Lipid, glycemic, and insulin responses to meals rich in saturated, cis-monounsaturated, and polyunsaturated (n-3 and n-6) fatty acids in subjects with type 2 diabetes mellitus

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Running Title: Postprandial response to fatty acids in T2DM

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Abstract

Objective: The recommendations for dietary fats in patients with type 2 diabetes (T2DM) are largely based on the impact of fatty acids on fasting serum lipid and glucose concentrations. How fatty acids affect postprandial insulin, glucose, and triglyceride concentrations remains unclear, however. The objective of this study is to study the effect of fatty acids on postprandial insulin, glucose, and triglyceride responses.

Research Design and Methods: Test meals rich in palmitic acid, linoleic acid, oleic acid, and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and containing 1,000 kcal each were administered in a randomized cross-over design to 11 T2DM subjects. Serum insulin, glucose, and triglycerides concentrations were measured for 360 minutes. All subjects received an isoenergetic diet of constant composition throughout the study.

Results: According to repeated measures ANOVA, insulin (p=0.0002) but not glucose (p=0.10) response was significantly different between meals. Insulin response was lower to meals rich in oleic acid or EPA and DHA than to meals rich in palmitic acid or linoleic acid (p<0.01). The triglyceride response did not reach statistical significance (p=0.06) but tended to be lower with EPA and DHA than with the other fatty acids. Similar trends were seen for area under the curve (AUC) and incremental AUC for serum insulin and triglycerides but the differences were not significant.

Conclusions: In comparison to palmitic acid and linoleic acid, oleic acid or EPA and DHA may modestly lower insulin response in patients with T2DM without deteriorating glucose response. EPA and DHA may also reduce triglyceride response.
Introduction

The dietary recommendations for fatty acid intakes to manage dyslipidemia and glycemia in patients with type 2 diabetes (T2DM) are largely based on the findings from studies on the impact of fatty acids on fasting serum lipid and glucose concentrations (1). How the different types of fatty acids affect postprandial lipid, glucose, and insulin concentrations is not clearly understood, however. This is important since most individuals in Western countries are in a postprandial state for most of the day (2; 3). Postprandial triglyceride concentrations are associated with cardiovascular disease (CVD) (4; 5), and this may be even more relevant in patients with T2DM given that these individuals have higher postprandial triglyceride response than individuals without T2DM (6; 7) even when baseline triglyceride concentrations are normal (7). How the different types of fatty acids affect postprandial glucose and insulin response also needs to be further examined especially since poor glycemic control is linked to diabetes complications including CVD and hyperinsulinemia is a risk factor for CVD (1).

Only two studies have examined the acute effect of different types of fats on postprandial insulin response in subjects with T2DM (8; 9). These studies compared meals rich in butter and olive oil and reported no difference in insulin levels. Studies in subjects without diabetes, reported either no difference in glucose response to meals rich in different fatty acids (10-15; 18; 21). Gatti et al (16) however, found a lower glucose response to meals rich in olive oil than to meals rich in corn oil or butter and Lardinois et al (19) found a higher glucose response to a meal rich in fish compared to a meal rich in corn oil or beef in individuals without diabetes. The number of studies examining the acute effect of meals on postprandial triglyceride response in T2DM subjects is also limited. West et al (20) reported a lower triglyceride response to a meal rich in both oleic acid and EPA and DHA compared to a meal rich in just oleic acid but only in subjects with high triglyceride concentrations. This difference may be due to decreased chylomicron production or secretion (22) and reduced synthesis of very low density lipoproteins (VLDL) (23) associated with very long-chain omega-3 fatty acids. Comparison of meals rich in butter and olive oil in subjects with T2DM revealed either no difference (8) or higher triglyceride response to the meal rich in butter (9). The results from studies
in subjects without diabetes have also been controversial with some studies showing no difference in postprandial triglyceride response to different fatty acids (11-15; 21; 24-27) and others showing either a lower response to meals rich in n-3 (17), n-6 (10) and n-9 (10; 18) fatty acids or a higher response to n-6 (28; 29) and n-9 (28; 29) fatty acids compared to saturated fatty acids.

Possible reasons for the conflicting results could be due to the fact that the order in which the meals were tested was not randomized by a number of studies (13; 14; 19; 24) and several studied did not provide a constant background diet (10-17; 19-21; 24; 27; 28). It has been reported that the fats and carbohydrates in the daily diet may influence the postprandial response to a meal (3; 30). In addition, some studies limited the energy content of the test meal (300-500 kcal) (16; 19) and the time during which the response was measured (180 minutes) (16; 19; 26) and this may make it difficult to detect significant differences between the different test meals. The controversial results may also be due to the use of butter as a source of saturated fat (8-10; 16; 18; 21; 25). Nearly 15% of the saturated fat content in butter is accounted for by medium chain fatty acids known to improve insulin sensitivity and glycemic control (31).

