Biochemical and Biophysical Research Communications **292,** 175–183 (2002)
doi:10.1006/bbrc.2002.6616, available online at http://www.idealibrary.com on IDEAL®

Smoking Has No Effect on the Amino Acid Composition of Apolipoprotein B100 of LDL While Directly Influencing the Antioxidant Status

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Received February 13, 2002

Previous studies have demonstrated increased plasma levels of oxidised low-density lipoprotein (oxLDL) in chronic smokers, which has been associated with the extent of endothelial dysfunction. In this study we examine the relationship between the amino acid composition of apolipoprotein B100 (apo B) of low-density lipoprotein (LDL), by reverse phase HPLC after precolumn derivatisation, between smokers (≥40 cigarettes/day) and nonsmokers in relation to their plasma and LDL antioxidant status. While there was a significant difference in the levels of plasma vitamin C and α-tocopherol between female smokers and nonsmokers, as well as in the levels of LDL α-tocopherol, there was no significant difference in the amino acid composition of apo B between the two groups.

Key Words: **smoking; vitamin C; α-tocopherol; amino acid analysis; protein hydrolysis; precolumn derivatisation; *o*-phthalaldehyde; apolipoprotein B100.**

There is increasing evidence that increased concentrations of low-density lipoprotein (LDL) and biologically modified lipoproteins, such as oxidised low-density lipoprotein (oxLDL), are associated in the pathogenesis of atherosclerosis [1–6]. Apolipoprotein B 100 (apo B) containing 4536 amino acid residues [7] is the major protein moiety of LDL. The altered recognition properties of the oxidised LDL particle, induced by the modification of specific amino acid residues on the apo B, are considered to be responsible for uptake of oxLDL by scavenger receptors on target macrophages, a process involved in the pathogenesis of the disease [1, 2].

Supplementation with antioxidant nutrients (vitamin E, vitamin C, carotenoids) as well as increased fruit and vegetable intake have been demonstrated to protect LDL from oxidation, when examined *ex vivo,* in nonsmokers, smokers and subjects exposed to passive smoking [8–16]. It has been reported that smoking is associated with elevations in plasma LDL levels and a decrease in plasma high-density lipoprotein cholesterol levels [17]. Other indications suggest that smoking cessation leads to elevated levels of HDL cholesterol [18].

It has been proposed that modifications to amino acids such as lysine, which alter the charge and the recognition properties of apo B, increase its recognition and uptake by the macrophage scavenger receptors [2]. To determine the structure of apo B many techniques such as sequencing via mRNA and cDNA [19] and more conventional methods of tryptic digestion [20–22] and cyanogen bromide [23] cleavage have been utilised. However, because of the relative insolubility of the protein and difficulty in separating the complex peptide fractions generated from such cleavage, estimation of the accurate composition of the protein has often been difficult.

In this study we apply an HPLC analytical approach for the determination of the protein composition of LDL-apo B, by quantitative analysis of the amino acids in protein hydrolysates as their *o*-phthalaldehyde/2-mercaptoethanol derivatives, and the influence of smoking (≥40 cigarettes/day) on such parameters. The results are compared with the modifications to the amino acid composition after copper-mediated oxidation of LDL, conventionally used to study LDL oxidisability and uptake. The method described here for amino acid analysis has been standardised through

Abbreviations used: LDL, low-density lipoprotein; OPA, *o*-phthalaldehyde; RP-HPLC, reversed-phase high-performance liquid chromatography; apo B, apolipoprotein B100.

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0006-291X/02 $35.00

the analysis of LDL protein hydrolysate from 33 healthy nonsmoking volunteers and 17 healthy smoking volunteers.

MATERIALS AND METHODS

Chemicals. Sodium dihydrogen orthophosphate dihydrate, disodium hydrogen orthophosphate dihydrate, potassium hydroxide, hydrochloric acid were obtained from Merck (Poole, Dorset, UK). Sodium acetate trihydrate, EDTA, standard amino acid solution, DL-β-amino butyric acid, *o*-phthalaldehyde, 2-mercaptoethanol, *o*-phenylenediamine, iodine solution, α-tocopherol, γ-tocopherol, δ-tocopherol and EDTA were purchased from Sigma-Aldrich Chemical Company (Poole, Dorset, UK). Rathburn Chemicals Ltd. (Walkerburn, Scotland) supplied the HPLC grade acetonitrile, tetrahydrofuran and methanol. All other reagents used were of analytical grade. All the reagents were prepared using 18 MΩ deionised water (Waters Milli-Q system).

