Effect Of Garlic, Fish Oil And Dietary Fibre On Postprandial Lipemia And Glycemia Of Type 2 Diabetes Subjects.
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Citation

Abstract
Objective: The present study was undertaken to evaluate the effect of garlic (deodourised powder), fish oil (Max EPA) and dietary fibre (wheat bran+pectin) on postprandial lipemic and glycemic profile in Type 2 diabetes mellitus subjects. Methods: Eight age matched male type 2 diabetes subjects with FBS of 144± 39.7 were randomly selected and anthropometric measurements were estimated. The study was divided into three phases, spaced 4 days apart; each phase consisting of 7 days supplementation of the each supplement in the breakfast and on the eight day the postprandial response was studied by drawing fasting and postprandial blood samples at every 1 ½ hour interval up to 6 hours. The serum was analyzed for composite lipid profile (total lipid, cholesterol, triglyceride, LDL-C, HDL-C and VLDL-C) and glucose levels. Results: The results showed that the fish oil supplement was most effective in controlling the peak rise of serum cholesterol (control 9%, fish oil 5%), LDL-C (control 53%, fish oil 6.7%) and increasing the levels of HDL-C (control 2.6%, fish oil 30%). Garlic supplement was observed to be effective in reducing the peak rise of serum triglyceride (control 91.6%, garlic 20%) and VLDL-C (control 106.5%, garlic 20.4%) and showed an early PP decline in lipid levels. Dietary fibre supplement only improved the postprandial glycemic response. Conclusion: The postprandial lipemic and glycemic response of the supplements have shown that while garlic is effective in controlling only postprandial lipemia and dietary fibre in controlling the postprandial glycemic response, fish oil is effective in controlling both the lipemic and glycemic response. It can therefore be suggested that a continuous supplementation with fish oil or a combination of fish oil and garlic is advisable for the management of type 2 diabetes to delay the onset of cardiovascular complications.

INTRODUCTION
Diabetes mellitus magnifies the risk of cardiovascular morbidity and mortality. Besides the well-recognized microvascular complications of diabetes, such as nephropathy and retinopathy, there is a growing epidemic of macrovascular complications, including diseases of coronary arteries, peripheral arteries, and carotid vessels, particularly in the burgeoning type 2 diabetic population. The factors commonly present in type 2 diabetic individuals which precipitate the risk of coronary artery diseases include hypercholesterolemia, hypertriglyceridemia, platelet function, alternations in vascular wall morphology, and higher ambient insulin concentration(1).

Postprandial (PP) lipemia, characterized by a rise in triglyceride-rich lipoproteins after eating, is a dynamic, non-steady state condition in which humans spend the majority of time. There are several lines of evidence suggesting that PP lipemia increases risk of atherogenesis. Clinical data show a correlation between postprandial lipoproteins and the presence/progression of coronary artery disease and carotid intimal thickness (2). The magnitude of the ensuing PP lipemia varies greatly among individuals, reflecting varying capacities to metabolize dietary fat and more so in diabetic subjects, where the fat metabolism is deviant due to the metabolic changes. Most of the studies reported so far on the PP response with various dietary interventions have been conducted on healthy and non-diabetic hyperlipidemic subjects(3). Several clinical studies have demonstrated a strong positive relationship between the progression and pathogenesis of atherosclerosis with magnitude and duration of postprandial triglyceridemia. Karpe etal reported that the PP lipoproteins might be particularly atherogenic, as they are metabolized on the endothelial surface of large arteries and their cholesterol becomes incorporated into the artery wall, where it stimulates formation of atherosclerotic lesions(4). Since limited evidence is available on the effect of dietary intervention on PP lipemia in type 2 diabetes subjects, and
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garlic, fish oil and dietary fibre have been established as potent hypolipidemic agents (5). This study was undertaken to evaluate the effect of garlic, fish oil and dietary fibre on PP lipemia and glycemia in type 2 diabetes subjects.

MATERIAL AND METHODS

Subjects: Eight male Type 2 diabetes subjects with mean fasting blood glucose levels of 144.5±39.7 mg/dl between the age of 50-63 years were selected for the study. A questionnaire was administered to exclude subjects allergic to foods used in the test meals, taking medications interfered with lipid metabolism and suffering from any liver or endocrine disorders.

