

# Diet-induced change in fatty acid composition of plasma triacylglycerols is not associated with change in glucagon-like peptide 1 or insulin sensitivity in people with type 2 diabetes<sup>1-3</sup>

Audrey E Brynes, C Mark Edwards, Arvind Jadhav, Mohammed A Ghatei, Stephen R Bloom, and Gary S Frost

## ABSTRACT

**Background:** Polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) have been shown to positively affect blood lipids; however, their comparative effects on insulin sensitivity are unclear.

**Objective:** Our objective was to investigate whether chronic intake of MUFAs or PUFAs improves insulin sensitivity in people with type 2 diabetes via stimulation of the endogenous gut hormone glucagon-like peptide 1 [7–36] amide (GLP-1).

**Design:** Nine overweight people with type 2 diabetes received isoenergetic high-MUFA (20.3 ± 3.5% of total energy) or high-PUFA (13.4 ± 1.3%) diets for 24 d in a randomized, double-blind crossover design.

**Results:** Weight and glycemic control remained stable throughout the study. Despite a significant change in the plasma triacylglycerol linoleic-oleic acid ratio (L:O) with both diets (MUFA: from 0.46 ± 0.03 to 0.29 ± 0.02,  $P < 0.005$ ; PUFA: from 0.36 ± 0.04 to 0.56 ± 0.05,  $P < 0.05$ ) and the phospholipid L:O (1.7 ± 0.1 to 2.0 ± 0.3;  $P = 0.04$ ) during consumption of the PUFA diet, this change was not associated with a change in insulin sensitivity, measured by the short-insulin-tolerance test. There was a significant reduction in the ratio of total to HDL cholesterol during consumption of the PUFA diet (5.2 ± 0.4 compared with 4.7 ± 0.3;  $P = 0.005$ ) but no change with the MUFA diet. There was no change in the fasting or postprandial incremental area under the curve in response to an identical standard test meal for glucose, insulin, triacylglycerol, nonesterified fatty acids, or GLP-1.

**Conclusions:** Over the 3-wk intervention period, diet-induced change in the triacylglycerol or phospholipid L:O was not associated with either increased stimulation of GLP-1 or a change in insulin sensitivity in people with type 2 diabetes. *Am J Clin Nutr* 2000;72:1111–8.

**KEY WORDS** MUFA, PUFA, insulin sensitivity, GLP-1, type 2 diabetes, monounsaturated fatty acids, polyunsaturated fatty acids, glucagon-like peptide 1 [7–36] amide, triacylglycerol, cholesterol

## INTRODUCTION

Diet is the mainstay therapy in people with type 2 diabetes, yet the ideal dietary guidelines for people with diabetes remain controversial. Recommendations aim to promote nutritional factors

that have been shown to improve outcome, such as good glycemic control (1) and maintaining ideal body weight (2), while reducing the risk of coronary heart disease through improved lipid profiles (3). UK recommendations (4) suggest that carbohydrate should make up 50–55% of daily energy intake while fat is reduced to 30–35% of energy. Of this, 10% should be saturated fat, 10% polyunsaturated fatty acids (PUFAs), and 10–15% monounsaturated fatty acids (MUFAs). However, there is concern that an intake of 50–55% of energy as carbohydrate may have an adverse effect on triacylglycerols and glycemic control (5); indeed, US American Diabetes Association guidelines (6) now recommend that 60–70% of energy be divided between carbohydrate and MUFAs, depending on patient preference and nutritional goals.

In a recent review by Garg (7), high-MUFA diets compared with high-carbohydrate diets reduced fasting triacylglycerol and VLDL cholesterol by 19% and 22%, respectively, with a modest increase in HDL and no adverse effect on LDL. It was also shown with use of the euglycemic clamp method that increasing MUFAs and decreasing carbohydrate intake improves insulin sensitivity and glycemic control while having no adverse effects on lipids (8, 9).

The lipid-lowering effects of MUFAs compared with those of PUFAs are well studied, suggesting that PUFAs may be more potent at lowering plasma LDL-cholesterol and triacylglycerol concentrations (10). However, information about the effect of specific fatty acids, especially n–6 PUFAs compared with MUFAs, on insulin sensitivity is scarce (11, 12). Many studies compare MUFAs with carbohydrate rather than with other fatty acids. The mechanism for the reported benefits of MUFAs on insulin sensitivity is unknown. New evidence suggests that gut hormones, which can be manipulated by nutrient intake, may be involved in the regulation of insulin sensitivity and insulin secretion (13).

<sup>1</sup>From the Nutrition and Dietetic Research Group, the Endocrine Unit, and the Lipoprotein Team, Imperial College School of Medicine, Hammersmith Hospital, London.

<sup>2</sup>Supported by a Small Project Grant from the British Diabetes Association and additional funding from the Sugar Bureau. CME is a BDA RD Lawrence research fellow.

<sup>3</sup>Address reprint requests to GS Frost, Department of Nutrition and Dietetics, Hammersmith Hospital, Du Cane Road, London, United Kingdom W12 0HS. E-mail: g.frost@ic.ac.uk.

Received November 19, 1999.

Accepted for publication April 19, 2000.

