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Very low density lipoprotein subfractions in Type II diabetes mellitus: alterations in composition and susceptibility to oxidation

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Abstract

Aims/hypothesis. Type II (non-insulin-dependent) diabetes mellitus is associated with raised triglycerides and increased very low density lipoprotein cholesterol. The aim of this study was to assess if very low density lipoprotein subfraction composition and potential to oxidise were altered in this condition.
Methods. Very low density lipoprotein was separated into four subfractions (A→D) by a novel, rapid ultracentrifugation procedure. Analysis of each subfraction included lipid and fatty acid composition. Preformed peroxides were measured spectrophotometrically and conjugated dienes were used as an indicator of in vitro lipid oxidation.
Results. In all results we compared patient and control subfractions. Mean fasting plasma glucose was 8.9 ± 2.0 mmol/l in patients vs 5.1 ± 0.4 mmol/l in control subjects ($p < 0.001$); patient HbA_{1c} was 7.6 ± 1.4 %. Patient total lipid standardised for apo B was higher than controls in subfractions A, B and C; A, 201 vs 60; B, 191 vs 40; C, 63 vs 21; D, 29 vs 34 μmol lipid per mg apo B ($p < 0.05$). Preformed peroxides were higher in all patient subfractions compared with controls: A, 340 vs 48; B, 346 vs 42; C, 262 vs 28; D, 54 vs 16 nmol per mg apo B ($p < 0.001$). Patient subfractions A and D were more susceptible to in vitro oxidation. Monounsaturated fatty acids were lower in patients subfractions, 35.2 vs 36.7; B, 35.1 vs 38.7; C, 34.4 vs 36.5; D, 33.0 vs 35.5 as per cent total ($p < 0.05$).
Conclusions/interpretation. These results indicate abnormalities in very low density lipoprotein subfraction composition and oxidation profile in Type II diabetic subjects, which are characteristic of more atherogenic particles and that may contribute to the development of cardiovascular disease in these patients. [Diabetologia (2000) 43: 485–493]

Keywords Lipid and fatty acid composition, lag time, preformed peroxides.

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Abbreviations: CETP, Cholesterol ester transfer protein; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; BHT, butylated hydroxy toluene; HPLC, high performance liquid chromatography; GC, gas chromatography; FOX, ferrous oxidation xylenol orange; d, density.

Death from cardiovascular disease is two to three times more common in patients with Type II (non-insulin-dependent) diabetes mellitus than in healthy subjects [1], and a strong correlation exists between serum lipids and atherosclerosis [2–4]. The abnormalities resulting from insulin resistance and Type II diabetes appear to affect the composition of lipoproteins in the absence of large changes in absolute concentrations [3–5]. Visceral obesity is closely associated with glucose intolerance and Type II diabetes. Studies have shown that this condition results in an increase in the VLDL apo B pool [6, 7]. In non-diabetic subjects, studies have also shown that insulin suppresses the release of VLDL 1 apo B from the liver. In Type

II diabetes, however, the production of this VLDL 1 apo B is not impaired, which may be one of the root causes of the characteristic hypertriglyceridaemia [8, 9]. One consequence of hypertriglyceridaemia is the release of large triglyceride rich VLDL subfractions from the liver [10, 11]. The metabolic cascade of these particles leads to the production of small dense LDL, a particle strongly associated with the development of atherosclerosis due in part to its increased susceptibility to oxidation [12–14]. Moderate changes occur in total serum cholesterol; HDL cholesterol is usually decreased whereas VLDL cholesterol is increased, the net result leading to normal or only moderately increased total serum cholesterol. The altered composition of lipoproteins in Type II diabetes is facilitated by the action of cholesterol ester transfer protein (CETP) [15]. Triglyceride rich particles have a prolonged residence time within the circulation and are exposed to the action of this enzyme for longer periods of time, the consequence of which is the net transfer of triglyceride from VLDL to HDL and LDL, with cholesterol moving in the opposite direction.

Diabetes is also associated with an accumulation of remnant particles within the circulation. These native particles are known to be atherogenic [16] due to their avid uptake by tissue macrophages, leading to the accumulation of cholesterol and resulting in the formation of foam cells [17], a prerequisite step in the development of atherosclerosis. Low density lipoprotein particles, however require oxidative modification before their uptake and deposition of cholesterol [18]. Quantitative alterations in VLDL are likely to be of particular importance, as VLDL particles contain up to 5 times more lipid than LDL particles, and consequently their uptake by macrophages or smooth muscle cells would lead to deposition of copious quantities of lipid within the artery wall [19]. Many proteins, including apolipoproteins, become glycated in patients with diabetes. Glycation can lead to alterations in the metabolic fate of VLDL particles and may alter their susceptibility to oxidation. Studies have shown that glycated LDL found in rabbits with induced diabetes has an altered metabolism and is removed from the circulation much faster than non-glycated LDL found in control rabbits [20].

