Implications of decreased serum adiponectin for type IIb hyperlipidaemia and increased cholesterol levels of very-low-density lipoprotein in Type II diabetic patients

Hiroshi YOSHIDA*†‡, Yuji HIROWATARI§, Hideo KUROSAWA‡ and Norio TADA*†

*Division of General Medicine, Department of Internal Medicine, Kashiwa Hospital, Jikei University School of Medicine, Chiba 277-856, Japan, †Institute of Clinical Medicine and Research, Jikei University School of Medicine, Chiba 277-856, Japan, ‡Department of Clinical Laboratory, Kashiwa Hospital, Jikei University School of Medicine, Chiba 277-856, Japan, and §Scientific Instrument Division, Tosoh Corporation, Kanagawa 252-1123, Japan

ABSTRACT

The present study was performed to investigate the relevance of cholesterol levels of plasma lipoproteins [HDL (high-density lipoprotein), LDL (low-density lipoprotein), IDL (immediate-density lipoprotein), VLDL (very-LDL) and chylomicrons] determined by a novel HPLC method, with adiponectin, which is decreased in Type II diabetes and assumed to be involved in dysregulated metabolism and atherogenesis. Type II diabetic patients who were not treated with insulin, statins and fibrates were enrolled. Study subjects included Type II diabetic patients with normolipidaemia (DM-NL; n = 15), type 4 hyperlipidaemia (DM-T4HL; n = 13), Type IIa hyperlipidaemia (DM-T2aHL; n = 15) and Type IIb hyperlipidaemia (DM-T2bHL; n = 13). Fasting blood samples were collected. The serum adiponectin level was lower in DM-T2bHL than in any of the other groups. Cholesterol levels of each lipoprotein fraction, serum triacylglycerol (triglyceride), remnant-like particle-cholesterol, fasting plasma glucose, HbA1c (glycated haemoglobin), age, gender difference and BMI (body mass index) were incorporated into a stepwise regression analysis as independent variables. VLDL-cholesterol correlated inversely with adiponectin independently of age, BMI, gender difference and glycemic control. Although the mechanisms remain to be explored, serum adiponectin was reduced particularly in Type II diabetics with type IIb hyperlipidaemia and correlated inversely with VLDL-cholesterol. Measuring VLDL-cholesterol may be helpful for understanding the pathological features of diabetic dyslipidaemia.

INTRODUCTION

CVD (cardiovascular disease) is major cause of morbidity and mortality in patients with diabetes, and diabetic patients have greatly increased risk for CVD compared with non-diabetic individuals [1–7]. One of the significant cardiovascular risk factors in Type II diabetes is dyslipidaemia. A characteristic of diabetic dyslipidaemia, observed frequently in Type II diabetes, consists of decreased HDL (high-density lipoprotein)-cholesterol, with non-diabetic individuals [1–7].
increased serum TG [triacylglycerol (triglyceride)] levels and increased levels of small dense LDL (low-density lipoprotein) [1,5,6,8]. This dyslipidaemia is usually not corrected with glycaemic control and should be monitored in clinical practice of diabetes [1,5,6,8].

Recently, we have developed a novel HPLC method with a column containing a non-porous polymer-based gel with diethylaminoethyl ligands to facilitate measurements of cholesterol levels in the major classes of serum lipoproteins [HDL, LDL, IDL (intermediate-density lipoprotein), VLDL (very-LDL) and CM (chylomicron)] [9]. TG-rich lipoprotein levels, including RLP (remnant-like particle)-cholesterol levels, have been found to be high in plasma of patients with CVD [10–14]. Lessons from clinical trials with fibrates administered to diabetic subjects confirm the findings that elevated serum TG is an important risk factor for CVD, as well as low HDL, and that TG reduction with HDL elevation can reduce the risk for CVD [1,2,15]. Therefore clinical monitoring of serum TG is conceivably important in diabetic patients prone to CVD. Major TG-rich lipoproteins consist of CM, VLDL and IDL. Our HPLC method conveniently determines the cholesterol levels of each TG-rich lipoprotein as well as HDL and LDL, as described previously [9].

