Abnormal Vascular Reactivity in Growth Hormone Deficiency

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Background—The reason why patients with growth hormone (GH) deficiency (GHD) are at increased risk for premature cardiovascular death is still unclear. Although a variety of vascular risk factors have been identified in GHD, little is known regarding vascular reactivity and its contribution to premature arteriosclerosis.

Methods and Results—We assessed vascular function in 7 childhood-onset, GH-deficient nontreated patients (age 22±3 years, body mass index [BMI] 25±1 kg/m²) and 10 healthy subjects (age 24±0.4 years, BMI 22±1 kg/m²) by using strain gauge plethysmography to measure forearm blood flow in response to vasodilatory agents. The increase in forearm blood flow following intrabrachial infusion of the endothelium-dependent vasodilator acetylcholine was significantly lower in GH-deficient nontreated patients than in control subjects (P<0.05). Likewise, forearm release of nitrite and cGMP during acetylcholine stimulation was reduced in GH-deficient nontreated patients (P<0.05 and P<0.002 versus controls). The response to the endothelium-independent vasodilator sodium nitroprusside was also markedly blunted in GH-deficient patients compared with control subjects (P<0.005). To confirm that abnormal vascular reactivity was due to GHD, we also studied 8 patients with childhood-onset GHD (age 31±2 years, BMI 24±1 kg/m²) who were receiving stable GH replacement therapy. In these patients, the response to both endothelium-dependent and -independent vasodilators, as well as forearm nitrite and cGMP, release was not different from that observed in normal subjects. Peak hyperemic response to 5-minute forearm ischemia was significantly reduced in GH-deficient nontreated patients (17.2±2.6 mL·min⁻¹·dL⁻¹, P<0.01) but not in GH-treated patients (24.8±3.3 mL·min⁻¹·dL⁻¹) compared with normal subjects (29.5±3.2 mL·min⁻¹·dL⁻¹).

Conclusions—The data support the concept that GH plays an important role in the maintenance of a normal vascular function in humans. (Circulation. 2001;103:520-524.)

Key Words: growth factors ■ hormones ■ vascular dysfunction ■ arteriosclerosis ■ nitric oxide

Extensive data support a role of growth hormone (GH) in cardiovascular physiology.¹ Patients with GH deficiency (GHD) show reduced left ventricular mass, low cardiac output, and decreased exercise tolerance, all abnormalities reversed by short-term GH replacement.² Beside its effects on the heart, GH also influences the vascular system by increasing blood flow in different vascular beds and by decreasing peripheral vascular resistance.³⁴

There also is evidence that the lack of GH is associated with increased cardiovascular morbidity and mortality rates in patients with hypopituitarism.⁵⁶ Indeed, patients with GHD have a 2-fold higher risk of death from cardiovascular disease than do age-matched control subjects.⁵ These patients present with a cluster of cardiovascular risk factors, including abnormal lipid profile, abdominal obesity, and insulin resistance, all of which may contribute to increase the atherosclerotic risk.⁷ Replacement therapy with GH exerts favorable effects on several of these abnormalities, although its long-term impact on cardiovascular mortality rates remains elusive.⁸ Recent studies have shown increased wall thickness of the carotid arteries in young adults with childhood-onset GHD, even in the absence of the classic risk factors for atherosclerosis.⁹ This finding suggests that GH deficiency per se, independent of the associated metabolic abnormalities, may increase the cardiovascular risk. In this regard, it is noteworthy that systemic NO synthesis is decreased in adults with hypopituitarism who have acquired GHD and that GH replacement increases NO production and concomitantly decreases peripheral vascular resistance.¹⁰ Because NO is essential for the integrity of the vessel wall,¹¹ it is possible that the deficit of biologically active NO in the vascular bed of patients with GHD leads to vascular abnormalities and premature atherosclerosis. However, very little information is available regarding the consequences of GHD on vascular function in humans.¹²¹³
Methods

