

Intake and home use of olive oil or mixed oils in relation to healthy lifestyles in a Mediterranean population. Findings from the prospective Pizarra study

Federico Soriguer^{1,2,3*}, M. Cruz Almaraz^{1,2,3}, J. M. García-Almeida^{1,3}, Isabel Cardona^{1,2,3}, Francisca Linares^{1,2,3}, Sonsoles Morcillo^{1,2,3}, Eva García-Escobar^{1,2,3}, M. Carmen Dobarganes⁴, Gabriel Olveira^{1,2,3}, Virginia Hernando¹, Sergio Valdes^{1,2,3}, M. Soledad Ruiz-de-Adana^{1,2,3}, Isabel Esteve¹ and Gemma Rojo-Martínez^{1,2,3}

¹*Servicio de Endocrinología y Nutrición, Hospital Universitario Carlos Haya, 29009 Malaga, Spain*

²*CIBER Diabetes and Metabolism (CIBERDEM), Instituto de Salud Carlos III (ISCIII), Alicante, Spain*

³*CIBER Physiology of Obesity and Nutrition (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Madrid, Spain*

⁴*Instituto de la Grasa, CSIC, Seville, Spain*

(Received 16 June 2008 – Revised 27 May 2009 – Accepted 28 May 2009 – First published online 14 September 2009)

Discordances exist in epidemiological studies regarding the association between the intake of nutrients and death and disease. We evaluated the social and health profile of persons who consumed olive oil in a prospective population cohort investigation (Pizarra study) with a 6-year follow-up. A food frequency questionnaire and a 7 d quantitative questionnaire were administered to 538 persons. The type of oil used in food preparation was determined by direct measurement of the fatty acids in samples obtained from the kitchens of the participants at baseline and after follow-up for 6 years. The fatty acid composition of the serum phospholipids was used as an endogenous marker of the type of oil consumed. Total fat intake accounted for a mean 40% of the energy (at baseline and after follow-up). The concordance in intake of MUFA over the study period was high. The fatty acid composition of the serum phospholipids was significantly associated with the type of oil consumed and with fish intake. The concentration of polar compounds and polymers, indicative of degradation, was greater in oils from the kitchens where sunflower oil or refined olive oil was used, in oils used for deep frying and in oils that had been reused for frying five times or more. Consumption of olive oil was directly associated with educational level. Part of the discordance found in epidemiological studies between diet and health may be due to the handling of oils during food preparation. The intake of olive oil is associated with other healthy habits.

Olive oil: Frying oil: MUFA: Lifestyle

The so-called Mediterranean diet has been proposed as a model for a healthy diet^(1–3). Although the common denominator in Mediterranean countries is a high consumption of olive oil, important differences nevertheless exist concerning overall mortality and mortality associated with coronary disease. These differences have been attributed to variations in social, religious and cultural customs that confound associations between health and diet and the risk for disease⁽⁴⁾. Over the second half of the 20th century, the populations of industrialised countries markedly increased fat intake. Although this increase has coincided in time with a rise in the prevalence of obesity, diabetes mellitus and CVD⁽⁵⁾, a causal role of dietary fats in the development of these conditions has been much debated^(5,6). Most public-health programmes in Western countries have aimed at reducing the proportion of energy obtained from fat in the diet to below 30%, an objective that should be very difficult to achieve in Mediterranean countries unless the intake of olive oil is reduced⁽⁷⁾. In fact, nutritional recommendations

are contradictory, as, on the one hand, they propose to reduce the amount of dietary fats and, on the other hand, to increase vegetable fats. Moreover, the recommendations on fats rich in PUFA are sometimes contradictory and the public are urged to consume less overall fat but more fats rich in MUFA, without taking into consideration the source of fat (virgin or refined olive oil, high oleic sunflower oil, etc)⁽⁸⁾. However, countries around the Mediterranean basin have witnessed important social, cultural and economic changes that have greatly influenced dietary habits⁽⁹⁾.

Studies carried out in Spain have shown that persons who consume a greater proportion of olive oil consume foods that differ from those consumed by people using less olive oil⁽⁷⁾. However, many studies on the intake of fats have been based either on 24 h food records, which have a poor sensitivity for representing the type of fatty acids consumed, or on FFQ, which reflect what people have bought or what they believe they consume, but not what they really consume⁽⁵⁾. Additionally, the information a person has about the type of

Abbreviation: P75, 75th percentile.

* **Corresponding author:** Federico Soriguer, fax +34 952286704, email federico.soriguer.sspa@juntadeandalucia.es

fat used in food is not always adequate, and during the cooking process, important changes are produced resulting in differences between what people say they consume and what they do, in fact, consume. In the present study, we considered the home kitchen as an uncontrolled experimental laboratory where important changes occur in the composition of the fats that are actually consumed. The aims of the study were: (1) to evaluate in a Mediterranean population the precision in people's awareness of the type of fat consumed, and the influence of food handling on the composition and quality of the fat, and (2) to assess the sociocultural and health profile of persons who preferentially consume olive oil.

Material and methods

Baseline study

The baseline study was undertaken in Pizarra, an inland town of 6600 inhabitants (5000 aged 18–65 years) located some 30 km (20 miles) from the city of Malaga, in southern Spain. The study characteristics have been reported in detail elsewhere^(10,11). Fig. 1 shows the flow of participants in the study. Exclusion criteria included people who were institutionalised, pregnant women and persons with a severe clinical problem or psychological disorder. The final sample

distribution by age and sex was not significantly different to the distribution of the general population.

Follow-up

The cohort was re-evaluated 6 years later in 2003–2004. Persons who had completed the baseline study (n 1119) were invited by letter or by phone to attend another clinical and anthropometric examination (Fig. 1).

