Diabetologia

Plasma lipoproteins and the incidence of abnormal excretion of albumin in diabetic American Indians: The Strong Heart Study

A. Fagot-Campagna[1], R. G. Nelson[1], W. C. Knowler[1], D. J. Pettitt[1], D. C. Robbins[2], O. Go[3], T. K. Welty[4], E. T. Lee[3], B. V. Howard[2]

[1] National Institutes of Diabetes and Digestive and Kidney Diseases, Phoenix, Arizona, USA
[2] Medlantic Research Institute, Washington, DC, USA
[3] Center for Epidemiologic Research, College of Public Health, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA
[4] Epidemiology Program, Aberdeen Area Indian Health Service, Rapid City, South Dakota, USA

Summary Animal studies suggest that lipids are risk factors for kidney diseases. Some prospective studies and clinical trials have reported predictive effects of lipoproteins on different stages of diabetic nephropathy in humans. We examined lipoprotein abnormalities to determine if they predict abnormal urinary excretion of albumin (≥ 30 mg albumin/g creatinine), using logistic regression. We followed 671 American Indians (211 men, 460 women) with Type II diabetes for a mean of 3.9 years (range 1.7–6.2). Participants were aged 45–74 years. They had normal excretion of albumin and normal serum creatinine at baseline. 67 men and 144 women developed abnormal excretion of albumin. In models controlled for age, treatment with oral hypoglycaemic agents or insulin, HbA$_{1c}$, study site, degree of Indian heritage, mean arterial blood pressure, albumin excretion at baseline and duration of diabetes, a high HDL cholesterol was a protector for abnormal excretion of albumin in women [odds ratio (OR) comparing the 90th with the 10th percentile = 0.56, 95 % confidence interval (CI) = 0.32–0.98], but not in men (OR = 1.5, 95 % CI = 0.66–3.4). Further adjustment for obesity, insulin concentration, alcohol consumption or physical activity did not change the results. There was a tendency for high values of VLDL and total triglyceride and small LDL size to predict abnormal excretion of albumin in women only. We conclude that low HDL cholesterol was a risk factor for abnormal excretion of albumin in women, but not in men. Sex hormones may be responsible for sex differences in the association between HDL cholesterol and abnormal excretion of albumin. [Diabetologia (1998) 41: 1002–1009]

Keywords Lipoproteins, albuminuria, nephropathies, Type II diabetes, Strong Heart Study.

Animal studies support the hypothesis that dyslipidaemia is a risk factor for diabetic nephropathy. Lipids could accelerate glomerulosclerosis [1–3] and the mechanisms could be analogous to atherosclerosis [4]. Oxidized LDL might not be recognized by the classic LDL receptor of the glomerular epithelial and mesangial cells but by the macrophage scavenger receptor. Foam cell uptake could be enhanced and LDL degradation impaired, causing LDL accumulation inside the foam cell, which would be toxic to mesangial cells. Oxidized LDL could also increase adhesion of monocytes to damaged endothelium, and glycated LDL might have a similar effect. Some lipoproteins (VLDL, LDL) could also bind with proteoglycans, which are constituents of the glomerular basement membrane, leading to changes in basement membrane total lipids and penetration of the glomerular basement membrane.

Studies in humans are still controversial. Many cross-sectional studies have reported a relationship between lipid or lipoprotein concentrations and nephropathy in people with Type I or Type II diabetes. Nevertheless, renal disease itself (e.g. nephrotic

syndrome) results in increased lipid concentrations and it is not possible to determine from cross–sectional studies whether the lipid abnormalities precede or are a consequence of diabetic nephropathy. In some prospective studies of people with Type I or Type II diabetes, total triglycerides [5, 6], LDL cholesterol [6, 7], total cholesterol [8–10] and apolipoprotein B [11] were risk factors for the progression of nephropathy. HDL cholesterol has not been reported as a risk factor [6, 7, 10–16] and no other lipid effect was found in several studies [12–18]. Clinical trials of angiotensin-converting enzyme inhibitors have also suggested an effect of total cholesterol on the development of nephropathy in participants with Type II [19] or Type I [20] diabetes. Two short-term controlled trials with simvastatin to reduce hypercholesterolaemia in participants with Type I [21] or Type II [22] diabetes did not show any improvement in kidney function. Two long-term randomized trials have, however, suggested a beneficial effect of hypolipidaemic treatment for microalbuminuria [23] or decreased glomerular filtration rate [24, 25] in Type II diabetes.

The Strong Heart Study was undertaken to estimate cardiovascular disease morbidity and mortality among American Indians aged 45 to 74 years, living in central Arizona, Oklahoma, and North and South Dakota [26, 27]. These groups of American Indians have a high prevalence of Type II diabetes [28] and diabetic nephropathy [29]. The present analysis examined the relationship between plasma lipoprotein concentrations and the risk of developing abnormal excretion of albumin in 671 American Indians with Type II diabetes who initially had normal urine albumin excretion and normal serum creatinine. They were followed for a mean of 3.9 years.

