Relationship Between Plasma Insulin Concentration and Plasma Remnant Lipoprotein Response to an Oral Fat Load in Patients With Type 2 Diabetes

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OBJECTIVES
The goal of this study was to evaluate the relative effects of hyperglycemia and hyperinsulinemia on postprandial remnant lipoprotein (RLP) concentrations in newly diagnosed type 2 diabetics.

BACKGROUND
Increases in fasting RLP concentration have been described in type 2 diabetics, as well as in insulin-resistant nondiabetics. Given the atherogenicity of RLPs, we have extended these observations by assessing postprandial RLP concentrations and observing that hyperglycemia was necessary for the increase in RLP concentrations.

METHODS
Patients with type 2 diabetes were subdivided on the basis of their plasma insulin response to oral glucose into hyperinsulinemic (H-DM) and normoinsulinemic (N-DM) groups of 15 patients each. Plasma triglyceride (TG), RLP-TG and RLP cholesterol (RLP-C) concentrations were determined before and 2 and 4 h after an oral fat load in these patients and 10 control (CTL) subjects.

RESULTS
Plasma TG, RLP-TG and RLP-C concentrations peaked 2 h after the fat load in the CTL group, returning to baseline within 4 h. In contrast, concentrations of these variables increased throughout the 4-h study in both groups of patients with type 2 diabetes. Total integrated plasma RLP-TG and RLP-C responses above baseline after the oral fat load were significantly higher in the H-DM group compared with the CTL (p < 0.019 and 0.009, respectively) or N-DM (p < 0.026 and 0.029, respectively) groups. Post-heparin lipoprotein lipase activities and apo E phenotypes were similar in the H-DM and N-DM groups.

CONCLUSIONS
Remnant lipoprotein response to an oral fat load is significantly increased in hyperinsulinemic patients with type 2 diabetes. These changes may increase the risk of coronary heart disease in these individuals. (J Am Coll Cardiol 2001;38:1628–32) © 2001 by the American College of Cardiology

The introduction of a technique based upon the use of monoclonal antibodies to apolipoproteins A-1 and B-100 (1–3) has provided a method for isolating and quantifying the plasma concentration of remnant lipoproteins (RLPs). Published results using this method have demonstrated that fasting RLP concentrations are increased in patients with either type 2 diabetes or impaired glucose tolerance (4), as well as in insulin-resistant, nondiabetic individuals (5). Given evidence that the postprandial accumulation of triglyceride (TG)-rich lipoproteins is atherogenic (6–11) and that concentration of these particles is elevated in insulin-resistant states (4,5,12), it seemed important to extend these observations of RLP concentrations beyond the fasting state. In particular, knowledge of the changes in postprandial RLP concentrations in patients with type 2 diabetes might be particularly interesting in lieu of our incomplete understanding of why the prevalence of atherogenesis is increased in these patients.

A second goal of this study was to gain additional insight into the relative importance of hyperglycemia versus hyperinsulinemia in the modulation of RLP metabolism. To accomplish this task, patients with newly diagnosed type 2 diabetes were subdivided into two groups on the basis of their plasma insulin response to an oral glucose challenge—hyperinsulinemic (H-DM) and normoinsulinemic (N-DM). A comparison of the RLP responses to an oral fat load of these two groups, as well as that of a third group of healthy, nondiabetic volunteers (CTL), forms the substance of this report.

METHODS
The study population consisted of 40 individuals: 30 patients with type 2 diabetes newly diagnosed at the time of an annual medical evaluation and 10 healthy, nondiabetic volunteers. The 30 patients with type 2 diabetes were selected from a larger group of 66 individuals who, as part of their annual evaluation, had been diagnosed as having type 2 diabetes on the basis of an oral glucose tolerance test (13).
From this group of 66 individuals, 15 were selected on the basis of having a plasma insulin concentration 120 min after the oral glucose load >80 μU/ml, and a sum of the six insulin concentrations determined before and 30, 60, 90, 120 and 180 min after the oral glucose load >300 μU/ml. From the remaining newly diagnosed patients, 15 additional individuals were selected with having insulin concentration 120 min after the oral glucose load >60 μU/ml, summed values during the glucose tolerance test >250 μU/ml and comparable to the first group in terms of age, gender and body mass index (BMI). The plasma glucose and insulin concentrations of the two groups of patients with type 2 diabetes are shown in Figure 1. It is apparent that the plasma glucose concentrations were essentially identical, whereas their insulin concentrations differed widely. The group with the highest insulin concentration (summed insulin concentration = 447 ± 34 μU/ml) was defined as being H-DM, and the other group (summed insulin concentrations = 198 ± 16 μU/ml) was classified as N-DM. In this fashion we were able to create two groups of patients with type 2 diabetes, dichotomous for only their plasma insulin response to an oral glucose load.

