PAPERS AND SHORT REPORTS

Plasma lipids and insulin in gall stone disease: a case-control study

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Abstract

Fasting plasma lipid and insulin concentrations were measured in 173 patients with gall stones and 284 hospital controls to investigate their relationship to this disease. Multivariate methods of analysis were used to estimate the net associations between individual plasma variables and the risk of developing gall stones. In both sexes increased plasma insulin values were associated with an increased risk of gall stones independently of plasma triglyceride values; increased plasma triglyceride concentrations were associated with an increased risk of gall stones in young subjects only; increased plasma total cholesterol concentrations were associated with a decreased risk of gall stones only after controlling for plasma insulin and triglyceride concentrations; while increased plasma high density lipoprotein cholesterol concentrations were associated with a decreased risk of gall stones, but were confounded by plasma insulin and triglyceride values. These associations were independent of obesity and dietary intake.

Introduction

Because cholesterol gall stones are known to result from an altered lipid metabolism, information on the relation of plasma lipids to gall stone disease may help elucidate the changes in lipid metabolism associated with the formation of gall stones.

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Interest in cholesterol was initially stimulated by Aschoff and Bacmeister in 1909, who proposed that an increase in blood cholesterol concentration resulted in a raised biliary cholesterol concentration, with consequent formation of gall stones.[1] Recent studies have generally found, however, that mean concentrations of plasma or serum total cholesterol are similar in patients with gall stones and controls.[2-7] Plasma high density lipoprotein cholesterol, which has been little studied in relation to gall stone disease, has been reported in women to be associated with a decreased risk of gall stones[8] and to have an inverse relation with bile cholesterol saturation.[9]

In contrast with cholesterol, plasma (or serum) triglyceride has typically been raised in patients with gall stones compared with controls.[6 7 10] The presence of gall stone disease is also positively associated with type IV[6 7 11] and type IIb[7] hyperlipoproteinaemia, which are both characterised by an increase in concentrations of triglyceride-rich very low density lipoprotein.

Insulin is relevant to gall stone disease because it stimulates triglyceride synthesis by the liver.[12] Its role was investigated in a small case-control study which found that patients with gall stones had a raised, albeit non-significantly so ($p > 0.05$), mean fasting plasma insulin concentration when compared with controls,[13] while an earlier case study reported that patients with gall stones typically had raised fasting serum insulin concentrations.[14]

Our purpose in this study was to examine these four plasma variables (total cholesterol, high density lipoprotein cholesterol, triglyceride, and insulin) in a large case-control study of patients with gall stones and to use multivariate analysis to control for possible confounding between the variables, as triglyceride is known to vary inversely with high density lipoprotein cholesterol[15] and directly with insulin.[12] This has not been done before.

Methods

MEASUREMENT OF PLASMA VARIABLES

Patients entered the study when they presented for oral cholecystography in the radiology departments of Flinders Medical Centre and Queen Elizabeth Hospital during December 1978 to September 1980. Blood for plasma lipid and insulin measurements was collected from 309 women and 148 men who had fasted overnight before under-

going oral cholecystography or cholecystectomy. About 20 ml of venous blood was taken from the forearm, placed in plastic tubes containing solid dipotassium ethylenediaminotetra-acetic acid at a final concentration of 1 mg/ml, and mixed by gentle inversion. Samples were kept at 4°C, and plasma was separated by low speed centrifugation within eight hours of collection. Low density lipoprotein and very low density lipoprotein were precipitated promptly with a combined heparin-manganese chloride reagent by method III[16] and the supernatant taken, after centrifugation, for high density lipoprotein cholesterol measurement.[16] An aliquot was immediately stored at −20°C for measurement of plasma insulin concentration.

Total cholesterol, triglyceride, and high density lipoprotein cholesterol concentrations were measured within a few days of collection by the AutoAnalyzer II according to the Lipid Research Clinics manual.[17] The Liebermann-Burchard colorimetric method was used to estimate total cholesterol concentration, and the Kessler-Lederer fluorimetric method for triglyceride. During the study periods the laboratory met the criteria of precision and accuracy as specified for standardisation by the World Health Organisation Collaborating Centre for Reference and Research in Blood Lipids, Atlanta, Georgia.