Subjects and methods

The Strong Heart Study comprises 4549 American Indians, aged 45 to 74 years at baseline, who are members of 13 tribes (7 tribes from southwestern Oklahoma, 2 from South Dakota, 1 from North Dakota, and 3 from Arizona) [30]. The study has been approved by the participating tribes and by the Indian Health Service Institutional Review Board, and each of the participants gave informed consent. The initial examinations were conducted between 1 July 1989 and 31 January 1992 and a second examination was done approximately 4 years later. Participants were interviewed about their Indian heritage, diabetes diagnosis, duration and family history, total physical activity during the past year (including leisure activity, occupational activity and walking, expressed as 'metabolic equivalent' per week [31]) and alcohol consumption. Participants were classified as non-drinkers or former drinkers, current drinkers (during past month, but less than 14 drinks a week) or heavy drinkers (5 or more drinks on one occasion during the last month or more than 14 drinks a week during the past month). Current use of hypolipidaemic, antihypertensive, oral hypoglycaemic medicines, insulin and estrogen were

reviewed. Height and weight were measured and body mass index (BMI) was calculated. Systolic and diastolic arterial blood pressures were measured three times while the participants were sitting and the mean of the last two measurements was used in these analyses. The mean arterial blood pressure was calculated as 2/3 of the diastolic plus 1/3 of the systolic pressure.

Venous blood samples were collected after a 12-h overnight fast. Participants were considered to have diabetes if they had a previous diagnosis, if their fasting plasma glucose was greater than 7.8 mmol/l (140 mg/dl) or if during a 75-g oral glucose tolerance test their 2-h plasma glucose was greater than 11.1 mmol/l (200 mg/dl) [32]. Measurements were done on fasting samples as well as on 2-h plasma glucose. Plasma lipoproteins (VLDL and total triglycerides; HDL, LDL, VLDL and total cholesterol) were measured by beta quantification. Cholesterol, triglyceride and glucose concentrations were determined by an enzymatic method using a Hitachi autoanalyser and reagents from Boerhinger Mannheim diagnostics (Indianapolis, Ind., USA). HDL cholesterol was isolated by precipitation with heparin and manganese chloride. Apolipoproteins A1 and B were measured by an automated immunoprecipitin assay (INCSTAR Co., Stillwater, Minn., USA) on a Hitachi autoanalyser. LDL particle size was measured by a gradient gel electrophoresis procedure using non-denaturing polyacrylamide gels. Participants were classified into three groups on the basis of the LDL predominant peak size: patterns B (< 254 Å), I (≥ 254 and < 257 Å) and A (≥ 257 Å). The mean and standard deviation sizes in the B, I and A patterns were, 248 ± 5, 255 ± 1 and 264 ± 5 Å, respectively. Lipoprotein (a) [Lp(a)] was measured by ELISA using commercial reagents (Terumo Medical Corporation, Newark, Del., USA). Insulin was measured by radioimmunoassay by the method of Morgan and Lazarow (Linco Research, St Louis, USA), and HbA$_{1c}$ using a high pressure liquid chromatography assay [33]. Fibrinogen was measured by the Von Clauss method [34]. A urine specimen, obtained on arrival at the clinic, was tested for albumin by nephelometry [35] and an albumin value less than 0.20 mg/l was assigned to undetectable albumin measurements. Serum and urine creatinine were measured by the alkaline picrate method [36]. An albumin (mg) to creatinine (g) ratio was calculated and abnormal excretion of albumin was defined as a urine albumin to creatinine ratio 30 mg/g creatinine or more, which included microalbuminuria ($30 \leq$ ratio < 300 mg/g creatinine) and macroalbuminuria (ratio $\geq$ 300 mg/g creatinine). Each measurement was performed in a central laboratory and standardized to outside reference values. Of the samples 5 % were masked and sent to the reference laboratories as duplicates for quality control purposes. The technical errors (coefficients of variation between masked pairs) for the plasma measurements were 5.8 % for total cholesterol, 9.5 % for HDL cholesterol, 10.2 % for total triglycerides, 13.6 % for fasting plasma glucose, 8.7 % for urine creatinine and 6.4 % for urine albumin.

Of the 4549 participants, 1380 had diabetes and complete information for lipoproteins, age, Indian heritage, mean blood pressure and HbA$_{1c}$ at baseline and had urine albumin to creatinine ratios and serum creatinine measured at baseline and at the second examination. Participants with abnormal excretion of albumin at baseline ($n = 674$) or elevated serum creatinine [> 132.6 µmol/l (1.5 mg/dl) for men and 114.9 µmol/l (1.3 mg/dl) for women, $n = 27$] and those with triglyceride concentration greater than 6.77 mmol/l (600 mg/dl, $n = 2$) or who were taking lipid lowering drugs ($n = 6$) were excluded. A total of 671 participants were included in the final analysis.

