PLASMA LIPOPROTEINS AND APOLIPOPROTEINS IN INSULIN-DEPENDENT AND YOUNG NON-INSULIN-DEPENDENT ARAB WOMEN

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Lack of standardized criteria and absence of non-diabetic control population have resulted in a paucity of data, more so regarding women with diabetes, with respect of prevalence and clinical manifestations of coronary heart disease (CHD) in different populations. However, a relatively greater risk of cardiovascular disease in diabetic women is well recognized, both in industrialized societies and in developing countries. Abnormalities in plasma lipids and lipoproteins are frequent and may contribute to the increased prevalence of atherosclerosis in diabetic patients. A number of studies reported conflicting levels of cholesterol and triglycerides in plasma and lipoprotein fractions of diabetic female patients depending on the type of therapy and degree of metabolic control. Normal cholesterol and triglycerides in plasma and lipoproteins were observed in well-controlled insulin-dependent diabetic patients on long-term insulin therapy. In non-insulin-dependent diabetes mellitus (NIDDM) patients, however, total triglyceride, VLDL and LDL triglyceride levels were elevated, but the levels of total HDL and LDL were reduced. It has been shown that HDL is composed of subfractions HDL and HDL and there is evidence that HDL may be the subfraction protecting against the subsequent development of atherosclerosis. In diabetics, only a few studies have reported the levels of HDL and HDL cholesterol and the results indicated a lack of general agreement on the HDL subfraction(s) which contribute the most to the observed changes in HDL-cholesterol.

Interest has lately been focused on the protein moiety of lipoproteins as a discriminating factor between patients with atherosclerotic heart disease and...
healthy subjects. Low levels of apoAI and raised levels of apoB have been reported in these patients. Concentration of apoAI and AI in female diabetics decreased, while that of apoB was varyingly reported as increased, decreased or normal.

The plasma enzyme lecithin cholesterol acyltransferase (LCAT; EC 2.3.1.45), which is activated by apoAI, plays an important role in the metabolism of lipoproteins since it represents a necessary cofactor for esterification of free cholesterol. LCAT activity in diabetes has been reported to be low, normal or increased.

Kuwait has one of the highest diabetes-related mortality rates in the world: a high mortality secondary to acute myocardial infarction (AMI) in the Kuwaiti diabetic population being a major contributory factor. Furthermore, mortality is significantly higher in the diabetic females following AMI. Therefore, we aimed to study plasma lipids, lipoprotein fractions and apolipoproteins AI, AII and B in young Arab female patients compared to control group matched for age, and body mass index (BMI), with a view to assessing lipid risk factors in relation to the possible future development of CHD in such subjects.

MATERIALS AND METHODS

Patients - Full informed consent was obtained from all subjects included in the study, the design of which was approved by the Ethics Committee of the Faculty of Medicine, Kuwait University. Blood samples were collected from Arab female diabetics aged 15–40 years after an overnight fast of 10–14 h. These patients were receiving insulin for metabolic control and were regularly followed up in diabetic polyclinics. Based on the criteria proposed by the WHO, the patients were then divided into two groups: (i) IDDM and (ii) insulin requiring (but) NIDDM. IDDM subjects were selected on the basis of early age at onset of symptoms, having a history of ketoadidosis and low C-peptide levels (less than 0.6 nmol/l) and were receiving treatment with insulin since the onset of the disease. NIDDM subjects were defined by older age at onset, C-peptide (greater than 0.6 nmol/l) and no history of ketoadidosis and initiation of insulin therapy for the sole purpose of achieving optimal metabolic control. Both groups had significantly higher levels of glucose and HbA1c as compared to their corresponding controls (tab. 1). Medical histories were reviewed to assess the present clinical status of the diabetic subjects. The mean total daily insulin requirement was 56 ± 22 U for the IDDM group and 46.12 ± 17 U for the NIDDM group. The mean duration of diabetes was 3.7 ± 1.2 years in IDDM and 4.6 ± 1.0 years in NIDDM. Subjects were selected from university personnel and were matched for age, and body mass index (BMI) to constitute appropriate subgroups to serve as controls for both diabetic groups. Mean BMI for IDDM and NIDDM patients and controls were comparable (tab. 1). While none of the subjects was considered obese (BMI > 30), 5 subjects were overweight (BMI < 30). The mean age of patients was 20.2 ± 4.7 and 42.8 ± 4.7 years in IDDM and NIDDM groups, respectively. None of the patients or controls was hypertensive, past or present smoker, or alcoholic, or pregnant at the time of study. In none of the subjects was there historical, clinical or electrocardiographic evidence of either CHD or peripheral vascular disease. All subjects led sedentary lives and were not participating in any physical exercise. All IDDM and NIDDM subjects were receiving insulin therapy, with appropriate dietary instruction.

