Elevated Serum Insulin Levels in Patients With Essential Hypertension and Microalbuminuria

Stefano Bianchi, Roberto Bigazzi, Carla Valtriani, Ivo Chiapponi, Gianpaolo Sgherri, Giorgio Baldari, Andrea Natali, Eleuterio Ferraninini, Vito M. Campese

Abstract
Hyperinsulinemia, insulin resistance, or both have been described in patients with essential hypertension. Previous work from our laboratory has shown that in hypertensive patients with microalbuminuria, dyslipidemia and abnormal patterns in the diurnal variations of blood pressure are frequently associated. Whether hyperinsulinemia and microalbuminuria are directly related has not been determined. To test this possibility, we measured the plasma insulin response to an oral glucose load in 25 patients with or without microalbuminuria and 20 normotensive control subjects. Serum lipid profile and 24-hour ambulatory blood pressure were obtained. In the hypertensive patients as a group, the plasma insulin response to glucose (evaluated as the insulin area under the curve) was significantly enhanced compared with a group of 20 normotensive healthy control subjects (4631±3745 and 27557±2563 pmol/L×2 hours, P<.01). When the hypertensive patients were subdivided according to their albumin excretion rate, the microalbuminuric patients had significantly higher plasma glucose (969±45.2 versus 762±28.7 mmol/L×2 hours, P<.01) and insulin (59172±5964 versus 37737±3422 pmol/L×2 hours, P<.01) area under the curve values. In addition, a significant direct correlation was found to exist between insulin area under the curve and the urinary albumin excretion rate (r=−0.63, P<.001). Serum levels of lipoprotein(a) were significantly greater (P<.01) in patients with than in those without microalbuminuria and in control subjects. Furthermore, daytime diastolic blood pressure and nighttime systolic and diastolic blood pressure values were greater in patients with than in those without microalbuminuria. We conclude that in patients with essential hypertension microalbuminuria is associated with an enhanced insulin response to glucose, altered lipid levels, and an abnormal circadian blood pressure pattern, thereby forming a cluster with pathogenic potential for cardiovascular complications.

Key Words: • hypertension, essential • albuminuria • insulin resistance • hyperinsulinism • lipoproteins

Microalbuminuria, defined as urinary albumin excretion (UAE) exceeding 30 mg per 24 hours, is present in 35% to 40% of patients with essential hypertension and is associated with an unfavorable serum lipid profile and increased cardiovascular morbidity and mortality. Recent studies have indicated that microalbuminuria is a marker of glomerular damage; in patients with insulin-dependent diabetes mellitus (IDDM), it predicts the development of overt proteinuria and progressive renal failure. Although the predictive value of microalbuminuria in non-insulin-dependent diabetes mellitus (NIDDM) is less well established, in both NIDDM and IDDM patients microalbuminuria predicts cardiovascular morbidity and mortality. Treatment of hypertension retards the progression of microalbuminuria and renal disease in patients with IDDM. Evidence also indicates that microalbuminuria can be considered an early sign of renal damage in patients with essential hypertension. More recently, several investigators have described the presence of insulin resistance and hyperinsulinemia in a substantial number of patients with essential hypertension. In addition, it has been suggested that hyperinsulinemia may be associated with a greater risk of atherosclerotic cardiovascular diseases.

The mechanisms by which insulin resistance, hyperinsulinemia, or both may increase the risk of cardiovascular disease are not well defined. It has been proposed that insulin resistance signals the presence of a cluster of metabolic and hemodynamic abnormalities with inherent atherogenic potential. In the present study, we tested the hypothesis that in patients with essential hypertension microalbuminuria is associated with evidence of insulin resistance and/or other cardiovascular risk factors.