Procedures

Both at baseline and at follow-up, all participants underwent an interview and a standardised clinical examination. In addition, several home visits were made to the participants to collect nutritional data. The same methodology was used for both the baseline and the follow-up studies. Data were recorded on education (no studies, primary education, secondary education or apprentice training and university studies), smoking (current smoker of more than one cigarette per day) and the degree of physical exercise: slight (sit down or stand almost all day without walking), moderate (frequent walking with light loads) or intense (strenuous physical effort). Measurements were made of weight and height,

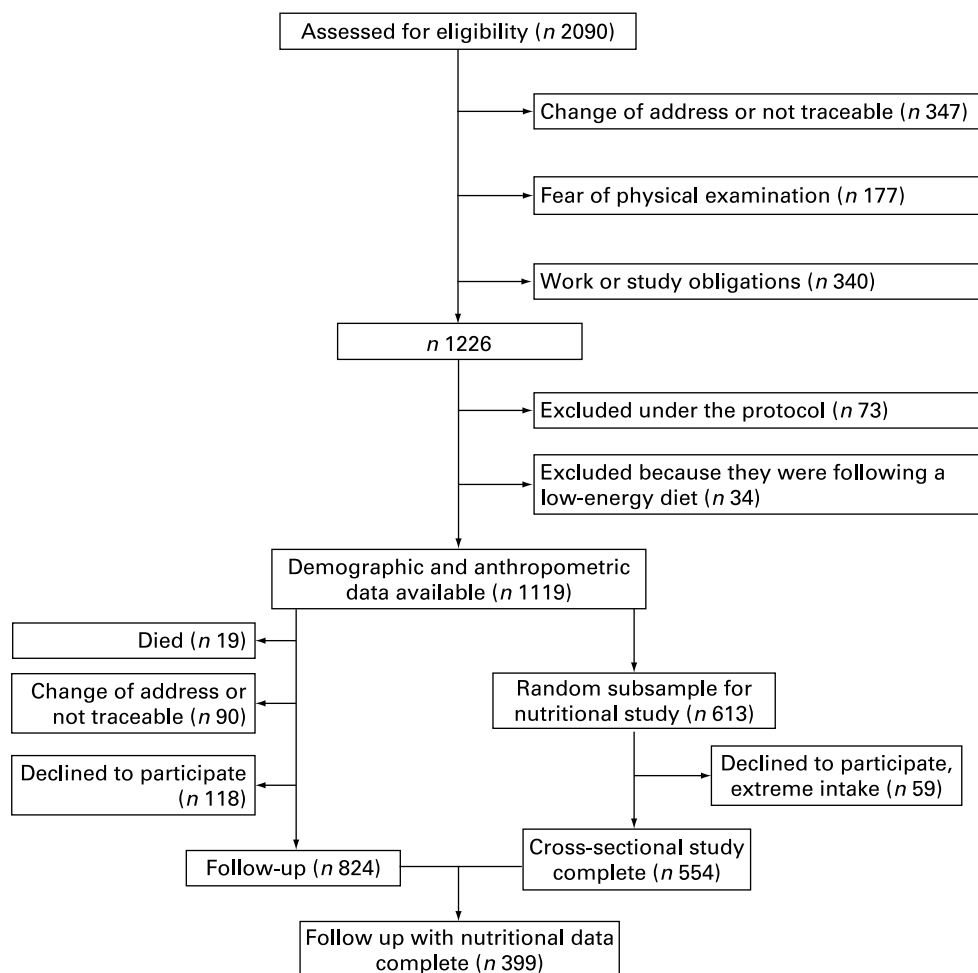


Fig. 1. Pizarra study sampling chart.

and BMI was calculated as weight in kg divided by height in square metres. A fasting blood sample was drawn at baseline and the serum stored at -70°C for later analysis.

The protocol was approved by the Ethics and Clinical Research Committee of Carlos Haya Hospital. All participants provided written informed consent.

Composition of serum fatty acids

The fatty acid composition of serum phospholipids was determined as described⁽¹²⁾. Briefly, after extraction of serum fat with chloroform–methanol (2:1) and butylated hydroxytoluene at 0.025%⁽¹³⁾ and phospholipid separation by TLC, fatty acid methyl esters were formed by heating the extracted fat for 30 min with 0.61 M H_2SO_4 in anhydrous methanol. After extraction with hexane, fatty acid methyl esters were analysed in a Hewlett–Packard chromatograph, equipped with a flame ionisation detector and using a BPX75 fused-silica capillary column (SGE, Villebon, France).

Evaluation of food intake

For the baseline study, 554 persons completed a prospective, 7 d quantitative food questionnaire at two different times during the year as well as a FFQ. We used questionnaires previously validated in a similar population⁽¹⁴⁾.

The surveys were given by experienced dietitians previously trained for this project. After making an appointment by telephone, the dietitians handed over the questionnaire in the home of the participant and provided information about the nature of the survey. The questionnaires were collected from the homes 7 d later, at which point any doubts about completing the questionnaire were resolved. Photographs were used for determinate portion sizes. The transformation to energy and macronutrients was done by a computer program that included the composition of local foods based on food composition studies, previously done by some of the authors^(15,16). For the follow-up study, 399 out of the 554 persons whose nutritional habits had been studied at baseline completed the same quantitative and FFQ, following the same procedure as before.

At the baseline study, four samples of cooking oil being used for frying were obtained from the kitchens of the participants at four different times of the year. For the second study, the samples were obtained only once.

Composition and quality of the fats used for frying

The fatty acids from frying fats were analysed by gas chromatography after derivatisation to fatty acid methyl esters with 2 M KOH in methanol and triheptadecanoin as an internal standard, according to the IUPAC standard method⁽¹⁷⁾. A HP 6890 chromatograph on a HP Innowax capillary column (Hewlett–Packard, Palo Alto, CA, USA) was used. Polar compounds were quantified by adsorption chromatography following the IUPAC standard methods 2.507⁽¹⁸⁾. TAG polymers were quantified by high-performance size exclusion chromatography following the IUPAC standard method⁽¹⁹⁾.

