Post-Prandial Hypertriglyceridemia in Patients with Type 2 Diabetes Mellitus with and without Macrovascular Disease

V Kumar*, SV Madhu**, G Singh**, JK Gambhir***

Abstract

Objectives: To study the postprandial hypertriglyceridemia in patients with type 2 diabetes mellitus with and without macrovascular disease.

Methods: Postprandial lipids were studied in 13 type 2 diabetic subjects with macrovascular disease (group I), 13 diabetic subjects without macrovascular disease (group II) and 13 age, sex and BMI matched healthy controls (group III) after an oral fat challenge which consisted of meal providing 729 kcal/m² body surface area with 65.2 g fat.

Results: All the three groups were age, sex and BMI matched. Average duration of diabetes was not significantly different between both the diabetic groups. Waist-hip ratio (WHR) was significantly more in group I and II as compared to group III. Also group I displayed significantly higher WHR than group II. Fasting total cholesterol and LDL levels were significantly higher in group I compared to group III. Fasting HDL was significantly lower in both group I and II vs group III. Fasting TG was not significant between any of the three study groups. Significant postprandial hypertriglyceridemia was observed in group I and group II compared to group III. When area under curves (iAUC) for different lipid parameters were adjusted for their respective fasting values, it was observed that only iAUC TG and iAUC VLDL remained significantly higher in group I and group II as compared to group III. Postprandial triglyceride levels at 6 and 8 hours in group I were significantly higher as compared to group III. Postprandial HDL-C levels at 6 and 8 hours were significantly lower group I and II as compared to group III. Postprandial triglyceride parameters showed significant correlation with fasting triglyceride in group I and II and no significant correlation was found with any of the anthropometric, glycemic and insulin resistance measures.

Conclusion: This study finds significant postprandial hypertriglyceridemia and significant delay in postprandial triglyceride clearance following a standardized fat meal challenge in patients with type 2 diabetes mellitus, particularly those with macrovascular disease. Persistent postprandial hypertriglyceridemia may result in a pro-atherogenic environment leading to atherosclerosis and macrovascular disease in type 2 diabetes subjects.

Introduction

Dyslipidemia that accompanies type 2 diabetes plays an important role in the pathogenesis of accelerated atherosclerosis in this population. The most important components of this dyslipidemia are an elevated very low density lipoproteins (VLDL) and total triglycerides (TGs) and a decreased high density lipoproteins (HDL) concentration in the serum.

While fasting hypertriglyceridemia may be a risk factor for atherosclerosis, particularly in the presence of diabetes mellitus, this association has not been consistent and fasting HDL-C appears to be a far more significant risk factor. However, when TGs are studied in postprandial state, they emerge as stronger and independent coronary risk factors than HDL-C.

Postprandial hypertriglyceridemia have been linked with asymptomatic and symptomatic macrovascular disease in both normo-and hypertriglyceridemic subjects and such abnormalities have been reported in type 2 diabetes, the increased risk of atherosclerosis among them, might therefore be related to the higher postprandial lipemia in them. Earlier studies from our institution clearly demonstrate the presence of postprandial hypertriglyceridemia among diabetic subjects, irrespective of fasting triglyceride levels.

It is not clearly known whether diabetic patients with macrovascular disease have greater abnormalities of postprandial TG metabolism than those without. Therefore, we investigated the postprandial lipid abnormalities in patients with and without macrovascular disease.

Material and Methods

Patients with type 2 diabetes mellitus aged more than 30 years and duration of diabetes more than one year on the basis of revised American Diabetic Association Criteria (Fasting plasma glucose ≥126 mg/dl and 2 hour postprandial plasma glucose ≥200 mg/dl) without history of inherited disorder of lipid metabolism, liver disease, endocrine diseases affecting lipids (hypothyroidism, cushings syndrome), hypertriglyceridemia, hypertension, congestive heart failure, smoking and on drugs affecting lipid metabolism were recruited for the study. Thirteen non diabetic individuals who were age, sex and body mass index (BMI) matched, who were non-smokers, non-alcoholic and do not have overt clinical evidence of coronary artery disease (CAD), cerebrovascular disease (CVD) or peripheral vascular disease (PVD) were recruited as controls.