Patients

Three groups of subjects were studied. The first group included 10 healthy volunteers (5 men and 5 women, mean age 24 ± 0.4 years, body mass index [BMI] 22 ± 1 kg/m²) who were recruited from our department. The second group included 7 patients with childhood-onset GHD (3 men and 4 women, mean age 22 ± 3 years, BMI 25 ± 2 kg/m²). GHD had been diagnosed during their childhood by means of ≥2 stimulation tests (ie, arginine infusion, exercise, insulin-induced hypoglycemia). In all patients, the diagnosis was confirmed with the GH-releasing hormone plus pyridostigmine test (GHRH+PD) and the clonidine test, as previously described.¹⁴ Incorporation criteria were a GH response after GHRH+PD and clonidine tests of <7 µg/L and insulin-like growth factor (IGF)-1 level below the normal range for age. GH therapy had been performed for a period of 1.8 to 4.6 years, until fusion of epiphyses or until a satisfactory height was attained. At the time of entry into the study, GH therapy had been withdrawn for ≥2 years. These patients are referred to here as GH-deficient nontreated patients. One of these patients had isolated GHD, whereas the remainder had multiple pituitary deficiency and were treated with multiple substitution therapy. The third group included 8 patients (3 men and 5 women, mean age 31 ± 2.4 years, BMI 24 ± 2 kg/m²) with childhood-onset GHD who were receiving stable GH replacement therapy for ≥2 years (GH-treated patients). GH administration was adapted on an individual basis to maintain stable GH replacement therapy for ≥2 years (GH-treated patients). GH administration was adapted on an individual basis to maintain stable GH replacement therapy for ≥2 years (GH-treated patients).

Experimental Procedure

All studies were performed at 7.30 AM, after an overnight fast, with the subjects lying supine in a quiet, air-conditioned room (22° to 24°C). Under local anesthesia (lidocaine [Xylocaine] 2%), a 20-gauge plastic cannula was inserted into the brachial artery of the nondominant arm for the regional infusion of the test substances, systemic blood pressure and heart rate monitoring, and arterial blood sampling. The ipsilateral deep antecubital vein was also cannulated for systemic blood pressure and heart rate monitoring, and arterial blood sampling. The net forearm balance of nitrite and cGMP were calculated and were receiving substitutive therapy at standard doses. The study protocol was approved by the institutional ethics committee, and informed consent was obtained from all participants.

Clinical and Metabolic Data

Systolic and diastolic blood pressures tended to be lower in GH-deficient nontreated patients, although the differences were not statistically significant (Table). The waist-to-hip ratio and fat mass were greater in GH-deficient nontreated patients than in control subjects (P < 0.01 to 0.005). Glucose, insulin, cholesterol, and triglyceride levels were similar in the 2 groups. No difference was found in fibrinogen, plasminogen activator inhibitor (PAI)-1, and homocysteine concentrations, whereas LP(a) was significantly higher in GH-deficient nontreated patients than in control subjects (14 ± 4 versus 5 ± 2 g/L, P < 0.05). In the group of patients receiving GH replacement therapy, blood pressure, waist-to-hip ratio, and fat mass values were similar to those of control subjects. Glucose and insulin concentrations were also similar in the 2

