

Plasma insulin, serum lipids and lipoproteins in gall stone disease in non-insulin dependent diabetic subjects: a case control study

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Abstract

Fasting insulin, lipids and lipoproteins were measured in 22 middle aged female non-insulin dependent diabetics with gall stone disease (cases) and in 22 non-insulin dependent diabetics without gall stone disease (controls). The groups were matched for sex, age, obesity, and fasting glucose concentrations. No differences were observed between the cases and controls in duration of diabetes, glycated haemoglobin A₁, alcohol intake, smoking, use of cardiovascular drugs or a history of myocardial infarction. Diabetics with gall stone disease had higher fasting insulin concentrations ($p < 0.05$), lower total ($p < 0.01$) and low density lipoprotein cholesterol ($p < 0.01$) and high density lipoprotein cholesterol (not statistically significant) concentrations than diabetics without gall stone disease. These changes in insulin, lipids and lipoproteins are similar to reported changes in non-diabetic subjects with gall stone disease. Therefore, they are characteristic for gall stone disease and not as such explanatory to an increased risk of gall stones in patients with non-insulin dependent diabetes.

Numerous studies have confirmed the clinical impression that gall stones occur more frequently among obese than normal weight subjects).^{1,3} Because patients with non-insulin dependent diabetes are often obese this population is at a high risk in developing gall stones although the higher frequency of gall stone disease in diabetes has remained controversial.^{3,4} Lipid abnormalities associated with non-insulin dependent diabetes favouring the development of gall stones are of particular interest in this respect. No studies have, however, been published on lipids and lipoproteins in patients with non-insulin dependent diabetes and with gall stone disease compared with patients with non-insulin dependent diabetes and without gall stone disease. In non-diabetic subjects, increased plasma insulin values, high serum triglyceride and low total and HDL cholesterol concentrations have been associated with gall stone disease.⁵⁻⁸ Whether or not these changes in insulin, lipid and lipoprotein concentrations are associated with gall stone disease also in patients with non-insulin dependent diabetes are largely unknown. We, therefore, carried out a case control study in order to investigate the relationship between fasting plasma insulin, serum lipids, and lipoproteins in patients with non-insulin dependent diabetes with and without gall stone disease.

Methods

PATIENTS

The subjects were 22 middle aged non-insulin dependent diabetics with gall stone disease and 22 non-insulin dependent diabetics without gall stone disease. All subjects were women and fulfilled the WHO criteria of diabetes mellitus.⁹ Patients treated with diet only or with oral drugs were studied in order to exclude patients with insulin dependent diabetes. The subjects were randomly drawn from a large study investigating the prevalence of atherosclerotic vascular disease and its risk factors in non-insulin dependent diabetes.¹⁰ Only women were studied because of sex difference in serum lipids and lipoproteins and in the occurrence of gall stone disease. The study was based on a case control design. The pairs were matched for sex, age, obesity (measured by body mass index, BMI) and fasting glucose concentration in order to control the effects of these variables on plasma insulin, serum lipids, and lipoproteins.

Table I shows the characteristics of the cases and the controls. No difference between the groups was found with respect to age, body mass index, duration of diabetes, fasting plasma glucose, glycated haemoglobin A₁, use of oral hypoglycaemic drugs, alcohol intake (patient's own estimate transformed to absolute alcohol in grams/week), smoking, use of cardiovascular drugs and a history of myocardial infarction. In 11 diabetic patients the diagnosis of gall stone disease had been made before and in 11 patients after the diagnosis of diabetes.

DIAGNOSIS OF GALL STONE DISEASE

The diagnosis of gall stone disease was verified on the basis of medical records showing either a history of cholecystectomy or a diagnosis of gall stone disease based on cholecystography or ultra-

TABLE I Characteristics of the study population

	Controls	Cases
Subjects (n)	22	22
Age (yrs)	58.7 (0.9)	58.9 (1.0)
BMI (kg/(m) ²)	30.7 (1.1)	31.2 (1.3)
Duration of diabetes (yrs)	8.7 (0.9)	8.0 (0.8)
P-glucose (mmol/l)	12.0 (0.9)	12.4 (0.9)
GHbA ₁ (%)	10.1 (0.4)	9.9 (0.4)
Alcohol intake (g/week)	10.0 (10.0)	4.4 (3.3)
Use of oral hypoglycaemic drugs (%)	68.2	63.6
Current smokers (%)	9.1	13.6
Use of betablockers (%)	40.9	36.4
Use of diuretics (%)	36.4	63.6
History of myocardial infarction (%)	0	9.1

Results are given as mean (SEM) with the exception of prevalence estimates which are expressed as percentages. BMI = body mass index; GHbA₁ = glycated haemoglobin A₁.

