Acromegalic Patients Show the Presence of Hypertrophic Remodeling of Subcutaneous Small Resistance Arteries

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Abstract—Structural alterations of small resistance arteries in patients with essential hypertension (EH) are mostly characterized by inward eutrophic remodeling. However, we have observed the presence of hypertrophic remodeling in patients with renovascular hypertension, as well as in patients with noninsulin-dependent diabetes mellitus, suggesting a relevant effect of humoral growth factors on vascular structure. Growth hormone may stimulate in vitro proliferation of vascular smooth muscle cells. However, no data are presently available about small artery structure in acromegalic patients. Therefore, we have investigated the structure of subcutaneous small arteries in 12 normotensive (NT) subjects, in 12 EH subjects, and in 9 acromegalic patients (APs). All subjects underwent biopsy of the subcutaneous fat; then, small resistance arteries were dissected and mounted on a micromyograph. The normalized internal diameter, media thickness, media-to-lumen ratio, the media cross-sectional area together with remodeling, and growth indices were calculated. Demographic variables were similar in the three groups, except for blood pressure. The media-to-lumen ratio was significantly greater in EH and AP, compared with NT. No difference was observed between EH and AP. The media cross-sectional area was significantly greater in AP compared with EH and with NT. The calculation of remodeling and growth index suggests the presence of eutrophic remodeling in EH (growth index 0%) and of hypertrophic remodeling in AP (growth index 40%). In conclusion, our data suggest the presence of hypertrophic remodeling of subcutaneous small resistance arteries of AP, probably as a consequence of growth-stimulator properties of IGF-1. (Hypertension. 2004;43:561-565.)

Key Words: arteries ■ hypertrophy ■ remodeling ■ growth hormone

Structural alterations of small resistance arteries (lumen diameter: 100 to 300 μm) of patients with essential hypertension (EH) are mostly characterized by inward eutrophic remodeling (rearrangement of the same wall material around a narrowed lumen).2,3 At variance, we have observed the presence of hypertrophic remodeling (vascular smooth muscle cell hypertrophy or hyperplasia) in subcutaneous small arteries of patients with renovascular hypertension.4 Neurohumoral factors are probably involved in the genesis of vascular structural alterations, and growth factors, such as angiotensin II, seem to be able to induce smooth muscle cell hypertrophy.5,6

Also, patients with noninsulin-dependent diabetes mellitus showed the presence of hypertrophic remodeling of subcutaneous small arteries,7 suggesting, again, a relevant effect of humoral growth factors on vascular structure. In fact, insulin and insulin-like growth factor-1 (IGF-1) seem to be able to stimulate cardiac and vascular smooth muscle cell growth.8 A weak but statistically significant correlation between media-to-lumen ratio of subcutaneous small arteries and levels of circulating insulin was observed in patients with diabetes mellitus.7

It was previously demonstrated that growth hormone (GH) may stimulate in vitro and in vivo proliferation of muscle cells,9,10 either directly or via its peripheral mediator IGF-1. In addition, acromegalic patients (APs) show an increased prevalence of hypertension (∼33%) and of glucose disorders (∼25%).11 The deleterious effects of GH/IGF-1 excess on cardiac structure and function have been widely demonstrated by in vivo and in vitro studies,12 whereas very few data are available on the vascular consequences of acromegaly. An increased intima-media thickness of common carotid arteries was demonstrated by Colao et al,13 while some evidence of morphological alterations in the peripheral circulation was observed by a capillaroscopic approach.14 However, no data are presently available about small artery structure in APs.

Given all these considerations, we aimed to investigate the structure of subcutaneous small arteries of normotensive (NT) subjects, of patients with EH, and of APs using a precise and reliable micromyographic technique.
Patients and Methods

Twelve NT subjects, 12 patients with EH, and 9 patients with acromegaly caused by GH-secreting pituitary adenoma were included in the study. Their age range was 40 to 80 years. The presence of hypertension was established according to ISH/WHO Guidelines. The possible presence of diabetes mellitus was established according to the Guidelines of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Acromegaly was diagnosed after proper investigation by imaging techniques and humoral assessments.

Venous blood samples were taken with the participants in the supine position, after a therapeutic washout period of at least 2 weeks, for standard hematology and serum biochemistry tests (including triglycerides and total cholesterol). In addition, in Aps, GH and IGF-1 plasma levels were evaluated by radio immune assay.

