Lipoprotein Lipids and Apolipoproteins (AI, All, B, CII, CIII) in Type 1 and Type 2 Diabetes Mellitus in Young Kuwaiti Women

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Plasma lipid and apolipoprotein levels of Type 1 and Type 2 young Kuwaiti diabetic women on insulin therapy were investigated to elucidate the relationship between coronary artery disease risk factors and lipid levels. Forty Type 1 and 52 Type 2 diabetic women aged 45 and 62 corresponding control subjects (matched for age and body mass index) were investigated. In comparison with control subjects, both groups of diabetic patients showed marked increases in total-cholesterol, LDL-cholesterol, triglycerides, very low density triglycerides, apolipoprotein B, glucose, fructosamine, and glycosylated haemoglobin Hba1c (all p < 0.001). However, apolipoprotein CII was significantly elevated in Type 2 diabetic patients (p < 0.001) but not in Type 1 patients. Concentrations of apolipoproteins CII and All in both diabetic groups were not significantly different from those in control subjects. Levels of HDL1, HDL2 and HDL3-cholesterol and plasma apolipoprotein AI were markedly decreased in both the diabetic groups compared with their control groups (all p < 0.001 except HDL3-cholesterol in Type 1 diabetic vs control, p < 0.05). In Type 2 diabetic patients, Hba1c correlated positively with triglycerides (r = 0.70, p < 0.001), cholesterol (r = 0.60, p < 0.001), apolipoprotein B (r = 0.77, p < 0.001), and apolipoprotein CII (r = 0.55, p < 0.001) and negatively with apolipoprotein AI (r = -0.49, p < 0.001). In Type 1 diabetic patients Hba1c correlated positively only with apolipoprotein CII (r = 0.50, p < 0.001).

KEY WORDS Type 1 diabetes Type 2 diabetes Kuwait Women Lipids Lipoproteins Apolipoproteins Blood glucose control

Introduction

Epidemiological studies in several countries have suggested a relatively greater risk of coronary artery disease in diabetic women. Abnormalities in plasma and lipoprotein lipids are frequent and may contribute to the increased incidence of atherosclerosis in diabetic patients. A number of studies have yielded contradictory information regarding levels of cholesterol and triglycerides in plasma and lipoprotein fractions of female patients with diabetes. These alterations have been shown to be dependent on the control of diabetes.

In well-controlled Type 1 diabetic patients normal plasma cholesterol, triglycerides, and lipoproteins have been observed, while in Type 2 diabetic patients triglycerides, VLDL and LDL-triglyceride levels are elevated, while the levels of HDL and HDL-cholesterol are reduced. However, only limited data have been reported regarding levels of HDL and HDL-cholesterol in diabetic women and the results indicated disagreement as to which of the HDL subfractions contributed most to total HDL-cholesterol.

A number of studies suggest that plasma apolipoprotein levels, and the ratio of plasma apolipoprotein AI (the major HDL protein) and of apolipoprotein B (the major LDL protein) could provide additional information in predicting coronary artery disease. Apolipoproteins CII and CIII (the major VLDL proteins) are reported to play an important role in triglyceride catabolism, and may also contribute to coronary artery disease. Apolipoprotein CII is an activator of lipoprotein lipase which hydrolyses triglycerides, while apolipoprotein CIII reportedly inhibits lipoprotein lipase. Low levels of apolipoprotein AI and raised levels of apolipoprotein B have been reported in coronary artery disease. Concentrations of apolipoprotein AI, All, and CII in female Type 1 and Type 2 diabetic patients were reported to be normal or decreased, while apolipoprotein B and CIII levels have been found to be increased, decreased or normal.

Recent studies suggest that young diabetic Arab females with Type 2 diabetes treated with insulin have abnormal serum levels of lipids and apolipoproteins, and high mortality after myocardial infarction. Furthermore our preliminary studies showed that young normal Kuwaiti female subjects compared with the young normal Arab female subjects, independent of the presence of diabetes,
had slightly higher plasma cholesterol and LDL-cholesterol and lower HDL-cholesterol and apolipoprotein AI levels, (all risk factors for atherosclerosis). Therefore, we have investigated plasma and lipoprotein lipids, and apolipoproteins Al, All, B, CII, and CIII in Type 1 and Type 2 young diabetic Kuwaiti women treated with insulin, and compared them with normal subjects matched for age and body mass index.

