

## Original Research

# A MUFA-Rich Diet Improves Postprandial Glucose, Lipid and GLP-1 Responses in Insulin-Resistant Subjects

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**Objective:** To study the effects of three weight-maintenance diets with different macronutrient composition on carbohydrate, lipid metabolism, insulin and incretin levels in insulin-resistant subjects.

**Methods:** A prospective study was performed in eleven (7 W, 4 M) offspring of obese and type 2 diabetes patients. Subjects had a BMI > 25 Kg/m<sup>2</sup>, waist circumference (men/women) > 102/88, HbA<sub>1c</sub> < 6.5% and were regarded as insulin-resistant after an OGTT (Matsuda ISI<sub>M</sub> < 4). They were randomly divided into three groups and underwent three dietary periods each of 28 days in a crossover design: a) diet high in saturated fat (SAT), b) diet rich in monounsaturated fat (MUFA; Mediterranean diet) and c) diet rich in carbohydrate (CHO).

**Results:** Body weight and resting energy expenditure did not change during the three dietary periods. Fasting serum glucose concentrations fell during MUFA-rich and CHO-rich diets compared with high-SAT diets (5.02 ± 0.1, 5.03 ± 0.1, 5.50 ± 0.2 mmol/L, respectively, Anova < 0.05). The MUFA-rich diet improved insulin sensitivity, as indicated by lower homeostasis model analysis-insulin resistance (HOMA-ir), compared with CHO-rich and high-SAT diets (2.32 ± 0.3, 2.52 ± 0.4, 2.72 ± 0.4, respectively, Anova < 0.01). After a MUFA-rich and high-SAT breakfasts (443 kcal) the postprandial integrated area under curve (AUC) of glucose and insulin were lowered compared with isocaloric CHO-rich breakfast (7.8 ± 1.3, 5.84 ± 1.2, 11.9 ± 2.7 mmol · 180 min/L, Anova < 0.05; and 1004 ± 147, 1253 ± 140, 2667 ± 329 pmol · 180 min/L, Anova < 0.01, respectively); while the integrated glucagon-like peptide-1 response increased with MUFA and SAT breakfasts compared with isocaloric CHO-rich meals (4.22 ± 0.7, 4.34 ± 1.1, 1.85 ± 1.1, respectively, Anova < 0.05). Fasting and postprandial HDL cholesterol concentrations rose with MUFA-rich diets, and the AUCs of triacylglycerol fell with the CHO-rich diet. Similarly fasting proinsulin (PI) concentration fell, while stimulated ratio PI/I was not changed by MUFA-rich diet.

**Conclusions:** Weight maintenance with a MUFA-rich diet improves HOMA-ir and fasting proinsulin levels in insulin-resistant subjects. Ingestion of a virgin olive oil-based breakfast decreased postprandial glucose and insulin concentrations, and increased HDL-C and GLP-1 concentrations as compared with CHO-rich diet.

## INTRODUCTION

Increases in body fat are contributing to the increased prevalence of type 2 diabetes (T2D) and cardiovascular disease

[1,2]. Some subjects accumulate a constellation of major risk factors associated with central (intra-abdominal) obesity, such as hypertriglyceridemia, low HDL cholesterol, raised blood pressure and insulin resistance [3]. Overweight subjects with

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multiple risk factors ought to be given recommendations, including weight reduction and lifestyle changes. Lifestyle modification programs encourage patients to eat conventional foods that reduce saturated fat, sugar and energy intake by 500 to 1000 kcal/d [4,5]. However, most overweight patients and subjects at risk to T2D fail to maintain long-term weight loss [6], and the question of whether diets should be enriched in monounsaturated fats or carbohydrates during the weight-maintenance period is still a matter of discussion. The increased risk of cardiovascular disease in overweight insulin-resistant subjects identifies this group as one for whom dietary advice could be particularly important. Fasting and postprandial HDL cholesterol concentrations are usually low in insulin-resistant type 2 diabetic patients. Postprandial lipemia in these patients also seems to be a major determinant of plasma HDL cholesterol concentrations. It has been suggested that when saturated fat energy is replaced by MUFAs, the final diet has a higher total fat content (i.e. >35% of energy) but this does not result in raised triacylglycerols or lower concentrations of HDL cholesterol. Furthermore, a MUFA diet could prevent the deleterious effects of increasing plasma concentrations of glucose observed with-CHO enriched diets. The macronutrient composition of a diet may also directly modulate the integrated postprandial responses of insulin and glucagon-like peptide 1 (GLP-1) secretion. The biological activities of GLP-1 include stimulation of glucose-dependent insulin secretion and insulin biosynthesis, inhibition of glucagon secretion and inhibition of food intake. The findings that GLP-1 lowers blood glucose and that GLP-1 may restore  $\beta$ -cell sensitivity suggests that activation of GLP-1 signaling may become a useful strategy to treat diabetic patients. Macronutrient composition may also affect  $\beta$ -cell function though its effects on the conversion of proinsulin to insulin. *In vivo*, the ratio of proinsulin to insulin (PI/I) in plasma provides an accurate estimate of secretory granules, and an elevated PI/I ratio has been observed in conditions with impaired  $\beta$ -cell function, such as type 2 diabetes or impaired glucose tolerance. The objective of this study was to determine the metabolic effect of three weight maintenance-specific diets, a diet rich in saturated fat (SAT), a diet enriched in monounsaturated (MUFA: Mediterranean diet) fat and a carbohydrate-rich diet (CHO) on body weight, fasting and postprandial glucose and lipid metabolism and acute postprandial responses of insulin, GLP-1 and PI/I in a selected population of insulin-resistant patients without previous antidiabetic treatment.