All of the patients with type 2 diabetes were newly diagnosed and were taking no medications that affect carbohydrate or lipoprotein metabolism; the oral fat tolerance test (OFTT) was performed within 30 days of the positive oral glucose tolerance test. Glycosylated hemoglobin concentrations (HbA1c) (mean ± SEM) were 5.4 ± 0.1%, 5.2 ± 0.1% and 5.0 ± 0.2% in the N-DM, H-DM and CTL groups, respectively, and these differences were not statistically significant. The measurement of RLP concentrations in response to an oral fat load were performed as previously described (14) in the 30 patients with type 2 diabetes and 10 healthy, nondiabetic volunteers. Briefly, after an overnight fast, each subject ingested 17 g of fat/m² surface area (OFTT cream, Jomo Food Industry, Takashi, Japan) containing 57% water, 33% lipid, 3% protein and 7% carbohydrate (14). The fat distribution in the cream is 64.3% saturated, 29.3% monounsaturated and 3.5% polyunsaturated. Venous blood samples were obtained before and 2 and 4 h after the oral fat load to determine total cholesterol (C) (15), triglyceride (16), high-density lipoprotein (HDL) cholesterol (17), glucose (18), insulin (19) and remnant lipoprotein cholesterol (RLP-C) and remnant lipoprotein triglyceride (RLP-TG) concentrations (1–3). The decision to limit this study to 4 h was based on results in normal individuals, showing that RLP-C and RLP-TG concentrations peaked at 2 h after the fat load and had returned to baseline at 4 h. The plasma TG, RLP-C and RLP-TG responses to the fat load were quantified by determining the total integrated response above baseline during the 4 h of the study.

Plasma lipoprotein lipase (LPL) concentration was estimated on a separate day. After an overnight fast, subjects were injected intravenously with heparin (30 U/kg intravenous), and a blood sample was drawn 15 min later for measurement of lipoprotein lipase (20). Finally, apolipoprotein E phenotype was also analyzed (21) from the fasting plasma by isoelectric focusing electrophoresis (phenotyping Apo E IEF System, Yoko, Japan).

Data are expressed as mean ± SEM. The statistical significance of differences between the experimental groups was determined by one-way analysis of variance (ANOVA).
Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Group</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H-DM</td>
<td>N-DM</td>
<td>CTL</td>
</tr>
<tr>
<td></td>
<td>(n = 15)</td>
<td>(n = 15)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>57 ±  2</td>
<td>57 ±  2</td>
<td>33 ±  1</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>9/6</td>
<td>9/6</td>
<td>6/4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.7 ± 0.7</td>
<td>24.6 ± 0.6</td>
<td>22.9 ± 0.5</td>
</tr>
</tbody>
</table>

BMI = body mass index; CTL = normal volunteers; H-DM = hyperinsulinemic patients with type 2 diabetes; N-DM = normoinsulinemic patients with type 2 diabetes.

Scheffé F test method was used as a post hoc test if any differences were noted with ANOVA.

RESULTS

The baseline characteristics of the three study groups are shown in Table 1. It can be seen that the two groups of patients with type 2 diabetes were comparable in terms of age, gender distribution and BMI. Furthermore, the distribution of apo E phenotype was similar in the two diabetic patient groups. However, by selection, the two groups of patients with type 2 diabetes differed dramatically with regard to their plasma insulin response to oral glucose (Fig. 1).

Fasting lipid and lipoprotein concentrations of the three study groups are listed in Table 2. When analyzed by one-way ANOVA, differences were noted between the total C (p = 0.035), TG (p = 0.073) and RLP-C (p = 0.016) concentrations of the three experimental groups. Hyperinsulinemic patients with type 2 diabetes, as a group, had higher plasma C (p = 0.059) and RLP-C (p = 0.009) when compared with the N-DM group (Scheffé post hoc method). When two subgroups of patients with type 2 diabetes were compared with the CTL population, the only significant differences were higher values in the H-DM patients for total C (p = 0.016), TG (p = 0.025) and RLP-C (p = 0.025) concentrations. There were no differences between the N-DM group and the CTL group.

Since concentrations of total C, HDL-C, glucose and insulin did not change substantially during the OGTT, only the TG, RLP-C and RLP-TG responses to the fat load will be shown. These data are seen in Figure 2. Fasting concentrations of TG, RLP-TG and RLP-C were highest in the H-DM group before the fat load, and the results in Figure 2 indicate that this difference persisted 2 and 4 h after the oral fat load. The most obvious difference between the oral fat load responses of the three groups is that the concentrations of TG, RLP-TG and RLP-C increased progressively during the 4 h in both groups of patients with type 2 diabetes, whereas levels for the CTL group peaked 2 h after the fat load and returned to baseline levels within 4 h.

