Monounsaturated Fat–Rich Diet Prevents Central Body Fat Distribution and Decreases Postprandial Adiponectin Expression Induced by a Carbohydrate-Rich Diet in Insulin-Resistant Subjects

J.A. Paniagua, md, phd[1,2]
A. Gallego de la Sacristana, md[1]
I. Romero, phd[1]
A. Vidal-Puig, md, phd[3]
J.M. Latre, md, phd[4]
E. Sanchez, md[1]
P. Perez-Martinez, md, phd[1,2]
J. Lopez-Miranda, md, phd[1,2]
F. Perez-Jimenez, md, phd[1]

OBJECTIVE — Central obesity is associated with insulin resistance through factors that are not fully understood. We studied the effects of three different isocaloric diets on body fat distribution, insulin sensitivity, and peripheral adiponectin gene expression.

RESEARCH DESIGN AND METHODS — Eleven volunteers, offspring of obese type 2 diabetic patients with abdominal fat deposition, were studied. These subjects were considered insulin resistant as indicated by Matsuda index values <4 after an oral glucose tolerance test, and they maintained A1C <6.5% without therapeutic intervention. All subjects underwent three dietary periods of 28 days each in a crossover design: *1*) diet enriched in saturated fat (SAT), *2*) diet rich in monounsaturated fat (MUFA) (Mediterranean diet), and *3*) diet rich in carbohydrates (CHOs).

RESULTS — Weight, body composition, and resting energy expenditure remained unchanged during the three sequential dietary periods. Using dual-energy X-ray absorptiometry we observed that when patients were fed a CHO-enriched diet, their fat mass was redistributed toward the abdominal depot, whereas periphery fat accumulation decreased compared with isocaloric MUFA-rich and high-SAT diets (ANOVA $P < 0.05$). Changes in fat deposition were associated with decreased postprandial mRNA adiponectin levels in peripheral adipose tissue and lower insulin sensitivity index values from a frequently sampled insulin-assisted intravenous glucose tolerance test in patients fed a CHO-rich diet compared with a MUFA-rich diet (ANOVA $P < 0.05$).

CONCLUSIONS — An isocaloric MUFA-rich diet prevents central fat redistribution and the postprandial decrease in peripheral adiponectin gene expression and insulin resistance induced by a CHO-rich diet in insulin-resistant subjects.

Diabetes Care 30:1717–1723, 2007

From the [1]Lipids and Atherosclerosis Research Unit, Reina Sofía University Hospital, Córdoba, Spain; the [2]Ciber Fisiopatología Obesidad y Nutrición (CB06/03), Instituto Salud Carlos III, Madrid, Spain; [3]Molecular Mechanisms of Energy Balance, Department of Clinical Biochemistry, University of Cambridge, Cambridge, U.K.; and the [4]Nuclear Laboratory Service, Reina Sofía University Hospital, Córdoba, Spain.

Address correspondence and reprint requests to Juan A. Paniagua González and F. Pérez Jiménez Unidad de Lípidos y Arteriosclerosis, Hospital Universitario Reina Sofía, Avda Menéndez Pidal, s/n 14004, Córdoba, Spain. E-mail: japaniaguag@yahoo.es or md1pejif@uco.es.

Received for publication 1 November 2006 and accepted in revised form 16 March 2007.

Published ahead of print at http://care.diabetesjournals.org on 6 April 2007. DOI: 10.2337/dc06-2220.

Additional information for this article can be found in an online appendix at http://dx.doi.org/10.2337/dc06-2220.

Abbreviations: AUC, area under the curve; CHO, carbohydrate; DEXA, dual-energy X-ray absorptiometry; MUFA, monounsaturated fat; NEFA, nonesterified fatty acid; PUFA, polyunsaturated fat; SAT, saturated fat.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

A positive energy balance, which leads to obesity, is associated with insulin resistance and an increased risk of type 2 diabetes. According to our studies in rodents, adipose tissue expandability seems to be a key determinant linking obesity and its associated complications (1,2). Expansion of the intra-abdominal depot has been associated with increased insulin resistance, as well as with an increased incidence of fatty liver and β-cell failure (3,4).

The factors that regulate body fat distribution are not well understood. We hypothesize that the specific macronutrient composition of the diet may be an important environmental factor that controls nutrient partitioning to specific adipose tissue depots (5). It is now well established that adipose tissue is not simply an energy storage organ but is, in fact, the "largest" endocrine gland controlling energy homeostasis (6). It is conceivable that specific diet composition may directly influence the molecular events that govern gene expression in adipocytes (7), adipokine production, and adipocyte lipid and glucose metabolism (8).

Adiponectin adipocyte-secreted adipokine plays an important role in modulating insulin sensitivity and concentrations of circulating plasma glucose and nonesterified fatty acids (NEFAs) (9,10). A lower concentration of circulating plasma adiponectin seems to be a good predictor of reduced insulin sensitivity and increased risk of type 2 diabetes (11,12). Plasma adiponectin concentration is negatively correlated with body fat percentage and preferential central fat distribution (13).

In humans, the optimal diet for individuals at risk of developing type 2 diabetes is still controversial. It is unclear, for example, whether during weight-maintenance phases there is any metabolic advantage in administering diets preferentially enriched in monounsatu-

rated fat or carbohydrates, a question that becomes particularly relevant when most overweight patients fail to maintain long-tem weight loss.

