Independent Association Between Plasma Leptin Levels and Heart Rate in Heart Transplant Recipients

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Background—Leptin, the protein product of the ob gene, has been linked to a faster heart rate in animal and human studies. The interaction between leptin and heart rate in the denervated heart is not known. Therefore, we studied the relationship between plasma leptin levels and heart rate in heart transplant recipients.

Methods and Results—We studied 32 male patients (mean age, 56.5±9.3 years; range, 41 to 74 years) after orthotopic heart transplantation. All subjects underwent a physical examination, anthropometric measurements, blood chemistry analysis, and office blood pressure measurements. A blood sample was collected from each subject while fasting. In univariate analysis, heart rate was related to leptin levels (r=0.47, P=0.007) but heart rate was not related to systolic or diastolic blood pressure, mean arterial pressure, body mass index, or catecholamines. Leptin levels were only strongly associated with heart rate and body mass index (r=0.73, P<0.0001). In multivariate analysis, heart rate was independently and positively associated with leptin levels (F=2.61, P=0.017). We also observed a strong, independent association between leptin levels and body mass index (F=5.8, P<0.00001).

Conclusions—We show an independent association between leptin levels and heart rate in heart transplant recipients. We speculate that this may be due, in part, to a direct effect of leptin on heart rate, conceivably mediated through cardiac leptin receptors. (Circulation. 2001;104:384-386.)

Key Words: leptin ■ heart rate ■ transplantation

Heart rate is emerging as an important cardiovascular risk factor, and it may predict the development of sustained hypertension.1 Autonomic neural and humoral mechanisms contribute to the control of heart rate. Leptin, the 167 amino acid product of the ob gene,2 has also been linked to cardiovascular risk.3,4 Animal studies show that chronic leptin infusion increases heart rate and blood pressure.5,6 High leptin levels are linked to poor prognosis in the setting of myocardial infarction.4 In human studies, plasma leptin levels correlate strongly with heart rate, most strikingly during sleep.7–9 The effects of leptin on heart rate are presumably mediated by the central effects of leptin as it modulates autonomic neural control of the heart.5,6 However, there is emerging evidence that leptin may have direct, non-neural effects on cardiovascular function. Leptin receptors or their mRNA have been detected in blood vessels and in the heart.10–13 The interaction between leptin and heart rate in the denervated heart is not known. Therefore, we studied the relationship between plasma leptin levels and heart rate in heart transplant recipients.

Methods

Subjects
We studied 32 male patients (aged 56.5±9.3 years; range, 41 to 74 years). Subjects were selected across a broad spectrum of time intervals after transplantation (mean time after transplantation, 56±8 months; range, 1 to 132 months). Acute rejection, significant graft vasculopathy (stenoses ≥50%), and impaired systolic function were excluded by endomyocardial biopsy, coronary angiography, and ventriculography within 7 days before the study. The underlying cardiac disease before transplantation was dilated cardiomyopathy in 61% of cases and ischemic heart disease in the remaining 39%.

Medications
All patients were receiving maintenance immunosuppression with cyclosporine in combination with azathioprine (38%) and prednisone (54%). None were receiving β-blockers. Subjects also received calcium antagonists (46%), hydralazine (29%), clonidine (21%), angiotensin-converting enzyme inhibitors (21%), and other active drugs.

Procedures
General conditions in the experimental setting were maintained constant to limit the influence of extraneous variables. Experimental studies were performed during the same time of day to limit any potential influence of circadian neuroendocrine patterns on measurements.14 Subjects were asked to refrain from eating or drinking for 4
Group Data Showing Demographic, Hemodynamic, and Biochemical Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SEM (Median)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>56 ± 1.6 (57)</td>
<td>41–74</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29 ± 1 (29)</td>
<td>19.9–42.7</td>
</tr>
<tr>
<td>Time after transplantation, mo</td>
<td>56 ± 8 (53)</td>
<td>1–132</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>139 ± 2 (140)</td>
<td>115–180</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>91 ± 2 (90)</td>
<td>76–118</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>86.5 ± 3 (89)</td>
<td>55–124</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>19.6 ± 2.5 (16)</td>
<td>2–65</td>
</tr>
<tr>
<td>Norepinephrine, ng/mL</td>
<td>502 ± 36 (477)</td>
<td>214–879</td>
</tr>
<tr>
<td>Epinephrine, ng/mL</td>
<td>32 ± 9 (20)</td>
<td>10–236</td>
</tr>
</tbody>
</table>

*BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; and HR, heart rate.*

to 6 hours before the study and to refrain from nicotine, alcohol, and caffeine for at least 8 hours before testing.

The protocol was approved by the institutional Human Ethics Committee, and informed consent was obtained from all subjects. All subjects underwent a physical examination, anthropometric measurements, blood chemistry analysis, and office blood pressure measurements in the morning. Plasma leptin levels were measured by radioimmunoassay (Linco Research, Inc; intra-assay coefficient of variation, 3.4% to 8.3%; interassay coefficient of variation, 3.6% to 6.2%). Catecholamine levels were determined by high-performance liquid chromatography with electrochemical detection (interassay and intra-assay coefficients of variation were 3.4% and 3.1%, respectively).

