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Cholelithiasic Disease and Associated Factors in a Spanish Population

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In order to analyze the factors associated with cholelithiasic disease, 1268 participants of a population sample were studied. On univariate analysis, 11 of the 23 variables included showed a statistically significant association ($P < 0.05$). Five of these variables, including obesity, triglyceride level, intake of hypolipidemic drugs, and a diet rich in cholesterol and saturated fats in women, and physical exercise in men, remained significantly associated after controlling for age. On multivariate analysis among women, a positive association was found with age ($P < 0.001$), obesity, and the use of hypolipidemic agents ($P < 0.05$) and a negative one with a diet rich in cholesterol and saturated fats ($P < 0.05$). Among men, the same analysis revealed there was a positive association with age ($P < 0.001$) and triglycerides ($P < 0.05$) and a negative one with physical exercise ($P < 0.05$). In conclusion, obesity and the use of hypolipidemic agents in women and triglycerides in men, were positively associated with cholelithiasic disease, independent of age, while negative associations included the intake of cholesterol and saturated fats in women and physical exercise in men.

KEY WORDS: cholelithiasic disease; biliary lithiasis; ultrasound; epidemiology; associated factors.

A number of studies and reviews (1–4) on the etiology of biliary lithiasis (BL) have not only confirmed the influence of the classically known variables, but have also pointed towards a probable influence of new factors associated with a lithogenic process, the complexity of which is just becoming to be understood.

There are wide variations in the prevalence of BL in different geographical areas. This may be explained by racial differences (5–7), which in turn could reflect distinct genetic backgrounds, (8, 9). BL predominates in the female sex, most probably due to the influence of hormonal factors. A positive association with age, pronounced obesity, rapid weight loss, and multiparity has also been found in some studies. There remains, however, an important controversy regarding dietary or metabolic factors (diabetes, hyperlipemias), as findings are conflicting, mainly as a result of methodological issues (10–12). On the other hand, the positive or negative influence of pharmaceutical drugs or active principles is of clear interest in order to avoid iatrogenesis, and promote some drugs with a prophylactic purpose. Cholesterol-lowering agents, such as clofibrates, constitute compounds with a well-proven risk relation with BL. In contrast, studies of possible preventive substances, such as vitamin C or aspirin (13), have only been reported preliminarily and require confirmation.

We, therefore, conducted this case–control study with the aims of evaluating the prevalence of cholelithiasic disease (CD) in a Spanish population and

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defining the factors associated with CD in the same population.

MATERIALS AND METHODS

Patients. Between February 1991 and April 1993, 1803 adults, ranging in age from 20 to 75 years and belonging to two population nucleii linked to our hospital (El Real and Gandia, in the region of Valencia, Spain) were contacted and asked if they were to participate in a study aimed at determining the prevalence of CD and associated factors. Approximately 70% (1268 individuals) answered and completed the screening protocol. These individuals constitute the object of the study. Individuals or cases of CD were grouped in: (1) patients with ultrasound-proven biliary lithiasis (14); and (2) individuals with previous cholecystectomy (PC). The remainder constituted the control group.

Methods. Ultrasonography examination was performed in all individuals using a Toshiba 77 SL machine with a 3.5-MHz convex transducer. Additionally, all participants completed a questionnaire to investigate factors associated with CD. The questionnaire was conducted by members of the clinical staff or previously trained nurses. The variables collected were: sex, age, origin, (Valencian community, elsewhere in Spain, and foreigners), level of schooling, number of childbirths, (completed or miscarried), age at first pregnancy, body mass index (BMI, Quetelet index), periods of weight reduction (with weight loss greater than 5 kg), history of diabetes and hyperlipemia, intake of fiber, cholesterol saturated fat (according to a questionnaire of dietary habits in which the weekly frequency intake of different foods was surveyed), citric intake, consumption of alcohol (grams per day), coffee (cups per day), tobacco (cigarettes per day), use of hypolipidemic agents, aspirin, other non steroidal anti inflammatory drugs (NSAIDs), physical exercise (sedentary, moderate, active, and high), intestinal rhythm, and use of laxatives. Blood samples were collected at baseline, and the following parameters were evaluated: (1) hematological, (2) biochemical, and (3) serum values of glucose, triglycerides, total cholesterol, LDL and HDL. Cases with a glucose value greater than 140 mg/dl were considered as diabetics. The biochemistry was processed by means of an ERIS-6170 autoanalyzer, except for cholesterol HDL, which was obtained by Ependorf (Chod-Pap method). For technical reasons it was not possible to determine the HDL and LDL in 20% of the participants.

Statistical Methods and Validation of Data Base. The SPSS/PC V3.1 program was used to perform all statistical analysis. The function Statcalc of Epiinfo V5 was used to calculate the odds ratio (OR) in the 2×2 table. A check of the normality of the quantitative variables was carried out by means of the Kolmogorov-Smirnov method. The comparison of these variables was carried out with Student's *t* test. The qualitative variables were compared with the chi-square test (χ^2) using Fisher's exact test when necessary. For multivariate analysis, multiple logistic regression was used after separating women from men. The following models, which grouped variables in different ways, were used: (1) BMI, periods of weight reduction, physical exercise, and diabetes, (2) intake of fiber, cholesterol, saturated fats, and citrics; (3) intake of coffee, alcohol, and tobacco; (4) use of aspirin and NSAIDs; (5) defecation rhythm and consumption of laxatives; (6) total cholesterol, triglycerides, and use of hypolipidemic agents; and (7) LDL cholesterol, HDL cholesterol, triglycerides, and consumption of hypolipidemic agents. In women, the following additional models were also analyzed: (8) number of childbirths and use of oral contraceptives, and (9) age at first childbirth and use of oral contraceptives. The age was included in all models. The confidence interval (CI) used was 95%.

We validated the database by examining a random sample of 10% of the protocols. Only a 0.29% error or omission (CI 0.21 to 0.37) was obtained, without observation of accumulation in the groups comprising the main variables.

Ethical Aspects. The study was approved by the Committee of Investigation and Ethics of the Hospital Francesc de Borja (Gandia); all related documentation was of a confidential nature.

RESULTS

Univariate Analysis

BMI. The BMI average was lower in non lithiasic women than in lithiasic women (26.6 ± 4.70 vs 29.17 ± 4.47; $P < 0.001$). Among women, a linear relationship was found between CD and BMI levels ($\chi^2 = 35.64$, $P < 0.001$; Mantel Haenszel = 34.75, $P < 0.001$). Women with a BMI > 25 had a prevalence of CD of 19.1% as opposed to 6.3% in those with a BMI < 25 (OR = 3.55; CI: 1.99–6.41). Among women >40 years old, the significant correlation persisted ($P = 0.007$, OR = 2.79, CI: 1.23–6.57). In contrast, no significant association was found between the BMI and CD in men. Finally, there was no significant association between the number of weight loss periods and CD in both men and women.

Diabetes. There was a positive correlation between diabetes and CD. Subjects with glucose > 140 mg had a CD prevalence of 22.7% compared to 9.2% in those with lower levels of glucose ($\chi^2 = 14.19$, $P < 0.001$), differences which were maintained after separating men and women and after comparing diabetic individuals treated pharmacologically with those treated only by diet and with non diabetics. After controlling for age, nonsignificant differences were found in the prevalence of CD among diabetics and nondiabetics, both in women and in men.