The above studies have a number of design issues which makes it difficult to clearly interpret the results. Also there is paucity of data in patients with T2DM. We addressed these issues in our study which compared the postprandial triglyceride, glucose, and insulin response to meals rich in palmitic acid, oleic acid, linoleic acid, and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in subjects with T2DM. The postprandial response was measured for 360 minutes and the meals were administered in a randomized cross-over design. Each test meal was designed to provide 1,000 kcal and the percent energy from carbohydrate, protein, and fat was held constant. In addition, the subjects were fed an isoenergetic diet of constant composition throughout the study. We hypothesized that there will be no difference in postprandial insulin and glucose response to meals rich in different fatty acids. The secondary hypothesis was that the postprandial triglyceride response will be lower to meals rich in very long chain omega-3 fatty acids compared to the other fatty acids.

Research Design and Methods

Subjects

Eleven men with T2DM who had fasting blood glucose concentrations <200 mg/dL and were not on insulin therapy were studied at the General Clinical Research Center (GCRC) of UT Southwestern Medical Center at Dallas. The protocol was approved by the UT Southwestern Institutional Review Board, and each participant gave informed consent. Mean age was 54.6±12.2 years and mean body mass index was 33.2±3.7 kg/m². Six of the subjects were non-Hispanic Whites, 3 were African-American, and 1 each was Asian and Hispanic. None of the subjects had thyroid, renal, or hepatic disease, uncontrolled hypertension, anemia, or a history of ketosis.

Experimental Design

All subjects received an isoenergetic diet of constant composition throughout the study duration of 12 to 15 days. The subjects were instructed to maintain a constant level of physical activity.
At intervals of 3-4 days, after an overnight fast, each subject consumed a mixed test meal on four occasions in a randomized manner. The type of fat in the test meal varied on each occasion and was rich in palmitic acid, oleic acid, linoleic acid, or EPA and DHA.

**Daily Diet and Test Meals**

Daily energy intake of the subjects was estimated using the Harris Benedict equation (32). The subjects received an isoenergetic diet containing 15% of total energy as protein, 35% as fat, and 50% as carbohydrate throughout the study period which started 3-4 days before the first test meal was evaluated. The subjects picked up their daily meals every 3-4 days from the GCRC metabolic kitchen. Energy intake was adjusted to maintain a constant body weight. Alcohol was not allowed during the entire study period. Coffee was limited to one serving of reconstituted freeze-dried coffee (2 g dry coffee) at breakfast and tea was limited to one serving of reconstituted instant tea at lunch and one at dinner. Sugar free soft drink was allowed but only as a replacement for tea. No deviations from the above guidelines were reported.

Each test meal provided 1,000 kcal with 15% energy as protein, 35% as carbohydrate, and 50% as fat. The test meals contained farina, egg substitute, ham with 5% fat, white bread, skim milk, orange juice, and 51 g of test oil. The test meals containing palmitic acid, oleic acid, linoleic acid, and EPA and DHA were derived from palm oil, olive oil, safflower oil, and salmon oil, respectively. The fat content of the four test oils is shown in Table 1.

**Meal Tolerance Test**

The meal tolerance test was conducted after a 12 hour overnight fast. An intravenous canula was placed in a forearm vein for blood sampling. After collecting three baselines blood sample at -30, -15, and 0 minutes, patients were asked to consume the test meal in a 15 minute period and blood was collected every 30 minutes for the next 360 minutes for measurement of plasma glucose, insulin, and triglycerides concentrations.

**Biochemical Analysis**

Plasma glucose concentrations were assayed by the glucose oxidase method (Beckman Glucose Analyzer, Beckman Instruments, Fullerton, California). Plasma insulin concentrations were measured using a radioimmunoassay kit (Linco Research Inc., St. Louis, MO). Plasma triglycerides were measured enzymatically (Sigma Diagnostics, St. Louis, MO).

**Statistical Methods**

Repeated measures analysis of variance (ANOVA) model was used to assess the effect of the different meals on plasma glucose, insulin, and triglycerides responses following log transformations. The main effects and meal by time interaction effects were evaluated. Pairwise contrasts were made by comparing the least square means estimates and the p values were adjusted for multiple comparisons using the Bonferroni Holm method (33). Repeated measures ANOVA was also used to compare the effect of the meals following rank transformation of the glucose, insulin, and triglyceride response values after subtracting the respective baseline values. Peak response and peak time were compared across meals by a single factor repeated measures ANOVA model.

Area under the curve (AUC) and incremental AUC (iAUC), i.e., the area above baseline, were calculated using the
trapezoidal rule. The respective natural log values were compared by a single factor repeated measures ANOVA model.