Subjects. The present study was approved by the Guy's Research Ethics Committee (Code: 98/07/06). All subjects signed a statement of informed consent. The subjects were divided into two categories. The nonsmoking population consisted of 33 volunteers (males: n = 13, mean age = 32, BMI = 24; females: n = 20, mean age = 31, BMI = 23) and the smoking population (minimum of 40 cigarettes/day) consisted of 17 volunteers (males: n = 6, mean age = 37, BMI = 28; females: n = 11, mean age = 31, BMI = 24) and these volunteers were not on any food supplements or medication. To establish whether the self-reporting smoking levels of smokers was accurate at more than 40 cigarettes/day, plasma cotinine (a metabolite of nicotine) levels were measured using a standard protocol.

Isolation of LDL. Blood was withdrawn by venepuncture and plasma separated by centrifugation at 1000*g*. The separated plasma is analysed for vitamin E and vitamin C; LDL was separated from the remaining plasma. A density gradient ultracentrifugation method [24] a modification of the method described by Chung *et al.* [25], was utilised to isolate LDL from the plasma using a Beckman L-70 centrifuge with a fixed angle rotor (Ti-70) working at 150,000*g*. Protein concentration of the different LDLs was estimated using a modified Lowry method with bovine serum albumin as the standard [26]. LDL vitamin E concentration and total plasma lipid profile were also determined.

Estimation of plasma vitamin C concentration. Isolated plasma was immediately treated with four volumes of 0.3 M trichloroacetic acid. The deproteinised plasma was separated by centrifugation and stored at -70°C until vitamin C determinations were carried out. Total ascorbic acid levels were determined fluorimetrically using a modified fluorimetric analysis [27]. Briefly, ascorbic acid was oxidised to dehydroascorbic acid with iodine, followed by condensation with *o*-phenylenediamine (OPD) to form a fluorescent quinoxaline which was measured at an excitation wavelength of 348 nm, emission at 423 nm.

Estimation of plasma and LDL vitamin E concentration. Estimation of plasma and LDL vitamin E (both α- and γ-tocopherols) was carried out by RP-HPLC [28]. In brief, plasma and LDL samples were subjected to protein precipitation followed by the extraction of tocopherols with hexane. The hexane layer was transferred; evaporated to dryness under a stream of nitrogen and the tocopherols reconstituted using acetone. The samples were then analysed by RP-HPLC and the tocopherols were detected by fluorescence (excitation: 280 nm; emission: 340 nm).

Estimation of total plasma lipid profile. Total plasma cholesterol and triglycerides were measured according to the methods of Warnick [29]. HDL cholesterol was measured directly by precipitation with phosphotungstate [30] and LDL cholesterol was calculated by the Friedewald formula [31].

Hydrolysis of apolipoprotein B100. The amino acid composition of apo B was determined by HPLC after protein hydrolysis. LDL (0.125 mg of protein/ml) was treated with an equal volume of methanol to precipitate the protein and the samples centrifuged at 1000*g*. The supernatant was removed and the precipitated protein dissolved in 1 ml of 6N hydrochloric acid. This was followed by the addition of DL-β-amino butyric acid (50 μl of 1 mM solution) containing 25 μM phenol and 50 μl of Trolox (2.5 mM) to prevent any oxidation of amino acids from taking place during hydrolysis. The samples were transferred into vials and sealed and the solutions frozen at -70°C and subsequently evacuated using a high-pressure pump to remove all the dissolved oxygen. The samples were subjected to hydrolysis at 110°C for 24 h. After hydrolysis the vials were carefully opened and the pH of the samples adjusted exactly to 9 using sodium hydroxide solution. This was followed by HPLC analysis. All samples were analysed in triplicate.

