

Improvement of Endothelial Dysfunction in Acromegaly after Transsphenoidal Surgery

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Abstract. Flow-mediated vasodilatation (FMD) is a vascular functional test to detect endothelial dysfunction at the early stage of cardiovascular diseases. Patients with active acromegaly have higher morbidity and mortality due to cardiovascular events. To determine whether active acromegaly is associated with endothelial dysfunction, we studied 17 patients with active acromegaly for measurements of FMD, carotid intima-media thickness (IMT) and brachial-ankle pulse wave velocity (baPWV), and other biochemical parameters before and 3 months after transsphenoidal surgery (TSS). Baseline %FMD in patients with active acromegaly was significantly lower than that in age- and sex-matched control subjects. After TSS, the mean %FMD in acromegaly significantly increased from 5.3% to 7.4%; 12 patients had increased %FMD (responders), whereas 5 patients had decreased or unchanged %FMD (non-responders). However, neither carotid IMT nor baPWV changed after TSS. Serum levels of GH, insulin-like growth factor (IGF)-1, total cholesterol, low-density lipoprotein cholesterol (LDL-C), hemoglobin H_{A1c}, fasting plasma glucose and insulin levels, and homeostasis model assessment (HOMA)-R significantly decreased, whereas high-density lipoprotein cholesterol significantly increased. Responders had significantly lower baseline %FMD than did non-responders and both insulin levels and HOMA-R significantly decreased in responders, but not in non-responders after TSS. Simple regression analysis revealed that the change of %FMD showed a significant negative correlation with that of LDL-C, but not of IGF-1 or GH, in responders. In conclusion, it is suggested that endothelial dysfunction associated with active acromegaly improves soon after TSS, which is related to LDL-C and/or insulin resistance, but not to excess GH and/or IGF-1 itself.

Key words: Acromegaly, Endothelial dysfunction, Flow-mediated vasodilatation, Transsphenoidal surgery

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ACROMEGALY is associated with high morbidity and mortality mainly resulting from cardiovascular diseases (CVD) due to its higher prevalence of risk factors, including hypertension, diabetes and dyslipi-

demia [1]. Furthermore, excess GH and/or insulin-like growth factor (IGF)-1 has been suggested to contribute to the development of endothelial dysfunction in acromegaly [2]. Increased myocyte size, cardiac interstitial fibrosis and left ventricular hypertrophy, which are common even in young acromegaly with short duration, can be reversed by specific treatment [3]. Increased carotid artery intima-media thickness (IMT), an atherosclerotic marker, observed in acromegaly, can be decreased after disease control with lanreotide, a somatostatin analog, for 6 months [4]. Despite such association between acromegaly and cardiomyopathy, little information is available for the relationship of

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hormonal abnormalities, *i.e.* excess GH and IGF-1, to endothelial dysfunction.

The endothelial cells play a key role in vascular homeostasis through the production of nitric oxide (NO) [5]. NO, a key regulator of vascular tone, exerts its anti-atherogenic effects, including inhibition of monocyte and platelet adhesion, inflammatory cytokine production, smooth muscle cell proliferation, and oxidation of low density lipoprotein (LDL-C) [6]. Measurement of flow-mediated vasodilatation (FMD) is a sensitive and noninvasive vascular functional test to evaluate NO-dependent endothelial function, and considered as an early functional marker for CVD [7]. Patients with endocrine diseases, such as hypopituitarism, primary hyperparathyroidism, hypothyroidism and estrogen deficiency, have been reported to have endothelial dysfunction [8], suggesting its potential predictor for CVD events. It has been reported that FMD in cured acromegaly was higher than that in active acromegaly, but still lower than that in healthy controls [2]. This study, however, is a cohort transverse study, and no prospective study for comparison before and after disease control has been performed thus far.

The present study was designed to determine whether endothelium-dependent FMD is impaired in active acromegaly and improves after transsphenoidal surgery (TSS), and if so, to determine which hormonal and/or metabolic parameters are responsible for postoperative improvement of FMD.

