

**Coronary microvascular dysfunction may be induced by insulin-like growth factor-1 in acromegalic patients and can be restored by treatment with somatostatin analogues**

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## Abstract

**Context:** Acromegaly increases the risk of cardiovascular mortality. Data on the cardiovascular risk in asymptomatic acromegaly are limited. In particular, data on coronary microvascular abnormalities are lacking.

**Objective:** We assessed coronary flow reserve (CFR) as a marker of coronary microvascular function in asymptomatic acromegaly.

**Design and Setting:** This was a cross-sectional case control and observational study.

**Participants:** Forty acromegalic patients (23 male, age  $52 \pm 11$  years) without clinical evidence of cardiovascular disease, and 40 control subjects matched for age and sex.

**Outcome Measurements:** We compared CFR in patients and control subjects. We also explored if therapy with somatostatin analogues increase coronary microvascular function.

**Results:** CFR was lower in patients than in controls ( $2.9 \pm 0.8$  vs  $3.7 \pm 0.6$ ,  $p < 0.0001$ ) and was abnormal ( $\leq 2.5$ ) in 13 patients (32.5%) compared with any control subjects ( $p < 0.0001$ ). CFR was inversely related to insulin-like growth factor 1 (IGF-1) levels ( $r = -0.5$ ,  $p < 0.004$ ). In patients with  $CFR \leq 2.5$ , IGF-1 was higher ( $756 [381-898]$   $\mu\text{g/l}$  versus  $246 [186-484]$   $\mu\text{g/l}$ ,  $p < 0.007$ ) whereas growth hormone (GH) levels were similar ( $6.3 [2.8-13.7]$   $\mu\text{g/l}$  versus  $5 [2.8-8.9]$   $\mu\text{g/l}$ ,  $p = 0.8$ ). In multivariable linear regression analysis, IGF-1 was independently associated with CFR ( $p < 0.0001$ ). In multiple logistic regression analysis, IGF-1 independently increased the probability of  $CFR \leq 2.5$  ( $p = 0.009$ ). In patients with active disease, treatment with somatostatin analogues normalized CFR.

**Conclusions:** Acromegalic patients may have coronary microvascular dysfunction that may be restored by therapy with somatostatin analogues. IGF-1 seems to independently correlate with the coronary microvascular impairment, suggesting a potential role of this hormone in explaining the increased cardiovascular risk in acromegaly.

## Introduction

Acromegaly is a relatively rare disease characterized by growth hormone (GH) and insulin-like growth factor (IGF)-1 excess, which in most cases is caused by a GH-secreting pituitary adenoma (1).

Systemic complications of GH/IGF-1 excess include acromegalic cardiomyopathy, typical features of which are concentric biventricular hypertrophy and diastolic dysfunction and finally heart failure (2-3). Cardiovascular events are frequent, representing the first cause of death in acromegaly (2).

Coronary risk factors like hypertension, diabetes mellitus, and dyslipidemia are frequent in acromegaly, thus providing a possible link between GH/IGF-1 hypersecretion, vascular abnormalities and coronary artery disease (CAD) (4). IGF-1 is a potent mitogen for vascular smooth muscle cells (5) and stimulates the expression of adhesion molecules (6), a feature of endothelial dysfunction. On the other hand, IGF-1 stimulates nitric oxide production from both the endothelium and vascular smooth muscle cells (VSMC) (7). Therefore, data about the role of GH/IGF-1 on atherosclerosis in acromegalic patients are scanty and controversial.

It is not well known whether GH or IGF-1 alterations, *per se*, may compromise cardiovascular function. Several investigations have documented acromegaly-associated endothelial dysfunction and subclinical cardiac dysfunction (8). Indeed the reversal of cardiovascular dysfunction after successful treatment of acromegaly further supports the concept of a specific role of GH or IGF-1 in the pathogenesis of cardiovascular disease in acromegaly (9).