Analytical Methods

Total serum cholesterol, triglyceride, and HDL cholesterol concentrations were measured with commercially available kits. Serum LDL cholesterol was calculated with the Friedewald formula. Serum IGF-1 and insulin were determined by radioimmunoassay. Serum lipoprotein(a) was assayed with an ELISA [Macra Lp(a); Terumo Medical Corporation]. Nitrite concentration was measured in plasma samples with EDTA used as an anticoagulant. After collection, blood samples were immediately centrifuged at 2000 rpm at 4°C, and the plasma was stored at −20°C. Before the assay, plasma was ultralow-filtered through a 10-kDa molecular mass cutoff filter (Centricon 10; Millipore). Total plasma nitrites and nitroso compounds were measured with a spectrophotometric assay kit (Cayman Chemical Co). Nitrate was converted to nitrite by nitrate reductase and then nitrite was assayed according to the standard Greiss diazoreaction. All determinations were made in triplicate. The data are referred to as “nitrite concentrations,” but they reflect the sum of nitrate and nitrite. For the determination of cGMP, plasma samples were centrifuged at 4°C after the addition of cold 6% trichloroacetic acid. Supernatants were washed with 5 volumes of water-saturated diethyl ether and dried under a stream of nitrogen at 60°C. The dried extracts were dissolved in 0.5 mol/L acetate buffer, pH 5.8, and acetylated by a mixture of acetic acid anhydride/triethylamine. cGMP content was measured in duplicate with a radioimmunoassay (Amersham International).

Calculations

The net forearm balance of nitrite and cGMP were calculated according to the Fick principle: Net balance = (A − dV)/t F, where A and dV indicate arterial and deep venous concentrations of nitrite or cGMP, and F is the FBF.¹⁶ The differences in clinical and metabolic characteristics between groups were analyzed using unpaired Student’s t test. Vascular reactive data are expressed as absolute values of FBF in the basal state and during each step of drug administration. Two-way ANOVA for repeated measures was applied to test differences in dose-response curve between groups. Where the F ratio was significant, further comparisons were made using the Bonferroni multiple-comparisons procedure. Significance was defined at a value of P < 0.05. Results are expressed as mean ± SEM.

Results

In the present study, we assessed vascular reactivity in a group of patients with childhood-onset, untreated GHD by measuring the forearm vasodilatory response to intra-arterial infusion of endothelium-dependent- and -independent agonists. To better understand the role of GHD per se, we also studied a group of patients with childhood-onset GHD who were receiving stable GH replacement therapy.
GH-deficient nontreated (GHD), and GH-treated subjects.

Figure 1. Changes in FBF in response to ACh in control, GH-deficient nontreated (GHD), and GH-treated subjects.

2.7 mL of ACh, FBF rose to 8.0 compared with the control subjects. At the highest dose of ACh infusion induced a dose-dependent increase in FBF in both control subjects and GH-deficient nontreated patients (Figure 1). However, the vasodilatory response to ACh was significantly reduced in GH-deficient nontreated patients compared with the control group (Figure 1). At the maximal dose of SNP, the vasodilatory response in GH-deficient nontreated patients was 50% lower than that of normal subjects (8.1 ± 1.4 versus 16.1 ± 1.8 mL·dL⁻¹·min⁻¹ in the control and patient group, respectively; P < 0.005). In contrast, in GH-treated patients, no impairment was found in the vasodilatory response to SNP. The percent change in FBF of normal subjects, with a maximal increase in FBF to 19.9 ± 3.9 mL·dL⁻¹·min⁻¹ at the highest ACh dose. The percent change in FBF in response to the highest ACh dose was 780%, 320%, and 620% in control subjects, GH-deficient nontreated patients (P < 0.0005 versus controls), and GH-treated subjects (P < 0.005 versus GH-deficient nontreated patients), respectively. Forearm nitrite release during ACh infusion was significantly lower in GH-deficient nontreated patients than in control subjects (P < 0.005), whereas no difference in ACh-stimulated forearm nitrite release was found between GH-treated patients and control subjects.

