

Diet, physical activity, and gallstones—a population-based, case-control study in southern Italy^{1, 2}

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ABSTRACT

Background: Gallstones are a highly prevalent condition; however, the nutritional and lifestyle risk factors of this disease are not well understood.

Objective: We evaluated the association between diet, physical activity, and incident cases of gallstones diagnosed by ultrasound in a population-based, case-control study.

Design: One hundred patients with newly diagnosed gallstones and 290 randomly selected control subjects without gallstones were enrolled in the study. The presence of gallstones was determined by ultrasonography. Both patients and control subjects completed a questionnaire about their usual diet and physical activity for the 12 mo before the ultrasonography. The association between diet and physical activity and risk of gallstone formation was analyzed by using multiple logistic regression.

Results: Body mass index and intake of refined sugars were directly associated with risk of gallstone formation, whereas physical activity, dietary monounsaturated fats, dietary cholesterol, and dietary fibers from cellulose were inversely associated with risk of gallstone formation. Saturated fats were a risk factor for gallstone formation and the association appeared to be stronger for men than for women.

Conclusion: These findings suggest that a sedentary lifestyle and a diet rich in animal fats and refined sugars and poor in vegetable fats and fibers are significant risk factors for gallstone formation. *Am J Clin Nutr* 1999;69:120–6.

KEY WORDS Diet, physical activity, gallstones, case-control study, Mediterranean diet, southern Italy, monounsaturated fats, saturated fats, fiber, cholesterol, adults, humans

INTRODUCTION

Numerous epidemiologic studies have examined the role of diet as a potential risk factor for gallstone formation (1–31), but their findings are controversial, probably because of differences in study designs, methods of dietary exposure assessment, and methods used to determine the presence of gallstones. Most of the epidemiologic studies conducted to date on diet and gallstones consist of case-control studies performed on symptomatic patients with gallstones (1, 3, 4, 5, 8, 10, 11, 13, 14, 18, 19, 24, 25, 27); the assessment of dietary intake with this design could be influenced by changes in the diet secondary to the painful symptoms associated with gallstones; fur-

thermore, the association of the diet could be with symptoms and not with disease.

A smaller number of case-control studies used cholecystographic or echographic methods to evaluate the presence of gallstones (12, 17, 20, 21, 30); however, these studies all considered prevalent disease and therefore the temporal relation between exposure and disease could not be evaluated with certainty. A series of cohort studies (2, 22, 23, 26, 28, 29, 31) were also conducted; however, they also had shortcomings, both with regard to the assessment of exposure (ie, the diet was poorly assessed) and outcome (ie, in all but one study, outcome was assessed on the basis of symptoms and not disease). Only 2 dietary trials with postmortem findings of gallstones are available in the literature, and their results are contrasting (6, 7).

The present study addressed several methodologic shortcomings and biases: it is a population-based, case-control study of patients with new cases of symptomatic and asymptomatic gallstones; dietary intakes were assessed with a semiquantitative food-frequency questionnaire.

SUBJECTS AND METHODS

Study area

This case-control study was carried out in a small town in southern Italy (Castellana, province of Bari, in the Apulia region) with 18000 inhabitants at the 1991 census. The main occupation in the town (30% of the people at the 1991 census) was agriculture related.

Case and control subjects

Between May 1985 and June 1986, 3500 individuals (2000 men and 1500 women aged 30–69 y) were randomly selected

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from the electoral register of Castellana, and 70.6% (1429 men and 1043 women) of them participated in a survey about gallstones. As part of the survey, the participants had an ultrasonography of the gallbladder and a blood sample taken by venipuncture. The subjects who showed mobile echoes in the gallbladder lumen at ultrasonography, those cholecystectomized with no visualization of the gallbladder, and those with an abdominal scar at physical examination were considered to have a prevalent case of gallstones: 226 (9.2%) of 2472 subjects (92 men and 134 women). Of the remaining 2246 subjects, 11 had an uncertain diagnosis of gallstones, even after cholecystography, and were excluded from the cohort study (32).