To evaluate the statistical significance of the response curves shown in Figure 2, we calculated the total incremental response above baseline. These comparisons are shown in Table 3, and the ANOVA analysis demonstrated that there were no statistically significant differences between the total TG responses in the three groups. However, there were significant differences between the RLP-TG (p = 0.027) and RLP-C (p = 0.016) concentrations in the three groups. Furthermore, the post hoc analysis showed that the H-DM group had significantly higher RLP-TG and RLP-C concentrations than the N-DM (p = 0.026 and p = 0.029, respectively) or the CTL (p = 0.19 and p = 0.009, respectively) group. However, the post hoc analysis indicated that there were no differences between the CTL population and the N-DM group for any of the variables.

Despite the obvious changes in plasma concentrations of TG, RLP-TG and RLP-C concentrations during the fat tolerance test, the values for postheparin lipoprotein lipase activity were essentially identical in the H-DM (282 ± 26 ng/ml) and N-DM (255 ± 24 ng/ml) groups. Finally, apo E phenotypes were similar in the H-DM and N-DM groups, with 10 and nine individuals apo E 3/3 and five and six subjects apo E 4/3, respectively.

DISCUSSION

Postprandial RLP concentrations of the three groups. The results of this study have provided straightforward answers to the two questions posed in the introduction. In the most general sense, it was shown that the TG, RLP-TG and RLP-C concentrations before and after the oral fat load were highest in the H-DM group, lowest in the CTL group.

Table 2. Comparison of the Lipid and Lipoprotein Concentrations in the Three Study Groups* (Mean ± SEM)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>p Values</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>H-DM</td>
<td>N-DM</td>
</tr>
<tr>
<td></td>
<td>ANOVA</td>
<td>DM</td>
</tr>
<tr>
<td></td>
<td>(H vs. N)</td>
<td>H vs. CTL</td>
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<tr>
<td>Cholesterol (mg/dl)</td>
<td>220 ± 6</td>
<td>196 ± 10</td>
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<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>129 ± 10</td>
<td>116 ± 9</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>61 ± 4</td>
<td>57 ± 4</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>151 ± 24</td>
<td>118 ± 10</td>
</tr>
<tr>
<td>RLP-TG (mg/dl)</td>
<td>19.4 ± 4.6</td>
<td>14.0 ± 2.0</td>
</tr>
<tr>
<td>RLP-C (mg/dl)</td>
<td>4.9 ± 0.5</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.035</td>
<td>0.059</td>
</tr>
<tr>
<td></td>
<td>0.248</td>
<td>0.349</td>
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</tr>
<tr>
<td></td>
<td>0.462</td>
<td>0.278</td>
</tr>
<tr>
<td></td>
<td>0.016</td>
<td>0.009</td>
</tr>
</tbody>
</table>

*The results of each of the three groups are compared with the other two.

ANOVA: p values were calculated by one-way ANOVA and a value <0.05 indicates that a difference exists between the three groups. Scheffé's post hoc method was used to define the significance of the difference between any two of the three groups.

ANOVA = analysis of variance; C = cholesterol; CTL = normal volunteers; DM = patients with type 2 diabetes; HDL = high-density lipoprotein; H-DM = hyperinsulinemic patients with type 2 diabetes; LDL = low-density lipoprotein; N-DM = normoinsulinemic patients with type 2 diabetes; RLP = remnant lipoprotein; TG = triglyceride.
subjects, with values of the N-DM patients being intermediate. To a large extent, the differences between the three groups was due to the fact that the TG, RLP-TG and RLP-C concentrations continued to increase throughout the 4 h period after the fat load in the H-DM and N-DM groups, whereas the concentrations of all three variables peaked at 2 h in the CTL group, returning to baseline by the 4 h time point. As a consequence, the total integrated response above baseline after the oral fat load was significantly higher in the H-DM compared with the CTL group for the RLP-TG (p = 0.019) and RLP-C (p = 0.009) concentrations. Although the mean integrated responses of all three variables was somewhat higher than the CTL values in the N-DM group, none of the differences were of even marginal significance.

Differences between the RLP concentrations of the two diabetic groups. If we now focus on comparisons of the two groups of patients with type 2 diabetes, the impact of the differences in plasma insulin concentrations on the results of the fat tolerance test is apparent from Figure 2. First, the concentrations of TG, RLP-TG and RLP-C are higher at every time point in the H-DM patients compared with the N-DM patients. Furthermore, the total integrated RLP-TG (p = 0.026) and RLP-C (p = 0.029) responses above baseline were significantly higher in the H-DM group (Table 3). It should be noted that the fasting plasma TG concentrations were not significantly different in the two groups (Table 2), suggesting that the differences noted were not a simple function of an enlarged fasting plasma TG pool size. Furthermore, the fact that the LPL levels were the same in the two diabetic groups suggests that differences in the level of this enzyme was not the reason for the higher RLP levels in the H-DM group.