Collection of blood samples and analytical methods - Twenty-five ml of blood in EDTA (1.5 mg/ml) tubes (manufactured by Monocent, Division of Sherwood Medical ST. Louis, MO, USA) was collected after an overnight fast (12 ± 2 h) and divided into two portions. 2.5 ml was used for determination of glycosylated hemoglobin (HbA1c) and the rest was centrifuged at 4°C at 3,000 rpm and clear plasma was collected for immediate analysis of glucose, total cholesterol (TC), triglycerides (TG), phospholipids (Ph), LCAT, C-peptide, apoAI, AII, B and for the sequential ultracentrifugal separation of VLDL, LDL, HDL, and HLDL fractions. Hemoglobin A1c was separated by isoelectric focusing of electrophoresis hemolysates on polyacrylamide slab gel over a pH gradient of 5.3–7.5 (LKB kit Cat. n° 1804-131 and LKB 2197 power supply; LKB-producer Ah, Bromma, Sweden).
Tab. 1 - Clinical and biochemical characteristics of the controls and diabetic female subjects. (BMI = weight (kg)/height (m)^2. Values given as mean ± SD; significantly different from corresponding control values *p<0.001, **p<0.0001.)

Lipoprotein separation - The separation of lipoproteins was performed by modified sequential ultracentrifugation method in a preparative ultracentrifuge (Sorval, GTD 75 B, Biomedical Products Division, Washington, DC, U.S.A.) using a fixed-angle rotor (type TFF 48.6) with appropriate adaptations. The procedure is detailed elsewhere.

Lipid and apolipoprotein estimations - Triglycerides, cholesterol, phospholipids, glucose, HDL, LDL, and HDL cholesterol were analysed in triplicate by enzymatic methods (Wako Pure Chemical Industries Ltd., Osaka, Japan) using an Abbott super VP Analyzer (Abbott Diagnostic Products Gmb, Wiesbaden, Germany). Control plasma pools were supplied by the Wako Company and Centers for Disease Control (CDC, Atlanta, GA, U.S.A.). The values for normal controls were within the recommended range and the coefficient of variation (CV) within the batch analysis was less than 1.5%, and between the batches less than 4.5% for all of the above estimations including normal, low and high concentrations. Apolipoproteins A1 and AII, and B were analysed in duplicate by radial immunodiffusion method (Immuno, Vienna, Austria). Controls from CDC were used and all were within the expected ranges. For apoA, inter- and intra-assay CV were 5% at the level of 150 mg/dl and 6% at a level of 100 mg/dl. For apoAI the CV was 4.5% at a level of 70 mg/dl and 5.0% at a level of 40 mg/dl. For apoB it was 5.0% at a level of 100 mg/dl and 5.5% at a level of 70 mg/dl. The minimum detection limit for apoAI, AII and B were 2.9 mg/dl, 0.6 mg/dl and 15.4 mg/dl, respectively.

LCAT activity was measured by the method of Dieplinger and Kostner using the system enzymatic kit from E. Merck Darmstadt, (Germany). The assay range was linear for absorbance versus free cholesterol concentration in the range 0.13-4.8 mmol/l. The CV of 4.6% was observed for LCAT measurement in and between the runs. Copeptide estimation was done by RIA using reagent kit (Cat. n° 10582, Biodata Laboratories, Rome, Italy). The assay range was 0.1-6.0 nmol/l. The sensitivity of the method was 0.05 nmol/l. The inter- and intra-assay CV were within the suggested limits (below 8%) for low, normal and high ranges.

