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Comparison of LDL fatty acid and carotenoid concentrations and oxidative resistance of LDL in volunteers from countries with different rates of cardiovascular disease

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Within Europe there are differences in cardiovascular disease (CVD) risk between countries and this might be related to dietary habits. Oxidative modification of LDL is suggested to increase the risk of CVD and both the fatty acid and antioxidant content of LDL can affect its oxidation. In the present study, concentration of LDL fatty acid and antioxidant micronutrients (tocopherols and carotenoids) and *ex vivo* oxidative resistance of LDL (lag phase) was compared in volunteers from five countries with different fruit and vegetable intakes and reported rates of CVD. Eighty volunteers (forty males, forty females per centre), age range 25–45 years, were recruited from France, Northern Ireland, UK, Republic of Ireland, The Netherlands, and Spain, and their LDL composition and lag phase were measured. There were some differences in LDL carotenoid and α-tocopherol concentrations between countries. α-Tocopherol was low and β- + γ-tocopherol were high ($P<0{\cdot}001$) in the Dutch subjects. β-Carotene concentrations were significantly different between the French and Spanish volunteers, with French showing the highest and Spanish the lowest concentration. LDL lycopene was not different between centres in contrast to lutein, which was highest in French (twofold that in the Dutch and Spanish and threefold that in Northern Ireland and the Republic of Ireland, $P<0{\cdot}001$). However absolute LDL saturated, monounsaturated, polyunsaturated and total unsaturated fatty acid concentrations were different between countries ($P<0{\cdot}001$, total unsaturated highest in Northern Ireland) there was little difference in unsaturated:saturated fatty acid concentration ratios and no difference in polyunsaturated:saturated fatty acid concentration ratios. LDL from the Republic of Ireland (a region with a high rate of CVD) had greater resistance to Cu-stimulated oxidation than samples obtained from volunteers in other countries. In conclusion, LDL composition did not predict resistance to Cu-stimulated oxidation, nor is there evidence that LDL from volunteers in countries with lower rates of CVD have greater resistance to oxidation.

LDL oxidation: Fatty acids: Antioxidants: Tocopherols: Carotenoids

Oxidative modification of LDL has been associated with atherosclerosis and risk of cardiovascular disease (CVD). Oxidised LDL has properties that could explain the development of atherosclerotic plaque via formation and accumulation of 'foam' cells (lipid-laden monocyte-derived macrophage) in the arterial intima (Steinberg & Witztum, 1990; Witztum & Steinberg, 1991). Although there is no conclusive evidence suggesting that people with low

Abbreviations: CVD, cardiovascular disease; ES, Spain; FR, France; IE, Republic of Ireland; NL, The Netherlands.

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resistance to LDL oxidation have increased risk of CVD, the general consensus is that the two may be related. The oxidation of LDL can be affected by several factors and among these factors, the composition of the LDL particle is believed to have a marked influence on its susceptibility to oxidation (Spranger *et al.* 1998). LDL particle size (Chait *et al.* 1992; Tribble *et al.* 1995), antioxidant content (Jialal, 1993; Abbey *et al.* 1993; Tertov *et al.* 1998) and fatty acid composition (Reaven *et al.* 1993; Louheranta *et al.* 1996; Aviram & Eias, 1993), can affect its oxidation. A linoleate-rich diet has been reported to increase (Reaven *et al.* 1993; Louheranta *et al.* 1996), and an oleate-rich diet to reduce (Aviram & Eias, 1993), *ex vivo* oxidizability of LDL particles. Likewise, supplementation with α-tocopherol (Dieber-Rothender *et al.* 1991) and carotenoids (Levy *et al.* 1995; Agarwal & Rao, 1998; Hininger *et al.* 1997) has been reported to protect LDL from oxidation.

Epidemiological studies have indicated a significant inverse correlation between the concentration of vitamin E and carotenoids in plasma and mortality from CHD (Riemersma *et al.* 1990; Gey *et al.* 1991). Recently, plasma α-carotene and γ-tocopherol have been suggested as markers of atherosclerosis and a decrease in their concentration was shown to be associated with the presence of CHD (Kontush *et al.* 1999). Across Europe, large differences in mortality due to CVD have been reported (Sans *et al.* 1997). However, the evidence on differences in LDL composition and oxidation of LDL between countries with reported different risk of CVD is lacking.

