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## Effects on blood glucose, insulin, lipid and proatherosclerotic parameters in stable type 2 diabetic subjects during an oral fat challenge

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Published: 20 July 2004

Received: 09 July 2004

*Lipids in Health and Disease* 2004, **3**:17 doi:10.1186/1476-511X-3-17

Accepted: 20 July 2004

This article is available from: <http://www.lipidworld.com/content/3/1/17>

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### Abstract

**Background:** Restriction of fat intake has been effective in improving insulin sensitivity in obese and type 2 diabetic subjects, but what effects the recommended diet (less than 30% of total calories from fat) have not been elucidated in subjects with type 2 diabetes. The purpose of this study was to test the effects of oral fat challenge, composing 30% calories of a meal, on blood glucose, insulin, lipid, leptin, plasminogen activator inhibitor-1 (PAI-1) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ).

**Design and Methods:** Blood glucose, insulin, lipid, leptin, TNF- $\alpha$  and PAI-1 were compared in 14 type 2 diabetic patients and 10 normal subjects after an oral fat challenge upto 2 hours (fasting, 15 min, 30 min, 45 min, 60 min, 90 min and 120 min).

**Results:** Postprandial glucose, total cholesterol, leptin, PAI-1 levels did not differ significantly from levels at fasting. Serum triglyceride increased significantly from baseline only in diabetic patients ( $P = 0.042$ ). Serum insulin increased postprandially in both groups ( $P = 0.028$  in diabetic group and  $P = 0.055$  in normal group), with displaying a prolonged insulin response in diabetic subjects. TNF- $\alpha$  decreased postprandially in both groups without significant difference, although diabetic patients have higher baseline levels ( $P = 0.024$  compared to normal subjects).

**Conclusions:** Oral fat load does not have an acute effect on blood glucose, total cholesterol, leptin and PAI-1 levels in both type 2 diabetic and normal subjects. TNF- $\alpha$  value showed decreased trend in both diabetic and normal subjects. The tendency of a delayed postprandial insulin response and elevated serum triglyceride level in diabetic subjects might be related to insulin resistance at the level of adipose tissue. Additional research is needed to assess the impact of the use of fat contents on the macronutrient composition of the diet, and potentially healthy and nutritional benefits for patients with diabetes.

### Introduction

The incidence of diabetes mellitus has been increasing rapidly in the past 2 decades and this is accompanied by the notably high prevalence of associated disorders, such as hypertension, atherogenic lipid profile and metabolic

syndrome, which lead to significantly high cardiovascular morbidity and mortality in diabetic patients. For reducing the risk of developing cardiovascular disease in type 2 diabetic patients, there have been longstanding recommendations to reduce saturated fat intake to <10% of energy

intake and to limit total fat intake to <30% of total daily calories. The study was to elucidate the effects of oral fat challenge in stable type 2 diabetic subjects.

## Research design and method

### Subjects

There were 14 well controlled type 2 diabetic subjects (hemoglobin A1c level below 7.5% for 6 months at least) and 10 normal subjects enrolled in our study. All subjects had normal renal and liver function profiles, and they had no history of hyperlipidemia or thyroid disorders before. All subjects had informed the study protocol and had agreed for this study before and during study protocol.

### Study protocol and procedure

In Eastern Asia, the average total calories intake was around 1800 kcal in an average 60 kg subject. Dietary fat intake was recommended to less than 30% of total daily calories and average meal fat of each eating was around 180 kcal. In order to understand the effect of oral fat intake in Chinese type 2 diabetic subjects and normal subjects, we performed this study protocol in 2002.

After an overnight fast, an intravenous line was placed into an antecubital vein for blood sampling and analysis. All subjects received oral fat tolerance test by drinking 180 kcal of Lipovenos MCT 20% solution (Containing glycerol 25 g, soybean oil 200 g and phosphatidylcholine 12 g in 1000 ml emulsion, Fresenius Kabi). Blood samples were drawn at 0, 15, 30, 45, 60, 90 and 120 minutes. Blood were collected under -20°C immediately and sent for analyses later.

After completion of these tests, glucose, insulin, lipid profiles, plasminogen activator inhibitor 1 (PAI-1), leptin, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) were analysed.