**TABLE 1**  
Compliance with a high-MUFA or a high-PUFA diet monitored by three 7-d food diaries<sup>1</sup>

	Baseline diet	MUFA diet	PUFA diet
Total energy (MJ/d)	8.93 ± 3.58	9.81 ± 2.89	9.78 ± 2.54
(kcal/d)	2134 ± 855	2345 ± 691	2338 ± 606
Protein (% of energy)	15.0 ± 1.2	13.5 ± 2.3	12.4 ± 1.3 <sup>2</sup>
Carbohydrate (% of energy)	49.3 ± 9.2	47.2 ± 4.9	46.5 ± 5.1
Sugars (% of energy)	19.4 ± 3.4	22.9 ± 3.2	22.4 ± 3.0
Starch (% of energy)	29.7 ± 7.0	23.8 ± 6.2 <sup>3</sup>	24.1 ± 6.3 <sup>3</sup>
Glycemic index <sup>4</sup>	81.2 ± 9.0	83.3 ± 3.8	83.4 ± 3.0
Total fat (% of energy)	34.5 ± 8.9	39.1 ± 4.3 <sup>2</sup>	41.1 ± 4.6 <sup>2</sup>
Monounsaturated fat (% of energy)	9.1 ± 2.8	20.3 ± 3.5 <sup>3</sup>	11.7 ± 1.9 <sup>2,5</sup>
Polyunsaturated fat (% of energy)	5.2 ± 3.1	4.2 ± 1.1	13.4 ± 1.3 <sup>3,5</sup>
Saturated fat (% of energy)	10.6 ± 5.0	8.0 ± 2.9	9.2 ± 2.6
Unknown fats (% of energy)	9.7 ± 3.8	6.6 ± 3.0	6.8 ± 3.3

<sup>1</sup> $\bar{x} \pm$  SD. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Diaries were available for 8 of 9 subjects.

<sup>2,3</sup>Significantly different from baseline diet: <sup>2</sup> $P < 0.05$ , <sup>3</sup> $P < 0.005$ .

<sup>4</sup>Statistical analyses performed on log-transformed data.

<sup>5</sup>Significantly different from MUFA diet,  $P = 0.001$ .

Glucagon-like peptide 1 [7–36] amide (GLP-1) is the most potent known endogenous gut hormone stimulant of insulin secretion in humans. In addition to its insulin-stimulating effects, GLP-1 suppresses glucagon secretion, delays gastric emptying, and is postulated to increase peripheral insulin sensitivity (14–16). It is thus being investigated as a possible treatment for type 2 diabetes (17). A preprandial subcutaneous dose of 25 nmol GLP-1 was shown to reduce peak postprandial glycemia by 2 mmol/L in people with type 2 diabetes (18). GLP-1 is released from the L cells of the terminal ileum and colon in response to a mixed meal (19). It is not known whether increasing endogenous plasma GLP-1, via dietary manipulation, could improve glycemic control in people with type 2 diabetes, although there is some evidence that blocking endogenous GLP-1 with exendin 9–39 causes a deterioration in glycemic control (20).

We previously showed a trend toward increased GLP-1 release with an acute MUFA-rich meal in healthy lean volunteers, although the effect was not significant (21). Thomsen et al (22) examined the acute effect of different fats on postprandial lipemia and GLP-1 concentrations in healthy volunteers and showed that olive oil induced higher concentrations of GLP-1 and gastric inhibitory peptide than did butter (22). If a nutrient were to be identified that augments the chronic release of GLP-1, it would be a potential treatment for type 2 diabetes because it would improve glycemic control and insulin sensitivity.

## SUBJECTS AND METHODS

We aimed to recruit obese subjects with type 2 diabetes treated by diet alone and who were therefore insulin resistant with residual pancreatic  $\beta$  cell function. Fifteen people with type 2 diabetes were approached to take part in this study. After initial screening, 9 (5 male and 4 female) overweight people with type 2 diabetes were recruited from the Hammersmith Hospital diabetes clinic. All subjects fulfilled the World Health Organization criteria for the diagnosis of diabetes (23). They were not taking any medication or dietary supplements and had normal results on an electrocardiogram and physical examination. The mean length of time since

diagnosis of diabetes was  $3.0 \pm 2.1$  y. The mean ( $\pm$ SD) age was  $56 \pm 5.3$  y, body mass index (BMI; in  $\text{kg}/\text{m}^2$ ) was  $29.8 \pm 2.8$ , and waist-to-hip ratio was  $0.97 \pm 0.05$  for the men and  $0.93 \pm 0.01$  for the women. The subjects were all nonsmokers and had stable weights. Previous dietetic input was per British Diabetic Association (BDA) guidelines (4). Subjects' habitual baseline diets are described in **Table 1**; the subjects' macronutrient intakes compare favorably with the BDA guidelines. Alcohol intake and physical activity level did not differ significantly between subjects or between the 2 intervention periods. The Imperial College School of Medicine, Hammersmith Campus Research and Ethics Committee granted ethical approval. All volunteers gave written consent.

## Experimental protocol

A double-blind, randomized crossover study design was undertaken (**Figure 1**). After a 1-wk run-in period during which subjects recorded their normal habitual diet for 7 d, participants were randomly assigned to one of two 3-wk treatments: a diet rich in MUFAs or a diet rich in PUFAs. There was a minimum 2-wk washout period between the 2 diets ( $\bar{x}$ :  $25 \pm 6$  d). Subjects then started the other intervention. Each dietary period included a short-insulin-tolerance test (SITT) on days 1 and 23 and a standard test meal (STM) on days 2 and 24 of each intervention. The SITT was used to measure change in insulin sensitivity. Postprandial GLP-1, insulin, glucose, and lipid responses were measured after STMs eaten before and after subjects had consumed the MUFA or PUFA diets. Consistent exercise and daily routines were encouraged throughout the study. No alcohol or exercise was allowed on the days before the SITT or STM. On the basis of results from previous studies, alterations in glycemia, insulinemia, and lipemia were assumed to occur within the first 3 wk after crossover (24–26).

## Clinical and metabolic measurements

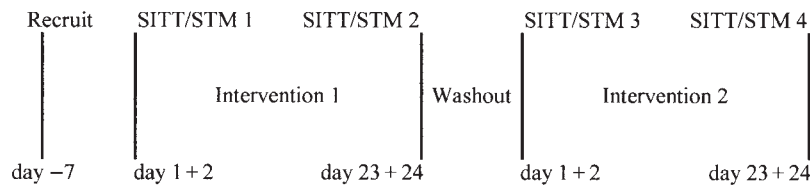
### Short-insulin-tolerance test

Volunteers were tested in the early morning after an overnight fast. Two intravenous cannulas were inserted: one in one arm for the sampling of arterialized blood, with the hand placed in a warm box at  $55^\circ\text{C}$ , and the other in the other arm to give insulin. Insulin (0.05 U Human Actrapid insulin/kg; Novo Nordisk Pharmaceuticals, Crowley, United Kingdom) was given intravenously at 0 min. Blood samples (2 mL) were taken at  $-15$ , 0, 3, 4, 6, 8, 10, 12, 14, and 15 min for the measurement of glucose. The test was terminated at 15 min with oral glucose (Lucozade; Smith Kline-Beecham Ltd, London) followed by a light breakfast. This procedure was done as described previously by Bonora et al (27) and modified by Gelding et al (28).