HPLC analysis of protein hydrolysate. The protein hydrolysates were analysed by HPLC by precolumn derivatisation using *o*-phthalaldehyde (10 mg dissolved in 0.1 ml methanol and made up to 1 ml with pH 9 borate buffer, 50 mM) and 2-mercaptoethanol (10% in pH 9 borate buffer, 50 mM). 10 μl each of the borate buffer (50 mM, pH 9), *o*-phthalaldehyde and 2-mercaptoethanol and 50 μl of the sample were drawn into the HPLC syringe needle mixed in the loop and injected directly on to the column within 30 sec. The amino acids form a fluorescent compound, which increases the sensitivity of the assay. A procedure that has been developed for derivatisation of amino acids using OPA and FMOC has been suitably modified for the amino acid analysis [32]. The analytical conditions used were: Novopak C_{18} column 250 mm $\times$ 4.6 mm i.d. (column temp. 25°C) (Waters Corp., Milford, MA) and the mobile phase consisted of: eluent A, sodium acetate trihydrate (30 mM) and 1 mM EDTA, pH 7.2 and 0.25% tetrahydrofuran in 18.2 MΩ water and eluent B, sodium acetate trihydrate (20 mM) and 1 mM EDTA, pH 7.2 in 80% acetonitrile in 18.2 MΩ water. A gradient system (min/%B: 0/5; 15/15; 20/18; 30/20; 50/45; 55/55; 56/5; 60/5) was used for eluting the samples. The derivatised amino acids were detected by fluorescence: excitation wavelength 340 nm and emission wavelength 450 nm at 110 Hz for better signal to noise ratio and with a 375-nm cutoff filter.

Calibration of amino acids. The amount of the amino acids present in the protein hydrolysate was determined from calibration plots constructed using a standard solution of amino acids obtained from Sigma-Aldrich Chemical Company. DL-β-Amino butyric acid (50 μM) was used as the internal standard. Calibration plots were constructed over the range of 0–10 μM (low calibration) and 0–100 μM (high calibration). Known concentrations of the amino acids standard were spiked in pH 9 borate buffer, 0.05 M to which was added 50 μl of internal standard. Peak area ratios (PAR) of each amino acid:internal standard were calculated and plotted against the spiked concentration of the amino acid. The assay developed for the quantification of amino acids was found to be precise and accurate over the calibration range of 0–100 μM with CV% and M%D less than 5%. The minimum quantifiable limit of all amino acids is less than 1 μM. Similar results were obtained on the amounts of amino acids detected whether calibration samples were taken through the whole hydrolysis procedure akin to the preparation of the test samples or whether the spiked samples were directly analysed.

Amino acid concentration calculated for each subject LDL is converted into Mol% of amino acid based on the total amino acid composition (μM) of that subject's apo B.

Copper-mediated oxidation of low-density lipoprotein. Copper-mediated oxidation of low-density lipoprotein was carried out and the amino acid composition of apo B was estimated by HPLC. Copper sulphate (3 μM final concentration) was added to a solution containing LDL (0.125 mg protein/ml) in 20 mM phosphate buffer pH 7 and incubated for 3 h at 37°C. The reaction was terminated by the addition of methanol. Hydrochloric acid (6 M) was added, followed by

TABLE 1

Plasma Antioxidant Composition of the Two Groups of Volunteers with Relation to the Plasma Cholesterol Concentration

	n	Plasma vitamin C μM	Plasma α-tocopherol μM	Plasma γ-tocopherol μM	Plasma α-tocopherol:cholesterol ratio
Total					
Nonsmokers	33	69.6 ± 18.6	24.16 ± 6.33	1.38 ± 0.77	0.00736 ± 0.00154
Smokers	17	52.0 ± 20.6	20.06 ± 7.42	1.11 ± 0.92	0.00631 ± 0.00147
		$P \leq 0.005$	$P \leq 0.05$	N.S.	N.S.
Male					
Nonsmokers	13	63.5 ± 21.7	24.46 ± 6.23	1.30 ± 0.66	0.00746 ± 0.00113
Smokers	6	56.2 ± 20.3	22.41 ± 10.42	1.34 ± 0.42	0.00679 ± 0.00227
		N.S.	N.S.	N.S.	N.S.
Female					
Nonsmokers	20	74.1 ± 15.1	23.94 ± 6.56	1.44 ± 0.86	0.00728 ± 0.00181
Smokers	11	49.7 ± 21.4	18.78 ± 5.36	0.99 ± 1.10	0.00604 ± 0.00080
		$P \leq 0.001$	$P \leq 0.05$	N.S.	N.S.

Note. Results are expressed as means ± standard deviation.

DL-β-amino butyric acid and phenol as described above. Protein hydrolysis and HPLC analysis of the amino acids is carried out as described above. To compare the extent of protein modification with that of lipid peroxidation, LDL oxidation was followed by monitoring the increased formation of conjugated dienes at 234 nm as described previously [33]. The concentration of conjugated dienes were calculated using the extinction coefficient of 2.8×10^4 M^{-1} cm^{-1} [34].