Vascular Reactivity Studies
ACh infusion induced a dose-dependent increase in FBF in both control subjects and GH-deficient nontreated patients (Figure 1). However, the vasodilatory response to ACh was significantly reduced in GH-deficient nontreated patients compared with the control subjects. At the highest dose of ACh, FBF rose to 8.0 ± 2.7 mL·dL⁻¹·min⁻¹ in GH-deficient nontreated patients and to 14.1 ± 1.4 mL·dL⁻¹·min⁻¹ in control subjects (P < 0.05). In contrast, in GH-treated patients, the vasodilatory response to ACh was not different from that of normal subjects, with a maximal increase in FBF to 19.9 ± 3.9 mL·dL⁻¹·min⁻¹ at the highest ACh dose. The percent change in FBF in response to the highest ACh dose was 780%, 320%, and 620% in control subjects, GH-deficient nontreated patients (P < 0.0005 versus controls), and GH-treated subjects (P < 0.005 versus GH-deficient nontreated patients), respectively. Forearm nitrite release during ACh infusion was significantly lower in GH-deficient nontreated patients than in control subjects (P < 0.005), whereas no difference in ACh-stimulated forearm nitrite release was found between GH-treated patients and control subjects (Figure 2). Similarly, in GH-deficient nontreated patients, forearm cGMP balance remained unchanged during ACh infusion, whereas a net cGMP release was observed both in control subjects (P < 0.002 versus GH-deficient nontreated patients) and in GH-treated subjects (P < 0.04 versus GH-deficient nontreated patients) (Figure 3).

The dose-response curve to the endothelium-independent vasodilator SNP was significantly reduced in GH-deficient nontreated patients compared with the control group (Figure 4). At the maximal dose of SNP, the vasodilatory response in GH-deficient nontreated patients was 50% lower than that of normal subjects (8.1 ± 1.4 versus 16.1 ± 1.8 mL·dL⁻¹·min⁻¹ in the control and patient group, respectively; P < 0.005). In contrast, in GH-treated patients, no impairment was found in the vasodilatory response to SNP. The percent change in FBF...
in response to the highest SNP dose was 750%, 210%, and 780% in control, GH-deficient nontreated patients (P<0.0005 versus controls), and GH-treated subjects (P<0.05 versus GH-deficient nontreated), respectively. The individual data for the blood flow response to the maximal dose of ACh and SNP are presented in Figure 5. Blood pressure and heart rate remained stable in all subjects throughout the reactivity studies.

Maximal, postischemic vasodilation was significantly lower in GH-deficient nontreated patients (17.2±2.6 mL·dL^{-1}·min^{-1}) compared with control subjects (29.5±3.2 mL·dL^{-1}·min^{-1}; P<0.01). The response of GH-treated patients (24.8±3.3 mL·dL^{-1}·min^{-1}) was not different from that of the control subjects (Figure 6).

Discussion

The results of the present study provide direct evidence for impaired vascular function in GH-deficient nontreated patients, as shown by the blunted increase in FBF in response to vasodilatory agents. Interestingly, both endothelium-dependent and -independent responses were altered, indicating the presence of a complex defect that involved the entire pathway of NO-mediated vasodilation.

Recent studies have shown impaired endothelial function in patients with GHD, based on ultrasound measurement of flow-mediated changes in the brachial artery diameter. Our studies are consistent with those observations and demonstrate a novel defect at the level of the vascular smooth muscle cells. This is supported by the finding that the response to SNP, a direct NO donor, was markedly attenuated in GH-deficient nontreated patients, indicating a reduced sensitivity of smooth muscle cells to NO. In view of this reduced response to NO, one could argue that the impaired response to ACh is entirely the consequence of abnormalities in the vascular smooth muscle rather than a manifestation of primary endothelial defect. However, an examination of nitrite and cGMP data across the forearm also supports the presence of a defect in endothelial function. Although a net forearm release of nitrite and cGMP occurred in normal and GH-treated subjects during ACh infusion, no increment was observed in GH-deficient nontreated patients, a pattern consistent with a defect in NO generation in the endothelial cells. Indirect support of this conclusion comes from recent studies by Serri et al that demonstrate an increased monocyte adhesion to endothelium in GHD patients, a process normally inhibited by NO. Thus, the blunted vasodilatory capacity of GH-deficient nontreated patients appears to be the consequence of a dual defect (ie, impaired NO generation in the endothelium and reduced responsiveness of the vascular smooth muscle to NO).