Between May 1992 and June 1993, 2235 subjects free of gallstones at the baseline examination were reexamined. Ultrasound evaluation of gallstones was carried out by trained echographers according to a standardized protocol (33). The same echograph [a real time machine, Aloka SSD-202, with a 3.5-MHz (87.7%) linear transducer; Aloka Co, Ltd, Tokyo] was used in both the 1985–1986 and 1992–1993 examinations. Respondents completed a questionnaire, pertaining to the year before the study, with 4 areas of interest: sociodemographic status, medical history, dietary habits, and physical activity. A blood sample was also taken at the reexamination. Respondents were considered to have diabetes if it had been diagnosed by a physician and was reported in the medical history portion of the questionnaire.

One hundred four (55 men and 49 women) of 1962 respondents had developed gallstones. The overall incidence rate was 7.9/1000 person-years (the number of persons at risk of the disease multiplied by the number of years of follow-up). Only 5 of 104 (5%) subjects (4 men and 1 woman) had been operated on for gallstones (32). Ninety-nine subjects with gallstones and a gallbladder were offered a direct X-ray of the abdomen and a cholecystography. Of the 79 subjects who agreed to have an X-ray, 70 had radiotransparent or mixed gallstones and 9 had radioopaque gallstones. Thus, 88.6% of the gallstones in the population were composed of cholesterol.

Three control subjects, frequency-matched by sex and season to each patient, were selected from the gallstone-free population. Completed questionnaires from 100 patients with new cases of gallstones and from 290 control subjects were analyzed. Four patients and 22 control subjects refused to fill in the semiquantitative food-frequency questionnaire. Control subjects who refused to fill out the questionnaire were excluded from data analysis and were not replaced.

Dietary and physical activity measurements

The reproducibility and accuracy of the food-frequency portion of the questionnaire (96 food items) was documented in a validation study in which the questionnaire was compared with two 7-d dietary records completed over 6 mo (34). The list of foods and beverages were grouped into 12 separate sections, according to principal food groups (35).

Physical activity was ascertained with the use of 10 questions designed to measure both leisure time and work activities. The items were selected on the basis of a previous report on activity patterns of elderly populations in rural areas (36) and from a local survey of individuals attending the outpatient department in our hospital. These questions aimed to quantify the time (hours and minutes) spent in bed (sleeping and resting), performing household activities (cooking and cleaning), and performing discretionary activities (eg, gardening, walking, bicycling, and exercising).

Residual time (time not accounted for by the listed activities) was assumed to be spent in light-to-moderate activities. Daily energy expenditures were calculated from these items according to the procedures described by James and Schofield (37). Briefly, each activity was assigned a physical activity ratio (PAR) based on the amount of energy (kJ/min) expended and the estimated basal metabolic rate. These PARs were then converted into integrated energy indexes (IEIs), which take into account the amount of time spent in pauses during the performance of these activities. Finally, the activity-specific IEIs were converted to kilojoules by multiplying by the time spent in the activity (hours and minutes) and the average basal metabolic rate.

The reproducibility of the physical activity portion of the questionnaire was assessed in a subsample of the cohort: the intraclass correlation for the 10 items ranged from 0.45 (energy expenditure during bicycling) to 0.93 (energy expenditure during sleeping). Overall, the intraclass correlation for total energy expenditure, calculated with data from the 2 physical activity questionnaires in the cohort sample, was 0.77.

Statistical analysis

All respondents were included in the analysis of diet because all questionnaires were $\geq 90\%$ complete. Total energy and macro- and micronutrient intakes were calculated by using Italian food-composition tables (38, 39). Energy-adjusted nutrient intakes were computed as the residuals from the regression model, with total energy intake as the independent variable and absolute nutrient intake as the dependent variable (40). Subjects were classified by quartiles of energy-adjusted nutrient intake and by quartiles of total energy expenditure.

The odds ratios and 95% CIs for the risk of gallstone formation by quartiles of nutrient intake [adjusted for age, sex, body mass index (BMI; in kg/m^2), and all other nutrients] were calculated by using unconditional multiple logistic regression (41). The linear trend of the associations was assessed by assigning a score (1, 2, 3, etc) to each of the percentiles (tertiles or quartiles) of the nutrient of interest. The reported *P* values were always two sided.

The backward multiple logistic regression method was used to select dietary and nondietary factors significantly associated with gallstones (the statistical level of significance determined with the log-likelihood ratio test was set at $P < 0.10$) after forcing age and sex in the model. Finally, the first-order interactions of nutrients with sex and age were evaluated. All statistical computations were made by using STATA 4.0 statistical software (Stata Corporation, College Station, TX).