The subjects were divided into three groups of thirteen each: Group I – subjects with type 2 diabetes mellitus with...
macrovascular disease (CAD, CVD and PVD), group II – subjects with type 2 diabetes mellitus without macrovascular disease, group III – non-diabetic healthy individuals. CAD was defined on the basis of electrocardiography (ECG) evidence of CAD; viz. ST-T and Q-wave changes. Treadmill test (TMT) evidence on the basis of electrocardiography (ECG) evidence of CAD; group III – non-diabetic healthy individuals. CAD was defined with type 2 diabetes mellitus without macrovascular disease, macrovascular disease (cAD, cVD and PVD), group II – subjects containing 729 kcal/m² body surface areas (BSA) and with 5.3 hr). The subjects were then given a standardized fatty meal blood was collected for various biochemical parameters (0 hr). It was given over 10-15 minutes. Blood samples were drawn at baseline, 2, 4, 6, and 8 hours after the oral fat challenge. Serum was separated in all the samples by centrifuging it immediately after collection and stored at -20° C for various biochemical estimations.

Table 1: Demographic profile of the patients in three study groups

<table>
<thead>
<tr>
<th></th>
<th>Group I mean±SD</th>
<th>Group II mean±SD</th>
<th>Group III mean±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54.5±9.5</td>
<td>54.6±7.4</td>
<td>51.1±9.7</td>
<td>a &gt;0.05</td>
</tr>
<tr>
<td>Male:Female</td>
<td>1:3.3 (3/13)</td>
<td>1:3.3 (3/13)</td>
<td>1:3.3 (3/13)</td>
<td>b &gt;0.05</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>7.3±5.4</td>
<td>4.6±3.6</td>
<td>NA</td>
<td>c &gt;0.05</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.5±2.3</td>
<td>22.9±3.3</td>
<td>23.4±2.1</td>
<td>a &gt;0.05</td>
</tr>
<tr>
<td>Waist (cms)</td>
<td>89.6±5.4 b</td>
<td>92.0±14.6 c</td>
<td>78.2±5.2</td>
<td>b &gt;0.05</td>
</tr>
<tr>
<td>Waist : Hip ratio</td>
<td>1.0±0.1 a</td>
<td>0.9±0.1 b</td>
<td>0.8±0.0</td>
<td>c &gt;0.05</td>
</tr>
</tbody>
</table>

Group I vs II = a, Group I vs III = b, Group II vs III = c
p>0.05 (not significant), p<0.05 (significant)

Study Design

An informed consent was obtained from each subject prior to entering the study. A detailed history and physical examination was carried out for every subject who entered in the study as per pre-designed proforma. This included history of duration of diabetes, family history of premature atherosclerosis, history of macrovascular disease in the form of CAD, CVD or PVD, dyslipidemia, history regarding complications of diabetes as well as history and details of treatment received.

Physical examination included assessment of vital parameters, anthropometry (height, weight, waist, and hip measurement) and the thorough systemic examination.

Screening investigations included hemoglobin concentration, glycated hemoglobin, fasting and postprandial blood glucose, blood urea, serum creatinine, liver function tests, fasting lipid profile, chest X-ray, routine urine analysis, ECG and fundus examination by ophthalmologist.

After 14 hours fast, during which water intake was allowed, blood was collected for various biochemical parameters (0 hr). The subjects were then given a standardized fatty meal containing 729 kcal/m² body surface areas (BSA) and with 5.3 gm protein, 24.75 gm carbohydrates, 240 gm cholesterol and 65.2 gm fat. This was in the form of whipped cream and fruits. It was given over 10-15 minutes. Blood samples were drawn at baseline, 2, 4, 6, and 8 hours after the oral fat challenge. Serum was separated in all the samples by centrifuging it immediately after collection and stored at -20° C for various biochemical estimations.