Radioimmunoassay was used to measure plasma insulin concentration, with the modification of second antibody precipitation for separating the bound and free hormones,[18] after plasma samples had been stored for one to two years at −20°C. Because of the large number of subjects blood samples were randomly allocated to one of nine assay runs. Eight samples from the first assay, covering a range of insulin values, were measured in each subsequent assay. The mean values of the nine assays for each of the eight samples were used to standardise the other insulin values measured in the separate assays.

The results from 13 plasma samples with insulin concentrations greater than 30 mU/l from subjects who did not have diabetes were not included in the insulin and triglyceride data analyses, as we thought they were unlikely to be fasting specimens.

The gall stone status of the patients, which was not known by those who measured the plasma variables, was determined by the results of oral cholecystography and confirmed by cholecystectomy when carried out. Details of their selection into the study, as well as the methods of measurement of dietary intake and level of obesity, have been reported elsewhere.[19]

STATISTICAL ANALYSES

The *t* test for independent samples was used to compare case and control means.[20] Multiple logistic regression was used to estimate the net association between individual variables and the risk of gall stones after controlling for other confounding variables. Regression coefficients were estimated by the method of maximum likelihood,[21] and standardised coefficients were calculated by dividing a regression coefficient by its standard error. The unconditional model was used, and model building proceeded by forward selection, with the likelihood ratio test used to determine goodness of fit. All variables were treated as continuous, and non-linear relationships between individual variables and the relative risk were investigated by square and log transformations, and by interaction terms.

Results

UNIVARIATE ANALYSES

Plasma insulin and lipid concentrations are shown in table I for patients with gall stones and their controls according to sex and age. Mean plasma insulin concentration was higher in patients of both sexes than in their respective controls in either age group, although the difference was significant only when the age groups were combined. With triglyceride concentrations, however, there was evidence of age interaction, since the means for patients of both sexes aged under 50 years were raised compared with the values for their respective controls, while there was almost no case-control difference in mean triglyceride concentration among subjects aged 50 or over. In contrast, mean concentrations of plasma total cholesterol were similar for cases and controls of either sex aged under 50, while among subjects aged 50 or over mean cholesterol concentrations were lower in patients than in controls, but significantly so only in women. There was no evidence of age interaction with high density lipoprotein cholesterol, which was lower in patients than in controls in both sexes and both age groups, although the difference was significant only for women aged 50 or over.

The relative risk associated with each plasma variable was estimated using unconditional logistic regression analysis, after we had controlled for age (table II). Insulin was associated with an increased risk of gall stones in both sexes, as was triglyceride, although to a lesser extent. The regression coefficients for total cholesterol were not significantly ($p > 0.05$) different from zero in univariate analyses. High density lipoprotein cholesterol, however, was strongly associated with a decreased risk of gall stones in both sexes.

TABLE II—*Univariate* logistic regression in which the variables were entered in separate equations*

Variable	Regression coefficient (standardised coefficient†)	
	Women	Men
Insulin (mU/l)	0·0447 (2·02)	0·0646 (1·96)
Triglyceride (mmol/l)	0·2180 (1·50)	0·2204 (1·48)
Cholesterol (mmol/l)	−0·1061 (−0·88)	−0·1346 (−0·75)
High density lipoprotein cholesterol (mmol/l)	−0·8682 (−1·91)	−2·228 (−2·38)

* After controlling for age.
† Standard coefficient = regression coefficient/SE: if > ±1·96, $p < 0.05$.

MULTIVARIATE ANALYSES

Because some of the plasma variables were correlated with each other, multiple logistic regression was used to estimate the net association each variable had with the risk of gall stones, after we had controlled for possible confounding by other variables. The statistical models (equations) which contained the combination of variables which best described the female and male data sets showed consistency between the sexes (table III).

