Effect of growth hormone replacement therapy on plasma diacron-reactive oxygen metabolites and endothelial function in Japanese patients: The GREAT clinical study

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Abstract. Patients with growth hormone deficiency (GHD) have an increased risk of atherosclerosis and vascular mortality. Evidence suggests that endothelial dysfunction is involved in all stages of atherogenesis. This study examined the effect of growth hormone (GH) replacement therapy on diacron-reactive oxygen metabolites (d-ROMs) and endothelial function in Japanese patients with GHD, using peripheral arterial tonometry. This was an open-label, prospective, case-control study. Nine patients with GHD who had not previously received any GH replacement therapy were enrolled. The following parameters were evaluated at baseline (before treatment), and after 24 weeks of GH replacement therapy: endothelial function using the reactive hyperemia index (RHI; EndoPAT® system), d-ROMs, blood pressure, and fasting lipid levels. Plasma GH and insulin-like growth factor-1 (IGF-1) levels were measured at baseline and after 24 weeks of GH replacement therapy. We also enrolled eight controls with pituitary disease but no GH deficiency. Over 24 weeks of GH replacement therapy, the serum IGF-1 levels normalized with significant improvement in the RHI (from 1.65 ± 0.33 to 1.92 ± 0.26, \( p < 0.05 \)) and decreased d-ROM levels (from 356.8 ± 64.1 to 303.1 ± 43.3 U.CARR, \( p < 0.05 \)). There were no significant improvements in the RHI or d-ROM levels in controls. GH replacement therapy in Japanese patients with GHD may be mediated by the reduced oxidative stress and the d-ROMs associated with the treatment.

Key words: Growth hormone deficiency, Growth hormone replacement, Peripheral arterial tonometry, Diacron-reactive oxygen metabolites levels

THE ASSOCIATION between the growth hormone/insulin-like growth factor-1 (GH/IGF-I) axis and the cardiovascular system has been confirmed through the assessment of the functional and the morphological cardiac abnormalities in patients with either excess, or deficient, GH [1, 2]. Sherlock et al. reported that patients with hypopituitarism have reduced life expectancy and a 2-fold higher risk of death from cardiovascular diseases when compared to their healthy counterparts [3]. GH deficiency (GHD) may underlie the increased mortality

Plasma Diacron-reactive Oxygen Metabolite levels and improved Endothelial Function as Assessed by Peripheral Arterial Tonometry; HbA1c, Hemoglobin A1c; HDL-C, High density lipoprotein cholesterol; IGF-1, Insulin-like growth factor-1; IRMA, Immunoradiometric assay; LDL-C, Low-density lipoprotein cholesterol; NO, Nitric oxide; PAT, Pulse amplitude tonometry; QOL, Quality of life; RHI, Reactive hyperemia index; Rh-GH, Recombinant human growth hormone; RH-PAT, Reactive hyperemia peripheral arterial tonometry; ROS, Reactive oxygen species; S, Sex steroid (testosterone); SBP, Systolic blood pressure; SD, Standard deviation; SEM, Standard error of the mean; TG, Triglycerides; U.CARR, Caratelli units.

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observed in patients receiving adequate replacement therapy with other pituitary hormones [3, 4]. While altered body composition, abnormal lipid profile, insulin resistance, and impaired glucose metabolism increase cardiovascular risk among adults with GHD, endothelial dysfunction may also play an important role [5, 6].

Endothelial dysfunction signals the start of the atherosclerotic process. Atherosclerosis is an inflammatory disease of the arterial walls. There is increasing evidence suggesting that endothelial dysfunction is involved in all stage of atherogenesis [7]. Therefore, therapy that reduces inflammation and improves endothelial function may have therapeutic value in the prevention of atherosclerotic cardiovascular disease [8].

GH/IGF-1 deficiency is associated with vascular endothelial dysfunction and premature vascular atherosclerosis. The mechanisms that underlie endothelial dysfunction in patients with GHD remain unclear. One hypothesis is that decreased nitric acid (NO) formation occurs in patients with GHD who are untreated [9, 10]. Specifically, local production of IGF-I causes endothelial-dependent vasodilatation, via the stimulation of NO production [1, 4]. Therefore, it is reasonable to hypothesize that reduced NO synthesis may contribute to the endothelial dysfunction observed in patients with GHD. Reports indicate that endothelial function is altered in patients with GHD, many of whom demonstrate reduced aortic distensibility and impaired vasodilatory flow [11]. Recombinant human (rh) GH replacement therapy significantly increases flow-mediated dilation, a marker of endothelial function and arterial compliance [4, 10].