Adiponectin is a unique and essential adipocytokine that is produced abundantly in adipocytes and secreted in the circulation [16,17]. Serum adiponectin levels have been reported to be lower in obese subjects than in non-obese subjects, and hypoadiponectinaemia has been reported to be observed in patients with Type II diabetes, hypertension, hypertriglyceridaemia and ischaemic heart disease [16–24]. In non-diabetic subjects, decreased serum adiponectin was associated with increased serum TG and decreased HDL-cholesterol, but not with LDL-cholesterol levels [25]. Another study [26] reported a significant inverse correlation of LDL with adiponectin in subjects without diabetes and hyperlipidaemia independent of BMI (body mass index). Serum adiponectin was also reported to be associated inversely with serum TG in Type II diabetic patients [21]. However, results concerning the relevance of adiponectin to dyslipidaemia determined by means of assessment of lipoproteins separated by ultracentrifugation or HPLC have not been reported. Thus, the precise relationship of serum adiponectin levels to each lipoprotein fraction have not been clarified. In addition, although decreased adiponectin has been confirmed in Type II diabetes, studies in which hyperlipidaemia is related to low adiponectin in Type II diabetes have not been reported.

Therefore the present study was performed to examine cholesterol levels of each lipoprotein, determined by a novel HPLC method, in Type II diabetic patients and to investigate the relevance of the fractionated lipoprotein levels to adiponectin, a serum marker related to diabetic dysregulated metabolism.

**METHODS**

Diabetic patients undergoing insulin therapy and taking statins, fibrates, thiazolidinedione and other hypoglycaemic agents, except for sulphonylurea and α-glucosidase inhibitors, were not enrolled in the present study. Patients with liver or kidney dysfunction, endocrine disease and cardiovascular disease were also excluded. The study subjects were composed of Type II diabetic patients with normolipidaemia (DM-NL; n = 15), type IV hyperlipidaemia (DM-T4HL; n = 13), type IIa hyperlipidaemia (DM-T2bHL; n = 13) and type IIa hyperlipidaemia (DM-T2aHL; n = 15). The study procedure was explained and informed consent was obtained from all the subjects. This study was approved by the Ethics Committee of Jikei University. Clinical characteristics of study subjects are shown in Table 1, and all the female subjects were postmenopausal.

Fasting blood samples were collected, and lipoprotein-cholesterol levels were measured by the HPLC method reported previously [9]. Briefly, serum lipoproteins were separated by elution on a column containing a non-porous polymer-based gel with diethylaminoethyl ligands with a step gradient of sodium perchlorate, and were detected by post-column reaction with a reagent containing cholesterol esterase and cholesterol oxidase. RLP-cholesterol was determined with a commercially available kit (Jimro-II; Japanese Immunoresearch Laboratories). Serum TG, fasting plasma glucose and HbA1c (glycated haemoglobin) were measured conventionally. Serum adiponectin was measured by ELISA (Otsuka Pharmaceutical Co.) [19,25].

The skewed frequency distributions of serum adiponectin and TG were confirmed by Z-score histogram.
analysis, and thereby their levels were log-transformed to achieve normal distributions. Data are expressed as means ± S.D. Significant differences between groups (using \( P < 0.05 \)) were compared by one-way ANOVA, followed by the Bonferroni multiple comparison method (statistical significance was accepted with \( P < 0.0083 \)). Multiple stepwise regression analysis was performed to assess the independent relationship of the studied variables in whole study population, and gender differences were dummy coded (male, 1; female, 0) in this analysis. Simple correlations between log-transformed adiponectin and VLDL-cholesterol in the respective study groups were determined by Pearson product-moment correlation coefficient. \( P < 0.05 \) was accepted as statistically significant in the regression analyses.

### RESULTS

As shown in Table 1, BMI was higher in the DM-T4HL and DM-T2bHL groups compared with the DM-NL group. In terms of HbA1c, these four groups were kept in similar glycaemic control. There was no significant difference in the number of subjects who were smokers, were on diabetic medication and had hypertension among the four study groups.