Patients and Methods

Patients

Twelve- to 14-h fasting blood samples were collected from female insulin-treated patients aged 16–40 years attending diabetic out-patient clinics for their regular check-ups. A mixture of short- and extended-acting insulin (Novo, Bagsvaerd, Denmark) was then administered. Based on the criteria proposed by the WHO, the patients were then divided into two groups. Type 1 (insulin-dependent) patients were selected on the basis of onset of symptoms, a history of ketoacidosis, low fasting serum C-peptide levels (< 0.60 nmol l⁻¹), and treatment with insulin since the onset of the disease. Type 2 (non-insulin-dependent) patients were defined by strong family history of diabetes mellitus at onset, fasting serum C-peptide > 0.60 nmol l⁻¹ 32,33 and no history of ketoacidosis. Both groups had significantly higher levels of plasma glucose, serum fructosamine, and HbA₁c compared with their corresponding control groups (Table 1). Medical histories were reviewed to assess the present clinical status of the diabetic patients, as detailed in Table 1. The control subjects were selected from university personnel and were matched for age, height, and body weight for both diabetic groups. Body mass index (BMI) of Type 1 and 2 diabetic patients was comparable with their control group (Table 1). None of the patients or control subjects were hyperventilators, smokers, alcoholics, or pregnant, and their sole medication was insulin. In none of the study populations were there historical, clinical or electrocardiographic evidence of macrovascular disease, nor were there any detectable retinopathy. All subjects led sedentary lives and none was taking regular physical exercise.

Analytical Methods

Blood (25 ml) was collected in EDTA and immediately sent to the laboratory. An aliquot was taken for glycosylated haemoglobin (HbA₁c) determination. Plasma was separated from the remainder for immediate analysis of glucose, fructosamine, cholesterol, triglycerides, apolipoproteins AI, All, B, CII and CIII, and for the sequential ultracentrifugal fractionation of VLDL, LDL, total HDL, HDL₂ and HDL₃ fractions. Haemoglobin A₁c, was separated by isoelectric focusing of erythrocyte haemolysates on polyacrylamide slab gel over a pH gradient of 5.3–7.5 (LKB kit, Bromma, Sweden). The concentration of fructosamine was assayed using a commercial kit (Hoffmann La Roche, Basle, Switzerland). The intra- and inter-assay precision were 2.2% and 2.6% respectively.

Separation of lipoproteins was performed by modified sequential ultracentrifugation in a preparative ultracentrifuge (Sorvall, Washington, DC, USA) using a fixed-angle rotor (type TFT 48.6) with appropriate adaptors. The procedure has been described in detail elsewhere. Triglycerides, cholesterol, glucose, HDL₁, HDL₂, and HDL₃-cholesterol were analysed in triplicate by enzymatic methods (Wakopure, Osaka, Japan) using an Abbott Super VP Analyzer (Abbott, Wiesbaden Delkenheim, Germany). Control plasma pools were

<table>
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<tr>
<th>Table 1. Clinical and biochemical characteristics of the control and diabetic female subjects</th>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Height (m)</td>
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<tr>
<td>Weight (kg)</td>
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<tr>
<td>Body mass index (kg m⁻²)</td>
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<tr>
<td>Duration of diabetes (years)</td>
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<tr>
<td>Insulin required (U day⁻¹)</td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
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<tr>
<td>Diastolic blood pressure (mmHg)</td>
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<tr>
<td>Plasma glucose (mmol l⁻¹)</td>
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<tr>
<td>Glycosylated haemoglobin (HbA₁c, %)</td>
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<tr>
<td>C-peptide (nmol l⁻¹)</td>
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<td>Fructosamine (mmol l⁻¹)</td>
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Mean ± SD.
* p < 0.001 vs corresponding control values.
* p < 0.001 vs Type 1.
HbA₁c, normal range 4.5–7.5%.