After analysis, samples were classified according to two criteria: fatty acid composition and level of polar compounds. Three groups of oils were defined: oils having levels of

linoleic acid higher than 50% were classified as sunflower oils (this is the seed oil principally commercialised and used in Spain), oils having less than 25% linoleic acid were classified as olive oils, while those containing between 25 and 50% linoleic acid were classified as mixtures. Two levels of polar compounds were considered in the present study, depending on whether they were higher or lower than 20%. This value was selected as indicative of significant degradation, because current official regulations in Spain establish that frying fats and oils have to be discarded for human consumption when polar compound levels are close to 25%⁽¹⁹⁾.

Statistical analyses

The results are presented as mean, standard deviation and proportions. The hypothesis contrast for qualitative variables was performed with the χ^2 test and for continuous variables with Student's *t* test or ANOVA statistics. The concordance between discrete variables was calculated with the κ index. The strength of associations between one variable (dependent) and other potentially explanatory variables was measured by calculating the OR from the regression coefficients of a multivariable logistic model. The criteria of Kleimbaum *et al.*⁽²⁰⁾ were followed for the inclusion of the independent variables, and 95% CI were calculated by the method of Miettinen⁽²¹⁾. In all cases, the level of rejection of a null hypothesis was $\alpha = 0.05$ for two tails.

Results

Macronutrient intake

No significant sex differences in age or BMI were detected in the baseline study. As expected, men consumed greater daily amounts of carbohydrate, protein and fat than women. The proportion of energy from protein and carbohydrate was similar in men and women, but the overall contribution of fats to daily energy intake was significantly greater in women, at the expense of MUFA and *n*-3 fatty acids (Table 1). BMI increased during the 6-year follow-up, but energy intake decreases in both sexes. This decrease was at expense of carbohydrate and PUFA, while MUFA increased significantly.

Influence of diet on the fatty acid composition of serum phospholipids

The composition of SFA, MUFA and *n*-3 fatty acids in serum phospholipids was significantly associated with age and the type of cooking oil used, especially MUFA, which were significantly higher in persons using olive oil for frying (Fig. 2).

Serum *n*-3 fatty acids were higher in persons who consumed fish at least once a day (Fig. 3). There were no sex differences in fatty acid composition, although *n*-6 PUFA were slightly higher in women (data not shown). Similar results were observed regarding the concentration of MUFA in serum phospholipids depending on the type of oil used for cooking or seasoning (data not shown).

The concentration of MUFA in serum phospholipids of persons who consumed margarine at least once a day, compared with those who consumed it less often, was 11.37 ± 2.39 v. 12.14 ± 2.64 %, $P=0.01$. None of the other foods or food

Table 1. Age, BMI and intake of macronutrients in the study population at baseline and after a 6 year follow-up (Mean values and standard deviations)

	Men				Women				P value (sex)	P value (Baseline v. follow-up)	r (Baseline v. follow-up, P value)
	Baseline study		Follow-up study		Baseline study		Follow-up study				
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
Age (years)	40.1	14.3	46.8	14.4	39.3	13.4	45.7	13.6	0.9	—	—
BMI (kg/m ²)	27.2	4.3	28.7	4.2	27.5	5.6	28.6	5.8	0.8	0.001	0.87 (<0.0001)
Energy (kJ/d)	10 991.37	2897.37	9267.56	3493.64	8183.90	2384.88	7727.84	2585.71	<0.0001	<0.0001	0.4 (<0.0001)
Protein (% En)	15.3	3.0	15.6	4.5	15.16	3.1	15.4	4.6	0.7	0.7	0.18 (<0.0001)
Carbohydrates (% En)	45.0	7.0	40.9	10.1	44.6	7.0	41.9	10.4	0.8	<0.0001	0.12 (0.04)
Total fat (% En)	39.6	5.8	42.7	11.0	41.9	5.7	43.4	10.4	0.003	<0.0001	0.11 (0.05)
SFA (% En)	9.5	3.1	10.2	4.1	9.9	2.2	9.7	4.2	0.7	0.1	0.10 (0.04)
MUFA (% En)	17.2	4.2	18.9	7.7	18.6	4.0	19.3	7.6	0.05	0.02	0.03 (0.5)
PUFA (% En)	7.7	4.4	4.5	2.3	12.8	5.7	4.8	2.6	0.1	<0.0001	0.07 (0.1)

% En, nutrient intake as percentage of daily energy.

* P value, ANOVA for repeated measures with adjustment by sex.

groups studied was associated with the concentration of MUFA in serum phospholipids (data not shown).

Type of cooking oil used

At the baseline study, most people used olive oil for frying (64.8%), braising (85.1%) and seasoning their food (91.5%). The concordance between the type of oil used for frying as reported during the survey and that estimated from chemical analysis was: olive oil, 70.5%; sunflower oil, 46.6%; a mixture of olive and sunflower oils, 7.1%. This concordance between the two procedures (questionnaire and laboratory analysis) to evaluate the type of oil used for frying was greater than expected by chance ($\chi^2 = 92.06$; $P < 0.0001$; $\kappa = 0.50$). Among persons who used olive oil for frying, 98.9% and 95.8% also used it for braising and seasoning, respectively, while only 29.0% and 10.5% of those who used sunflower oil for frying also used it for braising or seasoning.

After obtaining a first sample of cooking oil at baseline (sample 1 (olive oil = 54.3%)), three more samples were also taken from the homes over a 12-month period (samples 2 (olive oil = 55.0%), 3 (olive oil = 57.3%) and 4 (olive oil = 61.4%)). The concordance for olive oil of sample 1 with sample 2 ($n = 395$) was $\kappa = 0.68$, for sample 3 ($n = 101$) it was $\kappa = 0.34$, and for sample 4 ($n = 47$) it was $\kappa = 0.40$. In all cases, concordance was significantly greater than expected by chance ($P < 0.05$).