The SITT is a simple, safe, accurate, and reproducible test (28). The test measures blood glucose decline over a 15-min period in response to a bolus of insulin and before the onset of counter-regulatory hormones. The SITT was adapted from the insulin tolerance test (29), which involves the measurement of blood glucose concentration over a 15-min period in response to a bolus of insulin providing 0.1 U/kg body wt. Because this dose increased the risk of hypoglycemia, we instead used 0.05 U/kg, as used in other studies with insulin-resistant subjects (30). The results of the SITT correlate well with those of the euglycemic-hyperinsulinemic clamp method ( $r = 0.81$ ,  $P < 0.005$ ) (28).

### Standard test meals

Volunteers were tested in the early morning after an overnight fast. An intravenous cannula was inserted into the



**FIGURE 1.** Schematic diagram of study design.  $n = 9$ . SITT, short-insulin-tolerance test; STM, standard test meal. Monounsaturated fatty acid and polyunsaturated fatty acid interventions were assigned in random order.

antecubital fossa for blood sampling. Blood samples were taken at  $-15, 0, 5, 15, 30, 45, 60, 90, 120, 150,$  and  $180$  min. The meal was consumed within 10 min on each occasion. A supplement drink (Liquid Ensure Plus; Abbott Laboratories Ltd, Kent, United Kingdom) was used for the STM. This was nutritionally complete (400 mL contains 20 g fat, of which the main fatty acids were 16:0, 6%; 18:0, 3%; 18:1, 59%; 18:2, 25%; and 18:3, 5%; 80 g carbohydrate; 25 g protein; and 2.5 MJ), was palatable, and produced reproducible data. We showed previously that a liquid meal stimulates a significantly greater amount of GLP-1 than does an identical solid one (21). We used identical test meals to assess the long-term background effects of ingesting MUFAs compared with PUFAs, and measured the plasma fatty acid profiles of subjects at the beginning and end of each intervention to show that the interventions had induced a dietary change in background plasma and phospholipid triacylglycerol profiles.

#### Dietary interventions

The aim of the dietary interventions was to test the effect of a chronic increase in MUFAs (mainly oleic acid, 18:1n-9) or

PUFAs (mainly linoleic acid, 18:2n-6) in the diet by substituting them for high-glycemic index carbohydrates (ie, bread and potatoes) while keeping the total energy and protein intakes constant. To aid compliance, an oil high in MUFAs (olive oil) or an oil high in PUFAs (corn oil) was incorporated into a carrot cake, which was portioned to provide an additional 10%, above habitual intake, of the total daily energy intake as corn oil or olive oil, while keeping the diets isoenergetic. In practice, this corresponded to an increase from  $9.1 \pm 2.8\%$  to  $20.3 \pm 3.5\%$  with the MUFA diet and from  $5.2 \pm 3.1\%$  to  $13.4 \pm 1.3\%$  with the PUFA diet (Table 1). Total energy intake was assessed by a 7-d diary during the run-in period and validated according to estimated basal metabolic rate multiplied by an activity factor of 1.3 (2). The cakes were weighed into portions and then frozen to take home. Volunteers were instructed to include 3 slices of cake/d in their diet, ideally before the 3 main meals, and then to eat to satiety.

#### Laboratory analysis

Plasma glucose concentrations were measured by using a glucose oxidase-based autoanalyzer (Technicon; Axon Bayer

**TABLE 2**

Biochemical test results of 9 people with diet-controlled type 2 diabetes with a high-MUFA and a high-PUFA diet<sup>1</sup>