## MATERIALS AND METHODS

### Subjects

Participants were recruited from the diabetic patients records at primary care centers in Cordoba. Fifty-nine potential subjects were recruited in 2003 and 2004, and invited to attend a screening session at which body weight, height, waist circumference, blood pressure, and blood biochemistry indices

were measured in order to determine their eligibility for the study. Twenty-seven patients either did not meet the inclusion criteria ( $n = 18$ ) or refused ( $n = 9$ ) to participate in the study. Qualifying subjects ( $n = 22$ ) underwent an oral glucose-tolerance test (with a 75-g oral glucose load). A catheter was inserted in an antecubital vein to collect blood samples obtained at 0', 15', 30', 45', 60', 90' and 120 min. Samples were analyzed for glucose and immunoreactive plasma insulin concentrations. Finally, eleven insulin-resistant subjects (four men and seven women) assessed as having an insulin sensitivity index ISI (composite) of less than 4.0 (indicating insulin resistance; lower values indicate greater insulin resistance) were included in this study [7].

**Clinical Inclusion Criteria.** All subjects should be  $35 < 75$  years old and should have a history of fasting glycemia  $< 125$  mg/dL with glycosylated hemoglobin ( $HbA_{1c}$ )  $< 6.5\%$  without any drug or previous insulin treatment. A body-mass index (BMI), calculated as weight (in kilograms) divided by height (in square meters)  $> 25$  Kg/m<sup>2</sup> and a waist circumference of  $\geq 102$  cm  $\geq 88$  cm (men/women) [8,9]. These indices had not changed for at least six months prior to the study and remained constant throughout it. Menopause was confirmed in women by follicle-stimulating hormone concentrations  $> 40$  IU/L and the absence of menses for at least 1 year.

**Clinical Exclusion Criteria.** Signs of diabetic retinopathy, nephropathy, neuropathy, or macrovascular complications; cigarette smoking and alcohol consumption; use of diuretics, steroids and  $\beta$ -blockers or use of medications that might affect glucose metabolism. All subjects had normal results on screening blood tests of hepatic, renal, thyroid and adrenal function. Hypertension in three patients was treated with Enalapril 10 mg/d (two) or 20 mg/d (one).

The purpose and risks of the study were carefully explained to all subjects before they gave their informed consent to participate. The study protocol was reviewed and approved by the RSUH Ethics Committee. Four days before completion of his third period (on CHO rich diet), one patient presented an acute coronary syndrome which required his admission to an intensive coronary care unit; the patient did not complete this period.

### Experimental Diets

Subjects were fed three different diets; a low-fat high-carbohydrates diet that consisted of 65% CHO and 20% fat: (6% SAT, 8% MUFA, 6% PUFA), a Mediterranean diet: 47% CHO and 38% fat (9% SAT, 23% MUFA, 75% of which was provided in the form of extra virgin olive oil and 6% PUFA), and a diet rich in SAT: (47% CHO, 15% PROT and 38% fat, of which 23% SAT, 9% MUFA, 6% PUFA) (Table 2). Our diet design was guided by the objective of replacing saturated fats with carbohydrates in the low-fat diet, whereas in the high-fat diet, monounsaturated fats replaced saturated fats. Total energy was determined by using the Atwater factors of 16.74 kJ (4

kcal/g for protein, 16.74 kJ (4 kcal)/g for carbohydrate, and 37.66 kJ (9 kcal)/g for fat. A randomized Williams Latin Square crossover study design was employed. Subjects were randomly allocated to three groups and underwent three dietary periods of 28 days each in the crossover design. Dietary cholesterol was kept constant (3 mmol/4800 kJ) during the three periods. The mean fiber content of the SAT-rich and MUFA-rich diets were 27 and 29 g/d respectively, and the mean fiber content of the CHO-rich diet was 32 g/d. Dietary composition during the intervention period was calculated using the program DIETSOURCE v. 1.2 (Novartis, Barcelona, Spain) based on United States Department of Agriculture food tables and Spanish food composition tables for local foodstuffs. Adherence to the dietary protocols was determined by measuring the fatty acids at the end of each dietary period by gas chromatography.

### Experimental Design for Weight-Maintenance Diets

Before the start of the study, energy expenditure was measured in the fasting state by indirect calorimetry. Each subject also completed a 3-d food-intake diary to estimate his usual energy intake. Daily energy needs were defined between the subject's resting energy expenditure and his calculated energy requirements on the basis of individual activity. In order to allow *ad libitum* consumption of the diets, 125% of the subjects' estimated energy needs were provided during the experimental protocol. Twenty menus were prepared based on common foodstuffs and administered on a rotational basis. Subjects were instructed to eat until they felt satiated. Experimental subjects stayed at home and prepared their own food as indicated. A dietician provided subjects with food scales accurate to 1 g and diet record booklets and explained in detail the weighed intake method. During the protocol experimental subjects attended the research clinic twice a week to discuss their energy and macronutrient intakes with the programme dietician. Subjects were encouraged to maintain a regular schedule of meals and were instructed to maintain their usual exercise regime during the study. Body weight was measured twice weekly and was not permitted to vary by more than 1 kg. Total dietary intake was estimated weekly and a 3-d weighed food records were collected on six occasions during the study (two per period) [10]. None of the experimental subjects was known to consume alcohol and any occasional consumption was carefully recorded.

### Breakfast Meals Used for Postprandial Studies

A standardised breakfast was consumed on the last day of each diet period as a test breakfast. At 8 A.M. the subjects met at the laboratory after an overnight fast. An intravenous catheter was inserted in a brachial vein and a basal blood sample was obtained, followed by administration of the test meal. The meal contained 443 Kcal and its composition was as follows; CHO-rich breakfast: 200 mL skim milk, 50 g bread and 75 g marmalade; MUFA-rich breakfast: 200 mL skim milk, 50 g

bread and 27 cc olive oil; SAT-rich breakfast: 200 mL whole milk, 50 g bread and 25 g butter. Venous blood samples were taken before and after the meal at the following points in time: - 10', -5', 0', 15', 30', 60', 90', 120', 150', 180'. Plasma concentrations of glucose, triacylglycerol, HDL cholesterol, insulin, and GLP-1 were determined.