We have established methods for the rapid isolation of VLDL and its four subfractions. The latter can be directly related to subfractions isolated by other published methods thus: (i) subfractions A and B correspond to VLDL1; (ii) subfraction C corresponds to VLDL2 and (iii) subfraction D corresponds to VLDL3 [21, 22]. Our rapid method enables identification of quantitative changes within the particles and assessment of susceptibility to (copper-mediated) oxidation. These methods have been applied to VLDL isolated from patients with Type II diabetes who have been compared with healthy control subjects. The aim of this study was to establish if the dyslipidaemia found in Type II diabetes is associated with compositional changes in VLDL that would result in more atherogenic VLDL particles.

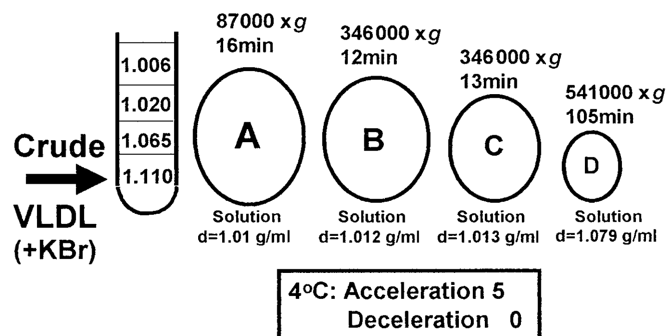

Fig. 1. VLDL subfractionation by sequential ultracentrifugation

Subjects and methods

Subjects. This study was approved by the research ethics committee, Altnagelvin Hospital, Londonderry and informed consent was given by all participants.

Study group. Fifteen patients with uncomplicated Type II diabetes were matched for age and sex with fifteen healthy control subjects. Patients with a clinical history of cardiovascular disease, retinopathy, microalbuminuria or peripheral neuropathy were excluded from the study, as were those on lipid lowering drugs or vitamin supplements.

Plasma isolation. Fasting peripheral venous blood was collected into heparinised tubes (Li Heparin 500 U/10 ml) on ice and centrifuged (Jouan CR 412) at $950 \times g$ for 10 min at 4 °C. Plasma was removed within 30 min from venepuncture and stored frozen at –70 °C in 2 ml aliquots until utilisation.

Isolation and subfractionation of VLDL. To facilitate the subfractionation of VLDL, 'crude' VLDL was isolated from frozen heparinised plasma (–70 °C) by a rapid ultracentrifugation method [23, 24]. Four subfractions of VLDL were then isolated in a Beckman Tabletop Ultracentrifuge, TL100 (Beckman Instruments, Highwycombe, UK) using a fixed angle rotor (TL100.3). This method is a rapid modification of prolonged isolation procedures [25–27]. Very low density lipoprotein was subfractionated by cumulative rate centrifugation to float particles with a diameter greater than 75 nm (Sf > 400, subfraction A); 50–75 nm (Sf 175–400, subfraction B); 37–50 nm (Sf 100–175, subfraction C); 20–37 nm (Sf 20–100, subfraction D). This rapid isolation requires less than 4 h in comparison to 20 h required for the shortest of the prolonged procedures [27]. The ultracentrifugation variables (speed and time) used for the four ultracentrifugations for this rapid method are shown in Figure 1. The density of whole VLDL was adjusted to 1.11 g/ml by the addition of 0.1225 g KBr to 0.875 ml VLDL. This was overlaid with three solutions of decreasing density in the following volumes 0.688, 0.688 and 0.75 ml. On completion of each ultracentrifugation step, 0.75 ml of the isolated subfraction was removed from the top section of the ultracentrifuge tube by careful aspiration. This volume was then replaced by an equal volume of NaCl solution (d = 1.006 g/ml)

prior to the next ultracentrifugation step. Each subfraction was stored on ice until completion of the four ultracentrifugation steps prior to oxidation; this was carried out immediately after the final ultracentrifugation step. The remainder of the samples (subfractions A→D and crude VLDL) were then stored frozen in aliquots for subsequent analyses. To determine the density of the solutions in which each subfraction was isolated, a blank solution containing NaCl (d = 1.006 g/ml) replaced VLDL. Potassium bromide was added to this solution as described in the subfractionation of VLDL, together with the overlaying solutions (Fig. 1). On completion of each ultracentrifugation step the solutions were assessed for density. We found that subfraction A was isolated from a solution of density 1.010 g/ml, subfraction B from a density of 1.012 g/ml, subfraction C from a density of 1.013 g/ml and subfraction D from a density of 1.079 g/ml.

