Dietary factors in relation to rheumatoid arthritis: a role for olive oil and cooked vegetables?[1–3]

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ABSTRACT

Background: Although several studies showed that risk of rheumatoid arthritis (RA) is inversely associated with consumption of n−3 fatty acids, the one study showing that olive oil may have a protective role has not yet been confirmed.

Objective: We examined the relation between dietary factors and risk of RA in persons from southern Greece.

Design: We studied 145 RA patients and 188 control subjects who provided information on demographic and socioeconomic variables, prior medical and family history, and present disease status. Subjects responded to an interviewer-administered, validated, food-frequency questionnaire that assessed the consumption of >100 food items. We calculated chi-square statistics for linear trend and odds ratios (ORs) for the development of RA in relation to the consumption of olive oil, fish, vegetables, and a series of food groups classified in quartiles.

Results: Risk of developing RA was inversely and significantly associated only with cooked vegetables (OR: 0.39) and olive oil (OR: 0.39) by univariate analysis. A significant trend was observed with increasing olive oil (chi-square: 4.28; $P = 0.03$) and cooked vegetable (chi-square: 10.48; $P = 0.001$) consumption. Multiple logistic regression analysis models confirmed the independent and inverse association between olive oil or cooked vegetable consumption and risk of RA (OR: 0.38 and 0.24, respectively).

Conclusions: Consumption of both cooked vegetables and olive oil was inversely and independently associated with risk of RA in this population. Further research is needed to elucidate the underlying mechanisms of this finding, which may include the antioxidant properties or the high n−9 fatty acid content of the olive oil. *Am J Clin Nutr* 1999;70:1077–82.

KEY WORDS Diet, rheumatoid arthritis, olive oil, vegetable consumption, food-frequency questionnaire, Greek diet, Greek Orthodoxy, humans

INTRODUCTION

The etiology of rheumatoid arthritis (RA), a chronic inflammatory disease, remains largely unknown, although microbiological, immune, genetic, hormonal, and dietary factors have been implicated in its pathogenesis (1). Specifically, dietary factors affect experimentally induced polyarthritis in rats (2) and accumulating evidence from intervention studies in humans suggests that supplementation of the diet with fish oil (3–16) or olive oil (7) improves the symptoms of RA, possibly by altering the production of mediators of immune and inflammatory responses (1). Thus, because an increase in n−3 fatty acid consumption ameliorates the symptoms of RA (3–16), much attention has been focused on the effect of dietary factors, particularly the effect of dietary n−6 and n−3 polyunsaturated fatty acids, in the pathogenesis and clinical course of RA (3, 17, 18).

In the traditional Greek diet, vegetables, fish, and olive oil are consumed frequently. We reported previously that lifelong consumption of fish and olive oil as well as adherence to the traditional dietary restrictions of the Greek Orthodox Lent may have independent protective effects on the development or severity of RA (1). However, the interaction between adherence to the Greek Orthodox Lent, which prescribes long periods of fasting that limit the consumption of meat, fish, and olive oil, and the effect of dietary factors has not been studied. Because our previously published study generated a hypothesis that has not yet been confirmed, we designed another, independent, case-control study in Athens, Greece. The present study examines the consumption of >100 food items, including olive oil, fish, and vegetables, in relation to risk of developing RA. Furthermore, it examines the association between consumption of these food items and risk of developing RA after adherence to the Greek Orthodox Lent was controlled for.

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SUBJECTS AND METHODS

We performed a hospital-based, serially matched case-control study of 145 consecutively enrolled case subjects [24 men and 121 women who met the criteria of the American Rheumatism Association (19)] and 188 control subjects (41 men and 147 women). The case subjects' ages ranged from 18 to 84 y ($\bar{x}$: 54.9 ± 14.53 y) and the control subjects' from 18 to 80 y ($\bar{x}$: 54.5 ± 12.9 y). The study was approved by the Committee on Human Studies.

All case and control subjects were seen in 2 major hospitals or an outpatient clinic located in the Athens metropolitan area that serve a large segment of the population in southern Greece (1). Case and control subjects were enrolled in the study over a 2-y period. Case subjects were examined clinically and radiologically and blood samples were taken for laboratory examinations as described previously (1). Control subjects were matched with the case subjects by sex, age (±5 y), and health care facility. To be selected, a control subject had to be hospitalized at the same time as the case subject if the case subject was hospitalized. If the case subject was an outpatient, then an outpatient was selected as a control subject. In this way, we selected case and control subjects from the same general population and avoided a potential source of bias. We selected mainly persons with minor eye or ear-nose problems to serve as control subjects. Control subjects with illnesses that could affect dietary habits, such as metabolic diseases or peptic ulcers, were excluded. We also excluded persons with a known diagnosis of rheumatic disease.