The aim of our study was to determine the influence of acromegaly on coronary microvascular function, assessed by coronary flow reserve (CFR) by transthoracic Doppler echocardiography (TDE), in patients with asymptomatic acromegaly without evidence for epicardial CAD as assessed by multislice computed tomography (MSCT) coronary angiography.

## Materials and Methods

### Study population

In this cross-sectional case control and observational study, we enrolled 40 consecutive acromegalic patients (23 male; aged 52±11 years). Active acromegaly was defined in patients presenting with the typical clinical features, by high serum GH levels (mean of three serum GH baseline samples >2.5

µg/l) and/or GH not suppressible by the glucose tolerance test (GH >0.4 µg/l), in association with high IGF-1 levels (according to age- and gender-adjusted values) and evidence of a pituitary adenoma on magnetic resonance imaging. Patients who had recently undergone a neurosurgery or radiation treatment, who had recently been hospitalized for a cardiovascular event were excluded from this study. Thyroid and parathyroid function were normal in all patients. Acromegaly was considered cured when safe hormone levels were achieved, *i.e.* fasting or glucose-suppressed GH levels below 2.5 µg/l or 0.4 µg/l, respectively, together with normal IGF-1 levels. Baseline evaluation included physical examination and collection of clinical and laboratory data (Table 1). The median time from diagnosis was 5 years (range 2-10 years). Patients with a history or evidence of cardiopulmonary, renal (serum creatinine >133 µmol/l in men and >120 µmol/l in women), or hepatic disease and malignant or infectious disease were excluded. The nonrandomized control group consisted of 40 healthy volunteers recruited from institutional personnel who were matched for age and sex. Characteristics of control group and cardiovascular risk factor definition are provided in the Supplementary material online.

In a subgroup of 8 patients with active acromegaly, CFR was repeated after 10 months (range 7.5-14 months) of therapy. The study was approved by the institutional ethics committee, and all patients gave written informed consent.

#### **Doppler echocardiography and CFR assessment**

Transthoracic Doppler echocardiography was performed with a commercially available ultrasound system (Vivid 7, GE Medical System, Inc., Hortem, Norway). All images were analyzed offline by two investigators (F.T. and G.F.), blinded to clinical data. Coronary images were obtained in the distal part of the left anterior descending artery (LAD) with 7.MHz transducer. Coronary blood flow was obtained by color Doppler flow-mapping guidance, and sample volume was positioned within the color signal in the LAD artery by pulse wave Doppler. For Doppler echocardiographic methods and CFR assessment see Supplementary material online. A  $CFR \leq 2.5$  was considered abnormal.

#### **MSCT coronary angiography protocol and interpretation**

Patients with abnormal CFR underwent to MSCT coronary angiography to exclude epicardial

significant CAD and calcium score. The MSCT protocol is detailed in the Supplementary material online.

### **Laboratory methods**

Fasting serum samples were collected at baseline and at the end of the study and stored at -80°C until they were analysed. For laboratory methods see Supplementary material online.

### **Statistical analyses**

Continuous variables with no/mild skew were presented as mean  $\pm$  SD; skewed measures as median were represented with first and third quartiles (Q1-Q3). Discrete variables were summarized as frequencies and percentages. The distribution of the data was analyzed with a 1-sample Shapiro-Wilk test. Logarithmic transformation was performed to achieve normal distribution for skewed variables. Categorical variables were compared by the  $\chi^2$  test or the Fisher exact test as appropriate. Continuous data were compared by use of the 2-tailed paired or unpaired  $t$  test (for normally distributed data sets) or the Mann-Whitney  $U$  or Wilcoxon signed-rank test (for skewed variables). Bivariate correlations were assessed by the Pearson coefficient ( $r$ ). Unadjusted and multiple linear regression analyses were performed between CFR and risk factors or clinical conditions. Stepwise logistic regression analysis was used to model normal versus abnormal CFR as a function of IGF-1 and other coronary risk factors or clinical conditions. Baseline characteristics were chosen for entry into multivariable models on the basis of their discrimination between low and high CFR and on unadjusted association with CFR  $\leq 2.5$  of  $p \leq 0.1$ . A combination of forward and backward selection procedures was used to aid in determining the best model of factors independently associated with CFR. This was followed by forcing potential confounders into the models and determining their effect on the relationship of interest. Risk factors were removed if they did not significantly add to the model. Summary statistics for the regression models included the C statistic (a measure of association of predicted probabilities and observed prevalence of a binary outcome) and  $R^2$  (rescaled for use in logistic regression by the Cox and Snell method). Intra-observer and inter-observer reproducibility of CFR was evaluated by linear regression analysis and expressed as the correlation of coefficients ( $r$ ) and standard error of estimates and by the intraclass correlation coefficient (ICC). Reproducibility is considered satisfactory if the ICC is between 0.81 and 1.0. Intra-observer and inter-observer reproducibility measurements