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sound investigation. All diabetic patients forming the control group underwent an ultrasound investigation (Aloka Fansonc 190 with a convex probe operating at 3.5 MHz). All patients fasted at least eight hours before the examination. Only those whose ultrasound investigation excluded a gall stone disease were accepted for controls. Altogether 10 patients primarily selected as controls had symptomless gall stone disease and they were therefore excluded from the final analyses.

DETERMINATION OF BIOCHEMICAL PARAMETERS

Serum lipids and lipoproteins were determined from fresh serum samples drawn after a 12 hour overnight fast. Lipoprotein fractionation was carried out using ultracentrifugation and selective precipitation with modifications¹¹ to the method originally described by Havel *et al.*¹² All spinings were done at 10°C using a Kontron TGA-65 ultracentrifuge (Kontron International, Switzerland). Serum samples were centrifuged at density (d)=1.006 (105 000 g, for 18 hours), and very low density lipoprotein triglycerides (d<1.006) were recovered as the top fraction. Total high density lipoprotein cholesterol was separated by spinning serum samples at d=1.063 (105 000 g, for 18 hours). Low density lipoprotein cholesterol (d=1.006–1.063, including intermediate density lipoprotein) was calculated as a difference between the bottom fractions. On average, the mean day-to-day variation in high density lipoprotein cholesterol measurements was 3.3%, and the daily variation was 0.95%. HDL₂ and HDL₃ cholesterol subfractions were separated running total high density lipoprotein cholesterol fraction at d=1.125 (105 000 g, for 40 hours) and the top and bottom fractions were isolated by the tube slicing technique. Cholesterol and triglycerides from the whole serum and from lipoprotein fractions were assayed by automated enzymatic methods (Boehringer-Mannheim, West Germany). Glycated haemoglobin A_{1c}, normal range 5.5–8.5%; coefficient of variation=6% was determined by commercial column chromatography (Quick-Sep Fast Hemoglobin Test System, Isolab Inc, Akron, OH) after incubation in 0.9% saline solution for 12 hours. Blood samples for plasma insulin assay were taken into chilled tubes. Plasma was separated immediately and the samples were stored at –70°C until the assay. Fasting plasma insulin was determined by commercial double antibody solid phase radioimmunoassay (Phadeseph, Pharmacia Diagnostics, Sweden) with a detection limit of 2.5 mIU/l and the variation coefficient below 5.0%.

STATISTICAL ANALYSIS

The results are expressed as mean (SEM). The differences between the cases and the controls were assessed by Student's two-tailed *t* test for paired data and by the χ^2 test (uncontinuous variables). Logarithmic values for total triglycerides and fasting insulin concentrations were used in statistical analyses. The relationships between variables were calculated using Pearson

TABLE II Fasting plasma insulin (mU/l), serum total and lipoprotein cholesterol (mmol/l) and triglycerides (mmol/l) in diabetics with (cases) and without gall stone disease (controls)

	Controls	Cases
Fasting plasma insulin	22.6 (2.3)	30.0 (3.3)*
Total cholesterol	7.43 (0.28)	6.51 (0.28)†
HDL cholesterol	1.24 (0.05)	1.09 (0.09)
HDL ₂ cholesterol	0.84 (0.05)	0.70 (0.10)
HDL ₃ cholesterol	0.39 (0.03)	0.39 (0.03)
LDL cholesterol	5.02 (0.22)	3.97 (0.22)†
VLDL cholesterol	1.17 (0.09)	1.45 (0.16)
Total triglycerides	2.46 (0.25)	2.51 (0.18)
HDL triglycerides	0.16 (0.02)	0.16 (0.02)
LDL triglycerides	0.47 (0.04)	0.44 (0.04)
VLDL triglycerides	1.83 (0.23)	1.91 (0.15)

Results are given as mean (SEM). Comparison between cases and controls by Student's paired *t* test and χ^2 test.

**p*<0.05; †*p*<0.01

HDL=high density lipoprotein; LDL=low density lipoprotein; VLDL=very low density lipoprotein.

correlation coefficients. The comparison of more than two groups was performed by the analysis of covariance (ANCOVA) after adjustment for confounding factors.

Results

Table II shows plasma insulin, serum lipid and lipoprotein concentrations in cases and controls. Fasting plasma insulin was higher among patients with gall stone disease than among those without (*p*<0.05). Cases had lower total and low density lipoprotein cholesterol concentrations than controls. High density lipoprotein and HDL₂ cholesterol tended to be lower in cases than in controls but the difference was not statistically significant. Total triglycerides and triglyceride fractions were almost identical between the two groups. Seventeen of the cases had undergone cholecystectomy and five had a gall stone disease diagnosed by radiograph or ultrasound investigation.