After they had given their informed consent to participate, case and control subjects responded to an interviewer-administered, detailed, previously validated questionnaire (1) that covered personal data and information on socioeconomic variables, prior medical history, family history, diet, and present disease. The interviewers were not blinded to the subjects' status but were unaware of the specific hypothesis of the study. Case and control subjects were equally distributed among interviewers.

The dietary questionnaire was used to gather information on the frequency of consumption of >100 different food items and adherence to the traditional dietary restrictions of Orthodox fasting periods before the subjects' current diseases were diagnosed, as described previously (1, 20). Subjects were asked to state the average frequency of consumption of each food item (number of times per day, week, or month that they consumed each specific dietary item) from childhood until their current disease was diagnosed (RA for case subjects and the disease for which control subjects were seen at the time of the interview). For statistical analysis, the frequency of consumption of different food items was transformed into times per month that the food was consumed. Thus, daily consumption was multiplied by 30, weekly consumption was multiplied by 4, 0 was assigned to food items never consumed, and 0.5 was assigned to food items reported as rarely consumed (less frequently than once a month) (1, 20). The values for monthly consumption were added and the sums were approximately distributed into quartiles based on the distribution of control subjects (1, 20, 21). Food items were grouped in main food categories, eg, meat, fish, shellfish, dairy products, cereals, starchy roots, sugars or syrups, pulses, vegetables, fruit, nonalcoholic beverages, olive oil, other vegetable oils, and animal fat (1, 20). When suggested by prior hypotheses or knowledge, groups were further subdivided, eg, vegetables were divided into cooked and raw vegetables (1, 20).

In contrast with the other food items, consumption of olive oil was calculated only on the basis of adherence to Greek Orthodox Lent and abstinence or not from olive oil. Each subject was asked whether he or she adhered to the fast for each specific Lent period (Christmas: 6 wk, liberal; Easter: 7 wk, strict; Dormition of the Virgin: 2 wk, strict; Wednesday and Friday weekly: strict) at each age interval as described previously (1). The age intervals (ie, birth to 18 y, 18–30 y, and 30 y to time of diagnosis) were defined by using typical milestones at which dietary habits (and religious attitudes) may change (1). For example, at the age of 18 y, most Greeks leave home to study or work and at 30 y of age, most persons are married and form new habits.

According to the rules of the Greek Orthodox church, people abstain from meat and animal products during the periods of Lent (≈180 d/y), whereas olive oil and fish may be consumed during certain periods, as described previously (1). Thus, during Lent people consume mainly fruit, cereals, and vegetables. A small number of persons strictly adhere to religious rules banning any fat consumption (including olive oil); such strict adherence was recorded.

Olive oil is an ingredient in most Greek dishes (the only possible exception being meat and dairy foods, which are often cooked with butter or other animal fat). To document the type of oil consumed by the subjects, we asked specific questions about the type of oil used in salads, for cooking, or for frying during each age interval. Both olive oil consumption and adherence to the Greek Orthodox Lent were shown to be important predictors of risk of RA (1). Thus, we created an additional food consumption variable by multiplying the numbers of years a subject adhered to Greek Orthodox Lent (periods in years between the age milestones used) by the number of days of adherence to Lent per year and whether olive oil (or other oils) was consumed during the corresponding periods. In this way we created a new variable reflecting lifelong olive oil consumption that took into account not only the frequency of consumption but also the time of year olive oil was actually consumed.

Data on food consumption, adherence to the fasting periods, and lifelong consumption of food items were analyzed univariately and multivariately. For the univariate analysis, odds ratios (ORs) and the corresponding 95% CIs were calculated. The statistical significance and the value of chi-square tests for linear trends of various levels of food consumption and adherence to fasting compared with the lowest level of exposure were also computed.