were calculated in all patients. All tests were 2 sided and statistical significance was accepted if the null hypothesis could be rejected at  $p < 0.05$ . Data were analyzed with SPSS software version 22.0 (Chicago, SPSS, Inc., Chicago, Illinois). The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

## Results

### Baseline clinical features and CFR evaluation

Patients characteristics are presented in Table 1. CFR was lower in patients than in controls ( $2.9 \pm 0.8$  vs  $3.7 \pm 0.6$ ,  $p < 0.0001$ ). The prevalence of  $CFR \leq 2.5$  was higher in patients (32.5% vs 0%;  $p < 0.0001$ ). Overall, during adenosine infusion, heart rate increased ( $76 \pm 12$  vs  $93 \pm 17$  bpm/min;  $p < 0.0001$ ), systolic blood pressure decreased ( $126 \pm 18$  vs  $113 \pm 19$  mmHg;  $p < 0.0001$ ) and diastolic blood pressure decreased ( $80$  [70-80] vs  $70$  [60-80] mmHg;  $p < 0.0001$ ), whereas peak diastolic flow velocity (DFV) in the LAD coronary artery increased ( $22$  [19-26] vs  $68$  [53-79] cm/sec;  $p < 0.0001$ ). There were no significant electrocardiographic changes or LV wall motion abnormalities during adenosine infusion in any of our patients and controls. Diastolic dysfunction was moderate (impaired relaxation) in all patients. Six patients were on treatment with  $\beta$ -blockers, 10 with angiotensin-converting enzyme inhibitors, 7 with calcium-antagonists, 4 with AT1-receptor antagonists and 6 with diuretics and 11 patients with Pegvisomant.

### Hemodynamic parameters based on CFR value

Heart rate at rest and during adenosine infusion was similar in patients with  $CFR \leq 2.5$  and patients with  $CFR > 2.5$  ( $76 \pm 13$  vs  $76 \pm 12$  beats/min,  $p = 1$  and  $92 \pm 22$  vs  $93 \pm 15$  beats/min,  $p = 0.9$ , respectively). Systolic blood pressure at rest was higher in patients with  $CFR \leq 2.5$  ( $138 \pm 14$  vs  $122 \pm 13$  mmHg;  $p = 0.01$ ). Diastolic blood pressure at rest was similar in the two patient groups ( $76 \pm 10$  vs  $78 \pm 10$  mmHg;  $p = 0.6$ ). Systolic blood pressure during adenosine was similar in the two patient groups ( $107 \pm 12$  vs  $117 \pm 21$  mmHg;  $p = 0.2$ ) as well as diastolic blood pressure during adenosine ( $65 \pm 11$  vs  $67 \pm 11$  mmHg;  $p = 0.6$ ). Baseline peak DFV was higher in patients with  $CFR \leq 2.5$  ( $23 \pm 6$  vs  $20 \pm 5$  cm/s,  $p = 0.03$ ), reflecting the increase in blood pressure and, consequently, cardiac work and myocardial oxygen demand. However, this difference was still present even after normalization of resting DFV by

the rate-pressure product, as an index of cardiac work ( $26\pm 6$  vs  $22\pm 5$  cm/s,  $p=0.02$ ). Hyperemic peak DFV as well as CFR were significantly lower in patients with  $CFR\leq 2.5$  compared with patients with normal CFR ( $43\pm 5$  vs  $74\pm 4$  cm/s,  $p<0.002$  and  $1.9\pm 0.4$  vs  $3.4\pm 0.6$ ,  $p<0.0001$ , respectively). Also corrected CFR was lower in patients with  $CFR\leq 2.5$  ( $2.1\pm 0.5$  vs  $3.7\pm 0.6$ ,  $p<0.0001$ ).