Analyses were performed separately for men and women. Logarithms of VLDL and HDL cholesterol, VLDL and total

Table 1. Characteristics of study participants at baseline by sex

	Men (*n* = 211)	Women (*n* = 460)
	Mean ± SD	Mean ± SD
Age (years)	56 ± 7	56 ± 7
Degree of Indian heritage (%)[a]	100 (50–100)	100 (50–100)
Duration of diabetes (year)	9 ± 6	11 ± 8
Body Mass Index (kg/m^2)	32 ± 6	34 ± 6
Mean blood pressure (mm Hg)	96 ± 10	91 ± 10
Fasting glucose (mmol/l	9.6 ± 3.2	10.3 ± 4.1
Fasting insulin (pmol/l)[b]	120 (48–288)	144 (72–282)
HbA$_{1c}$ (%)	7.4 ± 2.0	7.9 ± 2.3
Urinary albumin excretion (mg/g)[b]	6 (1–21)	8 (3–23)
Serum creatinine (μmol/l)	84 ± 12	70 ± 11
Fibrinogen (g/l)[b]	2.7 (2.1–3.6)	3.0 (2.3–4.1)
Reported physical activity ('metabolic equivalent' past week)[a,c]	54 (0–204)	29 (0–156)
	Prevalence	Prevalence
Insulin treatment (%)	9	16
Oral glycaemic treatment (%)	41	38
Non or former drinker (%)	57	83
Current drinker (%)	9	6
Heavy drinker (%)	34	11

[a] Median [10th–90th percentiles]; [b] Geometric mean [10th–90th percentiles]; [c] Because of missing values, numbers of participants are 201 men and 446 women

triglycerides, Lp(a), insulin and fibrinogen concentrations and albumin to creatinine ratio and the square root of reported total physical activity per week were used to bring their distributions to normal. For the cross-sectional analysis, the population measurements were described at baseline by proportions, means or geometric means, standard deviation and 10th and 90th percentiles, depending on their distributions.

For the prospective analysis, the relation between baseline lipid measurements and the incidence of abnormal excretion of albumin was assessed in separate logistic regression models. The odds ratios of developing abnormal excretion of albumin were calculated to compare the 90th with the 10th percentile for each continuous variable. Interactions between lipids and other variables were evaluated by the likelihood ratio test [37] but were not significant and therefore not included in the models presented.

Principal component analysis [38] was used to extract a linear combination of LDL predominant peak size, HDL cholesterol and total triglyceride, which are the lipids described in the insulin resistance syndrome and abnormalities of these lipids would therefore represents an index associated with insulin resistance. These factors were selected to account for the maximum amount of variance in the data. Factor scoring coefficients were estimated to compute factor scores as a weighted sum of the values of the three standardized variables for each participant. The score was introduced as a continuous variable in a logistic model to predict abnormal excretion of albumin.

Generalized additive logistic regression [39] was used to examine non-linearities in the effect of lipids on the incidence of abnormal excretion of albumin. The linear and non-linear components were examined in each sex.

Results

Table 1 shows characteristics of the 671 diabetic participants (211 men and 460 women) at baseline. Table 2 shows lipid, lipoprotein and apolipoprotein con-

centrations and LDL predominant peak size at baseline. Pattern B of small LDL was present in 38% of the men and 25% of the women, whereas, pattern A of large LDL was present in 50% of the men and 61% of the women.

Microalbuminuria was developed by 61 (29%) men and 129 (28%) women, and by 6 (3%) men and 15 (3%) women during 3.9 (range: 1.7–6.2) years of follow-up. Median albumin excretions were 72 and 64 mg/g creatinine, respectively, in the 67 men and 144 women who developed abnormal excretion of albumin. High baseline concentrations of fasting glucose, HbA$_{1c}$ and albumin excretion, oral treatment for diabetes, and a low level of physical activity were risk factors for abnormal excretion of albumin when controlled for age. Other risk factors were degree of Indian heritage and insulin treatment in women, and low BMI and duration of diabetes at follow-up in men. Mean blood pressure was not a significant risk factor in men or women. When controlled for age, duration of diabetes at follow-up and albumin excretion at baseline, the statistically significant risk factors were fasting glucose, oral or insulin treatment for diabetes and Indian heritage in women only, and a low level of physical activity in both men and women.