In the present study, we have compared the concentration of α-tocopherol, carotenoid and fatty acid components of LDL isolated from human adult volunteers recruited from five European countries which differ in the rates of CVD; France (FR), Northern Ireland (UK), the Republic of Ireland (IE), The Netherlands (NL) and Spain (ES). We have examined whether there was a relationship between the concentration of variables mentioned earlier and the oxidation resistance of LDL to Cu determined by measuring the lag phase.

Volunteers and methods

Volunteers

Apparently healthy, non-smoking, male and female volunteers, aged 25–45 years, assessed as consuming diets typical of the region, were recruited at five European centres: Grenoble (FR); Coleraine, Nothern Ireland (UK); Cork (IE); Zeist (NL); and Madrid (ES). Volunteers consuming supplements or taking prescribed medication were not included in the study. Those participating in the study were asked to maintain their normal lifestyle during the course of the study. The recruitment objective set by organizing committee was eighty volunteers at each centre (forty male and forty female) and volunteers were checked for normal fasting blood biochemical profiles (haemoglobin concentration, red cell, white cell and platelet count, plasma glucose, cholesterol, triacylglycerol and retinol concentrations). Those included in the study were required to fulfil certain inclusion criteria, which included: plasma cholesterol <6·8 mmol/l; triacylglycerol <2·3 mmol/l and normal blood glucose <6 mmol/l (for exclusion of potential diabetics). Results from volunteers with serum retinol <1·0 μmol/l (three subjects from the UK) were also not included in the study. The reason for choosing a cut-off point for retinol was that low retinol levels indicate an increased susceptibility to infection. The final number of valid volunteers entering the study was (*n*): FR 75 (thirty-eight male, thirty-seven female); UK 65 (thirty-two male, thirty-three female); IE 73 (forty male, thirty-three female); NL 72 (thirty-three male, thirty-nine female); ES 64 (thirty-two male, thirty-two female). However, due to the centralization of analysis and time constraints, not all samples were analysed. The final number of volunteers for whom complete sets of data were obtained are displayed in each of the Tables. Various sex-related as well as plasma characteristics have been published in our previous report (Olmedilla *et al.* 2001), and are also shown in Table 1. The table also includes the information on CVD death rates published by Sans *et al.* 1997.

The volunteers signed a written consent form and all relevant ethical committees approved the study.

Blood samples

Fasting venous blood samples (5 ml) were added to tubes containing lithium heparin as anticoagulant, centrifuged, and plasma transferred to screw-capped Eppendorf tubes, frozen immediately at −70°C and then transported by air-courier over dry ice to F. Hoffmann-La Roche (Basle, Switzerland) for central analysis. The analysis was completed within 1 year of completion of the study.

LDL analysis: fatty acids, tocopherols and carotenoids

After thawing the plasma, LDL were isolated by density ultracentrifugation on a TL-100 ultracentrifuge (Beckman, Palo Alto, CA, USA) as described previously (Himber *et al.* 1995). LDL cholesterol concentration was determined using an enzymatic colorimetric test (Roche). LDL lipid, tocopherol and carotenoids were extracted by the method of Folch *et al.* (1957).

Fatty acid analysis. LDL lipids were extracted with chloroform–methanol, dried under N_2 transmethylated with methanolic HCl and separated by GC (HP 5890A; Hewlett-Packard, Palo Alto, CA, USA) as detailed previously (Raederstroff *et al.* 1991). In brief, a fused silica capillary column (50 mm length, 0·25 mm i.d., 0·1 mm layer thickness) was used with following conditions: injection at 55°C, 10°C/min from 55–177°C, 1°C/min from 177–218°C, 4°C/min from 218–270°C. C_{17} methyl ester was used as internal standard, and fatty acids were quantified by using commercial methyl ester standards.

