

CLINICAL STUDY

Oleic acid from cooking oils is associated with lower insulin resistance in the general population (Pizarra study)

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Abstract

Aim: To evaluate the relation between type of dietary fatty acid and degree of insulin resistance.

Design: A cross-sectional study.

Methods: Anthropometrical data were measured in 538 subjects, aged 18–65 years, selected randomly from the municipal census of Pizarra (Spain). An oral glucose tolerance test (OGTT) was given to all subjects and measurements were made of glycemia, insulinemia and the proportion of fatty acids in plasma phospholipids. Insulin resistance (IR) was estimated by homeostasis model assessment. Samples of cooking oil being used were obtained from the kitchens. The strength of association between variables was measured by calculating the odds ratio (OR) from logistic models, and the relationships were measured by linear correlation coefficients.

Results: Insulin resistance was significantly less in people who used olive oil compared with those who used sunflower oil or a mixture. Statistical significance remained in the group of people with normal OGTT after adjusting for obesity. In the whole sample, IR correlated negatively with the concentration of oleic acid ($r = -0.11$; $P = 0.02$) and positively with that of linoleic acid ($r = 0.10$; $P = 0.02$) from the cooking oil. In subjects with normal OGTT, IR correlated negatively with oleic acid from cooking oil ($r = -0.17$; $P = 0.004$) and from plasma phospholipids ($r = -0.11$; $P = 0.01$) and positively with the concentration of linoleic acid in cooking oil ($r = 0.18$; $P = 0.004$) and plasma phospholipids ($r = 0.12$; $P = 0.005$). The risk (OR) of having raised IR was significantly lower in people who consumed olive oil, either alone (OR = 0.50) or mixed (OR = 0.52) compared with those who consumed only sunflower oil.

Conclusion: There is an association between the intake of oleic acid, the composition of oleic acid in plasma phospholipids and peripheral insulin action.

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Introduction

Insulin resistance, glucose intolerance and hyperinsulinemia are the main components of the metabolic syndrome (1) and probably precede the onset of type 2 diabetes mellitus (2). The prevalence of obesity, type 2 diabetes mellitus and other risk factors associated with the metabolic syndrome have increased markedly in the developed world (3). The roles of genetics and lifestyle in this increase are under debate (4). Although Himsworth showed that macronutrients in the diet can influence glucose tolerance in healthy subjects (5), studies of the role of diet in the risk of diabetes have been inconsistent. Some authors have found no association (6, 7) whereas others have found some degree of association with a particular nutrient (8, 9). Most population studies have focused on evaluating the relationship between diet and risk of type 2 diabetes

mellitus. Overall, they indicate that intake of fats, especially saturated fats, has an adverse effect on the risk of diabetes, whilst intake of fibre is beneficial (10). There is general agreement about increased insulin resistance with saturated fatty acids (11) and reduced insulin resistance with n-3 fatty acids (12), although the effect of n-6 fatty acids on insulin resistance is controversial (13). The association between dietary intake of oleic acid and insulin resistance is less well understood. A relationship has been found between insulin resistance and levels of some monounsaturated fatty acids (MUFA), such as palmitoleic acid (14), but studies of oleic acid have reported contradictory results. Some studies have shown a reduction in insulin resistance with diets rich in oleic acid (15), whereas others have either found no relationship (16) or have even shown an inverse association between oleic acid and insulin sensitivity (17).

In a group of people from southern Spain who have a high intake of olive oil in their diet and who commonly cook their food by frying, we tested the hypothesis of a possible association between oleic acid intake and insulin resistance. Results suggest that the greater richness in oleic acid of frying oil is associated with lower insulin resistance.

Materials and methods

The study was undertaken in Pizarra, a small town in the province of Malaga, Andalusia, southern Spain. Details of the study design and sample have been reported previously (18). A total of 538 subjects, aged 18–65 years, were selected randomly from the municipal census. All institutionalized persons, for whatever reason, were excluded from the study, as were pregnant women, and those with a severe clinical problem or psychological disorder. The subjects were requested by mail to attend their local health centre for a medical examination. Those who failed to attend their first appointment were sent a second letter giving them another appointment, and all those still not attending were visited at home in order to ascertain the reason. The final sample distribution by age and sex was not significantly different from the population distribution (19).

Procedures

All participants were interviewed and given a standardized clinical examination by the same doctors (I E, M S R A, J M G A). Measurements were made of weight and height and the body mass index (BMI) was calculated ($\text{weight}/\text{height}^2$) (20). To determine the presence of carbohydrate metabolism disorders each person received an oral glucose tolerance test (OGTT) with 75 g glucose. Capillary glycaemia was measured at baseline and 120 min after the OGTT with Glucometer-Elite (Bayer, Barcelona, Spain). Venous blood samples were taken at base line and the serum stored at -70°C for later study of insulin and fatty acid composition in plasma phospholipids.