### Biochemical analyses

Plasma glucose was measured using glucooxidase method. Plasma insulin levels were determined by ELISA method. Plasma samples were assayed for PAI-1 antigen using colorimetric method (Sysmex CA-1500 and Berichrom PAI reagent, Japan) and for leptin using RIA (radioimmunoassay, LINCO Research Inc.) and TNF- $\alpha$  via EIA (enzyme immunoassay, Immunotech Comp.) method.

### Statistical analysis

Baseline data are expressed as means  $\pm$  SD. All statistical analyses were performed using the ANOVA test. Paired *t* test or appropriate nonparametric tests were used to estimate the effects of oral fat tolerance test on all variables. All tests were conducted using an  $\alpha$  level of 0.05.

## Results

There were no significant differences on blood glucose and cholesterol levels after oral fat challenge in both normal and type 2 diabetic subjects. (See figure 1) Concerning blood triglyceride concentration, there was steady increased triglyceride concentration and achieved significantly statistical difference in type 2 diabetic subjects ( $P = 0.042$ ). The insulin response showed early response in normal subjects up to 45 minutes, but persistent response with delayed returning to baseline value in type 2 diabetic subjects. (See figure 2).

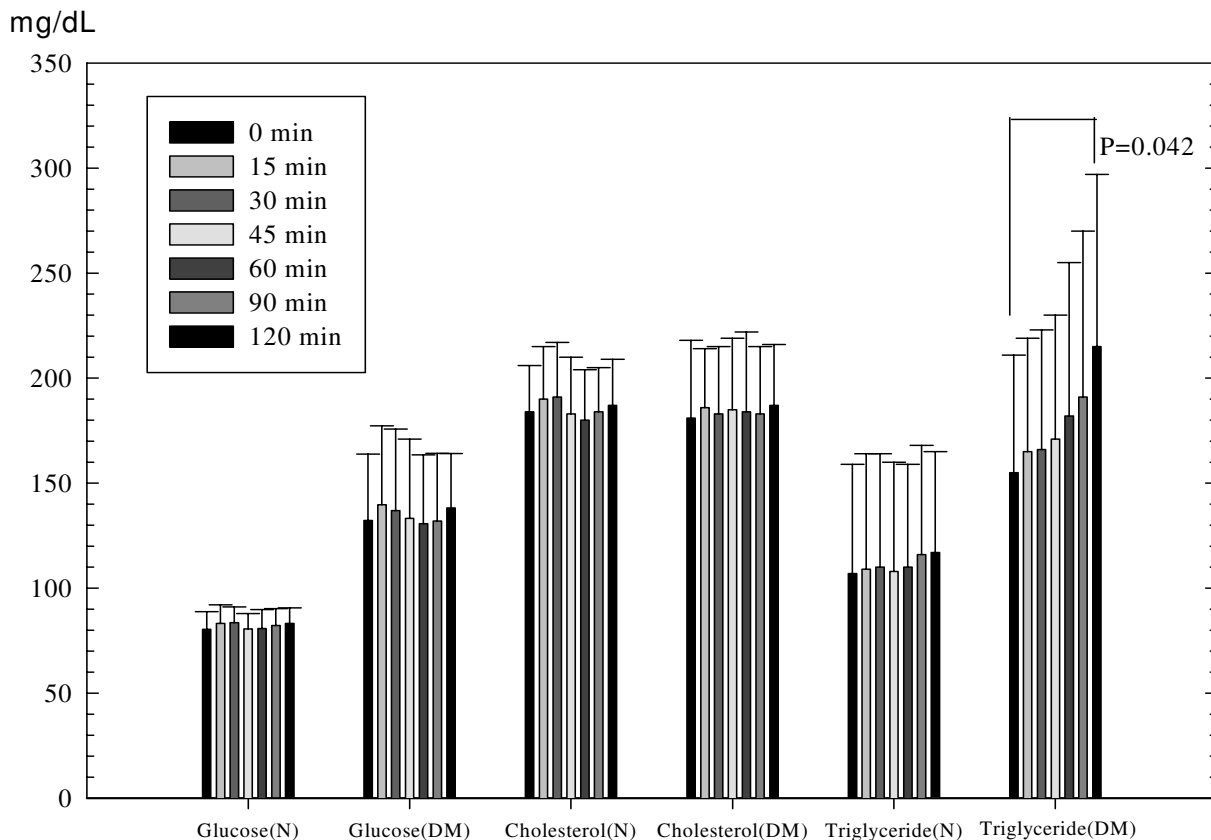
Type 2 diabetic subjects had higher values in baseline values of leptin and TNF- $\alpha$ , as compared with normal subjects (TNF- $\alpha$  achieved significant difference as  $p = 0.024$ ). There were no significant differences in both normal and type 2 diabetic subjects in leptin and TNF- $\alpha$  levels, although it seemed to decline after oral fat challenge, especially TNF- $\alpha$  in type 2 diabetic subjects ( $p = 0.12$ ). (See figure 3).