### **Characteristics of patients with $CFR\leq 2.5$**

The clinical characteristics of patients with  $CFR\leq 2.5$  and patients with  $CFR>2.5$  are given in Table 2. IGF-1 levels were higher in patients with  $CFR\leq 2.5$  ( $p<0.007$ ) (Figure 1). Patients with coronary microvascular dysfunction had a higher prevalence of active disease ( $p=0.03$ ). Diastolic dysfunction and plasma GH levels were comparable in the two groups as well as echocardiographic features but LV mass ( $p=0.03$ ). Left ventricular ejection fraction (LVEF) was lower but normal in patients with  $CFR\leq 2.5$  ( $p=0.02$ ). Therapy was not different between groups. 12 patients with  $CFR\leq 2.5$  had normal coronary arteries at MSCT. Only 1 patient had a mild LAD coronary stenosis ( $<50\%$ ). Calcium score was  $<50$  in all patients with  $CFR\leq 2.5$ .

### **Factors associated with CFR**

Factors associated with CFR were IGF-1 ( $p<0.0001$ ), active acromegaly ( $p<0.003$ ), hypertension ( $p=0.01$ ), and diabetes ( $p=0.002$ ). In multivariable analysis, only IGF-1 ( $p<0.0001$ ), hypertension ( $p=0.04$ ), and diabetes ( $p=0.01$ ) were independently associated with CFR. If diabetic patients were excluded from the analysis, IGF-1 ( $p<0.0001$ ), and time from diagnosis ( $p=0.01$ ) were independently associated with CFR.

### **Factors associated with coronary microvascular dysfunction ( $CFR\leq 2.5$ )**

In unadjusted logistic regression analysis, the significant or marginally significant ( $p<0.1$ ) risk factors were IGF-1 ( $p<0.01$ ), LVEF ( $p=0.04$ ), active phase of disease ( $p=0.03$ ), age ( $p=0.09$ ), left ventricular end-diastolic volume (LVEDV) ( $p=0.09$ ), hypercholesterolemia ( $p=0.06$ ), time from diagnosis ( $p=0.06$ ) and left ventricular hypertrophy ( $p=0.05$ ). Factors independently associated with abnormal CFR are summarized in Table 3. When modelled using a stepwise regression and adjusting for baseline differences, the factor independently associated with  $CFR\leq 2.5$  was only IGF-1 (odds ratio 6.2; 95% confidence interval, 2.7-10;  $p=0.009$ ) (Table 3). The inclusion of heart-rate product did not greatly affect the model and no other clinical characteristic entered as a significant covariate. To

exclude the modulating effect of other variables, we also added variables marginally significant in unadjusted analysis (model 2). In this model, left ventricular hypertrophy and age were also independently associated with  $\text{CFR} \leq 2.5$ , but their addition to the model did not significantly affect the relationship between IGF-1 and  $\text{CFR} \leq 2.5$  (Table 3). When other conditions (current smoking, history of hypertension or diabetes mellitus, obesity, and diastolic dysfunction) were forced into the model (potentially overfitting it), IGF-1 remained significantly associated with  $\text{CFR} \leq 2.5$  ( $p=0.01$ ) (model 3). The C statistic for model 1 was 0.71 (95% confidence interval, 0.55-0.87) without IGF-1 and 0.83 (95% confidence interval, 0.70-0.96) with IGF-1.