Isolation of LDL. Low density lipoprotein was isolated by a 1-h single spin ultracentrifugation method [28]. Purified LDL was oxidised immediately and the remaining sample was stored frozen in aliquots for subsequent analyses.

Lipoprotein composition

Lipid determination. Total cholesterol, triglyceride and phospholipids from plasma together with subfractions A→D, crude VLDL and LDL were measured using enzymatic assays (Boehringer Mannheim, Lewes, UK) on a Cobas Bio analyser.

Protein determination. The four subfractions of VLDL together with crude VLDL and LDL were analysed for total protein concentration using a commercial kit based on the Coomassie Blue reaction with proteins (Biorad, Hemel Hempstead, UK; 500–006), following the manufacturer's protocol. This was used to standardise samples for the oxidation experiments.

Apo B determination. The concentration of apo B in each VLDL subfraction together with crude VLDL and LDL were determined by single radial immunodiffusion as described [23, 24].

Fatty acid determination. The per cent distribution of fatty acids as monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) in subfractions A→D, crude VLDL and LDL was measured by gas chromatography using a Hewlett Packard GC system (Hewlett Packard, Wilmington, Delaware (DE) USA) and was done as follows:

Lipid extraction. Total lipid was extracted by a method described previously [29]. The following modifications were used: 500 μl of purified lipoprotein were extracted in glass tubes using 6 ml chloroform/methanol (2:1; BDH, Laboratory Supplies, Poole, UK, Analar Grade), containing 0.01 % butylated hydroxy toluene (BHT) for 30 min, with occasional mixing. After which 2 ml calcium chloride (0.2 %) was added, vortexed and then centrifuged at 2000 rev/min for 10 min.

Methyl ester preparation. This was carried out according to a published method [30], with the following modifications: the lower lipid layer was transferred into clean glass tubes and evaporated to dryness under nitrogen. This was then redissolved in 2 ml boron trifluoride methanol (14 % w/v) and incubated at 60 °C for 30 min. After allowing it to cool, 2 ml petroleum spirit (BDH, Analar; boiling point 40–60 °C, low in aromatic hydrocarbons) containing 0.01 % BHT, and 2 ml distilled water were added, mixed thoroughly and allowed to stand until the layers had separated. The top layer was transferred into pre-weighed auto-sampler vials and evaporated to dryness under nitrogen. The vials were re-weighed and the extract redissolved in petroleum spirit containing 0.01 % BHT; 250 μl petroleum spirit/mg extract.

Chromatography. Fatty acids were determined using a Hewlett Packard 6890 gas chromatograph equipped with an HP chemstation computing integrator, an autosampler and a flame ionisation detector. A 2 μl sample was analysed on a BP-20 fused silica capillary column, 25 m × 22 μm internal diameter, film thickness of 0.25 μm, (Scientific Glass Engineering, Milton Keynes, UK). The following gas chromotography conditions were used; helium carrier gas flow rate, 1.5–2.0 ml per min, injector temperature 240 °C, detector temperature 300 °C. The oven was temperature programmed from 70–160 °C at 25 °C per min, then 160–250 °C at 3 °C per min, and isothermal for 20 min. Fatty acid methyl esters were identified according to their retention times compared with known standards (Sigma and Supelchem, Herts, England).

Preformed hydroperoxides. Aqueous phase hydroperoxides in plasma were measured using the FOX I assay [31, 32]. Lipid phase hydroperoxides in isolated lipoproteins were measured by the FOX II assay, as described previously [24].

Susceptibility of isolated lipoproteins to copper mediated oxidation. The routine oxidation of VLDL subfractions was as follows: after each VLDL subfraction was standardised to 10 μg/ml protein with phosphate buffered saline (0.01 mol/l, pH 7.4; PBS), oxidation was initiated by the addition of 10 μmol/l copper II chloride (final concentration). Crude VLDL was oxidised as described previously [23, 24]. In brief, VLDL was standardised to 25 μg/ml protein with PBS and oxidation was initiated by the addition of 17.5 μmol/l copper II chloride (final concentration). Low density lipoprotein was standardised to 50 μg/ml protein with PBS; oxidation was initiated by the addition of 2 μmol/l copper II chloride (final concentration) [28].