RESULTS

Plasma Vitamin C Concentration in Nonsmokers and Smokers

Plasma vitamin C levels were measured according to the standard protocol. Data summarised in Table 1 show that there is a significant difference ($P \leq 0.005$) in plasma vitamin C concentration between nonsmokers (69.6 ± 18.6 μM) and smokers (52.0 ± 20.6 μM). While there was no significant difference in the male population between nonsmokers (63.5 ± 21.7 μM) and smokers (56.2 ± 20.3 μM), there was a significant difference ($P \leq 0.001$) in the vitamin C concentration in the female population of the two groups (nonsmokers: 74.1 ± 15.1 and smokers: 49.7 ± 21.4). It was observed that there was no significant correlation between plasma vitamin C and cotinine levels, in smokers.

Plasma Vitamin E Concentration and Complete Lipid Profile

Plasma α- and γ-tocopherols were measured in both the groups of volunteers by RP-HPLC (Table 1). It was observed that there was a significant difference ($P \leq 0.05$) in the levels of α-tocopherol in the two groups of volunteers (nonsmokers: 24.16 ± 6.33 μM and smokers: 20.06 ± 7.42 μM). While there was no significant difference in plasma α-tocopherol concentration in the male population of the two groups, there was a significant difference in the female population (nonsmokers: 23.94 ± 6.56 and smokers: 20.06 ± 7.42 μM). The total plasma lipid profile is given Table 2. The values observed are consistent with the reported literature val-

TABLE 2

Total Plasma Lipid Profiles of Nonsmokers and Smokers

	n	Total cholesterol mM	Triglyceride mM	HDL cholesterol mM	LDL cholesterol mM
Total					
Nonsmokers	33	3.29 ± 0.61	0.98 ± 0.42	0.99 ± 0.21	2.74 ± 0.68
Smokers	17	3.19 ± 0.89	1.13 ± 0.92	0.94 ± 0.24	2.76 ± 1.27
		N.S.	N.S.	N.S.	N.S.
Male					
Nonsmokers	13	3.31 ± 0.63	0.86 ± 0.21	1.04 ± 0.24	2.66 ± 0.61
Smokers	6	3.26 ± 0.6	1.14 ± 0.56	0.92 ± 0.16	2.86 ± 0.77
		N.S.	N.S.	N.S.	N.S.
Female					
Nonsmokers	20	3.16 ± 1.02	0.87 ± 0.53	1.03 ± 0.23	2.52 ± 1.29
Smokers	11	3.23 ± 0.67	1.61 ± 1.30	0.78 ± 0.14	3.19 ± 1.21
		N.S.	N.S.	$P \leq 0.005$	N.S.

Note. Results are expressed as means ± standard deviation.

TABLE 3

Comparison of LDL α-Tocopherol Concentration in Nonsmokers and Smokers with Relation to LDL Cholesterol

	n	LDL α-tocopherol (nmoles/mg LDL protein)	LDL α-tocopherol:cholesterol ratio
Total			
Nonsmokers	33	12.9 ± 2.5	0.00401 ± 0.00095
Smokers	17	10.2 ± 1.9	0.00343 ± 0.00096
		$P \leq 0.0005$	$P \leq 0.05$
Male			
Nonsmokers	13	12.7 ± 2.4	0.00387 ± 0.00082
Smokers	6	11.2 ± 1.6	0.00354 ± 0.00063
		N.S.	N.S.
Female			
Nonsmokers	20	13.0 ± 2.6	0.00412 ± 0.00105
Smokers	11	9.7 ± 1.9	0.00336 ± 0.00112
		$P \leq 0.001$	$P = 0.07$

Note. Results are expressed as means ± standard deviation.

ues [13, 16, 35–37]. However, there was no significant difference between the two groups in their lipid profile. It was also observed that there was no significant difference between the two groups in γ-tocopherol concentration. The observed values were consistent with the previously reported values [28]. Tocopherol concentration was corrected for total plasma cholesterol concentration. The plasma α-tocopherol:cholesterol ratios are given in Table 1. It was observed that correction of α-tocopherol levels for plasma cholesterol concentration resulted in the loss of any significant difference between the nonsmoking and smoking groups and also between the female populations of both the groups.