### Biochemical Procedures

Serum was immediately separated by centrifugation at 2500g for 15 minutes at 4°C, and aliquots were frozen and stored at -70°C. Plasma glucose was measured by the glucose oxidase method. Plasma insulin concentration was measured by microparticle enzyme immunoassay (MEIA; Abbott Diagnostics, Matsudo-shi, Japan; CV 2.5-6%). Total hemoglobin A1c (HBA1c) was determined in fresh samples by HPLC (Bio-Rad, Paris, France). The serum total cholesterol (TC) and triacylglycerols (TG) concentrations were determined by enzymatic techniques (CHOD-PAP and GPO-PAP, respectively; Boehringer, Mannheim, Germany). Commercially available quality controls (Precinorm and Precilip; Boehringer) were included in all the runs. High density lipoprotein cholesterol (HDL-C) was determined by a dextran sulfate-magnesium precipitation procedure. Low density lipoprotein cholesterol (LDL-C) concentrations were calculated from TC, TG and HDL-C concentrations by means of the Friedewald formula. Apo A-I and Apo B concentrations were determined by the immunoturbidimetric method (Boehringer). Plasma concentrations of GLP-1 were measured against standards of synthetic GLP-1 7-36 amide (proglucagon 78-106 amide) using antiserum code no. 89390, which can be used at a final dilution of 1:250 000 and gives the assay a detection limit of 1 pmol/L, with an intraassay CV < 5% at 20 pmol/L. This antiserum is highly specific for the COOH-terminus of proglucagon 78-107 amide and reacts neither with glycine-extended GLP-1 (proglucagon 78-108) nor with proglucagon 78-106. Thus, it mainly reacts with GLP-1 of intestinal origin. Before the analysis, plasma was extracted with ethanol (70% v:v) [11]. Serum intact proinsulin was determined at 30' and 60' using highly specific amplified Enzyme-Linked Immunoassay of Human Proinsulin (enzyme immunoassay; IBL, Hamburg, Germany; detection Limit: 0.1 pmol/L). Normal range in healthy subjects; fasting 1.3-2.5 pmol/L, 30' after SOG 6.4 pmol/L and 120' after SOG 14.8 pmol/L. The intrassay coefficient of variation was between 4.3-7.4%. The proinsulin assay has 0% cross-reactivity with human insulin. The insulin assay has 0% cross-reactivity with proinsulin. The proinsulin to insulin (PI/I) ratio was calculated as the mean proinsulin concentration divided by the mean insulin concentration at 30 and 60 min after each of diet period breakfast. ISI (composite) = 10.000/square root of [fasting glucose × fasting insulin × mean glucose during the OGTT × mean insulin during the OGTT] [7]. Insulin resistance was evaluated by the homeostasis model assessment method

(HOMA-IR) using fasting glucose (FG) and fasting insulin (FI) concentrations [12]. After 12 h of overnight fasting, two samples were obtained, 5 min apart, for glucose and insulin concentrations. Their mean values were calculated and used to estimate insulin resistance in the formula HOMA-IR ( $\text{mmol/L} \times \mu\text{U/ml}$ ) =  $\text{FG (mmol/L)} \times \text{FI} (\mu\text{U/ml})/22.5$ . The computer-based HOMA2 model calculator (Diabetes Trial Unit, Oxford, UK; available at [www.dtu.ox.ac.uk/homa](http://www.dtu.ox.ac.uk/homa)) was used to generate the index of insulin resistance and the index of  $\beta$ -cell function, HOMA-%B [13]. An ideal, normal-weight person less than 35 yr of age has a HOMA-IR of 1 and HOMA-%B of 100%.

### Plasma Fatty Acid Measurements

Fatty acids were extracted from plasma phospholipids and were measured using a fluorometric method [14]. After a 12-h fast the blood collected in test tubes containing EDTA was centrifuged and the plasma was dispensed into 0.5-mL aliquots and frozen at  $-70^\circ\text{C}$  until analysis for fatty acid content by a single assay. Total lipids from plasma were extracted into 0.5 mL methanol followed by 1.0 mL chloroform and the pooled solvent extracts were dried under a nitrogen atmosphere. Plasma phospholipids were separated by one-dimensional thin-layer chromatography in a 60-m fused-silica column (0.25 mm) SP-2380 (Supelco, Bellefonte, PA) and measured on a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with automatic injector (Agilent 6890 II), a flame ionization detector, and CHEMSTATION and MUSTANG software (Hewlett-Packard) for treatment of data. Hydrogen was used as carrier gas. The relative amount of each fatty acid (% of total fatty acids) was quantified by integrating the area under the peak and dividing the result by the total area for all fatty acids. The intrassay precision varied from 2% to 5%, depending on the peak size.

### Anthropometry

Height and weight were measured twice and the mean of the paired values was computed. Height was measured to 0.1 cm on a stadiometer and weight was measured to 0.1 kg on a balance scale.

### Indirect Calorimetry

Energy production (EP) and the respiratory quotient (Rq) were measured by continuous indirect calorimetry using a computerized, flow-through canopy gas analyzer system (Deltatrac; Datex, Helsinki, Finland). The system was calibrated against standard gas mixtures and calibration was verified at intervals throughout the collection periods. This system has a precision of better than 1% for oxygen consumption ( $\text{VO}_2$ ) or carbon dioxide production ( $\text{VCO}_2$ ). Energy expenditure was measured after an equilibration period of 10 min. The gas-exchange rate was recorded for 30 min in the fasting state [15].