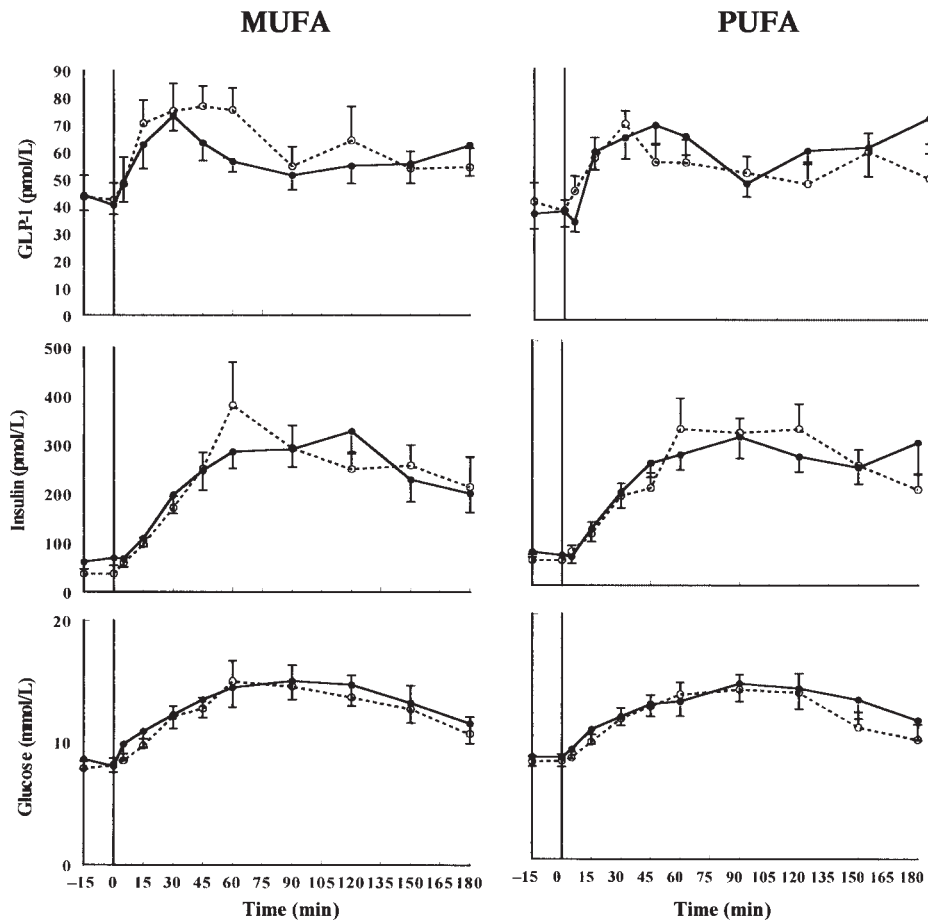
	MUFA diet		PUFA diet	
	Day 1	Day 24	Day 1	Day 24
<b>Fasting variables</b>				
Weight (kg) <sup>2</sup>	87.0 ± 4.8	87.3 ± 4.9	86.7 ± 5	87.0 ± 4.9
Hb A <sub>1c</sub> (%) <sup>2</sup>	6.6 ± 0.3	6.5 ± 0.3	6.7 ± 0.3	6.6 ± 0.3
Total cholesterol (mmol/L)	4.8 ± 0.3	4.9 ± 0.3	4.9 ± 0.4	4.6 ± 0.3
HDL cholesterol (mmol/L)	0.96 ± 0.1	0.97 ± 0.1	1.02 ± 0.1	1.00 ± 0.1
Total-HDL cholesterol ratio <sup>3</sup>	5.2 ± 0.4	5.2 ± 0.3	5.2 ± 0.4	4.7 ± 0.3 <sup>4</sup>
LDL cholesterol (mmol/L)	3.05 ± 0.3	2.87 ± 0.3	3.12 ± 0.3	2.82 ± 0.2
Triacylglycerol (mmol/L) <sup>2</sup>	1.51 ± 0.2	1.8 ± 0.3	1.66 ± 0.3	1.78 ± 0.3
Nonesterified fatty acids (mmol/L)	0.67 ± 0.1	0.59 ± 0.1	0.60 ± 0.1	0.67 ± 0.1
GLP-1 (pmol/L)	40.6 ± 6.9	42.2 ± 4.4	36.6 ± 5.1	36.2 ± 5.6
Insulin (pmol/L)	37.2 ± 6.8	57.6 ± 12.7	47.4 ± 10.7	56.2 ± 10.9
Glucose (mmol/L)	8.0 ± 0.6	8.3 ± 0.6	7.9 ± 0.5	8.2 ± 0.7
Short-insulin-tolerance test ( $\mu\text{mol}\cdot\text{L}^{-1}\cdot\text{min}^{-1}$ )	115 ± 13	113 ± 17	117 ± 17	125 ± 16
<b>Integrated area under the curve</b>				
Insulin (nmol·min/L)	37.2 ± 4.8	32.6 ± 3.9	35.2 ± 3.4	32.9 ± 4.9
Glucose (mmol·min/L)	845 ± 167	957 ± 205	705 ± 115	792 ± 139
GLP-1 (pmol·min/L)	3685 ± 699	3075 ± 898	3114 ± 930	4021 ± 1200
Nonesterified fatty acids (mmol·min/L) <sup>2</sup>	-39.3 ± 5	-39.7 ± 6.5	-46.1 ± 5.3	-42 ± 10
Triacylglycerol (mmol·min/L)	71.8 ± 13.1	65.4 ± 24	82.9 ± 16.6	99 ± 18.6

<sup>1</sup> $\bar{x} \pm \text{SEM}$ . MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; GLP-1, glucagon-like peptide 1 [7-36] amide; Hb A<sub>1c</sub>, glycated hemoglobin.

<sup>2</sup>Statistical analyses performed on log-transformed data.

<sup>3</sup>Significant interaction between diet and time,  $P < 0.005$ .

<sup>4</sup>Significantly different from day 1,  $P = 0.009$ .



**FIGURE 2.** Mean ( $\pm$ SEM) glucagon-like peptide 1 (GLP-1), insulin, and glucose response to a standard test meal eaten after people with type 2 diabetes ( $n = 9$ ) consumed diets containing either 20.3% of energy as monounsaturated fatty acids (MUFAs) or 13.4% of energy as polyunsaturated fatty acids (PUFAs) for 24 d. Open symbols indicate baseline values, filled symbols indicate values after 24 d of the diet.

Diagnostic, Newbury, United Kingdom). GLP-1 and insulin concentrations were measured in-house by using specific radioimmunoassays (19, 31). For both assays the interassay and intraassay CVs were  $<10\%$ . The assays were capable of detecting 2 pmol/L with 95% confidence. All samples were included in one assay and analyzed in duplicate after the first freeze-thaw. Triacylglycerol and nonesterified fatty acids were measured by the enzymatic colorimetric method using commercial kits (MPR2 Triacylglycerols GPO-PAP 701 912 kit; Roche Diagnostics Ltd, Lewes, United Kingdom; NEFA kit; WAKO Chemicals, Alpha Laboratories, Eastleigh, United Kingdom). Total and HDL-cholesterol concentrations were measured on a DAX-72 analyzer (Bayer Diagnostics, Basingstoke, United Kingdom).