There are several possible mechanisms for impaired endothelial function in patients with cardiovascular disease. As mentioned previously, decreased NO bioavailability (decreased NO production and/or increased NO inactivation) induces endothelial dysfunction. An alternative mechanism modulating endothelial function is oxidative stress, an imbalance between endothelium derived vasodilators, particularly NO, and reactive oxygen species (ROS) [12]. It is postulated that increased oxidative stress impairs endothelium-dependent vasodilation and contributes to the development of atherosclerosis. Increased oxidative stress may be associated with endothelial dysfunction in adults with GHD. Recently, a test evaluating serum derivatives of reactive oxygen metabolites, the (d-ROMs) test, has become available [13]. To investigate endothelial dysfunction and the increased oxidative stress in adults with GHD, we measured the former using the reactive hyperemia index (RHI; EndoPAT® system), and the latter using d-ROM levels. We examined the correlation between ROS production and endothelial function in adults with GHD undergoing rh GH replacement therapy. This clinical study is known as the GREAT Clinical Study (Clinical Impact of Growth Hormone Replacement Therapy: Decreased Plasma Diacron-reactive Oxygen Metabolite levels and improved Endothelial Function as Assessed by Peripheral Arterial Tonometry in Japanese Adult Patients with Growth Hormone deficiency).

**Patients and Methods**

**Subjects**

The GREAT clinical study included nine patients with adult-onset GHD, and eight control patients with stable pituitary disease but no GHD. Other pituitary hormone deficiencies were supplemented (hydrocortisone, thyroxine, testosterone, and vasopressin) for subjects in both groups, and their levels were stable at the start of our study. The diagnosis of severe GHD was confirmed by low levels of IGF-1, compared to levels adjusted for age and a peak GH response ≤9 ng/mL, induced by a 100 μg GHRP-2 tolerance test, and/or ≤3 ng/mL induced by arginine tolerance test. rh-GH replacement therapy was commenced at an initial dose of 0.021 mg/kg/week, and subsequently adjusted to achieve IGF-1 levels within normal age-adjusted ranges. We applied the following exclusion criteria: 1) type 2 diabetes, 2) a history of malignancy, 3) unable to self-administer rh-GH, 4) pregnancy, nursing, or subjects who may be pregnant, 5) patients deemed inappropriate by study investigators. All patients and controls were recruited from the outpatient Department of Endocrinology and Metabolism, Dokkyo Medical University Hospital. Patients were given detailed explanations of the study protocol. Informed consent was obtained from each patient. The study protocol was approved by the Ethical Committee of Dokkyo Medical University (no. #28136). The trial was registered with the University Hospital Medical Information Network (UMIN no. #000025223).

**Study protocol**

Fig. 1 summarizes the study protocol. The present study was an open-label, prospective, case-controlled study. Patients in the adult GHD group underwent assessments of body mass index, fasting glucose levels, hemoglobin A1c (HbA1c), renal function, adrenocortico-
tropic hormone (ACTH), thyroid function, lipid profiles, blood pressure, GH, IGF-1, RHI, and d-ROMs before commencing rh-GH replacement therapy, and after 24 weeks (after demonstrating normal range IGF-1 levels). Patients in the control group underwent examinations twice, at the start of observation period and after 24 weeks. No changes were made to either the antidysslipidemic agent or the hormone replacement type (e.g., hydrocortisone, thyroxine, testosterone, and vasopressin) or dose during the study period to avoid possible influences on the endothelial function and the oxidative stress. These drugs were prescribed for at least 6 months prior to the study commencement.

**Assessment of endothelial function**

Peripheral vasodilator response measurement with a fingertip pulse amplitude tonometry (PAT) device is emerging as a useful method for assessing vascular function. Reactive hyperemia peripheral arterial tonometry (RH-PAT), using an EndoPAT2000 device (Itamar Medical, Caesarea, Israel), is a validated methodology that is described in the literature [14-16]. A blood pressure cuff was placed on the subject’s upper arm, while the contralateral arm served as control [14]. PAT probes were placed on a finger in each hand. After a 5-minute equilibration period, the cuff was inflated to 60 mmHg above the systolic pressure (if systolic blood pressure was >140 mmHg) or to 200 mmHg (if systolic blood pressure was ≤140 mmHg) for 5 minutes, then deflated to induce reactive hyperemia. RH-PAT values were assessed at baseline and at 24 weeks. We defined endothelial dysfunction as a RHI <0.670. Previous studies indicate that RH-PAT technology has excellent reproducibility [15, 16].