Table 2 shows the lipid profiles of the study subjects. The levels of CM-, VLDL-, IDL-, LDL- and HDL-cholesterol were measured by HPLC. VLDL- and CM-cholesterol were significantly higher in the DM-T4HL and DM-T2bHL groups compared with the DM-NL and DM-T2aHL groups, consistent with the increased TG levels in former groups. In contrast, HDL-cholesterol was lower in the DM-T4HL and DM-T2bHL groups compared with the DM-NL and DM-T2aHL groups. LDL-cholesterol and total cholesterol levels were higher in the DM-T2aHL and DM-T2bHL groups compared with the DM-NL and DM-T4HL groups. In addition, RLP-cholesterol was higher in DM-T4HL and DM-T2bHL groups compared with the DM-NL and DM-T2aHL groups, consistent with increased TG levels in the former groups.

As shown in Figure 1, the levels of serum adiponectin were significantly lower in the DM-T2bHL group than the other study groups. To explore the reasons for decreased adiponectin in Type II diabetic patients with type IIb hyperlipidaemia, we performed multiple regression analysis with log-transformed serum adiponectin levels as the dependent variable. Total cholesterol, log-transformed TG, RLP-cholesterol, HbA1c, age, BMI, gender difference and each lipoprotein-cholesterol data measured by HPLC were incorporated as independent variables into the stepwise multiple regression analysis. VLDL-cholesterol was inversely correlated with adiponectin independently of age, BMI, gender difference and glycaemic control in the first step analysis (Table 3). Moreover, RLP-cholesterol was also inversely correlated with adiponectin in the first-step analysis. However, after employing VLDL-cholesterol as a primacy independent factor, no further factors were independently associated with adiponectin in the second-step analysis without VLDL-cholesterol. In addition, multiple regression analysis was performed further by using data in men and women separately. In men, VLDL-cholesterol independently correlated with adiponectin, as found in the whole study population (\( n = 37; r = -0.388, F = 6.219, P = 0.0175 \)); however, it did not reach statistical

### Table 2 Plasma lipid profiles of the study subjects

<table>
<thead>
<tr>
<th>Lipid levels (mg/dl)</th>
<th>DM-NL</th>
<th>DM-T4HL</th>
<th>DM-T2bHL</th>
<th>DM-T2aHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>184 ± 24</td>
<td>193 ± 20†</td>
<td>264 ± 54‡</td>
<td>238 ± 20*</td>
</tr>
<tr>
<td>CM-cholesterol</td>
<td>2.8 ± 1.3</td>
<td>7.1 ± 3.6‡</td>
<td>8.8 ± 4.7‡</td>
<td>3.7 ± 2.6</td>
</tr>
<tr>
<td>VLDL-cholesterol</td>
<td>12.1 ± 5.9</td>
<td>36.6 ± 11.5†</td>
<td>42.4 ± 19.6†</td>
<td>9.7 ± 6.0</td>
</tr>
<tr>
<td>IDL-cholesterol</td>
<td>7.2 ± 1.9</td>
<td>9.6 ± 6.9</td>
<td>11.7 ± 9.1</td>
<td>6.0 ± 2.3</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>106 ± 21</td>
<td>101 ± 13;</td>
<td>158 ± 38 †</td>
<td>154 ± 23 †</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>56 ± 16</td>
<td>39 ± 14 †</td>
<td>40 ± 6 †</td>
<td>55 ± 13</td>
</tr>
<tr>
<td>RLP-cholesterol</td>
<td>4.6 ± 2.0</td>
<td>12.8 ± 4.1 †</td>
<td>12.9 ± 9.3 †</td>
<td>5.6 ± 2.2</td>
</tr>
<tr>
<td>Log TG</td>
<td>2.0 ± 0.2</td>
<td>2.5 ± 0.2 †</td>
<td>2.4 ± 0.2 †</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>TG</td>
<td>99 ± 33</td>
<td>335 ± 148</td>
<td>284 ± 111</td>
<td>98 ± 33</td>
</tr>
</tbody>
</table>

