Effects of a plant based High carbohydrate-high fiber diet vs. High monounsaturated-low carbohydrate diet on postprandial lipids in type 2 diabetic patients.

Running title: High fiber diet and postprandial lipemia

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Objective To search for a better dietary approach to treat postprandial lipid abnormalities and improve glucose control in type 2 diabetic patients.

Research Design and Methods According to a randomized cross-over design, 18 type 2 diabetic patients (age 59±5 years; BMI 27±3 kg/m²)(mean±SD) in satisfactory blood glucose control on diet or diet+metformin, followed a diet relatively rich in CHO (52% total energy), rich in fiber (28g/1000 kcal) and with a low glycemic index (58%)(High CHO-high fiber diet), or a diet relatively low in CHO (45%) and rich in monounsaturated fat (23%) (Low CHO-high MUFA diet) for 4 weeks. Thereafter, they shifted to the other diet for 4 more weeks. At the end of each period, plasma glucose, insulin, lipids and lipoprotein fractions (separated by discontinuous density gradient ultracentrifugation) were determined on blood samples taken at fasting and over 6 hrs after a test meal having a similar composition as the corresponding diet.

Results In addition to a significant decrease in postprandial plasma glucose, insulin responses and glycemic variability, the High CHO-high fiber diet significantly improved also the primary endpoint, as it reduced the postprandial incremental areas (IAUC) of triglyceride-rich lipoproteins, in particular chylomicrons (cholesterol IAUC: 0.05±0.01 vs.0.08±0.02 mmol/l⋅6h; triglycerides IAUC: 0.71±0.35 vs.1.03±0.58 mmol/l⋅6h , p<0.05).

Conclusions A diet rich in CHO and fiber, essentially based on legumes, vegetables, fruit, and whole cereals may be particularly useful for treating diabetic patients, due to its multiple effects on different cardiovascular risk factors, including postprandial lipids abnormalities.
The clinical and scientific relevance of postprandial lipid abnormalities is based on the evidence of their association with a higher cardiovascular risk as recently shown by the results of two very large prospective studies (1,2). Patients with type 2 diabetes mellitus have more pronounced postprandial dyslipidemia (3,4), and this may account, at least in part, for their higher rate of cardiovascular diseases, not explained by hyperglycemia and the classical cardiovascular risk factors alone (5).

Despite the clinical relevance of postprandial lipid alterations, there is very little scientific evidence on the potential therapeutic interventions able to correct these abnormalities. If we consider that human beings spend most of their time in the postprandial condition and that all the lipid alterations in this state greatly outnumber those occurring in fasting conditions, diet is the natural approach to correct postprandial abnormalities. However, while it has been repeatedly demonstrated that dietary treatment in type 2 diabetic patients is able to improve glucose control and blood lipids at fasting (6), there are few data on the effects of various diets on postprandial lipemia (7). The Mediterranean diet is generally recommended as a useful nutritional tool for the prevention of cardiovascular disease because it is able to act positively on the main cardiovascular risk factors, including excess body weight (8). However, the two main components of the Mediterranean diet—olive oil rich in monounsaturated fat and foods rich in CHO and fiber—traditionally found in association in the Mediterranean diet of some decades ago, are now often considered in opposition. In particular, a diet rich in CHO is considered less effective on fasting lipid metabolism than one rich in monounsaturated fat (MUFA), as it induces higher plasma triglycerides concentrations (22%) and lower HDL cholesterol levels (4%) (9); these untoward effects, however, can be avoided by selecting CHO rich foods with a high fiber content and a low glycemic index (10).

It can be hypothesized that a diet containing these kinds of foods may be more beneficial on postprandial lipid metabolism compared to a MUFA- rich diet, which, given the higher fat content, could induce, a postprandial increase in triglyceride-rich lipoproteins, especially of exogenous origin.

On the basis of this working hypothesis, the aim of this intervention study was to evaluate in type 2 diabetic patients the effects on postprandial lipemia and glucose metabolism (both in everyday life conditions and after a standard test meal) of two diets, one moderately rich in CHO, rich in fiber and with a low glycemic index, and the other relatively low in CHO and rich in MUFA.

Since adipose tissue, mainly through its lipolytic activities, is considered as having a pivotal role in the buffering of lipid flux in the postprandial period (11), a further aim of our study was to evaluate the activities of lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) after the two diets, as possible mechanisms of different effects on postprandial lipid metabolism.