Similar trends were seen even after adjusting our analyses for treatment with lipid or glucose lowering medications or excluding the 4 men who were on one of these medications. All statistical analyses were performed using the SAS Version 9.13 (SAS Institute, Cary, NC).

Results

Body weight was maintained throughout the study period with the carefully controlled isoenergetic background diet of constant composition. According to the repeated measures ANOVA test following log transformation, the postprandial insulin response (Figure 1A) was significantly different between the various meals (p=0.0002) whereas the postprandial glucose (Figure 1C) and triglyceride (Figure 1E) responses did not differ significantly by the type of meal given (p=0.10 and p=0.06, respectively). Post-hoc analyses, adjusted for multiple comparisons, showed that the insulin response was significantly higher to the meal rich in palmitic acid than to the meals rich in oleic acid (p=0.002) or EPA and DHA (p=0.002) and to the meal rich in linoleic acid than to the meals rich in oleic acid (p=0.007) or EPA and DHA (p=0.006). There was no difference in peak time (p=0.62) which was reached at approximately 60 minutes for each meal. Peak insulin concentration was reached at approximately 60 minutes for each meal. Peak insulin concentration was significantly different (p=0.01) by meals (Figure 1A). It was higher following the meal rich in linoleic acid than the meals rich in oleic acid (p=0.04) or EPA and DHA (p=0.02) and not different between the meals rich in oleic acid and EPA and DHA and between the meals rich in palmitic acid and the other fatty acids.

Repeated measures ANOVA following rank transformation after subtracting the baseline values, showed a significantly different effect of the meals on postprandial triglyceride (p=0.004) and insulin (p=0.006) response but not on the glucose (p=0.58) response. Post-hoc analyses, adjusted for multiple comparisons, showed that the insulin response was higher to the meals rich in linoleic acid than to the meals rich in oleic acid (p=0.05) or EPA and DHA (p=0.02) and also higher to palmitic acid compared to EPA and DHA (p=0.05). Postprandial triglyceride response tended to be lower following the meals rich in EPA and DHA than the other fatty acids but the difference was significant only between EPA and DHA and oleic acid (p=0.003). Because the triglyceride response was delayed by 120 minutes, we conducted additional analysis after excluding the postprandial data obtained during the first 120 minutes and found that the response was significantly higher following the meal rich in oleic acid than the other meals. However, because the study was designed to look at the postprandial response for 360 minutes and because the typical response lasts for more than 120 minutes, our focus will be on the entire postprandial period.

AUC for insulin (Figure 1B) was higher for meals rich in palmitic acid or
linoleic acid than for meals rich in EPA and DHA or oleic acid and that for triglycerides (Figure 1 F) tended to be lower for the meal rich in EPA and DHA than the other meals but the differences did not reach statistical significance. AUC for glucose (Figure 1D) did not differ by meals. Similar results were seen for iAUC (data not shown).

**Discussion**

We examined the effect of different fatty acids on postprandial triglyceride, glucose, and insulin concentrations in subjects with type 2 diabetes. According to repeated measures ANOVA, insulin response was significantly different by the type of fatty acid consumed. It was significantly higher in response to the meals rich in palmitic acid or linoleic acid than to meals rich in oleic acid or EPA and DHA. A similar trend was seen for AUC and iAUC but the differences did not reach statistical significance possibly because of the small sample size. These results contradict most of the earlier studies which found no difference in insulin response to meals rich in different types of fatty acids in subjects with (8; 9) or without (10-18) diabetes.

A possible mechanism for the insulin response observed in our study may be due to the different insulinotropic potency of the different fatty acids. Stein et al (34) studied the influence of fatty acids on insulin secretion in the perfused rat pancreas and found that the glucose stimulated insulin release was higher with palmitic acid than with oleic acid which in turn was higher than with linoleic acid. Although we also found an increased postprandial insulin response with palmitic acid compared to oleic acid, our observation of higher insulin response to linoleic acid than to oleic acid is not consistent with the data of Stein et al (34). Holness et al (35) reported that acute replacement of some dietary saturated fatty acids with EPA and DHA, in rats made insulin resistant by high-saturated fat feeding for 4 weeks, reversed insulin hypersecretion in vivo, and during glucose perifusion of isolated islets. The lowered insulin secretion, however, was not accompanied by improved insulin action and glucose tolerance was adversely affected. In our study, the insulin lowering effect of EPA and DHA and oleic acid was not associated with deterioration in glucose tolerance as indicated by the lack of difference in postprandial glucose response to the different fatty acids and may suggest an increase in insulin sensitivity. In our study, we did not observe an improvement in insulin sensitivity following the meal rich in linoleic acid compared to palmitic acid. This conflicts with data from a 5-week study (36) in which improved insulin sensitivity was reported on a diet rich in linoleic acid compared to a diet rich in palmitic acid. Energy intake in the latter study, however, was lower during the linoleic acid rich phase compared to the other diet phase which may partly explain their results.