During a home visit a sample was taken of the oil being used for cooking at that time. To avoid the oil being swapped for newer oil, the family was unaware of the intention to request a sample of their oil until the time of the visit by the investigator. All participants authorized the collection of these samples of oil from their kitchens.

Laboratory measurements

Baseline insulinaemia was measured by radioimmunoassay (Coat A Count Insulin, DPC, Los Angeles, CA, USA). Insulin resistance was estimated by homeostasis model assessment (HOMA) (21), according to the

formula: $\text{insulin resistance index} = \text{fasting insulin } (\mu\text{U}/\text{ml}) \times \text{fasting glucose } (\text{mmol}/\text{l})/22.5$.

The fatty acid composition of serum phospholipids was carried out by extraction of the serum fat with chloroform:methanol 2:1 and butylated hydroxytoluene (BHT) at 0.025% (22) and phospholipid separation by TLC. Fatty acid methyl esters were formed by heating the extracted fat for 30 min with 0.61 mol/l H_2SO_4 in anhydrous methanol. After extraction with hexane, the fatty acid methyl esters were analyzed in a Hewlett Packard chromatograph, equipped with a flame ionization detector and using a BPX75 fused-silica capillary column (SGE, Villebon, France).

Composition and quality of cooking oil

Fatty acids from the cooking oil were analyzed by gas chromatography after derivatization to fatty acid methyl esters with 2 M KOH in methanol and using triheptadecanoin as internal standard according to the IUPAC standard method (23). An HP 6890 chromatograph on an HP Innowax capillary column (polyethylene glycol, 30 m \times 0.25 mm i.d., film thickness 0.25 μm) (Hewlett Packard), was used under the following temperature program: 180°C (4 min), $4^\circ\text{C}/\text{min}$ to 230°C (15 min). Samples were introduced into the column via a split injector (split ratio 1:40) at 250°C and the flow rate of hydrogen, used as carrier gas, was 1 ml/min. The temperature of both split injector and flame ionization detector was 250°C . After analysis, samples were classified according to fatty acid composition.

Classification criteria

Since only olive oil and sunflower oil are commercially available for domestic use in Spain, three groups of oils were defined: oils having levels of linoleic acid higher than 50%, which were classified as sunflower oil; oils having less than 25% linoleic acid, classified as olive oil; and those containing between 25% and 50% linoleic acid, which were classified as a mixture.

The American Diabetes Association (ADA) 1998 criteria were used for the classification of people with diabetes and carbohydrate metabolism disorders (24). A normal OGTT was considered to be baseline capillary glycaemia lower than 100 g/dl and post-OGTT glycaemia lower than 140 g/dl (24). Obesity was set at a BMI of 30 or above (25).

Statistical study

Statistical differences between means of continuous variables were studied with one-way ANOVA, and the chi-square test was used for qualitative variables. In all cases the rejection level for a null hypothesis was $\alpha = 0.05$ for two tails. The strength of association between variables was measured by calculating

the odds ratio (OR) from logistic regression models according to Kleimbaum *et al.* (26) and the confidence intervals of the OR according to Miettinen (27).

Ethical considerations

All subjects were informed of the nature of the study and gave their written consent to participate. The study was approved by the Ethics and Clinical Investigation Committee of Carlos Haya Hospital.

Results

The mean age of the participants was 39.97 ± 13.82 years, range: 17–68 years; 37.5% were men and 62.5% were women. The age and sex of the sample were not statistically different from those of the whole population. Of the participants, 53.6% consumed just olive oil, 21.6% just sunflower oil, and 24.8% a mixture of both. Table 1 shows the mean composition of the frying oils used. There were no differences in age, sex or BMI according to the type of oil consumed (data not shown).

The fatty acid composition of the plasma phospholipids followed the pattern expected from the fatty acid composition of the cooking oil used (Table 2), with a significant positive correlation for oleic acid

($r = 0.32$, $P = 0.0001$) and for linoleic acid ($r = 0.17$, $P = 0.001$).