Because cholecystectomy and the awareness of the diagnosis of gall stone disease could have had an influence of the results, lipid and lipoprotein concentrations were also compared between the following subgroups of patients: (i) diabetics with previously diagnosed gall stone disease who had undergone cholecystectomy (17); (ii) diabetics with previously diagnosed gall stone disease who had not been operated (five); (iii) diabetics with newly diagnosed gall stone disease (10 patients primarily selected as controls but excluded from all other analyses because of gall stone disease, see Methods). The cases of the study consist of groups i and ii. As the three subgroups differed with respect to age, body mass index, fasting plasma glucose, alcohol intake and the use of diuretics, lipid and lipoprotein levels were adjusted for the effects of these factors (ANCOVA). No difference in adjusted means between these subgroups was seen in total cholesterol (6.81, 5.86 and 6.60 mmol/l, respectively, *p*=NS) low density lipoprotein cholesterol (4.12, 3.75 and 4.01 mmol/l, respectively, *p*=NS), or in high density lipoprotein cholesterol (1.15, 0.90 and 1.10 mmol/l, respectively, *p*=NS). Neither did other cholesterol and triglyceride lipoprotein fractions differ between these three subgroups of patients.

Table III shows Pearson correlation coefficients between age, body mass index, alcohol

TABLE III Pearson correlation coefficients between age, body mass index, alcohol intake, duration of diabetes, plasma glucose, glycated haemoglobin A₁, fasting plasma insulin and lipids and lipoproteins in diabetics with (cases) and without gall stone disease (controls).

	Controls TOTC	HDLC	LDLC	VLDLC	TG	HDLTG	LDLTG	VLDLTG
Age	0.060	-0.112	0.068	0.084	0.193	0.223	-0.244	0.237
BMI	-0.258	-0.418*	-0.337	0.278	0.255	0.251	-0.089	0.277
Alcohol intake	-0.157	-0.185	-0.238	0.204	-0.004	-0.145	0.011	0.003
Duration of diabetes	-0.253	0.117	-0.281	-0.155	-0.019	-0.018	-0.386*	0.044
Plasma glucose	0.643	0.148	0.546†	0.527*	0.403*	0.197	0.394*	0.364*
GHbA ₁	0.383*	0.144	0.251	0.456*	0.296	0.111	0.287	0.270
Fasting plasma insulin	0.029	-0.469*	0.083	0.166	0.174	0.320	0.316	0.117

	Cases TOTC	HDLC	LDLC	VLDLC	TG	HDLTG	LDLTG	VLDLTG
Age	0.220	0.115	0.500†	-0.355	-0.218	-0.364*	-0.139	-0.177
BMI	-0.014	-0.450*	-0.016	0.244	0.296	0.275	0.442*	0.205
Alcohol intake	0.107	0.364*	0.110	-0.161	-0.213	0.050	-0.217	-0.205
Duration of diabetes	-0.012	0.119	0.216	-0.378*	-0.274	-0.289	-0.257	-0.224
Plasma glucose	-0.345	-0.336	-0.210	-0.135	0.079	-0.011	-0.011	-0.095
GHbA ₁	-0.086	-0.176	-0.109	0.094	0.191	0.008	-0.047	0.238
Fasting plasma insulin	-0.068	-0.395*	-0.274	0.468*	0.363*	0.290	0.368*	0.301

*p<0.05; †p<0.01

TOTC=total cholesterol; HDLC=high density lipoprotein cholesterol; LDLC=low density lipoprotein cholesterol; VLDLC=very low density lipoprotein cholesterol; TG=triglycerides; HDLTG=high density lipoprotein triglycerides; LDLTG=low density lipoprotein triglycerides; VLDLTG=very low density lipoprotein triglycerides; BMI=body mass index; GHbA₁=glycated haemoglobin.

intake, duration of diabetes, fasting glucose, glycated haemoglobin A₁, fasting insulin and lipids and lipoproteins in cases and controls. Age was correlated positively with low density lipoprotein cholesterol and negatively with very low density lipoprotein cholesterol in cases but not in controls. Body mass index correlated inversely with high density lipoprotein cholesterol in both groups and with low density lipoprotein triglycerides in cases. Alcohol intake correlated positively with high density lipoprotein cholesterol in cases. Duration of diabetes correlated negatively with low density lipoprotein triglycerides in controls and with very low density lipoprotein cholesterol in cases. Fasting glucose was positively correlated with total and low density lipoprotein cholesterol in controls, but not in cases. In controls, plasma glucose correlated significantly also with very low density lipoprotein cholesterol, total triglycerides, low density lipoprotein triglycerides, and very low density lipoprotein triglycerides. Glycated haemoglobin A₁ correlated positively with total and very low density lipoprotein cholesterol in controls. Fasting insulin had strong inverse correlation with high density lipoprotein cholesterol in both groups and a positive correlation with very low density lipoprotein cholesterol, total and low density lipoprotein triglycerides in cases.

