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Demonstration of an abnormality of C apoprotein of very low density lipoprotein in patients with gout

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SUMMARY The very low density (VLDL) apolipoproteins of 12 male patients with gout have been studied by analytical isoelectric focusing. The relative percentage distribution of the C apolipoproteins was calculated and compared with that from 12 normolipidaemic and 12 'lipid-matched' controls.

In the gout patients apolipoprotein CII (apo CII) represented 19·6% of the VLDL C proteins and the $CII/CIII_2$ ratio was 0·57. In the normolipidaemic controls apo CII represented 28·8% and in the lipid-matched controls 33·1% of VLDL apo C, and the $CII/CIII_2$ ratio approached one in each control group. These differences were significant. This suggests that a reduction in VLDL apo CII may predispose to the hypertriglyceridaemia that is commonly found in patients with gout.

Key words: gout, hyperlipidaemia, apolipoprotein CII.

Many studies have shown a high incidence of hyperlipidaemia in patients with gout, commonly hypertriglyceridaemia due to raised endogenous VLDL.[1–3] As well as lipid, VLDL particles contain B, C, and E apoproteins, and recent discoveries about the function of these have had considerable impact on the understanding of disorders of lipid metabolism. Lipoprotein lipase (LPL) catalyses the conversion of VLDL to LDL at capillary endothelial sites.[4 5] Apo CII is an activator[6] of LPL, and apo CIII has been reported to inhibit this enzyme in vitro.[7]

To date, studies of hypertriglyceridaemia in gout have concentrated on the lipid moiety. The purpose of this study was to look for an abnormality in the C apoprotein component of VLDL in men with gout, which might explain their predisposition to hypertriglyceridaemia.

SUBJECTS

Twelve men with gout (mean age of 48 years) were selected at random from the rheumatology clinic. They all gave informed consent to the study which had been approved by the local ethical committee. All their drug treatment was stopped for a period of six weeks, and they were also requested to reduce their alcohol intake as much as possible and avoid self medication. At the end of the six-week period serum was obtained after a 12-hour overnight fast.

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CONTROLS

Serum from fasting subjects was obtained from two control groups. One group consisted of 12 normolipidaemic, normouricaemic men (mean age 34·5 years) with a normal social alcohol intake drawn from the hospital laboratory and medical staff. The other consisted of 12 normouricaemic men (mean age 46) without a history of gout, who were matched in lipid status with the gout patients: type IV (five patients), type IIb (two patients), and five normolipidaemic patients.

ASSESSMENT OF LIPID STATUS

Cholesterol was measured by cholesterol oxidase and triglyceride by enzymatic assay after enzymatic hydrolysis (Boehringer Mannheim GmbH Diagnostica). Patients were classifed into Fredrickson hyperlipidaemia types according to the appearance of serum before and after standing for 24 hours at 4°C,

the serum lipid levels, and, where indicated, lipoprotein electrophoresis.[8]

PREPARATION OF VLDL APOPROTEINS

VLDL was isolated from serum of fasting patients by ultracentrifugation[9] in an MSE Prepspin 50 and was subjected to a further 18-hour period of ultracentrifugation at 40 000 rpm to remove any contaminating albumin. The washed VLDL (containing 1·5–3·0 g/l protein) was delipidated with tetramethylurea (TMU).[10]

300 µl VLDL was added to 300 µl 4·2 M TMU in a small glass tube. The contents were mixed and incubated at 37°C for 30 minutes. The tube was then centrifuged at 2500 rpm for 10 minutes, and the supernatant containing TMU-soluble apoproteins was removed and subjected to analytical isoelectric focusing in polyacrylamide gel rods.