#### Statistical analysis

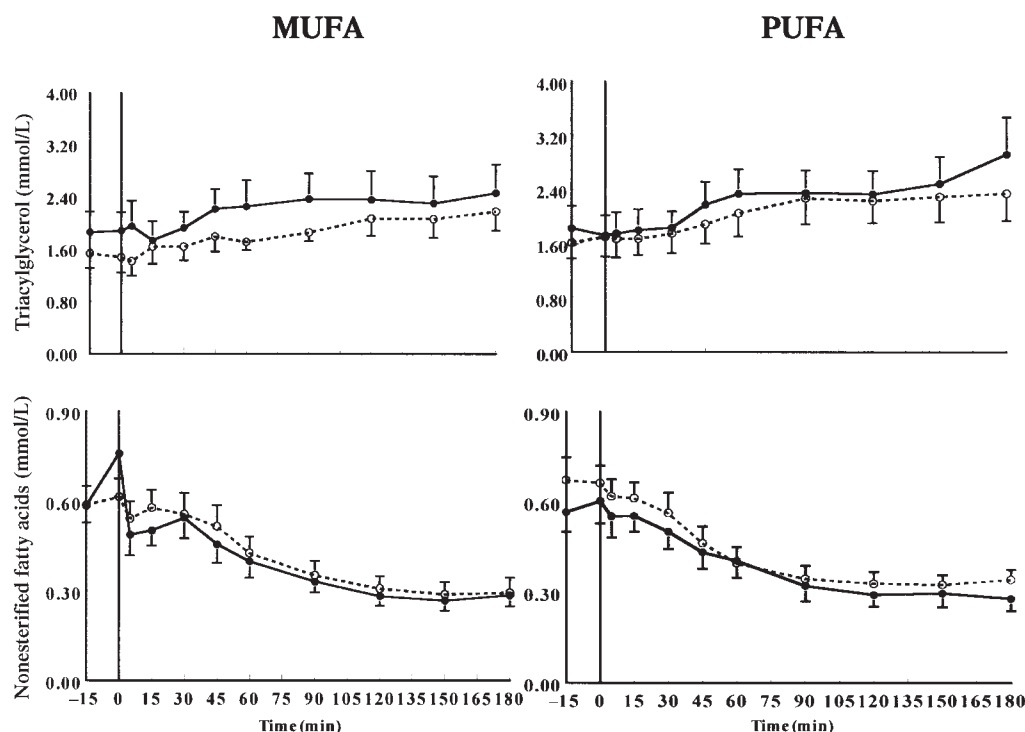
Sample size was estimated from a previous acute study in healthy volunteers for a power of 80% and an  $\alpha$  of 5% with an estimated difference in GLP-1 incremental areas under the curve (IAUC) of 1000 pmol $\cdot$ min/L and an SD of 1000 pmol $\cdot$ min/L (21). This suggested a minimum sample size of 10 pairs. All results are presented as means  $\pm$  SEMs unless stated otherwise. For **Table 2** and **Table 3** a repeated-measures design was used with a  $2 \times 2$  factorial structure for the repeated measures, with

factors of diet (MUFA and PUFA) and day (1 and 24). Individual means have been reported only if there was a significant interaction. In **Table 1**, a repeated-measures design, with one repeated-measures factor, diet (baseline, MUFA, or PUFA), was used. Log transformation was used for positively skewed variables.  $P < 0.05$  was taken as significant. The IAUC above baseline was calculated by using the trapezoidal rule.

#### RESULTS

Body weights did not change significantly over the study period (**Table 2**). Glycated hemoglobin (Hb A<sub>1c</sub>) did not change significantly over either of the 3-wk intervention periods (normal range: 4.3–5.7%). There was a significant reduction in the fasting ratio of total cholesterol to HDL cholesterol with the PUFA diet but no change with the MUFA diet. There was no change in fasting or postprandial GLP-1, insulin, or glucose concentration (**Table 2**, **Figure 2**). There was no change in fasting or postprandial triacylglycerol or nonesterified fatty acid concentrations (**Table 2**, **Figure 3**).

There was no change in insulin sensitivity measured by the SITT with either the MUFA or the PUFA diet (**Table 2**, **Figure 4**). The normal value is reported to be  $\approx 175 \pm 10 \mu\text{mol}\cdot\text{L}^{-1}\cdot\text{min}^{-1}$  in young, lean nondiabetic adults (28); the lower the result, the more



**FIGURE 3.** Mean ( $\pm$ SEM) triacylglycerol and nonesterified fatty acid response to a standard test meal eaten after people with type 2 diabetes ( $n = 9$ ) consumed diets containing either 20.3% of energy as monounsaturated fatty acids (MUFAs) or 13.4% of energy as polyunsaturated fatty acids (PUFAs) for 24 d. Open symbols indicate baseline values, filled symbols indicate values after 24 d of the diet.

insulin resistant a person is. Intersubject variation was low [ $\bar{x}$ :  $116 \mu\text{mol} \cdot \text{L}^{-1} \cdot \text{min}^{-1}$ ; 95% CI: 94, 138), 19%, which suggests that this lack of effect was not due to the sample size ( $n = 9$ ). The error SD between the 2 sets of baseline SITT data were also low (CV = 11.3%) (32). The SITT results are also supported by no significant change in the insulin or glucose IAUC after the test meals.

All subjects reported 100% compliance by returning the empty cake containers. Eight subjects returned a 7-d diet diary (one subject was excluded because of dyslexia). These were analyzed by using household portion measures and suggested that dietary intervention was achieved (Table 1). There was a tendency for a reduction in total energy from carbohydrate, although this was not significant, and a significant increase in energy from fat (Table 1). The only significant differences between the endpoints of the 2 intervention diets were that MUFA intake increased from  $9.1 \pm 2.8\%$  of total energy intake to  $20.3 \pm 3.5\%$  with the olive oil cake and PUFA intake increased from  $5.2 \pm 3.1\%$  to  $13.4 \pm 1.3\%$  with the corn oil cake (Table 1). Total energy intake did not change significantly over the study period.

Relative percentage fatty acid composition of the plasma triacylglycerols was measured on days 1 and 24 of both of the interventions (33). This confirmed compliance in both intervention periods (Table 3). Despite a difference in baselines, the linoleic-oleic acid ratio (L-O ratio) fell significantly with the MUFA intervention and increased significantly with the PUFA intervention. The fatty acid composition of phospholipids was measured in 5 of 9 subjects. There was a strong correlation ( $r = 0.74$ ,  $P = 0.0004$ ) between the phospholipids and the plasma triacylglycerol concentrations with both interventions and a significant increase in the L-O phospholipid ratio with the

PUFA diet (from  $1.7 \pm 0.1$  to  $2.0 \pm 0.3$ ;  $P = 0.04$ ). The decrease in the L-O phospholipid ratio with the MUFA diet did not reach significance ( $2.5 \pm 0.1$  to  $1.9 \pm 0.4$ ; NS) over the 3-wk period.