RESULTS

Selected nondietary characteristics of cases and control subjects are shown in **Table 1**. Age, BMI, prevalence of diabetes, and stool frequency $< 1/\text{d}$ were higher, whereas physical activity was lower in patients with gallstones than in control subjects. Dieting, coffee consumption, and smoking habits did not seem to be associated with the disease. Sex distribution was similar in patients and control subjects as a result of the matching procedure. Average dietary nutrient intakes by patients with gallstones and control subjects are shown in **Table 2**. Only mean alcohol consumption tended to be significantly lower in patients with gallstones than in control subjects.

The ORs of gallstone risk for various energy-adjusted nutrient quartiles (with the lowest quartile used as the reference category)

TABLE 1

Main nondietary characteristics of patients with gallstones and control subjects

	Patients with gallstones (n = 100)	Control subjects (n = 290)	P ¹
Men (%) ²	54.0	53.4	0.92
Age (y)	59.4 ± 10.8 ³	57.1 ± 10.2	0.06
Body mass index (kg/m ²)	29.5 ± 5.9	27.4 ± 4.1	0.0001
Dieting (%)	12.0	9.3	0.44
Stool frequency <1/d (%)	19.0	10.7	0.03
Coffee consumption (%)	72.0	78.6	0.17
Smokers (%)	23.0	19.7	0.47
Physical activity (MJ/d) ⁴	7.05 ± 3.16	7.73 ± 3.03	0.06
Diabetes (%)	14.0	6.6	0.02

¹t Test for continuous variables; chi-square test for categorical variables.²Patients and control subjects were sex-matched.³ $\bar{x} \pm SD$.⁴Energy expenditure.

and the results of the linear trend analysis are shown in **Table 3**. The results of 2 separate models are presented: model 1, which includes both age, sex, BMI, and energy, and model 2, which includes age, sex, BMI, energy, and all other nutrients. Energy and monounsaturated fat intakes were inversely related whereas intake of refined sugars was directly related to the risk of gallstone formation in both models. Saturated fat intake appeared to increase the risk of gallstones whereas intake of dietary cholesterol had an apparent protective effect after adjustment for the intake of other nutrients. Alcohol intake appeared to be significantly inversely related to risk only when model 1 was used. Results of the final logistic regression model with both dietary and nondietary variables included are summarized in **Table 4**. BMI and refined sugar and saturated fat intakes were associated with an increase in the risk of gallstones, whereas physical activity and monounsaturated fat intake were associated with a reduction in the risk of gallstone formation. There was a significant negative interaction between sex and saturated fat intake (**Figure 1**). Women appeared to have a greater risk of gallstone formation

TABLE 2

Daily intake of nutrients in patients with gallstones and control subjects, and upper cutoff points for quartiles of intake based on the distribution of nutrients in both groups

Nutrient	Patients with gallstones (n = 100)	Control subjects (n = 290)	Upper cutoff point for quartiles ¹		
			Q ₁	Q ₂	Q ₃
Energy (MJ)	9.08 ± 3.09 ²	9.53 ± 3.41	6.95	8.83	11.20
Protein (g)	69.3 ± 27.4	71.7 ± 29.0	50.9	66.9	86.5
Saturated fat (g)	20.6 ± 9.5	20.4 ± 9.2	14.0	18.6	24.6
Monounsaturated fat (g)	38.5 ± 11.6	40.3 ± 12.6	31.2	39.0	49.2
Polyunsaturated fat (g)	8.2 ± 2.9	8.6 ± 3.1	6.5	7.9	10.0
Cholesterol (mg)	179.9 ± 104.1	187.8 ± 105.1	120.1	163.3	231.8
Glycogen (g)	150.5 ± 73.9	159.6 ± 82.9	93.3	134.5	213.3
Refined sugar (g)	117.4 ± 70.6	115.3 ± 71.6	64.9	96.7	149.2
Fiber from cellulose (g)	8.2 ± 4.9	8.2 ± 4.8	4.8	6.9	10.5
Fiber from noncellulose (g)	19.9 ± 9.6	20.4 ± 10.2	12.9	18.5	25.5
Alcohol (g)	19.3 ± 26.3	25.2 ± 27.6 ^{3,4}	0.0	15.6	38.5 ⁵
Calcium (mg)	855.2 ± 440.9	836.6 ± 458.2	510.3	764.3	1061.6

¹Q₁, Q₂, and Q₃, quartiles 1, 2, and 3.² $\bar{x} \pm SD$.³Median intakes: patients, 10.4 g/d; control subjects, 15.6 g/d.⁴Significantly different from patients, P < 0.10 (t test).⁵Reference category was "no drinking alcohol" and drinking quantities in tertiles.

than men at all intakes of saturated fat, except for the highest quartile, for which men displayed a greater risk than women.