LDL α-Tocopherol Concentration

α-Tocopherol is one of the main defences for LDL against oxidative stress. LDL α-tocopherol concentration was measured by HPLC (Table 3). The mean LDL α-tocopherol concentration in non-smokers (12.9 ± 2.5 nmoles/mg LDL protein) was significantly different ($P \leq 0.0005$) from the smoking population (10.2 ± 1.9 nmoles/mg LDL protein). While there was no significant difference in LDL α-tocopherol concentration in the male population of the two groups, there was a very significant variation ($P \leq 0.001$) in the female population of the nonsmoking (13 ± 2.6 nmoles/mg LDL protein) and smoking (9.7 ± 1.9 nmoles/mg LDL protein) volunteers. It was observed that after correction of LDL α-tocopherol concentration to the LDL cholesterol concentration, a significant difference ($P \leq 0.05$) still existed. While there was no significant difference between the male population, there was a significant difference in the LDL α-tocopherol: cholesterol ratio between the female population of both groups.

Apolipoprotein B – Amino Acid Analysis

Amino acids interact with *o*-phthalaldehyde and 2-mercaptoethanol to form a fluorescent derivative, which is very sensitive and stable at pH 7.2 during the analysis [38]. Satisfactory separation was obtained for the amino acids with baseline resolution. Most of the amino acids gave a good and consistent response at the detection limits. The derivatising reagents OPA and 2-mercaptoethanol were stable for relatively prolonged periods of time in the autosampler as they were prepared separately in pH 9 borate buffer containing 10% methanol. In contrast, in the procedure described by Lewisch and Levine [38] and Fekkes *et al.* [39] premixing of these two reagents led to the formation of an intermediate, which was found to be unstable with reduced sensitivity in derivatising the amino acids. The approach described here involves the formation of the OPA/2-mercaptoethanol intermediate just before mixing with the protein hydrolysate, which improved the sensitivity and selectivity of amino acid quantification and also minimised the breakdown of the derivatising intermediate.

Amino acid analysis was performed on the hydrolysed apo B sample. The chromatogram of apo B (Fig. 1) confirms well-resolved peaks of all the quantifiable amino acids. The concentration of the amino acids was calculated from the calibration plots. Linear behaviour with correlation coefficients ≥0.995 were obtained. The concentration of each amino acid is expressed as mol% of amino acid when compared to the total sum of amino acids detected per sample. The results obtained are consistent between different individuals with low intra-individual variation in the main (Table 4). The data that were obtained from the individuals in each category were pooled and the mean mol% of the amino acids was calculated. It was observed that there was no significant difference in the individual amino acid concentration between the two main groups and in the male and female sub-groups.