The body weight and height were measured by the same person using an electronic balance and stadiometer with the recording nearest to 0.1kg and 0.1cm respectively. The Body Mass Index (BMI) was calculated accordingly.

Experimental design: the study was divided into three phases, each phase consisting of 7 days spaced with a gap of ≥ 4 days as a wash out period between the two consecutive phases.

The different supplements given in each phase were as follows

Phase 1 Diet I - Control diet (CD)
Phase 2 Diet II- CD+5 g deodourised garlic powder
Phase 3 Diet III- CD+4 capsule MaxEPA (1.2g n-3 PUFA)
Phase 4 Diet IV CD+5 g wheat bran+ 5 g pectin

Each subject served as his own control by first participating in the control test meal response then waiting for ≥ 4 days before returning to meal enriched with test supplement. The subjects were instructed not to deviate from their regular dietary, exercise habits before and between test meals. The experimental protocol and potential risks of participating were explained verbally and in a written consent form. Subjects were treated in accordance with the criteria outlined by the Regional Ethical Committee.

The control diet included Chapathi (unleavened pan baked bread), Vegetable sautéed (beans, carrot, tomato, egg plant), Pulse, boiled egg white and tea which contributed to 381kcal, 68g protein, 16g protein and 48 g fat.

The test supplements (Diet II) deodourised garlic powder and dietary fibre (Diet IV) (Both commercially available) were incorporated into the vegetable sautéed whereas fish oil (Diet III) was given in the form of capsules along with the meal.

The test supplement were given to the subjects along with control meal at breakfast for seven days, and on the eighth day postprandial response was studied by drawing fasting (8-10 hours) intravenous blood at every 1 ½ hours interval up to 6 hours after breakfast.

Drawing of blood: 2 ml of venous blood was drawn by a trained technician, centrifuged within 20 minutes at 2000-3000 rpm and the serum was stored in sterile glass vials at 2-8°C until analysis.

Analysis: Serum total lipids(6), triglyceride(7), Plasma glucose(8), Plasma total cholesterol and HDL-C (9) were estimated using standard methods; whereas LDL-C was calculated using Friewalds formula, and VLDL-C was calculated as follows:

\[ \text{VLDL-C mg%=Total Cholesterol-HDL-Cholesterol-LDL-Cholesterol} \]

Statistical analysis: The results were reported as means values and standard deviations; the group means were subjected to Analysis of variance (ANOVA) to compare the effect of each supplement to the control and in between the variables. A p value less than 0.05 were considered as statistically significant.

RESULTS

According to the BMI all the subjects were in the overweight category (24.3±0.8kg/m²) with respect to weight-for-height. Supplementation of garlic, dietary fibre and fish oil for a short period of 7 days did not decrease the fasting lipids significantly.

The mean postprandial total lipid levels decreased significantly (p<0.05) with garlic and dietary fibre supplement. The diet with garlic and fish oil reached an early peak at 1 ½ hours, followed by a decline to fasting levels by 6 hours. Diet enriched with dietary fibre supplement showed a sustained rise up to 4 ½ hours followed by a decline (fig 1).
Serum triglyceride and VLDL-C levels almost reached the fasting levels by 6 hours postprandially, and there was no significant difference (p<0.05) between the mean postprandial response of the control and the experimental diets. The per cent peak rise of PP triglyceride after the garlic and fish oil supplementation was 20% and 27.7% when compared to 91.6% for the control meal. The diet with garlic showed a biphasic response with two peak at 1 ½ hour and 4 ½ hour while PP triglyceride peak with dietary fibre and fish oil was reached at 3rd hour (fig 2).

Fig 3 shows the PP cholesterol response where in the peak levels were reached at 3rd hour with all the diets though the diet supplemented with fish oil showed a minimum rise and sustained the raised levels for a longer period. Similarly dietary fibre supplement also took longer to reach the fasting levels. Contrarily, garlic supplemented diet showed a sharp and earlier decline in the serum cholesterol levels. Although the peak rise with dietary fibre (18.5%) was higher than the control (9.6%) the mean PP serum cholesterol reduced significantly (p<0.05) with dietary fibre supplement (Table 1).