**Derivatives of reactive oxygen metabolites (d-ROMs) evaluation**

Oxidative stress results from an imbalance between ROM production and ROS removal, by a variety of endogenous and exogenous antioxidants. In the current study, we assessed oxidative stress using a simple and recently-developed method for evaluating ROMs [17]. This assay is relatively inexpensive, can be performed in minutes, and has been used to assess the effectiveness of various antioxidant treatment strategies [18]. The d-ROMs test evaluates free radical activity by measuring the serum hydroperoxides levels (Diacron, Grosseto, Italy). The results of the d-ROMs test are expressed in arbitrary units, so-called Caratelli units (U.CARR), where 1 U.CARR corresponds to 0.08 mg/100 mL H₂O₂ [19].

**Biochemistry**

Venous blood samples and urinary samples were taken from subjects in the morning, after an overnight fast.

We evaluated the following parameters at baseline, and after 24 weeks in each group: lipid profiles, measured using standard enzymatic methods (MetaboLead high density lipoprotein (HDL-C) and MetaboLead low density lipoprotein (LDL-C), KYOWA MEDEX CO., LTD., Tokyo, Japan); HbA1c levels, measured using the automated analyzer “MetaboLead HbA1c” (KYOWA MEDEX CO., LTD., Tokyo, Japan); GH and ACTH, measured using immunoenzymometric assay (AIA-360, TOSOH Bioscience, Inc., CA, USA); IGF-1 measured by immunoradiometric assay (“IGF-1 IRMA” FUJIREBIO Inc., Tokyo, Japan); free thyroxine
(FT4), free tri-iodothyronine (FT3) and thyroid stimulating hormone (TSH), measured by chemiluminescence immunoassay (The ARCHITECT Free T3, Free T4, TSH, ABBOTT JAPAN CO., LTD., Tokyo, Japan); estimated glomerular filtration rate (eGFR), calculated as $194 \times \text{serum creatinine}^{-1.094} \times \text{age}^{-0.287}$ in male subjects, and as $194 \times \text{serum creatinine}^{-1.094} \times \text{age}^{-0.287} \times 0.739$ in female subjects.

**Statistical analysis**
Differences in continuous variables were analyzed via paired $t$-test and Wilcoxon’s matched pairs test, as appropriate. Categorical variables were compared using the chi-square test. $p$ values <0.05 were considered significant. All analyses were performed using Prism 6 (GraphPad Software, Inc., San Diego, CA, USA) or StatMate V (Nihon 3B Scientific Inc., Niigata, Japan).

**Results**

**Clinical characteristics of the subjects**
Patient clinical data are shown in Table 1 and include

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>Diagnosis</th>
<th>IGF-1 (ng/mL)</th>
<th>SD score</th>
<th>Procedures</th>
<th>Duration of GHD (years)</th>
<th>Hormones</th>
<th>Dyslipidemia</th>
<th>Smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>18</td>
<td>Craniopharyngioma</td>
<td>71</td>
<td>–3.3</td>
<td>Surgery</td>
<td>2</td>
<td>C, T, S, D</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>M</td>
<td>38</td>
<td>Idiopathic</td>
<td>24</td>
<td>–5.22</td>
<td>—</td>
<td>22</td>
<td>T</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>M</td>
<td>36</td>
<td>Pituitary hypoplasia</td>
<td>109</td>
<td>–1.69</td>
<td>—</td>
<td>21</td>
<td>S</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>M</td>
<td>26</td>
<td>Craniopharyngioma</td>
<td>68</td>
<td>–3.36</td>
<td>Surgery</td>
<td>5</td>
<td>C, T</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>M</td>
<td>68</td>
<td>Lymphocytic panhypoplasia</td>
<td>41</td>
<td>–3.18</td>
<td>—</td>
<td>5</td>
<td>C, T, D</td>
<td>Rosuvastatin 2.5 mg</td>
<td>—</td>
</tr>
<tr>
<td>F</td>
<td>67</td>
<td>Idiopathic</td>
<td>26</td>
<td>–4.17</td>
<td>—</td>
<td>12</td>
<td>C, T</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>F</td>
<td>38</td>
<td>Craniopharyngioma</td>
<td>107</td>
<td>–1.84</td>
<td>Surgery</td>
<td>8</td>
<td>C, T, D</td>
<td>Atorvastatin 5 mg</td>
<td>—</td>
</tr>
<tr>
<td>M</td>
<td>67</td>
<td>Hypophysyal stalk rupture syndrome</td>
<td>10</td>
<td>–4.68</td>
<td>—</td>
<td>1</td>
<td>C, T</td>
<td>Rosvastatin 2.5 mg</td>
<td>—</td>
</tr>
</tbody>
</table>