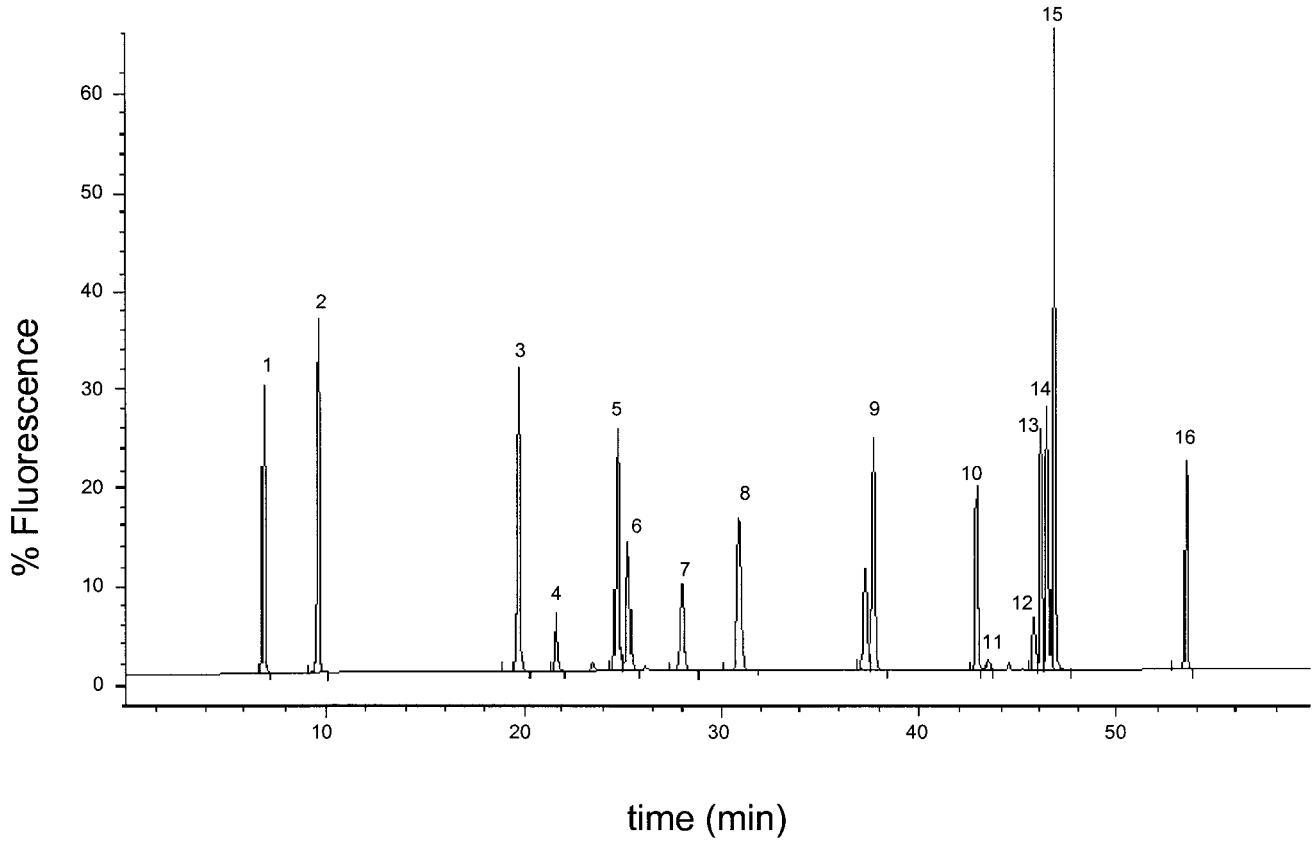

FIG. 1. Amino acid analysis of hydrolysed apolipoprotein B100. Chromatogram shows the baseline resolution of the amino acids. 1: asp, rt. 6.9 min; 2: glu, rt. 9.6; 3: ser, rt. 19.7; 4: his, rt. 21.6; 5: gly, rt. 24.7; 6: thr, rt. 25.3; 7: arg, rt. 27.9; 8: ala, rt. 30.9; 9: tyr, rt. 37.7; 10: val, rt. 42.9; 11: met, rt. 43.2; 12: trp, rt. 45.8; 13: ile, rt. 46.1; 14: phe, rt. 46.5; 15: leu, rt. 46.8; 16: lys, 53.4.

LDL subjected to copper-mediated oxidative stress undergoes the depletion of its inherent antioxidant defences followed by the modification of the lipid and protein moieties [33]. Subjecting LDL (0.125 mg LDL protein/ml) to 3 μM copper-mediated oxidative stress for 3 h resulted in extensive modification to the lipid and protein moieties as estimated by the formation of conjugated dienes and amino acid composition of the apo B, respectively. The level of conjugated diene formation was 305.6 $\pm$ 43 μmoles/mg LDL protein. The degree of modification to the individual amino acid residues of LDL-apo B is given in Fig. 2. It can be observed that the protein moiety has undergone considerable modification to all the amino acids with no specific selectivity to any one class of amino acid. The extent of modification of the amino acids was up to 80%, with lysine being marginally higher than the rest of the amino acids.

DISCUSSION

Cigarette smoking is a risk factor for cardiovascular diseases [40, 41]. Smoking is also associated with decreased levels of plasma antioxidant nutrients such as ascorbic acid, vitamin E, β-carotene and other carotenoids, ubiquinone and uric acid [14, 42–44]. The aim of this study was to focus on the changes to the apo B fraction of LDL in relation to the plasma and LDL antioxidants.

No significant difference was detected in the amino acid concentration between the nonsmoking and smoking groups (minimum 40 cigarettes/day) and between the sub-groups of males and females. Amino acid concentration (all the 50 volunteers data pooled) of the LDL-apo B by amino acid analysis is compared with the published cDNA and mRNA sequencing data of apo B (Table 5) [7, 19, 33, 45]. To calculate the mol% of amino acids by cDNA analysis, only those amino acids that were quantified in this analysis have been used. Values for aspartic acid and asparagine are pooled as are glutamic acid and glutamine and their mol% was calculated as asx and glx. The comparison reveals good consistency between the HPLC method and the results derived from protein sequencing based on cDNA sequencing procedure [19] for most of the amino acids. However, the HPLC method for amino acid analysis gives a range of 2.47 to 7.91 for lysine over the 50 individual LDL donors, although the mean is lower

TABLE 4

Mol% of Amino Acid Concentrations in Apolipoprotein B100 in Smokers and Nonsmokers after Acid Hydrolysis of the Protein