Insulin resistance was significantly lower in people who consumed olive oil than in those who consumed sunflower oil or a mixture, for both the overall sample, which included people with some glycemic disorder ($P = 0.01$) and for those with a normal OGTT ($P = 0.006$) (Table 3). As expected, subjects with an abnormal OGTT had higher IR-HOMA values than those with normal OGTT ($P < 0.0001$ between the three groups). Although IR-HOMA values were lower in the olive oil group, they were not statistically different from those of the sunflower oil group ($P = 0.23$) (Table 3). After adjusting for obesity and carbohydrate metabolism disorders this relationship was no longer significant for the whole sample ($P = 0.40$), but after adjusting for obesity the relationship was still significant in those with a normal OGTT ($P = 0.006$).

The degree of insulin resistance in the overall sample correlated negatively with the oleic acid ($r = -0.11$; $P = 0.02$) and positively with the linoleic acid concentration ($r = 0.10$; $P = 0.02$) of the cooking oils consumed. In subjects with a normal OGTT there was a significant negative association between insulin resistance and oleic acid from both cooking oil ($r = -0.17$; $P = 0.004$) and plasma phospholipids ($r = -0.11$; $P = 0.01$) and a positive association with linoleic acid in cooking oil ($r = 0.18$; $P = 0.004$) and plasma phospholipids ($r = 0.12$; $P = 0.005$).

Table 4 shows the risk (OR) of having an insulin resistance above the 75th percentile of the sample population ($P_{75} = 3.15$). This risk was significantly lower in people who consumed olive oil alone (OR = 0.50) or mixed oils (OR = 0.52) compared with those who just used sunflower oil. As expected, the risk of insulin resistance was related to obesity (OR = 1.98), impaired fasting glucose (IFG) (OR = 3.76), impaired glucose tolerance (IGT) (OR = 2.27) and diabetes mellitus (OR = 18.29). The strength of the association between the consumption of olive oil and the reduced risk of insulin resistance was hardly changed by the inclusion in the model of obesity, diabetes mellitus and other carbohydrate metabolism disorders (model 5). However, as can be seen in models 2 and 3, the association between risk of insulin resistance and concentration of oleic acid in cooking oil and plasma phospholipids, considered as continuous variables, suggests a dose-dependant reduction in risk. This negative dose-dependant association between MUFA and insulin resistance was also seen in people with normal OGTT (Table 5), even after inclusion of BMI in the model (Table 5, model 4).

Discussion

We evaluated the association in the general population between the composition of cooking oil and peripheral

Table 1 Concentrations (means \pm S.D.) of the fatty acids in the cooking oils.

	Type of oil used		
	Olive	Mixture	Sunflower
Palmitic acid (%)	11.2 \pm 2.1 ^a	8.3 \pm 1.8 ^b	7.6 \pm 1.2 ^c
Stearic acid (%)	2.9 \pm 0.4 ^b	3.5 \pm 0.5 ^a	3.7 \pm 0.4 ^a
Oleic acid (%)	73.0 \pm 4.8 ^a	45.7 \pm 7.0 ^b	29.6 \pm 4.1 ^c
Linoleic acid (%)	10.2 \pm 4.3 ^c	39.9 \pm 7.1 ^b	56.8 \pm 4.8 ^a

For each fatty acid, the means with a different letter were significantly different ($P = 0.0001$, one-way ANOVA).

Table 2 Fatty acid composition of the plasma phospholipids according to the type of cooking oil used.

	Type of oil		
	Olive	Mixture	Sunflower
Myristic acid (%)	0.39 \pm 0.47 ^a	0.41 \pm 0.52 ^a	0.50 \pm 0.59 ^a
Palmitic acid (%)	31.4 \pm 7.0 ^a	29.7 \pm 5.3 ^a	31.3 \pm 6.7 ^a
Palmitoleic acid (%)	0.52 \pm 1.01 ^a	0.49 \pm 0.45 ^a	0.51 \pm 0.53 ^a
Estearic acid (%)	13.9 \pm 2.2 ^b	14.4 \pm 1.9 ^a	14.7 \pm 2.1 ^a
Oleic acid (%)	12.2 \pm 2.6 ^a	11.1 \pm 1.8 ^b	10.5 \pm 2.0 ^b
Linoleic acid (%)	23.9 \pm 4.3 ^b	25.9 \pm 3.8 ^a	25.1 \pm 4.0 ^a
Arachidonic acid (%)	11.9 \pm 3.9 ^a	11.9 \pm 2.8 ^a	12.0 \pm 2.9 ^a
Eicosapentaenoic acid (%)	0.73 \pm 0.63 ^a	0.66 \pm 0.51 ^a	0.66 \pm 0.54 ^a
Docosahexaenoic acid (%)	4.7 \pm 1.4 ^a	5.0 \pm 3.2 ^a	4.5 \pm 1.4 ^a

For each fatty acid, the means with a different letter were significantly different ($P = 0.0001$, one-way ANOVA).