Positive Family History of CD. CD was more frequent (12.1%) among those with a family history of this pathology compared with those without a history of CD among direct relatives (9.6%); however, the differences did not reach statistical significance.

Childbirths. The prevalence of CD was found to be significantly correlated with the number of childbirths. The pregnancy average (completed and aborted) was higher among women with CD (2.61 ± 1.58) than in those without CD (1.96 ± 1.74; t = 3.35, P = 0.001). Likewise, the prevalence of CD was greater among women with pregnancies (16.3%) than in nuliparus (6.7%) (P = 0.002; OR = 2.69, CI: 1.35–5.50). After stratifying by age, there were no significant differences. The average age of the first pregnancy was similar in both groups (with or without CD).

Diet. The average frequency of intake of food rich in fiber was similar in persons with or without CD, with no significant differences after separating men and women. However, those whose fiber intake was very frequent, had a lower prevalence of CD compared to those whose fiber intake was low or medium (2.4% vs 8.8% vs 11.1%; χ^2 = 7.25, P = 0.27).

The average frequency of intake of food rich in cholesterol and saturated fats was lower in CD cases compared to the controls (11.12 ± 2.28 vs 12.6 ± 2.33; t = −4.74, P < 0.001). This difference persisted after stratifying by sex and following exclusion of subjects with previous surgery cases or known lithiasis. Both men and women had a higher prevalence of CD when the intake of this type of food was low, the effect still being present in women >40 years old (30% of CD in low consumers as opposed to 14.9% in high consumers, P < 0.001, OR = 0.41, CI: 0.24–to 0.70).

No association was found between the consumption of citrics and CD.

The prevalence of CD was higher in those who consumed non alcohol compared to occasional consumers or habitual consumers (14.7% vs 6.3% vs 7.0%, χ^2 = 22.21, P = 0.000). Subjects with CD drank an average of 8.32 ± 22.08 g/day compared to 18.44 ± 39.33 in controls (t = −441, P = 0.000). After stratifying by sex, men continued to show the same pattern, but this did not statistical significance, whereas in women, the average alcohol consumption was practically the same in cases and controls.

CD was less prevalent among coffee consumers than in nonconsumers (8.7% vs 12.8%; χ^2 = 5.21, P = 0.022). After stratifying by sex, this trend persisted without reaching statistical significance.

Tobacco Use. Smokers and exsmokers had a lower prevalence of CD than nonsmokers (5.1% vs 14.4%; χ^2 = 30.44, P < 0.001). This difference persisted after stratifying by sex, but did not reach statistical significance in men.

Oral Contraceptives. CD was more frequent among women who had not taken oral contraceptives than those who had (16% vs 7.1%; χ^2 = 7.82, P = 0.005). However in the group of women under 40,

TABLE 1. ORAL CONTRACEPTIVES (OC) AND CHOLELITHIASIC DISEASE (CD)

	CD prevalence				
Women studied	*OC yes [N (%)]*	*OC no [N (%)]*	P	*OR*	*95% CI*
All	11/155 (7.1)	81/507 (16.0)	0.0052	0.40	0.20–0.81
Age < 40	5/108 (4.6)	5/142 (3.5)	0.6577*	1.33	0.30–5.94
Age ≥ 40	6/47 (12.8)	76/366 (20.8)	0.1956	0.56	0.19–1.39

*Fisher: P = 0.7496.

CD was more frequent among those who had used oral contraceptives compared to those who had not used them (4.6% vs 3.5%) without reaching statistical significance (Table 1).

Hypolipidemic Drugs. The prevalence of CD was higher among subjects who had taken hypolipidemic drugs than those who had not (25% vs 9.1%; χ^2 = 19.03, P < 0.001). After stratifying by sex, differences were still observed in the group of women regardless of age (P < 0.001, OR = 3.42, CI, 1.61–7.25).

Aspirin and Other NSAIDs. CD was more prevalent among subjects who took aspirin than in those who did not (13.7% vs 9.3; χ^2 = 3.66, P = 0.056). After stratifying by sex, differences did not reach statistical significance. A higher frequency of CD was observed among people who consumed NSAIDs (different other than aspirin) when compared to those who did not (16.3% vs 9.4%; χ^2 = 5.10, P = 0.024). This pattern was also observed in subjects older than 40 (19.3% vs 14.4%) without reaching statistical significance

Exercise. CD was more frequent among sedentary people than in those who did some form of physical exercise (12.1% vs 9.1%) without reaching statistical significance. After stratifying by sex, differences reached statistical significance in men (10.8% vs 3.6%; χ^2 = 12.14, P < 0.001), regardless of age.

Bowel Habit. People with one or more defecations per day had a lower CD prevalence than those with a low defecation rhythm (9.3% vs 13.5%, χ^2 = 3.29, P = 0.070). After stratifying by sex and age, this trend was still observed without reaching statistical significance. CD was also more frequent among consumers of laxatives than in nonconsumers (17.6% vs 9.1%; χ^2 = 9.91, P = 0.002). This tendency continued after separating by sex and age, but was not statistically significant.

Lipid Metabolism. The total cholesterol average was higher among subjects with CD than in those

TABLE 2. TOTAL CHOLESTEROL AND CHOLELITHIASIC DISEASE (CD)

Group	No. of cases and Cholesterol (mean ± SD) CD yes	CD no	T	P
All	126	1136		
	239.05 ± 55.19	221.89 ± 48.38	3.35	0.001
Men	34	564		
	224.38 ± 44.14	223.08 ± 48.39	0.15	0.879
Women	92	572		
	244.47 ± 58.03	220.71 ± 48.38	3.72	0.000
Women >40	80	324		
	251.58 ± 58.61	242.58 ± 46.38	1.28	0.204

without (239.1 ± 55.19 vs 221.9 ± 48.38; $t = 3.35$, $P = 0.001$). After stratifying by sex, differences persisted in women but not in men (Table 2). The LDL cholesterol average was also higher among individuals with CD than those without (151.3 ± 49.46 vs 141.8 ± 41.66; $t = 1.84$, $P = 0.068$). This trend became significant in women under 40 years old ($t = 2.68$, $P = 0.009$), but not in those over 40. In men, the trends observed were the opposite to those observed in women without reaching statistical significance.

The HDL cholesterol average was higher in the CD group than in the controls without reaching statistical significance (62.1 ± 15.24 vs 60.6 ± 16.57; $t = 1.18$, $P = 0.240$).

The triglyceride average was higher in subjects with CD than in the controls. (131.1 ± 96.99 vs 111.7 ± 80.58; $t = 2.16$, $P = 0.03$). After stratifying by sex, the differences continued to be significant in women even in the over-40 group, but not in men (Table 3). Women over 40 with triglycerides >200 mg/dl, had a CD prevalence of 35.5% compared to 18.5% among those who had <200 mg/dl. ($P = 0.020$, OR = 2.43, CI: 1.04–5.63).

Multivariate Analysis

The variables that were independently associated with CD in the various models of logistic regression, apart from age, were: in women—positive association with obesity (Table 4) and consumption of hypolipidemic drugs, and negative association with consumption of food rich in cholesterol and saturated fats; and in men—positive association with the triglyceride level, and negative association with physical exercise (Table 5).

There was a trend between the number of childbirths in women and the LDL cholesterol in men, without reaching statistical significance.