**Statistical Analysis**

To assess simple relations between variables, a Pearson correlation with Bonferroni adjustments was used. To assess the independent association of heart rate and leptin, each of these variables was entered into a multiple linear regression model using heart rate as a dependent variable and age, body mass index, mean arterial pressure, time from transplantation (months), and leptin and catecholamine levels as covariates. After the initial analysis, we further adjusted the data to minimize the potential effects of medications on heart rate. Each medication was scored according to the expected influence on heart rate as follows: 1, drug decreased the heart rate (nondihydropyridine Ca²⁺ blockers and clonidine); 0, drug had no effect on heart rate (azathioprine, prednisone, angiotensin-converting enzyme inhibitors, and cyclosporine); and 1, drug expected to increase heart rate (hydralazine, nitrates, and dihydropyridines). The sum of this score, named the drug effect, was calculated for each subject and was used as a covariate in the analysis. To assess the relation between drug effect and heart rate, 1-way ANOVA was used.

Data are presented as mean ± SEM or mean ± SD, where appropriate. *P* < 0.05 was considered significant.

**Results**

Demographic data are presented in the Table. In univariate analysis, heart rate was related to leptin levels (*r* = 0.47, *P* = 0.007) and drug effect (*P* = 0.03), but heart rate was not related to systolic or diastolic blood pressure, mean arterial pressure, or body mass index. Importantly, heart rate was unrelated to catecholamine levels. Leptin levels correlated only with heart rate (*P* = 0.007) and body mass index (*r* = 0.73, *P* < 0.0001).

In a multivariate analysis using heart rate as the dependent variable and adjusting for all covariates except the drug effect, heart rate was independently and positively associated with leptin levels (*F* = 2.61, *P* = 0.017; Figure). Including the drug effect within the multivariate analysis revealed an even stronger independent association between heart rate and leptin (*F* = 3.13, *P* = 0.006). In addition, heart rate was independently associated with drug effect in this analysis (*F* = 2.12, *P* = 0.05). We also observed a strong, independent association between leptin levels and body mass index (*F* = 5.8, *P* < 0.0001).

**Discussion**

The novel finding of this study is the independent relationship between leptin levels and heart rate in the denervated heart in cardiac transplant patients. This interaction cannot be explained only on the basis of catecholamine levels. In the innervated heart, the mechanisms by which leptin may participate in the elevation of heart rate are unknown. Possible explanations include activation of the sympathetic nervous system or withdrawal of parasympathetic tone; evidence exists to support both these mechanisms. However, in heart transplant recipients, other factors may play a substantial role. Transplantation of the heart results in sympathetic and parasympathetic cardiac denervation. This may conceivably lead to enhanced direct effects of leptin on heart rate, possibly through cardiac leptin receptors. Alternatively, changes in the sensitivity of other cardiac receptors, particularly adrenergic receptors, may potentiate the relative effect of catecholamines on rate control.

The strength of the correlation between heart rate and plasma leptin levels in cardiac transplant recipients is more striking than that present in studies of humans with preserved cardiac neural innervation. Indeed, in autonomic heart rate seems independently linked to leptin in the setting of cardiac denervation.

Leptin binds to at least one receptor in the brain. The full leptin receptor (Ob-Rb) is a protein containing a single transmembrane domain. In mice, the gene for the leptin receptor seems to encode at least 6 alternatively spliced variants of the receptor. Interestingly, mRNA for the leptin receptor is expressed not only in the hypothalamus, but also in the choroid plexus, adipose tissue, heart, kidney, liver,
spleen, pancreatic islets, and testes, although the presence of the full-length receptor splice variant has not been demonstrated in all of these tissues.\textsuperscript{13,18} Leptin receptors have also been identified in blood vessels, where they promote angiogenesis.\textsuperscript{10,11} The functional effects of cardiac leptin receptors are unknown. Our data suggest they may conceivably be involved in heart rate control.

The small number of subjects did not allow us to establish any difference in the leptin–heart rate relationship early compared with late after heart transplantation. Partial autonomic reinnervation may occur in long-standing heart transplant recipients,\textsuperscript{19,20} although this is not invariable.\textsuperscript{21,22} Overall, the data suggest that functional reinnervation, if it occurs, is mediated by relatively small numbers of sympathetic or parasympathetic neurons. We tried to reduce the potential influence of reinnervation of the heart on heart rate by taking into account the time since transplantation in multiple regression analysis.

In conclusion, we show an independent association between leptin levels and heart rate in heart transplant recipients. We speculate that in the denervated transplanted heart, this association may be due, in part, to a direct effect of leptin on heart rate that may conceivably be mediated through cardiac leptin receptors. Alternatively, leptin levels may be a surrogate for one or more substances that influence the rate of contraction of the transplanted heart.

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References
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