In both sexes triglyceride was associated positively with the risk of

TABLE I—*Plasma insulin and lipid levels: mean (standard error)*

Plasma variable	Age <50 years		Age ≥50 years		All ages	
	Patients	Controls	Patients	Controls	Patients	Controls
			Insulin (mU/l)			
Women (No)	9·3 (0·7) (78)	8·1 (0·5) (111)	9·2 (0·7) (44)	7·6 (0·6) (60)	9·3 (0·5)* (122)	7·9 (0·4) (171)
Men (No)	8·5 (1·6) (19)	6·6 (0·6) (58)	10·6 (1·3) (26)	7·9 (0·9) (40)	9·7 (1·0)*(45)	7·1 (0·5) (98)
			Triglyceride (mmol/l)			
Women (No)	1·39 (0·09)† (80)	1·08 (0·05) (114)	1·37 (0·14) (44)	1·33 (0·09) (61)	1·39 (0·08)* (124)	1·17 (0·05) (175)
Men (No)	1·66 (0·12) (18)	1·32 (0·09) (59)	1·79 (0·34) (26)	1·74 (0·17) (40)	1·74 (0·20) (44)	1·49 (0·09) (99)
			Cholesterol (mmol/l)			
Women (No)	5·22 (0·10) (80)	5·15 (0·10) (117)	5·91 (0·14)* (47)	6·33 (0·14) (65)	5·48 (0·09) (127)	5·57 (0·09) (182)
Men (No)	5·22 (0·27) (20)	5·08 (0·12) (61)	5·56 (0·21) (26)	5·87 (0·18) (41)	5·41 (0·17) (46)	5·39 (0·11) (102)
			High density lipoprotein cholesterol (mmol/l)			
Women (No)	1·14 (0·03) (80)	1·19 (0·02) (117)	1·20 (0·04)*(47)	1·32 (0·04) (65)	1·16 (0·02)* (127)	1·23 (0·02) (182)
Men (No)	0·96 (0·04) (20)	1·06 (0·03) (61)	0·97 (0·04) (26)	1·05 (0·03) (41)	0·96 (0·03)*(46)	1·05 (0·02) (102)

Patients significantly different from controls in same age-sex category: *$p < 0.05$; †$p < 0.01$.

TABLE III—*Multivarate logistic regression analysis in which the plasma variables were entered together in the same equation*

Variables	Regression coefficient (standardised coefficient*)	
	Women	Men
Constant	−2·686 (−2·57)	−4·511 (−2·68)
Age (years)	0·0422 (2·23)	0·0997 (3·23)
Triglyceride (mmol/l)	1·078 (2·80)	0·3897 (1·96)
Insulin (mU/l)	0·1455 (3·50)	0·0749 (2·13)
Insulin × triglyceride	−0·0835 (−3·09)	†
Triglyceride × (age − 50)	−0·0308 (−2·30)	−0·0387 (−2·31)
Cholesterol (mmol/l)	−0·2327 (−1·76)	−0·4504 (−2·00)
p value for model	0·0027	0·0007

* Standardised coefficient = regression coefficient/SE; if standardised coefficient $> \pm 1{\cdot}96$, $p < 0{\cdot}05$.
† For men, the interaction term between insulin and triglyceride did not have a significant regression coefficient.

gall stones and also had an age interaction term with a negative regression coefficient. Insulin was also positively associated with the risk of gall stones, independently of triglyceride, although among women there was a significant interaction between insulin and triglyceride. In contrast, total cholesterol was negatively associated with the risk of gall stones in either sex, the regression coefficients being larger in comparison with table II after we had controlled for insulin and triglyceride. High density lipoprotein cholesterol was found to be collinear with both triglyceride and insulin among women and men and did not have a significant regression coefficient when included in models with these same two variables.

The associations described in table III were independent of Quetelet's index (weight/height^2) and also dietary intake.