Vascular dysfunction is known to be associated with all major risk factors for atherosclerosis, encompassing arterial hypertension, hypercholesterolemia, and diabetes mellitus, of which all may cause vascular damage by reducing NO generation or accelerating NO inactivation. It is worth noting that our GH-deficient nontreated patients exhibited only modest metabolic abnormalities. Apart from an increase in abdominal fat mass and serum LP(a) concentration, their cardiovascular risk factor profile was not of the atherogenic kind, because the lipid pattern and fibrinogen, homocysteine, and PAI-1 levels were normal. In addition, no impairment in insulin sensitivity was demonstrable on the basis of fasting glucose and insulin levels. Thus, the finding of impaired vascular function in young GHD patients, who were mostly free of metabolic abnormalities, suggests that the deficit of GH per se may play a role in this process. This hypothesis is also supported by the clinical finding...
that GH replacement reverses the increased intima-media thickness (IMT) of the carotid artery in GHD adults with hypopituitarism within a few months.\textsuperscript{12,20} The reduction of IMT in these patients was independent of changes in plasma lipids but was related to increases in IGF-1 levels, suggesting that the beneficial effect of IGF-1 may be due to its direct action on the vascular wall, and it is not mediated by correction of the metabolic disturbances.

The impairment of vascular function in GHD is the likely consequence of the lack of GH/IGF-1 vasodilatory effect, which has been ascribed to different mechanisms.\textsuperscript{3} Besides the stimulation of NO synthesis, GH/IGF-1 may act through the activation of the Na\textsuperscript{+}K\textsuperscript{+}-ATPase pump, with a consequent reduction in the intracellular Ca\textsuperscript{2+} concentration.\textsuperscript{21} In addition, an NO-independent mechanism has been proposed that involves GH/IGF-1 action on voltage-dependent potassium channels.\textsuperscript{22} Given the role that GH/IGF-1 plays as a physiological regulator of the vascular function, it is conceivable that chronically low levels of GH/IGF-1 may cause vascular dysfunction.

The blunted vasodilatory response to SNP observed in GH-deficient nontreated patients deserves some comments. One possible explanation for the low sensitivity of vascular smooth muscle to NO is the existence of a defect distal to the generation of cGMP. Once this mediator is formed, a G-kinase is activated that induces smooth muscle relaxation through the phosphorylation of unknown cellular substrates.\textsuperscript{23} Thus, it is possible that GH/IGF-1 participates in the regulation of the biochemical steps downstream of cGMP and that GH/IGF-1 deficiency may interfere with the NO signaling in the vascular smooth muscle. Another possibility is that the lack of GH, at the onset of the developmental period, may damage the contractile machinery of the vascular smooth muscle cells, leading to a generalized defect in vasodilatory function and a reduced response to vasorelaxing substances. However, on the basis of the results in the GH-treated group, this possibility seems unlikely, because these patients showed a normal response to vasodilatory agents as well as to forearm ischemia.

The finding of preserved vascular function in GHD patients treated with GH replacement therapy supports the concept that GH/IGF-1 deficiency per se is responsible for the impaired vascular reactivity in GHD and that the defect in NO-mediated vasodilation is reversible with therapy. This interpretation is also supported by the observation that short-term GH treatment is capable of reversing early atherosclerotic lesions of the carotid artery in adults with GHD.\textsuperscript{12,20} Because the impairment of vasodilatory capacity appears to precede structural changes of the arterial wall in the evolution of atherogenesis,\textsuperscript{24} it is likely that the restored vascular function by GH treatment may prevent the progression to more severe vascular damage.

In conclusion, our data support the presence of impaired vascular function in GH-deficient nontreated patients. This abnormality is not present in GHD patients who receive GH replacement therapy, indicating that the vascular defect is reversible. The data support the concept that GH plays an important role in the maintenance of a normal vascular function in humans.

References

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