Subjects and Methods

Patients and Subjects

We studied 17 patients (8 males and 9 females, aged 48.0 ± 3.2 years) with active acromegaly (Table 1), who consulted to our clinics and agreed to participate in this study during the period from July 2005 to February 2007. The study was approved by the Ethical Committee of our institute. Informed consent was obtained from each patient and control subject. Acromegaly was diagnosed on the basis of typical clinical features, elevated serum GH levels not suppressed below 1 ng/ml after 75 g oral glucose tolerance test (OGTT), and higher IGF-1 levels compared to those in age- and sex-matched control subjects [9]. Exclusion criteria were those with uncontrolled diabetes, severe

Table 1. Clinical characteristics of 17 acromegalic patients studied

Age (years)	48.0 \pm 3.2
Sex (male/female)	8/9
Body mass index (kg/m ²)	25.6 \pm 1.6
Disease duration (years)	10.2 \pm 1.8
Risk factors	
Obesity	7
Hypertension	5
Dyslipidemia	8
DM/IGT	4/2
Current smoker	6
Medication	
Anti-hypertensive drugs	2
Statin	1

DM: diabetes mellitus, IGT: impaired glucose tolerance

Data are shown as mean \pm SEM.

heart failure and history of ischemic heart disease. Thirteen age- and sex-matched healthy control subjects (6 males and 7 females, aged 50.2 ± 6.2 years) without cardiovascular risk factors (obesity, diabetes, dyslipidemia, smoking) were recruited in this study. Baseline clinical characteristics are shown in Table 1. Cardiovascular risk factors include obesity (body mass index (BMI) ≥ 25 kg/mm²) in 7 (41%), hypertension (systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg) in 5 (29%), dyslipidemia (triglyceride (TG) ≥ 150 mg/dl, LDL-C ≥ 140 mg/dl, high density lipoprotein (HDL-C) < 40 mg/dl) in 8 (41%), diabetes (fasting plasma glucose (FPG) ≥ 126 mg/dl at two consecutive measurements and/or ≥ 200 mg/dl 2 h after OGTT) in 4 (24%) and impaired glucose tolerance (FPG 100–125 mg/dl or 140–199 mg/dl 2 h after OGTT) in 2 (12%) and current smokers in 6 (35%). Two patients were treated with anti-hypertensive drugs (calcium-channel blockers, angiotensin receptor blockers), one with statin, but none with oral anti-diabetic drugs or insulin. One patient had recurrence at 12 years after unsuccessful TSS, while 16 patients had never been treated before this study; they received TSS before the completion of the study. Except for one patient with hyperprolactinemia, all patients had normal secretion of pituitary hormones other than GH.

Vascular functional studies

All vascular studies were carried out in the morning after an overnight fast in a quiet room at a constant

temperature of 23°C. All subjects were asked to abstain from smoking and intake of caffeine-containing food or beverage for at least 12 h before the study. All drugs were discontinued for at least 18 h before the study.

Endothelial function was measured by FMD according to the guideline by International Brachial Artery Reactivity Task Force as previously described [10]. FMD was measured as the change in diameter of the brachial artery after deflation of the cuff on the proximal position of the arm using SONOS (Philips Medical Systems, Andover, MA, USA) from B-mode ultrasound images using an 11-MHz linear artery transducer. The cuff is inflated to 250 mmHg for 5 minutes and the images of the brachial artery were recorded at 10, 30, 60, 90, 120 seconds after deflation. Measurements were taken from the anterior to the posterior interface between media and adventitia (M line) at the end of diastole measured by the R wave on continuously recorded electrocardiogram. The diameters were determined as an average diameter of triplicate measurements. Subsequently, the diameter was also measured 3 minutes after sublingual application of nitroglycerin (NTG) (0.3 mg). The response to NTG expressed as percent change relative to the diameter before the cuff inflation reflects endothelium-independent vasodilatation. Percent FMD (%FMD) of the brachial artery was expressed as the percent increase in maximal diameter after cuff deflation within 120 seconds to that of the baseline diameter. Percent NTG (%NTG)-mediated vasodilatation was expressed as the percent increase in the diameter 3 minutes after administration of NTG to that before cuff inflation. All scans for the same patients before and after treatment were performed by the same experienced operator. Inter- and intraobserver variability for repeated measurements of brachial artery in our laboratory is 0.05 ± 0.02 mm and 0.04 ± 0.02 mm, respectively.

Other vascular tests include measurements of carotid artery intima-media thickness (IMT) and brachial-ankle pulse wave velocity (baPWV) [11] using SNOS and an automated device from PWV/ABI (Omron Healthcare, Kyoto, Japan.), respectively. Scanning of the common carotid, the internal carotid and the external carotid arteries was performed bilaterally. IMT was the distance between the lumen-intima interface and the media-adventitia interface; the mean of the three determinations of right and left IMT was defined as the mean IMT [12].