Discussion

Our study based on a case control design in female non-insulin dependent diabetics showed that diabetics with gall stone disease had lower total and low density lipoprotein cholesterol than diabetics without gall stone disease. Lower total and low density lipoprotein cholesterol concentrations were not related to a history of cholecystectomy because no significant difference in cases in total and low density lipoprotein cholesterol was observed between those who had been operated and those who had not been operated. A statistically non-significant trend for lower high density lipoprotein and HDL₂ chole-

sterol in cases was seen compared to controls but total triglycerides were similar in both groups. Diabetics with gall stone disease had higher fasting plasma insulin than those without gall stone disease.

The only study measuring lipids and lipoproteins and fasting insulin in subjects with and without gall stone disease is the study of Scragg *et al.*⁵ They measured fasting plasma lipid and insulin concentrations in 173 non-diabetic patients with gall stones and 284 hospital controls. Increased plasma insulin was associated with an increased risk of gall stones in both sexes. Total and high density lipoprotein cholesterol were associated inversely and total triglycerides positively with the risk of gall stone disease. Our findings in patients with non-insulin dependent diabetes are quite similar; gall stone disease was related to high insulin and low total, low density lipoprotein and high density lipoprotein cholesterol.

The association of gall stone disease with low total cholesterol is of particular interest because it has also been reported in other studies.^{5,6} In previous studies low density lipoprotein cholesterol has not been measured. The lowering of total cholesterol in our patients was due to the lowering of low density lipoprotein cholesterol fraction. Lowered low density lipoprotein cholesterol might be a manifestation of a decreased rate of apo low density lipoprotein (corresponding to apolipoprotein B of low density lipoprotein⁴ synthesis.¹³ Decreased apo low density lipoprotein synthesis would result in a decreased cholesterol efflux from the liver into blood with a concomitant increase in cholesterol secretion into bile. Indeed, as can be calculated from the data of Grundy *et al.*,¹⁴ lower plasma cholesterol levels and higher biliary cholesterol output are characteristic features of southwestern American Indian women with cholesterol gall stones as compared with those without. None of the control group had diabetes but seven of the 22 gall stone patients had non-insulin dependent diabetes. On the other hand, in white women with gall stones blood (plasma or

serum) concentrations of cholesterol may be age dependent. Women with gall stones and under the age of 50 years tended to have higher cholesterol concentrations than the controls whereas women with gall stones and over 50 years of age have had lower concentrations than their controls.^{5,15} This is in accordance with the findings of our study. Low levels of total and low density lipoprotein cholesterol in patients with gall stone disease, however, would be at least in part related to the modification of their life styles, particularly to those related to diet. Lower intake of dietary saturated fat could lead to lowering of total and low density lipoprotein cholesterol as shown by several studies.¹⁶

Like Scragg *et al*⁵ we also found that patients with gall stone disease had higher fasting insulin concentrations than those without. Similar results have been reported in other studies,^{17,18} too. Non-insulin dependent diabetes is characterised by obesity and insulin resistance and raised fasting insulin concentrations in non-insulin dependent diabetes is an indication of insulin resistance. In insulin resistant states a positive correlation between obesity and insulin level is usually seen. In addition, high insulin concentration is associated positively with total triglyceride concentration and negatively with high density lipoprotein cholesterol concentration.¹⁹ In our study populations we also found a consistent positive correlation between insulin and body mass index, and a negative correlation between insulin and high density lipoprotein cholesterol. On the basis of these results it can be concluded that insulin resistance is a powerful risk factor for gall stone disease among patients with non-insulin dependent diabetes. This hypothesis is supported by an extraordinary high frequency of gall stone disease in Pima Indians having high concentrations of fasting insulin and marked insulin resistance.²⁰ Mechanisms by which hyperinsulinaemia could induce gall stone formation are largely unknown. High insulin concentrations can, however, activate low density lipoprotein receptors²¹ which enhance low density lipoprotein cholesterol transport from blood into the liver. This could decrease low density lipoprotein cholesterol concentration in serum and hepatocytes would then increase cholesterol output into bile. As a matter of fact, those non-insulin dependent diabetics with highest fasting plasma insulin concentrations have reported to have lowest plasma low density lipoprotein cholesterol concentrations.²²

In conclusion, our study shows that high fasting insulin, low total and low density lipoprotein cholesterol as well as a tendency to low high density lipoprotein and HDL₂ cholesterol level are characteristic for non-insulin dependent diabetics with gall stone disease as compared

with corresponding diabetics without gall stones the findings are related to gall stone disease itself. Our results suggest that mere changes in plasma insulin, serum lipid and lipoprotein levels in non-insulin dependent diabetics with gall stones do not account for increased prevalence of gall stone disease in non-insulin dependent diabetes. The role of other possible factors, such as the function of gall bladder, for instance, remains to be determined.

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