### **Active versus controlled acromegaly**

Acromegaly was active in 28 patients and controlled in 12 patients. IGF-1 was higher in patients with active disease (525 [376-780] vs 159 [117-198]  $\mu\text{g/l}$ ,  $p<0.0001$ ) as well as HOMA index ( $2.1 \pm 0.2$  vs  $1.1 \pm 0.2$ ,  $p=0.01$ ). Baseline peak DFV was higher in patients with active acromegaly ( $28 \pm 5$  vs  $23 \pm 5$   $\text{cm/s}$ ,  $p=0.03$ ). Hyperemic peak DFV as well as CFR were lower in patients with active disease ( $61 \pm 19$  vs  $78 \pm 16$   $\text{cm/s}$ ,  $p=0.01$  and  $2.6 \pm 0.8$  vs  $3.5 \pm 0.6$ ,  $p=0.003$ , respectively). Even corrected CFR was lower in patients with active acromegaly ( $2.8 \pm 0.5$  vs  $3.9 \pm 0.6$ ,  $p=0.007$ ).  $\text{CFR} \leq 2.5$  was more frequent in patients with active disease ( $p=0.02$ ).

### **Correlation between CFR, clinical and biochemical characteristics**

Bivariate correlation analysis revealed significant and inverse correlation between CFR and IGF-1 ( $r=-0.583$ ,  $p<0.0001$ ), whereas no correlation with GH was observed ( $p=0.6$ ) (Figure 2). A negative relationship between resting DFV and time from diagnosis ( $r=-0.339$ ,  $p=0.03$ ) and between hyperemic DFV and age ( $r=-0.411$ ,  $p=0.009$ ), glycaemia ( $r=-0.379$ ,  $p=0.01$ ), and FRS ( $r=-0.374$ ,  $p=0.01$ ) were found. A significant correlation was also observed between IGF-1, LV mass ( $r=0.342$ ,  $p=0.03$ ) and GH ( $r=0.347$ ,  $p=0.02$ ).

### **Effect of treatment on coronary microvascular dysfunction**

In a subgroup of 8 patients with active disease (5 patients with  $\text{CFR} \leq 2.5$  and 3 patients with  $\text{CFR} > 2.5$ ), CFR was repeated after 10 months (range 7.5-14 months) of therapy with Somatostatin analogues. IGF-1 levels dropped from 207 (160-780) to 159 (135-191)  $\mu\text{g/l}$  ( $p=0.002$ ). Taking into account all 8 patients, CFR increased from  $2.3 \pm 0.4$  to  $2.7 \pm 0.5$  ( $p=0.2$ ) and remained lower than in



controls ( $p=0.01$ ). However, in 4 patients (everyone with  $CFR \leq 2.5$ ) CFR increased from  $2.1 \pm 0.4$  to  $3.0 \pm 0.5$  ( $p < 0.01$ ) and was comparable to controls ( $p=0.2$ ). In the remaining 4 patients CFR decreased from  $2.6 \pm 0.3$  to  $2.3 \pm 0.4$  ( $p=0.1$ ) (Figure 3). Time from onset of symptoms to diagnosis was longer in patients in which CFR has not improved ( $12.2 \pm 2.6$  vs  $5.7 \pm 0.9$  years,  $p=0.004$ ).

### **Factors associated with CFR changes**

The median CFR change after therapy ( $\Delta CFR$ ) was  $+0.25$  (range  $-0.32$ - $+0.8$ ).  $\Delta CFR$  was inversely correlated with time from symptoms to diagnosis ( $r=-0.806$ ,  $p=0.01$ ), with LogGH ( $r=0.764$ ,  $p=0.02$ ) and LogIGF-1 before therapy ( $r=0.714$ ,  $p=0.02$ ).

### **Intra- and inter-observer reproducibility of CFR by transthoracic echocardiography**

Intra-observer and inter-observer reproducibility of CFR measurements were assessed by repeating CFR evaluation twice, 1 h apart, by the same operator (F.T.) in all patients and by another operator (G.F.) in all patients as well. The intra-observer reproducibility was high ( $r=0.93$ ,  $SEE=0.11$ ); ICC was 0.970. The inter-observer reproducibility was also high ( $r=0.90$ ,  $SEE=0.10$ ); ICC was 0.963.