RESEARCH DESIGN AND METHODS

Subjects. Nineteen type 2 diabetic patients were enrolled in the study, after giving their written informed consent. There was one drop-out due to concomitant family problems; therefore, 12 males and 6 women, aged 59±5 years (mean±SD), concluded the study. Patients were slightly overweight (Body Mass Index 27 ± 3 kg/m²), in satisfactory blood glucose control (HbA1c 6.9 ± 0.7%) on diet alone (n=13) or diet + metformin (n=5) and with normal fasting plasma lipid levels. They were all sedentary and did not change their physical activity level throughout the study. The protocol was approved by the Federico II University Ethics Committee.
Sample size. Based on differences between diabetic and control subjects in the triglycerides postprandial responses of chylomicrons and VLDL fractions (0.7 mmol/l·6h, standard deviation 0.85 mmol/l)(12), we considered as clinically relevant a 30% difference between the two treatments of the primary endpoint (postprandial incremental area of triglycerides content in chylomicrons and VLDL fraction). In order to detect this difference with an 80% power at a p-value of 5% (two-tailed) 13 patients had to be studied.

Study design. The study was performed according to a randomized crossover design. After a run-in period of 4 weeks, during which patients were stabilized on their own diet, they followed, in alternate order, two isoenergetic diets, each for 4 weeks. One diet was relatively rich in CHO, rich in dietary fiber both of soluble and unsoluble type and with a low glycemic index, the other was rich in MUFA, relatively low in CHO, low in dietary fiber and with a relatively high glycemic index (Table 1). The glycemic load of the high CHO/high fiber diet was lower than that of the high MUFA diet (155 vs. 205). The other components of the two diets, in particular the saturated fat content, were similar (Table 1). Calories and nutrients of the diets were calculated from tables of food composition of the Italian Institute of Nutrition utilizing the MetaDieta software (Meteda s.r.l., Ascoli-Piceno, Italy). The main components of the two diets were: 1) a portion of legumes 4 times a week, one serving of pasta twice a week and one serving of parboiled rice once a week, 2 servings of vegetables and 2 of fruit per day and whole meal bread for the High CHO-high fiber diet; 2) white bread, a serving of potatoes, rice or pasta each twice weekly, a serving of pizza once a week, and use of vegetables and fruit low in fiber for the Low CHO diet (a weekly meal schedule for both dietary treatment is shown in the online appendix, tables A1 and A2, available at http://care.diabetesjournals.org).

To improve dietary compliance patients were seen weekly by an experienced dietician who, in addition, called them up every two-three days to ensure that they followed the diet assigned. The adherence to the two dietary treatments was evaluated by a 3-day food record filled in by the patients at the end of the two periods.

Experimental procedures. At the end of each dietary intervention period, patients underwent the following procedures: 1) 12-hour fasting blood sampling for the determination of total, LDL and HDL cholesterol and triglycerides; 2) home self measurement of triglyceride and glucose levels with samples taken at fasting, immediately before and 2 and 3 hours after lunch and before dinner for two days at the end of each dietary treatment; 3) a test meal with a composition similar to the dietary treatment being followed, with blood samples taken at fasting (at least 12 hours) and 2, 4, and 6 hours after the meal to evaluate glucose, insulin, cholesterol and triglycerides in plasma and lipoprotein subfractions. The test meal was performed in 12 patients. The composition of the test meal performed at the end of the High-fiber diet was 52% CHO (41% starch, 11% soluble), 30% fat (7% saturated), 18% proteins, and consisted of beans and pasta soup + an apple. The composition of the test meal performed at the end of the Low CHO-high MUFA diet, was 45% CHO (34% starch, 11% soluble), 37% fat (7% saturated fat), 18% protein, and consisted of a potato gateau (a pie made of mashed potato, whole milk, eggs, cheese, ham and butter) + orange juice. The energy content of the two test meals was 948 kcal; 4) six hours after the test meal a needle biopsy of abdominal subcutaneous adipose tissue was taken for the determination of LPL and HSL activities.
Laboratory procedures. All laboratory measurements were made blind to the dietary treatments.

Lipoprotein separation. Samples were kept at +4°C before, during and after centrifugation. Fasting and postprandial lipoprotein subfractions were isolated by discontinuous density gradient ultracentrifugation, as previously described (5). Briefly, three consecutive runs were performed at 15°C and at 40000 rpm to float chylomicrons (Svedberg flotation unit, Sf, >400), large VLDL (Sf 60-400) and small VLDL (Sf 20-60). IDL (Sf 12-20) and LDL (Sf 0-12) were recovered from the gradient after the Sf 20-60 particles had been collected. HDL were isolated by a precipitation method.