A summary of variables associated with CD, both in univariate and multivariate analyses is shown in Tables 6 and 7.

TABLE 3. TRIGLYCERIDES AND CHOLELITHIASIC DISEASE (CD)

Group	No. of cases and triglycerids (Mean ± SD) CD yes	CD no	T	P
All	126	1136		
	131.08 ± 96.99	111.73 ± 80.58	2.16	0.033
Men	34	564		
	151.85 ± 131.87	133.20 ± 90.18	0.81	0.421
Women	92	572		
	123.40 ± 79.96	90.56 ± 63.11	3.76	0.000
Women >40	80	324		
	129.88 ± 82.40	107.11 ± 75.09	2.38	0.018

TABLE 4. CD-ASSOCIATED FACTORS: MULTIVARIATE ANALYSIS (WOMEN) OF OBESITY, SLIMMING, AND PHYSICAL EXERCISE

Variable	B	SE	Sig	OR	CI
Age (quant.)*	0.0496	0.0098	0.0000	1.05	1.03–1.07
BMI(Quetelet) (quant.)	0.0699	0.0276	0.0114	1.07	1.02–1.13
Slimming (No><Some)†	0.3537	0.2558	0.1668	1.42	0.86–2.35
Physical exercise (No><Yes)‡	0.2935	0.2660	0.2669	1.34	0.80–2.26

*Quant.: quantitative variable.
†Periods of voluntary or involuntary slimming with weight loss >5 kg.
‡No (sedentary) compared with yes (any of three levels: moderate, active, heavy).

DISCUSSION

Obesity and Periods of Weight Loss. The vast majority of authors consider obesity as a potent risk factor for CD in women (15–19), both in old (20–22) and young (23) individuals. The mechanisms described by which obesity can facilitate lithiasis include an increase in the biliary secretion of cholesterol, changes in nucleation factors, and alteration of the gallbladder motility. Results from our study are in

TABLE 5. CD-ASSOCIATED FACTORS: MULTIVARIATE ANALYSIS (MEN) OF OBESITY, SLIMMING, AND PHYSICAL EXERCISE

Variable	B	SE	Sig.	OR	CI
Age (quant.)*	0.0649	0.0147	0.0000	1.07	1.04–1.10
BMI(Quetelet) (quant.)	−0.0328	0.0539	0.5422	0.97	0.87–1.08
Slimming (No><Some)†	0.2773	0.4416	0.5301	1.32	0.56–3.14
Physical exercise (No><Yes)‡	−0.8216	0.3750	0.0284	0.44	0.21–0.92

*Quant.: quantitative variable.
†Periods of voluntary or involuntary slimming with weight loss >5 kg.
‡No (sedentary) compared with yes (any of three levels: moderate, active, heavy).

TABLE 6. UNIVARIATE ANALYSIS SUMMARY*

Variable	Global		Age > 40	
	Sig (P)	OR (CI)	Sig (P)	OR (CI)
Women				
Obesity	<0.001	3.86 (2.14–7.08)	0.007	2.79 (1.23–6.57)
Diabetes	0.017	2.47 (1.07–5.56)	NS	
Pregnancies	<0.001	3.09 (1.75–5.51)	NS	
Hypolipidemic agents	<0.001	4.79 (2.31–9.89)	<0.001	3.42 (1.61–7.25)
Total cholesterol	<0.001	2.20 (1.36–3.58)	NS	
LDL cholesterol	=0.013	3.00 (1.18–9.03)	NS	
Triglycerides	<0.001	3.76 (1.68–8.32)	=0.022	2.43 (1.04–5.63)
Tobacco	<0.001	0.26 (0.12–0.55)	NS	
Cholest-SF	=0.002	0.42 (0.23–0.77)	<0.001	0.41 (0.24–0.70)
Oral contraceptives	=0.005	0.40 (0.20–0.81)	NS	
Men				
Diabetes	=0.004	4.46 (1.62–11.83)	NS	
Cholest-SF	=0.002	0.35 (0.16–0.74)	NS	
Physical exercise	<0.001	0.30 (0.14–0.65)	=0.002	0.32 (0.14–0.72)

*Sig: statistical significance. Cholest-SF: diet rich in cholesterol and saturated fats.

accordance with those from the literature, with a linear correlation between BMI and CD (Figure 1). Moreover, obesity was one of the three factors associated with CD in the multivariate analysis. The importance of this factor is not as evident in men as it is in women (17), and while some studies have found a positive association, (14, 23–26), others, such as ours, were unable to demonstrate a real association (15, 27). The reasons for these discrepancies include the inadequacy of the Quetelet index to estimate obesity in men, as it also measures muscular mass, and the lack of power of the study due to the relatively low number of significantly obese men (15). Prolonged periods of fasting could be trigger a series of lithogenic phenomena. Indeed, it has been pointed out that nocturnal fasting is greater in young women with lithiasis compared to nonlithiasic women (28). The results of studies that analyze changes in the components of bile in obese cases fed with a low calorie diet (29) have described the following sequence: shortening of nucleation time, change from arachidonic acid to prostaglandin, and stimulus of biliary synthesis/secretion of glycoprotein, which in turn cause nucleation. This hypothesis is supported by the high incidence of lithiasis in obese patients who undergo a slimming diet (30, 31), an incidence which fluctuates between 10 and 25% in the first few months of diet (17), to higher proportions of lithiasic phenomena (bilial sludge, gallstones) during parenteral nutrition (32) or after surgical treatment of morbid obesity

TABLE 7. MULTIVARIATE ANALYSIS SUMMARY*

Variable	Sig.	OR	CI
Age (MW)	0.0000		
Chol-SF consumption (W)	0.0203	0.88	0.79–0.98
Hypolipidemic agents* (W)	0.0145	2.51	1.20–5.26
Obesity (W)	0.0114	1.07	1.02–1.13
Pregnancies (W)	0.1220	1.73	1.24–5.28
Physical exercise (M)	0.0280	0.44	0.21–0.92
Triglycerides (M)	0.0445	1.00	1.00–1.01
LDL Cholesterol (M)	0.0850	0.99	0.98–1.00

*M: men; W: women; MW: men and women; Chol-SF: cholesterol and saturated fats (dietary consumption). All variables were treated as quantitative except those marked with a dagger, which are dichotomous (no/yes). In the case of lipidemic agents, this refers to history of consumption, and in physical exercise, no activity or sedentary (no) compared to some (yes) in the scale (moderate, active, or heavy).