The aim of this study was to compare the effects of three isocaloric diet models that differed in fatty acid and carbohydrate (CHO) composition on body fat composition and distribution, insulin sensitivity, and adiponectin expression on peripheral adipose tissue. The diet models were a diet rich in saturated fat (SAT), a diet enriched in monounsaturated fat (MUFA) (Mediterranean diet), and a CHO-rich diet

RESEARCH DESIGN AND METHODS

— In 2003 and 2004, 59 potential subjects were recruited and invited to attend a screening session. The study protocol was reviewed and approved by the Ethics and Clinical Investigation Committee of the Reina Sofía University Hospital of Cordova, Spain. Clinical inclusion criteria were as follows. Subjects should have a history of fasting glycemia <125 mg/dl with A1C <6.5% without any drug or previous insulin treatment. A BMI >25 kg/m^2 and waist circumference ≥102 cm in men and ≥88 cm in women were required. Clinical exclusion criteria were microvascular or macrovascular complications, cigarette smoking and alcohol consumption, and use of diuretics, steroids, β-blockers, or any medications that might affect glucose metabolism.

Included in this study were 11 subjects with an insulin sensitivity index (S_I) <4.0, which is regarded as insulin resistant (lower values indicates greater insulin resistance) (14). The subjects were 4 men and 7 women aged 62 ± 9.4 (mean ± SD) years with BMI 32.6 ± 7.8 kg/m^2 and A1C 6.0 ± 0.5%. Their fasting serum glucose levels at presentation were 5.47 ± 0.5 mmol/l and 2 h after an oral glucose load were 8.75 ± 1.6 mmol/l, and fasting serum insulin levels were 87.5 ± 26.6 pmol/l and 2 h after an oral glucose load were 959 ± 486 pmol/l. The Matsuda index was 2.9 ± 0.9 (Supplementary Table 1 [available in an online appendix at http://dx.doi.org/10.2337/dc06-2220]).

In brief, each subject completed a 3-day food intake diary, and energy expenditure was measured in the fasting state, by indirect calorimetry to estimate normal energy intake. Patients were randomly divided into three groups (Williams Latin square) and underwent three dietary periods of 28 days each: the low-fat, high-CHO diet contained 65% CHO and 20% fat (6% SAT, 8% MUFA, and 6% polyunsaturated fat [PUFA]); the high-fat, MUFA-rich diet contained 47% CHO and 38% fat (9% SAT; 23% MUFA, 75% of which was provided as extra virgin olive oil; and 6% PUFA); and the diet rich in SAT contained 47% CHO, 15% protein, and 38% fat (23% SAT, 9% MUFA, and 6% PUFA). Subjects were monitored to maintain their weight and usual exercise intensity during the study. Dietary adherence was determined by measuring the fatty acid content extracted from plasma phospholipids at the end of dietary period by means of gas chromatography (5890 gas chromatograph; Hewlett Packard, Avondale, PA) (15).

Breakfast meals used for postprandial studies

Postprandial studies were conducted on the last day of each diet period. At 8 A.M., the subjects met in the laboratory after an overnight fast. The breakfast meals contained 443 kcal and were consumed within 15 min. The CHO-rich breakfast comprised 200 ml skim milk, 70 g bread, and 75 g marmalade; the MUFA-rich breakfast diet comprised 200 ml skim milk, 50 g bread, and 27 g olive oil; and the SAT-rich breakfast consisted of 200 ml whole milk, 50 g bread, and 25 g butter. Venous blood samples were taken before the breakfast and afterward, at the following time points: −10, −5, 0, 15, 30, 60, 90, 120, 150, and 180 min.

Insulin sensitivity calculations

A frequently sampled intravenous glucose tolerance test was performed as described previously (16). Values for glucose and insulin were entered into the Minmod Millenium 2002 computer program (version 6.02; Richard N. Bergman) for determination of the acute insulin response and S_I.

Anthropometry and body composition

Stature was measured to 0.5 cm on a stadiometer, and weight was measured to 0.1 kg on a balance scale. The waist circumference of each subject was measured at the umbilicus to the nearest millimeter with a fiberglass tape measure. Bioelectrical impedance was performed, and body fat mass was determined by subtracting lean body mass from body weight (17). For assessment of body fat distribution, all subjects underwent a dual-energy X-ray absorptiometry (DEXA) whole-body scan in a Lunar DPX-IQ 240 densitometer at baseline and at the end of each dietary treatment. Analysis of the total and regional body composition was performed using the extended research mode with Lunar software (version 1.3; Lunar Software, Madison, WI) to provide data on whole-body and regional data (limbs and trunk) (18). The trunk region or upper body fat includes the chest and abdomen, excluding the pelvis. The leg region includes the entire hip, thigh, and leg. The coefficient of variation (CV) for the DEXA scan is ≤2%.

Indirect calorimetry

The energy production and respiratory quotient were measured by continuous indirect calorimetry using a computerized, flow-through canopy gas analyzer system (Deltatrac; Datex, Helsinki, Finland) (19).