### Statistical Analysis

The results are presented as the mean and standard error from individually analyzed results from all 11 participants. Changes in anthropometry, calorimetry and biochemical determinations were analyzed using repeated-measures design, with one repeated-measures factor: diet (High-SAT, high-MUFA, High-CHO) was used. Individual P values are reported when statistically significant using the post hoc Tukey's test. [16]. In designing the study, preliminary data were used to obtain power calculations (80% power and an  $\alpha$  of 5%) for detecting a difference of 15% in fasting serum glucose and HOMA-ir. The data presented were all tested for normality of distribution. The Friedman test was used for variables that did not log transform. Plasma glucose, triacylglycerol, HDL-c, GLP-1 and Insulin were analyzed by calculating the incremental area under the curve (AUC) with a formula based on the trapezoid rule with adjustment for baseline concentrations.  $P < 0.05$  was taken as significant.

## RESULTS

### Clinical Characteristics of the Subjects

The mean ( $\pm$ SD) age of patients was  $62 \pm 9$  years (range 41 to 70) (Table 1). At baseline, the mean BMI was  $32.6 \pm 7.8$ , waist circumference 106.2 cm (men) and 112.8 cm (women). None of these parameters changed significantly in the course of

**Table 1.** Characteristics of Insulin-Resistant Subjects

	At baseline	
Gender	7 Female	4 Male
Age in years	$62.3 \pm 9.4$	
BMI ( $\text{kg/m}^2$ )	$32.6 \pm 7.8$	
	M	F
Waist circumference (cm)	106.2	112.8
Glycosilated hemoglobin %	$6.0 \pm 0.5$	
Serum glucose (mmol/L)		
Fasting <sup>1</sup>	$5.47 \pm 0.5$	
2 h after oral glucose load	$8.75 \pm 1.6$	
Serum insulin (pmol/L)		
Fasting <sup>1</sup>	$87.5 \pm 26.6$	
2 h after oral glucose load	$959 \pm 486$	
Matsuda index (ISIm) <sup>2</sup>	$2.9 \pm 0.9$	
Treatment of hypertension	3 Yes	8 No
Blood pressure (mm Hg)		
Systolic	$136 \pm 8.6$	
Diastolic	$87 \pm 5.8$	
Mean <sup>3</sup>	$103 \pm 3.7$	

Data are Mean  $\pm$  SD.

<sup>1</sup> Mean of basal determinations obtained on two separate occasions. To convert glucose to mg, divide mmol by 0.0555; to convert insulin to IU/ml divide by 6.945.

<sup>2</sup>  $\text{ISIm} = 10^4 / (\text{G0} \times \text{I0}) \times (\text{MPG} \times \text{MSI})$ . Values ranged from 1.49 to 3.96, with higher values indicating greater activity.

<sup>3</sup> Mean Blood Pressure =  $(\text{Systolic} - \text{Diastolic})/3 + \text{Diastolic}$ .

**Table 2.** Energy Expenditure, Dietary Intake and Body Weight

	SAT		MUFA		CHO		<i>p</i>
Weight, kg	83.2 ± 5.7		83.6 ± 5.8		81.8 ± 6.0		0.36
REE‡ (kJ · kg <sup>-1</sup> · d <sup>-1</sup> )	96.33 ± 5.6		91.5 ± 6.00		89.14 ± 3.9		0.18
Dietary offered	12477 ± 994		12470 ± 978		12482 ± 982		0.9
Dietary intakes (KJ/d)	9565 ± 769		9586 ± 743		9526 ± 716		0.7
Total fat (% , g · kg <sup>-1</sup> · d <sup>-1</sup> )	38%	1.64 g	38%	1.64 g	20%	0.86 g	0.00
Saturated fat (% , g/d)	23%	82 g	9%	32.4 g	6%	21.3 g	0.00
Monounsaturated fat (% , g/d)	9%	32.2 g	23%	81.5 g	8%	28.5 g	0.00
Polyunsaturated fat (% , g/d)	6%	21.4 g	6%	21 g	6%	21.0 g	0.00
Carbohydrate (g · kg <sup>-1</sup> · d <sup>-1</sup> )	47%		47%		65%		0.00
Protein (g · kg <sup>-1</sup> · d <sup>-1</sup> )	15%		15%		15%		0.7
Alcohol (g/wk)	< 10		< 10		< 10		0.4

‡ REE = Resting energy expenditure. To convert values for KJ to Kcal divide by 4.18.

Diets were offered at 125% of the estimated energy need. Dietary intakes were calculated. Data are Mean ± SE. *P* value, analysis of variance of repeated variables.

the study. Mean fasting serum glucose concentrations were 5.47 ± 0.5 mmol/L, fasting plasma insulin 87.5 ± 26.6 μU/ml and HbA1c concentration was 6.0 ± 0.5%. After 2 h of OGTT, serum glucose was 8.75 ± 1.6 mmol/L and plasma insulin concentration was 959 ± 486 pmol/L. The mean Matsuda index (ISIm) was 2.9 ± 0.9. Mean arterial blood pressure was 103 ± 3.7 mm Hg.

body weight remained unchanged during the three periods (Anova, *p* = 0.36). Diets were offered at 125% of the estimated energy need. The mean dietary intakes as estimated from the two food frequency questionnaires were not significantly different amongst the three dietary periods (*p* = 0.7). No significant events were recorded in the subjects' diaries.

**Energy Expenditure, Dietary Intake and Body Weight**

The resting energy expenditure (REE) at evaluation was 93.01 ± 5.02 kJ · kg<sup>-1</sup> · d<sup>-1</sup> and this did not change during the three dietary periods (Anova, *p* = 0.18) (Table 2). The mean

**Fatty Acid Composition**

Fatty acid composition in fasting plasma phospholipids (PLs) during the three dietary interventions are shown in Table 3. The dietary fatty acid profile reflects each dietary period. Thus, the proportions of myristic acid (14:0), palmitic acid