In women, a low HDL cholesterol was a risk factor for abnormal excretion of albumin when controlled for the potential confounders: age, treatment with oral hypoglycaemic agents or insulin, HbA$_{1c}$, study site, degree of Indian heritage, mean arterial blood pressure and albumin excretion at baseline, and duration of diabetes at follow-up (Table 3). No other lipid variable [including Lp(a)] significantly predicted ab-

Table 2. Lipid levels at baseline by sex

	Men (n = 211)		Women (n = 460)	
	Mean	[10th–90th percentiles]	Mean	[10th–90th percentiles]
Cholesterol Total (mmol/l)	4.78	[3.57–5.84]	4.83	[3.72–6.05]
VLDL (mmol/l)[a,b]	0.59	[0.26–1.52]	0.59	[0.31–1.32]
LDL (mmol/l)	2.89	[1.94–4.01]	2.89	[1.94–3.85]
HDL (mmol/l)[a]	1.04	[0.78–1.40]	1.12	[0.85–1.50]
Triglycerides Total (mmol/l)[a]	1.42	[0.70–3.01]	1.49	[0.86–2.87]
VLDL (mmol/l)[a,b]	0.88	[0.30–2.62]	0.88	[0.40–2.12]
Apolipoprotein A 1 (g/l)	1.39	[1.05–1.71]	1.49	[1.16–1.85]
Apolipoprotein B (g/l)	1.47	[0.67–1.46]	1.8	[0.71–1.49]
LDL predominant peak size (Å)[c]	257	[245–270]	259	[248–269]
Lp(a) (mg/dl)[a,d]	4.7	[1–28]	4.8	[1–26]

[a] Geometric mean.
Because of missing values, numbers of participants are [b] 187 men and 412 women, [c] 208 men and 456 women, [d] 202 men and 434 women

Table 3. Effect of lipids in different logistic models for risk of abnormal excretion of albumin among 211 diabetic men (67 cases) and 460 diabetic women (144 cases) with normal excretion of albumin at baseline. Odds ratios (OR) and 95% confidence intervals (CI), 90th compared with 10th percentile in men and women

	Men				Women			
	Model 1[a]		Model 2[b]		Model 1[a]		Model 2[b]	
	OR	95% Cl	OR	95% Cl	OR	95% Cl	OR	95% Cl
Total cholesterol	0.78	0.38–1.6	1.0	0.40–2.5	0.88	0.53–1.5	1.2	0.66–2.1
VLDL cholesterol[c]	0.77	0.36–1.7	0.62	0.24–1.6	1.4	0.82–2.4	1.5	0.84–2.7
LDL cholesterol	0.66	0.31–1.4	1.1	0.43–2.9	0.91	0.56–1.5	1.2	0.67–2.0
HDL cholesterol	1.7	0.83–3.3	1.5	0.66–3.4	0.59	0.36–0.99	0.56	0.32–0.98
Total triglycerides	0.77	0.36–1.7	0.67	0.27–1.7	1.5	0.88–2.4	1.7	0.98–2.9
VLDL triglycerides[c]	0.76	0.33–1.7	0.69	0.26–1.8	1.6	0.93–2.7	1.6	0.91–2.9
Apolipoprotein A 1	1.2	0.59–2.4	1.3	0.60–2.9	0.86	0.53–1.4	0.89	0.53–1.5
Apolipoprotein B	0.88	0.41–1.9	1.1	0.45–2.8	0.92	0.58–1.5	1.1	0.65–1.8
LDL predominant peak size[d]	1.5	0.66–3.2	1.1	0.98–1.2	0.65	0.39–1.1	0.96	0.90–1.0
Lp(a)[c]	0.63	0.27–1.43	0.73	0.27–2.0	0.83	0.48–1.4	0.92	0.51–1.7

[a] Models 1 are controlled for age.
[b] Models 2 are controlled for age, treatment with oral hypoglycaemic agents or insulin, HbA$_{1c}$, study site, degree of Indian heritage, mean arterial blood pressure and albumin excretion at baseline, and duration of diabetes at follow-up.

Because of missing values, numbers of participants are [c] 187 men (59 cases) and 412 women (128 cases), [d] 208 men (66 cases) and 456 women (142 cases), and [e] 202 men (62 cases) and 434 women (142 cases)

normal excretion of albumin in men or in women, although there was a tendency towards high total and VLDL triglyceride and a small LDL predominant peak size. The three LDL patterns (A, B, I) were also introduced in a model, by using two indicator variables, and did not predict abnormal excretion.

Further adjustments for serum insulin concentrations, BMI, fibrinogen, alcohol consumption (using indicator variables for each category) or reported total physical activity and analyses by site did not modify the estimated effect of HDL cholesterol in women (data not shown). The inverse association between HDL cholesterol and abnormal excretion of albumin was decreased slightly by the exclusion of 96 women taking antihypertensive medicines among whom 29 developed abnormal excretion of albumin

[odds ratio (OR) = 0.61, 95% confidence interval (CI) = 0.33–1.2].