In the follow-up study (sample 5), 6 years later, 81.9% of persons who had consumed olive oil initially still used it, whereas only 57.4% of those who had used sunflower oil still did so. The net result was an increase in the number of persons who consumed olive oil (71.4%). The concordance between baseline and follow-up samples was significant ($\chi^2 = 38.9$; $\kappa = 0.20$; $P < 0.0001$).

Concentration of polar compounds in the oils used

The concentration of polar compounds and polymers in the oils used for cooking was significantly greater when the cooking oil was sunflower oil or a mixture of sunflower and olive oils, in all four samples from the first study (data are average of the four samples) as well as in the study undertaken 6 years later in the homes of the same persons (Table 2). A high concentration of polar compounds (>20%) in the oils was significantly more frequent in the samples taken from families who used sunflower oil (16.5%) or a mixture of sunflower and olive oils (15.0%) than in the samples of those using olive oil (5.0%). The use of refined olive oil or an electric deep fryer and the number of times the oil was used for frying were significantly associated with an increase in polar compounds and polymers in the oils of all samples taken (Table 3).

An ANOVA model in which the dependent variable was the concentration of polar compounds in the cooking oils and the independent variables were those in Table 3 showed that the type of oil ($P = 0.05$) and the use of a frying pan or deep fryer ($P = 0.002$) contributed independently to the explanation of the variance in the concentration of polar compounds, with a significant interaction between the use of a frying pan or deep fryer and the number of times the oil was reused ($P = 0.01$).

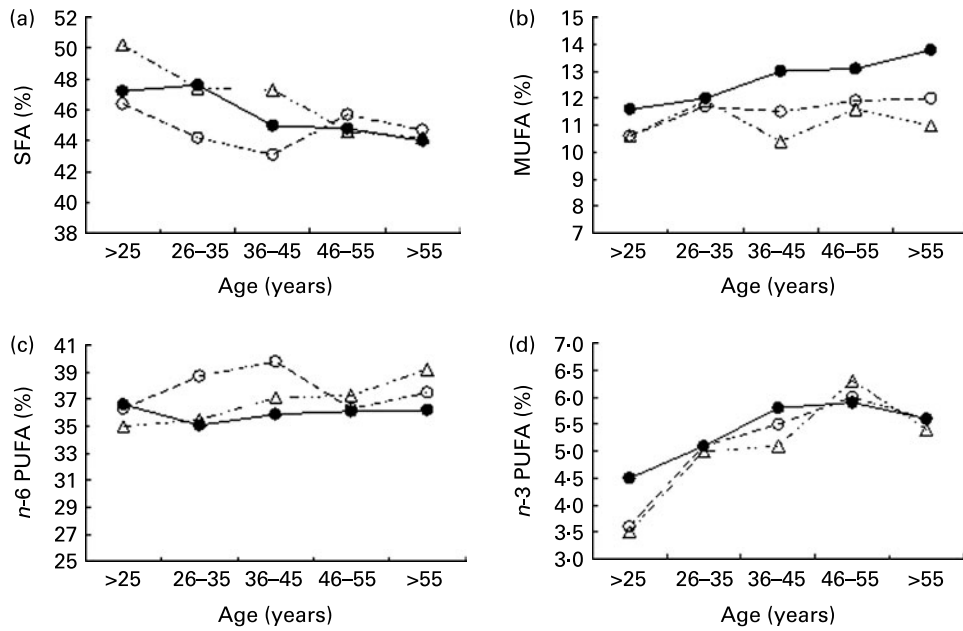


Fig. 2. Concentration of fatty acids (SFA, *n*-6, MUFA and *n*-3) in serum phospholipids according to age and type of oil commonly used in the kitchens. All *P* values are ANOVA. (a) Oil type, NS; sex, NS; age, <0.001. (b) Oil type, <0.001; sex, NS; age, <0.001. (c) Oil type, <0.05; sex, 0.03; age, NS. (d) Oil type, NS; sex, NS; age, <0.001 (●, olive oil; ○, mixed oils; △, sunflower oil).

Intake of macronutrients according to the amount of MUFA consumed

Table 4 summarises the intake of macronutrients according to the 25th percentile (P25) and the 75th percentile (P75) of the intake of MUFA, expressed as a percentage of daily energy (P75 = 20.5% of energy intake or P25 = 15.5% of energy intake) and adjusted for age, sex and BMI. Subjects with a higher proportion of energy intake from MUFA consumed less energy per day, at the expense of a lower proportion of carbohydrates. Those who had an intake of MUFA >P75 also consumed a greater proportion of saturated and *n*-3 fatty acids. Finally, subjects with the highest MUFA intake also consumed less fibre.

Nutritional habits according to the amount of MUFA consumed

The different behaviour in dietary habits according to the greater ($\geq P75$) or lesser ($\leq P25$) intake of MUFA is shown in Table 5. As the data are expressed as qualitative variables, they have been adjusted for age, sex and BMI in a logistic model in which the dependent variable was the intake of MUFA $\geq P75$ or $\leq P25$. The table only shows variables with significant differences between extremes of MUFA intake. Persons who consumed more MUFA used more olive oil for frying and less sunflower oil for braising and seasoning; ate more whole-meal bread, salads, asparagus, artichokes and cucumbers; and consumed less white bread, biscuits, nuts, peas, potatoes and sausages.