From the regression coefficients listed in table III we were able to calculate the change in risk of gall stones associated with a change in each plasma variable—that is, the relative risk associated with a change of Z units in a variable = antilog$_e$ (regression coefficient × Z). An increase in triglyceride concentration was associated with an increase in the risk of gall stones at younger ages only (table IV). An increase in insulin concentration of 10 mU/l, which was roughly the mean insulin concentration measured in this study, was associated with a relative risk for women of 1·9 (95% confidence limits 1·1, 3·0) when plasma triglyceride was 1·0 mmol/l and 2·1 (1·1, 4·2) for men. For total cholesterol an increase in concentration of 2 mmol/l was associated with a decrease in the risk of gall stones by a factor of 0·6 (0·4, 1·1) among women and 0·4 (0·2, 1·0) among men.

TABLE IV—*Estimated age specific relative risks* associated with an increase in plasma triglyceride of 0·5 mmol/l*

Age (years)	20	30	40	50	60	70
Women†						
Relative risk:	1·8	1·5	1·3	1·1	1·0	0·8
95% confidence limits‡:						
Upper	3·1	2·4	1·9	1·6	1·3	1·2
Lower	1·0	1·0	0·9	0·8	0·7	0·6
Men						
Relative risk:	2·2	1·8	1·5	1·2	1·0	0·8
95% confidence limits‡:						
Upper	3·5	2·5	1·8	1·5	1·3	1·3
Lower	1·4	1·3	1·2	1·0	0·8	0·5

* Regression coefficients from table III. † Insulin = 10 mU/l. ‡ Pooled variance.

Discussion

INSULIN

This is the first study to show conclusively that an increased fasting plasma insulin concentration is associated with an increased risk of gall stone disease in men and women; previous reports are consistent with this finding.[13 14] Furthermore, this association is supported by studies of bile metabolism which have shown that maturity onset diabetics, who typically have raised blood insulin values[22] due to obesity related insulin resistance,[23] also have a bile cholesterol saturation index higher than that of controls,[24 25] although the last study does not state whether it matched for age, sex, or level of obesity. The administration of insulin to maturity onset diabetics has also been reported significantly ($p < 0{\cdot}05$) to raise their bile saturation index.[26] In contrast, another study found no difference in cholesterol saturation between maturity onset diabetics and controls matched for age, sex, and level of obesity.[27]

The underlying cause of the raised fasting insulin concentration in our patients is not clear. If insulin resistance was implicated it was not related to obesity, since logistic regression analysis showed that the insulin associated increase in the risk of gall stones was independent of the value of Quetelet's index.

Sucrose intake, which increases blood insulin concentrations,[28 29] was greater in our patients than in our controls.[19] Logistic regression analysis of data for subjects from whom plasma was collected, however, showed that the association of insulin with the risk of gall stones was independent of all major dietary nutrients. Thus the raised insulin concentrations in our patients with gall stones do not appear to be related to diet.

Oestrogens may have been responsible for the raised fasting insulin values in our patients. Gall stones are more common in women[30] and in women taking oral contraceptives.[31] Oestradiol, which is secreted maximally by premenopausal women and is unrelated to obesity in them,[32] increases insulin secretion[33] and fasting plasma insulin concentrations[34] in the rat. Furthermore, in our study female controls aged under 50 had a higher mean fasting plasma insulin concentration than male controls under 50 ($p < 0{\cdot}10$), while there was little male-female difference in controls aged 50 or over (table I). In another study adolescent and young women (aged under 44) had a greater insulin response to glucose than men of the same age, a sex difference which was not apparent in young children or older adults.[35] Oral contraceptives also affect (variably) insulin secretion,[36] and the third trimester of pregnancy is associated with raised insulin concentrations because of insulin resistance.[37] Thus the observation in this study that raised plasma insulin concentrations were associated with gall stone disease may in part explain the increased prevalence of gall stones among women and the increased risk of gall stones associated with pregnancy and oral contraceptive use.