DISCUSSION

Our results indicate that many nutritional factors and physical activity may play an important role in the etiology of gallstones. In particular, a high intake of saturated fats and refined sugars may increase the risk of gallstone formation, whereas a high intake of monounsaturated fats, fiber from cellulose, and dietary cholesterol and high levels of physical activity may protect against gallstone formation. Evidence on the relation between diet and gallstone formation, however, is somewhat conflicting. After the first report by Malhotra in 1968 suggesting a possible association between gallstone formation and saturated fat intakes (42), a series of case-control and clinical investigations have shown contrasting results; however, they all focused on symptomatic gallstones (1, 3, 8). An exception is the study by Jørgensen and Jørgensen (21), which found a positive, nonsignificant association between total fat intake (mostly saturated fats) and prevalent gallstones (determined by ultrasound) in a cross-sectional survey of a population-based sample in Denmark. Of potential importance was the observed significant interaction in our study between sex and saturated fat intakes with regard to gallstone formation, indicating that the association between saturated fat intake and gallstone formation may be weaker in women than in men and that men in the highest quartile of saturated fat intake may be at greater risk than women. These findings seem to suggest that for women there may be factors other than saturated fat intake (eg, hormonal or metabolic) that are more powerful risk factors for gallstone formation.

The potential etiologic role of other fats has not been investigated thoroughly. Our study found no evidence of a link between polyunsaturated fats and risk of gallstone formation. The limited available evidence on the relation between these fats and gallstone formation is conflicting (6, 7). The link between monounsaturated fats and risk of gallstone formation has not been investigated. Monounsaturated fats, as all fats, have been shown to have a pow-



TABLE 3Odds ratios (ORs) and 95% CIs of the risk of gallstone formation, by quartiles 1–4 (Q_{1–4}) of nutrient intakes¹

Nutrients	OR (95% CI)			P ²
	Q ₂	Q ₃	Q ₄	
Protein				
Model 1	1.25 (0.64, 2.48)	1.54 (0.78, 3.06)	1.24 (0.62, 2.49)	0.45
Model 2	1.55 (0.66, 3.64)	2.29 (0.82, 6.41)	2.43 (0.73, 8.08)	0.13
Saturated fat				
Model 1	0.99 (0.50, 1.97)	1.37 (0.70, 2.64)	1.25 (0.64, 2.44)	0.37
Model 2	1.11 (0.43, 2.84)	2.65 (0.87, 8.06)	3.79 (0.86, 16.82)	0.03
Monounsaturated fat				
Model 1	0.89 (0.46, 1.72)	0.96 (0.50, 1.86)	0.47 (0.23, 0.96)	0.06
Model 2	0.73 (0.31, 1.70)	0.85 (0.31, 2.31)	0.30 (0.09, 1.04)	0.09
Polyunsaturated fat				
Model 1	0.77 (0.39, 1.50)	0.81 (0.42, 1.56)	0.68 (0.34, 1.33)	0.30
Model 2	0.68 (0.29, 1.62)	0.72 (0.26, 1.99)	0.77 (0.24, 2.52)	0.75
Cholesterol				
Model 1	0.96 (0.50, 1.83)	0.94 (0.48, 1.83)	0.75 (0.38, 1.49)	0.43
Model 2	0.53 (0.22, 1.25)	0.36 (0.13, 1.01)	0.24 (0.07, 0.82)	0.02
Refined sugar				
Model 1	1.34 (0.66, 2.71)	1.61 (0.80, 3.25)	2.10 (1.06, 4.16)	0.03
Model 2	1.91 (0.77, 4.74)	3.13 (1.05, 9.28)	6.34 (1.55, 25.98)	0.01
Glycogen				
Model 1	0.52 (0.26, 1.04)	0.83 (0.44, 1.59)	0.78 (0.41, 1.48)	0.73
Model 2	0.41 (0.18, 0.92)	0.71 (0.28, 1.84)	0.76 (0.21, 2.78)	0.62
Fiber from cellulose				
Model 1	1.03 (0.53, 2.0)	0.66 (0.33, 1.33)	1.2 (0.63, 2.32)	0.86
Model 2	0.85 (0.34, 2.10)	0.54 (0.16, 1.84)	0.76 (0.14, 4.02)	0.56
Fiber from noncellulose				
Model 1	0.69 (0.34, 1.36)	0.78 (0.40, 1.52)	1.14 (0.60, 2.17)	0.60
Model 2	0.42 (0.16, 1.09)	0.28 (0.08, 0.97)	0.33 (0.06, 1.85)	0.12
Calcium				
Model 1	1.17 (0.60, 2.27)	1.02 (0.52, 2.01)	1.35 (0.69, 2.63)	0.48
Model 2	0.77 (0.33, 1.80)	0.51 (0.20, 1.30)	0.40 (0.11, 1.43)	0.11
Alcohol³				
Model 1	0.66 (0.35, 1.25)	0.59 (0.31, 1.13)	0.38 (0.18, 0.78)	0.008
Model 2	0.83 (0.39, 1.78)	0.74 (0.32, 1.67)	0.42 (0.14, 1.28)	0.17
Energy				
Model 1 ⁴	0.92 (0.49, 1.73)	0.48 (0.24, 0.96)	0.58 (0.29, 1.16)	0.04
Model 2 ⁵	0.97 (0.44, 2.15)	0.33 (0.13, 0.81)	0.46 (0.18, 1.19)	0.03