ANALYTICAL ISOELECTRIC FOCUSING

Gel preparation

Polyacrylamide gels were prepared by mixing the following solutions: 2·4 ml of 13·75% acrylamide w/v–0·8% *N,N*-methylene bis(acrylamide) w/v in 8 M urea; 7·5 ml 8 M urea; 1·2 ml glycerol; 0·5 ml *N,N,N′N′*-tetramethylethylenediamine; and 0·6 ml ampholine pH 4·6 (LKB Produckter). Chemical polymerisation was carried out by adding 0·15 ml of a 1·07% solution of ammonium persulphate w/v in 8 M urea, and the gels were cast to a height of 10·5 cm in 0·5×12 cm glass tubes. The final concentration of urea was 6·8 M and of acrylamide 7·5%.

ELECTROFOCUSING

A disc gel electrophoresis apparatus (Shandon Scientific Co. Ltd) was adapted for isoelectric focusing by fitting a Perspex extension collar to accommodate the longer gels. The lower electrode chamber was filled with 250 ml of 0·1 M phosphoric acid and the upper with 240 ml 0·1 M NaOH. The gels were run at a constant voltage with a current of 1 mA per tube for one hour to remove the persulphate and partially establish the pH gradient. 50 µl of a solution consisting of 2·5% v/v ampholine pH 4·6 in 5% w/v sucrose solution was then carefully placed on top of each gel, and 100 µl of delipidated VLDL solution layered underneath this. A constant voltage of 44 V per tube was then applied for 24 hours at room temperature. Each sample was run in duplicate. No overheating was experienced with up to six tubes run simultaneously.

GEL FIXING AND STAINING

Gels were first fixed in a 11·5% w/v trichloracetic acid +3·46% w/v sulphosalicylic acid mixture for 30 minutes, then washed with 25% ethanol v/v +8% acetic acid v/v for a further 30 minutes to remove ampholine from the gel, and finally stained with 0·25% Coomassie brilliant blue according to the method of Weber and Osborn.[11]

IDENTIFICATION OF THE PROTEIN BANDS

Each protein band was identified by its isoelectric point (pI) from a standard graph prepared by running 20 samples under identical conditions and cutting the unfixed gels into 2 mm segments which were soaked for 24 hours in 1 ml deionised water. The pH of each segment was measured and a scatter diagram prepared by plotting the pH of each segment against the positions of that segment along the gel. A linear least mean square analysis generated a straight line in the pH region 4·2–6·2, and a pI was determined for each band by measuring the distance along the gel, making allowance for gel shrinkage that occurred during fixing and staining. The four major anodal bands were assigned on the basis of their pI to each of the four major apo C proteins by reference to the published literature[12] and previous work from this department.[13]

QUANTIFICATION OF APO C

The stained gels were scanned at 560 nm in a Pye Unicam (SP800) spectrophotometer fitted with a gel scanning attachment. The peak area (height×width at ½ peak height) was calculated for each apo C band, and the contribution of each individual band was expressed as a percentage of the total area of quantified apo C. The within-assay coefficient of variation was 2%, and between-assay coefficient of variation ranged from 2·7 to 7%.

Previous work in this department[13] has established that this method of quantification is valid. The absorption of the bands produced by the C apoproteins is linear over a wide range. This has also been confirmed by other workers.[14]

STATISTICAL ANALYSIS

The differences observed in the distribution of C apoproteins in the three groups were tested for statistical significance by Wilcoxon's rank sum test for unpaired data.

Results

Relevant clinical findings in the gout patients are shown in Table 1 and their serum and VLDL cholesterol and triglyceride levels in Table 2. The serum and VLDL cholesterol and triglyceride level of the lipid-matched controls are shown in Table 3.

Seven of the 12 gout patients (58%) had a hyperlipidaemia: five type IV and two type IIb.

