Impact of Hyperthyroidism and Its Correction on Vascular Reactivity in Humans

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Background—Although thyroid hormone (TH) exerts relevant effects on the cardiovascular system, it is unknown whether TH also regulates vascular activity in humans.

Methods and Results—We studied 8 patients with hyperthyroidism, basally (H) and 6 months after euthyroidism was restored by methimazole (EU). Thirteen healthy subjects served as control subjects (C). We measured forearm blood flow (FBF) by strain-gauge plethysmography during intrabrachial graded infusion of acetylcholine, sodium nitroprusside (SNP), norepinephrine, and L-NMMA (inhibitor of NO synthesis). Basal FBF (in mL · dL⁻¹ · min⁻¹) was markedly higher in H than in C (5.8±1.2 and 1.9±0.1, respectively; P<0.001) and was close to normal in EU (2.6±0.3, P<0.01 versus H). During acetylcholine infusion, FBF increased much more in H (+33±5) than in C (+14±3, P<0.01 versus H) and in EU (+20±5, P=0.01 versus H and P=NS versus C). In contrast, the response to SNP infusion was comparable in the patients and control subjects. During norepinephrine infusion, the fall in FBF was much more pronounced in H (−6±1) than in C (−0.7±0.3, P<0.005 versus H) and in EU (−1.5±0.3, P<0.01 versus H). Finally, inhibition of NO synthesis by L-NMMA decreased FBF by 2.8±0.6, 0.61±0.7, and 1.4±0.3 in H, C, and EU, respectively (H versus C and EU, P<0.05).

Conclusions—In hyperthyroidism, (1) the marked basal vasodilation is largely accounted for by excessive endothelial NO production, (2) vascular reactivity is exaggerated because of enhanced sensitivity of the endothelial component, (3) the vasoconstrictory response to norepinephrine is potentiated, and (4) this abnormal vascular profile is corrected when euthyroidism is restored by medical therapy. The data demonstrate that vascular endothelium is a specific target of TH.

Key Words: endothelium ■ nitric oxide ■ thyroid ■ vasodilation

Thyroid hormone (TH) exerts multiple effects on the heart and vascular system.¹–⁶ Patients with TH excess or deficiency present with relevant changes in cardiovascular hemodynamics.²,⁶ In particular, hyperthyroidism induces a high-output state, with a marked fall in peripheral vascular resistance, whereas hypothyroidism is characterized by opposite changes.

The mechanisms by which TH affects vascular physiology are largely unknown. An increased capillary density was reported in hyperthyroid rats and human subjects.⁷,⁸ Data obtained in rat aortic rings suggest that TH exerts part of its vascular effects through an endothelium-mediated mechanism.⁹ In contrast, other in vitro data suggest that the smooth muscle cell, rather than the endothelium, is the main target of TH.¹⁰,¹¹ No data are available in humans regarding the effects of TH on endothelial function or, more generally, on vascular reactivity. Such data would be important to define the role of TH in the regulation of vascular homeostasis and to clarify the mechanisms underlying the vascular abnormalities observed in thyroid diseases.

The primary objective of this study was to investigate the mechanisms by which TH affects vascular function. In particular, we tried to determine whether chronic TH elevation in hyperthyroid human subjects affects endothelium-mediated and non–endothelium-mediated vascular reactivity. We also studied the effects of restored euthyroid state by medical therapy on the abnormalities in vascular regulation observed during hyperthyroidism.

Several manifestations of hyperthyroidism resemble those of catecholamine excess and are ameliorated by β-adrenergic antagonists. Accordingly, the number of β-adrenergic receptors is increased in thyrotoxicosis.¹² Data obtained in experimental hyperthyroidism induced by administration of TH to healthy volunteers, however, indicate that the sensitivity to epinephrine is unaltered by TH.¹² Similarly, in experimental hyperthyroidism in baboons, the sensitivity of the heart to adrenergic stimulation is normal.¹³ No data are available regarding the effects of norepinephrine on vascular function in patients with hyperthyroidism. Therefore, an ancillary aim of the present study was to evaluate the effects of norepi-
Clinical Characteristics of the Subjects Studied