The oxidation process was carried out in a thermostatically controlled spectrophotometer at 37 °C (Hitachi U2000, Hitachi, Wickingham, UK), containing an automatic six cell positioner. Conjugated diene production was followed by change in absorbance at 234 nm. The lag time, a measure of the inherent resistance of the particle to copper mediated oxidation, was calculated as the intercept of the initial and rapid phases of the reaction and calculated by a specially written macro on a Microsoft Excel spreadsheet [28].

Urate detection. In previous work we have shown that crude VLDL and LDL isolated by a single spin are contaminated with urate [24], and other studies have shown that urate can greatly alter the susceptibility of lipoproteins to oxidation [33, 34]. The presence of urate was therefore measured in subfractions A→D, crude VLDL and LDL by HPLC with electrochemical detection by a modification of a reported method [35]. The detection limit of this assay was less than 0.5 μmol/l.

Urate was not detected in VLDL subfractions from either the patient or control group. Crude VLDL and LDL samples were contaminated with urate in both groups, which was successfully removed by size exclusion chromatography using PD10 columns containing Sephadex G25 (Amersham Pharmacia Biotech, St. Albans, UK).

Statistical analysis. Groups were compared using the unpaired Student's *t* test or the Mann Whitney U test when data were not normally distributed.

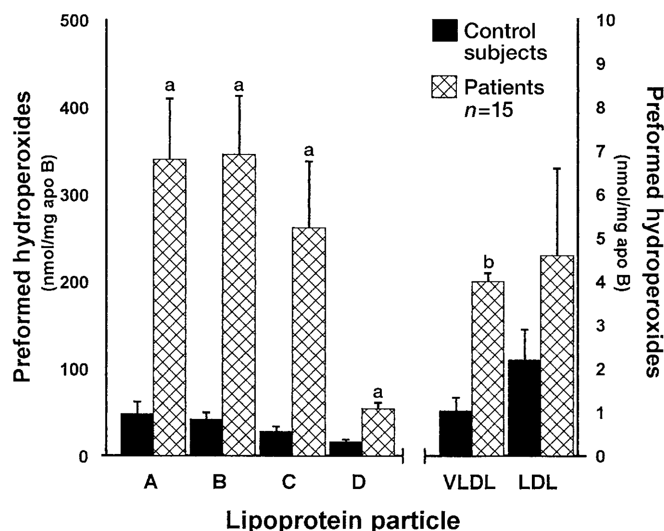

Fig. 2. Distribution of preformed hydroperoxides standardised for apo B found in VLDL subfractions (A→D), crude VLDL and LDL in patients with Type II diabetes compared with matched control subjects. (Data presented as mean ± SD: [a]$p < 0.001$; [b]$p < 0.05$)

Results

Subject profile. Each group (patient and control subject) consisted of 8 men and 7 women. Mean ages of patient and control subjects were 55 ± 7.0 and 52 ± 7.8 years (p = NS), respectively. Patients were all non-smokers but two of the 15 control subjects smoked. Exclusion of the two smokers from the analysis made no difference to the results. The patient and control groups had a mean fasting plasma glucose of 8.9 ± 2.0 mmol/l and 5.1 ± 0.4 mmol/l, respectively ($p < 0.001$), patient HbA_{1c} was 7.6 ± 1.4 %. Treatment for diabetes included a sulphonylurea [3], metformin [2], sulphonylurea and metformin [5], metformin and acarbose [1] and diet alone [4]. There had been no change in patient treatment in the 3 months prior to sampling.

Serum lipids. Serum total triglycerides were higher in the patient group (1.74 ± 0.6 vs 1.40 ± 0.6 mmol/l, $p < 0.05$) and total cholesterol lower (5.02 ± 0.8 vs 5.64 ± 0.8 mmol/l, $p = 0.02$). Phospholipids were not different between the groups (2.62 ± 0.34 vs 2.82 ± 0.5 mmol/l, $p = 0.337$). Low density lipoprotein and HDL cholesterol were also not significantly lower in the patient group (LDL, 3.12 ± 0.5 vs 3.71 ± 0.6 mmol/l, $p = 0.174$; HDL, 1.13 ± 0.2 vs 1.29 ± 0.4 mmol/l, $p = 0.305$). Patients receiving metformin treatment did not have lower triglyceride concentrations than those not receiving metformin [triglyceride concentrations: + metformin ($n = 8$) 1.71 ± 0.7 vs – metformin ($n = 7$) 1.72 ± 0.41, $p = 0.324$].

Preformed hydroperoxides

(i) FOX I. The FOX I assay used to measure preformed hydroperoxides in plasma showed that patient plasma contained higher concentrations of hydroperoxides when compared with controls (1.14 ± 0.21 vs 0.87 ± 0.27 μmol/l, $p = 0.026$).