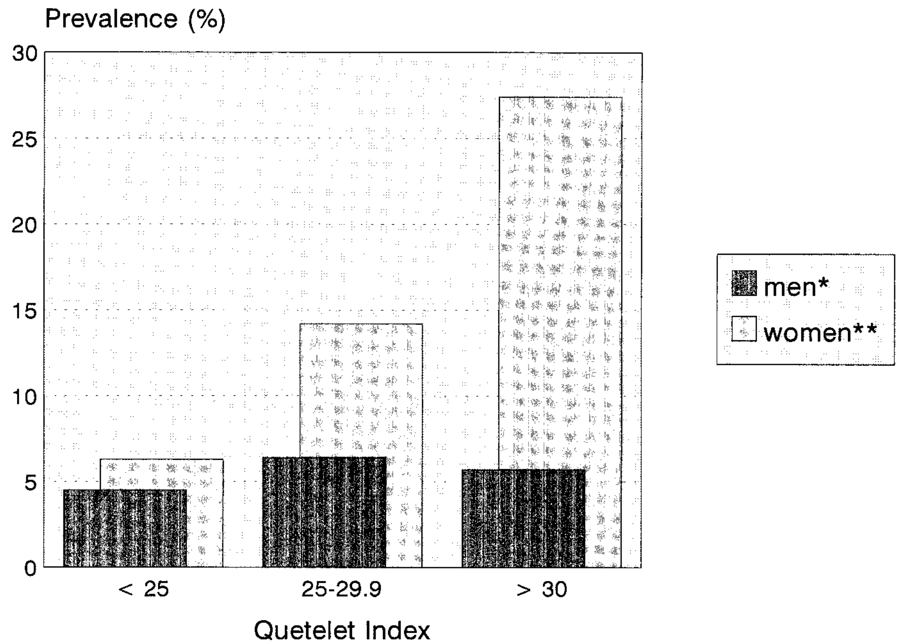

Fig 1. Obesity and cholelithiasic disease. Prevalence according to Quetelet index in men and women. *NS, **χ^2 = 35.6, $P < 0.0001$; Mantel-Haenszel = 34.7, $P < 0.0001$.

(33). In our series, a positive relationship was observed between the number of weight loss periods (loss >5 kg) and CD. However, after stratifying by BMI, or in the multivariate analysis, weight loss did not end up as an independent risk factor for CD, either in women or in men. The possible reasons for some of the discrepant results include: (1) incorrect study design of (2) lack of distinction between rapid and slow weight loss; and (3) difficulty in detecting unstable situations

Diabetes. Results on the influence of diabetes on CD are controversial (15). Some studies have detected several types of gallbladder alterations, ranging from increases in the saturation of cholesterol related with insulin (34) to a decrease of gallbladder motility in neuropathic diabetes (35). Others, however, have failed to find these changes (36). By combining several studies, diabetes does not appear as a predictive factor for CD. Indeed, in our series, although there was a clear positive association in the univariate analysis, it did not reach statistical significance when performing the multivariate analysis, suggesting that diabetes, has, if any, a weak influence on CD.

Positive Family History of Biliary Lithiasis. A greater frequency of biliary lithiasis in families with gallstone carriers has previously been described (1, 10). Contributing factors include both environmental (related to dietary habits) and genetic factors (greater predisposition of CD in carriers of APO E-4) (8, 9). In our study, a possible bias in the recollection of this variable must be taken into consideration, since data collection was only done at baseline and not at the end of the study where additional information on family history of lithiasis could have been obtained.

Multiparity and Age of First Childbirth. Although the number of pregnancies is a classically accepted risk factor, there have been a number of discrepancies among various studies, with some showing no relation (16, 18, 24, 31, 37) and some showing a positive association (14, 38–41). Recent investigations confirm the existence of a positive association (42–45). They have described that: (1) the population of nulliparous and primiparous women (before the tenth week) had an incidence of CD similar to that of men of the same age (42); (2) the prevalence of CD increases independently with number of pregnancies (43); (3) pregnancy is a key factor, since during this process, there is a higher incidence of lithiasis and biliary sludge, compared to controls, whereas in post-pregnancy some of these lithiasic phenomena disappear due to the normalization of metabolism (44, 45). The phenomena that take place during pregnancy include an increase in the saturation of cholesterol in bile; and biliary stasis secondary to the actions of estrogens and progesterone (46). Our data are in accordance to those published, since we found signif-

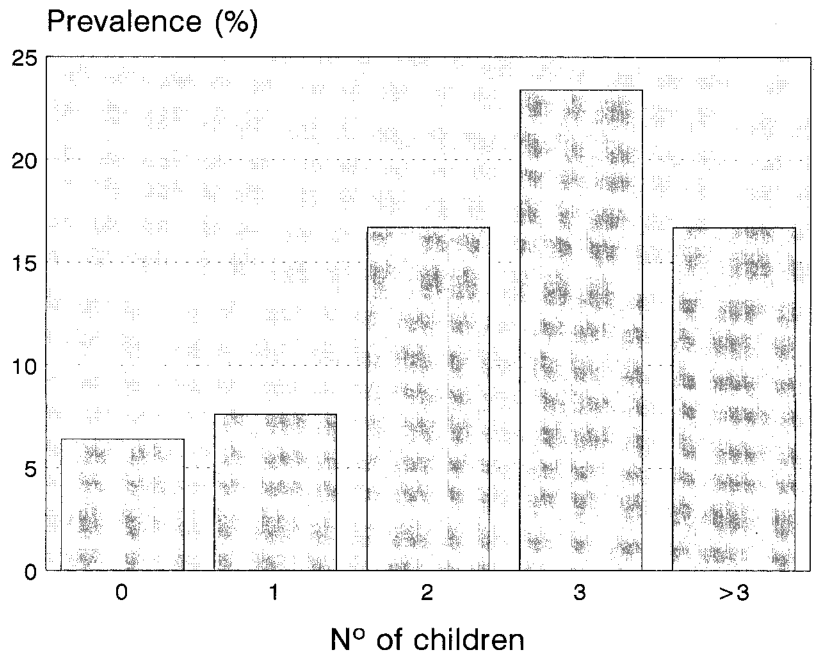

Fig 2. Multiparity and cholelithiasic disease. Prevalence according to number of children. $\chi^2 = 21$, $P = 0.0003$; Mantel-Haenszel = 15.6, $P = 0.0001$.

icant differences in CD prevalence between nulliparous woman and women with some pregnancy, and a positive linear relation with the number of children (Figure 2); a nearly significant statistical association in the multivariate analysis was observed. However, in contrast to a recent cohort study, we did not find any association between the age of the first pregnancy and CD.

Diet. Significant discrepancies exist as to whether diet plays a role in the pathogenesis of CD. Both fiber and saturated fats have been found to be either a risk factor or a protective one against CD. The reasons for this wide variation of results probably relates to the lack of homogeneity in dietary surveys, both in terms of methods and timing of data collection. For instance, diet may change as a consequence of knowledge of CD, and thus biased results may be obtained if the questions are not conducted at baseline. In that sense, cohort studies are more appropriate to study this type of association. In our study, we found a reduced prevalence of CD among high consumers of fiber compared to low or medium consumers, but this difference did not reach statistical significance in the multivariate analysis. There was also a negative linear relation between the prevalence of CD and the intake of foods rich in cholesterol and saturated fats. This protective effect was still maintained on multivariate analysis in the group of women but not in the group of men. The possible protective role of fiber and cholesterol and saturated fat in women constitutes a dietary pattern similar to that described in the study by Barbara et al (47). The reduced intake of fat and total calories in cholelithiasis might explain the increased prevalence of CD in men over a 10 year period in an English necropsy study. This increase could be attributed to the important dietary changes secondary to a program aimed at the prevention of coronary heart disease, which took place at the same time and place as the necropsy study (48). On the other hand, recent studies seem to suggest that an excessive reduction of dietary fat would disturb gallbladder motility, thus favoring biliary stasis and consequently the promotion of gallstones (49, 50).

According to experimental studies on animals, vitamin C may play a protective role. This, however, has yet to be proven in humans (51). On the other hand, various situations where there is usually a deficit of ascorbic acid (obesity, advanced age, diabetes, and treatment with estrogen) have been associated with a high prevalence of CD (52). A high consumption of citrics (and therefore of vitamin C) occurs in the study area. We therefore included this variable in the analysis but found no association.