Adipose tissue lipases activities. Heparin-releasable LPL, total LPL, and HSL activities were determined as previously described (13).

Other measurements. Cholesterol and triglyceride concentrations were assayed by enzymatic colorimetric methods; plasma insulin concentrations by ELISA. Home self measurements of triglyceride levels were performed by a GCT Accutrend instrument (Roche Diagnostics, Mannheim, Germany), a method already validated (3).

Statistical Analysis. Data are expressed as mean±standard deviation (mean±SD), unless otherwise stated. Postprandial incremental areas were calculated by the trapezoidal method as the area under the curve above the baseline value (IAUC). Coefficients of variations of plasma glucose at different points after test meals were evaluated as an index of postprandial glycemic variability (14). Differences between the two diets were evaluated by analysis of variance for repeated measures and t-test for paired data. Two-tailed tests were used and a p<0.05 was considered statistically significant. Variables not normally distributed were analyzed after logarithmic transformation or by nonparametric tests.

Since there was no carry-over effect, the results of patients starting with either diet were put together. The statistical analysis was performed according to standard methods using the Statistical Package for Social Sciences software (SPSS/PC, SPSS, Inc., Chicago, IL, USA).

RESULTS

There were no changes in body weight during the experiment (kg 73.0 after both dietary treatments). The compliance to the two diets was optimal, as shown by the average composition of the diet followed by subjects (Table 1). As expected, the two diets followed by patients were significantly different for total fat, MUFA, CHO, fiber, glycemic index and glycemic load (Table 1). Both diets were well accepted by patients.

Fasting plasma lipoproteins. At the end of the High CHO-high fiber diet there was a significant reduction of total cholesterol (4.20±0.70 vs. 4.40±0.78 mmol/l, p<0.05), LDL cholesterol (2.62±0.60 vs. 2.82±0.62 mmol/l, p<0.05), HDL cholesterol (0.98±0.25 vs. 1.06±0.26 mmol/l, p<0.05) and an increase in HDL triglycerides (0.20±0.05 vs. 0.18±0.05 mmol/l, p<0.05) in comparison with the Low CHO-high MUFA diet, while triglyceride levels in plasma (1.16±0.38 vs. 1.07±0.39 mmol/l) and LDL (0.41±0.11 vs. 0.41±0.09 mmol/l) were similar.

Home self monitoring. Blood glucose levels measured by patients (average of two daily profiles) were significantly lower during the High CHO-high fiber diet both 2 hours (7.2±1.1 vs. 9.2±2.9 mmol/l; p<0.05) and 3 hours (5.9±1.3 vs. 7.3±1.5 mmol/l; p<0.05) after lunch. Similarly, self monitored triglycerides levels (average of two daily profiles) were 30% lower 3 hours after lunch.

Plasma glucose and insulin responses to test meals. After the High CHO-high fiber
test meal performed at the end of the corresponding diet plasma glucose concentrations were lower in the first part of the postprandial curve, especially after two hours (p=0.06) (Fig.1A). The same pattern was observed for plasma insulin, significantly lower at the 2nd and 3rd hour (p<0.05 for both) than after the Low CHO/high MUFA test meal performed at the end of corresponding diet (Fig.1D). ANOVA for repeated measures was statistically significant for plasma glucose (p<0.05) and plasma insulin curves (p<0.008). The combination of the effects on plasma glucose and insulin led to a reduction of the insulin/glucose ratio both at the 2nd (0.44±0.30 vs. 0.52±0.46) and 3rd hour (0.39±0.29 vs. 0.49±0.34; p<0.05) after the High CHO-high fiber test meal. On the contrary, plasma glucose and insulin levels were both significantly higher 6 hours after the High CHO/high fiber meal than after the other test meal (p<0.05) (Fig. 1A, 1D). These patterns in the postprandial curve corresponded to: 1) a nearly 50% decrease in plasma glucose IAUC until the 3rd hour (p<0.05) (Fig. 1B); 2) a significant reduction in insulin IAUC by 14 and 21 %, at 3 and 6 hours respectively (p<0.05 ) (Fig. 1E); 3) a significant decrease of nearly 50 % in postprandial glycemic variability (p<0.02) (Fig.1C).