(ii) FOX II. The FOX II assay measures hydroperoxides in the lipid phase and is used on isolated lipoproteins. The patient group contained higher concentrations of hydroperoxides in subfractions A, B, C and D and crude VLDL. Low density lipoprotein isolated from the patient group had a trend to higher concentrations of hydroperoxides although this did not reach significance (Fig. 2).

Susceptibility of isolated lipoproteins to copper mediated oxidation. Within each group the susceptibility to oxidation increased as the subfractions increased in density, with subfraction A being statistically more resistant to oxidation compared with subfractions B, C and D (Fig. 3). Direct comparison of both groups showed that patient VLDL subfractions were more susceptible to oxidation; however, this only reached significance in subfractions A and D (Fig. 4). Susceptibility to oxidation of crude VLDL was not different between the two groups. As previously reported by other groups, LDL was more susceptible to copper mediated oxidation in diabetic patients (Fig. 4).

Lipoprotein composition

Lipid determination. The distribution of different lipids was found to change across subfractions in both patient and control groups when expressed as a per cent of total lipid (cholesterol + triglyceride + phospholipid; mmol/l) within each subfraction (Table 1). The per cent of cholesterol and of phospholipid increased and the per cent of triglyceride decreased from subfraction A through to subfraction D in both patient and control groups. Overall, therefore, the denser VLDL subfractions were enriched with cholesterol and phospholipids and tended to contain less triglyceride.

Direct comparison of the patient and control subjects showed that in the patients VLDL subfractions A, B and C were more enriched with cholesterol and depleted in triglyceride whereas phospholipid was similar in both groups in all subfractions (Table 1).

Apo B concentration. The patient group contained lower concentrations of apo B in VLDL subfractions B→D. Results expressed as mean ± SD were: subfraction A, 4.3 ± 0.6 vs 7.8 ± 0.7 mg/l; subfraction B, 6.0 ± 0.5 vs 11.3 ± 0.5 mg/l; subfraction C, 9.5 ± 0.9 vs

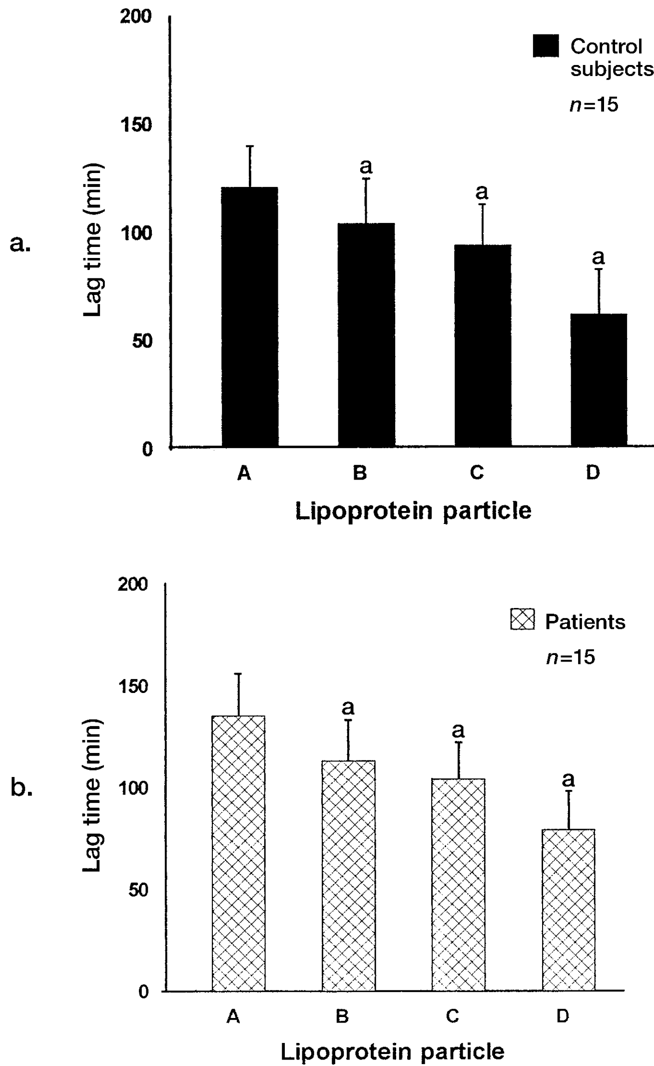

Fig. 3. Change in VLDL subfractions susceptibility to copper mediated oxidation with increasing density (A→D) (**a**) control (**b**) Type II diabetes. (Data presented as mean ± SD: [a]compared with subfraction A; $p < 0.004$)

17.7 ± 0.8 mg/l; subfraction D, 22.7 ± 0.9 vs 32.7 ± 1.2 mg/l, $p < 0.01$. Patient crude VLDL also contained lower concentrations of apo B (49.1 ± 4.6 vs 74.4 ± 7.9 mg/l, $p = 0.01$), while there was no difference in LDL apo B in patients and control subjects (297.3 ± 25 vs 330.3 ± 18 mg/l, $p = 0.305$).