The insulin finding may also partly explain the greatly raised prevalence of gall stones among Pima Indians, since they have a higher fasting mean insulin value than obese white people.[38]

Insulin may increase gall stone formation by increasing the activity of HMG-CoA reductase,[39] the rate limiting enzyme known to increase the synthesis of cholesterol in the liver.[40]

The observation that the association of insulin with the risk of gall stones was independent of plasma triglyceride concentration is paradoxical, given the well described association between insulin and liver triglyceride secretion.[41] Insulin has been reported to stimulate the bile acid-independent flow of bile in whole animals[42] and in perfused liver[43] and to increase triglyceride secretion in perfused liver.[44] Nevertheless, in experiments in perfused rat liver the stimulation of bile flow by insulin remains constant over a wide range of triglyceride secretion rates (D L Topping, personal communication). Thus the effect of insulin on bile flow appears to be independent of its effect on triglyceride secretion.

The statistically significant interaction term between insulin and triglyceride for women, and not for men (table III)—a finding for which we are unable to provide a biological explanation—may have been due to a bias that occurred only in the female data set.

TRIGLYCERIDE

Our finding that a raised plasma triglyceride concentration was associated with an increased risk of gall stones independently of obesity is consistent with the findings of previous case-control studies.[6 7 10] Our observation that a raised triglyceride concentration was a risk factor for gall stones only in younger people agrees with a report that the prevalence of gall stones in men with type IV hyperlipoproteinaemia decreased with age,[11] although another study of men and women did not find consistent age related differences in mean triglyceride values between cases and controls.[7]

The mechanism underlying the raised plasma triglyceride levels in patients with gall stones is not clear, since there are no reported studies on whether patients with gall stones have an increased triglyceride synthesis, decreased removal from plasma, or both. Nevertheless, the observation that patients with type IIb and type IV hyperlipoproteinaemia, who have an increased prevalence of gall stones, also have an increased triglyceride synthesis,[45] suggests that the raised plasma triglyceride concentration in patients with gall stones is the result of increased synthesis. Furthermore, the observation that endogenous cholesterol synthesis, which primarily occurs in the liver, is positively correlated with the plasma triglyceride concentration,[46] together with our finding that only patients aged under 50 had a raised mean plasma triglyceride concentration, suggests that young patients with gall stones typically have an increased cholesterol synthesis compared with their controls, while there is no difference between older patients and controls in their cholesterol synthesis.

Recent reports suggest that there is a subpopulation of people who are prone to gall stone formation if they take oral contraceptives,[47 48] are obese, or have an increased dietary intake.[19] Our observation that an increased plasma triglyceride concentration was associated with an increased risk of gall stones at young ages and no increased, or even a decreased risk, at older ages (table IV) is consistent with this notion.

Subjects with an increased plasma triglyceride concentration were reported to have an increased sensitivity to sucrose, which is possibly genetically mediated,[29] while patients with gall stones and pre-β hyperlipoproteinaemia who were fed with sucrose were found to have a decreased biliary concentration of chenodeoxycholic acid compared with normolipidaemic patients with gall stones.[49] Oral contraceptives can also induce hypertriglyceridaemia.[36] Thus the plasma triglyceride value may possibly be a biological marker of susceptibility to gall stone disease and, among those prone to stone formation, increased exposure to a causal factor, such as diet or oral contraceptives, may result in a raised plasma triglyceride concentration together with formation of gall stones early in life.

TOTAL CHOLESTEROL

Logistic regression analysis showed that increased plasma total cholesterol concentration was associated with a decreased risk of gall stones in both sexes, after we had controlled for plasma triglyceride and insulin concentrations. This finding was unexpected but is supported by other reports. A Swedish study reported that women with gall stones aged under 65 had a significantly ($p < 0.01$) lower mean serum cholesterol value than controls.[50] Pima Indians, who have a high prevalence of gall stones, also have lower serum cholesterol values than whites[51 52] owing to a decreased apo low density lipoprotein synthesis,[53] although serum cholesterol concentrations among Pimas are the same for controls and patients with gall stones.[5] The National Cooperative Gallstone Study found that chenodeoxycholic acid, which is used to reverse the process of gall stone formation, produced a higher serum cholesterol concentration than placebo.[54]