Among 70 pre-menopausal and 390 post-menopausal women, 8 and 36, respectively, received estrogen treatment. Menopause, estrogen treatment and the interaction terms between HDL cholesterol and menopause or estrogen treatment did not significantly predict abnormal excretion of albumin. Although, analyses among pre-menopausal women and post-menopausal women treated with estrogen and those among post-menopausal women not treated with estrogen suggested that, when adjusted for confounders, a low HDL cholesterol was a greater risk factor for abnormal excretion of albumin in the pre-menopausal group treated with estrogen (106 people and 31 cases of abnormal albumin excretion; OR = 0.29,
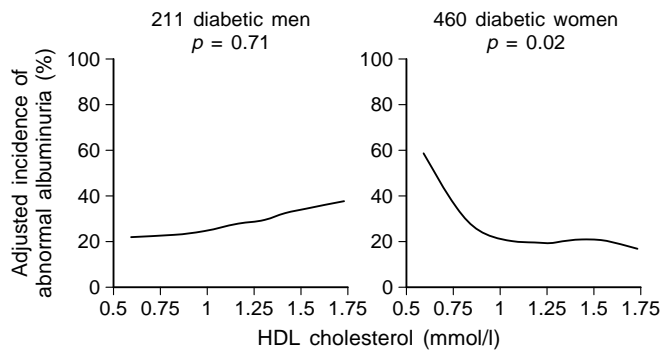
Fig. 1. Effect of HDL cholesterol concentration on the incidence of abnormal excretion of albumin (± 2 standard errors) in diabetic participants with normal albumin excretion at baseline. Results are given by sex and are controlled for age, treatment with oral hypoglycaemic agents or insulin, HbA_{1c}, study site, degree of Indian heritage, mean arterial blood pressure and urine albumin excretion at baseline, and duration of diabetes at follow-up. A generalized additive logistic regression model was used so that non-linearity in the relation could be examined. In each sex, the p-value is a test for any effect (linear or not) of HDL cholesterol

95 % CI = 0.08–1.0) than in the post-menopausal group receiving no estrogen (354 people and 113 of them had abnormal albumin excretion; OR = 0.62, 95 % CI = 0.33–1.2).

Predominant peak size of LDL, HDL cholesterol and total triglyceride, the lipid abnormalities described in the insulin resistance syndrome, were analysed together using principal component analysis. One factor was extracted for each sex, which accounted for 64 % of the variance in men and 61 % in women. The correlations between the factor and each variable were, in men and women respectively, 0.85 and 0.83 for LDL predominant peak size, 0.74 and 0.70 for HDL cholesterol and –0.82 and –0.81 for total triglyceride. A low score, which is the weighted sum of the three variables, would therefore reflect the lipid abnormalities of the insulin resistance syndrome. In a logistic model adjusted for confounders, a low score was a risk factor for abnormal excretion of albumin in women (OR = 0.55, 95 % CI = 0.32–0.94) but not in men (OR = 2.1, 95 % CI = 0.80–5.5).

A generalized additive logistic regression model was used to examine possible non-linear effects of lipids on the incidence of abnormal excretion of albumin. The analysis suggested that most of the predictive effect of HDL cholesterol in women, when controlled for potential confounders, was below 0.9 mmol/l with little additional effect associated at higher concentrations (Fig. 1). Of the women 15 % had a HDL cholesterol value below this approximate threshold. The effect of principal component analysis factor, which combined the effects of triglyceride, LDL predominant peak size and HDL cholesterol, was linear (data not shown). No significant linear or non-linear association with HDL cholesterol and the

principal component analysis factor was found in men and with other lipids in men and women when the generalized additive model was used (data not shown).

Discussion

In American Indian women with Type II diabetes, a low HDL cholesterol was a risk factor for abnormal excretion of albumin. This association was still significant when adjusted for age, duration of diabetes at follow-up, treatment with oral hypoglycaemic agents or insulin, HbA_{1c}, study site, degree of Indian heritage, mean arterial blood pressure, baseline albumin excretion, insulin concentration, BMI, alcohol consumption or physical activity. There was a tendency for high concentrations of VLDL and total triglyceride and a small LDL predominant peak size to predict abnormal excretion of albumin in women. On the other hand, high concentrations of LDL and total cholesterol, and apoprotein B, each of which has been reported previously to predict abnormal excretion of albumin, did not predict it in this population, either in men or in women.

Lipids could have a direct effect on diabetic nephropathy [1–4]. It is also possible that lipids, and HDL cholesterol in particular, are associated with endothelial dysfunction, which has been related to the development of microalbuminuria in patients with Type II diabetes [40]. Other factors related to lipid concentrations could, however, be responsible for the relation between HDL cholesterol and diabetic nephropathy and for an approximate threshold at 0.9 mmol/l above which the effect of HDL cholesterol was weaker. Women with low HDL cholesterol are probably more insulin resistant and at higher risk for diabetic nephropathy if insulin resistance is a risk factor for diabetic nephropathy, but this hypothesis does not explain why the same abnormality is not a risk factor in men. Moreover, adjusting for obesity, HbA_{1c}, duration of diabetes, type of diabetes treatment, and serum insulin concentration did not modify the conclusions. Serum insulin is not, however, a good estimate of insulin resistance among diabetic patients [41]. Therefore, and because of a tendency for high serum concentrations of triglyceride and small LDL predominant peak size to predict abnormal excretion of albumin, principal component analysis was used to find an index to the lipid abnormalities of the insulin–resistance syndrome. A low level of the factor extracted from a combination of HDL cholesterol, total triglyceride and LDL predominant peak size predicted abnormal excretion of albumin as well as a low HDL cholesterol alone. Therefore, it is not possible to determine whether low HDL cholesterol itself, insulin resistance, or some other correlated variable is the most important of these risk factors. This is espe-

cially difficult because this study lacked a good measurement of insulin resistance.