When lipid was standardised for apo B content, there was a significant alteration in composition of patient VLDL subfractions (Fig. 5). Again these results reflect lipid enrichment in patient subfractions A, B and C together with crude VLDL, suggesting patient VLDL synthesised by the liver contains more large lipid rich particles. There was a trend for the lipid to apo B ratio to be less in LDL isolated from the patient group, although this figure did not reach significance (8.9 ± 1.7 vs 9.4 ± 2.3 μmol/mg apo B).

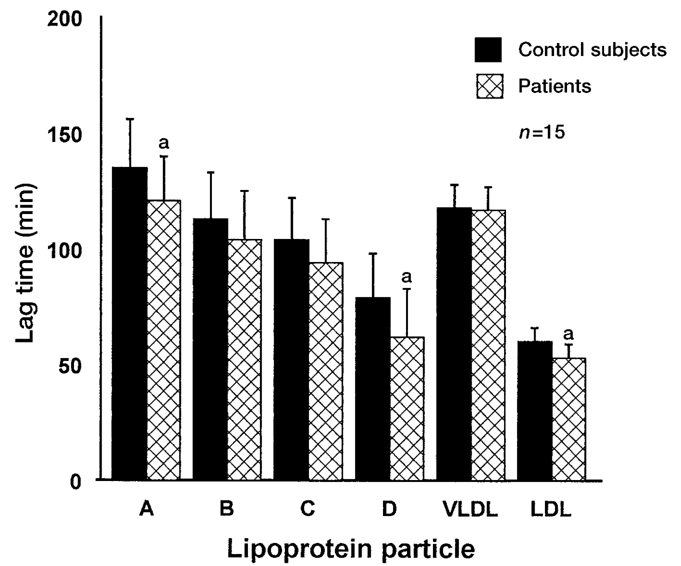

Fig. 4. Susceptibility of VLDL subfractions (A→D), crude VLDL and LDL to copper mediated oxidation in patients with Type II diabetes compared with matched control subjects. (Data presented as mean ± SD: [a]$p < 0.05$)

Fatty acid composition

(i) Between group comparison: analysis of the fatty acid content of VLDL subfractions showed that subfraction D contained higher concentrations of SFA in the patient group, whereas MUFAs were lower in all patient subfractions (A, B, C and D). Patient crude VLDL was higher in SFA, lower in MUFA and similar in PUFA content compared with control VLDL. Patient LDL was similar in PUFA and SFA and lower in MUFA compared with control (Table 2).

(ii) Within group changes (subfractions A→D): patient and control VLDL subfractions showed a different trend in the distribution of fatty acid. As the subfractions became smaller and more dense (A→D) patient SFAs remained unchanged (41.9 ± 2.8 → 40.8 ± 2.9). Monounsaturated fatty acids decreased (35.2 ± 3.0 → 33.0 ± 2.1) and PUFAs increased (22.8 ± 3.0 → 26.1 ± 2.8). Whereas the trend in control VLDL subfractions showed that SFAs decreased (41.5 ± 3.4 → 38.1 ± 2.5), MUFAs remained unchanged (36.7 ± 3.5 → 35.5 ± 2.1) and PUFAs increased (21.9 ± 3.7 → 26.3 ± 2.3) ($p < 0.05$).

Analysis of individual fatty acids within the two groups showed that patient oleic acid decreased as the subfractions increased in density (A→D) but oleic acid remained unchanged in the control group. As the subfractions became smaller and denser, linoleic and arachidonic increased in both groups (Table 3).

Table 1. Lipid composition in isolated lipoproteins; Type II diabetes vs control subjects

		Sub A	Sub B	Sub C	Sub D	VLDL
% Cholesterol[a]	Patients	24.21[b] ± 2.2	27.5[b] ± 1.9	30.8[b] ± 2.7	35.7 ± 2.3	28.9 ± 4.5
(of chol + trigs + PL)	Control subjects	11.98 ± 2.2	17.5 ± 1.5	18.1 ± 1.6	32.1 ± 1.2	28.5 ± 3.0
% Triglyceride[a]	Patients	65.2[c] ± 2.1	57.2[c] ± 6.4	49.8[c] ± 2.9	46.6 ± 2.3	56.3 ± 4.7
(of chol + trigs + PL)	Control subjects	74 ± 3.6	66.5 ± 3.3	59.8 ± 3.1	44.2 ± 2.1	51.9 ± 4.4
% Phospholipid[a]	Patients	10.6 ± 1.9	15.3 ± 1.2	19.4 ± 1.8	17.7 ± 2.6	14.8 ± 1.2
(of chol + trigs + PL)	Control subjects	14 ± 4.1	16 ± 3.6	22.1 ± 3.1	23.7 ± 2.4	19.5 ± 2.0