**M7/F2**: 43.8 ± 18.8

53.3 ± 37.1

–3.6 ± 1.3

10.2 ± 7.9

4/9 (44.4%)

0/9 (0%)

**Control patients**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>Diagnosis</th>
<th>IGF-1 (ng/mL)</th>
<th>SD score</th>
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<th>Smoking</th>
</tr>
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<tbody>
<tr>
<td>M</td>
<td>50</td>
<td>Non-functional pituitary adenoma (micro)</td>
<td>146</td>
<td>–0.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>F</td>
<td>21</td>
<td>Prolactinoma</td>
<td>321</td>
<td>0.6</td>
<td>—</td>
<td>—</td>
<td>PRL: 68 ng/mL</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>M</td>
<td>65</td>
<td>Non-functional pituitary adenoma (micro)</td>
<td>188</td>
<td>1.3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Atorvastatin 5 mg</td>
<td>—</td>
</tr>
<tr>
<td>F</td>
<td>41</td>
<td>Non-functional pituitary adenoma (micro)</td>
<td>116</td>
<td>–1.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>M</td>
<td>27</td>
<td>Prolactinoma</td>
<td>300</td>
<td>1.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>F</td>
<td>66</td>
<td>Isolated ACTH deficiency</td>
<td>99</td>
<td>–0.4</td>
<td>—</td>
<td>—</td>
<td>C</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>M</td>
<td>45</td>
<td>Isolated ACTH deficiency</td>
<td>114</td>
<td>–1.2</td>
<td>—</td>
<td>—</td>
<td>C</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>F</td>
<td>28</td>
<td>Non-functional pituitary adenoma (macro)</td>
<td>198</td>
<td>–0.3</td>
<td>Surgery</td>
<td>—</td>
<td>C, D</td>
<td>—</td>
<td>—</td>
</tr>
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</table>

**M4/F4**: 42.9 ± 17.4

185.3 ± 85.1

0.03 ± 1.0

2/8 (25.0%)

0/9 (0%)

C: hydrocortisone, T: thyroxine, S: sex steroid (testosterone), D: desmopressin
IGF-1: insulin-like growth factor, GHD: growth hormone deficiency
Data are represented as the mean ± SD or median and interquartile range

---

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IGF-1: insulin-like growth factor, GHD: growth hormone deficiency
Data are represented as the mean ± SD or median and interquartile range
sex, age, original diagnosis, IGF-1 levels before rh-GH replacement therapy, procedures, duration of GHD, and levels of other replacement hormones both in the GHD group and among the control subjects. No patient had diabetes mellitus and there were no smokers in both groups. However, some of the patients in both GHD group (44.4%) and among the control subjects (25.0%) had dyslipidemia.

Table 2 shows the clinical and biochemical parameters in the GHD group and control subjects. In both groups, thyroid hormone and ACTH were maintained within normal ranges. As expected, IGF-1 levels were significantly lower in the GHD group prior to rh-GH replacement therapy, and showed increased age-adjusted normal levels after 6 months of rh-GH replacement therapy. LDL-cholesterol levels decreased over 6 months with rh-GH replacement therapy.

Vascular endothelial function and derivatives of reactive oxygen metabolites

RHI values significantly improved (from 1.65 ± 0.33 to 1.92 ± 0.26; \( p = 0.038 \)) following rh-GH therapy over 6 months. RHI values, measured in controls, did not change significantly (from 1.76 ± 0.20 to 1.75 ± 0.14; \( p = 0.875 \)) (Fig. 2A, B). The percent change in RHI in the patients with GHD was significantly greater than that observed in the control group (Fig. 2C, \( p < 0.05 \)).

d-ROM levels, a biomarker of oxidative stress, also decreased in the rh-GH therapy group (from 356.8 ± 64.1 to 303.1 ± 43.3 U.CARR; \( p = 0.020 \); Fig. 3A). There were no changes in controls d-ROMs when comparing levels before and after 24 weeks (from 371.4 ± 95.1 to
361.0 ± 94.3 U.CARR; \( p = 0.383 \); Fig. 3B). The percent change in d-ROMs in the GHD group was significantly lower than that in the control group (Fig. 3C). The percent changes in RHI (the value at the start of the trial minus the value after 24 weeks divided by the starting value) following rh-GH treatment showed a strong inverse correlation with the percent change in d-ROMs, but not with LDL-cholesterol and IGF-1 levels (\( r = 0.778; p < 0.05 \); Fig. 4). In a stepwise regression analysis, the change in RHI levels appeared to be determined by the change of d-ROMs, although this was with a borderline significance (\( \beta = -0.7137, p = 0.055 \)); there was no significant change in LDL-cholesterol levels (\( \beta = -0.2842, p = 0.3073 \)).