Table 1 *Clinical and laboratory features of the gout patients*

Patient	*Age*	*SUA* (mmol/l)*	*Obesity†*	*Alcohol intake*	*Type‡*
1	38	0·44	−	+	N
2	53	0·42	−	+	N
3	30	0·54	−	++	N
4	56	0·54	−	+	N
5	73	0·63	+	±	N
6	42	0·5	+	+	IV
7	43	0·42	+	±	IV
8	61	0·62	+	±	IV
9	40	0·44	−	+	IV
10	52	0·55	−	0	IV
11	56	0·5	−	0	IIb
12	37	0·54	−	0	IIb

*Serum uric acid.
†Weight/height index >115.
‡Fredrickson type: N=normolipidaemic.
0=non-drinker; ±=occasional drinker; +=moderate regular drinker; ++=heavy regular alcohol intake.

Table 2 *Serum and VLDL lipid levels in gout patients*

Patient	*Triglyceride (mmol/l)*		*Cholesterol (mmol/l)*		*Type**
	Total	*VLDL*	*Total*	*VLDL*	
1	1·76	0·79	7·76	0·57	N
2	1·59	0·99	7·0	0·64	N
3	1·43	0·9	6·57	0·78	N
4	1·78	1·07	8·81	1·25	N
5	2·50	1·06	7·31	1·1	N
6	4·51	3·4	6·57	0·99	IV
7	3·21	1·86	9·45	1·69	IV
8	3·59	2·41	6·61	0·7	IV
9	7·52	4·93	9·53	1·47	IV
10	4·51	2·58	8·50	1·34	IV
11	2·99	2·1	9·33	2·0	IIb
12	3·84	2·45	10·5	1·87	IIb
Mean±SD	3·26±1·7	2·0±1·2	8·16±1·36	1·2±0·48	

*Fredrickson type.

Table 3 *Serum and VLDL levels in normouricaemic lipid-matched controls*

Matched control	*Triglyceride (mmol/l)*		*Cholesterol (mmol/l)*		*Type**
	Total	*VLDL*	*Total*	*VLDL*	
1	1·68	0·84	6·42	0·46	N
2	1·48	1·09	6·84	0·53	N
3	1·36	1·06	7·23	0·71	N
4	1·52	0·94	8·23	0·92	N
5	2·24	0·76	6·92	1·10	N
6	3·35	2·66	7·41	1·30	IV
7	2·87	1·78	8·64	1·42	IV
8	4·88	2·22	6·84	0·68	IV
9	6·40	4·34	8·23	1·45	IV
10	3·86	2·02	7·30	1·09	IV
11	3·42	1·87	8·90	2·30	IIb
12	3·04	1·94	11·40	1·60	IIb
Mean±SD	3·0±1·5	1·79±1·0	7·8±1·36	1·13±0·52	

*Fredrickson type.

Three patients with hyperlipidaemia and one with normal serum lipids were obese. The correlation between hypertriglyceridaemia and alcohol consumption was not absolute: one patient who was a non-drinker had type IV.

The relative distribution of the C apoproteins is given in Table 4. The gout patients had a lower mean CII and a higher mean $CIII_2$ than the normolipidaemic controls and a lower mean CII than the lipid-matched controls. The ratio $CII/CIII_2$ was lower in the gout patients than in both control groups. These differences were all statistically significant.

Analysis of the apo C distribution in the gout patients by lipid status (Table 5) showed that the low $CII/CIII_2$ ratio was not just confined to those with type IV hypertriglyceridaemia but was also present in those with type II hyperlipidaemia and in subjects with normal lipid levels.

Table 4 *Relative distribution of C apoproteins (%±SD)*

	Number	*$CIII_2$*	*$CIII_1$*	*CII*	*$CIII_0$*	*$CII/CIII_2$*
Gout patients	12	28·7±5·7	44·6±5·4	16·4±2·7	9·86±5·2	0·59±0·21
Normolipaemic controls	12	23·3±3·7*	42·0±4·4	22.4±3·6*	9·6±2·2	0·96±0·3*
Lipid-matched controls	12	25·0±4·3	39·6±4·1	24·9±2·6*	10·4±4·7	1·09±0·24*

*Significantly different from the gout patients (p=0·02).