Tocopherol and Carotenoid analysis. A sample of 250 μl was mixed with 250 μl distilled water, deproteinized by addition of 500 μl ethanol and mixed on a vortex mixer. To the mixture, 1 ml hexane was added and following mechanical shaking for 10 min tubes were centrifuged at 2000 ***g*** for 10 min. A sample of the hexane layer (400 μl) was evaporated to dryness and samples were re-suspended in mobile phase (acetonitrile–tetrahydrofuran–methanol–ammonium acetate (10 g/l) (684:220:68:28, by vol.)), and

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Table 1. Subject characteristics* and population-based cardiovascular disease (CVD) risk figures†

	Age (years)				Plasma triacylglycerol (mmol/l)				Total plasma cholesterol (mmol/l)				CVD death rate (average per 10 000) †	
	m		f		m		f		m		f			
	Mean	Range	Mean	Range	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	m	f
FR	35	24–45	36	25–45	1·08	0·92, 1·24	0·83	0·73, 0·93	5·12	4·85, 5·39	4·92	4·65, 5·19	102	30
ES	36	25–45	36	25–45	1·06	0·90, 1·22	0·74	0·66, 0·82	5·14	4·85, 5·43	5·06	4·79, 5·33	126	39
NL	31	24–45	33	24–43	1·08	0·96, 1·20	1·04	0·92, 1·16	4·66	4·39, 4·93	4·98	4·76, 5·20	137	45
IE	31	24–44	32	24–45	1·15	0·99, 1·31	0·86	0·74, 0·98	4·79	4·54, 5·04	4·72	4·50, 4·94	196	56
UK	35	26–46	35	19–45	1·04	0·88, 1·20	0·92	0·80, 1·04	5·25	4·98, 5·52	4·99	4·70, 5·28	225	76

m, male; f, female; FR, France; ES, Spain; NL, The Netherlands; IE, Republic of Ireland.
* Also reported in Olmedilla *et al.* (2001).
† From Sans *et al.* (1997).

separated by C_{18} reverse-phase HPLC with either fluorimetric (tocopherol) or visible wavelength (carotenoids) spectrophotometric detection (Hess *et al.* 1991). The stock solutions for calibration were prepared in hexane–butylated hydroxy toluene using pure standards of α- and β-carotene, lycopene, lutein and α- and β-tocopherol. The solutions were stable at −20°C for up to 1 month (except lycopene, which was stored up to 1 week only).

LDL oxidation

Cu-induced LDL oxidation lag-time was measured using the method of Esterbauer *et al.* (1989) and modified by Himber *et al.* (1995). Briefly, heparinized plasma was adjusted to a density of 1·21 g/ml by addition of solid KBr and overlaid with 150 mmol NaCl/l. The samples were centrifuged in a TL-100 ultracentrifuge (Beckman) for 25 min at 10°C and 440 000 ***g***. The LDL layer was removed by slicing the polycarbonate tube and used directly for the LDL oxidation. Cu-initiated oxidation of LDL was followed up to 5 h by measuring diene conjugate formation at 234 nm. The lag phase was determined as the intercept of the tangents drawn to the segments of the absorbance curve corresponding to propagation phase, as described by Frei & Gaziano (1993).

Statistical analysis

All data was $\log_{10}$-transformed to normalize distributions and/or to produce homogeneous variances. A comparison within sex, at baseline, between centres (FR, UK, IE, NL and ES), of plasma LDL tocopherols, carotenoids, fatty acids, and Cu-induced LDL-oxidation lag-times, was undertaken by one-way ANOVA. Where the variance ratio (F test) was significant ($P<0·05$), comparison between the means for all centres was undertaken by using the Scheffé post-hoc test. Within each centre, the mean for females for each variable was compared with the male counterpart, using Student's unpaired t test. Associations between Cu-induced LDL-oxidation lag-times and facets of LDL composition (i.e. tocopherol, carotenoid and fatty acid components) were assessed by regression analysis.