**Table 3.** Serum Fatty Acid Composition after the Three Diet Periods

Fatty acids		SAT	MUFA	CHO	<i>P</i>
		% of total fatty acids			
Saturated fatty acids	Myristic acid, (14:0)	0,59 ± 0,07	0,42 ± 0,05 <sup>a</sup>	0,50 ± 0,05	<0,05
	Palmitic acid, (16:0)	23,30 ± 0,47	21,40 ± 0,46 <sup>b,*</sup>	23,46 ± 0,51	<0,01
	Margaric acid, (17:0)	0,30 ± 0,01	0,30 ± 0,02	0,31 ± 0,03	0,9
	Stearic acid, (18:0)	10,00 ± 0,27	9,23 ± 0,28 <sup>b</sup>	10,07 ± 0,37	<0,05
	Arachidic acid, (20:0)	0,36 ± 0,04	0,39 ± 0,03	0,34 ± 0,02	0,7
	Behenic acid, (22:0)	0,60 ± 0,04	0,53 ± 0,04	0,55 ± 0,04	0,06
	Lignoceric acid, (24:0)	1,33 ± 0,07	1,22 ± 0,08	1,27 ± 0,14	0,1
	Total saturated fatty acids	36,5 ± 0,44	33,51 ± 0,45 <sup>b,*</sup>	35,7 ± 0,35	<0,01
MUFA	Palmitoleic acid, (16:1, n-7)	1,0 ± 0,11	1,0 ± 0,09	1,13 ± 0,11	0,2
	Oleic acid, (18:1, n-9)	20,37 ± 1,22	24,91 ± 0,67 <sup>b,**</sup>	21,83 ± 0,59	<0,01
	Vaccenic acid, (18:1, n-7)	1,73 ± 0,06	1,76 ± 0,04	1,67 ± 0,11	0,5
PUFA	Erucic acid, (22:1)	6,17 ± 0,44	5,53 ± 0,39	5,73 ± 0,43	0,2
	Linoleic acid, (18:2, ω-6)	23,41 ± 1,22	22,58 ± 0,62	22,04 ± 0,81	0,6
	Alpha-Linoleic acid, (18:3, ω-3)	0,23 ± 0,02	0,24 ± 0,02	0,28 ± 0,03	0,3
	Stearidonic acid, (18:4, ω-3)	0,06 ± 0,02	0,11 ± 0,02	0,11 ± 0,02	0,3
	Arachidonic acid, (20:4, ω-6)	0,28 ± 0,02	0,28 ± 0,02	0,28 ± 0,02	0,6
	Eicosapentaenoic acid, (20:5, ω-3)	0,64 ± 0,17	0,48 ± 0,07	0,56 ± 0,13	0,7
	Docosapentaenoic acid, (22:5)	0,43 ± 0,09	0,29 ± 0,02 <sup>a</sup>	0,35 ± 0,02	<0,05
Docosahexaenoic acid, (22:6)	2,23 ± 0,15	2,08 ± 0,15	2,19 ± 0,13	0,9	

MUFA = Monounsaturated fatty acids PUFA = Polyunsaturated fatty acids.

Data are Mean ± SEM. *P* value, analysis of variance of repeated variables.

● \* < 0.05; \*\* < 0.01, compare MUFA vs CHO.

● <sup>a</sup> < 0.05; <sup>b</sup> < 0.01, compare MUFA and CHO vs SAT.

**Table 4.** Biochemical Determinations after the Three Dietary Periods

	SAFA	MUFA	CHO	P
HBA <sub>1c</sub> , %	5,74 ± 0,137	5,39 ± 0,10 <sup>b</sup>	5,40 ± 0,10 <sup>b</sup>	<0.01
Glucose				
Fasting, mmol/L	5,50 ± 0,19	5,02 ± 0,14 <sup>a</sup>	5,03 ± 0,13 <sup>a</sup>	<0.05
AUC, mmol · 180 min/L	5,84 ± 1,2	7,82 ± 1,3	11,9 ± 2,7*, <sup>b</sup>	<0.05
Plasma insulin,				
Fasting, pmol/L	64,1 ± 9,6	60,72 ± 12,42	75,21 ± 12,33	0.3
AUC, pmol · 180 min/L	1253 ± 140	1004 ± 147	2667 ± 329**, <sup>a</sup>	<0.01
HOMA-ir, mmol/L × μU/mL	2,72 ± 0,37	2,32 ± 0,35*, <sup>a</sup>	2,52 ± 0,37	<0.01
HOMA-i%β	103,1 ± 12,2	109,1 ± 12,5	126,6 ± 18,4	0.2
GLP-1				
Fasting, pmol/L	6,47 ± 0,59	6,34 ± 0,51	5,67 ± 0,49	0.4
AUC, pmol · 180 min/L	4,34 ± 1,10	4,22 ± 0,66	1,85 ± 1,14*, <sup>a</sup>	<0.05
Proinsulin				
Fasting, pmol/L	11,0 ± 2,6	8,72 ± 2,3 <sup>a</sup>	9,52 ± 1,39	<0.05
60 min PII ratio (%)	1,64 ± 0,57	2,59 ± 1,21	1,44 ± 0,43	0.17
Total cholesterol, mmol/L	6,24 ± 0,31	5,96 ± 0,31	5,58 ± 0,30	0.2
Triacylglycerols, mmol/L	1,36 ± 0,15	1,31 ± 0,12	1,39 ± 0,17	0.4
HDL-cholesterol, mmol/L	1,22 ± 0,12	1,22 ± 0,14	1,09 ± 0,11*, <sup>a</sup>	<0.05
LDL-cholesterol, mmol/L	4,24 ± 0,25	3,99 ± 0,22	3,85 ± 0,28	0.6
Apo A-1, mg/dL	151 ± 9	156 ± 9,41	136 ± 9,87*, <sup>a</sup>	<0.05
Apo B <sub>100</sub> , mg/dL	104 ± 6	100 ± 6	92 ± 8 <sup>a</sup>	<0.05

HDL is high density lipoprotein and LDL is low density lipoprotein. LDL cholesterol concentration was calculated from total cholesterol, triacylglycerols and HDL-C values with the Friedewald formula. To convert values for glucose, cholesterol and triacylglycerols to mg/dl, divide mmol by 0.0555, 0.02586 and 0.01129, respectively. To convert values for insulin to IU/ml, divide by 6.945.