supplied by Wako and the Center for Disease Control (Atlanta, GA, USA). The values for these controls were within the recommended range and coefficient of variation within batch was < 1.8%, and between batches < 4.5%, for all of the above estimations for normal, low and high concentrations. Apolipoproteins AI and AlII\(\text{III}\) and B\(\text{IV}\) were determined by radial immunodiffusion. Plates were purchased from Immuno (Vienna, Austria) and were analysed in duplicate including standards and controls. Controls from the Center for Disease Control were used, and all were within the expected ranges. For apolipoprotein Al inter- and intra-assay coefficients of variation were 3.5% at the level of 1.5 g l\(^{-1}\) and 4.5% at the level of 1.00 g l\(^{-1}\). For apolipoprotein AlII, the coefficient of variation was 4.5% at the level of 0.70 g l\(^{-1}\). Apolipoprotein CII and CIII values were determined using plates from Diichi (Tokyo, Japan).\(^{40}\) Inter- and intra-assay coefficients of variation were below 10%. C-peptide estimation used reagent kits from Biodata (Rome, Italy);\(^{42}\) assay range was 0.1–6.0 nmol l\(^{-1}\). The inter- and intra-assay variations were below 8% throughout the assay range.

Statistical Methods

Statistical significance for differences in lipoprotein and apolipoprotein compositions between groups was tested using one-way analysis of variance and Student’s two-tailed t-test. Pearson’s correlation coefficients were calculated. All statistical tests were performed at the computer department of the Kuwait Medical School using standard SPSS programmes (Texas, USA).

Results

Both female Type 1 and Type 2 diabetic patients had significantly increased cholesterol, VLDL-cholesterol, LDL-cholesterol, triglyceride, VLDL-triglyceride, LDL-triglyceride, and HDL-triglyceride when compared with their corresponding control group (Table 2). In Type 1 diabetic patients triglyceride and VLDL-triglyceride concentrations were lower than in Type 2 diabetic patients. Levels of HDL-cholesterol, HDL\(_2\)-cholesterol and HDL\(_3\)-cholesterol were significantly decreased in both diabetic groups compared with control subjects, but higher levels of these fractions were observed in Type 1 than in Type 2 diabetic patients.

Apolipoprotein AI levels were significantly decreased, but apolipoprotein B levels significantly increased in both diabetic groups compared with their corresponding control groups (Table 3). Apolipoprotein CIII levels were significantly increased in Type 2 diabetic patients compared with their control group and Type 1 diabetic patients, but apolipoprotein CIII levels did not differ between Type 1 patients and their normal control group. There was no significant difference in apolipoprotein CII in either diabetic group compared with their control group.

For the groups combined HDL-cholesterol correlated positively with apolipoprotein AI, \(r = 0.61, p < 0.001\), and negatively with triglycerides \(r = -0.62, p < 0.001\) and apolipoprotein B, \(r = -0.45, p < 0.001\). Triglycerides correlated negatively with apolipoprotein AI, \(r = -0.60, p < 0.001\) and positively with apolipoprotein B, \(r = 0.62, p < 0.001\). All these correlations were significant in either group analysed individually.

In Type 1 diabetic patients HbA\(_1\)c correlated positively with apo CIII only \(r = 0.50, p < 0.001\). In Type 2 patients HbA\(_1\)c correlated positively with cholesterol \(r = 0.60, p < 0.001\), triglycerides \(r = 0.70, p < 0.001\), apolipoprotein B \(r = 0.77, p < 0.001\), and apolipoprotein CIII \(r = 0.55, p < 0.001\), and negatively with apolipoprotein AI \(r = -0.47, p < 0.001\). Cholesterol correlated positively with apolipoprotein CII \(r = 0.41, p < 0.001\) only in the Type 2 diabetic group. Triglycerides correlated positively with apolipoprotein CII \(r = 0.53, p < 0.001\) only in Type 2 patients. Apolipoprotein CII and apolipoprotein CIII correlated positively to each other in both diabetic groups \(r > 0.50, p < 0.001\).