Results

Blood sample collection was done between the months of January and March. During this period minimum fluctuations are known to occur in plasma concentration of antioxidant micronutrients (Van de Vijver *et al.* 1997). In addition, seasonal and day-to-day variations in the LDL lag-phase, micronutrients and the fatty acid composition of LDL are reported to be low in unsupplemented subjects (Van de Vijver *et al.* 1997; Cantilena *et al.* 1992; Chopra *et al.* 2000). The subjects were advised to maintain their normal dietary habits. The results of the present study are therefore unlikely to be influenced by the seasonal and day-to-day variations in variables.

LDL concentration of tocopherols and carotenoids

Within centres (except for ES), there were generally no

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Table 2. Male (m) and female (f) fasting LDL tocopherols (μmol/mmol LDL cholesterol), β-carotene, lycopene and lutein (nmol/mmol LDL cholesterol) for centres in France (FR), Northern Ireland (UK), Republic of Ireland (IE), The Netherlands (NL) and Spain (ES)†

(Mean values and 95 % confidence intervals)

		Centre 1 (FR)			Centre 2 (UK)			Centre 3 (IE)			Centre 4 (NL)			Centre 5 (ES)			Statistical significance of effect (one-way ANOVA, *F* test): *P*<
Variable	Sex	*n*	Mean	95 % CI	*n*	Mean	95 % CI	*n*	Mean	95 % CI	*n*	Mean	95 % CI	*n*	Mean	95 % CI	
α-Tocopherol‡	m	23	$4{\cdot}34^{ab}$	3·97, 4·75	32	$4{\cdot}46^{a}$	4·15, 4·78	39	$4{\cdot}15^{ab}$	3·89, 4·42	33	$3{\cdot}74^{b}$	3·56, 3·92	32	$3{\cdot}84^{b}$	3·67, 3·99	0·001
	f	24	$4{\cdot}55^{x}$	4·13, 5·01	33	$4{\cdot}72^{x}$	4·43, 5·02	33	$4{\cdot}30^{xy}$	4·01, 4·63	38	$3{\cdot}93^{y}$	3·75, 4·11	32	$4{\cdot}70^{x*}$	4·50, 4·90	0·001
β- + γ-tocopherol	m	23	$0{\cdot}16^{a}$	0·13, 0·18	32	$0{\cdot}19^{a}$	0·16, 0·23	39	$0{\cdot}19^{a}$	0·16, 0·21	33	$0{\cdot}39^{c}$	0·31, 0·49	32	$0{\cdot}10^{b}$	0·08, 0·11	0·001
	f	24	$0{\cdot}15^{xy}$	0·11, 0·18	33	$0{\cdot}19^{y}$	0·16, 0·23	33	$0{\cdot}19^{y}$	0·16, 0·21	38	$0{\cdot}39^{z}$	0·33, 0·45	32	$0{\cdot}10^{x}$	0·09, 0·12	0·001
Total tocopherol‡	m	23	$4{\cdot}52^{ab}$	4·12, 4·94	32	$4{\cdot}68^{a}$	4·36, 5·01	39	$4{\cdot}34^{ab}$	4·07, 4·63	33	$4{\cdot}21^{ab}$	3·95, 4·47	32	$3{\cdot}92^{b}$	3·76, 4·09	0·01
	f	24	$4{\cdot}72^{x}$	4·29, 5·20	33	$4{\cdot}93^{x}$	4·63, 5·25	33	$4{\cdot}51^{x}$	4·20, 4·84	38	$4{\cdot}35^{x}$	4·15, 4·57	32	$4{\cdot}81^{x*}$	4·60, 5·01	0·05§
β-Carotene	m	23	130^{a}	104, 162	32	93^{ab}	73, 117	39	95^{ab}	76, 119	33	116^{ab}	97, 138	32	73^{b}	60, 88	0·01
	f	24	206^{x*}	144, 293	33	111^{y}	112, 138	33	130^{xy*}	113, 149	38	108^{y}	90, 128	32	101^{y*}	87, 118	0·001
Lycopene‡	m	23	70	58, 83	32	82	69, 96	39	80	65, 97	33	72	60, 86	32	58	49, 68	NS
	f	24	84	64, 111	33	81	65, 101	33	65	53, 79	38	68	56, 81	32	62	50, 78	NS
Lutein‡	m	23	42^{a}	34, 52	32	16^{c}	13, 18	39	16^{c}	14, 18	33	21^{bc}	18, 23	32	26^{b}	23, 30	0·001
	f	24	56^{x*}	46, 67	33	18^{z}	16, 20	33	17^{z}	15, 20	38	26^{y}	23, 29	32	29^{y}	26, 33	0·001
β-Carotene+	m	23	214	159, 287	32	201	170, 238	39	210	170, 244	33	220	195, 248	32	164	143, 187	NS
lycopene + lutein‡	f	24	362^{x}	260, 502	33	224^{y}	189, 265	33	221^{y}	198, 247	38	216^{y}	192, 244	32	206^{y*}	185, 230	0·001