Analytic procedures

Plasma glucose was measured by the glucose oxidase method. Plasma insulin concentrations were measured by microparticle enzyme immunoassay (Abbott Diagnostics, Matsudo-shi, Japan) (CV 2.5–6%). Serum human glucagon peptide was measured by competitive format using an ELISA (Wako Chemicals, Osaka, Japan). Human ELISA kits (Linco, St. Charles, MO) were used for measurement of adiponectin protein (CV 4.3%), serum leptin concentrations (CV 4.8%), and resistin (CV 3.2%). Serum fatty acids (free fatty acids and NEFAs) concentrations were measured in triplicate in each sample using an enzymatic colorimetric assay (Roche Molecular Biochemicals, Mannheim, Germany) (CV 7.8%). Fasting blood samples were analyzed for cortisol, testosterone, estrone, 17-OH-progesterone, dehydroepiandrosterone sulfate, and androstenedione by specific radioimmunoassays (Diagnostic Systems Laboratories, Sinsheim, Germany).

Peripheral adipose adiponectin quantification with RT–PCR

At 7:00 A.M. patients ingested a 443-cal breakfast in each dietary period and after 180 min, samples of peripheral fatty tissue (~300 mg) were obtained from the outer upper quadrant of the buttocks, following local anesthesia (scandicain) without adrenalin. A Bard Magnum pistol (MG1522; Bard) with Bard Magnum core tissue biopsy needles (MN1410) was used

Table 1—*Composition and body fat distribution after three dietary interventions in insulin-resistant subjects*

	Baseline	High-SAT diet	High-MUFA diet	High-CHO diet	*P* value
Energy expenditure (kJ/min)	5.36 ± 0.40	5.49 ± 3.90	5.23 ± 0.37	5.02 ± 0.36	0.30
Anthropometry					
Weight (kg)	84.4 ± 5.7	83.2 ± 5.7	83.6 ± 5.8	81.8 ± 6.03	0.3
Total body fat (kg)	36.8 ± 4.1	35.0 ± 4.0	35.6 ± 4.0	34.9 ± 4.3	0.1
Lean body mass (kg)	47.5 ± 2.5	48.1 ± 2.5	48.9 ± 2.6	46.8 ± 2.1	0.2
Waist-to-hip ratio	0.99 ± 0.01	0.99 ± 0.01	0.98 ± 0.01	0.98 ± 0.01	0.9
DEXA analysis					
Total body trunk (g)	—	37,101 ± 2.026	38,154 ± 1,911	39,134 ± 2,104	0.3
Fatty body trunk (g)	—	14,313 ± 1.362	14,842 ± 1,437	16,459 ± 1,653	<0.05
Total body limb (g)	—	36,420 ± 3.886	36,239 ± 3,862	32,887 ± 3,825	0.7
Fat in arm (g)	—	7,097 ± 1.528	7,652 ± 1,339	7,225 ± 1,830	0.4
Fat in leg (g)	—	8,517 ± 1.588	8,036 ± 1,398	7,358 ± 1,253	<0.05
Fat trunk-to-fat leg ratio	—	1.9 ± 0.3	2.1 ± 0.2	2.50 ± 0.2	<0.05

Data are means ± SEM. *P* value determined by ANOVA for repeated variables.

to obtain samples, which were submerged in an mRNA preserving solution (RNA-Later; AMBION, Austin, TX) and finally stored at −20°C. Total RNA was isolated using Tri Reagent (MRC, Cincinnati, OH), homogenized with Ultra-Turrax T25 (Wolf Laboratories, York, U.K.), and quantified by spectrophotometry (Nano-Drop, Wilmington, DE). First-strand cDNA was generated from 1 μg RNA by means of an iScript cDNA synthesis kit (Bio-Rad) and cDNA synthesis using a Mastercycler gradient (Eppendorf, Westbury, NY). The cDNA obtained was used as a template in quantitative real-time PCR using QuantiTect SYBR Green PCR kit (QIAGEN, Hilden, Germany) and a LightCycler system (Roche Molecular Biochemicals). Primer and probes were designed with the aid of Primer Express Software (version 1.0; Applied Biosystems, Foster City, CA) from gene sequences obtained from GenBank (http://www.ncbi.nih.gov/blast/; National Library of Medicine, Bethesda, MD). The forward primer used for adiponectin was 5′GGAGATCCAGGTCTTATTGG3′, and the reverse primer used was 5′GTAAAGC GAATGGGCATGTT3′. The PCR product obtained was 194 base pairs and was analyzed by melting curves and in agarose gels electrophoresis. The signal for β-*actin* (GenBank X00351) was used to normalize against differences in RNA isolation and degradation and the efficiencies of the reverse transcription and PCRs. Samples were run in duplicate, were quantitated by normalizing the adiponectin signal with the β-*actin* signal, and are expressed in arbitrary units, with the values for adiponectin mRNA concentrations during SAT-rich diet arbitrarily set at 1.00.

Statistical analysis

The data presented were all tested for normality of distribution. Data for mRNA adiponectin expression were log transformed to normalize the distribution. Changes in anthropometry, plasma lipids and lipoproteins, and glycemic variables were analyzed by means of a two-factor ANOVA model with repeated measures. The postprandial excursion of plasma glucose, fatty acids, insulin, and glucagon were analyzed by calculating the incremental area under the curve (AUC) for 180 min after the meal intake, with a formula based on the trapezoid rule with adjustment for baseline concentrations. Results are presented as means ± SEM, unless otherwise indicated.