For PAI-1 response after oral fat challenge, there was no statistical difference in normal and type 2 diabetic subjects. The value of PAI-1 in type 2 diabetic subjects was lower than normal subjects, although not statistically significant. (probably due to ACE inhibitor usage in 8/14 stable diabetic subjects) [1]

## Discussions

Prevention of chronic complications is a key issue in the management of diabetes mellitus because of the huge premature morbidity and mortality associated with it. Recent studies, the Finnish Diabetes Prevention Study [2] and the Diabetes Prevention Program [3], strongly confirm the hypothesis that alter diet and physical activity to achieve weight loss can prevent or postpone the development of type 2 diabetes in high-risk individuals.

In our study, there was no significant effect on blood glucose, cholesterol, triglyceride, leptin, TNF- $\alpha$  and PAI-1 levels up to 120 minutes in normal subjects, except showing diminished TNF- $\alpha$  level trend (no significant difference,  $p > 0.05$ ). This means such oral intake of fat did not exert abnormal response in normal subjects. In blood insulin response, it displayed a peak response from 15 minutes to 45 minutes both in normal and type 2 diabetic subjects, with delayed return to baseline level in type 2 diabetic subjects. This response may be due to neural or hormonal factors as insulin is also secreted in response to amino acids and fatty acid with the magnitude of response being modulated by a variety of neural and hormonal factors (as glucagons, glucagons-like peptide 1, gastric inhibitory polypeptide and somatostatin).