Means with different superscript are significantly different (p<0.05)

The mean serum PP LDL-C levels decreased significantly after the supplementation of meal with garlic and dietary fibre and contrarily increased significantly (p<0.05) on supplementation of fish oil (Table 2).
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**Table 2: Mean Postprandial serum LDL-Cholesterol response after supplementation (mg/dl)**

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Control</th>
<th>Garlic</th>
<th>Fish Oil</th>
<th>Dietary Fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting (0)</td>
<td>165.1±35.1</td>
<td>107.3±51.1</td>
<td>188.5±16.6</td>
<td>88.3±34.3</td>
</tr>
<tr>
<td>1 ½</td>
<td>117.0±52.1</td>
<td>96.8±35.2</td>
<td>179.1±24.9</td>
<td>82.3±43.4</td>
</tr>
<tr>
<td>3</td>
<td>117.6±51.5</td>
<td>118.1±21.3</td>
<td>183.6±33.3</td>
<td>94.3±27.9</td>
</tr>
<tr>
<td>4 ½</td>
<td>98.1±40.5</td>
<td>81.6±48.4</td>
<td>191.1±17.3</td>
<td>60.6±31.5</td>
</tr>
<tr>
<td>6</td>
<td>103.3±33.6</td>
<td>81.5±27.3</td>
<td>149.3±12.5</td>
<td>67.8±26.6</td>
</tr>
<tr>
<td>Mean</td>
<td>142±</td>
<td>97±</td>
<td>176±</td>
<td>80±</td>
</tr>
</tbody>
</table>

% peak rise: 53, 10, - , 6.7

Means with different superscript are significantly different (p<0.05)

Fig 4 shows that unlike control which showed a rise, a slow decline in LDL-C levels with all the three supplements below the fasting levels was observed at 1 ½ hour. Garlic and dietary fibre supplement reached the peak levels by 3rd hour and then declined rapidly while fish oil supplement showed a sustained level up to 4 ½ hours.

**Figure 6**

Fig 4. Serum LDL-Cholesterol Postprandial response (0-6 hr)

The PP glucose response to the supplementation was compared to the control and this showed that the % peak rise was highest with garlic(77.8%) followed by fish oil(68.4%) and dietary fibre(62.7%) (fig. 6). Garlic and dietary fibre supplement showed a slow decline towards fasting values whereas fish oil supplement brought about a sharp decline in the PP glucose levels after 1 ½ hours proving to be most effective. Although the mean PP glucose levels did not change significantly for garlic, fish oil or dietary fibre compared to the control.

**Figure 8**

Fig 6. Plasma Glucose Postprandial response (0-6 hr)

DISCUSSION

The results of this study indicate that incorporation of garlic, fish oil and dietary fibre into the test meal alters the postprandial lipemic and glycemic response.

Although there was no significant decrease in the fasting lipid profile or glucose levels after the supplementation of for a short period of 7 days, dietary fibre supplementation
showed a 8.6%, 36% and 46% reduction in total lipids, cholesterol and LDL-C respectively. The mechanism underlying the hypocholesterolemic effect could be attributed to viscosity, bile salt binding capacity and fermentability. The products of colonic fermentation i.e. short chain fatty acids, acetate and propionate inhibit HMG-Co reductase by feedback mechanism that results in decreased hepatic cholesterol synthesis (18). However, studies have been reported that the long term supplementation has been reported to be beneficial hypolipidemic agents (5).

The meals with all the test supplements showed a plateau response compared to a sharp peak rise with control meal; however, garlic and fish oil supplementation gave a quick rise at 1 ½ hour and significant decline by 4 ½ hours. From the above observations it was evident that garlic and fish oil supplements could be more effective in terms of reducing the level and length of diet induced hyperlipidemia.

The results of the present study indicate that garlic and fish oil supplements were effective in reducing the PP triglyceridemia when compared to dietary fibre supplement.

Several factors have been shown to influence the degree of alimentary triglyceridemia (19). Serum responses can be influenced by the basal diet consumed before the experimental diet (13); however, in this study the subjects were instructed not to deviate from normal dietary or exercise habits before and between the experimental periods. Previous reports indicate that the degree of alimentary triglyceridemia is directly proportional to fasting plasma triglyceride concentrations (20) but the similar effect was not elucidated in the present study.

Triglyceride lowering effect of garlic appears to be due to inhibition of de novo fatty acid synthesis and the second peak of the biphasic response with garlic represented triglycerides ingested with the test meal, whilst the first represented previously ingested triglycerides (10). The mechanism of the hypotriacylglycerolaemic effect of n-3 PUFA (Polyunsaturated fatty acid) remains obscure but could be attributed to reduced triglyceride-rich lipoprotein synthesis (TRL), increased TRL removal or a combination of both. Reduced chylomicron synthesis could be unlikely as there is no evidence of reduced fat absorption associated with fish oil supplementation (11).