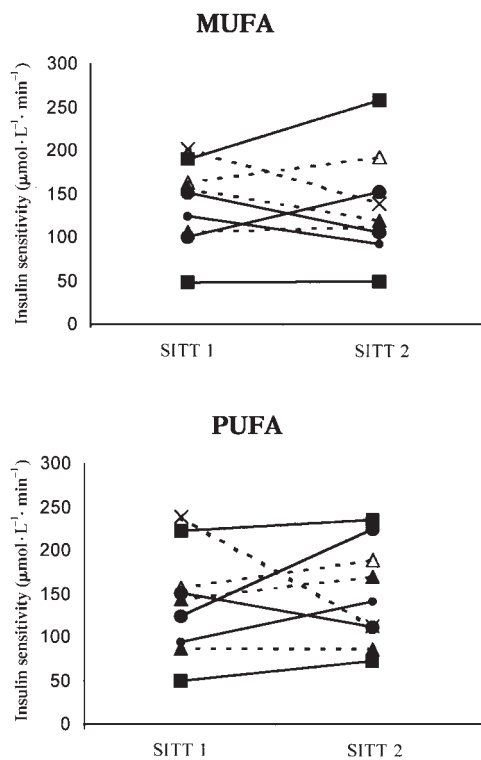
## DISCUSSION

In this study, we investigated the effect of increasing the proportion of MUFAs compared with PUFAs on insulin resistance in people with type 2 diabetes. Insulin resistance is thought to explain some of the increased risk of coronary heart disease in type 2 diabetes. It is important to explore the role of dietary change in insulin resistance. Previous evidence suggests that MUFAs increase insulin sensitivity (7); however, there is inconclusive evidence of the effects of n-6 PUFAs on insulin sensitivity (12).

We did not find an increase in insulin sensitivity with either the MUFA intervention or the PUFA intervention despite showing compliance with the dietary intervention by a change in the composition of plasma triacylglycerols and phospholipids. This observation is also supported by the lack of effect of the diets on insulin or glucose IAUCs after the test meals.

We also did not see a change in GLP-1. Because there was also no effect on insulin sensitivity, we speculate that a diet-induced change in GLP-1 is still an exciting possibility—if a different nutrient were to be identified that augments the chronic release of endogenous GLP-1, that nutrient could still be a potential treatment for type 2 diabetes.

Other diet studies that suggested a positive effect on insulin sensitivity have been conducted over a similar length of time (9). In our study we were able to independently verify changes in plasma triacylglycerol that support the reported compliance. In the subset



**FIGURE 4.** Short-insulin-tolerance test (SITT) of change in insulin sensitivity with 24 d of a high-monounsaturated fatty acid (MUFA) or high-polyunsaturated fatty acid (PUFA) diet in people with type 2 diabetes ( $n = 9$ ).

of subjects we analyzed for plasma phospholipid content, there was already a significant increase in the L-O phospholipid ratio with the PUFA intervention; however, we suggest that the interventions may not have been of sufficient length to enable us to see a change in the skeletal muscle phospholipids, in which increasing unsaturation is associated with increasing insulin sensitivity (34).

We chose to give an identical test meal with each intervention so that we could directly compare the effects that the change in the background plasma triacylglycerols and phospholipids had on the various variables we measured. The fact that there was a significant change in the composition of plasma triacylglycerol

at the endpoints in the study confirms a change in the metabolic environment. This does not tell us whether differences would have been measured if the composition of the test meals had been high in the corresponding intervention MUFAs or PUFAs.

As can be seen from the SITT results (Figure 4), there was no consistent change in insulin sensitivity despite high concentrations of corresponding plasma triacylglycerols and phospholipids at the time of the test. It may be that some individuals are more sensitive to dietary effects than are others; however, there was no correlation between the percentage change in the L-O ratio and the percentage change in SITT or GLP-1 release.

Total fat intake (% of energy) increased by 5% and 7% with the MUFA and PUFA interventions, respectively. Although the intake of saturated fat did not change, the increase in total fat per se may have influenced the results of the SITT (35). However, the recent American Diabetic Association guidelines suggest no clear upper limit on the amount of energy from MUFAs (36), suggesting a split between MUFAs and carbohydrate depending on the individual.

The typical UK MUFA intake is reported to be 12%, whereas that of a typical current Mediterranean diet is 17%, although at the time of the Seven Countries Study a 24% MUFA intake was recorded in Crete (37). We achieved an adequate 11.2% increase in MUFAs to 20.3% and an increase in PUFAs to 8.2%, 7% above the UK average (38), while keeping the diet palatable. An intake of 20.3% of energy as MUFAs is in line with 2 other prescribed dietary intervention studies in free-living subjects, which did show a benefit with a high-MUFA diet (39, 40), although neither of those studies measured insulin sensitivity directly.

Total cholesterol and triacylglycerol were surprisingly low considering that the group was insulin resistant with diet-controlled diabetes. The total cholesterol concentration at baseline was  $4.8 \pm 0.3$  mmol/L. There was no significant change in total cholesterol with either intervention; however, there was a reduction in the ratio of total to HDL cholesterol with the PUFA diet. This is in agreement with the results of previous studies (10).

Garg's (7) meta-analysis of previous studies on insulin sensitivity and MUFAs suggests that MUFAs also reduce triacylglycerol. Baseline triacylglycerol and nonesterified fatty acids were within the normal range and there was no significant change in fasting values or IAUCs for these during either intervention. It is impossible to know whether this effect would have been different had the volunteers been less well controlled metabolically.