	Nonsmokers		Smokers	
Amino acid	Male (n = 13)	Female (n = 20)	Male (n = 6)	Female (n = 11)
Asx	15.5 ± 1.6	15.4 ± 1.4	14.5 ± 0.9	15.9 ± 1.3
Glx	15.1 ± 1.0	15.7 ± 1.2	16.0 ± 0.3	16.0 ± 0.4
Ser	8.9 ± 0.5	8.8 ± 0.6	7.6 ± 0.3	8.5 ± 0.7
His	2.3 ± 0.6	2.3 ± 0.4	2.6 ± 0.5	2.2 ± 0.4
Gly	4.6 ± 1.9	4.7 ± 3.4	4.5 ± 0.6	3.8 ± 0.6
Thr	6.6 ± 0.6	6.4 ± 0.5	6.3 ± 0.4	6.6 ± 0.5
Arg	1.9 ± 0.1	2.0 ± 0.2	2.2 ± 0.1	2.0 ± 0.2
Ala	7.3 ± 0.8	7.4 ± 0.8	6.7 ± 0.7	7.5 ± 0.6
Tyr	2.9 ± 0.1	2.9 ± 0.1	3.1 ± 0.1	3.0 ± 0.1
Val	5.1 ± 0.7	5.2 ± 0.6	5.4 ± 0.4	5.4 ± 0.4
Met	0	0	0	0
Trp	1.8 ± 0.8	1.5 ± 0.9	1.2 ± 0.3	1.2 ± 0.7
Ile	5.5 ± 0.6	5.4 ± 0.6	5.8 ± 0.4	5.6 ± 0.5
Phe	4.8 ± 0.8	4.6 ± 0.5	5.3 ± 0.5	4.6 ± 0.5
Leu	12.7 ± 0.6	12.8 ± 0.7	12.8 ± 0.5	12.9 ± 0.3
Lys	4.9 ± 1.6	4.7 ± 1.5	6.1 ± 1.4	4.6 ± 1.3

Note. Results are expressed as means ± standard deviation.

than that for the cDNA sequencing procedure, as is also the case for arginine (range 1.61 to 2.45).

Oxidation of the LDL particle in the presence of copper (II) has been shown to go through various phases. The initial lag phase is reported to consist of the loss of antioxidants with no structural variation of apo B [33, 46]. During the propagation phase, which corresponds to the formation of lipid hydroperoxides, changes in the secondary structure of the protein's α-helix has been reported [46]. During the aldehyde formation, which corresponds to the termination phase, extensive changes to the α-helix and β-sheet of the apo B resulting in structural changes have been observed using infrared spectroscopy [46]. Amino acid analysis of the apo B sample has shown that there is considerable modification to all the amino acids with no specific selectivity to any one class of compounds. It is possible that depletion/modification of the apo B amino acids occur by protein fragmentation via the mediation of lipid peroxyl radicals as a result of the formation of LDL aggregates with the onset of oxidation [47]. However, it has been suggested that during metal ion-catalysed oxidation of LDL, protein and lipid oxidation are concurrent and may take place even while much vitamin E remains in the particles [Reviewed in 48, 49].

The findings also reveal that smokers had significantly lower levels of plasma and LDL antioxidants compared with nonsmokers. While plasma vitamin C levels were comparatively lower in smokers compared to the nonsmoking population, the observation was very significant in the female population between the groups. LDL and plasma α-tocopherol concentration in the smoking population was lower compared to the nonsmoking population. There was also a considerable difference in the LDL α-tocopherol:cholesterol ratios in the nonsmoking and smoking female population. It was observed that there was no correlation between