Methods

Patients
Twenty-eight patients with essential hypertension stage 1 to 3 and 20 normotensive control subjects were included in this study. Hypertensive patients were included if their diastolic blood pressure was consistently between 95 and 120 mm Hg during three clinic visits, if their creatinine clearance was greater than 80 ml/min per 1.73 m², and if their urinalysis was normal. Hypertensive patients and control subjects with a family history of diabetes mellitus and women of childbearing potential were excluded. Eighteen hypertensive patients had never received any antihypertensive drugs before enrollment, and the remaining 10 patients discontinued their antihypertensive drugs at least 4 weeks before the study. Six of these patients had been treated with a calcium channel blocker and 4 with a converting enzyme inhibitor. All subjects were kept on their habitual diet. The study protocol was approved by the Human Research Committee of the Spedali Riuniti of Livorno, Italy, and all subjects gave their informed consent.

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Takeda A and D model TM-2420. The TM-2420 uses the Korotkov method, the first and the fifth phases representing systolic and diastolic pressure, respectively. Two microphones within the pneumatic cuff can differentiate between Korotkoff sounds and noise. The TM-2420 was calibrated against a mercury sphygmomanometer before each recording. Left arm readings were taken with a standard-sized cuff beginning at 9 AM. Measurements were made every 20 minutes from 7 AM to 11 PM (daytime period) and every 30 minutes from 11 PM to 7 AM (nighttime period). All patients underwent ambulatory blood pressure monitoring as outpatients during a working day without any changes in their habits or diet. All subjects kept a diary to report the time of going to bed as well as every significant episode that could have happened during the recordings (such as physical and emotional stress and nocturnal awakenings and their causes). All patients awoke at 7 AM with an alarm.

**Data Analysis**

From the 24-hour blood pressure profile the following values were calculated: 24-hour average systolic values, 24-hour average diastolic values, and daytime and nighttime average systolic and diastolic values. The recorder automatically discarded artifactual readings; computerized analysis excluded isolated diastolic readings less than 40 mm Hg, systolic values greater than 240 mm Hg, and differential pressure less than 20 mm Hg.

The [STATVIEW+GRAPHICS](http://hyper.ahajournals.org) was used for statistical analysis. Because of the possibility of outliers, nonparametric and robust statistical techniques (the Kruskal-Wallis rank sum test) were used to evaluate serum lipid levels and microalbuminuria; the values are expressed as medians and ranges. One-way ANOVA and Scheffe's F test were used to evaluate ambulatory blood pressure; the data are expressed as mean±SEM. Spearman’s rank correlation technique was used to estimate correlations between different parameters. Multiple regression analysis was performed to examine the individual contribution of different factors on UAE. Glucose and insulin areas under the oral glucose tolerance test curve were computed by trapezoidal integration.

**Results**

With individuals in the fasting state, plasma glucose and insulin levels were similar in patients and control subjects. In response to oral glucose loading, plasma glucose increased similarly in essential hypertensive patients and control subjects, whereas the patients showed significantly higher plasma insulin levels (Fig 1). The mean insulin area under the curve of hypertensive pa-

| TABLE 1. Clinical Characteristics of Hypertensive Patients With and Without Microalbuminuria and Control Subjects |
|-------------------------------------------------|-------------------------------------------------|-----------------|
| Hypertensive Without Microalbuminuria | Hypertensive With Microalbuminuria | Control Subjects |
| n | 15 | 10 | 20 |
| Males/females | 5/10 | 6/4 | |
| Age, y | 44 (21-60) | 50 (42-63) | 45.5 (36-58) |
| BMI, kg/m² | 25.2 (22.2-26.8) | 25.3 (23-28.2) | 25.4 (22.4-33) |
| Duration of hypertension, mo | 24 (1-144) | 15 (1-120) | NS |
| Microalbuminuria, mg/24 h | 14.9 (7-22) | 53 (36-110) | 7.6 (2-15) |
| Office systolic BP, mm Hg | 160 (137-185) | 167 (155-180) | 129 (117-144) |
| Office diastolic BP, mm Hg | 105 (99-117) | 108 (102-120) | 74.5 (66-84) |

BMI indicates body mass index; BP, blood pressure. Values are medians and ranges. Statistical analysis for microalbuminuria was performed by nonparametric techniques using the Kruskal-Wallis test.