Values are means ± S.D. Lipoprotein-cholesterol levels were measured by HPLC. Log TG was used for statistical analysis instead of TG. † \( P < 0.0083 \) compared with DM-NL; ‡ \( P < 0.0083 \) compared with DM-T4HL; * \( P < 0.0083 \) compared with DM-T2aHL.
Table 3 Multiple stepwise regression analysis between log-transformed data for serum adiponectin and 13 clinical variables

Lipoprotein cholesterol levels were measured by HPLC. Total cholesterol shows the aggregate data of each lipoprotein-cholesterol levels. Log-transformed adiponectin and log-transformed TG were used for the present analysis. VLDL-cholesterol was employed as an independent factor associated with serum adiponectin in the first-step analysis. After employing VLDL-cholesterol, further significantly associated factors were not found in the second-step analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Partial correlation coefficient</th>
<th>F value (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-cholesterol</td>
<td>0.180</td>
<td>1.802 (0.185)</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>-0.201</td>
<td>2.264 (0.138)</td>
</tr>
<tr>
<td>IDL-cholesterol</td>
<td>-0.171</td>
<td>1.631 (0.207)</td>
</tr>
<tr>
<td>VLDL-cholesterol</td>
<td>-0.367</td>
<td>7.399 (0.005)</td>
</tr>
<tr>
<td>CM-cholesterol</td>
<td>-0.101</td>
<td>0.560 (0.457)</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-0.261</td>
<td>3.504 (0.069)</td>
</tr>
<tr>
<td>RLP-cholesterol</td>
<td>-0.304</td>
<td>4.251 (0.029)</td>
</tr>
<tr>
<td>Log TG</td>
<td>-0.215</td>
<td>2.621 (0.111)</td>
</tr>
<tr>
<td>Fasting plasma glucose</td>
<td>0.095</td>
<td>0.487 (0.488)</td>
</tr>
<tr>
<td>HbA1c</td>
<td>-0.011</td>
<td>0.067 (0.993)</td>
</tr>
<tr>
<td>Age</td>
<td>-0.063</td>
<td>0.213 (0.646)</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.232</td>
<td>3.079 (0.085)</td>
</tr>
<tr>
<td>Gender difference</td>
<td>0.220</td>
<td>2.554 (0.103)</td>
</tr>
</tbody>
</table>

Significance in women (n = 19; r = -0.409, F = 3.42). When examining the simple correlation between adiponectin and VLDL-cholesterol in the DM-T4HL group, a significant inverse relationship was observed (n = 13; r = -0.62, P = 0.024). However, when using data from the DM-T2bHL group, no significant correlation between VLDL-cholesterol and adiponectin was observed (n = 13; r = -0.269; P = 0.3223). Likewise, no significant correlations were found between VLDL-cholesterol and adiponectin in the DM-T2aHL or DM-NL groups (results not shown).

**DISCUSSION**

The results of the present study demonstrate that adiponectin levels in Type II diabetic patients with type IIB hyperlipidaemia were reduced to approximately half of those from the other Type II diabetic study groups, and that, among the fractionated lipoproteins, VLDL-cholesterol level was inversely correlated with serum adiponectin independently of age, BMI, gender difference and glycemic control. Previous reports have demonstrated that adiponectin is associated with atherosclerosis [17,24]. In particular, hyperadiponectinaemia is considered to be relevant to atherosclerosis. It is noteworthy that serum adiponectin was reduced predominantly in patients with type IIB hyperlipidaemia among the Type II diabetic groups in the present study, who already have a decreased level of plasma adiponectin compared with non-diabetic subjects. Taken together, increased VLDL-cholesterol may reflect hypoadiponectinaemia in relation to atherogenesis in Type II diabetes, but this relationship remains to be elucidated.