For the multivariate analysis, a multiple logistic model as developed by Breslow and Day (22) was used. We used presence or absence of RA as a dependent variable and age, sex, body mass index, years of schooling, and consumption of major food groups (olive oil, meat, fish, shellfish, dairy products, raw and cooked vegetables, cereals, fruit, starchy roots, sugars or syrups, pulses, and nonalcoholic beverages) as independent variables, as described previously (20). The reported *P* values are two-tailed.

RESULTS

A total of 145 case and 188 control subjects were interviewed. The subjects' ages at onset of disease ranged from 18 to 80 y ($\bar{x}$: 49.2 y). Rheumatoid factors (as ascertained by nephelometry) were present in 75% of case subjects, whereas the results of tests in 25% of case subjects were persistently negative. Of the case subjects, 61% had bone erosions and 8.3% had subcutaneous nodules. Felty's syndrome was not detected in any case subjects.

The number of days per year of adherence to Greek Orthodox fasting periods during which olive oil consumption is allowed

TABLE 1
Distribution of case and control subjects and risk of developing rheumatoid arthritis by quartiles of frequency of consumption of raw vegetables, cooked vegetables, fish, and olive oil[1]

Food groups	Quartiles				*P* for trend
	1 (low)	2	3	4 (high)	
Raw vegetables (servings/mo)[2]	40	85	120	180	
Case subjects	43	29	42	31	
Control subjects	47	47	54	40	
OR (95% CI)	1	0.67 (0.34, 1.28)	0.89 (0.47, 1.61)	0.85 (0.44, 1.67)	0.78
Cooked vegetables (servings/mo)[2]	20	45	60	85	
Case subjects	58	37	28	22	
Control subjects	48	45	48	47	
OR (95% CI)	1	0.68 (0.37, 1.26)	0.48 (0.25, 0.92)	0.39 (0.20, 0.77)	0.001
Fish (servings/mo)[2]	3	4	6	10	
Case subjects	35	45	39	26	
Control subjects	46	49	57	36	
OR (95% CI)	1	1.21 (0.64, 2.29)	0.90 (0.47, 1.71)	0.95 (0.46, 1.96)	0.65
Olive oil (d)[3,4]	292	2500	3500	19500	
Case subjects	47	30	43	19	
Control subjects	42	43	42	43	
OR (95% CI)	1	0.62 (0.32, 1.22)	0.91 (0.48, 1.73)	0.39 (0.19, 0.82)	0.03

[1] OR, odds ratio.
[2] Consumption calculated on the basis of monthly consumed portions (servings).
[3] Cumulative number of days that olive oil was consumed, calculated on the basis of lifelong adherence to Greek Orthodox Lent.
[4] Complete data on olive oil consumption were available for only 139 case subjects and 170 control subjects.

ranged from 0 to 180. The corresponding mean for the lowest quartile was 1 d/y, that for the second quartile was 12 d/y, that for the third quartile was 48 d/y, and that for the fourth quartile was 130 d/y. In this population, the mean daily consumption of cooked vegetables was 0.85 servings for subjects in the lowest quartile, 1.5 servings for subjects in the second quartile, 2 servings for subjects in the third quartile, and 2.9 servings for subjects in the fourth quartile. A simple correlation analysis of cooked vegetable consumption and adherence to Lent (in days) was not significant ($r = 0.006$, $P > 0.10$).

Shown in **Table 1** is the distribution of case and control subjects by quartiles of frequency of monthly consumption of raw vegetables, cooked vegetables, and fish and lifelong consumption of olive oil (calculated on the basis of adherence to the Greek Orthodox Lent). The risk of developing RA decreased significantly with increased lifelong consumption of olive oil (chi-square: 4.28; $P = 0.03$). Moreover, persons in the highest category of olive oil exposure had an OR of 0.39 (95% CI: 0.19, 0.82) when compared with the corresponding lowest category of consumption.

The OR for developing RA also appeared to be reduced when consumption of raw vegetables, cooked vegetables, and fish increased, but this was significant only for cooked vegetables. The ORs for subjects in the highest and second-highest quartile of consumption of cooked vegetables were 0.39 (95% CI: 0.20, 0.77) and 0.48 (95% CI: 0.25, 0.92), respectively. The corresponding trend test for increased consumption of cooked vegetables was also highly significant (chi-square: 10.48; $P = 0.001$).