Micromyography

All participants underwent biopsy of subcutaneous fat from the gluteal region (3-cm long, 0.5-cm wide, 1.5-cm deep). Small arteries (~100 to 280 μm of average diameter in relaxed conditions; 2-mm-long) were dissected from the subcutaneous fat of the biopsy results and mounted as a ring preparation on an isometric myograph (410 A; JP Trading, Aarhus, Denmark) by threading onto 2 stainless-steel wires (40-μm diameter). The following morphological parameters were measured: normalized internal diameter, media thickness, wall thickness, media-to-lumen ratio, and media cross-sectional area. Details about the micromyographic technique of evaluation of small arteries were previously reported.

A calculation of the remodeling and growth indices was then performed according to Heagerty et al. The remodeling index quantifies how much of the vascular structural alteration may be explained by a rearrangement of the same material around a narrowed lumen, without cell growth. The remodeled internal diameter may be calculated from the normalized external diameter of the hypertensive patients and from the cross-sectional area measured in the group of NT subjects. The remodeling index can be calculated from the remodeled internal diameter and from the normalized internal diameters observed in the NT subjects and in the hypertensive patients. The growth index can be calculated from the media cross-sectional area observed in NT subjects and in hypertensive patients. Because of the different method of calculation of remodeling index and growth index, the sum of the values is usually >100%.

The presence of a remodeling index <100% means that not all the difference in media-to-lumen ratio may be explained by eutrophic remodeling. The presence of a growth index >0% means that a component of vascular growth participates in the development of vascular structural alterations.

The average values obtained from two vessels in each experiment were considered. Correlation coefficients between media-to-lumen ratio measured in the two arteries obtained from each biopsy was 0.79. The protocol of the study was approved by the ethics committee of our institution (Medical School of University of Brescia), and informed consent was obtained from each participant. The procedures followed were in accordance with institutional guidelines.

Statistical Analysis

All data are expressed as mean±SEM, unless otherwise stated. One-way ANOVA was used to evaluate differences among groups. The relation between continuous variables was evaluated by linear regression. Pearson and Spearman rank correlation coefficients were calculated. All analyses were performed with the BMDP statistical package (BMDP software programs 7D, 8D, 3S 1V; BMDP Statistical Software Inc., Los Angeles, Calif).

Results

Demographic Data

The demographic, hemodynamic, and humoral data are reported in Table 1. As expected, systolic and diastolic blood pressure were significantly higher in essential hypertensive subjects than in NT subjects, whereas APs show the presence of higher systolic blood pressure values compared with NT subjects. Fasting glucose and body mass index were similar in subjects. Fasting glucose and body mass index were similar in the three groups of subjects, albeit APs tended to have greater fasting glucose values. Serum cholesterol or triglycerides were not significantly different among the groups.

No signs of renal impairment were observed. Acromegaly was diagnosed from 1 month to 20 years before the present investigation. Seven of 9 APs were previously treated with octreotide or lanreotide. Five were responders to treatment whereas 2 were partial responders. Four of 9 of the APs showed signs of glucose disorders (impaired glucose tolerance or diabetes mellitus). Six of 9 were previously considered borderline hypertensive subjects, whereas according to blood pressure values measured in the present study after a

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensive Subjects n=12</th>
<th>Essential Hypertensive Subjects n=12</th>
<th>Acromegalic Patients n=9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>54±3.9</td>
<td>55±1.3</td>
<td>57±2.6</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>6 mol/L, 6F</td>
<td>6 mol/L, 6F</td>
<td>5 mol/L, 4F</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>1.80±0.05</td>
<td>1.83±0.04</td>
<td>1.86±0.03</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>5.54±0.21</td>
<td>5.69±0.24</td>
<td>6.28±0.62</td>
</tr>
<tr>
<td>Serum nitrogen (mmol/L)</td>
<td>13.5±1.24</td>
<td>13.0±0.60</td>
<td>12.8±0.85</td>
</tr>
<tr>
<td>Serum creatinine (μmol/L)</td>
<td>81.2±5.4</td>
<td>84.1±3.55</td>
<td>87.7±6.17</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>5.05±0.44</td>
<td>5.66±0.32</td>
<td>5.20±0.25</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.47±0.19</td>
<td>1.66±0.20</td>
<td>1.31±0.17</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>127±2.4</td>
<td>160±1.5*</td>
<td>139±2.6†</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>79±2.1</td>
<td>99±1.7*</td>
<td>84±2.4‡</td>
</tr>
<tr>
<td>Duration of the disease (months)</td>
<td>77±23</td>
<td>134±31</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.001 vs essential hypertensives; †P<0.01 and ‡P<0.001 vs normotensives.
short washout of previous treatments, only 3 of 9 patients had blood pressure values above the normal limits. Six of the APs were previously treated for various periods of time with diuretics, ACE inhibitors, calcium antagonists, doxazosin, or angiotensin receptor blockers. In APs, GH circulating levels at the time of the biopsy were 9.48 ± 7.02 ng/mL, whereas IGF-1 levels were 306 ± 63 ng/mL.