<table>
<thead>
<tr>
<th>Sex, M/F</th>
<th>Age, y</th>
<th>Body Mass Index, kg/m²</th>
<th>Body Weight, kg</th>
<th>Systolic Blood Pressure, mm Hg</th>
<th>Diastolic Blood Pressure, mm Hg</th>
<th>Heart Rate, bpm</th>
<th>Thyroid-Stimulating Hormone, µU/mL</th>
<th>Free Triiodothyronine, pg/mL</th>
<th>Free Thyroxine, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (n=13)</td>
<td>7/6</td>
<td>32±2</td>
<td>24.5±0.7</td>
<td>70±2</td>
<td>121±1</td>
<td>58±1</td>
<td>62±3</td>
<td>1.1±0.1</td>
<td>2.9±0.2</td>
</tr>
<tr>
<td>Untreated hyperthyroidism (n=8)</td>
<td>4/4</td>
<td>37±4</td>
<td>23.7±1.6</td>
<td>66±4</td>
<td>144±7*</td>
<td>60±2</td>
<td>100±7*</td>
<td>&lt;0.1</td>
<td>16.0±3*</td>
</tr>
<tr>
<td>Treated hyperthyroidism (n=8)</td>
<td>...</td>
<td>...</td>
<td>26.0±1.4†</td>
<td>73±4†</td>
<td>133±5*†</td>
<td>61±4</td>
<td>65±2†</td>
<td>1.1±0.3</td>
<td>3.4±0.4†</td>
</tr>
</tbody>
</table>

*P<0.01 vs control group. †P<0.05 vs patients with untreated hyperthyroidism.

Methods

Subjects
We studied 2 groups of subjects. One was made up of 13 healthy volunteers (7 male, 6 female, 32±2 years of age, body mass index 24.5±0.7 kg/m²), and the other included 8 patients affected by Graves’ disease (4 male, 4 female, 37±4 years of age, body mass index 23.7±1.6 kg/m²) who had been hyperthyroid for 6.5±1.6 months, according to their medical history. The subjects were studied basally and 6 months after correction of thyrotoxicosis by methimazole treatment. Informed consent was obtained by all participants, and the study protocol was approved by the Ethics Committee of the Federico II University School of Medicine.

Experimental Procedure
All the studies were performed, as previously described,14 in the morning in a quiet room kept at 22°C to 24°C. The subjects were studied in the supine position after a 12- to 15-hour overnight fast. The day of the study, a plastic cannula (20-gauge) was inserted into the brachial artery of the nondominant arm under local anesthesia (xylocaine 2%) and used for the infusion of the test substances, the monitoring of arterial blood pressure and heart rate, and arterial blood sampling. Systolic and diastolic blood pressure and heart rate were recorded by a transducer. Forearm blood flow (FBF) was measured in both the forearms by strain-gauge plethysmography with a calibrated mercury-in-Silastic strain gauge applied around the forearm and connected to a Hokanson plethysmograph (Hokanson 045 EC4, PMS Instruments). The data were monitored continuously with MacLab software. Both arms were supported slightly above the heart level. During the measurement of FBF and blood sampling, a pediatric cuff was inflated around the wrist 100 mm Hg above systolic blood pressure to exclude hand circulation from the measurements.

Each subject underwent the following stepwise infusions into the brachial artery: (1) acetylcholine (ACh) infused at the rate of 15, 30, 45, and 60 µg · L of forearm -1 · min -1 to assess endothelium-mediated vasodilation; (2) sodium nitroprusside (SNP) infused at the rate of 1, 3, and 9 µg · L -1 · min -1 to assess non–endothelium-mediated vasodilation; (3) norepinephrine infused at the rate of 140, 280, and 560 ng · L -1 · min -1 to assess the vascular sensitivity to noradrenergic stimulation; and (4) NG-nomonomethyl-L-arginine (L-NMMA), a competitive, inactive analogue of L-arginine, infused at the rate of 1 ng · L -1 · min -1 to assess the role of endothelial NO release in the maintenance of the basal vascular tone. The test substances were infused in the order listed above in all subjects. Each dose of the test substances was infused for 5.5 minutes, and FBF was measured during the last 1.5 minutes of infusion. At least 30 minutes of washout time was allowed between each substance. The infusion rates were adjusted according to the forearm volume of each subject, measured by water displacement. To determine the peak flow, FBF was measured after 5 minutes of ischemia induced by inflating a sphygmomanometer cuff around the upper arm. FBF was measured simultaneously in both arms to ensure that no systemic effects occurred during the experiment. Each FBF value represents the mean of 6 consecutive measurements performed at 10-second intervals.

Calculations
The differences in clinical and metabolic characteristics between groups or between pretreatment and posttreatment values in the patients with hyperthyroidism were analyzed with the unpaired or the paired Student’s t test, as appropriate. Vascular reactivity data are expressed as absolute values of FBF. Results are expressed as mean±SEM.

Results
The clinical characteristics of the subjects studied are shown in Table. As expected, the body weight and body mass index of hyperthyroid patients increased significantly after methimazole therapy (P<0.001). Heart rate was markedly increased in the untreated patients (P<0.001 versus control subjects [controls]) and was normalized by methimazole (P<0.01 versus pretreatment). Systolic but not diastolic blood pressure was significantly higher in the untreated patients than in controls (P<0.01) and was reduced by correction of hyperthyroidism (P<0.05).