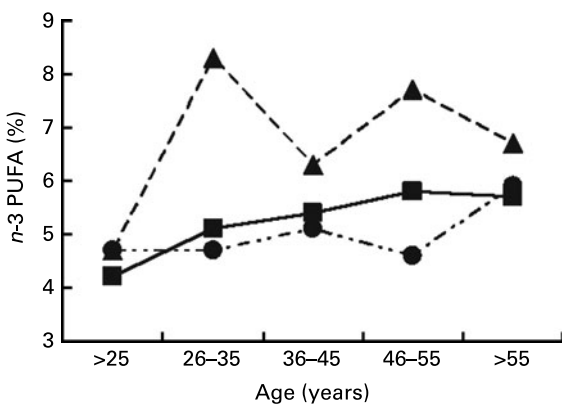


Fig. 3. Concentration of *n*-3 fatty acids in serum phospholipids according to fish intake. *P* (ANOVA): intake frequency, 0.001; sex, NS; age, <0.001 (●, once a week or less; ■, 2–6 times per week; ▲, at least once a day).

Table 2. Proportion of polar compounds and polymers in the oils from the kitchens sampled during the first-field study and in the second study 6 years later

(Mean values and standard deviations)

	Baseline study		Follow-up study	
	Mean	SD	Mean	SD
Polar compounds (%)				
Olive	8.3	3.8	9.6	4.5
Mixture	10.8	4.4	15.4	7.6
Sunflower	10.9	4.8	14.6	5.7
<i>P</i> *	<0.0001		0.001	
Polymers (%)				
Olive	1.8	2.2	1.9	2.2
Mixture	3.2	2.4	5.3	4.0
Sunflower	3.2	2.6	4.6	2.7
<i>P</i> *	<0.0001		<0.0001	

* *P* value, differences among oil types by one-way ANOVA.

Table 3. Polar compounds and polymers according to the quality of the oil, the cooking utensil used for frying and the number of times the same oil was used for frying

(Mean values and standard deviations)

	Polar compounds (%)		Polymers (%)	
	Mean	SD	Mean	SD
Quality of oil				
Virgin or virgin extra	9.6	4.5	1.9	2.1
Refined	14.5	6.9	4.8	3.6
<i>P</i> *	<0.0001		<0.0001	
Frying methods				
Pan frying	10.1	4.4	2.3	2.2
Deep frying	12.6	6.8	3.4	3.1
Electric deep frying	19.3	9.9	7.1	5.5
<i>P</i> *	<0.0001		<0.0001	
Number of times oil used for frying				
1–2	10.1	4.5	2.3	2.3
3–4	11.6	6.4	3.1	3.3
5 or more	21.9	5.7	8.4	5.6
<i>P</i> *	<0.0001		<0.0001	

* *P* value, one-way ANOVA.*Educational level and health habits according to the type of cooking oil used*

Persons who used only olive oil (as evaluated by chemical analysis of the fats used for cooking) had a higher level of education, more often consumed alcoholic drinks and were more physically active (Table 6). The differences remained significant after calculating the probability of consuming olive oil *v.* sunflower oil (alone or mixed with olive oil) in a logistic model adjusted for age, sex and BMI.

Discussion

Mediterranean countries show differences in dietary habits, food preparation and other health-related habits⁽²²⁾, but the common denominator in Mediterranean diets is the consumption of olive oil. The present report stems from the 'Pizarra study'^(11,14,23,24), a prospective investigation of diet and

health in a population from Southern Spain with a high prevalence of obesity and type 2 diabetes. The nutritional pattern in this population is defined by a higher intake of energy than recommended, and a high intake of energy from fat (average 41.1% of daily energy), especially MUFA, accounting for 18.2% of the daily energy consumption. This intake of MUFA, which can be equated to olive oil, is similar to that reported for Southern Italy⁽²⁵⁾. The present study shows that in this population from Southern Spain, persons who consumed more MUFA consumed less energy per day, fewer carbohydrates and more total fat (some 47% of the daily energy). They also consumed more SFA and *n*-3 PUFA than those who consumed less MUFA. These results differ in part from other studies undertaken in Northern Spain, which found that persons who consumed more MUFA also consumed a lower proportion of saturated fats⁽⁷⁾. Despite this high intake of MUFA, the *n*-6:*n*-3 fatty acid ratio was very high, similar to that of other Western countries (>10:1)⁽²⁵⁾ and far higher than that of diets in primitive societies⁽²⁶⁾. As expected, the *n*-6:*n*-3 fatty acid ratio was greater in persons who consumed less MUFA. On the other hand, persons with the greatest intake of MUFA (olive oil) consumed less white bread and sugar, fewer potatoes and sausages and more greens, such as salads or asparagus. Additionally, the persons who used olive oil for cooking had a higher level of education, drank more wine with meals and exercised more. The results are in partial agreement with those of other authors^(7,25), and confirm that the nutritional behaviour in the Mediterranean populations studied thus far is closely associated with the consumption of olive oil and depends in part on cultural level.

In a large, population-based cohort study, Trichopoulou *et al.*⁽²⁾ found that a higher degree of adherence to the Mediterranean diet, measured on a nine-point scale, was associated with a reduction in total mortality, even though no strong association was evident for the individual components of the Mediterranean diet score. These authors suggested various explanations for this finding. However, the quantification of the amount and type of fat in population studies are particularly difficult⁽⁵⁾, due, among other reasons, to both intra- and inter-person variability. In the present study, we administered a 7 d quantitative food survey at two different times of the

Table 4. Intake of macronutrients according to the intake of MUFA, expressed as percentage of daily energy (% En)

(Mean values and standard deviations)

	MUFA intake percentile						<i>P</i> *
	<P25		P25–P75		>P75		
	Mean	SD	Mean	SD	Mean	SD	
Energy (kJ/d)	9740.35	3192.39	9405.63	2870.22	8183.90	2573.16	0.00001
Proteins (% En)	15.4	3.2	15.5	2.8	15.5	3.4	NS
Carbohydrates (% En)	50.0	6.7	45.1	5.0	38.8	6.0	0.00001
Total fat (% En)	35.6	4.5	40.8	3.7	47.0	4.9	0.00001
SFA (% En)	8.2	2.1	10.1	2.7	10.7	2.0	0.00001
MUFA (% En)	13.3	1.9	17.9	1.4	23.5	3.1	0.00001
PUFA <i>n</i> -6 (% En)	4.9	2.1	5.0	1.8	5.1	1.4	NS
PUFA <i>n</i> -3 (% En)	0.3	0.1	0.4	0.1	0.5	0.1	0.00001
<i>n</i> -6: <i>n</i> -3 ratio	15.7	10.2	12.5	6.3	10.8	4.5	0.00001
Fibre (g/d)	16.3	6.9	16.5	6.7	14.6	5.8	0.04

25th Percentile (P25)=13.5% and 75th percentile (P75)=20.5%.