Plasma lipid and lipoprotein responses to test meals. At the end of the High CHO-high fiber diet postprandial IAUC of plasma triglycerides decreased by 31% compared to the Low CHO/high MUFA diet (1.87±1.22 vs. 2.70±1.58 mmol/l-6h), while no significant differences were observed for plasma cholesterol IAUC. In terms of peak postprandial changes the 4-hour increments were 0.4±0.3 mmol/l for the High CHO-high fiber diet and 0.6±0.4 mmol/l for the other diet (p<0.05).

The postprandial responses of triglyceride-rich lipoproteins are reported in Fig. 2. Both triglycerides and cholesterol in chylomicrons were lower after the test meal performed at the end of the High CHO-high fiber diet, and the IAUC was significantly reduced by 31 and 46%, respectively (p< 0.05 for both)(Fig.2B, 2A). Similarly reduced was the triglycerides content of large VLDL (IAUC 1.46±0.94 vs. 1.97±1.22 mmol/l-6h, p<0.06) (Fig. 2D), small VLDL (IAUC -0.21±0.24 vs.-0.15±0.21 mmol/l-6h, p=0.07), and LDL (IAUC -0.05±0.06 vs. 0.17±0.05 mmol/l-6h, p<0.05). No differences were observed in the postprandial response of the other lipoproteins (data not shown).

Lipases activities. Both total and heparin releasable LPL activities in adipose tissue were not significantly different at the end of the two diets (total LPL activity: 2792±958 vs. 3375±2251 nmol FA/g /h; heparin releasable LPL activity: 146±70 vs. 184±117 mmol FA/g/h). Likewise, no difference was observed in HSL activity (616±237 vs. 676±210 mmol FA/g/h).

DISCUSSION

This study clearly shows, for the first time, that a diet moderately rich in CHO, rich in dietary fiber and, consequently, with low glycemic index and glycemic load, essentially based on consumption of legumes, vegetables, fruit, and whole cereals, induces a significant reduction of postprandial lipoproteins, particularly chylomicrons, in type 2 diabetic patients. This effect was observed in experimental conditions, i.e. after a standard test meal, but also in everyday life, where there was a 30% reduction of plasma triglycerides 3 hours after lunch, despite the well known high day-to-day triglyceride variability (20% in our data).

Moreover, our results confirm that this kind of diet is to be preferred to a diet low in CHO and rich in MUFA for its effects on:

1) postprandial blood glucose control, with lower peaks in the first part of the postprandial period. This blunted
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The postprandial profile implies a reduced variability of blood glucose levels (almost halved in our study), considered so important in the prevention of cardiovascular disease in diabetic patients (15), as well as a lower risk of late postprandial hypoglycemia (16);

2) postprandial insulin levels, which are significantly reduced concomitantly with the presence of lower blood glucose levels, suggesting an improvement in insulin action;

3) LDL cholesterol levels, significantly reduced also in normocholesterolemic type 2 diabetic patients, reinforcing the data already obtained in patients with higher LDL cholesterol levels (10). This result, which may be considered of small entity (a 9% reduction), is, instead, important from a clinical point of view considering the need to constantly lower LDL cholesterol values, especially in type 2 diabetic patients (6).

The only drawback of the diet rich in CHO and fiber is the lower levels of fasting HDL cholesterol obtained compared to the diet low in CHO and rich in MUFA. Moreover, it is important to underline that any intervention able to reduce LDL cholesterol levels generally leads also to a reduction in HDL cholesterol. Furthermore, a previous study has shown that the decrease in HDL cholesterol after a low fat/high CHO diet is limited to HDL₃ (17), which is the HDL subfraction with less antiatherogenic properties.

Our results emphasize the clear importance of the quality of CHO, beside that of their amount (18). Indeed, the few studies that have so far looked at the effects of high CHO vs. high MUFA diets on postprandial lipid metabolism compared diets rich in MUFA with diets rich in CHO, but not in dietary fiber, and with a low glycemic index (19-21).