Discussion

Elevations in the level of plasma total- and LDL-cholesterol, apolipoprotein B, total- and VLDL-triglycerides in our young Type 1 and Type 2 diabetic Kuwaiti women is in agreement with some of the previously reported data in women with Type 1 or Type 2 diabetes. The results of the present study showed that diabetic control was poor as indicated by the high HbA\(_1\)c, fructosamine, and high fasting plasma glucose levels in both groups of patients despite insulin therapy and strict dietary advice. Thus the above observations may reflect either inadequate insulinization, or non-compliance with instructions regarding diet and exercise, and/or a combination of factors.\(^{6,12,18,41,44}\)

In our study the observed significantly low levels of the HDL- and LDL\(_2\)-cholesterol and its main apolipoprotein AI, and their inverse correlation with triglyceride and VLDL-triglycerides levels may indicate a possible metabolic link.\(^{8,9,13,15,28}\) In Type 1 diabetic patients, this could be due to diminished removal due to decreased lipoprotein lipase activity as a result of inadequate insulin levels,\(^{8,14,41,45}\) which in Type 2 diabetes these changes may reflect increased production of VLDL-triglycerides related to influx of non-esterified fatty acids into liver as excess substrate for hepatic triglyceride synthesis, or to hyperglycaemia or hyperinsulinaemia,\(^{12,41,42,46}\) or due to decreased clearance of triglyceride as a result of impairment of the lipoprotein lipase activity associated with insulin insensitivity.

The observed high levels of apolipoprotein B and its correlation with other plasma lipids reported in the present study in both Type 1 and Type 2 diabetic groups, may be related to either increased production and/or impaired catabolism of LDL possibly by glycosylation of...
Table 2. Plasma lipids and lipoprotein concentrations (mmol L⁻¹) in control and insulin-treated diabetic female subjects

<table>
<thead>
<tr>
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<th>Control</th>
<th>Type 1</th>
<th>Control</th>
<th>Type 2</th>
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<tr>
<td></td>
<td>45</td>
<td>40</td>
<td>62</td>
<td>52</td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total</td>
<td>4.54 ± 0.44</td>
<td>5.73 ± 0.52*</td>
<td>4.52 ± 0.20</td>
<td>5.81 ± 0.52*</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.43 ± 0.08</td>
<td>0.53 ± 0.10*</td>
<td>0.45 ± 0.10</td>
<td>0.57 ± 0.10*</td>
</tr>
<tr>
<td>LDL</td>
<td>2.54 ± 0.36</td>
<td>4.04 ± 0.66*</td>
<td>2.56 ± 0.29</td>
<td>4.31 ± 0.41*</td>
</tr>
<tr>
<td>HDL</td>
<td>1.58 ± 0.13</td>
<td>1.23 ± 0.13*</td>
<td>1.56 ± 0.16</td>
<td>0.98 ± 0.11**</td>
</tr>
<tr>
<td>HDL2</td>
<td>0.65 ± 0.08</td>
<td>0.45 ± 0.09*</td>
<td>0.64 ± 0.10</td>
<td>0.34 ± 0.09**</td>
</tr>
<tr>
<td>HDL3</td>
<td>0.93 ± 0.08</td>
<td>0.78 ± 0.17*</td>
<td>0.91 ± 0.08</td>
<td>0.64 ± 0.10**</td>
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<tr>
<td>Triglycerides</td>
<td></td>
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<tr>
<td>Total</td>
<td>0.73 ± 0.12</td>
<td>1.93 ± 0.32*</td>
<td>0.89 ± 0.17</td>
<td>2.17 ± 0.42**</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.43 ± 0.08</td>
<td>1.32 ± 0.25*</td>
<td>0.49 ± 0.11</td>
<td>1.54 ± 0.26**</td>
</tr>
<tr>
<td>LDL</td>
<td>0.24 ± 0.07</td>
<td>0.42 ± 0.12*</td>
<td>0.26 ± 0.08</td>
<td>0.41 ± 0.08**</td>
</tr>
<tr>
<td>HDL</td>
<td>0.13 ± 0.02</td>
<td>0.18 ± 0.03**</td>
<td>0.14 ± 0.03</td>
<td>0.22 ± 0.02**</td>
</tr>
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</table>

Mean ± SD.  
* p < 0.001;  
* p < 0.05 compared with control group.  
* p < 0.001;  
* p < 0.05 between diabetic groups.

Lipoprotein densities: VLDL, d < 1.006 g ml⁻¹; LDL, d 1.006-1.063 g ml⁻¹; HDL, d 1.063-1.21 g ml⁻¹.