There are no apparent explanations for the inverse association between plasma total cholesterol and the risk of gall stones and it is not clear whether this association is causal or casual. If the association is causal, one possible explanation is that low density lipoprotein, which transports most of the cholesterol (as an ester) in blood, may inhibit cholesterol synthesis by the liver, since this has been shown in vivo in rats.[55 56] Thus, a lowered plasma cholesterol concentration in patients with gall stones could, by itself, result in increased cholesterol synthesis by the liver. Alternatively, a lowered plasma cholesterol concentration in patients, particularly those aged 50 or over (table I), might be a manifestation of a decreased rate of apo low density lipoprotein synthesis as described in Pima Indians.[53] In the presence of normal or increased cholesterol synthesis, decreased apo low density lipoprotein synthesis would result in a decreased cholesterol efflux from liver into blood with a concomitant increased cholesterol secretion into bile.

HIGH DENSITY LIPOPROTEIN

High density lipoprotein cholesterol was found in both sexes to be negatively associated with the risk of gall stones only in univariate logistic regression analyses. In multivariate models, which also contained triglyceride and insulin, high density lipoprotein cholesterol was not significantly associated with the relative risk. Because of the collinearity which high density lipoprotein cholesterol had with these two variables we could not decide on statistical grounds which was the preferred variable.

One possible explanation of the inverse association between plasma high density lipoprotein cholesterol and the relative risk is that, in a fashion similar to that described above for low density lipoprotein, high density lipoprotein may inhibit liver cholesterol synthesis, since this has been observed in rats.[57] Alternatively, there is evidence that cholesterol from high density lipoprotein, which is thought to contribute about 70% of the cholesterol secreted into bile,[58] is the preferred substrate for bile acid synthesis in man[59] and primates.[60] If so the observation of an inverse association between plasma high density lipoprotein cholesterol and the risk of gall stone disease suggests that the free cholesterol in high density lipoprotein is preferentially metabolised to bile acids rather than secreted into bile as cholesterol.

In summary, we have found that raised plasma insulin and triglyceride concentrations were independently associated with an increased risk of gall stones; raised total plasma cholesterol concentration was associated with a decreased risk after we had controlled for insulin and triglyceride; while increased plasma high density lipoprotein cholesterol was also associated with a decreased risk of gall stones, but was confounded by plasma insulin and triglyceride.

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Omeprazole in duodenal ulceration: acid inhibition, symptom relief, endoscopic healing, and recurrence

COOPERATIVE STUDY*

Abstract

In a preliminary study to compare the effects of different doses of omeprazole 44 patients with endoscopically diagnosed duodenal ulceration randomly received omeprazole 20 mg, 30 mg, 40 mg, or 60 mg daily for four weeks. After four weeks the ulcer had healed in 41 of the 43 patients who completed the course of treatment; the proportions of patients whose ulcer healed were similar between the four dosage groups. Most patients were symptom free after one week of treatment. Seven patients reported a total of eight adverse events. With the exception of one patient who had persistent nausea and was withdrawn from the study, all the adverse events resolved spontaneously during continued treatment with the same dose of omeprazole. Pentagastrin tests were performed before the study and after four weeks' treatment. The mean inhibition of peak acid output measured 24 hours after the last dose was 61%, 94%, 91%, and 81% with omeprazole 20 mg, 30 mg, 40 mg, and 60 mg respectively. During the first six months after the end of treatment 11 out of 36 patients had a symptomatic, endoscopically diagnosed recurrence of ulceration; the median time to relapse was 10 (range 6-23) weeks.

Introduction

Omeprazole (H168/68) is a substituted benzimidazole that inhibits secretion of gastric acid by interacting with the enzyme H^+, K^+-ATPase, believed to be the proton pump of the parietal cell.[1-3] Studies in healthy subjects have shown that omeprazole produces a dose dependent inhibition of basal and pentagastrin stimulated acid secretion that is long lasting and maximal after five to seven days of treatment.[4-6] In patients with duodenal ulceration omeprazole 30 mg daily for one week caused a 95% decrease in 24 hour intragastric acidity.[7] Recently it was reported that duodenal ulcers healed in 93% of patients after two weeks'

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