### Subcutaneous Small Arteries

In APs, media-to-lumen ratio, media thickness, and wall thickness were significantly greater in comparison with NT subjects, whereas media thickness, wall thickness, and internal diameter were significantly greater in comparison with patients with EH (Table 2). In patients with EH, media-to-lumen ratio and media thickness were significantly greater, and internal diameter was significantly smaller in comparison with NT subjects (Table 2).

The media cross-sectional area was significantly greater in APs, compared with NT subjects and with EH subjects. EH subjects showed the presence of vascular eutrophic remodeling, as suggested by a remodeling index close to 100% whereas APs showed a remodeling index of 52%, indicating a relevant component of vascular smooth muscle cells growth.

A weak, but statistically significant, correlation between media-to-lumen ratio and levels of circulating IGF-1 (Pearson correlation coefficient: r = 0.63; P < 0.05; Spearman rank correlation coefficient: 0.60; P < 0.05) was observed in the APs (Figure). No significant correlation was observed between GH circulating levels and vascular structure (r = 0.20, P = NS).

Then, we subdivided our APs according the presence or absence of previous specific treatment, according to the type of drugs used, according to the duration of the disease (above or below the group average value), according to GH and IGF-1 circulating levels (below or above the whole group median value, respectively, 1.5 and 230 ng/mL), and also according to the presence or absence of hypertension or glucose disorders (Table 3). No difference in the media-to-lumen ratio or in the media cross-sectional area of subcutaneous small arteries between the subgroups was observed, apart from those with IGF-1 circulating values below or above the median value (Table 3).

### Discussion

For the first time to our knowledge, this study has evaluated small artery structure in APs using a direct, reliable, and well-assessed technique. The main result of our study is that APs show the presence of structural abnormalities in the resistance arteries, as indicated by an increased media-to-lumen ratio, and that these alterations are characterized, at least in part, by an inward hypertrophic remodeling, rather than by an eutrophic remodeling, which is usually observed in patients with EH. It is not presently known whether eutrophic and hypertrophic remodeling may have different underlying pathogenetic mechanisms, or a different natural history, or whether they may be differently modified by an appropriate treatment. It was, however, demonstrated that remodeling of subcutaneous small resistance arteries is an important determinant of prognosis, over and above other cardiovascular risk factors.

The presence of a hypertrophic remodeling was previously observed in patients with renovascular hypertension, in whom a pronounced activation of the renin–angiotensin system is present, as well as in patients with noninsulin-dependent diabetes mellitus. It has been suggested that insulin, or other related substances such as IGF-1, may have a role in
promoting vascular cell growth. In acromegaly, the elevated circulating concentrations of IGF-1 are closely correlated with 24-hour concentrations of GH, as well as with other clinical and biochemical indices of activity of the disease. It is therefore possible that also in APs, the observation of a vascular hypertrophic remodeling may be ascribed to the growth-promoting properties of GH or IGF-1. The observation of a significant correlation between plasma IGF-1 concentrations and media-to-lumen ratio of subcutaneous small arteries in our APs suggests, but does not prove, an important role of the hormone in the genesis of vascular structural alterations in such patients. We have measured GH/IGF-1 circulating levels at the time of the biopsy; therefore, in at least 7 of 9 patients, the effect of previous therapy for acromegaly should be considered in interpreting the data.25 In addition, because we did not measure smooth muscle cell size, we cannot safely attribute vascular hypertrophic remodeling to either cell hypertrophy or hyperplasia.

A possible confounding factor in the results obtained in our study could be ascribed to the duration of acromegaly and the duration as well as the characteristics of pharmacological treatment (type of drugs, extent of GH suppression during treatment). However, in our study, 2 of 9 cases of acromegaly were newly diagnosed, and the vascular structure of these 2 patients with newly diagnosed acromegaly was absolutely similar to the average of the remaining 7 patients. In addition, a careful look at the characteristics of the APs (subdivision according to the type of drug or according to the average duration of the disease) did not show any evidence of a strong relationship with the structural data.