**TABLE 3**

Relative fatty acid composition of the plasma triacylglycerols at baseline and day 24 with the MUFA and PUFA diets<sup>1</sup>

Fatty acid	Baseline, day 1	MUFA, day 24	Baseline, day 1	PUFA, day 24
	% by wt of total fatty acids			
14:0	2.17 ± 0.2	2.61 ± 0.3	2.56 ± 0.4	2.5 ± 0.5
16:0	28.5 ± 1.7	30.7 ± 1.5	30.9 ± 2.1	29.7 ± 2.2
16:1 <sup>2</sup>	4.37 ± 0.4	4.70 ± 0.4	4.84 ± 0.5	3.78 ± 0.3 <sup>3</sup>
18:0	4.36 ± 0.8	3.97 ± 0.3	3.56 ± 0.3	3.75 ± 0.4
18:1 <sup>4</sup>	38.1 ± 1.4	42.5 ± 1.6 <sup>5</sup>	38.3 ± 1.4	36.0 ± 1.6
18:2 <sup>6</sup>	17.6 ± 1.4	12.2 ± 1.1 <sup>5</sup>	13.8 ± 1.5	20.3 ± 3.5 <sup>5</sup>
18:3	1.09 ± 0.4	0.39 ± 0.2	0.86 ± 0.5	0.88 ± 0.9
L:O <sup>7</sup>	0.46 ± 0.03	0.29 ± 0.02 <sup>3</sup>	0.36 ± 0.04	0.56 ± 0.05 <sup>5</sup>
P:S	0.56 ± 0.1	0.35 ± 0.05 <sup>5</sup>	0.43 ± 0.08	0.68 ± 0.2


<sup>1</sup> $\bar{x} \pm$  SEM. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; L:O, ratio of linoleic (18:2n-6) to oleic (18:1n-4) acid; P:S, ratio of polyunsaturated to saturated fatty acids.

<sup>2,4,6,7</sup>Significant interaction between diet and time: <sup>2</sup> $P = 0.049$ , <sup>4</sup> $P = 0.008$ , <sup>6</sup> $P = 0.02$ , <sup>7</sup> $P = 0.03$ .

<sup>3,5</sup>Significantly different from day 1 within diet: <sup>3</sup> $P < 0.005$ , <sup>5</sup> $P < 0.05$ .



Note that the cake increased the sucrose content of the diet by a mean of 68 g/d (13% of overall energy intake); however, there was no significant increase in the total sugar intake during the study. This had no effect on the glycemic indexes of the diets (baseline,  $81.2 \pm 9.0$ ; MUFAs,  $83.3 \pm 3.8$ ; PUFAs,  $83.4 \pm 3.0$ ), which were calculated as described previously (41). We saw no effect of sucrose (75 g) on triacylglycerol, nonesterified fatty acids, insulin, or glucose profiles in insulin-resistant men at risk of coronary heart disease or in matched control subjects (42). In a review of the sucrose content of diabetic diets, Ha et al (43) reported that an intake of 10% of energy as sucrose had no effect on triacylglycerol concentrations.

This is the first study to investigate the effect of MUFAs compared with PUFAs on GLP-1 and insulin sensitivity in people with type 2 diabetes. Neither MUFAs nor PUFAs had an effect on the postprandial stimulation of GLP-1. We found no advantage in recommending increases in the diet of MUFAs in preference to PUFAs in terms of increased insulin sensitivity for people with type 2 diabetes over this 3-wk intervention; however, PUFAs did significantly reduce the ratio of total to HDL cholesterol, a recognized benefit in the prevention of coronary heart disease. Over the 3-wk intervention period, diet-induced change in the plasma triacylglycerol or phospholipid L-O ratio was not associated with either increased stimulation of GLP-1 or a change in insulin sensitivity in obese, insulin-resistant people with type 2 diabetes. 

We thank Caroline Doré from the Medical Statistics Department, Imperial College School of Medicine, for her helpful advice and discussion.