*Hypertensive with vs without microalbuminuria and control subjects.
†Hypertensive with and without microalbuminuria vs control subjects.

Analytic Procedures

Serum concentrations of total cholesterol, triglyceride, high-density lipoprotein cholesterol, and apolipoproteins A and B were measured in fresh samples. Plasma for the assay of lipoprotein(a) [Lp(a)] and insulin was frozen and stored at −30°C and measured within 15 days. Serum total and high-density lipoprotein cholesterol and triglyceride concentrations were measured by enzymatic methods. The concentrations of low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) were calculated with the formula of Friedewald et al.27 Apolipoproteins A and B were measured by nephelometry with antisera and standard reagents from Behringwerke AG. Lp(a) was measured by enzyme immunnoassay [Immunozym Lp(a)] and plasma insulin by radioimmunoassay. Twenty-four-hour urinary protein, creatinine, and sodium excretion were measured by standard methods. Albumin concentration in the urine was measured by an immunoturbidimetric method.28 Body mass index was calculated as weight (kilograms) divided by height (meters) squared.

Ambulatory Blood Pressure Monitoring

Ambulatory blood pressure monitoring was performed by a Takeda A and D model TM-2420. The TM-2420 uses the
Serum Insulin and Microalbuminuria

Bianchi et al

Glucose

Insulin

FIG 1. Line graphs show serum levels of glucose and insulin after oral glucose tolerance test (75 g) in 28 patients with stage 1 and 2 hypertension and 20 normotensive control subjects. *P<.01.

patients and control subjects, respectively, was 46 311 ± 3745 and 27 557 ± 2563 pmol/L x 2 hours (P<.01). Fig 2 shows the plasma glucose and insulin profiles of hypertensive patients divided into two groups (microalbuminuric and normoalbuminuric) according to their UAE rate. Both glucose and insulin increased more in patients with than in those without microalbuminuria (P<.01). The area under the curve of insulin was 59 172 ± 5964 pmol/L x 2 hours in patients with microalbuminuria and 37 737 ± 3422 pmol/L x 2 hours in hypertensive patients without microalbuminuria (P<.01). The area under the curve of glucose in hypertensive patients with microalbuminuria (969 ± 45.2 mmol/L x 2 hours) was also significantly greater (P<.01) than in hypertensive patients without microalbuminuria (762 ± 28.7 mmol/L x 2 hours) and control subjects (837 ± 31.2 mmol/L x 2 hours). In addition, a significant correlation was found between the insulin area under the curve and UAE rate (r=.63, P<.001) (Fig 3).

Plasma Lp(a), VLDL, and triglyceride levels were significantly greater in patients with than in those without microalbuminuria or control subjects (Table 2), and a significant correlation was present between UAE and Lp(a) (r=.36, P<.02) and between UAE and VLDL (r=.38, P<.01). A significant correlation was also present between insulin area under the curve and serum LDL (r=.49, P<.002) and VLDL (r=.34, P<.03) (Table 3).

Office blood pressure was not significantly different between the two groups of hypertensive patients (Table 1). However, when blood pressure was monitored for 24 hours, hypertensive patients with microalbuminuria had a significantly higher mean 24-hour and daytime diastolic blood pressure (Table 4). Also, the circadian blood pressure profile was altered in patients with microalbuminuria, who showed higher systolic and diastolic blood pressure levels during the night (Table 4). In the hypertensive patients, a significant correlation was present between daytime diastolic blood pressure and UAE (r=.41, P<.05) and between nighttime systolic or diastolic blood pressure and UAE (Table 3). A significant correlation was also present between 24-hour diastolic blood pressure and insulin area under the curve or UAE (Table 3 and Fig 4).