Measurements of vascular functional tests, physical and biochemical parameters were performed before and 3 months after TSS. Serum GH and IGF-1 levels were determined by immunoradiometric assays (Daichi Radioisotope Institute, Tokyo, Japan and Mitsubishi Chemical Medience Corp., Tokyo, Japan, respectively). Adiponectin and highly-sensitive C-reactive protein (hsCRP) were determined by ELISA (Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan.) and by latex-enhanced immunonephelometry (Dade Behring, Deerfield, IL, USA.), respectively. Other biochemical parameters were determined by commercially available kits.

Statistical analysis

Data were expressed as mean \pm SEM. The difference in prevalence was evaluated with the chi-square test. The correlation between two continuous variables was examined by linear regression analysis. Simple regression analysis was employed to determine factors that were independently associated with categories of a dependent variable. P values less than 0.05 were considered significant. All statistical analyses were performed using Windows software StatView 5.0 (SAS Corp., Cary, NC, USA).

Results

After TSS, elevated serum GH and IGF-1 levels significantly decreased from 17.8 ± 5.5 ng/ml to 1.7 ± 0.3 ng/ml, and from 1059 ± 108 ng/ml to 240 ± 20 ng/ml, respectively (Table 2). Among 17 patients, 14 were cured (nadir GH after OGTT <1 ng/ml) after surgery, but 3 were not. Biochemical parameters, including TC, LDL-C, FPG, HbA_{1c} and fasting insulin levels as well as HOMA-R significantly decreased after TSS, while HDL-C level significantly increased. By contrast, TG, adiponectin and hsCRP levels, BMI, SBP, DBP, carotid IMT and baPWV remained unchanged after TSS; these physical and vascular functional parameters were almost comparable to those in normal control subjects (data not shown). Preoperative medications (anti-hypertensive drugs and statin) given to some patients were continued after surgery.

The mean %FMD in 17 patients with acromegaly ($5.3 \pm 0.7\%$) was significantly ($P = 0.0001$) lower than that of age- and sex-matched 13 control subjects

Table 2. Changes of biochemical, physical and vascular parameters before and after transsphenoidal surgery (TSS)

	Baseline	After TSS	P value
Biochemical parameter			
GH (ng/ml)	17.8 ± 5.5	1.7 ± 0.3	0.010
IGF-1 (ng/ml)	1058.6 ± 111.2	239.6 ± 19.7	<0.001
TC (mg/dl)	194.8 ± 9.1	179.8 ± 7.2	0.018
LDL-C (mg/dl)	116.6 ± 7.7	99.1 ± 6.3	0.026
HDL-C (mg/dl)	59.8 ± 4.3	72.1 ± 4.4	0.004
TG (mg/dl)	113.1 ± 16.5	86.8 ± 22.8	0.074
FPG (mg/dl)	113.3 ± 8.5	98.1 ± 3.2	0.042
HbA _{1c} (%)	6.0 ± 0.3	5.5 ± 0.2	0.022
Insulin (μU/ml)	10.8 ± 2.2	4.1 ± 0.5	0.002
HOMA-R	2.9 ± 0.5	1.0 ± 0.1	0.001
Adiponectin (μg/ml)	12.7 ± 2.1	13.6 ± 1.9	0.472
hsCRP (mg/dl)	0.066 ± 0.056	0.022 ± 0.010	0.388
Physical parameter			
BMI (kg/mm ²)	25.6 ± 1.6	25.5 ± 1.5	0.851
SBP (mmHg)	125.5 ± 5.2	124.0 ± 3.6	0.705
DBP (mmHg)	74.3 ± 4.2	80.7 ± 3.8	0.189
Vascular parameter			
MeanIMT (mm)	0.65 ± 0.03	0.62 ± 0.03	0.142
baPWV (cm/s)	1304.6 ± 118.0	1296.9 ± 88.8	0.946
%FMD (%)	5.3 ± 0.7	7.4 ± 0.6	0.023
%NTG (%)	17.7 ± 1.9		

IGF-1: insulin like growth factor-1, TC: total cholesterol, LDL-C: low-density cholesterol, HDL-C: high-density cholesterol, TG: triglyceride, FPG: fasting plasma glucose, HbA_{1c}: hemoglobin A_{1c}, HOMA-R: homeostasis model assessment, hsCRP: highly sensitivity C-reactive protein, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, Mean IMT: intima-media thickness, baPWV: brachial-ankle pulse wave velocity, %FMD: Flow-mediated vasodilatation, %NTG: %percent nitroglycerine-mediated vasodilatation

Data are shown as mean ± SEM.