## REFERENCES

- Turner RC. The UK Prospective Diabetes Study—a review. *Diabetes Care* 1998;21(suppl) 3:C35–8.
- Lean ME, James WP. Prescription of diabetic diets in the 1980s. *Lancet* 1986;1:723–5.
- Laakso M. Lipids and lipoproteins as risk factors for coronary heart disease in non-insulin-dependent diabetes mellitus. *Ann Med* 1996;28:341–5.
- Dietary recommendations for people with diabetes: an update for the 1990s. Nutrition Subcommittee of the British Diabetic Association's Professional Advisory Committee. *Diabet Med* 1992;9:189–202.
- Garg A, Bantle JP, Henry RR, et al. Effects of varying carbohydrate content of diet in patients with non-insulin-dependent diabetes mellitus. *JAMA* 1994;271:1421–8.
- Nutrition recommendations and principles for people with diabetes mellitus. *Diabetes Care* 1999;22(suppl):S42–5.
- Garg A. High-monounsaturated-fat diets for patients with diabetes mellitus: a meta-analysis. *Am J Clin Nutr* 1998;67(suppl):577S–82S.
- Garg A, Grundy SM, Unger RH. Comparison of effects of high and low carbohydrate diets on plasma lipoproteins and insulin sensitivity in patients with mild NIDDM. *Diabetes* 1992;41:1278–85.
- Parillo M, Rivellese AA, Ciardullo AV, et al. A high-monounsaturated-fat/low-carbohydrate diet improves peripheral insulin sensitivity in non-insulin-dependent diabetic patients. *Metabolism* 1992; 41: 1373–8.
- Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. *Arterioscler Thromb* 1992;12:911–9.
- Saris WHM. Functional foods and substrate metabolism. *Br J Nutr* 1998;80(suppl):S47–75.
- Storlien LH, Baur LA, Kriketos AD, et al. Dietary fats and insulin action. *Diabetologia* 1996;39:621–31.
- Herrmann C, Goke R, Richter G, Fehmann HC, Arnold R, Goke B. Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. *Digestion* 1995; 56:117–26.
- Gutniak M, Orskov C, Holst JJ, Ahren B, Efendic S. Antidiabetogenic effect of glucagon-like peptide-1 (7–36)amide in normal subjects and patients with diabetes mellitus. *N Engl J Med* 1992;326:1316–22.
- Nathan DM, Schreiber E, Fogel H, Mojsov S, Habener JF. Insulinotropic action of glucagon like peptide-I-(7–37) in diabetic and nondiabetic subjects. *Diabetes Care* 1992;15:270–6.
- D'Alessio DA, Kahn SE, Leusner CR, Ensinnck JW. Glucagon-like peptide 1 enhances glucose tolerance both by stimulation of insulin release and by increasing insulin-independent glucose disposal. *J Clin Invest* 1994;93:2263–6.
- Todd JF, Edwards CMB, Ghatei MA, Mather HM, Bloom SR. Subcutaneous glucagon-like peptide-I improves postprandial glycemic control over a 3-week period in patients with early Type 2 diabetes. *Clin Sci* 1998;95:325–9.
- Jutti-Berggren L, Pijon J, Karpe F. The antidiabetic effect of GLP-1 is maintained during a 7-day treatment period and improves diabetic dyslipoproteinemia in NIDDM patients. *Diabetes Care* 1996;19:1201–6.
- Kreymann B, Williams G, Ghatei MA, Bloom SR. Glucagon-like peptide-1 7–36: a physiological incretin in man. *Lancet* 1987;2:1300–4.
- Edwards CMB, Todd JF, Mahmoudi M, et al. GLP-1 has a physiological role in the control of postprandial glucose in humans. *Diabetes* 1999;48:86–93.
- Brynes AE, Frost GS, Edwards CMB, Ghatei MA, Bloom SR. Plasma glucagon-like peptide-1 (7–36) amide (GLP-1) response to liquid phase, solid phase, and meals of differing lipid composition. *Nutrition* 1998;14:433–6.
- Thomsen C, Rasmussen O, Lousen T, et al. Differential effects of saturated and monounsaturated fatty acids on postprandial lipemia and incretin responses in healthy subjects. *Am J Clin Nutr* 1999; 69:1135–43.
- WHO Study Group. Prevention of diabetes mellitus. *World Health Organ Tech Rep Ser* 1994;844.
- Mensink RP, Katan MB. Effect of dietary *trans* fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *N Engl J Med* 1990;323:439–45.
- Coulston AM, Hollenbeck CB, Swislocki AL, Reaven GM. Persistence of hypertriglyceridemic effect of low-fat high-carbohydrate diets in NIDDM patients. *Diabetes Care* 1989;12:94–101.
- Coulston AM, Hollenbeck CB, Swislocki AL, Chen YD, Reaven GM. Deleterious metabolic effects of high-carbohydrate, sucrose-containing diets in patients with non-insulin-dependent diabetes mellitus. *Am J Med* 1987;82:213–20.
- Zavaroni I, Bonora E, Pagliara M, et al. Risk factors for coronary artery disease in healthy persons with hyperinsulinemia and normal glucose tolerance. *N Engl J Med* 1989;320:702–6.
- Gelding SV, Robinson S, Lowe S, Nithyananthan R, Johnston DG. Validation of the low dose short insulin tolerance test for evaluation of insulin sensitivity. *Clin Endocrinol (Oxf)* 1994;40:611–5.
- Shen SW, Reaven GM, Farquhar JW. Comparison of impedance to insulin-mediated glucose uptake in normal subjects and in subjects with latent diabetes. *J Clin Invest* 1970;49:2151–60.
- Gelding SV, Nithyananthan R, Chan SP, et al. Insulin sensitivity in non-diabetic relatives of patients with non-insulin-dependent diabetes from two ethnic groups. *Clin Endocrinol (Oxf)* 1994;40:55–62.
- Albano JD, Ekins RP, Maritz G, Turner RC. A sensitive, precise radioimmunoassay of serum insulin relying on charcoal separation of bound and free hormone moieties. *Acta Endocrinol (Copenh)* 1972;70:487–509.
- Alberti KG, Daly ME, Robinson A, Marshall SM, Mathers JC. The short insulin tolerance test is safe and reproducible. *Diabet Med* 1999;16:352–3.
- Jensen RG, Lammi KC, Henderson RA, Bush VJ, Ferris AM. Effect of dietary intake of n–6 and n–3 fatty acids on the fatty acid composition of human milk in North America. *J Pediatr* 1992;120:S87–92.
- Storlien LH, Pan DA, Kriketos AD, et al. Skeletal muscle membrane lipids and insulin resistance. *Lipids* 1996;31:S261–5.
- Hannah JS, Howard BV. Dietary fats, insulin resistance and diabetes. *J Cardiovasc Risk* 1994;1:31–7.

36. ADA. Nutrition recommendations and principles for people with diabetes mellitus. *Diabetes Care* 1994;17:519-22.
37. Aravanis C, Corcondilas A, Dontas AS, Lekos D, Keys A. Coronary heart disease in seven countries. IX. The Greek islands of Crete and Corfu. *Circulation* 1970;41:188-100.
38. Department of Health. Report of the Cardiovascular Review Group Committee on Medical Aspects of Food Policy. Nutritional aspects of cardiovascular disease. London: Her Majesty's Stationery Office, 1994. (RHSS 46.)
39. Lerman-Garber I, Ichazo-Cerro S, Zamora-Gonzalez J, Cardoso-Saldana G, Posadas-Romero C. Effect of a high-monounsaturated fat diet enriched with avocado in NIDDM patients. *Diabetes Care* 1994;17:311-5.
40. Campbell LV, Marmot PE, Dyer JA, Borkman M, Storlien LH. The high-monounsaturated fat diet as a practical alternative for NIDDM. *Diabetes Care* 1994;17:177-82.
41. Frost G, Wilding JP, Beecham J. Dietary advice based on the glycemic index improves dietary profile and metabolic control in type 2 diabetic patients. *Diabet Med* 1994;11:397-401.
42. Brynes AE, Edwards, Frost G. Postprandial lipaemia in response to sucrose intake in middle-aged men with CHD risk factors and matched controls. *Proc Nutr Soc* 1998;57:151 (abstr).
43. Ha TK, Lean MJ. Recommendations for the nutritional management of patients with diabetes mellitus. *Eur J Clin Nutr* 1998;52:467-81.