Alcohol, Coffee, and Tobacco. A study of volunteers showed a lowering of biliary cholesterol saturation after a 39 g/day intake of alcohol (53). The protective

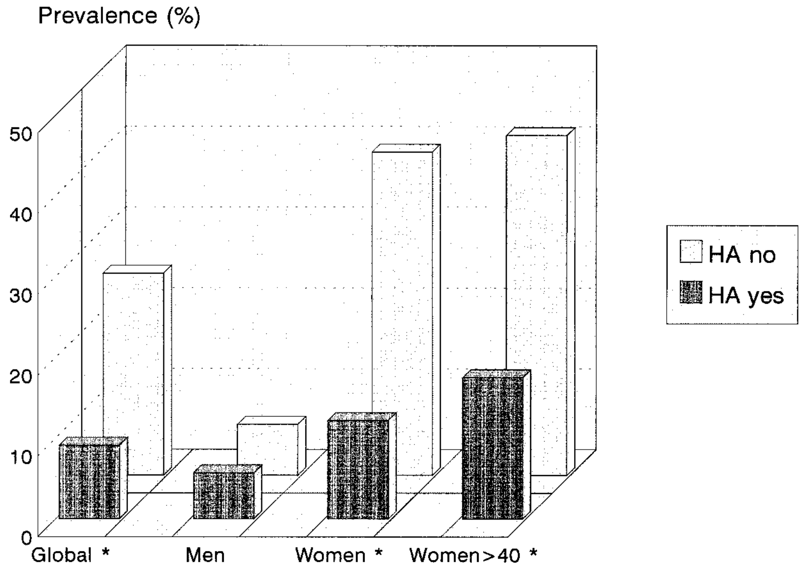

Fig 3. Hypolipidemic agents (HA) and cholelithiasic disease. Prevalence according to use in the entire group and separated by sex and women over 40. *$P < 0.001$.

effect of alcohol has been reported in some studies (12, 18, 24, 25, 54, 55), but not in all (27, 35, 56). Alcohol consumption was found as a protective factor of CD in men in the univariate analysis. Multivariate analysis, however, did not confirm this. Such an association was not observed in women, but we acknowledge possible bias due to a tendency of women to hide it.

Only one study (15) has evaluated coffee intake as a risk factor for CD, due to its hypothetical action on biliary acids on enterohepatic circulation. Our study, in accordance to that by Jorgensen, did not find an association between coffee intake and CD.

Tobacco is another substance that has been associated with CD through a reduction in HDL cholesterol (15). Results are controversial, however. We did not find an association between tobacco and CD.

Oral Contraceptives. The possible lithogenic role of oral contraceptives is a subject of continued controversy, given the disparity of results in prospective and cohort studies (10). Most physiopathological studies have shown an increase in biliary cholesterol saturation among women taking oral contraceptives. A recent review, however, has suggested that: (1) small doses of estrogen do not have this effect, (2) the chemical nature of progestagen has a clear role in biliary secretion, and (3) the role of oral contraceptives on gallbladder motility is still debatable (3). Our results confirm previous studies, which showed that age was a confounding variable (24). By controlling for this variable, we found a positive, although nonsignificant association between oral contraceptives and CD in women under the age of 40. In a meta-analysis, which included nine of 25, studies the association between oral contraceptives and CD was found to be weak and transitory, with an OR = 1.36 (use vs nonuse). There also seemed to be a dose effect, suggesting that modern oral contraceptives, which have lower doses, are less lithogenic than previous ones (57).

Hypolipidemic Agents. The positive association of clofibrate with biliary lithiasis seems well established. This drug provokes an increase in the biliary saturation of cholesterol (1, 10), in that patients who use it have a 2.4 greater risk of CD than control cases (4). Other hypolipidemic agents, however, such as HMG-CoA reductase inhibitors, lower the biliary saturation of cholesterol and thus could have a beneficial effect (58, 59). Few studies have analyzed this variable, and those that have included it lack the power to establish an association due to the low number of patients who take the drug. It is therefore interesting that an evident, positive association between hypolipidemic drugs and CD in women was observed in the present study (Figure 3). Unfortunately, the type of drug used was not included in the questionnaire. We nonetheless believe that given the time frame of data collection, the impact of new drugs such as inhibitors of HMG-CoA reductase seems unlikely.

Aspirin and Other NSAIDs. A study on laboratory animals showed that aspirin at doses of 100 mg/kg, inhibits the secretion of mucus in the gallbladder

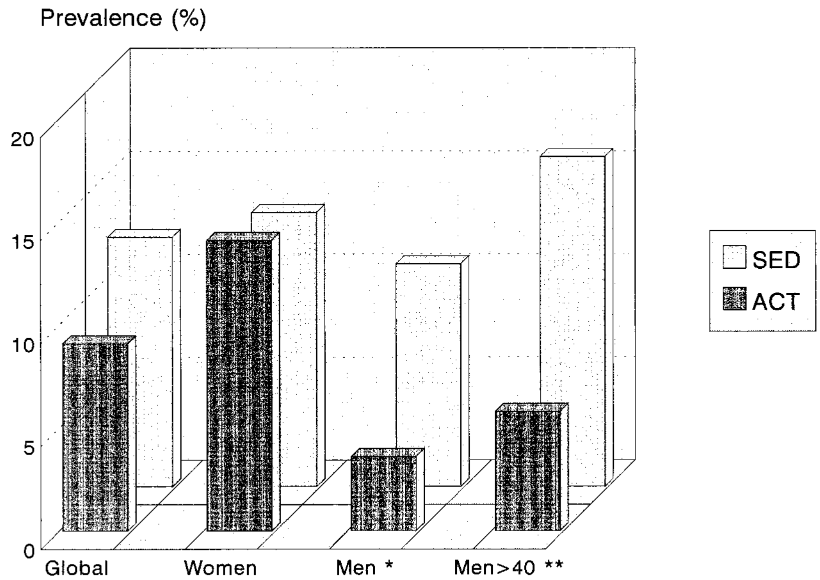

Fig 4. Physical activity and cholelithiasic disease. Prevalence according to activity or no-activity in the entire group and separated by sex and men over 40. SED: sedentary; ACT: activity (moderate, active, or heavy). *$P < 0.001$; **$P < 0.005$.

through a reduction in the synthesis of prostaglandin, and although it has no effect on the saturation of cholesterol, it impedes nucleation and prevents the appearance of gallstones (13). Clinical studies have, however, been unable to find a significant relationship (60–62). These studies, however, had methodological problems that could account for the lack of association. On the other hand, a British–Belgian group has found a preventive role for NSAIDs in the relapse of dissolved gallstones through oral administration of biliary acids (63, 64). In the present series, neither aspirin nor other NSAIDs had an association on multivariate analysis.

Physical Activity. Low physical activity may lead to a decrease in HDL (65, 66), which in turn may produce an increase in lithogenic index (67), and this leads to a greater frequency of gallstones (68). On the other hand, physical activity may be inversely related to levels of triglycerides (69), an increase of which could be a risk factor for cholelithiasis. In that sense, physical activity could be considered a protective factor against CD, whereas a sedentary state is a risk factor (1, 10). One extensive Japanese study (26), showed that physical activity was a protective factor against CD with a dose-related manner. In accordance to the previous study, we observed a negative association between physical activity and CD in men (Figure 4). However, we failed to find a dose-related response, perhaps due the relatively small size of the sample.