RESULTS

Effects of macronutrient diet composition on energy balance

As expected, the mean energy consumed remained unchanged throughout the three dietary phases (9,565 ± 769 kJ [SAT], 9,586 ± 743 kJ [MUFA], and 9,526 ± 716 kJ [CHO]; ANOVA $P = 0.7$). The resting energy expenditure at the initial evaluation of 93.01 ± 5.02 kJ · kg^{-1} · day^{-1} was not changed during the three dietary periods (5.49 ± 0.38, 5.23 ± 0.36, and 5.02 ± 0.37 kJ/min, respectively; ANOVA $P = 0.3$). The fasting fat oxidation rate was increased during the high-SAT and MUFA-rich diets compared with the high-CHO diet (1.60 ± 0.11, 1.82 ± 0.11, and 1.38 ± 0.13 kJ/min; ANOVA $P < 0.05$). CHO and protein oxidation rates were unchanged (2.45 ± 0.18, 1.88 ± 0.13, and 1.98 ± 0.13 kJ/min and 1.44 ± 0.09, 1.53 ± 0.12, and 1.66 ± 0.11 kJ/min; ANOVA $P = 0.5$ and $P = 0.07$, respectively) (Supplementary Table 2). The efficacy of the dietary treatment was confirmed as the proportions of palmitic, stearic, and total saturated fatty acids were increased after consumption of SAT-rich diets (ANOVA $P < 0.01$, $P < 0.05$, and $P < 0.01$, respectively); the proportions of oleic (18:1, n-9) and total monounsaturated fatty acids were higher after eating MUFA-rich diets (ANOVA $P < 0.01$ and $P < 0.05$, respectively); and the proportion of total polyunsaturated fatty acids was unchanged (Supplementary Table 3).

Diet-specific effects on adipose depot fat deposition

The mean BMI (32.5 ± 2.4 kg/m^2) and mean waist circumference at baseline (109.5 ± 5 cm) were not modified during the study (Table 1). Body composition remained unchanged, as indicated by stable total body fat and lean body mass. We observed increased accumulation of fat deposited in the trunk depot when experimental subjects were fed the CHO-enriched diet. This was associated with a decrease in the amount of fat mass deposited in the leg when subjects were fed the CHO-rich diet compared with the MUFA- and SAT-rich diets (16,459 ± 1,653, 14,842 ± 1,437, and 14,313 ± 1,362 g and 7,358 ± 1,253, 8,036 ± 1,398, and 8,517 ± 1,588 g, respectively, ANOVA $P < 0.05$). The result was an increase in the abdominal fat–to–leg fat ratio after the CHO-rich diet compared with the MUFA- and SAT-rich diets (2.50 ± 0.2, 2.1 ± 0.2, and 1.9 ± 0.3, respectively, ANOVA $P < 0.05$).

Table 2—*Hormonal, adipokine, and metabolic parameters after the three dietary periods in insulin-resistant subjects*

	High-SAT diet	High-MUFA diet	High-CHO diet	*P* value
Glucose				
Fasting (mmol/l)	5.5 ± 0.2	5.0 ± 0.1	5.0 ± 0.1	<0.05
AUC (mmol · 180 min^{-1} · l^{-1})	5.8 ± 1.2	7.8 ± 1.3	11.9 ± 2.7	<0.05
Insulin				
Fasting (pmol/l)	64.1 ± 9.6	60.7 ± 12.4	75.2 ± 12.3	0.3
AUC (pmol · 180 min^{-1} · l^{-1})	142.4 ± 28.9	113.8 ± 29.6	259.3 ± 74.2	<0.05
S_i [× 10^{-4} min^{-1}/(μU/ml)]	3.3 ± 0.5	3.9 ± 0.6	3.5 ± 0.4	<0.05
Acute insulin response (μU/ml)	264.7 ± 82.7	335.6 ± 85	347.4 ± 75.5	<0.05
Glucagon				
Fasting (μg/l)	322 ± 75	436 ± 110	495 ± 102	<0.05
AUC (ng · 180 min^{-1} · ml^{-1})	125 ± 162	466 ± 210	−55 ± 67	0.07
Adiponectin (mg/l)				
Fasting	8.6 ± 1.7	9.4 ± 1.5	12 ± 1.7	0.08
Postprandial (180 min)	11.1 ± 2.3	11.2 ± 1.9	10.7 ± 2.4	0.8
Resistin (μg/l)				
Fasting	10.3 ± 1.1	10.5 ± 1.3	11.5 ± 1.8	0.8
Postprandial (180 min)	10.3 ± 1.0	10.4 ± 1.2	10.4 ± 0.9	0.8
Leptin (μg/l)				
Fasting	31.6 ± 9.4	23.7 ± 7.4	32.1 ± 10.3	<0.05
Postprandial (180 min)	20.3 ± 4.4	21.1 ± 7.0	24.7 ± 7.4	0.4

Data are means ± SEM. *P* value determined by ANOVA for repeated variables.