## **Discussion**

The present study demonstrate that acromegaly is associated with coronary microvascular dysfunction in patients without CAD and that IGF-1 seems to be the principal determinant of this microvascular dysfunction. Indeed, the lower CFR in patients with active acromegaly and normalization of CFR in some patients after treatment suggest a novel role of IGF-1 in the pathophysiology of cardiovascular disease.

The poor prognosis of acromegaly is also due to a specific cardiomyopathy (2-3). IGF-1 is thought to play an important role in the pathogenesis of vascular damage under various conditions (10). It has been suggested that IGF-1 may increase the risk of the development of angiopathy as well as cardiovascular morbidity and mortality (11). However, other studies suggest a protective effect of IGF-1 on the development of vascular damage (12). Anyhow, the involvement of IGF-1 in the development of vascular damage is generally accepted. However, the detailed mechanism of its action is not completely understood and the conclusions of various studies are rather contradictory. Some experimental studies have shown that IGF-1 caused vasoconstriction (13). Other studies have shown

vasodilation that is nitric oxide synthase (NOS) dependent (14) or nitric oxide (NO) independent (15). IGF-1 may influence endothelial and VSMC (16-18).

On the basis of these previous findings, we have pointed out the coronary microcirculation as an IGF-1 target. Baseline coronary DFV, even after normalization by the rate-pressure product, was higher in patients with  $CFR \leq 2.5$  and in patients with active acromegaly. This is consistent with a resting microvascular vasorelaxation and a reduction of arteriolar resistance, and could alone account for the lower CFR.

The notion that IGF-1 cause an increase in blood flow and a decrease in vascular resistance supports our findings (15, 19). IGF-1 has effects on the regulation of vascular tone that are similar to those of insulin, with regional differences in vascular responses (15). Unlike insulin, however, IGF-1 is produced in cardiovascular tissue, where it exerts autocrine/paracrine effects (20). In particular, IGF-1 reduce  $Ca^{2+}$  influx into VSMCs by attenuating both voltage- and receptor-operated  $Ca^{2+}$  channels in conjunction with reductions in VSMC contractile responses (21). Another mechanism by which IGF-1 may modulate VSMC  $[Ca^{2+}]_i$ , and thus vascular contractility, is through stimulation of the  $Na^+, K^+$ -ATPase pump, which would reduce  $[Ca^{2+}]_i$  via changes in  $Na^+-Ca^+$  exchange. IGF-1 stimulates  $Na^+, K^+$ -ATPase activity in several tissues, including VSMCs (21-22).

The physiological consequences of IGF-1 action are potentially altered by several mechanisms (21). Indeed, IGF-1 regulate vascular tone, in part, by decreasing vasoconstrictor responses to agonists such as angiotensin II, norepinephrine and vasopressin (23), in part through vascular production of NO (24). Other studies in animal models, have shown that in coronary microvessels relaxation to IGF-1 is not mediated by NO/cyclooxygenase pathways, but rather by hyperpolarization through  $K^+$  channels (15). Moreover, IGF-1 may stimulate production of NO via induction of inducible NOS in VSMC, as well as by stimulation of endothelial NOS (24). Furthermore, it has been demonstrated that IGF-1 may serve as an endogenous regulator of endothelin-mediated vasoconstriction, attenuating endothelin A-receptor-mediated coronary contraction through an endothelium-independent mechanism (25).

The inverse relationship between resting DFV and time from diagnosis suggests that the conceivable role of IGF-1 as a vasodilating factor decreases over time and other mechanisms are involved in the pathogenesis of a low CFR in the prolonged exposure to IGF-1. Interestingly, we also

observed a reduced CFR caused by abnormal DFV increase during adenosine. Although CFR assessment by adenosine does not allow us to distinguish between endothelium-dependent and -independent abnormalities (26), this functional alteration can be recognized as the earliest detectable impairment in the process leading to the microvasculopathy. The occurrence of proliferative wall thickening of small intramural vessels in acromegalic hearts has been recognized previously, even if in untreated patients and with a very long duration of the disease (27). We could not exclude the presence of microvascular structural remodeling by means of currently available imaging techniques. However, the normal CFR in patients with controlled acromegaly suggests a predominant “functional remodeling” due to a direct effect of IGF-1 on microvascular function and potentially reversible.