Animal studies have shown that n-3 PUFA inhibit triglyceride synthesis through the inhibition of 1,2 diacylglycerol transerase. It is suggested that a background diet rich in n-3 PUFA reduces VLDL-synthesis (12). Chylomicrons and VLDL compete for lipoprotein lipase (LPL) mediated removal. Therefore, when VLDL synthesis is diminished, chylomicron particles have greater opportunity to interact with LPL, allowing more effective clearance of postprandial TRL, suggesting that n-3 PUFA supplementation increases LPL-mediated TRL clearance (13).

The PP VLDL-C response towards each supplement was similar to serum triglyceride response. It is reported that the reduction in plasma triglyceride rich lipoproteins from the liver or small intestine (VLDL and chylomicron), increased rates of their removal, or a combination of these could be responsible for low levels with the supplementation of fish oil (13).

In the present study, the mean serum PP LDL-C levels decreased significantly after the supplementation of meal with garlic and dietary fibre. It has been reported that the sources of fibre can slow the digestion and absorption of dietary fat because of their ability to interfere with lipase activity, bind micellar components, and increase the viscosity of the contents in the small intestine (21,22). Likewise, other investigators reported that cholestyramine, which binds micellar components in the intestinal lumen and slows lipid disappearance from the intestine (23). In addition viscous fibre may slow the digestion and absorption of other macronutrients.

Considering the two factors i.e. total quantum of increase over fasting levels and time taken to come to fasting levels, garlic and fish oil supplement could be considered effective in reducing the PP hypercholesterolemia. Though reports have indicated that garlic has been effective in significantly preventing fat induced hyperlipidemia (14, 15), fish oil supplements have not been effective in checking fat induced rise in serum cholesterol in normal subjects. A similar study has been documented wherein the n-3 fatty acid from Max EPA significantly reduced the LDL-C level following a fatty meal (16).

The PP response suggesting that garlic and fish oil supplementation was beneficial in sustaining elevated HDL-C levels postprandially. Increased HDL-C fraction following a full fat diet with fish oil supplementation has also been reported, showing that fish oil could prevent the deleterious effects of high fat diet on the cardio protective HDL-C.
The glycemic response to the test supplements demonstrated that a short time supplementation for 7 days did not change the fasting glucose levels; however the peak rise was less than the control. There was a peak rise ant 1 ½ hour and a sharp decline was seen with fish oil when compared to dietary fibre and garlic. Contrarily, it has been reported that although fish oil had no harmful effect on glucose tolerance in normal weight type 2 diabetes subjects, in overweight type 2 diabetes subjects it leads to mild glucose intolerance; however, it is suggested that the results may vary based on various parameters (dose of fish oil, EPA and DHA ratio, n-3 to n-6 ratio). (24). The effect of dietary fibre on the PP glycermia is well established and fibre rich diet especially the soluble fibre has been reported to improve the glucose tolerance through the slow access of glucose to the small intestine’s absorptive epithelium, thereby blunting PP glucose peak and may delay gastric emptying, slowing the carbohydrate uptake, another mechanism that may contribute to the PP effect is the sequestration of carbohydrates ingested with the meal, retarding carbohydrates access to digestive enzymes. However, the design of this study does not allow these mechanisms to be distinguished(17,24).

CONCLUSION
Management of type 2 diabetes mellitus should aim at preventing or at least delaying and minimizing the more serious and debilitating long term macro vascular and micro vascular complications and their metabolic manifestations that eventually occur in diabetes. This makes it necessary to improve both lipid and glucose tolerance in type 2 diabetes subjects. The three test supplements garlic, fish oil and dietary fibre have shown to be potent hypolipidemic agents on long term supplementation and their effect on PP lipemic and glycemic response have shown that while garlic is effective in controlling only PP lipemia and dietary fibre in controlling the PP glycermic response, fish oil is effective in controlling both the lipemic and glycemic response. It can therefore be suggested that a continuous supplementation with fish oil or a combination of fish oil and garlic is advisable for the management of type 2 diabetes to delay the onset of cardiovascular complications.

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