Table 2: Basal glycemic parameters, fasting lipids profile and insulin levels in three study groups

<table>
<thead>
<tr>
<th></th>
<th>Group I mean±SD</th>
<th>Group II mean±SD</th>
<th>Group III mean±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose (mg/dl)</td>
<td>157.2±45.3</td>
<td>145.9±46.2</td>
<td>84.8±9.8</td>
<td>a &gt;0.05</td>
</tr>
<tr>
<td>Post-prandial plasma glucose (mg/dl)</td>
<td>250.2±41.2</td>
<td>227.1±74.9</td>
<td>113.8±9.0</td>
<td>b &lt;0.001</td>
</tr>
<tr>
<td>Glycosylated hemoglobin (%)</td>
<td>8.6±1.9</td>
<td>8.5±1.6</td>
<td>6.1±0.98</td>
<td>c &lt;0.001</td>
</tr>
<tr>
<td>Fasting serum insulin (0 hr) (µIU/ml)</td>
<td>8.3±4.9</td>
<td>10.8±6.5</td>
<td>4.5±2.6</td>
<td>b &gt;0.05</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>186.3±42.2</td>
<td>153.1±20.4</td>
<td>163.6±11.6</td>
<td>b &lt;0.008</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>145.0±85.3</td>
<td>93.3±25.8</td>
<td>95.8±22.4</td>
<td>b &lt;0.001</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>34.0±3.5</td>
<td>39.0±7.1</td>
<td>46.9±5.5</td>
<td>a &gt;0.05</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>29.0±17.0</td>
<td>19.6±5.1</td>
<td>19.2±4.4</td>
<td>a &gt;0.05</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>123.2±40.8</td>
<td>96.2±23.4</td>
<td>85.4±10.8</td>
<td>b =0.004</td>
</tr>
</tbody>
</table>

Group I vs II = a, Group I vs III = b, Group II vs III = c
p>0.05 (not significant), p<0.05 (significant)

Lipid Estimation

Total serum cholesterol was estimated by enzymatic method using cholesterol esterase. HDL cholesterol was estimated by method of Burstein et al (1970), using kits from Accurex Biomedical Private Limited, Mumbai. Serum triglycerides were estimated by an enzymatic method. Serum VLDL was estimated by using VLDL = TG/5 based on the average ratio of TG to cholesterol in VLDL. Serum LDL was determined from the Friedwald’s and Fredrickson’s formula (1972).

Insulin Estimation

The insulin was analysed at 0 hour and at the time of the peak triglyceride response in the paired serum samples by radioimmunoassay using Diasorin INSI-CTK kit (sensitivity 2.0 µIU/ml).

Statistical Analysis

The data were expressed as mean ± SD for all the study groups. The significance of difference was determined using ANOVA followed by Tukey’s test and correlation between different parameter was determined by Pearson correlation coefficient.

Results

As seen in Table 1, all the three study groups are age, sex and BMI matched. Central obesity as indicated by waist and waist/hip ratio was significantly higher in the both diabetic groups with (p<0.001) and without macrovascular disease (p<0.001) when compared with controls. Also, those with macrovascular disease displayed significantly higher waist/hip ratio than...
Table 3: Postprandial lipid area under curves (AUC) and incremental area under curves (i AUC) in three study groups

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC AUC</td>
<td>1672.1</td>
<td>±392.5</td>
<td>1436.0</td>
<td>±179.8</td>
</tr>
<tr>
<td></td>
<td>a &gt;0.05</td>
<td>b =0.003</td>
<td>c &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>TC i AUC</td>
<td>162.9</td>
<td>±208.0</td>
<td>187.2</td>
<td>±98.4</td>
</tr>
<tr>
<td></td>
<td>a &gt;0.05</td>
<td>b =0.005</td>
<td>c &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>LDL AUC</td>
<td>966.5</td>
<td>±333.7</td>
<td>828.9</td>
<td>±170.6</td>
</tr>
<tr>
<td></td>
<td>a &gt;0.05</td>
<td>b =0.027</td>
<td>c &lt;0.05</td>
<td></td>
</tr>
<tr>
<td>VLDL-C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1: Area under curves (AUC) for postprandial triglyceride in three groups