Diet-specific effects on carbohydrate and lipid metabolism

Fasting glucose concentrations decreased during MUFA-rich and CHO-rich diets compared with the SAT-rich period (ANOVA $P < 0.05$) (Table 2). Insulin sensitivity assessed as the S_i was improved by the MUFA-enriched diet compared with the SAT-rich and CHO-rich diets [3.9 ± 0.6, 3.3 ± 0.5, and 3.5 ± 0.4 $\times 10^{-4}$ min^{-1}/(μU/ml), respectively; ANOVA $P < 0.05$]. Insulin secretion assessed as acute insulin response fell during the SAT-rich diet compared with the MUFA-rich and CHO-rich diets (264.7 ± 82.7, 335.6 ± 85, and 347.4 ± 75.5, respectively; ANOVA $P < 0.05$). The postprandial integrated AUCs for glucose and insulin concentrations were higher in response to the standard CHO-rich breakfast than to standard high-MUFA and high-SAT breakfasts (ANOVA $P < 0.05$ and $P < 0.01$, respectively). Fasting fatty acid concentrations remained constant throughout the three dietary periods, but postprandial excursions of NEFAs were lower after the CHO-rich breakfast than after the high-MUFA and SAT-rich breakfasts (ANOVA $P < 0.05$). The aspartate aminotransferase–to–alanine aminotransferase ratio was unchanged after high-SAT, high-MUFA, and CHO-rich diets (1.01 ± 0.05, 1.14 ± 0.07, 1.04 ± 0.05, respectively; ANOVA $P = 0.13$).

Diet-specific effects on plasma hormone and adipokine concentrations

Fasting and postprandial serum adiponectin concentrations remained unchanged during the three dietary phases (Table 2). The fasting serum leptin concentration was lower after the MUFA-rich dietary period than after the SAT-rich and CHO-rich diets (23.7 ± 7.4, 31.6 ± 9.4, and 32.1 ± 10.3 μg/l, respectively; ANOVA $P < 0.05$). Postprandial leptin concentrations fell in response to the three breakfasts and finally were similar after the three diets. Fasting serum resistin concentrations after the three dietary periods and in responses to the three breakfasts remained unchanged. Because changes in steroid metabolism might have been responsible for the observed changes in adipose tissue distribution, we also examined the effects of these diets on fasting serum concentrations of cortisol, androstenedione, testosterone, 17-OH-progesterone, estrone, and dehydroepiandrosterone sulfate. None of these hormones changed in response to the macronutrient composition of the diet. Finally, we looked at whether postprandial adiponectin mRNA expression in peripheral adipose tissue (gluteus) of insulin-resistant subjects was affected by the macronutrient composition of the three breakfast models (Fig. 1). The mRNA abundance of adiponectin was lower after a CHO-rich breakfast than after MUFA-rich and SAT-rich breakfasts (ANOVA $P < 0.05$). The expression of the reference gene β-*actin* was not modified during the three dietary periods.

CONCLUSIONS — In this controlled, randomized cross-over trial, we investigated the effects of ad libitum consumption of a high-CHO diet versus a high-MUFA diet on body fat distribution, postprandial adiponectin gene expression on peripheral adipose tissue, and insulin sensitivity in normoglycemic insulin-resistant first-degree relatives of type 2 diabetic patients.

In our opinion, the most interesting finding of our research is the demonstration that specific macronutrient composition affects the topography of fat distribution. After a low-fat CHO-enriched diet, our patients presented a preferential redistribution of their body fat from peripheral adipose tissue in the leg to central body depots in the trunk compared with what occurred when an isocaloric high-MUFA diet was fed. The consumption of CHO-rich diets has been associated with weight loss that could partially explain some of its metabolic effects (20). On the other hand, it has also been shown that low-CHO diets may have beneficial effects by reducing body