Intestinal Rhythm and Use of Laxatives. In an experimental study, an increase of deoxycholate and cholesterol saturation index was observed in a group of constipated people treated with senna laxatives and in another group of normal volunteers treated with loperamide (70). In a study at Bristol (71), intestinal transit was compared between 15 women with lithiasis without obesity (BMI < 25) and 15 controls. A significantly slow intestinal transit and reduced stool output was found in the lithiasic group (72). The possible relationship of intestinal transit to lithiasis remains unanswered. We observed a trend towards a positive association between constipation and CD, without reaching statistical significance. After stratifying by sex and BMI, a greater prevalence of CD was observed among women with BMI < 25 with marked constipation (<2 bowel movements per week) compared to the rest, but the differences did not reach statistical significance. The use of laxatives was also positively associated with CD, but the association was not seen after controlling for sex and age (Figure 5). Further studies are needed in order to confirm these results.

Plasma Lipids. There is no a typical lipid profile associated with CD. Case–control studies, moreover, are not suitable to evaluate such association as, with time, the levels of lipids may change. We acknowledge that findings related to LDL and HDL cholesterol should be interpreted with caution in the present study, as they were unavailable in 20% of the cases. Regarding total cholesterol, while the majority of studies have not found an association between total

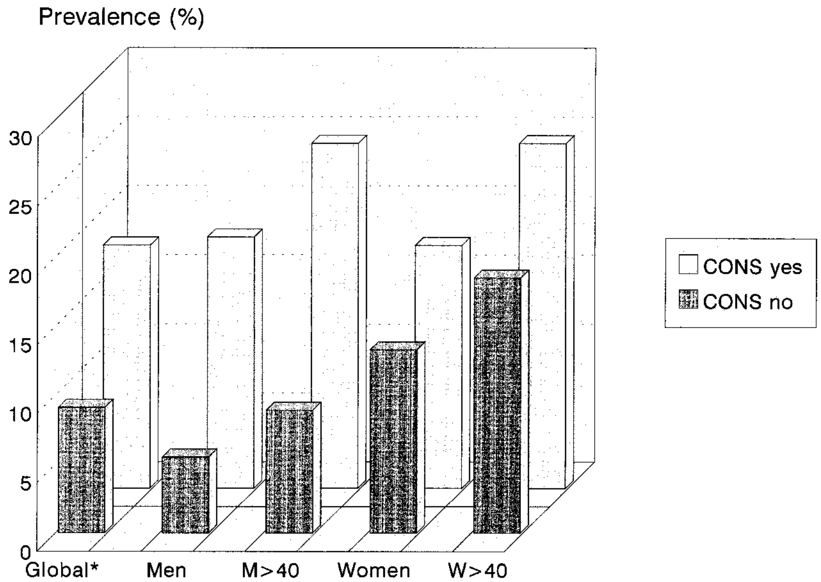

Fig 5. Laxatives and Cholelithiasic Disease. Prevalence according to consumption (yes/no) in the entire group and separated by sex and age over 40. CONS: consumption; M: men; W: women. *$P = 0.0016$.

cholesterol and CD (14, 26, 73–75), some authors have found a negative association (68, 76) and, finally others, such as ours, have found some positive association among women, at univariate analysis only (77). The studies that have included LDL cholesterol have generally not found a relationship between LDL cholesterol and CD (24, 73, 77). We also did not find an association between these two variables, although a trend was observed in univariate analysis between higher LDL cholesterol levels and CD in women. Regarding HDL cholesterol; as in other studies (14, 24, 75) we found no significant relationship between HDL cholesterol and CD. Larger sample studies, however, have observed a completely opposite relationship with an increase in biliary saturation of cholesterol and the risk of lithiasis (68, 69). Results regarding triglyceride levels and CD are also controversial, the larger studies reporting a positive association (14, 26, 27, 73, 76). This positive association was confirmed in our study in women, even after controlling for age; on multivariate analysis, however, differences did not reach statistical significance. In men, triglycerides were found to have an independent positive effect, at multivariate analysis.

In summary, although a number of factors have been evaluated as to their possible implication in the pathogenesis of CD, results are generally controversial, mainly as a consequence of small sample size, incorrect study design, lack of homogeneity, and bias due to confounding factors. In our study, five factors—obesity a diet rich in cholesterol and saturated fats, intake of hipolipidemic agents, physical exercise, and triglyceride level—were associated with CD, independent of age. Both a diet rich in cholesterol and saturated fats in women and physical exercise in men were inversely correlated with CD. Due to their probable protective effect against CD, they should be kept in mind. These and other possible associations should be confirmed by further and larger studies in order to clarify the complex mechanisms influencing the development of this disease.

REFERENCES

1. Davion T, Capron J-P: Epidemiologie et facteurs de risque de la lithiase biliaire. S Erlinger (eds). *In* La lithiase biliaire, Paris, Doin Editeurs, 1991, pp 1–15.
2. Bennion LJ, Grundy ST: Risk factors for the development of cholelithiasis in man. N Engl J Med 299:1221–1227, 1978
3. Davion T, Capron JP: Facteurs de risque de la lithiase biliaire cholestérolique. Bilan du septennat. Gastroenterol Clin Biol 12:649–660, 1988
4. Teres J: Epidemiologia, factores de riesgo y patogenia de la litiasis biliar. Gastroenterol Hepatol 16:630–635, 1993
5. Ros Rahola E: Litiasis biliar: Evolucion conceptual y revolucion terapeutica. Med Clin (Barc) 90:297–305, 1988
6. Maurer KR, Everhart JE, Ezzati TE, Johannes RS, Knowler WC, Larson DL, Sanders R, Shawker TH, Roth HP: Prevalence of gallstone disease in Hispanic populations in the United States. Gastroenterology 96:487–492, 1989
7. Sampliner RE, Bennett PH, Comess LJ, Rose FA, Bruch TA: Gallbladder disease in Pima indians. Demonstration of high