The difference in insulin response in our study may be due to secretion of incretin hormones, glucagon-like-peptide (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). However, how the incretin hormones respond to different fatty acids remains to be studied.

Our results on glucose response are similar to the results from most studies in subjects with (9; 20) and without (10-15; 18; 21) T2DM which found no difference in glucose response to different fatty acids. It is important to note that
although the insulin lowering effect of EPA and DHA and oleic acid did not adversely affect glucose response, the latter remained in the diabetes range. This indicates a need for more aggressive control of postprandial glucose levels using several treatment strategies.

According to the repeated measures ANOVA with rank transformed values after subtracting the baseline values, triglyceride response tended to be lower to the meal rich in EPA and DHA than to the other meals but the difference was only significant between the meals rich in EPA and DHA and oleic acid. A similar, albeit not significant, trend was seen for AUC or iAUC. These results are corroborated by studies examining the acute effect of different fatty acids on postprandial triglyceride concentrations in T2DM subjects with high baseline triglyceride concentrations (20) and in persons without diabetes (17). Other acute studies in healthy subjects (15; 24), however, found no difference in postprandial triglyceride response following meals rich in oleic acid or EPA and DHA. Our data are similar to that from a long-term intervention study (37) which reported reduced postprandial triglyceride concentration in healthy subjects when diets rich in saturated fatty acids or monounsaturated fatty acids were supplemented with fish oil (37). The composition of the test meals was similar to that of the diets that the subjects were assigned to (37).

It has been reported that the postprandial triglyceride response may be dependent on insulin status (38). We looked at the influence of insulin resistance, estimated using the HOMA insulin resistance calculator 2.2 (39), on triglyceride response by repeated measures ANOVA, and found no evidence of an interaction between insulin resistance status and triglyceride response to meals. Nevertheless, a larger sample may help to better examine this relationship.

Possible mechanisms by which EPA and DHA lower triglyceride levels include decreased chylomicron production or secretion (22) and reduced synthesis of hepatic very low density lipoproteins (VLDL) (23) seen after chronic feeding of EPA and DHA. The reduced VLDL concentrations would result in less competition for lipoprotein lipase for hydrolysis of chylomicrons. It is not known whether acute consumption of EPA and DHA would result in the above mechanisms, however.

In order to accurately distinguish the effect of different types of fatty acids on the postprandial responses, we tested meals that contained 1,000 kcal and 50% of energy as fat. Although these meals are more energy dense than the diet that is typically consumed by Americans (3; 40), we believe that the test meal rich in oleic acid may be acceptable over the long-term based on the strong adherence that we have observed in our earlier studies (3; 41) testing high monounsaturated fat diets for 6-12 weeks. Whether large doses of fish oil are acceptable over the long-term remain to be studied. Also whether meals with a lower fat content would lead to reduced or similar postprandial responses compared to the meals administered in our study will require additional studies. Our test meals also contained some carbohydrate sources such as white bread which has a high glycemic index. This should not preclude us from distinguishing the effect of the different fatty acids on postprandial response, however, since the type and amount of carbohydrate was held constant across the test meals.
In conclusion, our study shows that meals containing a high percentage of energy from oleic acid or EPA and DHA, the fatty acids that patients with T2DM are encouraged to consume by the American Diabetes Association (1), may be beneficial in lowering postprandial insulin response in comparison to meals rich in palmitic acid or linoleic acid with a comparable postprandial glucose response. Meals containing a high percentage of energy from EPA and DHA may also be beneficial in lowering postprandial triglyceride response compared to meals rich in oleic, palmitic, or linoleic acid.

**Acknowledgments**

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References


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Table 1. Fatty Acid Composition of the Various Test Oils

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<th>Saturated</th>
<th>Cis-monounsaturated</th>
<th>Polyunsaturated</th>
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**Fig. 1:** Postprandial insulin, glucose, and triglyceride responses to meals. Median values of postprandial insulin, glucose, and triglyceride responses to meals rich in palmitic acid (diamonds), oleic acid (squares), linoleic acid (triangles), and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (circles) are shown in panels A, C, and E, respectively. The baseline values are the mean of the values collected at -30, -15, and 0 minutes. To convert insulin values from pmol/L to µU/mL divide by 6.0, and glucose and triglyceride values from mmol/L to mg/dL, divide by 0.05551 and 0.01129, respectively. Area under the curve values (medians and 25th and 75th percentiles) for insulin, glucose, and triglycerides to different meals are shown in panels B, D, and F, respectively.
Figure 1