Data are Mean ± SEM. P value, analysis of variance of repeated variables.

• \* < 0.05; \*\* < 0.01, compare MUFA vs CHO.

• <sup>a</sup> < 0.05; <sup>b</sup> < 0.01, compare MUFA and CHO vs SAT.

(16:0), stearic acid (18:0) and total saturated acids were significantly increased after the SAT diets compared to the MUFA and CHO dietary periods (Anova, p < 0.05, p < 0.01, p < 0.05 and p < 0.01, respectively). The proportion of oleic acid (18:1, n-9) was significantly increased after eating high MUFA diets than CHO-rich and SAT rich diets (Anova, p < 0.01).

**Biochemical Results**

Fasting glucose was decreased during the MUFA and CHO periods compared with the SAT period (5.02 ± 0.1, 5.03 ± 0.1, 5.50 ± 0.2 mmol/L, Anova, p < 0.05) (Table 4). Fasting insulin concentrations were unchanged during the three diet periods (Anova, p > 0, 5). The mean HOMA-ir decreased during the MUFA dietary period as compared with the SAT and CHO diets (2.32 ± 0.35, 2.72 ± 0.37 and 2.52 ± 0.37 mmol/L × μU/ml, respectively; Anova p < 0.01). Fasting total cholesterol, triacylglycerols and LDL-cholesterol concentrations did not change during the three dietary periods. HDL-cholesterol and Apo A1 and Apo B concentrations fell during the CHO diet (Anova, p < 0.05, in all cases). The postprandial integrated AUCs of glucose and insulin were significantly higher in response to the standard CHO breakfast than to the MUFA and SAT breakfasts (11.9 ± 2.7, 7.8 ± 1.3, 5.84 ± 1.2 mmol · 180 min/L Anova, p < 0.05; and 2667 ± 329, 1004 ± 147, 1253 ± 140 pmol · 180 min/L, p < 0.01, respectively;

Figs 1 and 2). The postprandial AUC of total triacylglycerols fell during the CHO diet compared with both of the fat diets (Anova, p < 0.05), while postprandial HDL cholesterol concentrations were increased at 15, 60, 120 and 180 min following the MUFA and SAT diets (Fig. 1). Fasting plasma GLP-1 concentrations were similar during the three diet periods but the postprandial AUC of plasma GLP-1 in response to the CHO-rich meal was significantly lower than following the MUFA and SAT breakfasts (1.85 ± 1.1, 4.22 ± 0.7, 4.34 ± 1.1, Anova, p < 0.05, Fig. 2). Fasting serum proinsulin concentrations decreased after the MUFA diet as compared with the CHO and SAT diet periods (Anova < 0.05). The circulating proinsulin to insulin (PII) plasma ratio at 30 and 60 min after the three breakfasts remained unchanged (Fig. 2).

**DISCUSSION**

The main purpose of our study was to investigate the effects of three isocaloric diet models, a high-carbohydrate (CHO) diet, one that was enriched in monounsaturated fat (MUFA) and a third high in saturated fat (SAT), on postprandial glucose and lipid metabolism, insulin, proinsulin and incretin responses in free-living insulin-resistant, non diabetic subjects who were first-degree relatives of type 2 diabetic patients.

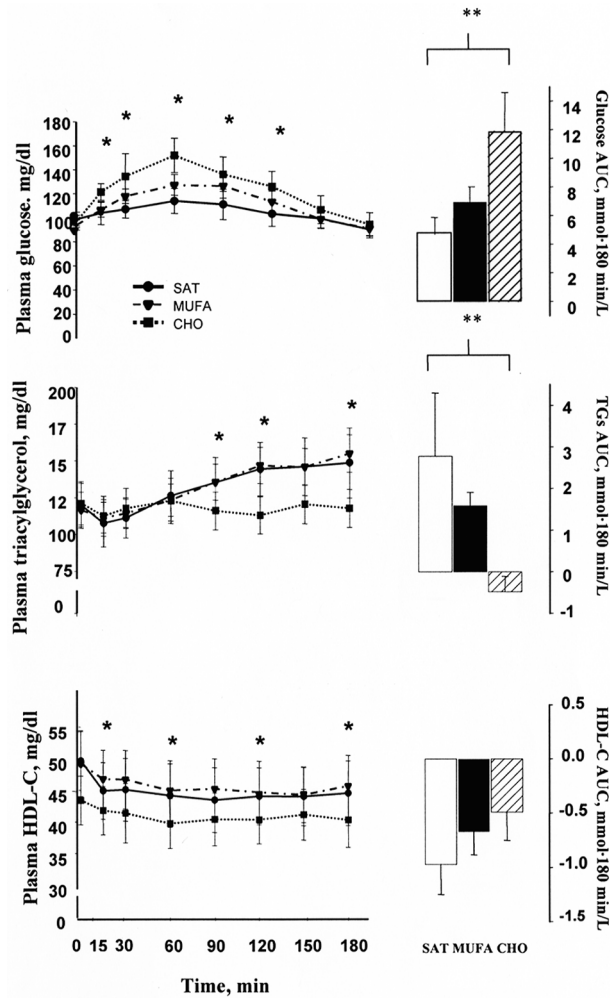


Fig. 1. Mean (±SE) postprandial responses of glucose, triacylglycerol (TG) and HDL cholesterol concentrations in 11 insulin-resistant subjects to three isocaloric (443 Kcal) standard breakfasts: a breakfast rich in carbohydrates (■ - CHO), a Mediterranean breakfast enriched in virgin olive oil (▼ - MUFA) and a standard breakfast high in saturated fat (● - SAT). The incremental area under the curve (AUC) was calculated by the formula based on the trapezoid rule with adjustment for baseline concentrations. Repeated measures ANOVA and Tukey's test. \*p < 0.05; \*\* p < 0.01.