Statistical methods - Statistical significance for differences in lipoprotein and apolipoprotein compositions between groups was tested using one-way analysis of variance and the two-tailed t-test. Pearson's correlation coefficients was also analysed. All statistical tests were performed at the computer department of the Kuwait Faculty of Medicine, using standard SPSS programs.

RESULTS

Plasma lipids in controls, IDDM and NIDDM (tab. 2) - Plasma concentrations of TC, VLDL, LDL, HDL, and HDL cholesterol, TG, VLDL, LDL and HDL triglycerides, phospholipids and LCAT in IDDM, NIDDM and their cor-
### Plasma Lipoproteins and Apolipoproteins in Young IDDM and NIDDM Arab Women

<table>
<thead>
<tr>
<th></th>
<th>controls (n = 40)</th>
<th>IDDM (n = 50)</th>
<th>controls (n = 60)</th>
<th>NIDDM (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>164.7 ± 16.2</td>
<td>219.3 ± 16.1*</td>
<td>170.5 ± 10.2</td>
<td>221.9 ± 21.4*</td>
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<tr>
<td>VLDL cholesterol</td>
<td>144 ± 2.3</td>
<td>166 ± 3.5*</td>
<td>166 ± 5.0</td>
<td>201 ± 3.5*</td>
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<tr>
<td>LDL cholesterol</td>
<td>90.1 ± 12.7</td>
<td>132.2 ± 26.6*</td>
<td>98.3 ± 10.9</td>
<td>165.8 ± 17.4*</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>50.2 ± 4.8</td>
<td>46.7 ± 4.9**</td>
<td>58.8 ± 5.3</td>
<td>57.1 ± 5.3**</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>55.6 ± 2.5</td>
<td>50.1 ± 5.6*</td>
<td>54.9 ± 2.6</td>
<td>54.5 ± 5.4**</td>
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<tr>
<td>Total triglyceride</td>
<td>65.4 ± 11.8</td>
<td>164.0 ± 27.7*</td>
<td>72.8 ± 16.0</td>
<td>189.2 ± 56.6*</td>
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<tr>
<td>VLDL triglyceride</td>
<td>55.2 ± 7.6</td>
<td>113.0 ± 20.6*</td>
<td>56.6 ± 10.1</td>
<td>135.9 ± 54.3*</td>
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<tr>
<td>LDL triglyceride</td>
<td>19.2 ± 5.3</td>
<td>35.4 ± 9.7*</td>
<td>22.3 ± 6.2</td>
<td>38.3 ± 6.3*</td>
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<tr>
<td>HDL triglyceride</td>
<td>100 ± 1.5</td>
<td>15.6 ± 2.0*</td>
<td>11.6 ± 3.3</td>
<td>20.0 ± 2.0**</td>
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<tr>
<td>Total phospholipid</td>
<td>176.0 ± 18.2</td>
<td>225.6 ± 28.2*</td>
<td>188.9 ± 22.3</td>
<td>237.5 ± 26.5*</td>
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<tr>
<td>LCAT activity</td>
<td>120.6 ± 50.0</td>
<td>110.6 ± 36.0</td>
<td>126.4 ± 29.8</td>
<td>118.5 ± 54.5</td>
</tr>
</tbody>
</table>

Tab. 2 - Plasma lipid, lipoprotein concentrations (mean ± SD mg/dl) and LCAT activity (nmol/ml/h) in controls and diabetic female subjects. (Significantly different from their corresponding control values: *p<0.001; **p<0.05; ***p<0.01; significantly different between IDDM group and NIDDM group: $^*$p<0.001; conversion factor from mg/dl to SI units for cholesterol is $\times 0.026$ and triglycerides is $\times 0.017$).