Fig. 2: Incremental area under curve (iAUC) for postprandial triglyceride in the three study groups

Table 4: Post-prandial TG peak, HDL nadir and their clearance in the three study groups

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG 6 hr PP</td>
<td>346.5±248.0</td>
<td>206.0±66.4</td>
<td>121.4±25.4</td>
<td>a &gt;0.05</td>
</tr>
<tr>
<td>TG 8 hr PP</td>
<td>294.1±249.2</td>
<td>188.8±72.9</td>
<td>106.3±17.4</td>
<td>b =0.008</td>
</tr>
<tr>
<td>HDL 6 hr PP</td>
<td>36.5±5.3</td>
<td>38.9±7.0</td>
<td>58.4±3.8</td>
<td>c &lt;0.001</td>
</tr>
<tr>
<td>HDL 8 hr PP</td>
<td>34.8±4.5</td>
<td>38.8±6.7</td>
<td>49.4±4.8</td>
<td>b &lt;0.001</td>
</tr>
</tbody>
</table>

Group I vs II = a, Group I vs III = b, Group II vs III = c
p>0.05 (not significant), p<0.05 (significant)

Table 2 shows that fasting, postprandial plasma glucose and glycosylated hemoglobin was significantly higher in both the diabetic groups (group I and II) as compared to controls (group III). Fasting insulin levels were also higher in both the diabetic groups as compared to controls. However, this reached statistical significance only for group II vs group III.

Fasting level of HDL was significantly lower in both the diabetic groups (group I and II) as compared to controls (group III). Fasting TC and LDL levels were significantly higher in diabetic subjects with macrovascular disease as compared to controls. However, it only reached statistical significance in group II vs group III.

Table 3 shows postprandial area under curves (AUC) for different lipids - AUC TC and AUC TG, AUC HDL, AUC VLDL and AUC LDL was significantly higher in diabetic subjects with macrovascular disease as compared to controls (Fig. 1). Significant difference was also found for AUC HDL and UAC-VLDL between diabetic subjects without macrovascular disease and healthy controls. When each of these curves were adjusted for their respective fasting lipid values, it was observed that only the incremental area under curve (iAUC) TG and iAUC VLDL remained significantly higher in both diabetic groups compared to controls (Fig. 2).

Table 4 shows significantly higher postprandial triglyceride levels at 6 and 8 hours in diabetic patients with macrovascular disease indicating a delayed postprandial TG clearance in this group. Similarly, there were significantly lower HDL-C levels at 6 and 8 hours in both the diabetic groups compared to controls.

There was no significant correlation observed between any value of HDL-C and TG levels at any time point.
of the postprandial triglyceride parameter including TG AUC, TG iAUC, TG6 and TG8 with any of the anthropometric (BMI, waist, WHR), glycemic [blood glucose fasting and postprandial and glycosylated hemoglobin (GHb)] or insulin resistance parameters (insulin at 0 hour) either in the combined diabetic groups or in those with macrovascular disease. However, all these postprandial lipid abnormalities significantly correlated with fasting triglyceride level both in the combined diabetic groups as well as those with macrovascular disease.

Discussion

This study demonstrates significant hypertriglyceridemia as indicated by significantly higher iAUC for TGs after standard oral fat challenge in patients of type 2 diabetes mellitus with and without macrovascular disease (MVD) as compared to controls. Further, diabetic patients with macrovascular disease also showed a significantly delayed postprandial TG clearance as compared to controls. Also, postprandial hypertriglyceridemia tended to be higher and postprandial TG clearance tended to be delayed in diabetic subjects with MVD compared to those without MVD. However, this difference did not reach statistical significance. Difference in postprandial TG metabolism occurred despite the fact that there was no significant difference in fasting TG level between any of the three study groups. It would thus appear that while postprandial-TG abnormality occur in T2DM patients regardless of presence or absence of macrovascular complications, the magnitude of the abnormalities tend to be higher in those with MVD suggesting that higher postprandial-TG burden may be associated with higher MVD.