Most earlier studies that have evaluated the role of various diet models on glycemic control have presented inconsistencies due to the fact that the dietary interventions involved were associated with changes in body weight and were not well controlled for caloric intake. In order to avoid the confounding effects of weight loss, this study was designed to ensure that changes in nutritional composition were isocaloric, and involved close supervision to maintain stable body weight. Dietary fats and MUFA-rich diets have been criticized because of their potential to promote weight gain when consumed *ad libitum* [17,18]. In addition, short-term studies in healthy and diabetic patients have also suggested that an *ad libitum* low-fat

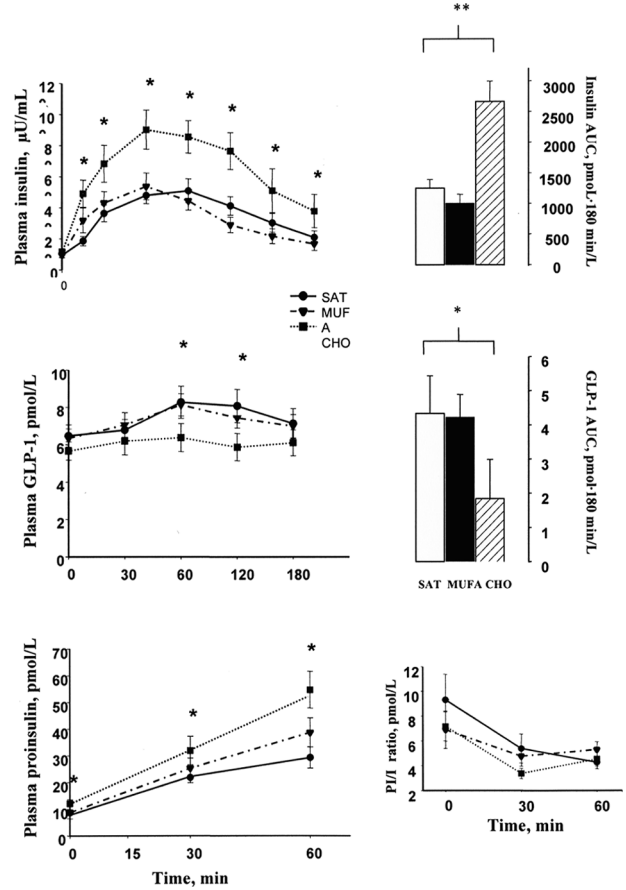


Fig. 2. Mean (±SE) postprandial responses of insulin and glucagon-like peptide 1 (GLP-1) in 11 insulin-resistant subjects to three isocaloric (443 Kcal) standard breakfasts: a breakfast rich in carbohydrates (■ - CHO), a Mediterranean breakfast enriched in virgin olive oil (▼ - MUFA) and a standard breakfast high in saturated fat (● - SAT). The incremental area under the curve (AUC) was calculated by the formula based on the trapezoid rule with adjustment for baseline concentrations. Repeated measures ANOVA and Tukey's test. \*p < 0.05; \*\*p < 0.01.

diet induces weight-loss more efficiently than high-fat diets [19,20]. However, the question of whether dietary fat is a major determinant of body fat is still controversial [21].

The present study in free-living subjects elicited excellent dietary compliance from its experimental subjects. Their fasting resting energy expenditure and body weight remained unchanged through the three dietary periods, indicating that changes in macronutrient composition *per se* are not the major determinant of fat deposition. Increasing the percentage of fat in the diet to approximately 40% of total energy did not increase body weight or adiposity when energy balance was maintained. Fatty acid composition in fasting plasma PLs reflects the composition of the diet during the weeks to months before the collection of the sample, and is a good marker of the type of fats that have been consumed by an individual [22]. In our study, compliance was biochemically confirmed by SAT

and MUFA concentrations in plasma CE being proportional to dietary intake.

Our results showed that isocaloric replacement of saturated fat by supplements of virgin olive oil, (the Mediterranean Diet), significantly improved fasting glycemic concentrations in insulin-resistant prediabetic patients. The mechanisms of the improved glycemic control associated with a high-MUFA diet remain undefined. There is some evidence that a proportion of total dietary fat in excess of 40% worsens insulin sensitivity, particularly when the diet includes high saturated fat [23–25]. In fact, the beneficial effects of monounsaturated fat compared to saturated fat seems to be lost in subjects who obtain more than 37% of their energy from fat [26]. In our study, insulin-resistant patients who were fed 38% as MUFA improved fasting glucose and HOMA-ir compared to patients who consumed a SAT-rich diet. The beneficial effects of an olive oil-enriched diet were also observed during the postprandial state, as indicated by lower glucose and insulin AUCs, as compared with the CHO-rich diet. As a matter of interest, HOMA-ir was not improved in patients on CHO-rich diets, suggesting that increased blood glucose concentrations induced by CHO-enriched diets stimulate fasting and postprandial insulin release. These findings thus underline the fact that postprandial glucose concentrations are primarily determined by the amount of carbohydrates ingested [27].

Patients with type 2 diabetes have lower postprandial GLP-1 responses than healthy subjects [28]. GLP-1 is secreted from gut endocrine cells in response to nutrient ingestion and plays a number of different roles in metabolic homeostasis following their absorption. The carbohydrate and fatty acid components of a meal may directly influence the GLP-1 postprandial responses. Monounsaturated fatty acids appear to be powerful stimulators of GLP-1 secretion both in enterocytes cultured from rats and *in vivo* in Zucker (genetically obese) rats [29,30]. A differential effect of saturated and monounsaturated fats on postprandial GLP-1 responses in healthy subjects and patients with type 2 diabetes has recently been reported [31,32]. Our study was performed in insulin-resistant prediabetic subjects. We found that following the consumption of a standard breakfast, the GLP-1 response was significantly enhanced in those patients that had taken an olive oil-enriched meal and with high SAT meal compared to those fed a CHO-rich meal, further supporting the idea that saturated and unsaturated fatty acids may act as secretagogues of GLP-1. The possible effects of the previous 28 days of different diets on the acute responses of intestinal cells were not examined in this study. The biological activities of GLP-1 include stimulation of glucose-dependent insulin secretion, lowering of blood glucose and lipids, inhibition of gastric emptying and food intake, the stimulation of  $\beta$ -cell proliferation and inhibition of their apoptosis [33]. Furthermore, the postprandial metabolic effects of unsaturated fat may reinforce the direct and indirect action of GLP-1 on  $\beta$ -cell function. It is well established that hyperglycemia has a negative effect on normal  $\beta$ -cell function [34,35]. Thus, the