Another possible confounder could have been the presence of hypertension and glucose disorders in our APs. Again, we subdivided our patients according to the presence or absence of associated conditions. No evidence of a relationship with vascular structure was observed. The characteristics of antihypertensive treatment (duration, type of drugs, etc) were similar in hypertensive APs and in patients with EH. However, heterogeneity of our patients remains a limitation of the study.

The calculation of remodeling and growth indices may give some semi-quantitative insight into the characteristics of small artery structural alterations. There are some caveats concerning their interpretation, because an unbiased identification of the presence of hypertrophy or hyperplasia at the cellular level should be based on other morphological techniques, such as “the dissector.”26 However, the presence of a growth index >40% was previously demonstrated to be associated to a real cell growth evaluated with alternative techniques.26

**Perspectives**

Our data support an important role of humoral growth factors in the development of vascular structural changes. Because the media-to-lumen ratio of subcutaneous small arteries may have an independent prognostic value in cardiovascular diseases, insights into the mechanisms possibly involved in the development (and probably in the regression) of vascular structural alterations may be relevant from a pathophysiological and clinical point of view. In addition, the demonstration of selective effects of GH or IGF-1 on vascular structure, independently of blood pressure elevation, may suggest some concern about the long-term use of GH as a therapeutic tool. GH was recently approved in the United States for use in normal children with short stature. The levels of GH achieved in treated individuals are, however, much lower than those seen in acromegaly. In conclusion, our data suggest the presence of hypertrophic remodeling in subcutaneous small resistance arteries of APs, probably as a consequence of growth-stimulator properties of IGF-1. The effects of acromegaly and EH on small artery morphology are quantitatively similar (despite the presence of a different hemodynamic load) but qualitatively different (hypertrophic versus eutrophic remodeling).

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TABLE 3. Media-to-Lumen Ratio and Media Cross-Sectional Area in Subgroups of Acromegalic Patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Media-to-Lumen Ratio</th>
<th>Media Cross-Sectional Area (μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previously treated (n=7)</td>
<td>0.099±0.005</td>
<td>22 196±2233</td>
</tr>
<tr>
<td>Newly diagnosed (n=2)</td>
<td>0.105±0.001</td>
<td>20 419±902</td>
</tr>
<tr>
<td>Treated with lanreotide (n=5)</td>
<td>0.101±0.006</td>
<td>20 034±2262</td>
</tr>
<tr>
<td>Treated with octreotide (n=2)</td>
<td>0.097±0.011</td>
<td>27 601±3552</td>
</tr>
<tr>
<td>Average duration of the disease &gt;11 years (n=4)</td>
<td>0.104±0.006</td>
<td>19 901±2916</td>
</tr>
<tr>
<td>Average duration of the disease ≤11 years (n=5)</td>
<td>0.098±0.005</td>
<td>23 321±2097</td>
</tr>
<tr>
<td>Circulating GH levels &gt;1.5 ng/ml (n=4)</td>
<td>0.104±0.006</td>
<td>21 735±3303</td>
</tr>
<tr>
<td>Circulating GH levels ≤1.5 ng/ml (n=5)</td>
<td>0.099±0.005</td>
<td>21 854±2061</td>
</tr>
<tr>
<td>Circulating IGF-1 levels &gt;230 ng/ml (n=4)</td>
<td>0.109±0.002</td>
<td>18 826±1052</td>
</tr>
<tr>
<td>Circulating IGF-1 levels ≤230 ng/ml (n=5)</td>
<td>0.094±0.005*</td>
<td>24 181±2635</td>
</tr>
<tr>
<td>Hypertensives (n=6)</td>
<td>0.102±0.005</td>
<td>21 625±2178</td>
</tr>
<tr>
<td>Normotensives (n=3)</td>
<td>0.099±0.006</td>
<td>22 154±3489</td>
</tr>
<tr>
<td>With glucose disorders (n=4)</td>
<td>0.101±0.005</td>
<td>23 365±1970</td>
</tr>
<tr>
<td>Without glucose disorders (n=5)</td>
<td>0.100±0.006</td>
<td>20 550±2755</td>
</tr>
</tbody>
</table>

*P<0.05 vs IGF-1>230 ng/ml.
References

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