combined pituitary hormone deficiency frequently have a micropenis and or cryptorchidism [89]. Peaks of ALP, ALT and AST occur at 2–4 weeks of life, while GGT levels and functional liver parameters are seldom elevated [89]. Liver biopsies in combined pituitary hormone deficiency showed canalicular cholestasis and mild portal eosinophilic infiltration [89]. Substitution with L-thyroxin, hydrocortisone and GH cure cholestasis in congenital combined pituitary hormone deficiency [89].

GHD can be already diagnosed by GH levels <8 ng/ml in the neonatal screening or in hypoglycaemia of the newborn without any stimulation test [91]. It is well known that during the newborn period, GH serum levels are higher than in childhood (neonatal hypersomatotropism) [92] which enable the diagnosis of GHD without the use of pharmacological stimulants [91]. In this context, it has been recommended to use serum IGF binding protein-3 as an additional diagnostic parameter which is reduced in neonatal GHD as well [93].

Interestingly, GH content in newborn screening cards stored for almost 3 years was not different from the content found in recently used screening cards indicating high immunological stability of GH over time [91]. Therefore, the newborn screening cards can be used as a reliable sample many months later to enable the diagnosis of neonatal GHD at a time when GH serum levels have already reached the low levels of early infancy [91]. This is of practical relevance because most screening laboratories store these cards for a couple of months.

Hypothyroidism

Normal thyroid hormone secretion or appropriate L-thyroxine substitution is crucial for the optimal effect of GH on the growth rate. The decrease of free thyroxine (FT4) levels during GH therapy has been reported in several studies [94]: Hypothyroidism associated to GH replacement treatment occurs in up to 23% [94]. The underlying mechanisms are not fully understood.

Neurological changes

GHD in childhood seems also to lead to metabolic changes in the neurological system [95]: Using MR spectroscopy (MRS), a decrease of the N-acetylaspartate (NAA) to Creatine (Cr) ratio in the posterior cingulate gyrus and in the left parietal white matter could be demonstrated in children with GHD and not in healthy control children, while the ratio choline (Cho)/Cr did not differ. However, the pathogenesis of these possible cerebral metabolic alterations in GHD in children remains unclear as well as the clinical impact. Therefore, much more research is needed.

Summary

GH therapy does not only have a promoting effect on children's growth but also important metabolic effects. GH has both insulin-like and anti-insulin-like effects on cells and tissues reducing body fat and increasing LBM by involving lipid and glucose homoeostasis. GH promotes insulin resistance and hyperglycaemia, decreases lipogenesis and stimulates lipolysis and lipid oxidation.

A summary with recommendations for the clinical practice based on our knowledge concerning metabolic alterations in GHD, and the needed research is enclosed. GHD is associated with a metabolic profile similar to the clinical picture of MetS including dyslipidemia, insulin resistance, haemostatic alterations, oxidative stress, and chronic inflammation. Epigenetics and metabolomics profiling will probably help us to understand the underlying mechanism in the future.

GH replacement treatment in GHD children improves these cardiovascular risk factors while cessating GH therapy at the final height in severe GHD is associated with a deterioration of most of these risk factors. However, the changes of these risk factors are less obvious in non severe GHD. Most importantly, it is unclear to date whether finishing GH replacement treatment is associated with an increased risk of CVD in later life. Furthermore, GH treatment itself can lead to insulin resistance probably also influencing the cardiovascular health status. Therefore, longitudinal studies with the primary outcome cardiovascular diseases are needed in GHD children with and without cessation of GH at the final height.

Disclosure

All authors have nothing to disclosure.

Conflict of interest

All authors declare that there is no conflict of interest.

Practice points (clinical consequences are highlighted in italics)

1. GHD as a part of combined hypopituitarism manifests in the neonatal phase with hypoglycaemia.
   - *Neonatal GHD can be diagnosed by growth hormone determination in the neonatal screening cards or during hypoglycaemia.*
2. The metabolic alterations of untreated GHD in childhood are similar to features of the Metabolic Syndrome (dyslipidemia, insulin resistance).
   - *However, the metabolic alterations are only mild in GHD and therefore no guiding symptom of GHD in contrast to adult GHD.*
3. Growth hormone (GH) treatment improves the lipid profile in GHD.
   - *Cessation of GH treatment can lead to an unfavourable lipid profile. Therefore, lipids should be measured before and after cessation of GH treatment. Continuing GH treatment should be considered if GHD is severe and dyslipidemia occurs.*
4. GH treatment increases insulin resistance.
   - *GH treatment leads to increased insulin requirement in type 1 diabetes.*
   - *GH should be given in the evening, since the GH related insulin resistance is physiologically at night in order to switch metabolism from glucose and protein to lipid utilization.*
   - *The higher pancreas capacity in children to counterbalance insulin resistance with higher insulin secretion explains that there is no or only minimal increased risk of diabetes mellitus or hyperglycaemia in GH replacement treatment.*
   - *Even type 2 diabetes mellitus is very rare in treatment of GHD, GHD patients with risk factors for diabetes such as high BMI and dyslipidemia or type 2 diabetes mellitus in relatives should be monitored by periodical evaluations of their glucose metabolism (fasting glucose, oGTT, HbA1c) to detect diabetes.*
5. Continuation of GH in adolescent GHD after reaching the final height has positive effects on the body composition in severe GHD.
   - *Body composition and weight status should be followed after cessation of GH. Continuing of GH treatment should be considered if GHD is severe and body composition deteriorates.*
6. The initial phase of GH treatment is sometimes associated with hypothyroidism.
   - *Hypothyroidism should be treated since even transient thyroid hormone has a negative effect on the growth rate.*

Open questions

1. Even though the cardiovascular risk factors trended to improve after initiating the replacement therapy with growth hormone (GH) in children with severe GHD, much further longitudinal research is necessary to prove that these cardiovascular risk factors are associated with an increased cardiovascular risk. Especially more long-term studies and endpoint studies analysing the body composition, lipids after cessation of GH treatment in relation to cardiovascular diseases are needed. The changes of the carotid intima-media thickness may be an interesting surrogate marker for cardiovascular diseases.
2. GHD may be associated with a change of cardiac function and mass. However, much more research is needed before drawing any clinical conclusions.
3. We do not know enough about the complex interplay between adipokines, inflammatory markers, haemostatic alterations, oxidative stress, GH, and the development of alterations in insulin sensitivity in GHD children. Epigenetic studies and metabolomics will probably help us to understand the underlying mechanisms
4. GH replacement treatment not administered in the fasting state such as performed by long-acting GH therapy may be associated with an increased risk of insulin resistance and diabetes mellitus type 2. Longitudinal studies of long-acting GH analysing the changes of insulin resistance and the diabetes risk are necessary before these drugs are given to children with GHD.
5. High IGF-1 and GH concentrations may be associated with an increased cardiovascular risk. Longitudinal studies on the cardiovascular risk are necessary in children, especially when supraphysiological doses are administered. Until the long-term cardiovascular risk has been proven or excluded GH treatment should be monitored by IGF-1 measurement and supraphysiologically GH applications and increased IGF-1 levels should be avoided in GHD children.
6. GHD in childhood may be associated with neurological changes. However, much more research is needed before drawing any clinical conclusions.

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