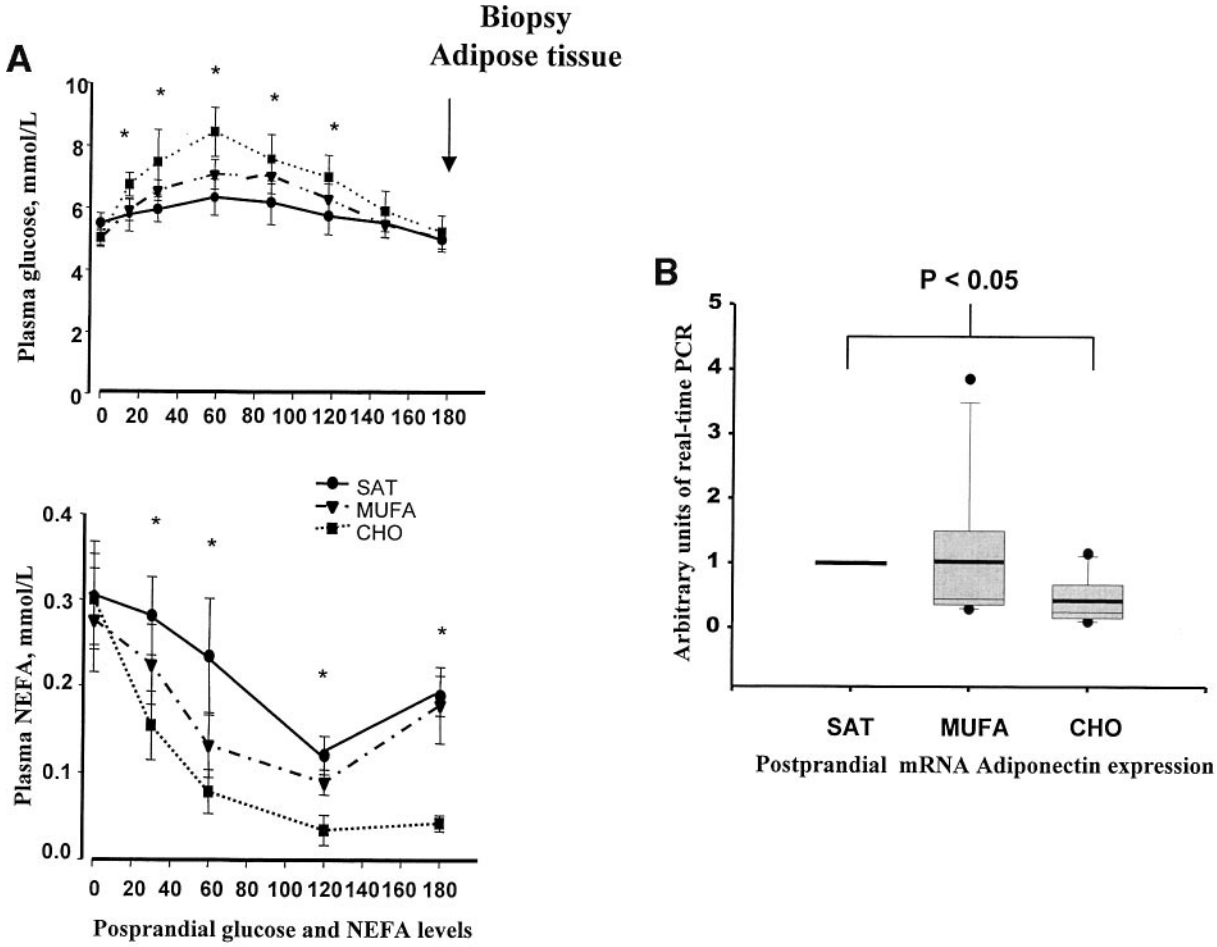

Figure 1—A: *Diet-specific postprandial responses to glucose and NEFA levels. Shown are mean ± S.E.M. postprandial responses of glucose and NEFA to the three isocaloric (443 kcal) standard breakfasts: a breakfast rich in CHOs (■), a Mediterranean breakfast enriched with virgin olive oil (MUFA, ▼) and a standard breakfast high in SAT (●). Repeated-measures ANOVA and Tukey's tests were used.* B: *Postprandial adiponectin gene expression in peripheral adipose tissue at 180 min after each isocaloric breakfast. Values represent means ± SE with the values for adiponectin mRNA concentrations in the SAT-rich diet arbitrarily set at 1. After log transformation, the data were normalized, and ANOVA for repeated measures was used for overall comparison. A 5% α risk concentration was required for statistical significance* ($P < 0.05$).

weight and improving insulin resistance in type 2 diabetic patients (21). There is evidence that type 2 diabetic patients in weight maintenance programs with a very low-fat CHO-enriched diet tend to have a disproportionate loss of lower body to upper body fat compared with patients fed a diet high in monounsaturated fats (22). However, consumption of hypocaloric diets, leading to weight loss, has not revealed preferential regional specific fat mass loss, either with high-fat or very low–fat diets (23). Our study was designed to specifically address the effects of three diets with different macronutrient composition but with similar caloric intake. As expected, at least in the short run, the macronutrient compositions of isocaloric diets did not have significant effects on body weight.

Because different diet composition may have heterogeneous effects on glucose, fatty acid, hormone, and adipokine concentrations, we investigated the effect on these parameters. Compared with high-fat diets, subjects consuming CHO-rich diets displayed, during the postprandial phase, higher glucose and insulin concentrations and lower fatty acid and glucagon levels. However, in the fasting state after a period of consumption of a CHO-rich diet, the glucagon concentration rose, whereas fatty acid concentrations remained similar compared with those for high-fat diets. In the fasting state, adipose tissue is the most important contributor (~80%) to the plasma NEFA concentrations, and it is well known that subcutaneous adipocytes have high lipolytic activity (24,25). We hypothesized that a glucagon-mediated lipolytic effect on peripheral adipose tissue may occur in the fasting state when patients are fed a CHO-rich diet, relocating fatty acids from peripheral to central body fat depots (26,27). Alternatively, it is possible that hepatic lipogenesis may have contributed to the postprandial increase in plasma concentrations of glucose and insulin observed in individuals fed CHO-rich diets that may stimulate de novo lipogenesis (28,29).

Several mechanisms may have been implicated in the changes in insulin sensitivity observed during these nutritional manipulations. These include nutritionally induced differences in hormone secretion, as well as differences in glucose- and lipid-buffering capacity (18,30,31). Regional differences in patterns of adipokine production and/or fatty acid handling may also influence the relative effects on adipose tissue deposition and

insulin sensitivity. Interestingly, our results show that fasting plasma leptin concentrations fell during MUFA-rich periods. Plasma leptin is an important signal for regulation of energy stores and has been associated with improved insulin action (32,33), and its concentrations seems to be increased by high-CHO diets (34). However, this finding should be interpreted with caution because elevated leptin concentrations in the context of obesity are generally viewed as evidence for leptin resistance and insulin resistance (35).

Our study has also documented differential postprandial regulation of adiponectin gene expression on peripheral adipose tissue in response to differences in the macronutrient composition of diets. After a CHO-rich breakfast, lower adiponectin mRNA expression levels were detected than after MUFA-rich and SAT-rich diets were ingested. However, fasting and postprandial adiponectin plasma concentrations did not differ significantly among the three diets. It can be speculated that despite similar levels of adiponectin in plasma, the paracrine effects of adiponectin may facilitate depot-specific effects that promote adipocyte differentiation and peripheral lipid accumulation (10). In fact, direct adiponectin action on adipose tissue expandability seems to be an important factor for the differences in insulin sensitivity in responses to the macronutrient composition of diets, in the context of energy balance.