(9.4 ± 0.6%); %NTG in patients with acromegaly (17.7 ± 1.9%) was almost comparable to that in control subjects (19.4 ± 1.2%). The mean %FMD showed a significant increase from 5.3% to 7.4% after TSS (Table 2). Vascular endothelial responses observed among all 17 patients consisted of two groups; 12 patients showed increased %FMD (responders) and 5 patients showed decreased or unchanged %FMD (non-responders). Responders had significantly lower baseline %FMD than did non-responders (Fig. 1); baseline fasting insulin levels and HOMA-R in responders tended to be greater, but not significant, than those in non-responders. There were significant differences between responders and non-responders in % changes of fasting insulin levels and HOMA-R before and after TSS (Fig. 1). However, there were no significant differences in other clinical, biochemical or vascular parameters between responders and non-responders.

To determine an independent predictor for improvement of %FMD in acromegaly after TSS, simple re-

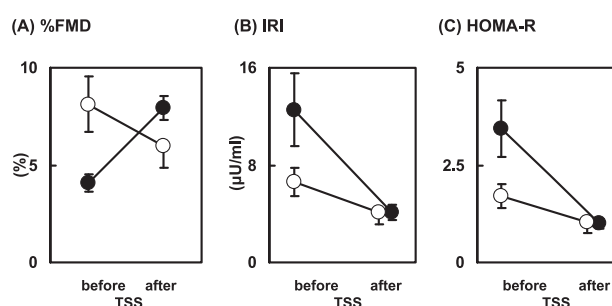


Fig. 1. Changes of %FMD, fasting insulin (IRI) and HOMA-R before and after transsphenoidal surgery (TSS). (A) Baseline %FMD in responders (●) was significantly ($P = 0.003$) lower than that in non-responders (○), which significantly ($P < 0.0001$) increased after TSS. (B) IRI levels ($P = 0.006$) and (C) HOMA-R ($P = 0.001$) in responders (●) significantly decreased after TSS, but not in non-responders (○). Each point is the mean; bar shows SE.

gression analysis was performed in 12 responders. The change of LDL-C showed a strong negative corre-

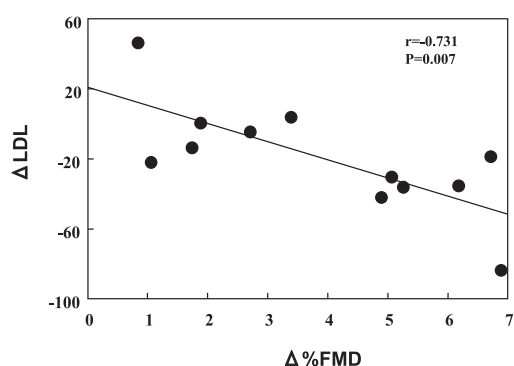


Fig. 2. Correlation between changes of %FMD and LDL-C after transsphenoidal surgery (TSS) in responders. The change of LDL-C showed strongest ($P = 0.007$) negative correlation with that of %FMD in responders. ($Y = 20.782 - 10.341X$, $P = 0.007$)

lation ($r = -0.731$, $P = 0.007$) with that of %FMD in the responders (Fig. 2). LDL-C tended to decrease, although not significantly, from 120.2 ± 9.6 mg/dl to 100.9 ± 8.9 mg/dl ($P = 0.057$); other biochemical parameters did not show any significant correlations.

Discussion

Measurement of FMD is currently regarded as an early marker of endothelial dysfunction and atherosclerosis [7]. Impaired FMD has been reported in various endocrine and metabolic diseases, such as diabetes, obesity, essential hypertension and insulin resistance [8]. Patients with acromegaly have higher incidence of CVD due to increased prevalence of cardiovascular risk factors, such as hypertension, diabetes, dyslipidemia, and insulin resistance [1]. Excess GH/IGF-1 has also been reported to cause endothelial dysfunction [2]. The present study clearly demonstrates that patients with active acromegaly had impaired FMD compared to control subjects, which improved soon after the removal of pituitary tumors. It has been reported that patients with cured acromegaly had lower %FMD than healthy subjects, but higher %FMD than those with active acromegaly [2]. Our data from the prospective study by comparison between pre- and post-treatment in the same patients are in agreement with those of a previous cohort transverse study [2].