Earlier studies of postprandial-TG metabolism in T2DM patients have reported on the diabetic group as whole and have not investigated its relationship with presence or absence of macrovascular complications. While some of these studies reported abnormal postprandial triglyceridemia in type 2 DM subjects, others failed to demonstrate the same.17 To the best of our knowledge this is the first study to report altered postprandial TG response in diabetic patients with and without MVD.

In this study, in addition to higher postprandial-TG AUC, higher AUCs were also observed for TC, LDL-C and HDL-C in T2DM with MVD. However, once these are corrected for their respective fasting values and postprandial incremental curves are compared for different lipemic parameters, it becomes clear that only postprandial-TG remains significantly higher in the macrovascular disease positive diabetic groups. This suggests that hypertriglyceridemia is the dominant lipid abnormality in the postprandial phase in this group of patients.

It is hypothesized that elevated postprandial TG levels may lead to an alteration in oxidative stress and consequent endothelial dysfunction that may finally lead to atherosclerosis and MVD in diabetic patients.18

Several mechanisms have been hypothesized to cause postprandial TG abnormalities in type 2 diabetes subjects. The finding of significant delay in TG clearance in diabetic patients with MVD in this study suggests that this is an important underlying defect in postprandial TG metabolism which predispose to postprandial hypertriglyceridemia in the face of a high fat meal.

Postprandial abnormalities in diabetic patients with MVD were not significantly related to fasting or postprandial glucose, glycosylated hemoglobin levels but were significantly associated with fasting TG levels. Similar observations were reported earlier in diabetic patients overall.17

While it would be difficult to say whether fasting TG level determine postprandial triglyceride or vice-versa, the finding of significant postprandial increment of TG in the presence of normal fasting TG levels in this study would suggest that is an earlier abnormality and as the magnitude of postprandial hypertriglyceridemia increases, TG levels would continue to remain high till the fasting state is reached.

PP hypertriglyceridemia and delayed TG clearance did not show any clear relation with central adiposity or insulin resistance. Some of the earlier studies in literature have reported significant association of postprandial lipemic and insulin resistance in both diabetic and non-diabetic subjects.19,20

In conclusion, this study finds significant postprandial hypertriglyceridemia and significant delay in postprandial-triglyceride clearance following a standardized fat meal challenge in patients with type 2 diabetes mellitus, particularly those with macrovascular disease. Persistent postprandial hypertriglyceridemia may result in a pro-atherogenic environment leading to atherosclerosis and macrovascular disease in type 2 diabetes subjects.

References


15. Friedwald WT, Levy RI, Fredrickson DS. Estimation of the 
   concentration of low-density lipoprotein cholesterol in plasma, 
   without use of the preparative ultracentrifuge. *Clin Chem* 

16. Barrett-Connor E, Grundy SM, Holdbrook MJ. Plasma lipids 
   and diabetes mellitus in an adult community. *Am J Epidemiol* 
   1982;115:657-663.

17. Lewis GF, O’Meara NM, Soltys PA. Fasting Hypertriglyceridemia in 
   non-insulin dependent diabetes mellitus is an important predictor 
   of postprandial lipid and lipoprotein abnormalities. *J Clin Endocrinol 

18. Blann AD, McCollum CN. Circulating endothelial cell/leukocyte 
   4.

   S, et al. Insulin resistance is independently associated with 
   postprandial alterations of triglyceride-rich lipoproteins in type 2 

20. Ntyintyane LM, Panz VR, Rall FJ, Gill GV. Postprandial lipoaemia, 
   metabolic syndrome and LDL-particle size in urbanized South 