prevalence and early onset by cholecistography. N Engl J Med 283:1358–1364, 1970
8. Bertomeu A, Ros E, Zambon D, Vela M, Perez-Ayuso RM, Targarona E, Trias M, Sanllehy C, Casals E, Ribo JM: Apolipoprotein E Polymorphism and Gallstones. Gastroenterology 111:1603–1610, 1996
9. Van Erpecum KJ, Carey MC: Apolipoprotein E4: Another risk factor for cholesterol gallstone formation ? Gastroenterology 111:1764–1767, 1996
10. Strom BL, West SL: Epidemiologia de la litiasis biliar. *In* Colelitiasis. S Cohen, S, RD Soloway (eds). Madrid, SANED, 1985, pp 1–26
11. Teres J: Epidemiologia y factores de riesgo de la litiasis biliar. *In* Actualidades en Gastroenterologia y Hepatologia (13), Litiasis biliar. J Rodes, C Chantar (eds). Barcelona, JR Prous, 1993, pp 35–41
12. Jorgensen T, Jorgensen LM: Gallstones and diet in a Danish population. Scand J Gastroenterol 24:821–826, 1989
13. Lee SP, Carey MC, LaMont JT: Aspirin prevention of cholesterol gallstone formation in prairie dogs. Science 211:1429–1431, 1981
14. Barbara L, Sama C, Labate AML, Taroni F, Rusticali AG, Festi D, Sapio C, Roda E, Banterle C, Puci A, Formentini F, Colasanti S, Nardin F: A population study on the prevalence of gallstone disease: The Sirmione study. Hepatology 7:913–917. 1987
15. Jorgensen T: Gall stones in a Danish population. Relation to weight, physical activity, smoking, coffe consumption, and diabetes mellitus. Gut 30:528–534, 1989
16. Walker ARP, Segal I, Posner R, Shein H, Tsotetsi NG, Walker AJ: Prevalence of gallstones in elderly black women in Soweto, Johannesburg, as assessed by ultrasound. Am J Gastroenterol 84:1383–1385, 1989
17. Everhart JE: Contributions of obesity and weight loss to gallstone disease. Ann Intern Med 119:1029–1035, 1993
18. Maclure KM, Hayes KC, Colditz GA, Stampfer MJ, Speizer FE, Willett WC: Weight, diet, and the risk of symtomatic gallstones in middle-aged women. N Engl J Med 321:563–569, 1989
19. Stampfer MJ, Maclure MK, Colditz GA, Manson JE, Willet WC: Risk of symptomatic gallstones in women with severe obesity. Am J Clin Nutr 55:652–658, 1992
20. Mellström D, Asztély M, Svanvik J: Gallstones and previous cholecystectomy in 77- to 78-year-old women in an urban population in Sweden. Scand J Gastroenterol 23:1241–1244, 1988
21. Attili AF, Carulli N, Roda E, Barbara B, Capocaccia L, Menotti A, Okoliksanyi L, Ricci G, Capocaccia R, Festi D, Lalloni L, Mariotti S, Sama C, Scafato E, M.I.COL. Group: Epidemiology of gallstone disease in Italy: Prevalence data of the Multicenter Italian Study on Cholelithiasis (M.I.COL.). Am J Epidemiol 141(2):158–165, 1995
22. Jorgensen T, Kay L, Schultz-Larsen K: The epidemiology of gallstones in a 70-year old Danish population. Scand J Gastroenterol 25:335–340, 1990
23. Palasciano G, Portincasa P, Vinciguerra V, Velardi A, Tardi S, Baldasarre G, Albano O: Gallstone prevalence and gallbladder volume in children and adolescents: An epidemiological ultrasonographic survey and relationship to body mass index. Am J Gastroenterol 84:1378–1382, 1989
24. Martinez de Pancorbo M: Litiasis biliar: Prevalencia y factores asociados en poblacion general. Doctoral thesis. Universidad Alcala de Henares. Fac. de Medicina, 1992
25. Kono S, Shichi K, Ikeda N, Yanai F, Imanishi K: Prevalence of gallstone disease in relation to smoking, alcohol use, obesity, and glucose tolerance: A study of self-defense officials in Japan. Am J Epidemiol 136:787–794, 1992
26. Kato I, Nomura A, Stemmermann GN, Chyou P: Prospective study of clinical gallbladder disease and its association with obesity, physical activity, and other factors. Dig Dis Sci 37:784–790, 1992
27. GREPCO (Rome Group for Epidemiology and prevention of cholelithiasis): The epidemiology of gallstone disease in Rome, Italy, Part II, Factors associated with the disease. Hepatology 8:907–913, 1988
28. Capron JP, Delamarre J, Herve MA, Dupas JL, Poulain P, Descombes P: Meal frequency and duration of overnight fast: A role in gall-stone formation. Br Med J 283:1435, 1981
29. Marks JW, Bonorris GG, Albers G, Schoenfield LJ: The sequence of biliary events preceding the formation of gallstones in humans. Gastroenterology 103:566–570, 1992
30. Kamrath R, Plummer LJ, Sadur CN, Adler MA, Strader WJ, Young RL, Weinstein RL: Cholelithiasis in patients treated with a very-low-calorie diet. Am J Clin Nutr 56:255S–257S, 1992
31. Yang H, Petersen GM, Roth MP, Schonefield LJ, Marks JW: Risk factors for gallstone formation during rapid loss of weight. Dig Dis Sci 37:912–918, 1992
32. Messing B, Boris C, Kunstlinger F, Bernier JJ: Does total parenteral nutrition induce gallbladder sludge formation and lithiasis? Gastroenterology 84:1012–1019, 1983
33. Shiffman ML, Sugerman HJ, Kellum JM, Brewer WH, Moore EW: Gallstone formation after rapid weight loss: a prospective study in patients undergoing gastric bypass surgery for treatment of morbid obesity. Am J Gastroenterol 86:1000–1005, 1991
34. Bennion LJ, Grundy SM: Effects of diabetes mellitus on cholesterol metabolism in man. N Engl J Med 296:1365–1371, 1977
35. Fiorucci S, Bosso R, Scionti L, Disanto S, Annibale B, Delle Fave G, Morelli A: Neurohumoral control of gallbladder motility in healthy subjects and diabetic patients whith or without autonomic neuropathy. Dig Dis Sci 35:1089–1097, 1990
36. Wedmann B, Droge C, Amoiridis G, Schmidt-Heinevetter G, Wegener M, Ricken D: Gallbladder motility in diabetic patients with and without cardiovascular neuropathy. Z Gastroenterol 30:519–524, 1992
37. Abdel-Rahman HA, Hafez AS, Maymoun NM, el-Gendy SD, Abdo AS, Yonis AM, Abdel-Aziz S: Risk factors of gallstone disease in a sample of patients in Benha City. J Egypt Public Health Assoc 68:205–227, 1993
38. GREPCO (Rome Group for Epidemiology and Prevention of Cholelithiasis): Prevalence of gallstone disease in an italian adult female population. Am J Epidemiol 119:796–805, 1984
39. Khuroo MS, Mahajan R, Zargar SA, Javid G, Sapru S: Prevalence of biliary tract disease in India: A sonographic study in adult population in Kashmir. Gut 30:201–205, 1989
40. Rusticali AG, Sama C, Morselli Labate AM, Frabboni R, Nacchiero MC, Tassinari G, Barbara L: The Boario project. A study of the prevalence of lithiasis in a spa population. Minerva Med 81:301–306, 1990
41. Jorgensen T: Gall stones in a Danish population: Fertility