[a] Lipid distribution, expressed as per cent (mean ± SD) of cholesterol + triglyceride + phospholipid; mmol/l, in each subfraction of VLDL (A → D) and crude VLDL from patients with Type II diabetes compared with their matched control subjects. ($n = 15$ for each group)
[b] $p < 0.001$: [c] $p < 0.05$

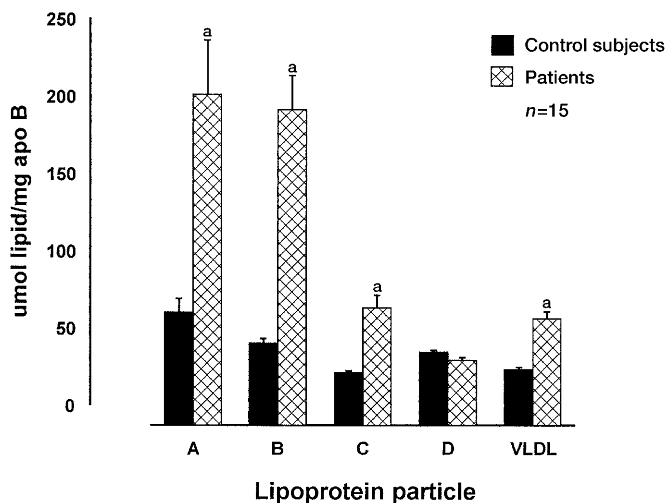

Fig. 5. Distribution of lipid (cholesterol + triglyceride + phospholipid) in VLDL subfractions (A→D) and crude VLDL when standardised for apo B content in patients with Type II diabetes compared with matched control subjects. (Data presented as mean ± SD: [a]$p < 0.05$)

Discussion

In a group of patients with Type II diabetes in whom glycaemic control was good and in whom the total cholesterol and triglyceride concentrations were unremarkable, VLDL subfractions had an altered composition which is likely to make them more atherogenic. Lipid analysis showed that patient VLDL and its subfractions were enriched in both cholesterol and triglyceride. This altered lipid composition was further enhanced when each particle was standardised for apo B content. This study indicates that patients with Type II diabetes have much larger lipid rich VLDL particles than control subjects. Studies have shown that a conformational change occurs in the apo B moiety of large VLDL particles, resulting in a decreased affinity for native receptors and an increased residence time within the circulation.

As expected, total triglyceride concentrations were higher in patients than control subjects. Fatty acid distribution changed in both the patient and control groups as VLDL subfractions became smaller and more dense (A→D). Patient subfractions decreased in MUFA and increased in PUFA whereas SFA remained constant. In control subfractions the MUFA remained constant, PUFA increased and SFA decreased. When these fatty acids were directly compared between the two groups, patient MUFAs were lower in all four subfractions, but SFA was higher in patient subfraction D. Monounsaturated fatty acids are more resistant to oxidative modification [36]. These alterations in fatty acid composition will tend to increase the susceptibility of patient VLDL to oxidation in comparison with control VLDL, consistent with the oxidation profile found in this study. Comparison of fatty acids in LDL gave a similar trend to crude VLDL in that patient LDL had lower concentrations of MUFA, similar concentrations of PUFA and increased concentrations of SFA.

The hydroperoxide results, both in the aqueous phase (plasma) and in the lipid phase (VLDL) show increased in vivo peroxidation in the patient group, indicating that patients with Type II diabetes are under increased oxidative stress. One of the major inhibitors of the peroxidation process in vivo is ascorbic acid. Although ascorbic acid was not measured in this study, we and others have shown that patients with Type II diabetes have decreased concentrations compared with control subjects [37, 38]. Ascorbate is a key antioxidant, particularly protecting lipids from peroxidative damage by aqueous oxidants. Studies have shown that in the presence of ascorbate depletion lipid hydroperoxides are

Table 2. Fatty acid composition in isolated lipoproteins; Type II diabetes vs control subjects