Table 3 Insulin resistance (IR) index according to type of dietary fat. Results are means \pm s.d.

Type of oil	Insulin resistance (whole sample)	Insulin resistance (only those with normal OGTT)	Insulin resistance (only those with IGT, IFG or UKDM)
Olive	3.07 \pm 2.94 ^b	2.05 \pm 1.23 ^b	3.96 \pm 3.73
Mixture	3.07 \pm 2.46 ^b	2.17 \pm 1.52 ^a	4.99 \pm 3.17
Sunflower	3.90 \pm 3.75 ^a	2.75 \pm 1.76 ^a	5.38 \pm 4.75

In each column the means with a different letter were significantly different (one-way ANOVA) – column 1, $P < 0.01$; column 2, $P = 0.006$; column 3, $P = 0.23$.

insulin sensitivity. The study was undertaken in Pizarra, a town in the province of Malaga, Spain, near the Mediterranean coast in Andalusia, a region with a great production of olive oil (28). It is not surprising, therefore, that 75% of the study subjects used olive oil for cooking, either alone (53.6%) or in combination with sunflower oil (22.6%), figures which are similar to those for the region as a whole (29).

The body distribution of fatty acids and their incorporation into tissues from the diet is organ- and tissue-specific (30) and numerous studies have shown that plasma fatty acids are, to a certain degree, representative of dietary fatty acids (31, 32). This was also seen in our study in which those people who consumed olive oil had greater concentrations of oleic acid in their plasma phospholipids whereas those who consumed sunflower oil had greater concentrations of linoleic acid.

Numerous epidemiological, clinical, and experimental studies have demonstrated the different biological importance of fats, especially saturated and polyun-

saturated n-6 fats used for human consumption (33). The observations concerning olive oil are more recent, although the Seven Countries study had already shown an inverse relationship between consumption of monounsaturated fats and the incidence and prevalence of cardiovascular disease (34). Recent prospective studies indicate that a high intake of fats, especially fats rich in saturated fatty acids, contributes to the risk of abnormal glucose tolerance and type 2 diabetes mellitus and that fish, legumes, potatoes and vegetables may have a protective effect (8). However, the individual role of fatty acids in their different families (saturated, n-9, n-6 and n-3) is under investigation (33, 34, 35).

The metabolic syndrome is the result of an interaction between genetic predisposition and environmental factors, with insulin resistance at the crossroads of the various metabolic problems making up the syndrome (36). The levels of insulin resistance in our study were lower in people who used olive oil than in those who used sunflower oil. We have already reported

Table 4 Risk of having an insulin resistance value above the 75th percentile of the sample population according to the composition of cooking oil and other variables. Logistic regression models with the whole study population. Dependent variable: insulin resistance $> P_{75}$ (no = 0, yes = 1).

Model	Independent variables ¹	Beta	Beta error	OR	95% CI	P value
1	Olive vs sunflower cooking oil	-0.75	0.25	0.47	0.30-0.75	0.003
	Mixture vs sunflower cooking oil	-0.61	0.32	0.54	0.32-0.92	0.05
	Age	0.06	0.008	1.06	1.04-1.08	0.0001
2	Level of oleic acid in cooking oil	-0.015	0.0057	0.98	0.97-0.99	0.007
	Age	0.06	0.009	1.06	1.04-1.08	0.0001
3	Level of oleic acid in serum phospholipids	-0.14	0.03	0.87	0.82-0.92	0.0001
	Age	0.05	0.006	1.06	1.04-1.06	0.0001
4	Olive vs sunflower cooking oil	-0.64	0.27	0.53	0.33-0.87	0.02
	Mixture vs sunflower cooking oil	-0.56	0.34	0.57	0.29-1.12	0.09
	Presence of obesity	1.41	0.29	4.11	2.32-7.24	0.0001
	Age	0.04	0.009	1.04	1.02-1.06	0.0001
5	Olive vs sunflower cooking oil	-0.85	0.33	0.43	0.23-0.81	0.008
	Mixture vs sunflower cooking oil	-0.66	0.40	0.51	0.27-1.00	0.09
	Presence of obesity ²	1.19	0.33	3.29	1.72-6.30	0.0003
	Presence of IFG vs OGTT-N ³	1.84	0.36	6.32	3.10-12.8	0.0001
	Presence of IGT vs OGTT-N	0.77	0.41	2.16	0.96-4.85	0.06
	Presence of UKDM vs OGTT-N	2.75	0.41	15.79	6.95-35.16	0.0001
	Age	0.02	0.01	1.02	1.0-1.04	0.14

CI, confidence intervals.