Cardiovascular risk factors in acromegaly may also account for coronary microvascular dysfunction. Hypercholesterolemia, diabetes and obesity have been well documented in acromegaly, and there is also evidence that the structure and function of coronary microvessels can be altered by these conditions (2, 28). Emerging evidence indicates that a low-grade systemic inflammation that results from risk factor-induced cell activation and cell-cell interactions, may underlie the microvascular changes induced by risk factor exposure (28). In particular IGF-1 increase has been associated with metabolic syndrome (2, 4). The metabolic syndrome is associated with a state of coronary vascular dysfunction that significantly impairs the ability of the coronary microcirculation to match myocardial oxygen delivery with myocardial metabolism (28).

Systemic hypertension and concentric hypertrophy are the most common feature of cardiac involvement in acromegaly (2-3). Functional and structural alterations of the coronary circulation have been well documented in all forms of pathologic left ventricular hypertrophy, in hypertensive cardiomyopathy as well as in acromegaly (3, 28). As a consequence CFR is reduced and minimal coronary resistance is increased significantly. The reduction of CFR in hypertrophy is caused both by a concomitant increase of resting myocardial blood flow, due to higher workload and oxygen consumption, and a reduction of hyperemic response to endothelial-dependent and -independent stressors (28).

### **Study limitations**

This study has some limitations. First, our study is cross-sectional, and conclusions about causal and

temporal order between IGF-1 and CFR cannot be drawn. Second, sample variability in GH and IGF-1 assays may increase random error. However, all assays used in the present study have acceptable levels of precision. Nevertheless, it is possible that the lack of significant results from GH is due in part to random error. Third, we measured total serum IGF-1 as an accepted measure of IGF-1 status. A recently reported bioassay based on activation of the IGF-1-specific kinase receptor may provide a means of assessing circulating bioactive IGF-1, but nowadays this method is not generally used. Furthermore, biological effects and bioavailability of IGF-1 are modulated through IGF binding proteins (IGFBPs) (11). Unfortunately, we did not have data about IGFBPs, and therefore our results do not fully represent biologically active IGF-1. Fourth, the relatively small number of patients, in particular patients with abnormal CFR, limits statistical power and creates the risk of overfitting the models when adding covariates. However, the addition of covariates, in any case, has influenced the relationship between IGF-1 and CFR. Fifth, the effects of GH on CFR cannot be completely excluded because its measurements have been partly biased in the small group of patients on treatment with Pegvisomant. Finally, neither any of the control subjects nor any of patients with normal CFR underwent MSCT or coronary angiography. It would be unethical to submit asymptomatic subjects with normal CFR to invasive diagnostic procedures.

### **Conclusions**

Our findings unmask a potential specific role of IGF-1 excess in coronary microvascular dysfunction and show that the control of the acromegaly may contribute to improve this impairment. In our study, IGF-1 correlates with CFR independently of established cardiovascular risk factors and other determinants. Our data can help to explain why IGF-1 is associated with increased cardiovascular risk, although the molecular mechanism involved need to be investigated further.

## Disclosures

None

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## Figure legends

**Figure 1:** IGF-1 in patients with normal and abnormal CFR. Each plot displays individual values, 25<sup>th</sup> percentile, median and 75<sup>th</sup> percentile.

**Figure 2:** Correlation between CFR and LogIGF-1.

**Figure 3:** Plot of individual changes in CFR before and after therapy. In 4 patients (everyone with CFR  $\leq 2.5$ ; red dots) CFR increased from  $2.1 \pm 0.4$  to  $3.0 \pm 0.5$  ( $p < 0.01$ ). In 4 patients (white dots) CFR decreased from  $2.6 \pm 0.3$  to  $2.3 \pm 0.4$  ( $p = 0.1$ )