		Sub A	Sub B	Sub C	Sub D	VLDL	LDL
SFA	Patients	41.9 ± 2.8	39.6 ± 2.9	40.7 ± 2.6	40.8[a] ± 2.9	37.9[a] ± 3.1	33.6 ± 2.1
	Control subjects	41.5 ± 3.4	37.5 ± 2.9	38.8 ± 2.9	38.1 ± 2.5	35.7 ± 3.1	32.7 ± 3.3
MUFA	Patients	35.2[a] ± 3.0	35.1[a] ± 2.7	34.4[a] ± 2.2	33.0[a] ± 2.1	35.5[a] ± 2.5	22.9[a] ± 3.3
	Control subjects	36.7 ± 3.5	38.7 ± 1.8	36.5 ± 3.8	35.5 ± 2.1	37.9 ± 5.0	26.7 ± 2.2
PUFA	Patients	22.8 ± 3.0	25.1 ± 3.1	24.9 ± 2.8	26.1 ± 2.8	26.6 ± 3.5	43.6 ± 3.2
	Control subjects	21.9 ± 3.7	23.3 ± 3.6	23.8 ± 3.1	26.3 ± 2.3	26.5 ± 3.7	41.3 ± 7.2

[a] $p < 0.05$ compared with control subjects
Data presented as per cent (mean ± SD) total fatty acid in each subfraction, VLDL and LDL

Table 3. Oleic, linoleic and arachidonic acid content in isolated lipoproteins; Type II diabetic patients and control subjects

	Fatty acid	Sub A	Sub B	Sub C	Sub D	VLDL	LDL
Type II diabetic patients	Oleic	32.0 ± 2.7	32.1 ± 2.6	31.2 ± 2.1	30.0[a] ± 2.1	32.0 ± 2.9	20.4 ± 2.0
	Linoleic	16.2 ± 2.9	17.9 ± 2.7	17.5 ± 2.8	18.3[a] ± 2.8	19.2 ± 3.3	31.6 ± 3.9
	Arachidonic	1.8 ± 0.4	2.2 ± 0.4	2.2 ± 0.5	2.5[a] ± 0.5	2.7 ± 0.4	5.9 ± 1.0
Control subjects	Oleic	32.3 ± 2.8	34.4 ± 2.2	33.0 ± 2.8	31.7 ± 2.5	33.0 ± 2.6	21.6 ± 2.2
	Linoleic	14.6 ± 3.4	16.7 ± 3.2	16.5 ± 3.5	18.1[a] ± 2.9	18.8 ± 3.7	31.3 ± 6.3
	Arachidonic	1.6 ± 0.4	1.8 ± 0.5	1.9 ± 0.5	2.4[a] ± 0.5	2.5 ± 0.6	5.6 ± 1.1

[a] $p < 0.004$
Data presented as per cent (mean ± SD) of individual fatty acids in each subfraction, VLDL and LDL.
(p = subfraction A vs B/C/D within each group)

formed, even when other antioxidants are still present [33].

This study has shown for the first time that as VLDL subfractions decreased in size and increased in density (A→D), there was a significant trend for the particles to become more susceptible to copper mediated oxidation. A similar trend has previously been reported for LDL subfractions [39, 40]. Comparison of the patient and control group indicates that all four VLDL subfractions from the patient group were more readily oxidised. As in vitro oxidation is a multifaceted process, it can be influenced by many factors such as lipid and antioxidant content of the purified lipoprotein particle. The increased susceptibility of subfractions A and D to oxidation compared with subfractions C and B may be explained in part by differences found in patient fatty acid content. Subfraction A contained significantly more linoleic acid whereas subfraction D contained significantly less oleic acid. Both of these fatty acids influence the oxidation process in different ways, with linoleic acid being a molecule more readily oxidised and oleic acid being a molecule less readily oxidised [41]. Therefore, these changes in subfractions A and D would enhance the already present oxidative burden due to the increased concentrations of preformed hydroperoxides. Although only subfractions A and D were statistically more susceptible to in vitro oxidation, a trend of increased susceptibility to oxidation was found in all four subfractions of VLDL.

The changes in VLDL composition that are described here will contribute to the development of atherosclerosis in two main ways. Firstly, as a consequence of the altered metabolic cascade described above, the increased preponderance of large triglyceride-rich VLDL species will result in the production of small, dense LDL and small, lipid poor HDL [14, 15]. Secondly, demonstrated here, VLDL in Type II diabetes carries an increased burden of lipid hydroperoxides and shows increased susceptibility to oxidation. Both of these changes will result in increased delivery of oxidised lipids to the arterial wall. This will be accentuated in the presence of the increased endothelial permeability characteristic of Type II diabetes [42], which will increase the penetration of VLDL into the arterial intima. These changes in

VLDL will therefore operate to promote the development of atherosclerosis in diabetes.

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