**Discussion**

The results of this Japanese study, comparing between patients with GHD and controls, showed that, over 24 weeks, rh-GH replacement therapy maintained IGF-1 levels within normal ranges and improved RHI values, as demonstrated by the reduced d-ROMs in subjects with GHD. Furthermore, there was a strong inverse correlation between improvement in the RHI and d-ROM levels despite the relatively small study population.

There are several reports on the association between adults with GHD and endothelial dysfunction [6, 10, 20-22]. In these studies, flow-mediated dilation (FMD) was used to evaluate endothelial function. However, the evaluation of endothelial function using FMD is dependent on the ability of the person operating the procedure [23, 24]. Conversely, Endo-PAT is a noninvasive, quantitative technique, characterized by less operator-dependency and greater repeatability that captures a beat-to-beat recording of the finger arterial pulse-wave amplitude with pneumatic probes. Consequently, Endo-PAT combines clinical utility, practicality, and convenience [25]. Earlier reports indicate that RHI values

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**Fig. 2** Changes in the RHI in the rh-GH replacement therapy group (A) and the control group (B). Comparison of RHI improvements between the treatment and the control groups (C).

Percent change in the RHI: \[ \text{RHI after 24 weeks} - \text{RHI at the start of the trial} \] / \text{RHI at the start of the trial}. RHI values significantly improved in the rh-GH replacement therapy group; the percent change in the RHI was significant in the control group. (RHI, reactive hyperaemia index). Data are mean ± SEM.
can be used to evaluate vascular conditions and rh-GH replacement efficacy. This is the first study comparing Japanese patients with GHD and controls with other pituitary diseases, to evaluate the efficacy of rh-GH replacement therapy on endothelial function by measuring RHI values, and assessing antioxidative effects by measuring d-ROMs.

We directly observed the oxidative effects of rh-GH replacement therapy as measured by d-ROM levels, a novel global oxidative stress marker that is easy to measure. The d-ROMs test is a simple assay marketed for analyzing the total amount of hydroperoxides in serum, via Fenton’s reaction. A portion of the signal detected in the assay derives from sources other than metabolites generated by oxidative stress [26]. Ceruloplasmin (CP) activity may interfere with the detection of accurate concentrations of hydroperoxides [26]. CP interferes with the d-ROMs test, and previous studies have established the usefulness of this test for evaluating the global oxidative status of a biological sample [27]. Measuring oxidative stress using the d-ROMs test is controversial; however GH deficiency induces oxidative damage, interfering with normal endothelial function via ROS overproduction [6]. The present study involved a relatively small number of patients with GHD; however, the reduction of d-ROM levels following rh-GH replacement therapy was associated with RHI improvement.

GH replacement therapy is known to act as an antioxidant because it upregulates the expression of important intracellular antioxidant enzymes, such as catalase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase [28]. It has been shown that IGF-1 reduces oxidative

Fig. 3 Changes in d-ROMs in the rh-GH replacement therapy group (A) and the control group (B). Comparison of the RHI improvements between the rh-GH replacement therapy and the control groups (C). Percent change in d-ROMs: [d-ROMs after 24 weeks of treatment–d-ROMs at the start of the trial]/baseline d-ROMs value. d-ROMS, a biomarker of oxidative stress, significantly decreased in the rh-GH replacement therapy group. The percent change in d-ROMs in the rh-GH replacement therapy group was significantly lower than that in the control group. Data are mean ± SEM.
stress and atherosclerosis. IGF-1 enhances NO production by increasing endothelial NO synthase. IGF-1 also upregulates anti-oxidant enzymes [29]. Additionally, Thum T et al. [30] reported that IGF-1 in response to GH administration elevated circulating endothelial progenitor cell levels associated with increased NO bioavailability, thereby preventing endothelial function. However, we did not evaluate NO bioavailability and the percent changes in RHI and d-ROMs following rh-GH treatment did not show a correlation with IGF-1 levels in this clinical study. This study has a relatively small number of patients. Additional information from future experimental studies, as well as clinical studies, with regard to rh-GH treatment is needed to address these results in patients with GHD.