¹ Adjusted for sex (not significant in any model).

² Presence of obesity when BMI > 30 kg/m².

³ OGTT-N; OGTT normal, when glycemia basal (capillary) < 100 mg/dl and glycemia post-OGTT (capillary) < 140 mg/dl. IFG, impaired fasting glucose; IGT, impaired glucose tolerance. UKDM, unknown diabetes mellitus, diagnosed by OGTT during the study.

Table 5 Risk of having an insulin resistance value above the 75th percentile of the sample population according to the composition of cooking oil and other variables. Logistic regression models with persons with a normal OGTT only (baseline capillary glycemia < 100 mg/dl and post-OGTT glycemia < 140 mg/dl). Dependent variable: IR > P₇₅ (no = 0, yes = 1).

Model	Independent variables ¹	Beta	Beta error	OR	95% CI	P value
1	Olive vs sunflower cooking oil	-1.40	0.51	0.24	0.09-0.67	0.006
	Mixture vs sunflower oil	-0.87	0.59	0.42	0.13-1.33	0.14
	Age	0.04	0.02	1.04	1.01-1.08	0.03
2	Level of oleic acid in cooking oil	-0.03	0.01	0.97	0.95-0.99	0.02
	Age	0.04	0.01	1.04	1.02-1.06	0.02
3	Level of oleic acid in serum phospholipids	-0.20	0.06	0.81	0.73-0.92	0.001
	Age	0.04	0.01	1.04	1.02-1.06	0.02
4	Body mass index	0.14	0.03	1.15	1.08-1.22	0.0001
	Level of oleic acid in serum phospholipids	-0.17	0.06	0.84	0.75-0.95	0.01
	Age	0.002	0.01	1.00	0.98-1.02	0.8

¹ Adjusted for sex (not significant in any model).
CI, confidence interval.

the prevalence of diabetes mellitus and carbohydrate metabolism disorders in this same population (18). Insulin resistance is known to precede in time the onset of type 2 diabetes mellitus, and people with IGT and type 2 diabetes mellitus (at least during the early phase as is the case of people with unknown diabetes mellitus (UKDM)), as well as obese people, have higher IR-HOMA levels (37, 38). Interestingly, the association in our study between type of dietary cooking oil and pattern of insulin resistance was seen in the general population, to a certain extent independently of the presence of obesity and carbohydrate metabolism disorders. Numerous studies over recent years, both experimental and clinical, have demonstrated a relationship between the type of dietary fat and insulin sensitivity (17, 39). Diets rich in saturated fatty acids are generally accepted to induce insulin resistance *in vitro* and *in vivo* (11, 39) and increased dietary levels of n-3 fatty acids raise peripheral insulin sensitivity (12, 40), with the role of n-6 fatty acids remaining controversial (13). Recent studies of the role of MUFA in insulin resistance have differed in their observations, with some finding that a MUFA-rich diet increases peripheral insulin sensitivity in both diabetic patients (15, 41) and healthy subjects (42, 43), whereas others have found that dietary oleic acid influences fat oxidation (16) and other clinical parameters such as blood pressure (44), and that it may have a negative influence on insulin sensitivity (17).

Experimental studies may help explain this effect of oleic acid on peripheral insulin action. The concentration of oleic acid is very high in tissue membranes, such as muscle or liver where the action of insulin is important (30) and changes in concentration affect cell membrane fluidity and functionality (45) and might also affect peripheral insulin sensitivity. Previous studies in rats by our group have shown that an olive-oil-enriched diet contributes to the redistribution of body fat (46) and modifies lipolytic efficiency of fat cells (47). Furthermore, n-9 fatty acids may regulate gene expression related to peripheral insulin sensitivity,

as occurs with other fatty acid families (48). Increased endothelium-dependent flow vasoreactivity (15), induction of an up-regulating effect on uncoupling protein mRNA in adipose tissue and muscle (49), and expression of GLUT2 in the liver (50) are also associated with olive oil enrichment in different experimental models. Nevertheless, it still remains necessary to elucidate the mechanisms by which enrichment with oleic acid favours peripheral insulin action.

In summary, reduced insulin resistance plays a central role in primary prevention of the metabolic syndrome and type 2 diabetes mellitus. Our study, in a Mediterranean population with a high consumption of olive oil, demonstrates an association between the amount of oleic acid in frying oils, the composition of oleic acid in plasma phospholipids and insulin resistance. The results suggest the convenience of increasing the proportion of olive oil in the daily diet of the general population and support the proposed increase in oleic acid in the dietary management of type 2 diabetes mellitus (51).

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