How can the two dietary approaches act on the postprandial response of triglyceride-rich lipoproteins? We looked at the possible effects of the two diets on lipolysis in adipose tissue, considering the crucial role of this tissue in partitioning the postprandial lipids (11). However, both LPL and HSL activities were not different at the end of either diet. This suggests that the two diets might act on other sites, particularly on those involved in the absorption of dietary fatty acids and cholesterol and the production of chylomicrons and VLDL, not only through their nutrient composition but also for their different combination of foods and for the presence of other components. On the one hand, since low-CHO/high-MUFA diets are richer in the amount of fat, which is one of the main determinants of postprandial lipid response (22), they may induce a higher absorption of dietary fatty acids, which would result in a higher intestinal production of chylomicrons. On the other hand, since the High CHO-high fiber diet is at the same time rich in CHO and dietary fiber, it may act in different ways, i.e., by slowing down gastric emptying, reducing the absorption of glucose, cholesterol and fatty acids and, finally, reducing the intestinal production and secretion of chylomicrons, as suggested by studies performed both in vitro and in vivo (23). Moreover, since the High CHO-high fiber diet also reduces postprandial plasma glucose and insulin, it is likely that both effects may reduce de novo lipogenesis, which is stimulated by glucose and insulin levels (23) and may be particularly relevant in type 2 diabetic patients, where it accounts for 25% of VLDL synthesis (24). Moreover, our High CHO-diet, being rich in fiber, induced also an improvement in insulin resistance, as suggested by the lower Insulin/Glucose ratios, which is relevant given the well known key role played by insulin resistance in determining postprandial lipoprotein abnormalities (25).
Clinical relevance of our results lies on the fact that postprandial lipemia is, according to recent prospective studies (1,2), an independent cardiovascular risk factor. On the basis of these studies a difference in postprandial triglycerides of 0.25 mmol/l such as that observed between our two diets, could mean a reduction in cardiovascular risk of about 25%. It has to be underlined that our results have been obtained with a non pharmacological intervention comparing two diets both recommended and in individuals with quite low lipid levels.

Limitations. Participants in our study were type 2 diabetic patients in relatively good blood glucose control, with quite normal plasma lipid levels, who already used a diet relatively rich in CHO and fiber. Therefore, we cannot extrapolate our results to all type 2 diabetic patients. However, it has to be considered that a similar type of high CHO-high fiber diet induced similar, if not better, results on blood glucose control and fasting lipid metabolism in type 2 diabetic patients not in satisfactory blood glucose control and with hyperlipidemia (10,18). Another point to be stressed is the length of the intervention: although one month for each diet cannot be considered a long term experiment, it is certainly sufficiently long to induce changes in glucose and lipid metabolism.

In conclusion, a diet relatively high in carbohydrates, rich in dietary fiber, with a relatively low glycemic index and a low glycemic load, essentially based on consumption of legumes, vegetables, fruit, and whole cereals, may be particularly useful for the treatment of type 2 diabetic patients on the basis of its multiple effects on different cardiovascular risk factors, including postprandial lipid abnormalities.

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Disclosure. The authors have no conflict of interest to disclose.
REFERENCES
Table 1. Composition of the two isoenergetic diets recommended and followed by the patients

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<th>High CHO-high fiber diet</th>
<th>Low CHO-high MUFA diet</th>
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<tr>
<td><strong>Total energy (kcal/day)</strong></td>
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<td><strong>Proteins (%)</strong></td>
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<tr>
<td><strong>Total fat (%)</strong></td>
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<td>37±1 *</td>
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<td>Saturated fat (%)</td>
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<td>7±1</td>
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<tr>
<td>Monounsaturated fat (%)</td>
<td>17±1 *</td>
<td>23±1</td>
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<td>Polyunsaturated n-6 fat (%)</td>
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<td><strong>Cholesterol (g/day)</strong></td>
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<td><strong>Glycemic index (%)</strong></td>
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<td><strong>Glycemic load</strong></td>
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Data are expressed as means±SD, *p<0.05 vs. Low CHO- high MUFA diet.

**Figure legends**

Figure 1. Plasma glucose and insulin curves (A and D), plasma glucose and insulin incremental area under the curve (IAUC) (B and E), and glycemic variability (C) after a High CHO-high fiber test meal (white squares and white bars) or a Low CHO-high MUFA test meal (black triangles and black bars) performed at the end of the corresponding diet (means±SEM, significance for paired T test *p<0.05, $p=0.06$; significance for repeated measures ANOVA p<0.05 for plasma glucose and p<0.008 for plasma insulin).

Figure 2. Incremental areas under the curve for cholesterol and triglycerides in chylomicrons (A and B) and large VLDL (C and D) after a High CHO-high fiber test meal (white bars) or a Low CHO-high MUFA test meal (black bars) performed at the end of the corresponding diet (means±SEM, *p<0.05, §p=0.06).
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Figure 1

A. Glucose

B. IAUC

C. Glycemic variability

D. Insulin

E. IAUC

Figure 2

Chylomicrons

A. Cholesterol

B. Triglycerides

Large VLDL

C. Cholesterol

D. Triglycerides