Basal FBF was >3-fold higher in the untreated patients than in controls (5.8±1.2 and 1.9±0.1 mL · dL⁻¹ · min⁻¹, respectively; P<0.001). Methimazole treatment drastically reduced basal FBF to nearly control values (2.6±0.3 mL · dL⁻¹ · min⁻¹, P<0.01 versus pretreatment; Figure 1). Infusion of ACh, an endothelium-dependent vasodilator, elicited a progressive vasodilatory response in all subjects (Figure 1). Hyperthyroid patients were demonstrated to be extremely sensitive to ACh, however, and their FBF response was consistently higher than that of control subjects at all steps (39±5 and 16±3 mL · dL⁻¹ · min⁻¹ at the highest ACh infusion rate, respectively; P<0.001, Figure 1). After methimazole treatment, FBF response to ACh was markedly attenuated (23±5 mL · dL⁻¹ · min⁻¹, P<0.01 versus pretreatment) and no longer differed from that of controls. Because the basal FBF was different in the 3 groups, we also analyzed the response to ACh in terms of absolute increments in FBF above the basal level. This analysis is shown in Figure 1B and confirms that the response to ACh was greater in the untreated hyperthyroid patients than in controls and

ephrine on vascular reactivity in patients with hyperthyroidism, basally and after restoration of euthyroidism.
methimazole-treated patients (32 ± 3, 14 ± 3, and 20 ± 5 mL · dL⁻¹ · min⁻¹ at the highest ACh infusion rate, respectively; P < 0.01 for untreated hyperthyroid patients versus both controls and methimazole-treated patients).

The response to ACh was also analyzed by use of the slope of the dose-response curves. This analysis shows that in untreated hyperthyroid patients the slope is markedly steeper than in the control group (0.53 ± 0.08 and 0.25 ± 0.06 mL · dL⁻¹ · min⁻¹ · μg⁻¹, respectively; P < 0.01). After restoration of euthyroidism, the slope was significantly reduced and no longer differed from control values (0.35 ± 0.09, P < 0.05 versus pretreatment values and P = NS versus controls).

The dose-response curve for SNP, an endothelium-independent vasodilator, is shown in Figure 2. Compared with controls, hyperthyroid patients showed a significantly higher response at all infusion rates. At the maximal dose of SNP, FBF was 30 ± 3 and 19 ± 2 mL · dL⁻¹ · min⁻¹ in patients and controls, respectively (P < 0.01). If we analyze the data as absolute changes from baseline (Figure 2B), however, it becomes evident that the FBF response to each infusion rate of SNP was virtually identical in the 3 groups of subjects. This is also confirmed by the slopes of the dose-response curves (1.67 ± 0.13, 2.18 ± 0.28, and 2.02 ± 0.14 mL · dL⁻¹ · min⁻¹ · μg⁻¹ in controls, untreated hyperthyroid, and methimazole-treated hyperthyroid patients, respectively; P = NS).

As shown in Figure 3, infusion of norepinephrine reduced FBF in all groups. Compared with control subjects, who showed a 20% reduction in their basal FBF, patients with hyperthyroidism showed a much more pronounced fall in FBF (0.7 ± 0.3 and 6.0 ± 1.3 mL · dL⁻¹ · min⁻¹ from the basal FBF, respectively, at the maximal norepinephrine infusion rate; P < 0.0001). Correction of hyperthyroidism normalized the vasoconstrictive response to norepinephrine (1.5 ± 0.3 mL · dL⁻¹ · min⁻¹ reduction; P = 0.005 versus untreated patients and P = NS versus controls).

As shown in Figure 4, inhibition of basal NO bioactivity by intra-arterial infusion of L-NMMA reduced FBF in all groups. This effect, however, was more prominent in the untreated hyperthyroid patients than the methimazole-treated patients and control healthy subjects (−2.9 ± 0.6, −1.4 ± 0.3, and −0.6 ± 0.6 mL · dL⁻¹ · min⁻¹, respectively; P < 0.05, untreated versus both treated patients and controls).

The peak FBF response to ischemia was significantly higher in the untreated hyperthyroid patients than in controls before (44 ± 5 and 31 ± 3 mL · dL⁻¹ · min⁻¹, respectively; P < 0.05) but not after (37 ± 3 mL · dL⁻¹ · min⁻¹, P = NS versus controls) methimazole treatment.

Discussion

This study shows that TH exerts profound effects on vascular reactivity in humans. The main findings obtained in our
hyperthyroid patients are that (1) an increased NO production plays a role in the marked vasodilation and the elevated FBF in the basal state; (2) the endothelial component of vascular reactivity, but not the smooth muscle cell component, is strongly potentiated; and (3) the sensitivity of the resistance vessels to the vasoconstrictive action of norepinephrine is enhanced. After 6 months of medical therapy with methimazole, the hormonal levels and the clinical status were fully corrected, and concomitantly, vascular reactivity was restored to normal. The data support the conclusion that TH elevation is specifically responsible for the vascular abnormalities observed in untreated hyperthyroidism.