The cholesterol sub-fractions HDL_3 and HDL_2 are higher in response to alcohol consumption [42] and physical activity increase [43], respectively. A specific increase in one of the HDL sub-fractions (e.g. HDL_3) could be protective for nephropathy, as it could be less protective for atherosclerosis. Such an effect could be responsible for sex discrepancies if alcohol consumption or physical activity differ between men and women, or is under-estimated in one group. No difference was observed, however, for the effect of HDL cholesterol between participants who were non, current or heavy drinkers, or who had different levels of physical activity.

A lower HDL cholesterol was associated with a higher incidence of nephropathy only in women. Women have higher HDL cholesterol than men and sex hormones could affect several enzymes involved in lipid metabolism [44]. A low HDL cholesterol could then be a reflection of sex hormone activity in that a low HDL cholesterol is associated with a lower concentration of sex hormone binding globulin in women [45] and a high concentration in men [46]. The fact that the relation between HDL cholesterol and diabetic nephropathy might be stronger among pre–menopausal women or women taking estrogen than among post–menopausal women favours this hypothesis but development of menopause during follow-up could be responsible for some confounding. Moreover, sex discrepancies have been found among the Pima Indians for prediction of diabetes, i.e. a low HDL cholesterol was a risk factor for diabetes in women only [47], as well as in one other study where LDL cholesterol was a weak predictor of microalbuminuria in men and total triglyceride a stronger one in women [6]. No effect of sex hormones on diabetic nephropathy has, however, been reported previously, and no measurements of sex hormones were available in the Strong Heart Study.

Animal studies suggest that it is mainly LDL and VLDL that are risk factors for kidney diseases [1–4]. Some prospective studies [5–11] and two clinical trials [23–25] have reported predictive effects of total and LDL cholesterol and triglyceride on different stages of diabetic nephropathy. Some limitations of our study could explain why, among the lipoproteins, only low HDL cholesterol was a risk factor for abnormal excretion of albumin. Because we studied older members of a population at high risk for diabetic nephropathy and excluded those with abnormal albumin excretion at baseline, we may have introduced an incidence-prevalence bias [48] by excluding subjects at highest risk. Therefore, it is possible that other lipids (e.g. LDL cholesterol, small LDL or triglyceride, which in women were almost significantly positively predictive) influence diabetic nephropathy but were not detected as risk factors because of our study de-

sign. Limited power could also explain the negative results in men, among whom only 67 developed abnormal excretion of albumin, compared with 144 women. In addition, only two examinations were done per participant and new cases of abnormal excretion of albumin were identified by a single urine test at each examination.

In conclusion, in this large prospective study of American Indians with Type II diabetes, we found that a low HDL cholesterol predicted abnormal excretion of albumin. There was also a tendency for high concentrations of VLDL and total triglyceride and a small LDL predominant peak size to predict abnormal excretion of albumin. The effect of HDL cholesterol was observed in women only and, for most of it, below approximately 0.9 mmol/l. A HDL cholesterol concentration below this threshold could be used as a marker for women at high risk for diabetic nephropathy, at least among the Pima Indian population. The HDL cholesterol effect in women tended to be greater before menopause, suggesting a sex hormone effect. Nevertheless, residual confounding due to insulin resistance, obesity, glucose control, alcohol consumption, physical activity, or unknown factors, could be partially responsible for the association. Other prospective studies are needed to explore the role of lipids in the development of diabetic nephropathy and their relationship to sex hormones. Nevertheless, if lipids are risk factors for diabetic nephropathy, their effect is probably weak and treatment should be directed primarily towards glucose and blood pressure control to prevent or delay diabetic nephropathy since improving these two risk factors has been shown in randomized clinical trials to slow the progression of diabetic nephropathy [49, 50]. Treatment of dyslipidaemia among American Indians with diabetes is important, however, because elevated total cholesterol [51] is a risk factor for mortality from cardiovascular diseases.