Table 5 *Apo C distribution in gout patients related to Fredrickson type (%±SD)*

Fredrickson type	*Number*	*$CIII_2$*	*$CIII_1$*	*CII*	*$CIII_0$*	*$CII/CIII_2$*
Normolipaemic	5	29·2±7·4	48·02±3·5	14·9±4·0	7·78±3·4	0·57±0·3
Type IV	5	27·2±6·4	44·2±8·1	15.6±2·3	10·5±7·6	0·59±0·21
Type IIb	2	29·3	3·6	19·0	8·4	0·67

Discussion

The results of this study suggest a mechanism which may predispose patients with gout to hypertriglyceridaemia. Apo CII activates the breakdown of triglyceride by lipoprotein lipase (LPL),[5] and apo CIII appears to have an inhibitory effect on this process, at least in vitro.[7] Apo CII attaches to LPL at capillary endothelium and facilitates penetration of the enzyme into the lipid core of VLDL.[15] It is not certain how apo CIII opposes this process, but it is known that the addition of CIII protein to triglyceride emulsions has a pronounced inhibitory effect on hepatic removal.[16] The degree of sialylation of glycoproteins such as apo CIII may affect this inhibitory potential,[17] and it could be relevant that apo $CIII_2$ contains 2 mol/mol sialic acid whereas apo $CIII_1$ and $CIII_0$ contain 1 and 0 mol/mol respectively.[12] Thus an alteration in the ratio of $CII/CIII_2$ could render the VLDL relatively resistant to the action of LPL. It is of interest that in the present study the abnormality was also found in the normolipidaemic gout patients. This suggests that the gouty diathesis itself is associated with an abnormal distribution of VLDL apo C which does not necessarily show itself in hypertriglyceridaemia.

A deficiency of apolipoprotein CII has been discussed in a kindred with severe hypertriglyceridaemia.[21 22] Homozygotes with this condition have marked hypertriglyceridaemia, but heterozygotes with only 50% or normal levels of apo CII do not have raised levels of triglycerides. Thus it appears that in this kindred, with its particular environment and possible low fat intake,[22] apo CII levels of half those in a control preparation are sufficient to keep serum triglycerides within the normal range.

An excess of apo $CIII_2$ has been shown by Stocks *et al.*[23] in a patient with hypertriglyceridaemia and glucose intolerance and also in some patients with chronic renal failure.[24] In both these groups apo CII levels were essentially normal, but the authors suggest that the increased levels of apo $CIII_2$ may have produced a VLDL particle with abnormal substrate properties for lipoprotein lipase.

Thus a decrease in ratio of apo CII to apo $CIII_2$, as in our gout patients (0·59 compared with 0·96 and 1·09 in normolipidaemic and hyperlipidaemic controls respectively), may predispose to hypertriglyceridaemia. This has also been suggested by the studies of Kashyap *et al.*[14]

We do not have any data from this study that help to clarify the cause of the apo C abnormality. VLDL is assembled and secreted by the liver without apo C, which is acquired later from high density lipoproteins (HDL).[18] As triglyceride-rich VLDL is transformed into cholesterol-rich LDL by LPL, apo C transfers back to HDL.[19] Hyperuricaemia itself could possibly interfere with the recirculation of the apo C proteins, but many patients with hyperuricaemia do not have hypertriglyceridaemia. Conversely many people with raised uric acid levels do not have gout. It has been suggested that an additional factor may be required to facilitate nucleation of the solute into crystal, and an abnormality of proteoglycans has been proposed as the predisposing factor.[20] In this regard it may be relevant that the LPL is attached to capillary endothelial cells via the proteoglycan heparan sulphate,[18] and an alteration here could affect the rate and efficiency of apo C-mediated VLDL on metabolism. Such a hypothesis would be in favour of the concept of a gouty diathesis and provide an explanation for the high incidence of hypertriglyceridaemia in gout. Studies on the release of LPL following heparin in patients with gout may help to clarify this.

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