Physiological relevance. The results presented show that both hyperglycemia and hyperinsulinemia modulate post fat load RLP concentrations. Specifically, RLP-TG and RLP-C concentrations increased progressively throughout the OFTT in both groups of patients with diabetes, whereas concentrations had returned to baseline within 4 h in the CTL group, irrespective of the degree of hyperinsulinemia present in patients with type 2 diabetes. Thus, in this case, hyperglycemia, per se, appeared to modulate the RLP response to an oral fat load. On the other hand, although the two groups of patients with type 2 diabetes were equally hyperglycemic, RLP-TG and RLP-C concentrations were higher before and after the fat load in the H-DM compared with the N-DM group. Since the only obvious difference between the two groups of patients with type 2 diabetes was in the plasma insulin concentrations, the possibility that the degree of hyperinsulinemia will lead to increases in RLP concentrations, independently of the degree of glycemia, is worthy of consideration.

Clinical relevance. The results provide potentially important clinical information concerning the link between hy-

**Table 3.** Comparison of the Plasma Triglyceride and Remnant Lipoprotein Responses in the Three Study Groups (Mean ± SEM) to the Oral Fat Load*  

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>p Values</th>
<th>ANOVA</th>
<th>DM (H vs. N)</th>
<th>H-DM vs. CTL</th>
<th>N-DM vs. CTL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H-DM</td>
<td>N-DM</td>
<td>CTL</td>
<td>H-DM vs. N-DM</td>
<td>H-DM vs. CTL</td>
<td>N-DM vs. CTL</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>134.9 ± 26.8</td>
<td>119.3 ± 17.5</td>
<td>74.6 ± 19.1</td>
<td>0.254</td>
<td>0.604</td>
<td>0.102</td>
</tr>
<tr>
<td>RLP-TG (mg/dl)</td>
<td>150.2 ± 25.9</td>
<td>86.9 ± 14.2</td>
<td>70.0 ± 14.9</td>
<td>0.027</td>
<td>0.026</td>
<td>0.019</td>
</tr>
<tr>
<td>RLP-C (mg/dl)</td>
<td>9.1 ± 1.4</td>
<td>5.5 ± 1.0</td>
<td>3.9 ± 0.9</td>
<td>0.016</td>
<td>0.029</td>
<td>0.009</td>
</tr>
</tbody>
</table>

*The results of each of the three groups are being compared with the results of the other two groups.

ANOVA: p values were calculated by one-way ANOVA and a value <0.05 indicates that a difference exists between the three groups. Scheffe’s post hoc method was used to define the significance of the difference between any two of the three groups.

ANOVA = analysis of variance; CTL = normal volunteers; DM = patients with type 2 diabetes; H-DM = hyperinsulinemic patients with type 2 diabetes; N-DM = normoinsulinemic patients with type 2 diabetes; RLP-C = remnant lipoprotein cholesterol; RLP-TG = remnant lipoprotein triglyceride; TG = triglyceride.

**Figure 2.** Plasma triglyceride, remnant lipoprotein triglyceride (RLP-TG) and remnant lipoprotein cholesterol (RLP-C) concentrations before and after an oral fat load in normal volunteers (CTL) and in hyperinsulinemic patients with type 2 diabetes (H-DM) and normoinsulinemic patients with type 2 diabetes (N-DM).
perinsulinemia and coronary heart disease (CHD) in patients with type 2 diabetes. The results of the United Kingdom Prospective study (22) demonstrated that improved glycemic control was much more effective in reducing microangiopathy than macrovascular disease in patients with type 2 diabetes. In this context, the recent results of a prospective study (23) showing that hyperinsulinemia significantly predicted CHD in patients with type 2 diabetes is of interest. The notion that atherogenesis is a postprandial phenomenon has received considerable support in the past several years (7–11). The current results have extended our previous finding, that fasting RLP concentrations are elevated in patients with type 2 diabetes (4), and have shown that this finding is accentuated in response to a oral fat load. More specifically, it appears that significant increases in fasting and postprandial plasma RLP concentrations are confined to the subset of patients with type 2 diabetes with the highest plasma insulin concentrations. As such, our findings offer a possible mechanism to account for the association described in patients with type 2 diabetes between hyperinsulinemia and CHD (23).

Acknowledgment

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