The Funagata study [27] and studies by other groups [17,28] have reported that decreased adiponectin is a risk factor for the development of Type II diabetes. In addition, correlations of serum adiponectin levels with insulin resistance index, HDL-cholesterol and TG are significantly independent of BMI [26,28]. By contrast, BMI correlated independently and inversely with serum adiponectin and positively with TG and the presence of diabetes, and weight reduction significantly increased serum adiponectin levels in diabetic patients as well as non-diabetic subjects [17,21,24]. However, the present study demonstrates the inverse correlation of VLDL-cholesterol with serum adiponectin independently of BMI. Nevertheless, because BMI does not necessarily imply body fat accumulation, it would be inappropriate to consider directly the inter-relationship between BMI and adiponectin, a molecule secreted from adipocytes. From the present study, it could be interpreted that adiponectin may primarily be involved in the metabolism of TG-rich lipoproteins.

In addition to the relevance of decreased adiponectin with increased VLDL-cholesterol, the reason why serum adiponectin was reduced, particularly in Type II diabetic patients with type IIB hyperlipidaemia, should be considered as an intriguing question. Recently, decreased plasma lipoprotein lipase has been found in hypoadiponectinaemia [29,30]. Thus adiponectin may play a role in increasing the activity and concentration of lipoprotein lipase, implicating that the relationship between adiponectin and lipoprotein lipase may be influenced by insulin resistance and overt diabetes [29,30]. The significant inverse association between adiponectin and VLDL-cholesterol found in Type II diabetic subjects with type IV hyperlipidaemia in the present study could be involved in decreased levels of lipoprotein lipase, but this remains to be determined. The mechanism linking serum adiponectin and dyslipidaemia is presently unknown, although it could be attributed to insulin resistance. In this regard, several studies have demonstrated that adiponectin administration to obese and diabetic mice reduced TG content in muscle and liver by increasing fatty acid oxidation, and that hepatic fat content was inversely correlated with adiponectin levels in human [31,32]. Taken together, adiponectin might reduce serum TG and VLDL-cholesterol, in part by inhibiting some of the steps in VLDL and TG production or by stimulating lipolysis of TG. However, the reason why adiponectin was reduced in Type II diabetic subjects with type IIB hyperlipidaemia but not with type IV hyperlipidaemia in the present study remains to be elucidated. Unravelling the mechanisms of the contribution of adiponectin to VLDL metabolism needs further investigation.
In conclusion, decreased levels of serum adiponectin were confirmed in Type II diabetic patients with type IIb hyperlipidaemia. VLDL-cholesterol, determined by a novel HPLC method, was inversely associated with serum adiponectin, which is assumed to be a key factor relating atherogenesis via dysregulated metabolism of lipid and glucose in diabetes. Although the decreased level of adiponectin has been reported to be correlated with increased serum TG, the present study demonstrates for the first time that, among the fractionated lipoproteins, VLDL-cholesterol is inversely correlated with adiponectin independently of age, BMI, gender difference and glycaemic control. From the results using a novel HPLC method to quantify fractionated lipoprotein levels, measurement of VLDL-cholesterol may provide some useful information in understanding the pathological features of diabetic dyslipidaemia and for exploring the mechanisms of adiponectin-mediated control of lipid metabolism. Thus this novel HPLC method may be a suitable tool in future clinical and basic science studies to reveal the mechanisms of adiponectin-mediated regulation of TG-rich lipoproteins.

ACKNOWLEDGMENTS

The present study was supported by grants from the Tosoh Corporation and the Sanko Company. We thank Professor Masayuki Kobayashi (Department of Laboratory Medicine, Kashiwa Hospital, Jikei University School of Medicine, Chiba, Japan) for helpful comments, and Ms Noriko Sato (Division of General Medicine, Department of Internal Medicine, Kashiwa Hospital, Jikei University School of Medicine, Chiba, Japan) for helpful assistance.

REFERENCES

27 Daimon, M., Oizumi, T., Saitoh, T. et al. (2003) Decreased serum levels of adiponectin are a risk factor for the progression to type 2 diabetes in the Japanese population. Diabetes Care 26, 2135–2140