Recently, several lines of evidence suggest the negative effect of chronic GH and IGF-I excess on cardiac

function [13, 14]. However, it is controversial whether excessive secretion of GH and IGF-1 and/or multiple risk factors associated with acromegaly contribute to the development of CVD. It has been shown that recombinant GH induces endothelial NO synthase expression and NO production in human endothelial cells [15], and that IGF-1 stimulates NO production in rat vascular smooth muscle cell [16] and diminishes vascular contractility [17]. These data strongly suggest that GH and IGF-1 have a direct and acute effect on vascular endothelial and smooth muscle cells to stimulate NO production. On the other hand, it has been reported that GH-deficient patients have a 1.9-fold higher risk of death from CVD than age-matched controls [18], and that decreased NO formation [19] and lower FMD [20] in these patients improved after treatment with recombinant human GH concomitantly with decreased visceral adipose tissue and improved lipid profile. It has been reported that bovine GH transgenic mice with chronic excess GH/IGF-1, develop hypertension and hypercholesterolemia associated with time- and vessel-specific deterioration of endothelial function [21]. Taken together, it is suggested that chronic GH and/or IGF-1 excess has an indirect deleterious effect on endothelial function by increasing serum cholesterol levels and elevating blood pressure.

The present study also shows that improvement of excess GH and IGF-1 after TSS is concomitantly associated with a significant improvement in abnormal lipid profile, that is, with decreases in TC and LDL-C and increase in HDL-C. It has been reported that patients with acromegaly have increased plasma concentrations of cholesterol [22], lipoprotein (a) and small dense LDL [23, 24], and patients with hypercholesterolemia have impaired endothelium-dependent vasodilatation [25, 26]. In fact, it has been shown that oxidized LDL-C impairs NO production [27], which plays a key role in the development of endothelial dysfunction and atherosclerosis [28] by production of reactive oxygen species [29].

The present study further shows that normalization of excess GH and IGF-1 after TSS is accompanied by decreases in HbA_{1c}, FPG, insulin and HOMA-R, *i.e.* a significant improvement in impaired glucose tolerance and insulin resistance. To determine the major parameters responsible for the improvement of FMD, patients were divided into two groups; those with increased %FMD after TSS (responders) and those with unchanged or decreased %FMD after TSS (non-

responders). In responders, fasting insulin levels and HOMA-R significantly decreased after TSS than in non-responders. This finding is consistent with a previous study showing that insulin resistance and hyperinsulinemia are independent predictors for decreased endothelium-dependent vasodilatation [30]. There are several possible mechanisms by which insulin resistance and hyperinsulinemia induce deterioration of endothelium-dependent vasodilatation. First, insulin has been shown to decrease eNOS production [31]. Second, insulin has been shown to increase synthesis of potent vasoconstrictor endothelin-1 [32] and oxidative stress [33] in endothelial cells. The present results with greater decreases in fasting insulin levels and HOMA-R in responders than in non-responders strongly suggest that a group of active acromegaly with insulin resistance is more likely to have endothelial dysfunction than a group without insulin resistance. Thus, it is possible to speculate that insulin resistance may play a pivotal role in the development of endothelial dysfunction in acromegaly.

It has been reported that carotid IMT was greater in acromegaly than in healthy control [34], which was reduced after lanreotide treatment for 6 months [4]. In the present study, however, there was no significant

difference of carotid IMT between active acromegalic patients and control subjects, which did not change after TSS. Measurement of FMD is superior to that of carotid IMT for detection of the functional vascular change during the early process of atherosclerosis. The different results on carotid IMT in the present and other studies [4, 34] may be accounted for by the patient population studied, including accumulation of risk factors, disease duration, follow-up periods and so forth.

In conclusion, patients with active acromegaly have endothelial dysfunction which improved soon after TSS largely resulting from decrease in LDL-C and/or improvement of insulin resistance rather than decrease in GH and/or IGF-1 per se.

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