Responding controls are shown in Tab. 2. Both groups of diabetic patients had significantly higher VLDL-TG, LDL and HDL-TG than in the controls. Furthermore, in IDDM, plasma TG and VLDL-TG concentrations were lower than in NIDDM patients. Plasma TC, LDL-C and VLDL-C levels were significantly higher in both diabetic groups compared to controls. In contrast, HDL, LDL and HDL cholesterol concentrations were significantly decreased in both diabetic groups compared to control subjects, although higher levels of these

<table>
<thead>
<tr>
<th></th>
<th>controls (n = 40)</th>
<th>IDDM (n = 50)</th>
<th>controls (n = 60)</th>
<th>NIDDM (n = 50)</th>
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<tbody>
<tr>
<td>Plasma ApoAI</td>
<td>133.5 ± 16.2</td>
<td>120.0 ± 14.0*</td>
<td>146.8 ± 23.1</td>
<td>104.0 ± 15.2*</td>
</tr>
<tr>
<td>HDL ApoAI</td>
<td>116.3 ± 14.5</td>
<td>79.4 ± 11.3*</td>
<td>120.8 ± 26.6</td>
<td>82.2 ± 10.5*</td>
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<tr>
<td>HDL ApoAI</td>
<td>48.6 ± 5.4</td>
<td>33.4 ± 7.2*</td>
<td>49.5 ± 13.8</td>
<td>24.2 ± 3.5*</td>
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<tr>
<td>HDL ApoAI</td>
<td>64.2 ± 7.0</td>
<td>59.1 ± 8.0</td>
<td>70.9 ± 9.9</td>
<td>66.8 ± 15.2</td>
</tr>
<tr>
<td>Plasma ApoAI</td>
<td>51.3 ± 6.8</td>
<td>48.8 ± 5.0</td>
<td>50.0 ± 9.5</td>
<td>50.4 ± 10.5</td>
</tr>
<tr>
<td>HDL ApoAI</td>
<td>41.0 ± 6.4</td>
<td>40.0 ± 5.5</td>
<td>41.1 ± 7.9</td>
<td>45.7 ± 8.6</td>
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<tr>
<td>HDL ApoAI</td>
<td>13.5 ± 5.6</td>
<td>12.5 ± 4.6</td>
<td>10.4 ± 2.0</td>
<td>9.1 ± 3.2</td>
</tr>
<tr>
<td>HDL ApoAI</td>
<td>56.8 ± 7.0</td>
<td>53.4 ± 6.4</td>
<td>54.8 ± 7.8</td>
<td>54.3 ± 6.4</td>
</tr>
<tr>
<td>Plasma ApoB</td>
<td>68.0 ± 8.6</td>
<td>100.1 ± 8.4*</td>
<td>76.8 ± 12.9</td>
<td>112.0 ± 9.4*</td>
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<tr>
<td>LDL ApoB</td>
<td>58.9 ± 9.0</td>
<td>94.2 ± 3.1*</td>
<td>60.9 ± 12.4</td>
<td>90.2 ± 11.7*</td>
</tr>
</tbody>
</table>

Tab. 3 - Apolipoprotein A1, AII and B concentrations (mean ± SD mg/dl) in controls and diabetic female subjects. (Significantly different from their corresponding control values: *p<0.001).
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Controls (n = 40)</th>
<th>IDDM (n = 60)</th>
<th>NIDDM (n = 50)</th>
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</thead>
<tbody>
<tr>
<td>Total cholesterol/HDL cholesterol</td>
<td>2.74 ± 0.04</td>
<td>4.68 ± 0.45**</td>
<td>2.92 ± 0.07</td>
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<tr>
<td>LDL cholesterol/HDL cholesterol</td>
<td>1.56 ± 0.22</td>
<td>3.56 ± 0.30**</td>
<td>1.33 ± 0.24</td>
</tr>
<tr>
<td>apo B/apoAI</td>
<td>0.52 ± 0.19</td>
<td>1.03 ± 0.14*</td>
<td>0.50 ± 0.10</td>
</tr>
<tr>
<td>LDL cholesterol/HDL cholesterol</td>
<td>3.51 ± 0.66</td>
<td>8.32 ± 1.30**</td>
<td>4.08 ± 0.77</td>
</tr>
<tr>
<td>HDL cholesterol/HDL cholesterol</td>
<td>1.67 ± 0.08</td>
<td>0.60 ± 0.08</td>
<td>0.65 ± 0.08</td>
</tr>
</tbody>
</table>