References

1. Diamond JR (1991) The role of cholesterol in glomerular injury. In: Keane WF, Stein JH (ed) Contemporary Issues in Nephrology. 2nd edn, New York, Churchill Livingstone pp 109–126
2. Keane WF, Mulcahy WS, Kasiske BL, Kim Y, O'Donnel MP (1991) Hyperlipidemia and progressive renal disease. Kidney Int 39:S41–S48

3. Moorhead JF (1991) Lipids and progressive kidney disease. Kidney Int 39:S35–S40

4. Diamond JR (1991) Effect of lipid abnormalities on the progression of renal damage. Analogous pathobiologic mechanisms in glomerulosclerosis and atherosclerosis. Kidney Int 31:S29–S34

5. Hasslacher C, Bostedt-Kiesel A, Kempe HP, Wahl P (1993) Effect of metabolic factors and blood pressure on kidney function in proteinuric Type II (non-insulin-dependent) diabetic patients. Diabetologia 36: 1051–1056

6. Coonrod BA, Ellis D, Becker DJ et al. (1993) Predictors of microalbuminuria in individuals with IDDM. Pittsburgh Epidemiology of Diabetes Complications Study. Diabetes Care 16: 1376–1383

7. Ellis D, Lloyd C, Becker DJ, Forrest KYZ, Orchard TJ (1996) The changing course of diabetic nephropathy: low-density lipoprotein cholesterol and blood pressure correlate with regression of proteinuria. Am J Kidney Dis 27: 809–818

8. Nelson RG, Knowler WC, Pettitt DJ, Hanson RL, Bennett PH (1995) Incidence and determinants of elevated urinary albumin excretion in Pima Indians with NIDDM. Diabetes Care 18: 182–187

9. Krolewski AS, Warram JH, Christlieb AR (1994) Hypercholesterolemia – A determinant of renal function loss and deaths in IDDM patients with nephropathy. Kidney Int 45:S125–S131

10. Wirta OR, Pasternack AI, Mustonen JT, Koivula TA, Harmoinen A (1996) Urinary albumin excretion rate and its determinants after 6 years in non-insulin-dependent diabetic patients. Nephrol Dial Transplant 11: 449–456

11. Samuelsson O, Aurell M, Knight-Gibson C, Alaupovic P, Attman PO (1993) Apolipoprotein-B-containing lipoproteins and the progression of renal insufficiency. Nephron 63: 279–285

12. Wirta O, Pasternack A, Laippala P, Turjanmaa V (1996) Glomerular filtration rate and kidney size after 6 years disease duration in non-insulin-dependent diabetic subjects. Clin Nephrol 45: 10–17

13. Yokoyama H, Jensen JS, Myrup B, Mathiesen ER, Ronn B, Deckert T (1996) Raised serum sialic acid concentration precedes onset of microalbuminuria in IDDM. A 10-year follow-up. Diabetes Care 19: 435–440

14. Yokohama H, Tomonaga O, Hirayama M et al. (1997) Predictors of the progression of diabetic nephropathy and the beneficial effect of angiotensin-converting enzyme inhibitors in NIDDM patients. Diabetologia 40: 405–411

15. Niskanen LK, Parviainen M, Penttilä I, Uusitupa MIJ (1996) Evolution, risk factors, and prognostic implications of albuminuria in NIDDM. Diabetes Care 19: 486–493

16. Kordonouri O, Danne T, Hopfenmüller W, Enders I, Hövener G, Weber B (1996) Lipid profiles and blood pressure: are they risk factors for the development of early background retinopathy and incipient nephropathy in children with insulin-dependent diabetes mellitus. Acta Paediatr 85: 43–48

17. Yokota C, Kimura G, Inenaga T, Kawano Y, Matsuoka H, Omae T (1995) Risk factors for progression of diabetic nephropathy. Am J Nephrol 15: 488–492

18. Maser RE, Usher D, Becker DJ, Drash AL, Kuller LH, Orchard TJ (1993) Lipoprotein(a) concentration shows little relationship to IDDM complications in the Pittsburgh Epidemiology of Diabetes Complications Study Cohort. Diabetes Care 16: 755–758

19. Ravid M, Neumann L, Lishner M (1995) Plasma lipids and the progression of nephropathy in diabetes mellitus Type II: effect of ACE inhibitors. Kidney Int 47: 907–910

20. The Microalbuminuria Captopril Study Group (1996) Captopril reduces the risk of nephropathy in IDDM patients with microalbuminuria. Diabetologia 39: 587–593

21. Hommel E, Andersen P, Gall MA et al. (1992) Plasma lipoproteins and renal function during simvastatin treatment in diabetic nephropathy. Diabetologia 35: 447–451

22. Nielsen S, Schmitz O, Moller N et al. (1993) Renal function and insulin sensitivity during simvastatin treatment in Type II (non-insulin-dependent) diabetic patients with microalbuminuria. Diabetologia 36: 1079–1086

23. Smulders YM, Van Eeden AE, Stehouwer CDA, Weijers RNM (1997) Can reduction of hypertriglyceridaemia slow progression of microalbuminuria in patients with non-insulin-dependent diabetes mellitus? Eur J Clin Invest 27: 997–1002

24. Lam KSL, Cheng IKP, Janus ED, Pang RWC (1995) Cholesterol-lowering therapy may retard the progression of diabetic nephropathy. Diabetologia 38: 604–609