In hypertensive patients, multiple regression analysis using microalbuminuria as the dependent variable and insulin area under the curve, Lp(a), and diastolic blood

FIG 2. Line graphs show serum levels of glucose and insulin after oral glucose tolerance test (75 g) in 12 hypertensive patients with and 16 patients without microalbuminuria (μA). *P<.01.

FIG 3. Plot shows correlation between insulin area under the curve and urinary albumin excretion (UAE) in hypertensive and normotensive subjects combined.
pressure as independent variables showed the strongest correlation with nighttime diastolic blood pressure (P < 0.04) and Lp(a) (P < 0.04).

Discussion

This study confirms that nonobese patients with untreated essential hypertension are hyperinsulinemic in response to oral glucose compared with normotensive control subjects (Fig 1). The new finding is that, within the hypertensive group, patients with microalbuminuria have significantly higher insulin and glucose levels than patients without microalbuminuria (Fig 2). Furthermore, a direct relation between UAE and serum insulin levels was evident in these patients (Fig 3).

Hyperinsulinemia, insulin resistance, or both are found in an estimated one third to one half of patients with essential hypertension. The present observation implies that microalbuminuria identifies those hypertensive patients who are more likely to be hyperinsulinemic and, presumably, insulin resistant. With regard to this, Doria et al have reported that a high rate of Na+-Li+ activity in essential hypertensive patients is impaired by some but questioned by others on the basis of two large prospective studies that showed no significant difference in Lp(a) levels between patients with myocardial infarction and control subjects.52,53 This observation supports the notion that the increased serum levels of LDL and Lp(a) may in part be responsible for the greater cardiovascular morbidity and mortality in hypertensive patients with microalbuminuria than in those without microalbuminuria.

The current studies also confirm that patients with microalbuminuria manifest significant alterations of the circadian pattern of blood pressure. In 63 patients with essential hypertension, we have previously shown that glucose are both genetically determined and cosegregate with the hypertensive status. Support for this notion derives from the observation that nondiabetic normotensive individuals with a genetic risk for hypertension may manifest microalbuminuria and insulin resistance. Another explanation is that insulin resistance, hyperinsulinemia, or both are causally related to hypertension on the one hand and to abnormal membrane permeability on the other, thereby leading to renal damage and microalbuminuria. The possibility that long-standing hypertension causes both insulin resistance and microalbuminuria (the latter via changes in the renal microcirculation) seems less likely, because insulin resistance and microalbuminuria may precede the appearance of high blood pressure and are not found in all patients with established hypertension. However, a positive correlation was present between blood pressure and UAE; multiple regression analysis showed that UAE was more strongly correlated with blood pressure and serum Lp(a). Finally, hypertension, enhanced plasma insulin response to glucose, and microalbuminuria may all be the consequence of the same pathogenic factor or factors.

In the present experiments we confirmed our previous observation that patients with essential hypertension and microalbuminuria show alterations of serum lipoprotein levels compared with patients with microalbuminuria. These alterations consist of increased serum levels of Lp(a), LDL cholesterol, and triglycerides. The atherogenic role of LDL cholesterol is well established, whereas the role of Lp(a) has been proposed by some but questioned by others on the basis of two large prospective studies that showed no significant difference in Lp(a) levels between patients with myocardial infarction and control subjects. This observation supports the notion that the increased serum levels of LDL and Lp(a) may in part be responsible for the greater cardiovascular morbidity and mortality in hypertensive patients with microalbuminuria than in those without microalbuminuria.
TABLE 3. Correlation Coefficients Between Urinary Albumin Excretion or Insulin Area Under the Curve and Other Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Urinary albumin excretion and</th>
<th>Hypertensive and Normotensive</th>
<th>Hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>Urinary albumin excretion and</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Office SBP</td>
<td>.61</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Office DBP</td>
<td>.66</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Day SBP</td>
<td>.58</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Day DBP</td>
<td>.66</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Night SBP</td>
<td>.63</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Night DBP</td>
<td>.69</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>24-Hour SBP</td>
<td>.63</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>24-Hour DBP</td>
<td>.70</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Insulin area under the curve</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipoprotein(a)</td>
<td>.36</td>
<td>&lt;.02</td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>.49</td>
<td>&lt;.002</td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>.38</td>
<td>&lt;.1</td>
<td></td>
</tr>
<tr>
<td>Apoprotein B</td>
<td>.29</td>
<td>&lt;.06</td>
<td></td>
</tr>
</tbody>
</table>