Tab. 4 - Ratios of lipid fractions in controls and diabetic female subjects. (Significantly different from their corresponding control values: *p<0.001; significantly different between IDDM group and NIDDM group: **p<0.0001). Fractions were found in IDDM compared to NIDDM subjects. Phospholipids were found to be significantly increased in both diabetic groups compared to their corresponding controls. There was no significant difference in LCAT activity between both the diabetic groups and the controls (tab. 2).

**Plasma apolipoproteins in controls, IDDM and NIDDM** (tab. 3) - Apolipoprotein AI levels were significantly decreased in total plasma, and in HDL, HDL$_c$ and HDL$_d$ fractions in both diabetic groups as compared to corresponding controls. There was no significant difference between apoAI level in IDDM and NIDDM patients. ApoAI levels did not differ between diabetic patients and controls either in the plasma or in HDL, HDL$_c$ and HDL$_d$ fractions. Plasma and LDL-apoB concentrations were significantly increased in both diabetic groups compared to corresponding controls.

**Ratios of lipid fractions in controls, IDDM and NIDDM** (tab. 4) - In IDDM and NIDDM patients, there was a significant increase in the ratios of TC/HDL-C, LDL-C/HDL-C, LDL-C/HDL$_d$-C and apoB/apoAI compared to the controls.

**Correlations between plasma glucose, HbA$_1c$, lipids and apolipoproteins** - Obesity (BMI) and indices of metabolic control (plasma glucose, HbA$_1c$) did not show significant correlation with plasma lipids, lipoproteins, apolipoproteins and LCAT activity in the diabetic groups. Plasma TG and VLDL-TG correlated positively with plasma apoB and LDL apoB ($r = 0.61$, $p<0.01$ for both) and negatively with plasma HDL-cholesterol, HDL$_c$-cholesterol ($r = -0.43$, $p<0.001$ for both), plasma apoAI, HDL apoAI ($r = -0.42$, $p<0.01$ for both) in the diabetic groups. In contrast, plasma cholesterol and LDL-cholesterol correlated positively with triglycerides, plasma apoB, LDL apoB ($r = 0.50$, $p<0.001$ for both) and negatively with HDL-cholesterol, HDL$_c$-cholesterol ($r = -0.55$, $p<0.001$ for both), plasma apoAI, and HDL apoAI ($r = -0.53$, $p<0.001$ for both) in the diabetic groups.

**DISCUSSION**

The significant elevation of TC, LDL-C, VLDL-C, TG, VLDL, LDL- and HDL-TG as well as phospholipids in young IDDM and insulin requiring NIDDM Arab women is in agreement with some of the previously reported
data in women with IDDM \(^6\) \(^,\) \(^{25-41}\), and in NIDDM \(^6\) \(^,\) \(^{15-18,47}\) with suboptimal glycemic control. Indeed the diabetic control was poor as indicated by the high HbA\(_1\), and high fasting plasma glucose levels in all diabetic in spite of insulin therapy and strict dietary advice particularly in IDDM patients. These changes may reflect either inadequate insulinization, or non-compliance to instructions regarding diet (40-50\% carbohydrate, 20\% protein and 20\% fat), calories were calculated according to weight and activity and exercise (walking, bicycling, swimming), or a combination of factors \(^{16,15,40,50}\).