In obese patients, adiponectin mRNA in peripheral adipose tissue can respond acutely to the short-term energy restriction associated with increased insulin sensitivity (36). In animals, dietary factors, such as fish oil and linoleic acid, have been associated with higher plasma concentrations and gene expression of adiponectin, which is consistent with these fatty acids exerting a protective effect on the development of diabetes (8,37). Similarly, adiponectin has been suggested as a potent insulin enhancer linking adipose tissue and whole-body glucose metabolism (38). Furthermore, plasma adiponectin concentrations progressively decrease with age in plasma in a rhesus monkey model of progressive development of type 2 diabetes (12). Moreover, insulin resistance in lipoatrophic mice was completely reversed by the combination of physiological doses of adiponectin and leptin but only partially by either adiponectin or leptin alone (39).

In summary, our results indicate that the macronutrient composition of diets may influence body fat distribution and carbohydrate metabolism without affecting total body weight. After a low-fat carbohydrate-rich diet, insulin-resistant patients presented a redistribution of their body fat from peripheral adipose tissue to central body depots. Conversely, a MUFA-rich diet improved insulin sensitivity, and this was associated with increased postprandial adiponectin mRNA gene expression.

Acknowledgments— This work was supported by grants to J.A.P. from the Spanish Arteriosclerosis Foundation (AstraZeneca, 2003); the Pharmaceutics Foundation AVENZOAR of Seville (2004); the Medical College of Cordova Foundation (2004); and the Secretaría General de Calidad y Eficiencia, Junta de Andalucía (78/02 and 240/04).

References

1. Barroso I, Luan J, Middelberg RP, Harding AH, Franks PW, Jakes RW, Clayton D, Schafer AJ, O'Rahilly S, Wareham NJ: Candidate gene association study in type 2 diabetes indicates a role for genes involved in β-cell function as well as insulin action. *PLoS Biol* 1:E20, 2003
2. Vidal-Puig AJ, Considine RV, Jimenez-Linan M, Werman A, Pories WJ, Caro JF, Flier JS: Peroxisome proliferator-activated receptor gene expression in human tissues: effects of obesity, weight loss, and regulation by insulin and glucocorticoids. *J Clin Invest* 99:2416–2422, 1997
3. Kriketos AD, Carey DG, Jenkins AB, Chisholm DJ, Furler SM, Campbell LV: Central fat predicts deterioration of insulin secretion index and fasting glycaemia: 6-year follow-up of subjects at varying risk of type 2 diabetes mellitus. *Diabet Med* 20:294–300, 2003
4. Shen W, Punyanitya M, Chen J, Gallagher D, Albu J, Pi-Sunyer X, Lewis CE, Grunfeld C, Heshka S, Heymsfield SB: Waist circumference correlates with metabolic syndrome indicators better than percentage fat. *Obesity (Silver Spring)* 14:727–736, 2006
5. Kaput J, Rodriguez RL: Nutritional genomics: the next frontier in the postgenomic era. *Physiol Genomics* 16:166–177, 2004
6. Vidal-Puig A, Jimenez-Linan M, Lowell BB, Hamann A, Hu E, Spiegelman B, Flier JS, Moller DE: Regulation of PPAR γ gene expression by nutrition and obesity in rodents. *J Clin Invest* 97:2553–2561, 1996
7. Duplus E, Glorian M, Forest C: Fatty acid regulation of gene transcription. *J Biol Chem* 275:30749–30752, 2000
8. Flachs P, Mohamed-Ali V, Horakova O, Rossmeisl M, Hosseinzadeh-Attar MJ, Hensler M, Ruzickova J, Kopecky J: Polyunsaturated fatty acids of marine origin induce adiponectin in mice fed a high-fat diet. *Diabetologia* 49:394–397, 2006
9. Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE, Lodish HF: Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci USA* 98:2005–2010, 2001
10. Fu Y, Luo N, Klein RL, Garvey WT: Adiponectin promotes adipocyte differentiation, insulin sensitivity, and lipid accumulation. *J Lipid Res* 46:1369–1379, 2005
11. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA: Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* 86:1930–1935, 2001
12. Hotta K, Funahashi T, Bodkin NL, Ortmeyer HK, Arita Y, Hansen BC, Matsuzawa Y: Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes* 50:1126–1133, 2001
13. Sargin H, Sargin M, Gozu H, Orcun A, Baloglu G, Ozisik M, Seker M, Uygur-Bayramicli O: Is adiponectin level a predictor of nonalcoholic fatty liver disease in nondiabetic male patients? *World J Gastroenterol* 11:5874–5877, 2005
14. Matsuda M, DeFronzo RA: Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 22:1462–1470, 1999
15. Wang ST, Peter F: Gas-liquid chromatographic determination of fatty acid composition of cholesteryl esters in human serum using silica Sep-Pak cartridges. *J Chromatogr* 276:249–256, 1983
16. Bergman RN: Lilly Lecture 1989: Toward physiological understanding of glucose tolerance: minimal-model approach. *Diabetes* 38:1512–1527, 1989
17. Bioelectrical impedance analysis in body composition measurement: National Institutes of Health Technology Assessment Conference Statement. *Am J Clin Nutr* 64:524S–532S, 1996
18. Snijder MB, Dekker JM, Visser M, Bouter LM, Stehouwer CD, Yudkin JS, Heine RJ, Nijpels G, Seidell JC: Trunk fat and leg fat have independent and opposite associations with fasting and postload glucose levels: the Hoorn study. *Diabetes Care* 27:372–377, 2004
19. Ferrannini E: The theoretical bases of indirect calorimetry: a review. *Metabolism* 37:287–301, 1988