¹Quartile 1 (low) is the reference category. In model 1, age, sex, BMI, and energy were controlled; in model 2, age, sex, BMI, energy, and all other nutrients were controlled for.

²Chi-square test for trend.

³Reference category was “no drinking alcohol” and drinking quantities in tertiles.

⁴Controlled for age, sex, and BMI.

⁵Controlled for age, sex, BMI, and all other nutrients.

erful effect on the rate of gallbladder emptying (43). However, the effect is different from that of saturated fats because monounsaturated fats increase the ratio of HDL to LDL cholesterol (44) and therefore may have important protective effects against gallstone formation.

A positive association between intake of refined sugars and risk of gallstone formation has been reported consistently (13, 14, 21, 29). However, a diet rich in refined sugars is usually poor in complex carbohydrates and fiber; therefore, whether refined sugars and fiber have independent effects on gallstone risk remains to be fully clarified (13, 14, 21, 26, 29). Our findings suggest that refined sugars and fiber from cellulose may have independent effects.

The statistical independent association found in our study is supported by physiologic evidence suggesting possible different mechanisms through which refined sugars and fibers may affect

the risk of gallstone formation. A high intake of refined sugars may increase the risk of gallstone formation because of the resultant increase in the synthesis of cholesterol in the liver secondary to an increase in insulin (45–48), whereas low fiber intakes have been associated with an increase in the risk of gallstone formation because of the resultant increase in secondary bile acid secretions due to decreased colonic motility (49, 50).

Our finding that BMIs were higher in patients with gallstones than in control subjects confirms the results from previous epidemiologic investigations (51). There have been fewer studies of the relation between physical activity and risk of gallstone formation; however, our finding of a negative relation confirms the findings of previous studies by Williams and Johnston (12) and Kato et al (28). Other studies, which focused mainly on symptomatic disease, found no association with physical activity (1, 3, 8, 20). Several mechanisms might account for the association

TABLE 4Final multiple logistic regression model of all dietary and nondietary risk factors for gallstone formation¹