A prominent feature of hyperthyroidism is the hyperkinetic state, characterized by high cardiac output, marked fall in peripheral vascular resistance, and consequent increase of tissue blood perfusion. The mechanisms underlying this hemodynamic pattern are, to a large extent, still unclear. In particular, no attempt has previously been made to clarify the impact of chronic TH elevation on vascular reactivity in humans. The present study provides evidence that inhibition of endothelial release of bioactive NO reduces FBF drastically. This implies that an increased production of NO by the endothelium contributes substantially to the augmented basal FBF and the reduced vascular resistance in hyperthyroidism. When euthyroidism was restored by methimazole treatment, basal FBF was nearly normalized. This was accompanied by marked attenuation of the fall in FBF in response to L-NMMA, in agreement with previous animal studies. Our data are apparently only in contrast with previous in vitro studies showing little or no effect of TH on the endothelium, because those data were obtained after acute TH stimulation of isolated vessels or smooth muscle cells and, therefore, were not adequate to clarify the role of persistent TH elevation in humans with hyperthyroidism.

We show for the first time that human hyperthyroidism is associated with a marked increase in ACh-mediated vascular reactivity. This exaggerated response is equally demonstrable if the effect of the augmented basal FBF is removed and the data are analyzed in terms of absolute flow increments. In addition, the slope of the FBF response curve to ACh in our hyperthyroid patients was twice as steep as that of control subjects. Because this analysis explores the sensitivity of the endothelial mechanism, regardless of the FBF values attained, the data provide another convincing argument that chronic elevation of TH in humans exerts a major influence on the endothelial component of vascular reactivity.

The dose-response curve to SNP, which stimulates the smooth muscle cells by directly yielding NO, runs at higher levels in hyperthyroid patients than in controls. The dose-response curves are parallel in the 2 groups, however, and accordingly, there is no statistical difference between them in terms of the slopes. This is clearly apparent in Figure 2B, in which the effect of the different basal flow is removed. Overall, the data support the concept that in hyperthyroidism, the sensitivity of the smooth muscle cells to NO is unchanged and that the exaggerated response to ACh is primarily due to a mechanism residing in the endothelium. In agreement with this view, recent data obtained in isolated arteries from hyperthyroid rats showed that 6 weeks of TH elevation increased ACh-mediated vasodilation without affecting the response to SNP. The upward shift of the whole response of FBF to SNP in our hyperthyroid patients is likely to reflect the consequence of chronic endothelial overactivity that resets the contractile status of the vascular smooth muscle cell.

Endothelial dysfunction plays a relevant role in the genesis and progression of atherosclerosis and related cardiovascular diseases. Congestive heart failure and myocardial infarction are also associated with abnormal endothelium-mediated vascular reactivity. Recent data obtained in hypertensive patients showed that abnormalities in endothelial function are associated with poor prognosis and worse progression of the disease. Conversely, decreased TH levels have been reported in a variety of nonthyroidal illnesses, including congestive heart failure and myocardial infarction. This is not a minor point, because hypothyroidism, even if subclinical, acts as an independent risk factor for atherosclerosis. In view of this finding and the present demonstration that TH exerts prominent effects on endothelial function, correction of subtle TH deficiency in the context of cardiovascular diseases might be a sound strategy to target endothelial dysfunction.

Many manifestations of thyrotoxicosis resemble those of catecholamine excess and are ameliorated by administration of β-adrenergic antagonists. Although these observations suggest that the sensitivity to catecholamines is enhanced in hyperthyroidism, the few studies performed so far have not clarified the issue. In particular, there are no data regarding the vasomotor response to norepinephrine in hyperthyroid humans. The present study provides evidence that norepinephrine infusion in hyperthyroid patients induces marked vasoconstriction. This response was much more pronounced than in control subjects and was corrected by restoring euthyroidism. The explanation for this finding and its signif-
icance is not easy. It is pertinent that in hyperthyroidism the circulating catecholamine levels are decreased, both at rest and during physical exercise, and therefore, increased sensitivity to catecholamines might be a compensatory mechanism to preserve hemodynamics during stress.

In conclusion, the present data in untreated hyperthyroidism and those obtained after euthyroidism has been restored lead to the conclusion that the endothelium is a specific target of TH in humans. The basally increased endothelial function and its hypersensitivity to vasodilating stimuli provide an explanation for the reduced peripheral vascular resistance and the maintenance of a hyperkinetic state in hyperthyroidism.

References
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