favorable effects of MUFA-rich meals may result from preferential decreases in serum glucose elevations compared to CHO meals. On the other hand, has been observed the failure of insulin secretion to compensate the insulin resistance in overweight and obese subjects who fed SAT diets [36]. Similarly, high long-term serum fatty acid levels have been associated with reduced  $\beta$ -cell function, but oleic fatty acids appear to have specific protective effects [37,38]. We investigated the influence of the three model diets on  $\beta$ -cell function, measuring fasting and postprandial proinsulin processing. Our results suggest that in insulin-resistant subjects, after four weeks of specific modification, fasting PI concentrations are associated with their final state of sensitivity to insulin (MUFA < CHO < SAT). It has been suggested that fasting intact proinsulin is a highly specific predictor of insulin resistance [39]. Postprandial circulating PI concentrations also increased progressively in the following order of macronutrient enriched meals: CHO > MUFA  $\approx$  SAT. However, we found that the stimulated PI/I ratio was unchanged with the three model meals, suggesting that acute stimulation with different macronutrient content diets had no effects on proinsulin processing.

Finally, the study also investigated the effects of these dietary interventions on the plasma lipid profile of these insulin-resistant subjects independently of weight loss. The plasma lipid changes observed are consistent with the classical assumption that a low-fat (15% of energy) CHO-rich (68%) diet is associated with reductions in total plasma cholesterol and HDL-C [40]. Serum total cholesterol and Apo B concentrations thus tended to decrease after the CHO diet, but we also observed a potentially detrimental effect of the lower HDL-C concentrations (<11%). Conversely, the MUFA diet was associated with significantly higher HDL-C concentrations. Fasting serum triacylglycerols concentrations were not altered by any of the three diets. These results may be related to the fact that body weight remained stable during the three dietary periods, suggesting that triacylglycerol concentration is primarily associated with total body fat [41]. Postprandial triacylglycerol response presented a similar increase after fed both SAT and MUFA-rich diets. However, when patients were fed a CHO-rich diet triacylglycerol concentrations were not changed in comparison with the fasting state and were lower than with the high-fat diets. These patterns may largely reflect the amount of fat ingested during each period. However, exogenous administration of insulin and GLP-1 lowers the postprandial rise in triglyceride concentrations and lowers levels of non-esterified fatty acids [42,43]. Thus, it is possible that the postprandial changes observed in GLP-1 during high-fat diets and insulin responses after CHO-rich diet can also had a certain effect on the rise in triglyceride concentrations, but this differential effect was not examined in our study. Finally, since the nutritional composition was different between breakfast, lunch and dinner, interpretation of the biochemical postprandial results in this study must be limited to breakfast or similar meals. In

conclusion, our results have shown that in prediabetic insulin-resistant subjects an olive oil-enriched Mediterranean Diet taken at weight-maintenance levels improves HOMA-ir and fasting proinsulin levels in insulin-resistant subjects. Ingestion of a virgin olive oil-based breakfast lowered postprandial glucose and insulin concentrations, and increased HDL-C and GLP-1 concentrations compared with a CHO-rich diet.

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## APPENDIX A

### Sample daily menus for the high saturated fat, monounsaturated-rich fat and carbohydrate-rich diets

Menu	SAT	MUFA	CHO
Breakfast			
Whole milk	200 cc	-	-
Skimmed milk	-	200 cc	200 cc
sugar	-	-	18 g
Whole-wheat bread +	50 g	50 g	50 g
Butter	25 g	-	-
Virgin olive oil	-	27 cc	-
Marmalade with sugar	-	-	50 g
Fresh fruit	225 g	200 g	225 g
Lunch			
Pasta +	60 g	110 g	110 g
Vegetables (onion. marrow. mushroom. etc)	60 g	120 g	120 g
+ York ham	-	50 g	80 g
+ Dressing made with butter	20 g	-	-
+ Dressing made with virgin olive oil	-	7 cc	8 cc
Roasted chicken +	100 g	100 g	100 g
lettuce	60 g	60 g	-
Whole-wheat bread +	65 g	35 g	80 g
Butter	20 g	-	-
Olive oil	-	7 cc	-
Sweet drink (100 cc = 10.5 g sugar)	-	-	200 cc
Fresh fruit	225 g	225 g	225 g
Dinner			
Skimmed soup + rice	40 g	40 g	40 g
Mixed salad (onion + lettuce + tomatoes. etc)	160 g	100 g	100
+ dressing with virgin olive oil	-	7 cc	-
dressing with sunflower oil	-	-	8 cc
Stuffed turkey	60 g	-	60 g
Butter	15 g	-	-
Jam	-	40 g	-
Fresh fruit	225 g	225 g	225 g
Whole-wheat bread +	65 g	30 g	70 g
Butter	20 g	-	-
Virgin olive oil	-	7 cc	-
Marmalade with sugar	-	-	40 g
Total Energy, kJ/d	8684 Kj	8686 Kj	8698 Kj
Protein	72.1 g = 14%	72.8 g = 14%	78.5 g = 15%
Lipids	91.2 g = 38%	89.5 g = 38%	49.1 g = 21%
Carbohydrates	239.8g = 46%	245.3g = 47%	334 g = 64%
SAT/MUFA	52.9g/18.2g	17.7g/52.2g	14.2g/14.6g
Total fiber, g/d	34.7 g	28 g	34.5 g

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