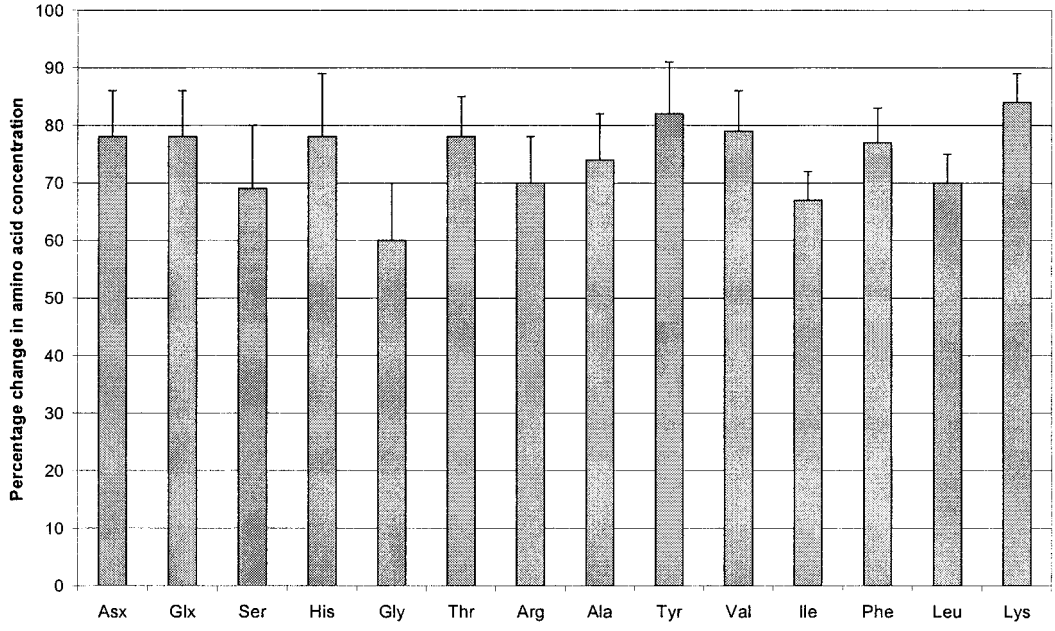

FIG. 2. Copper-mediated modification of LDL. Percentage change in amino acid composition. Methionine and tryptophan were not detected in the samples.

TABLE 5

Amino Acid Composition of Apolipoprotein B100

Amino acid	Mol% $n = 50$ Protein hydrolysis	Mol% Source:cDNA [19]	Mol% Source:cDNA [7]	Number of residues according to cDNA analysis [19, 33]
Asx	15.46 ± 1.43	11.00	11.01	480
Glx	15.63 ± 0.97	12.20	12.18	528
Ser	8.60 ± 0.69	9.05	9.03	392
His	2.33 ± 0.46	2.60	2.65	115
Gly	4.44 ± 2.31	4.80	4.77	207
Thr	6.49 ± 0.48	6.90	6.86	300
Arg	2.00 ± 0.18	3.40	3.41	150
Ala	7.32 ± 0.76	6.10	6.15	275
Tyr	2.94 ± 0.13	3.50	3.50	151
Val	5.25 ± 0.53	5.80	5.78	252
Met	0.00	1.80	1.80	79
Trp	1.49 ± 0.79	0.90	0.85	37
Ile	5.53 ± 0.55	6.60	6.63	285
Phe	4.76 ± 0.60	5.10	5.16	224
Leu	12.81 ± 0.56	12.00	12.04	535
Lys	4.92 ± 1.50	8.20	8.20	357

Note. The data expressed are the mean of all the 50 volunteers (nonsmokers and smokers). Results are expressed as means ± standard deviation. Amino acid compositions are expressed as mol% of the sum of all amino acids except proline (171 residues) and cysteine (25), which we did not measure. Asx represents aspartic acid (233) + asparagine (247) while Glx represents glutamic acid (298) + glutamine (230). Methionine (79) was not detected in any of the LDL samples that we measured.

plasma antioxidant and cotinine concentrations. The results were found to be consistent with the previously published studies [42–44, 50]. In a study involving 10 smokers and 27 nonsmokers, plasma vitamin E was measured to be 1.68 μg/mg total plasma lipid content in smokers compared to 2.78 μg/mg total plasma lipid content in nonsmokers, $P \leq 0.05$ [44]. Similar results were also shown in another study involving 10 smokers and 17 nonsmokers, where the smokers had significantly lower levels of vitamin E compared to the nonsmokers [43]. It has been suggested that α-tocopherol supplementation may provide important protection against oxidative stress in smokers, as they have greater requirements of vitamin C and E to normalise levels in plasma [51]. Analysis of the plasma lipid composition has shown values consistent with the published values. However, it was observed that there was no significant difference between the plasma lipid profiles of the two groups.

In summary, the results indicate no difference in the amino acid profile within the apo B100 of the circulating plasma LDL particles for smokers (≥40 cigarettes/day) compared with nonsmokers. It was also found that there was no significant correlation between plasma ascorbic acid levels and amino acid composition. The vitamin C levels and the LDL α-tocopherol:cholesterol ratios were significantly lower in the female smokers when compared to the nonsmoking female population. However, there was no significant difference between the male populations of the smoking and nonsmoking groups.

ACKNOWLEDGMENT

The authors thank the Food Standards Agency, UK (Grant NO4006) for financial support.

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