* *P* value, one-way ANOVA adjusted by age, sex and BMI.

Table 5. Probability of intake of different foods by extremes of MUFA intake (OR and 95% CI values)

	MUFA intake percentile		OR*	95% CI
	<P25	>P75		
Olive oil just used for frying (%)	60.9	72.5	1.94	1.06, 3.59
Sunflower oil for seasoning (%)	13.1	2.2	0.14	0.04, 0.51
Sunflower oil for braising (%)	13.5	2.2	0.14	0.04, 0.51
Sunflower oil for frying (%)	37.1	25.6	0.51	0.37, 0.93
Whole-meal bread daily (%)	11.9	24.6	1.95	1.06, 3.61
White bread one or more times per day (%)	72.2	51.8	0.45	0.17, 0.87
Potatoes daily (%)	42.2	27.6	0.45	0.17, 0.87
Biscuits (%)	13.3	7.2	0.53	0.38, 0.94
Sausages daily (%)	38.0	26.1	0.52	0.37, 0.93
Peas one or more times per week (%)	58.8	51.8	0.59	0.41, 0.98
Nuts one or more times per week (%)	13.9	8.0	0.42	0.16, 0.86
Salad daily (%)	54.3	65.0	1.29	1.02, 3.00
Asparagus one or more times per week (%)	18.8	32.8	1.93	1.04, 3.50
Cucumber one or more times per week (%)	38.7	53.4	2.02	1.19, 3.50

* Likelihood of consuming MUFA % En \geq P75, OR adjusted by age, sex and BMI. Only foods with a significant association with MUFA intake are shown. The following foods were also evaluated but were not associated with MUFA: ice cream; cured cheese; caramel cream and custard; white cheese; semi-cured cheese; milk and yoghurt; lentils; beans; cereals; rice; pasta; margarine; butter; commercial mayonnaise; lard, eggs; marmalade; pastries; tinned fish; sea food; meat; mixed vegetables; braised vegetables; Swiss chard and spinach; artichokes; green beans; carrots; aubergine; courgette; onions; mushrooms; cabbage; broad beans; maize; pepper; beetroot; tomato; fresh fruit; coffee; legumes; fish.

year, accompanied by a FFQ. In addition to identifying the oil used for cooking, we measured the fatty acids in samples of cooking oil taken from the homes of the participants. The questionnaire was validated by measuring the fatty acid composition of the serum phospholipids. Also, the study was undertaken in the context of a longitudinal study, which enabled us to evaluate the consistency of nutritional habits over time. The greatest source of MUFA was olive oil, which is used both for frying and braising, as well as for seasoning. On the other hand, many of the participants who used sunflower oil for frying used olive oil for braising or seasoning. The fatty acid composition in tissues is specific for each tissue, although it may be modified by the type of dietary fatty acid^(27–29). Subjects who consumed olive oil had a greater proportion of MUFA in their serum phospholipids and those who consumed sunflower oil had a more *n*-6 PUFA, while the *n*-3 fatty acids' content was associated with the intake of fish, indicating that the questionnaires were sufficiently sensitive to correspond with the biological markers. The results of the present study nevertheless warn of the need to use objective measurements beyond food records, as 16% of subjects who thought they were using olive oil were in fact cooking with a mixture of olive oil and sunflower oil, which led to an overrepresentation of the intake of MUFA in the overall evaluation. However, fatty acid exchange between the cooking oil and the food being cooked could explain in part the compositional differences in home oils⁽³⁰⁾. On follow-up, there was an increase in the intake of olive oil, which may be due to the Hawthorne

effect in population-based studies⁽³¹⁾ or to campaigns by the health authorities on its healthy properties.

Fried foods are popular all over the world due to their crunchiness and smell, and to their ease of cooking. Even though a certain amount of potentially toxic products are produced during frying (such as polar compounds or polymers), fried foods are considered safe^(32,33). Previous laboratory studies have shown that olive oil is more resistant than sunflower oil to deterioration by reuse in frying^(14,34–36). The present study confirms that in a natural environment, as is the home, persons who use sunflower oil more often have a high proportion of polar compounds and polymers in their cooking oil. The study also shows that the concentration of polar compounds and polymers in the oils depends on the way they are used with oil from kitchens using a deep fryer having a higher proportion. The evaluation of potentially toxic compounds produced by the inadequate use of vegetable oils in the kitchen is not usually contemplated in nutritional surveys. Recent experimental studies have shown that oxidised frying oil can have important effects on lipid metabolism⁽³³⁾, and we have recently reported an association between the concentration of polar compounds in the oil used for frying in the kitchens of family homes and the risk of hypertension⁽¹²⁾.

The present study also shows that persons with a greater intake of MUFA from olive oil had a higher educational level, were more physically active and consumed more vegetables than those who consumed sunflower oil, which resulted in a lower daily energy intake. This is a particularly important point if we consider that the prevalence of obesity and a sedentary lifestyle is very high in this population⁽³⁷⁾, although similar to that reported in other countries^(25,38).