period, pregnancies, and exogenus female sex hormones. Gut 29:433–439, 1988

42. Glasinovic JC, Marinovic I, Mege R, Vela P, Fernandez de la Reguera P, Ahumada E, Valdivia MT, Alvarado A, Scrivanti M: Prevalencia de colelitiasis en mujeres nuliparas en edad fertil. Gastroenterol Hepatol 16:111–115, 1993
43. Glasinovic JC, Marinovic I, Mege R, Vela P, Ferreiro O, Ahumada E, Tobar E, Valdivia MT, Alvarado A: Efecto del embarazo sobre la incidencia y la prevalencia de colelitiasis. Gastroenterol Hepatol 16:61–66, 1993
44. Valdivieso V, Covarrubias C, Siegel F, Cruz F: Pregnancy and cholelitiasis: Pathogenesis and natural course of gallstones diagnosed in early puerperium. Hepatology 17:1–4, 1993
45. Maringhini A, Ciambra M, Baccelliere E, Raimondo M, Orlando A, Tinè F, Grasso R, Randazzo MA, Barresi L, Gullo D, Musicò M, Pagliaro L: Biliari sludge and gallstones in pregnancy: Incidence, risk factors, and natural history. Ann Intern Med 119:116–120, 1993
46. Everson GT: Pregnancy and gallstones. Hepatology 17:159–161, 1993
47. Barbara L, Cornia GL, Festi D, Frabboni R, Morselli-Labate AM, Nacchiero MC, Parro S, Pollini G, Roda E, Rossi M, Rusticali AG, Sama C, Taroni F, Tassinari G, Banterle C, Colasanti S, Formentini G, Moreni O, Nardin F, Pilia MC, Puci C: Dietari habit and incidence of gallstones in the "Sirmione Study." Hepatology 10:736, 1989 (abstract)
48. Bates T, Harrison M, Lowe D, Lawson G, Padley N: Longitudinal study of gall stone prevalence at necropsy. Gut 33:103–107, 1992
49. Festi D, Orsini M, Colecchia A, Sottili S, Sangermano A, Mazzella G, Villanova N, Petroni ML, Pavesi S, Roda E: Gallbladder motility and gallstone formation during rapid weigth loss induced by very low calorie diets: A present confirmation of an old clinical observation. Gastroenterology 110:A663, 1996
50. Pallotta N, Cicala M, Frandina C, Corazziari E, and Torsoli A: Gallbladder recycling of hepatic bile after meals with different fat contents. Gastroenterology 110:A728, 1996
51. Lefkof IR, Marks JW, Schonfield LJ: La dieta en la formacion y tratamiento de los calculos biliares de colesterol. *In* Colelitiasis. S Cohen, RD Soloway (eds). Madrid, SANED, 1985, pp 113–127
52. Simon JA: Ascorbic acid and cholesterol gallstones. Med Hypoth 40:81–84, 1993
53. Thornton J, Symes C, Heaton K: Moderat alcohol intake reduces bile cholesterol saturation and raises HDL cholesterol. Lancet 2:819–822, 1983
54. Pastides H, Tzonou A, Trichopoulos D, Katsouyanni K, Trichopoulos A, Kefalogiannis N, Manousos O: A case-control study of the relationship between smoking, diet, and gallbladder disease. Arch Intern Med 150:1409–1412, 1990
55. Scraag RKR, McMichael AJ, Baghurs PA: Diet, alcohol and relative weight in gallstone disease: A case control study. Br Med J 40:81–84, 1984
56. Pixley F, Mann J: Dietary factors in the aetiology of gall stones: A case control study. Gut 29:1511–1515, 1988
57. Thijs C, Knipschild P: Oral contraceptives and the risk of gallbladder disease: A meta-analysis. Am J Public Health 83:1113–1120, 1993
58. Duane WC, Hunninghake DB, Freeman ML, Pooler PA, Schlasner LA, Gebhart RL: Simvastatin, a competitive inhibitor of HMG-CoA reductase, lowers cholesterol saturation index of gallbladder bile. Hepatology 8:1147–1150, 1988
59. Hoogerbrugge Van Den Linden N, Linden N, de Rooy FWM, Jansen H, Van Blankenstein M: Effect of pravastatin on biliary lipid concentration and bile acid synthesis in familial hypercholesterolaemia. Gut 31:348–350, 1990
60. Broomfield PH, Chopra R, Sheinbaum RC, Bonorris GG, Silverman A, Schoenfield LJ, Marks JW: Effects of ursodesoxycholic acid and aspirin on the formation of lithogenic bile and gallstones during loss of weight. N Engl J Med 319:1567–1572, 1988
61. Adamek HE, Buttmann A, Weber J, Riemann JF: Can aspirin prevent gallstone recurrence after successful extracorporeal schockwave lithotripsy? Scand J Gastroenterol 29:355–359, 1994
62. Kurata JH, Marcks J, Abbey D: One gram of aspirin per day does not reduce risk of hospitalization for gallstone disease. Dig Dis Sci 36:1110–1115, 1991
63. Hood KA, Gleeson D, Ruppin DC, Dowling RH, British-Belgian Gall Stone Study Group: Gall stone recurrence and its prevention: The British/Belgian gall stone study group's post-dissolution trial. Gut 34:1277–1288, 1993
64. Hood K, Ruggpin DC, Gleeson D, Dowling RH: Prevention of gallstone recurrence by non-steroidal anti-inflammatory drugs. Lancet. 2:1223–1225, 1988
65. Criqui MH, Wallace RB, Heiss G, Mishkel M, Schonfeld G, Jones GTL: Cigarette smoking and plasma high-density lipoprotein cholesterol. The lipid research clinics program prevalence study. Circulation 62(suppl 4):70–76, 1980
66. Stamford BA, Matter S, Fell RD, Sady S, Cresanta MK, Papanek P: Cigarette smoking, physical activity, and alcohol consumption: Relationship to blood lipids and lipoproteins in premenopausal females. Metabolism 33:585–590, 1984
67. Thornton JR, Heaton KW, MacFarlane DG: A relation between high-density-lipoprotein cholesterol and bile cholesterol saturation. Br Med J 283:1352–1354, 1981
68. Scragg RKR, Calvert GD, Oliver JR: Plasma lipids and insulin in gallstone disease: A case–control study. Br Med J 289:521–525, 1984
69. Hartung GH, Foreyt JP, Mitchell RE, Vlasek I, Gotto AM Jr: Relation of diet to high-density-lipoprotein cholesterol in middle-aged marathon runners, joggers, and inactive men. N Engl J med 302:357–361, 1980
70. Marcus SN, Heaton KW: Intestinal transit, deoxycholic acid and the cholesterol saturation of bile: Three inter-related factors. Gut 27:550–558, 1986
71. Heaton KW, Braddon FEM, Mountford RA, Hughes AO, Emmett PM. Symptomatic and silent gall stones in the community. Gut 32:316–320, 1991
72. Heaton KW, Emmet PM, Symes CL, Braddon FEM: An explanation for gallstones in normal-weight women: slow intestinal transit. Lancet 341:8–10, 1993
73. Jorgensen T: Gallstones and plasma lipids in a Danish population. Scand J Gastroenterol 24:916–922, 1989
74. Janzon L, Aspelin P, Eriksson S, Hildell J, Trell E, Ostenberg H: Ultrasonographic screening for gallstone disease in middle-age women: Detection rate, symptoms and biochemical features. Scand J Gastroenterol 20:706–710, 1985
75. Montalto G, Soresi M, Carroccio A, Averna MR, Muratore R, Li Castri C, Barbagallo CM, Cavera G, Sapienza M, Notarbartolo A: Prevalence of biliary lithiasis in the elderly people of small town in Sicily. Age Ageing 21:338–342, 1992

76. Thijs C, Knipschild P, Brombacher P: Serum lipids and gallstones: A case–control study. Gastroenterology 99:843–849, 1990
77. Nomura H, Kashiwagi S, Hayashi J, Kajiyama W, Ikematsu H, Noguchi A, Tani S, Goto M: Prevalence of gallstone disease in a general population of Okinawa, Japan. Am J Epidemiol 128:598–605, 1988
78. Holzbach RT, Kibe A, Thiel E, Howel JH, Marsh M, Hermann RE: Biliary Proteins. J Clin Invest 73:35–45, 1984