It has also been shown that GH replacement therapy reduces inflammatory cytokines such as interleukin-6 and C-reactive protein [20]. These results suggest that GH replacement therapy reduces vascular risks by modulating the pro-inflammatory pathway. Moreover, it was also reported that, after 6 months of GH replacement therapy, a reduction in both systolic and diastolic blood pressure occurs [20]. It has been shown in many cardiovascular trials, that small improvements in blood pressure may induce significant benefits on cardiovascular diseases. Although, we did not measure pro-inflammatory cytokines levels and there were no changes in blood pressure after GH replacement therapy, an improvement in endothelial function in this study might be closely associated with the cardio-protective effect of GH.

There were no quantitative differences in the baseline lipid profiles between patients with GHD and controls. rh-GH replacement therapy reverses abnormal lipid profiles and body composition [31-33]. Adult GHD induced abnormal body composition including, increased fat mass and reduced lean body mass. In this study, body mass index (BMI) levels in the GHD group were lower than in the control subjects, although it was difficult to explain the reasons for this. However, we did not mea-
sure waist circumference, body fat mass, and the percent body fat using the Body Composition Analyzer, widely used in many clinical and epidemiological studies [34]. Therefore, the possibility cannot be denied that lower BMI levels compared to the control subjects was affected the results of this trial.

We observed a significant reduction in LDL-C levels, but not in TG or HDL-C levels, after rh-GH replacement therapy. Evans et al. reported a strong inverse association between LDL-C and endothelial function [10]. Furthermore, LDL-C predicts endothelial function and cardiovascular disease [35]. Therefore, the ability of rh-GH replacement therapy to markedly decrease lipoxygenase expression, and LDL oxidation, could play a critical role in its vascular antioxidant effects [36]. We could not measure oxidized low-density lipoprotein levels, or establish any significant correlations between LDL-C levels and endothelial function, owing to the relatively small number of subjects. Further clinical studies are needed to assess the mechanisms underlying the ability of rh-GH replacement therapy to prevent atherosclerosis.

We also investigated patients’ liver enzyme levels, there were no differences in the liver enzyme levels after GH replacement therapy. Matsumoto R et al. reported that 24 months GH replacement therapy improved serum liver enzyme levels in patients with GHD [33]. We reasoned that this study has a relatively small number of patients and lower BMI levels to detect improved hepatic steatosis.

There are limitations to the present study. First, in this controlled study, placebo injection therapies were not administered to the patients in the control group. Further, this trial was not a single-blinded study. The number of participants was relatively small, and the study duration was short, because there are few patients in Japan, with adult-onset GHD, who consent to rh-GH replacement therapy. Nevertheless, the number of patients in our study is comparable to that assessed in previous clinical studies [5, 6, 10] on endothelial dysfunction associated with GHD. Therefore, the 17 participants evaluated in this study may be sufficient to assess the effects of rh-GH replacement therapy on endothelial function, and the production d-ROMs using the Endo-PAT and d-ROMs tests. The times taken, in some clinical investigations, to observe the effect of GH replacement therapy on endothelial functions, were similar to that in our study [10, 37]. In addition, the period of improvement of RHI and d-ROMs by reducing some risk factor for the onset of cardiovascular disease (i.e. hypertension, diabetes, dyslipidemia) were generally 3–6 months in another clinical study [14, 16-17, 38]. Therefore, we thought 24 weeks after demonstrating the normal range of IGF-1 levels might be adequate to verify the effects of GH replacement therapy. We did not also evaluate body composition, other oxidative stress biomarkers, or quality of life (QOL) using the adult hypopituitarism questionnaire. It is important to note that adult patients with GHD often have a low perceived QOL. rh-GH replacement therapy elicited significant changes in body composition, and the physical and psychosocial QOL [39]. These measures may further validate the efficacy of rh-GH replacement therapy. Finally, we were unable to verify endothelial function by comparing measurements using Endo-PAT and FMD, because of technical limitations.

In conclusion, rh-GH replacement therapy improves endothelial function, as measured by finger arterial pulse-wave amplitude, and decreased d-ROMs and LDL-C levels. There was a strong inverse correlation between the RHI and changes in d-ROMs levels, but not LDL-C levels. Further research is required to determine if d-ROM levels are a valid oxidative biomarker in rh-GH replacement therapy.

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Disclosure

None of the authors have any potential conflicts of interests associated with this research.

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