[a,b,c]Mean values for m groups within a row with unlike superscript letters were significantly different (Scheffé's post-hoc test $P<0{\cdot}05$).
[x,y,z]Mean values for f groups within a row within unlike superscript letters were significantly different (Scheffé's post-hoc test $P<0{\cdot}05$).
*Mean values for f groups were significantly different from those of m groups (Student's *t* test): $^{*}P<0{\cdot}05$.
† For details of subjects and procedures, see Table1 and p. 23.
‡ Geometric mean and 95 % CI back-transformed from mean and 95 % CI of '$\log_{10}$-transformed' skewed data.
§ Though variance ratio is significant, Scheffé's post-hoc test indicates no difference between individual means.

Table 3. Male (m) and female (f) fasting saturated (S), monounsaturated (M), polyunsaturated (P) and total unsaturated (U) LDL fatty acids (mmol/ mmol LDL cholesterol), and monounsaturated:saturated (M:S), polyunsaturated:saturated (P:S) and total unsaturated:saturated (U:S) LDL fatty acid ratios for centres in France (FR), Northern Ireland (UK), Republic of Ireland (IE), Republic of the Netherlands (NL) and Spain (ES)†

(Mean values and 95 % confidence intervals)

		Centre 1 (FR)			Centre 2 (UK)			Centre 3 (IE)			Centre 4 (NL)			Centre 5 (ES)			Statistical significance of effect (one-way ANOVA, *F* test): *P*<
Variable	Sex	*n*	Mean	95 % CI	*n*	Mean	95 % CI	*n*	Mean	95 % CI	*n*	Mean	95 % CI	*n*	Mean	95 % CI	
S‡	m	26	$2{\cdot}94^{a}$	2·65, 3·25	20	$2{\cdot}96^{a}$	2·73, 3·20		$2{\cdot}77^{ab}$	2·53, 3·03		$2{\cdot}39^{bc}$	2·23, 2·55		$2{\cdot}17^{c}$	1·95, 2·42	0·001
	f	26	$2{\cdot}84^{x}$	2·63, 3·07	22	$2{\cdot}92^{x}$	2·71, 3·14		$2{\cdot}56^{x}$	2·26, 2·90		$2{\cdot}47^{x}$	2·35, 2·59		$2{\cdot}65^{x*}$	2·52, 2·78	0·05§
M‡	m	26	$2{\cdot}10^{ab}$	1·89, 2·34	20	$2{\cdot}34^{a}$	2·17, 2·52		$2{\cdot}18^{a}$	1·97, 2·40		$1{\cdot}73^{bc}$	1·60, 1·87		$1{\cdot}67^{c}$	1·48, 1·87	0·001
	f	26	$2{\cdot}11^{xy}$	1·93, 2·30	22	$2{\cdot}49^{x}$	2·30, 2·68		$1{\cdot}95^{y}$	1·76, 2·16		$1{\cdot}81^{y}$	1·70, 1·92		$1{\cdot}93^{y*}$	1·76, 2·12	0·001
P‡	m	26	$5{\cdot}14^{ab}$	4·64, 5·67	20	$5{\cdot}51^{a}$	5·04, 5·99		$4{\cdot}89^{ab}$	4·43, 5·41		$4{\cdot}43^{bc}$	4·17, 4·70		$3{\cdot}94^{c}$	3·55, 4·35	0·001
	f	26	$5{\cdot}01^{x}$	4·62, 5·42	22	$5{\cdot}03^{x}$	4·69, 5·41		$4{\cdot}44^{x}$	4·06, 4·84		$4{\cdot}58^{x}$	4·37, 4·78		$4{\cdot}58^{x*}$	4·58, 4·84	0·05§