20. Gerhard GT, Ahmann A, Meeuws K, McMurry MP, Duell PB, Connor WE: Effects of a low-fat diet compared with those of a high-monounsaturated fat diet on body weight, plasma lipids and lipoproteins, and glycemic control in type 2 diabetes. *Am J Clin Nutr* 80:668–673, 2004
21. Boden G, Sargrad K, Homko C, Mozzoli M, Stein TP: Effect of a low-carbohydrate diet on appetite, blood glucose levels, and insulin resistance in obese patients with type 2 diabetes. *Ann Intern Med* 142:403–411, 2005
22. Walker KZ, O'Dea K, Johnson L, Sinclair AJ, Piers LS, Nicholson GC, Muir JG: Body fat distribution and non-insulin-dependent diabetes: comparison of a fiber-rich, high-carbohydrate, low-fat (23%) diet and a 35% fat diet high in monounsaturated fat. *Am J Clin Nutr* 63:254–260, 1996
23. Clifton PM, Noakes M, Keogh JB: Very low-fat (12%) and high monounsaturated fat (35%) diets do not differentially affect abdominal fat loss in overweight, nondiabetic women. *J Nutr* 134:1741–1745, 2004
24. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ: Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest* 115:1343–1351, 2005
25. Tchernof A, Belanger C, Morisset AS, Richard C, Mailloux J, Laberge P, Dupont P: Regional differences in adipose tissue metabolism in women: minor effect of obesity and body fat distribution. *Diabetes* 55:1353–1360, 2006
26. Cavallo-Perin P, Bruno A, Scaglione L, Gruden G, Cassader M, Pagano G: Feedback inhibition of insulin and glucagon secretion by insulin is altered in abdominal obesity with normal or impaired glucose tolerance. *Acta Diabetol* 30:154–158, 1993
27. Henkel E, Menschikowski M, Koehler C, Leonhardt W, Hanefeld M: Impact of glucagon response on postprandial hyperglycemia in men with impaired glucose tolerance and type 2 diabetes mellitus. *Metabolism* 54:1168–1173, 2005
28. Parks EJ: Dietary carbohydrate's effects on lipogenesis and the relationship of lipogenesis to blood insulin and glucose concentrations. *Br J Nutr* 87 (Suppl. 2):S247–S253, 2002
29. Aarsland A, Chinkes D, Wolfe RR: Hepatic and whole-body fat synthesis in humans during carbohydrate overfeeding. *Am J Clin Nutr* 65:1774–1782, 1997
30. Paradisi G, Smith L, Burtner C, Leaming R, Garvey WT, Hook G, Johnson A, Cronin J, Steinberg HO, Baron AD: Dual energy X-ray absorptiometry assessment of fat mass distribution and its association with the insulin resistance syndrome. *Diabetes Care* 22:1310–1317, 1999
31. Lelliott C, Vidal-Puig AJ: Lipotoxicity, an imbalance between lipogenesis de novo and fatty acid oxidation. *Int J Obes Relat Metab Disord* 28 (Suppl. 4):S22–S28, 2004
32. Ruige JB, Dekker JM, Blum WF, Stehouwer CD, Nijpels G, Mooy J, Kostense PJ, Bouter LM, Heine RJ: Leptin and variables of body adiposity, energy balance, and insulin resistance in a population-based study: the Hoorn Study. *Diabetes Care* 22: 1097–1104, 1999
33. Shimomura I, Hammer RE, Ikemoto S, Brown MS, Goldstein JL: Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. *Nature* 401:73–76, 1999
34. Fox C, Esparza J, Nicolson M, Bennett PH, Schulz LO, Valencia ME, Ravussin E: Plasma leptin concentrations in Pima Indians living in drastically different environments. *Diabetes Care* 22:413–417, 1999
35. Wang J, Obici S, Morgan K, Barzilai N, Feng Z, Rossetti L: Overfeeding rapidly induces leptin and insulin resistance. *Diabetes* 50:2786–2791, 2001
36. Liu YM, Lacorte JM, Viguerie N, Poitou C, Pelloux V, Guy-Grand B, Coussieu C, Langin D, Basdevant A, Clement K: Adiponectin gene expression in subcutaneous adipose tissue of obese women in response to short-term very low calorie diet and refeeding. *J Clin Endocrinol Metab* 88:5881–5886, 2003
37. Nagao K, Inoue N, Wang YM, Yanagita T: Conjugated linoleic acid enhances plasma adiponectin level and alleviates hyperinsulinemia and hypertension in Zucker diabetic fatty (*fa/fa*) rats. *Biochem Biophys Res Commun* 310:562–566, 2003
38. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE: The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 7:947–953, 2001
39. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T: The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 7:941–946, 2001