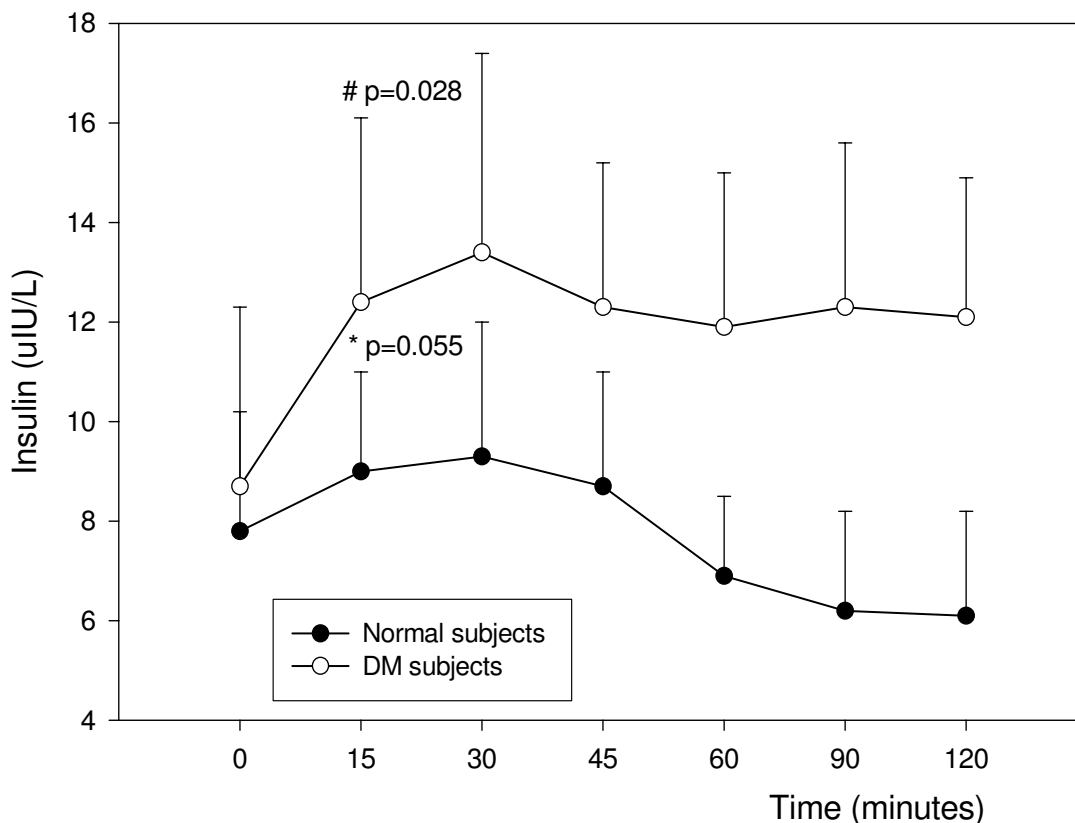


**Figure 1**

The responses of glucose, cholesterol and triglyceride in normal and diabetic subjects after oral fat challenge. The triglyceride response showed significant difference in type 2 diabetic subjects as p value of 0.042. (N: normal subjects; DM: diabetic subjects)

PAI-1, a member of the serine protease inhibitor family, can promote both thrombosis and fibrosis that play a key role in the pathogenesis of cardiovascular events. As these cardiovascular events are increased in patients with diabetes mellitus, it has been suggested that PAI-1 may be an important culprit in diabetic vascular complications. Also meal-related lipid excursions can potentially contribute to the atherosclerosis in type 2 diabetic subjects via elevation of variable inflammatory factors or cytokines, and several factors can stimulate PAI-1 gene transcription and its plasma levels, such as insulin [4], TNF- $\alpha$  [5], triglyceride [6], glucose [7], and angiotensin II [8]. These suggest that elevated plasma PAI-1 levels in type 2 diabetic patients are associated with multiple components, as hyperglycemia, hypertriglyceridemia, hyperinsulinemia and elevated

TNF- $\alpha$ , and substantially contribute to the prothrombotic and proatherosclerotic processes. Our study revealed elevated parameters in stable diabetic subjects as hyperglycemia, hypertriglyceridemia, elevated TNF- $\alpha$ , and hyperinsulinemia. The lower PAI-1 level in stable type 2 diabetic subjects as compared with normal subjects (2.50 IU/ml vs 3.07 IU/ml) was probably explained by ACE inhibitor usage (8 in 14 diabetic patients), resulting diminished angiotensin II formation. But this need further studies to address this potentially important issue. Moreover, there was no acute effect on blood leptin and PAI-1 values in stable type 2 diabetic subjects after oral fat challenge as shown in our study. The diminished TNF- $\alpha$  level may be due to fat source (mainly polyunsaturated fatty acid in our study), but it needed further evaluation to