80% of those with microalbuminuria show no nocturnal fall in blood pressure. Conversely, most hypertensive patients without microalbuminuria displayed a normal nocturnal dipping in blood pressure. Microalbuminuria was correlated with nighttime systolic and diastolic blood pressures. This abnormality in the circadian

TABLE 4. Ambulatory Blood Pressure Monitoring in Hypertensive Patients With and Without Microalbuminuria and Control Subjects

<table>
<thead>
<tr>
<th>BP Measurement</th>
<th>A Hypertensive Without Microalbuminuria</th>
<th>B Hypertensive With Microalbuminuria</th>
<th>C Control Subjects</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-Hour SBP</td>
<td>148±3.4</td>
<td>154±2.4</td>
<td>113±2.7</td>
<td>NS A vs B; &lt;.01 A,B vs C</td>
</tr>
<tr>
<td>24-Hour DBP</td>
<td>89.7±2.60</td>
<td>98.6±2.16</td>
<td>74±1.3</td>
<td>.022 A vs B; &lt;.01 A,B vs C</td>
</tr>
<tr>
<td>Day SBP</td>
<td>152±4.1</td>
<td>159±2.2</td>
<td>119±2.4</td>
<td>NS A vs B; &lt;.01 A,B vs C</td>
</tr>
<tr>
<td>Day DBP</td>
<td>93.9±2.45</td>
<td>101.6±2.18</td>
<td>77±0.9</td>
<td>.035 A vs B; &lt;.01 A,B vs C</td>
</tr>
<tr>
<td>Night SBP</td>
<td>136±4.7</td>
<td>150±4.9</td>
<td>105±3.7</td>
<td>.05 A vs B; &lt;.01 A,B vs C</td>
</tr>
<tr>
<td>Night DBP</td>
<td>82.7±3.10</td>
<td>94.6±3.12</td>
<td>64±1.6</td>
<td>.016 A vs B; &lt;.01 A,B vs C</td>
</tr>
</tbody>
</table>

BP indicates blood pressure; SBP, systolic blood pressure; and DBP, diastolic blood pressure. Values are mean±SEM. Comparisons among groups were performed by ANOVA and Scheffé's F test.
blood pressure pattern could contribute to the greater incidence of cardiovascular complications in hypertensive patients with microalbuminuria as well as to the renal damage responsible for the increase in UAE. Our observation that the plasma insulin response was directly related to daytime and nighttime diastolic blood pressure appears to provide evidence that microalbuminuria, abnormal blood pressure pattern, and hyperinsulinemia form a cluster of interrelated changes with unfavorable prognostic significance.

Finally, our patients with microalbuminuria had a worse tolerance to oral glucose than patients without microalbuminuria, despite the fact that all patients had normal glucose tolerance by conventional clinical criteria.

In summary, whatever the underlying mechanisms, microalbuminuria in hypertensive patients signals the presence of additional metabolic (enhanced plasma insulin response to glucose, abnormal glucose tolerance and lipid levels) and hemodynamic (higher blood pressure, absence of nocturnal pressure dip) defects with pathogenic potential. It should be mentioned that hyperinsulinemia itself has been regarded as a risk factor for the development of cardiovascular disease independent of blood pressure and plasma lipid levels.18-24

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