25. Lam KSL, Lauder IJ (1996) Response from the authors. Diabetologia 39: 367–373 (Letter)

26. Welty TK, Lee ET, Yeh JL et al. (1995) Cardiovascular risk factors among American Indians. Am J Epidemiol 142: 269–287

27. Howard BV, Lee ET, Cowan LD et al. (1995) Coronary heart disease prevalence and its relation to risk factors in American Indians: the Strong Heart Study. Am J Epidemiol 142: 254–268

28. Lee ET, Howard BV, Savage PJ et al. (1995) Diabetes mellitus and impaired glucose tolerance in three American Indian populations: the Strong Heart Study. Diabetes Care 18: 599–610

29. Robbins DC, Knowler WC, Lee ET et al. (1996) Regional differences in albuminuria among American Indians: an epidemic of renal disease. Kidney Int 49: 557–563

30. Lee ET, Welty TK, Fabsitz RR et al. (1990) The Strong Heart Study – A study of cardiovascular disease in American Indians: design and methods. Am J Epidemiol 132: 1141–1155

31. Kriska AM, Knowler WC, LaPorte RE et al. (1990) Development of questionnaire to examine the relationship of physical activity and diabetes in Pima Indians. Diabetes Care 13: 401–411

32. World Health Organization Study Group on Diabetes Mellitus (1985) Technical report series No 727, WHO, Geneva

33. Little RR, England JD, Wiedmeyer HM et al. (1986) Interlaboratory standardization of glycated haemoglobin determinations. Clin Chem 32: 358–360

34. von Clauss A (1957) Gerinnungsphysiolgische Schnellmethode zur Bestimmung des Fibrinogens. Acta Haematol 17: 237–240

35. Vasquez B, Flock EV, Savage PJ et al. (1984) Sustained reduction in proteinuria in Type II (non-insulin dependent) following diet-induced reduction of hyperglycaemia. Diabetologia 26: 127–133

36. Chasson AL, Grady HJ, Stanley MA (1960) Determination of creatinine by means of automatic chemical analysis. Tech Bull Register Med Tech 30: 207–212

37. Breslow NE, Day NE (1980) Unconditional logistic regression for large strata. In: IARC (International Agency for Research on Cancer) Publication. Statistical Methods in Cancer Research, Vol I, The Analysis of Case-Control Studies, No.32, Lyon, pp 191–259

38. Stevens J (1986) Applied multivariate statistics for the social sciences. Lawrence Erlbaum Associates, Hillsdale, N.J., pp 337–350

39. Hastie TJ, Tibshirani RJ (1990) Generalized Additive Models. Chapman and Hall, London and New York, N.Y.

40. Stehouwer CDA, Nauta JJP, Zeldenrust GC, Hackeng WHL, Donker AJM, Den Ottolander GJH (1992) Urinary albumin excretion, cardiovascular disease, and endothelial dysfunction in non-insulin-dependent diabetes mellitus. Lancet 340: 319–323

41. Laakso M (1993) How good a marker is insulin level for insulin resistance? Am J Epidemiol 137: 959–965

42. Srivastava LM, Vasisht S, Agarwal DP, Goedde HW (1994) Relation between alcohol intake, lipoproteins and coronary heart diseases: the interest continues. Alchol Alchol 29: 11–24

43. Goldberg L, Elliot DL (1994) The use of exercise to improve lipid and lipoprotein levels. In: Goldberg L, Elliot DL (eds) Exercise for prevention and treatment of illness. F. A. Davis, Philadelphia, Pa., pp 189–210

44. Haffner SM, Valdez RA (1995) Endogenous sex hormones: impact on lipids, lipoproteins, and insulin. Am J Med 98: 40S–47S

45. Haffner SM, Katz MS, Stern MP, Dunn JF (1989) Association of decreased sex hormone binding globulin and cardiovascular risk factors. Arteriosclerosis 9: 136–143

46. Haffner SM, Mykkänen L, Valdez RA, Katz MS (1993) Relationship of sex hormones to lipids and lipoproteins in nondiabetic men. J Clin Endocrinol Metab 77: 1610–1615

47. Fagot-Campagna A, Narayan KMV, Hanson RL et al. (1997) Plasma lipoproteins and incidence of non-insulin-dependent diabetes mellitus in Pima Indians: protective effect of HDL cholesterol in women. Atherosclerosis 128: 113–119

48. Sackett DL (1979) Bias in analytic research. J Chron Dis 32: 51–63

49. Ohkubo Y, Kishikawa H, Araki E et al. (1995) Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. Diabetes Res Clin Pract 28: 103–117

50. The Euclid Study Group (1997) Randomised placebo-controlled trial of lisinopril in normotensive patients with insulin-dependent diabetes and normoalbuminuria or microalbuminuria. Lancet 349: 1787–1792

51. Fagot-Campagna A, Hanson RL, Narayan KMV et al. (1997) Serum cholesterol and mortality rates in a Native American population with low cholesterol concentrations: a U-shaped association. Circulation 97: 1408–1415