**Figure 2**

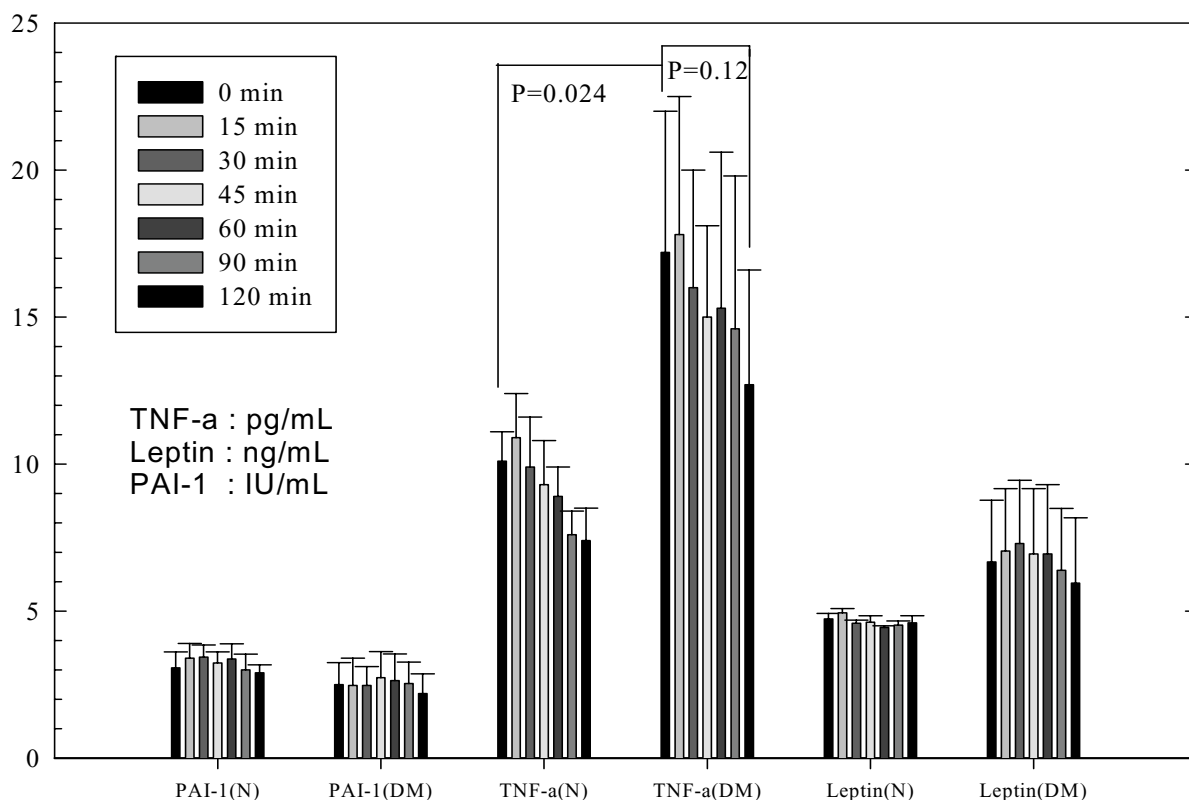
The response of insulin in normal and diabetic subjects after oral fat challenge. It showed persisted insulin response in diabetic subjects. ( $p = 0.028$ ).

confirm this or to elucidate the impact of fat intake on inflammatory factors on long-term effects of stable type 2 diabetic subjects or poorly controlled diabetic subjects.

Infusion of free fatty acids (as intralipid) causes increased oxidative stress in the healthy [9] and the diabetic subjects [10]. Simultaneously, elevated portal levels of FFA by oral routine may impair liver function, causing increased gluconeogenesis, increased triglyceride production and reduced insulin clearance, resulting in hyperglycemia, dyslipidemia and hyperinsulinemia in type 2 diabetic subjects. Also consumption of a high-fat diet and high intakes of saturated fat are associated with an increased risk of type 2 diabetes [11]. This means that both the total amount and types of dietary fat play an important role in insulin action, weight maintenance, and prevention of diabetes. Thus it has led to almost universal dietary rec-

ommendations to decrease the amount of dietary fat, aiming for a total fat intake of no more than 30% of calories and choosing foods low in saturated fat. It had been reported that postprandial dyslipidemia (hypertriglyceridemia) has high correlation to coronary heart and/or carotid artery disease [12-14] and may deteriorate the insulin resistance [15,16]. In our study, oral fat challenge induces delayed postprandial insulin response and elevated triglyceride level in stable type 2 diabetic subjects as compared to normal subjects. It will be important when recommending particular dietary source or pattern in the management of medical nutrition therapy in diabetic subjects to diminished postprandial hypertriglyceridemia.

However, the type of fat, rather than the amount of fat, in the diet may be more important in terms of determining health outcomes. As many studies had showed that the



**Figure 3**

The responses of PAI-1, TNF- $\alpha$  and leptin in normal and diabetic subjects after oral fat challenge. The values of TNF- $\alpha$  and leptin were higher in type 2 diabetic subjects as compared to normal subjects ( $p=0.024$  for TNF- $\alpha$ ). TNF- $\alpha$  decreased after oral fat challenge in both groups although no statistical difference. (N: normal subjects; DM: diabetic subjects; TNF- $\alpha$  TNF- $\alpha$ )

long chain n-3 polyunsaturated fatty acid found in oily fish and fish oil possessed cardioprotective effects [17,18] and  $\alpha$ -lipoic acid possesses antioxidant property with improving insulin sensitivity [19]. It is the future debate about the relative effects of the importance of fat quality, rather than fat quantity, in determining risk of developing insulin resistance, obesity, metabolic syndrome and cardiovascular disease.

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