The results of a multiple logistic regression analysis model controlled for the effect of several potential confounders, as indicated in Methods, are shown in **Table 2**. All variables were entered into the model simultaneously. Both cooked vegetable and olive oil consumption had an independent effect on risk of developing RA, whereas no other food group appeared to play a role of comparable significance (data not shown). Exclusion of the variables of sex, age, social class, occupation, and education from the model did not alter the effect estimates or *P* values.

DISCUSSION

Microbiological, immune, genetic, hormonal, and, recently, dietary factors have been implicated in the pathogenesis of RA (1); one or several of these factors may be responsible for the significant differences in disease frequency and severity that have been documented in different populations (23). Genetic factors alone cannot account for these differences because the documented genetic differences in these populations are minor (24–28). In contrast, great differences exist in dietary habits between Western and Mediterranean populations. Thus, because experimental evidence has suggested that consumption of fish oil and olive oil may affect the severity of RA, the distinct possibility exists that dietary factors are also involved in the etiology of this disease. The Greek diet is based mainly on fruit and vegetables, either raw or cooked with olive oil, and contains less meat

TABLE 2
Dietary factors associated with rheumatoid arthritis in a multiple logistic regression analysis model controlled for potential confounders as indicated in Methods[1]

Factors	OR (95% CI)	*P*
Olive oil consumption		
Quartile 2	0.60 (0.30, 1.22)	NS
Quartile 3	0.95 (0.48, 1.91)	NS
Quartile 4	0.38 (0.17, 0.85)	0.02
Cooked vegetable consumption		
Quartile 2	0.55 (0.28, 1.08)	0.08
Quartile 3	0.41 (0.20, 0.87)	0.02
Quartile 4	0.24 (0.11, 0.53)	0.0003

[1] OR, odds ratio.

and more fish and pulses than the Western diet, food items that may influence risk of RA.

The results of this case-control study confirm previously published data indicating that dietary factors may affect the development or course of RA (1). Furthermore, in the present study we attempted to quantify olive oil consumption more accurately by estimating the number of days of consumption per year and adding these numbers to estimate lifelong consumption. Persons in the lowest category of olive oil consumption had a 2.5 times higher risk of developing RA than did persons in the highest category of consumption. The excess daily olive oil consumption in this instance was ≈43 g/d. In addition, we observed a reduction in risk of 75% (OR: 0.24) in the highest category of consumption of cooked vegetables compared with the lowest category. It is possible that heat destroys the cell walls of cooked vegetables, helping the to body absorb more of a potentially beneficial substance. The specific beneficial substances in cooked vegetables remain to be identified, however.

Thus, this study confirms the findings of the previous study in Greece, which showed that risk of RA is inversely associated with consumption of olive oil and adherence to the Greek Orthodox Lent up to the time of diagnosis. Moreover, this study extends these observations by showing that lifelong consumption of olive oil and consumption of cooked vegetables is independently associated with risk of developing RA. Finally, similar to the previous case-control study in Greece (1), consumption of fish was not an independent predictor of risk of RA.

Although these results confirm our previous observations (1), they do not provide any indication of the mechanism that may underlie the protective effect of olive oil and vegetables. There is a strong possibility that this protective effect is attributed to the relatively high unsaturated fatty acid content of olive oil. The n−3 polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid, which are the major fatty acids in marine organisms (18), are metabolized to prostaglandins [eg, prostaglandin E_3 (PGE_3)] and leukotrienes [eg, leukotriene B_5 (LTB_5)]. The latter exert an inhibitory effect on PGE_2 and LTB_4, metabolic products of n−6 fatty acids (2, 15, 16), which are abundant in Western diets. Thus, the n−3 fatty acids are metabolized to competitive inhibitors of n−6 prostaglandins and leukotrienes and suppress the production of the inflammatory cytokines tumor necrosis factor α interleukin 1β (3).