Table 3. Plasma apolipoprotein AI, AII, B, CII, CIII concentrations (g L⁻¹) in the control and diabetic female subjects studied

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Type 1</th>
<th>Control</th>
<th>Type 2</th>
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<tr>
<td></td>
<td>45</td>
<td>40</td>
<td>62</td>
<td>52</td>
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<tr>
<td>Plasma apolipoproteins</td>
<td></td>
<td></td>
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<tr>
<td>AI</td>
<td>1.40 ± 0.17</td>
<td>1.19 ± 0.15*</td>
<td>1.41 ± 0.22</td>
<td>1.07 ± 0.14*</td>
</tr>
<tr>
<td>AII</td>
<td>0.56 ± 0.07</td>
<td>0.52 ± 0.06</td>
<td>0.560 ± 0.100</td>
<td>0.56 ± 0.01</td>
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<tr>
<td>B</td>
<td>0.78 ± 0.09</td>
<td>1.07 ± 0.08*</td>
<td>0.82 ± 0.11</td>
<td>1.16 ± 0.10**</td>
</tr>
<tr>
<td>CII</td>
<td>0.042 ± 0.002</td>
<td>0.030 ± 0.002*</td>
<td>0.045 ± 0.002</td>
<td>0.040 ± 0.001</td>
</tr>
<tr>
<td>CIII</td>
<td>0.070 ± 0.002</td>
<td>0.060 ± 0.002</td>
<td>0.070 ± 0.002</td>
<td>0.110 ± 0.004**</td>
</tr>
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Mean ± SD.  
* p < 0.001 compared with control group.  
* p < 0.001 between diabetic groups.

apolipoprotein B. On the other hand, low levels of plasma apolipoprotein AI and normal apolipoprotein All levels are observed in both the diabetic groups, in support of earlier reports. Furthermore, apolipoprotein AI showed a positive correlation with HDL- and HDL-cholesterol and negative correlation with VLDL-triglycerides, which may support possible metabolic link between apolipoprotein AI, HDL-cholesterol, and VLDL transport.

We found that Type 2 diabetic patients had increased total- and VLDL-triglycerides and that this was accompanied by an increase in apolipoprotein CII (an inhibitor of lipoprotein lipase) but not apolipoprotein CII (an activator of lipoprotein lipase) levels. In addition, the lower HDL- and HDL-cholesterol and apolipoprotein AI similar to those found in Type 1 diabetic patients may indicate that treatment with insulin does not abolish the abnormality of lipoproteins and apolipoproteins in these diabetic patients as suggested by others. Therefore, the above observations may support the finding reported by others  that regardless of the type of treatment, diabetic women have higher VLDL-triglyceride, LDL-cholesterol, and lower HDL- and HDL-cholesterol.

The relationship between metabolic control and plasma lipoproteins and apolipoproteins is important. Thus, our finding that HbA1c and glucose correlated with most of the lipoproteins, particularly apolipoproteins B, AI, and CII, in Type 2 diabetic patients suggests an influence of blood glucose control on lipid metabolism. The correlation observed between HbA1c and apolipoprotein CII in both diabetic groups may suggest abnormal composition of apolipoprotein CII in these patients.

In Type 1 patients our failure to find significant
correlations between fasting glucose, HbA1c, or triglyceride levels and some of the lipid-related parameters in this study could be partly explained by the inconsistency in the day-to-day metabolic control.

In conclusion, the main finding of the present study was of abnormal levels of cholesterol, triglycerides, and apolipoproteins AI, B, and CII in young insulin-treated Type 1 and Type 2 diabetic Kuwaiti females. Despite insulin therapy, strict dietary instructions, and regular follow-up, the abnormalities persist and might partly explain the finding reported by others that mortality is significantly higher in these female patients following myocardial infarction.

Acknowledgements

We thank A.H. Parker, B. Al-Ghouti, N.A. Hadi and S. Sobeh, for excellent technical assistance, and A.G. Pinto for typing the manuscript. The research was supported by MM011 Grant from the Research Management Unit, Kuwait University.

References


33. Rendel M. C-peptide as a clinical assay. Ligand & Q 1979; 24: 30-34.


48. Hollebeck CB, Idachen YD, Greensfield MS, Lardinois CK, Reaven GM. Reduced plasma HDL cholesterol concentration need not increase when hyperglycaemia is controlled with insulin in NIDDM. J Clin Endocrinol Metab 1986; 63: 605-608.