The association found between a greater intake of MUFA and a higher educational level suggests that the concept of a 'Mediterranean diet' is mainly associated with the intake of olive oil, as well as with other healthy habits linked to adequate health information. Important changes have occurred over recent years in the nutritional patterns of countries about the Mediterranean basin. As pointed out by Willett⁽³⁹⁾ paradoxically, the discoveries concerning the biological

Table 6. Educational level and health habits according to the oil used for cooking, as identified by measurement of the fatty acids by GC in the oils taken from the kitchen (OR and 95% CI values)

	Olive oil	Mixture or sunflower oil	OR of consuming olive oil v. mixture or sunflower oil*	95% CI
Education				
No studies (%)	50.3	49.7	6.11	1.93, 15.18
Primary (%)	40.5	50.5	4.81	1.64, 11.36
Secondary (%)	54.2	45.8	2.97	1.04, 6.56
Apprentice (%)	66.7	33.3	1.75	0.61, 4.69
University (%)	78.6	21.4	1.0	Reference
Intake of alcohol (at least 1 drink/d) (%)	59.1	51.1	1.39	0.75, 1.84
Smoker (at least 1 cigarette/d) (%)	34.4	34.9	0.97	0.94, 1.21
Physical activity (%)				
Moderate + Intense	15.6	8.8	1.0	Reference
Slight	84.4	91.2	1.86	1.05, 5.10

* All logistic regression models adjusted by age, sex and BMI.

value of the Mediterranean diet are in fact proving more useful for other countries that are changing their nutritional habits to approach the pattern of a Mediterranean diet than for the Mediterranean countries themselves. The increased prevalence of obesity in Mediterranean countries^(24,25) is an example of this change in lifestyle. The present study shows the difficulty reconciling the usual recommendations in Western countries to reduce dietary fats with the traditional nutritional behaviour associated with the Mediterranean diet, in which there is a high intake of fat as olive oil. However, none of the studies undertaken in Mediterranean populations has shown an association between the intake of olive oil and increased BMI^(7,24,40). Additionally, the role of fat in the genesis of the epidemic of obesity in industrialised countries is still unsettled^(5,6). The increased intake of saturated fats associated with the higher intake of MUFA found in the present study points to the need of designing selective educational strategies depending on the dietary and cooking habits of each region at any one time^(41,42).

The results show concordance in the use and consumption of olive oil in the same population over time, but also reflect imprecision in the awareness of the type of fat or vegetable oil being used. They also show that the preferential intake of MUFA from olive oil is associated with other health and nutritional habits. This association was conditioned by the educational level of the study population. The study also suggests that nutritional epidemiology would benefit from incorporating techniques for controlling home cooking, with the kitchen being considered as a small, non-controlled experimental laboratory. Finally, the results support the idea that the general recommendations on changes of what is known as the 'Western diet' should be adjusted to the Mediterranean countries, and to their particular culinary and health habits.

Acknowledgements

We are grateful to Ian Johnstone for his help with the English language version of the manuscript. The present study was supported by Fondo de Investigación Sanitaria (PI041883, PI051307), Junta de Andalucía (0124/2005, P06-CTS-01 684) and Fundación Centro de Excelencia en Investigación sobre Aceite de oliva y Salud (CEAS). CIBERDEM and CIBEROBN are initiatives of Instituto de Salud Carlos III, Spain.

Conflicts of interest. None disclosed.

Contribution of each author. Design and coordination: F. S. Provision of original data: M. C. A., I. C., J. M. G.-A., F. L., S. M., E. G.-E., M. C. D., G. O., V. H., S. V., M. S. R.-A. and I. E. Analysis of data and writing the draft of the manuscript: F. S., G. R.-M. Interpretation of results and contributions to the writing of the manuscript: all authors. *Sources of support.* Grants from ISCIII (PI041883, PI051307), Andalucía Government (0124/2005) and Asociación Maimónides.