Variables	Coefficient (β)	Standard error (β)	P (Wald test)	Odds ratio	95% CIs for odds ratio
Sex (male: 0, female: 1)	1.33	0.64	0.04	3.78	(1.08, 13.2)
Age (y)	0.01	0.01	0.31	1.01	(0.99, 1.04)
BMI (kg/m ²)	0.13	0.03	0.00	1.13	(1.07, 1.20)
Physical activity (MJ)	-0.30	0.13	0.02	0.74	(0.58, 0.96)
Saturated fat (g)	0.69	0.20	0.00	2.01	(1.35, 2.97)
Saturated fat \times sex interaction	-0.59	0.23	0.01	0.55	(0.35, 0.87)
Monounsaturated fat (g)	-0.34	0.14	0.01	0.71	(0.55, 0.93)
Cholesterol (mg)	-0.31	0.16	0.05	0.73	(0.54, 1.00)
Refined sugar (g)	0.47	0.18	0.01	1.60	(1.13, 2.25)
Fiber from cellulose (g)	-0.33	0.17	0.05	0.72	(0.51, 1.00)

¹Backward method, age and sex forced in the model; first order interaction of all risk factors with age and sex explored; all nutrients and physical activity in quartiles coded as 1, 2, 3, and 4.

between high physical activity levels and the reduced risk of gallstone formation, including a direct effect on colonic motility (52); possible secondary mechanisms are a reduction in insulin and insulin resistance (53).


In the present study, it is of interest that diabetes ceased to be a significant risk factor for gallstone risk when BMI and physical activity were included in the model, suggesting that some of the mechanisms linking diabetes to gallstone risk may be the metabolic abnormalities associated with overweight, obesity and physical inactivity (eg, insulin and insulin resistance) (54, 55).

Finally, in the present study, alcohol appeared to be a significant risk factor for gallstone formation only before the inclusion of other nutrients in the model. Therefore, our findings suggest that alcohol may not be an independent risk factor for gallstone formation; however, the problems due to collinearity among nutritional factors does not exclude the possibility that alcohol may still play an important physiologic role in gallstone formation. The findings from previous studies of the effects of alcohol intake on the gallbladder are conflicting (2, 3, 14, 17, 21, 23, 24, 56, 57); however, several potential mechanisms have been identified to explain the potential protective effect of alcohol against gallstone formation. These mechanisms include the well-known HDL cholesterol-raising effect of alcohol and the associated reduction in bile cholesterol saturation (58).

As already indicated, previous studies on nutritional factors and risk of gallstone formation have provided conflicting results. These discrepancies, however, may have been due to weaknesses

in study design, particularly the reliance on symptoms as a measure of gallstone disease and the inclusion of prevalent cases of gallstones. The possibility exists that gastrointestinal symptoms may affect dietary recall and that dietary recall after symptoms have developed may reflect changes in the diet secondary to the peripheral symptomatology.

Our study had many strengths however. In particular, we focused on new cases of gallstones only and we ascertained the presence of gallstones through echography. Our ascertainment of diet was, however, performed at the time of diagnosis; therefore, we cannot exclude the possibility that symptoms may have affected dietary intakes. However, only 6 of 100 patients and 7 of 290 control subjects reported biliary pain. Furthermore, when models 1 and 2 were tested in asymptomatic individuals only, the results did not change (data not shown).

In conclusion, our study indicated that nutritional factors may play an important role in the etiology of gallstones and that most of these factors have been shown to play an important role in the etiology of other chronic diseases, such as cardiovascular disease (59) and cancer (60). Thus, gallstone disease is one of a cluster of diseases that characterize affluent societies and that most likely share common pathophysiologic links and mechanisms. Preventive strategies aimed at improving nutrition and energy imbalance may have a powerful effect on a series of pathologic conditions that represent a major source of morbidity and mortality in our society. 

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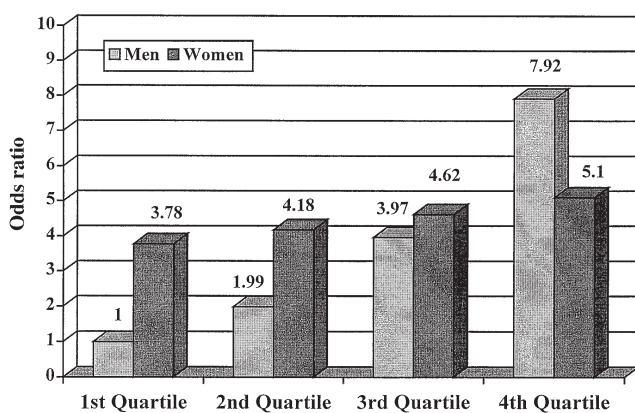


FIGURE 1. Odds ratios for risk of gallstone formation by quartiles of saturated fat intake in men and women separately.

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