The observed low levels of HDL-C and HDL\(_2\)-C and high levels of TG and VLDL-TG may indicate a possible metabolic link \(^4\) \(^,\) \(^{4,15,26,48}\). In IDDM, this could be due to diminished removal due to decreased lipoprotein lipase activity as a result of inadequate insulin levels \(^{15,18,44}\), while in NIDDM it may be due either to increased production of VLDL-TG related to influx of free fatty acids into liver as excess substrate for hepatic triglyceride synthesis, or to hyperinsulinemia or hyperglycemia \(^{34,36,44}\), or due to decreased clearance of TG as a result of impairment of the lipoprotein lipase activity associated with insulin resistance.

Irrespective of the underlying mechanism(s), the increased ratios of LDL-C/HDL-C, LDL-C/HDL\(_2\)-C, and T-C/HDL-C as observed in both diabetic groups may be considered as predisposing to increased risk of cardiovascular disease in such diabetes \(^5\) \(^,\) \(^{34}\).

Elevated apoB level and its correlation with other plasma lipids in both the diabetic groups may indicate either increased production and/or impaired catabolism of LDL probably by glycosylation of apoB \(^5\) \(^,\) \(^{34,36,41}\). In contrast to apoB increment, a reduction in apoAI and normal apoAII levels in plasma are observed in both the diabetic groups, which support earlier reports \(^6\) \(^,\) \(^{16,20}\).

The failure to find significant correlations between fasting glucose or HbA\(_1\), 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.

It is recognized that the duration of diabetes and the type of treatment greatly influence plasma lipids, lipoproteins and apolipoproteins. The published data on IDDM suggest that rise in lipoprotein and apolipoprotein levels is reversible following adequate insulinisation \(^6\) \(^,\) \(^{15,27}\); however, such reversibility is slower in NIDDM \(^6\) \(^,\) \(^{15,29}\). Regardless of the type of treatment women have been reported to have higher VLDL-TG, LDL-C and lower HDL-C and HDL\(_2\)-C \(^{26,40,41}\). The present study confirms these observations in Arab women.

In conclusion, the present study for the first time documents abnormal levels of cholesterol, triglyceride and apolipoproteins in plasma and in lipoprotein fractions, in insulin-treated young IDDM and NIDDM Arab females. In spite of insulin therapy, strict dietary instruction and regular follow-up, particularly in IDDM patients, the observed abnormalities could in part be due to lack of physical activity, day-to-day inconsistencies in food intake, or hitherto uncharacterised genetic factors. Long-term studies have been initiated to investigate the effects of strict dietary compliance, a regular exercise regimen, and optimized insulin therapy, both on lipid parameters and on cardio-vascular morbidity and mortality.
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SUMMARY

Plasma lipids, lipoproteins and apolipoproteins (apo) were analyzed in 30 young Arab IDDM and 50 young insulin-requiring NIDDM women. The mean age of IDDM and NIDDM groups was 20.2 and 24.5 years, and mean duration of diabetes was 5.7 and 4.6 years, respectively. Two groups of 40 and 60 healthy women (matched for age and BMI) provided corresponding control groups. In comparison with control subjects, diabetics showed marked increases in lipids and lipoproteins (LDL) cholesterol, triglycerides,IDL cholesterol, triglycerides (TG), very low density lipoprotein (VLDL) triglycerides, phospholipids, apoB, LDL apoB, glucose and glycated hemoglobin (HbA1c) as well as the ratio of total cholesterol/high density lipoprotein (HDL) cholesterol, LDL-cholesterol/HDL-cholesterol, LDL cholesterol/high density lipoprotein 2 (HDL) cholesterol and apoB/apoAI. Plasma LCAT activity, concentrations of HDL, apoAI and apoAII in plasma and lipoprotein fractions were normal in both the diabetic groups. Levels of C-peptide, HDL, LDL, cholesterol, plasma apoAI, HDL apoAI and HDL apoAII were markedly decreased in both the diabetic groups as compared to their respective controls. There was no significant correlation between fasting glucose or HbA1, and any of the above parameters. Despite insulin therapy in both the diabetic groups studied, abnormalities in lipids, apoB and apoAI still persisted. Our data suggest a possible higher risk of atherosclerosis in these patients.

REFERENCES

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