References

1. Willett WC, Sacks F, Trichopoulos A, *et al.* (1995) Mediterranean diet pyramid: a cultural model for healthy eating. *Am J Nutr* **61**, Suppl., 1402–1406.
2. Trichopoulos A, Costacoy T, Bamia Ch, *et al.* (1993) Adherence to a mediterranean diet and survival in a Greek Population. *N Engl J Med* **348**, 2599–2608.
3. Keys A (1995) Mediterranean diet and public health: personal reflections. *Am J Clin Nutr* **61**, Suppl., S1321–S1323.
4. Hu FB (1993) The Mediterranean diet and mortality. Olive oil and beyond. *N Engl J Med* **348**, 2595–2596.
5. Willett WC (1998) Is dietary fat a major determinant of body fat? *Am J Clin Nutr* **67**, Suppl., 556S–562S.
6. Bray GA & Popkin BM (1998) Dietary fat intake does affect obesity. *Am J Clin Nutr* **68**, 1157–1173.
7. Serra-Majem LI, Ngo de la Cruz J, Ribas L, *et al.* (2003) Olive oil and the mediterranean diet: beyond the rhetoric. *Eur J Clin Nutr* **57**, Suppl. 1, S2–S7.
8. Alvarez de Cienfuegos G, Badimon L, Barja G, *et al.* (2005) International conference on the healthy effect of virgin olive oil. Consensus report. *Eur J Clin Invest* **35**, 421–424.
9. Soriguer F, Rojo G, Rodriguez F, *et al.* (2007) Obesity and the metabolic syndrome in Mediterranean countries: A hypothesis related to olive oil. *Mol Nutr Food Res* **51**, 1260–1267.
10. Soriguer F, Esteva I, Rojo G, *et al.* (2002) Prevalence of diabetes mellitus type LADA in South Spain. *Diabetes Res Clin Pract* **56**, 213–220.
11. Soriguer F, Rojo-Martínez G, Almaraz MC, *et al.* (2008) Incidence of type 2 diabetes in South Spain (Pizarra Study). *Eur J Clin Invest* **38**, 126–133.
12. Soriguer F, Rojo G, Dobarganes MC, *et al.* (2003) Hypertension is related to the degradation of dietary frying oils. *Am J Clin Nutr* **78**, 1092–1097.
13. Soriguer F, Gonzalez-Romero S, Esteva de Antonio I, *et al.* (1992) Validación de una encuesta nutricional. *Nutrición Clínica* **12**, 33–41.
14. Mataix J, Garcia L, Mañas M, *et al.* (2003) *Tabla de composición de alimentos*. Granada: Editorial Universidad de Granada.
15. Soriguer F, Serna S, Valverde E, *et al.* (2007) Lipid, protein, and calorie content of different atlantic and mediterranean fish, shellfish and mollusc commonly eaten in the south of Spain. *Eur J Epidemiol* **13**, 451–463.
16. IUPAC (1992) *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 1st Supplement to the 7th Edition [International Union of Pure and Applied Chemistry, IUPAC, editor]. Oxford: Pergamon Press.
17. Wolf JP, Mordret FX & Dieffenbacher A (1991) Determination of polymerized triglycerides in oils and fats by high performance liquid chromatography. *Pure Appl Chem* **63**, 1163–1170.
18. Firestone D (1996) Regulation of frying fats and oils. *Deep Frying: Chemistry, Nutrition and Practical Applications*, pp. 323–334, chap. 19 [EG Perkins and MD Erickson, editors]. Champaign, IL: Publisher: AOCS Press.
19. Kleimbaum DG, Kupper LL & Keith EM (1988) *Applied Regression Analysis and Other Multivariable Methods*. Boston, MA: PWS-Kent Publishing.
20. Miettinen OS (1976) Stimability and stimation in case-referent studies. *Am J Epidemiol* **103**, 226–235.
21. Nestle M (1995) Mediterranean diet: historical and research overview. *Am J Clin Nutr* **61**, Suppl., S1313–S1320.
22. Soriguer F, Esteva I, Rojo G, *et al.* (2004) Oleic acid from cooking oils is associated with lower insulin resistance in the general population (Pizarra study). *Eur J Endocrinol* **150**, 33–39.
23. Morcillo S, Cardona F, Rojo G, *et al.* (2005) Association between MspI polymorphism of the APO AI gene and type 2 diabetes mellitus. *Diabetes UK. Diabetic Med* **22**, 782–788.
24. Soriguer F, Rojo G, Esteva I, *et al.* (2004) Prevalence of obesity in south-east Spain and its relation with social and health factors. *Eur J Epidemiol* **19**, 33–40.
25. Bargallo CM, Cavera G, Sapienza M, *et al.* (2002) Nutritional characteristics of a rural southern Italy population: The Ventimiglia di Sicilia Project. *J Am Coll Nutr* **21**, 523–529.

26. Eaton SB, Konner MJ & Shostak M (1996) An evolutionary perspective enhances understanding of human nutritional requirements. *J Nutr* **126**, 1732–1740.
27. Soriguer F, Tinahones F, Monzón A, *et al.* (2000) Varying incorporation of fatty acids in muscle, adipose, pancreatic exocrine tissue and thymocytes in adult rats fed with diet rich in different fatty acids. *Eur J Epidemiol* **16**, 585–594.
28. Rojo G, Soriguer FJ, González S, *et al.* (2000) Serum leptin and habitual fatty acids dietary intake in diabetes mellitus type 1 patients. *Eur J Endocrinol* **142**, 263–268.
29. Oliveira G, Oliveira C & Dorado A (2003) Serum phospholipid fatty acid profile and dietary intake in a Mediterranean adult population with cystic fibrosis. *Clin Nutr* **22**, Suppl. 1, S18–S190.
30. Small DM, Oliva C & Tercyak A (1991) Chemistry in the kitchen. Making ground meat more healthful. *N Engl J Med* **324**, 73–77.
31. McCarney R, Warner J, Iliffe S, *et al.* (2007) The Hawthorne Effect: a randomised, controlled trial. *BMC Med Res Methodol* **7**, 30.
32. Artman NR (1969) The chemical and biological properties of heated and oxidized fats. *Adv Lipid Res* **7**, 245–330.
33. Chao PM, Hsu SC, Lin FJ, *et al.* (2004) The up-regulation of hepatic acyl-CoA oxidase and cytochrome P450 4A1 mRNA expression by dietary oxidized frying oil is comparable between male and female rats. *Lipids* **39**, 233–238.
34. Cuesta C, Sánchez-Muñiz FJ & Varela G (1988) *Frying of Food*, pp. 112–128 [G Varela, AE Bender and ID Morton, editors]. Chitester: Ellis Horwood, Ltd.
35. Marquez G, Pérez MC & Dobarganes MC (1990) Evaluación nutricional de grasas termooxidadas y de fritura. *Grasas y Aceites* **6**, 432–4390.
36. Pérez-Camino MC, Márquez Ruiz G, Ruiz-Mendez MV, *et al.* (1991) Lipid changes during frying of frozen prefried foods. *J Food Sci* **56**, 1644–1650.
37. Soriguer F, Rojo Martínez G, Esteva I, *et al.* (2003) Physical activity and cardiovascular and metabolic risk factors in general Population. *Med Clin (Barc)* **121**, 565–569.
38. Martínez MA, Varo JJ & Santos JL (2001) Prevalence of physical activity during leisure time in the European Union. *Med Sci Sport Excer* **33**, 1142–1146.
39. Willett WC (2006) The Mediterranean diet: science and practice. *Public Health Nutr* **9**, 105–110.
40. Trichopoulou D, Gnardellis C & Benetou V (2002) Lipid, protein and carbohydrate intake in relation to body mass index. *Eur J Clin Nutr* **56**, 37–43.
41. Serra-Majen L, Ferro-Lizzi A, Bellizzi MC, *et al.* (1997) Nutrition policies in Mediterranean Europe. *Nutr Rev Suppl. II*, **55**, S39–S54.
42. World Health Organization (1998) Preparation and use of food-based dietary guidelines: report of a joint FAO/WHO consultation. *Technical Report Series*, no. 880 Geneva: WHO.