Although there is evidence from intervention studies that olive oil may be effective in relieving arthritis symptoms (7, 29), the literature concerning the effects of regular consumption of olive oil on RA is limited. Olive oil contains a high proportion of oleic acid, ranging from 68.8% to 82.8% ($\bar{x}$: 76.9%); a low proportion of linoleic acid ($\bar{x}$: 7.5%), and a very small proportion of linolenic acid (0.6%), arachidonic acid (0.4%), and eicosenoic acid (0.3%) (30). Linoleic acid, an n−6 polyunsaturated fatty acid that is abundant in the Western diet, is converted to arachidonic acid, which is the biosynthetic precursor of the n−6 series of PGE_2 and LTB_4. These molecules have potent proinflammatory activity and can cause vasodilation and increased vascular permeability (PGE_2) as well as neutrophil chemotaxis and neutrophil activation (LTB_4) (31). Oleic acid is an n−9 monounsaturated fatty acid that is converted to 8,9,11-eicosatrienoic acid (20:3n−9; ETA) under restriction of n−6 fatty acids (32) by desaturation and elongation. ETA is converted to LTA_3 (33), which is a potent inhibitor of LTB_4 synthesis (34). Thus, oleic acid, which is abundant in olive oil, and its metabolite ETA may exert an antiinflammatory effect through a mechanism similar to that of fish oil, which contains EPA, an n−3 fatty acid that acts competitively with n−6 fatty acids. Because ETA is substantially less unsaturated than EPA, it may have greater chemical stability, which would be an advantage for use as a dietary constituent or supplement (32).

The dietary benefits of olive oil may also be attributed, at least in part, to the presence of natural antioxidants. Tocopherols are important constituents of olive oil and are found in higher quantities in the unrefined, unbleached, and undeodorized olive oil that is mainly consumed by the Greeks. Tocopherols contribute to the remarkable stability of the oil and have a beneficial biological role as radical quenchers (30).

Our findings of the effect of fish consumption on RA risk, although in the same direction as previous studies in Western populations, were not significant. In a previous case-control study in Greece, we reported a protective effect of fish consumption by univariate analysis but no significant effect in the multivariate analysis model (1). No other epidemiologic studies have assessed risk of RA in a Mediterranean population. However, it was observed that the prevalence of RA in ethnic groups that consume diets rich in deep ocean fish is low (35–37); additionally, the therapeutic effect of dietary fish-oil supplementation of RA patients was documented in several intervention studies (4–16).

Several factors may explain the discrepancies between the present and previous studies. First, the consumption of deep ocean fish, which are rich in n−3 fatty acids, is low in Mediterranean populations. In contrast, Mediterranean populations commonly consume *Scomber japonicus*, *Boops boops*, *Mullus barbatus*, *Mullus surmuletus*, *Merluccius merluccius*, *Pagelinus erythrinus*, *Pagrus pagrus*, and *Sparus aurata*, in which the proportion of n−3 fatty acids ranges from 12.6% to 28.3% and the ratio of n−6 to n−3 fatty acids ranges from 0.2 to 0.7 (38). Second, Mediterranean populations consume large amounts of olive oil, which is rich in oleic acid and thus increases the formation of n−3 fatty acids. The large amounts of n−3 fatty acids derived from the n−9-rich olive oil may override the protective effect of the n−3 fatty acids derived from fish.

This study has several limitations, including its retrospective nature and the fact that lifelong assessment of dietary patterns may be affected by memory bias. We tried to limit this bias by using lifetime milestones. In addition, it is easier for a person to remember his or her lifelong religious habits than his or her lifelong consumption of specific dietary items. Thus, to the extent that adherence to Lent influences the consumption of specific foods, we corrected for the effect of these periods by creating a new variable. Furthermore, we examined several potential sources of bias and appropriately controlled for potential confounders. Our case subjects, although not representative of all incident cases diagnosed in the Greek population, were drawn from an outpatient clinic and 2 large general hospitals serving a large part of southern Greece. There was no indication that social class, education, or intensity of religious beliefs played a role in the decision of a patient to receive care in one of the above health care facilities. Greek Orthodoxy is the religion of >95% of the Greek population and all subjects in this study had received obligatory religious education at school and had similar possibilities of adhering to the Greek Orthodox Lent. Religious practice in Greece may be affected by age and sex and minimally, if at all, by social class, occupation, or education. No significant differ-

ences in any of these variables were observed between our case and control subjects and the inclusion of these variables in our logistic models of analysis did not alter the results. In addition, control subjects were selected from the same health care facilities as the case subjects and were matched with the case subjects by age and sex. Social class was controlled for in the analysis by including years of schooling in the multivariate model. Thus, at most, overmatching (introducing bias by matching case and control subjects according to the variables under study) with regard to religious traditions may have taken place, leading to underestimation of the effect of olive oil. Bias due to data collection methods during the interviews was also eliminated because the interviewers were completely blinded to the study aims and hypotheses. In conclusion, olive oil and cooked vegetable consumption may exert a protective effect on RA in the Mediterranean population studied.

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