U‡	m	26	$7{\cdot}26^{ab}$	6·57, 7·99	20	$7{\cdot}85^{a}$	7·24, 8·51		$7{\cdot}10^{ab}$	6·44, 7·80		$6{\cdot}18^{bc}$	5·82, 6·54		$5{\cdot}62^{c}$	5·08, 6·21	0·001
	f	26	$7{\cdot}13^{xy}$	6·57, 7·71	22	$7{\cdot}55^{x}$	7·04, 8·07		$6{\cdot}41^{y}$	5·87, 6·98		$6{\cdot}41^{y}$	6·12, 6·70		$6{\cdot}55^{xy*}$	6·21, 6·92	0·01
M:S‡	m	26	$0{\cdot}72^{a}$	0·69, 0·74	20	$0{\cdot}79^{bc}$	0·76, 0·82		$0{\cdot}79^{c}$	0·76, 0·81		$0{\cdot}73^{ab}$	0·70, 0·75		$0{\cdot}77^{abc}$	0·72, 0·81	0·001
	f	26	$0{\cdot}74^{x}$	0·72, 0·76	22	$0{\cdot}85^{y*}$	0·82, 0·87		$0{\cdot}76^{xy}$	0·70, 0·82		$0{\cdot}73^{x}$	0·71, 0·75		$0{\cdot}73^{x}$	0·68, 0·77	0·001
P:S‡	m	26	1·75	1·69, 1·81	20	1·86	1·79, 1·93		1·77	1·70, 1·83		1·85	1·78, 1·93		1·81	1·74, 1·88	NS
	F	26	1·76	1·70, 1·82	22	1·73*	1·65, 1·80		1·73	1·60, 1·86		1·86	1·80, 1·91		1·73	1·66, 1·80	NS
U:S‡	m	26	$2{\cdot}47^{a}$	2·41, 2·53	20	$2{\cdot}65^{b}$	2·58, 2·72		$2{\cdot}56^{ab}$	2·49, 2·62		$2{\cdot}59^{ab}$	2·51, 2·66		$2{\cdot}59^{ab}$	2·51, 2·66	0·05
	f	26	2·51	2·45, 2·56	22	2·58	2·51, 2·65		2·50	2·33, 2·68		2·59	2·54, 2·65		2·47*	2·41, 2·54	NS

[abc]Mean values for m groups within a row with unlike superscript letters were significantly different (Scheffé's post-hoc test $P<0{\cdot}05$).
[xyz]Mean values for f groups within a row with unlike superscript letters were significantly different (Scheffé's post-hoc test $P<0{\cdot}05$).
*Mean values for f groups were significantly different from those of m groups (Student's *t* test): $^{*}P<0{\cdot}05$.
† For details of subjects and procedures, see Table 1 and p. 23.
‡ Geometric mean and 95 % CI back-transformed from mean and 95 % CI of '$\log_{10}$-transformed' skewed data.
§ Though variance ratio is significant, Scheffé's post-hoc test indicates no difference between individual means.

Table 4. Male (m) and female (f) fasting plasma LDL copper-oxidation lag-phase (min), for centres in France (FR), Northern Ireland (UK), Ireland (IE), The Netherlands (NL) and Spain (ES)† (Mean values and 95 % confidence intervals)

		Centre 1 (FR)			Centre 2 (UK)			Centre 3 (IE)			Centre 4 (NL)			Centre 5 (ES)			Statistical significance of effect (one-way ANOVA, *F* test: *P*<
Variable	Sex	*n*	Mean	95 % CI	*n*	Mean	95 % CI	*n*	Mean	95 % CI	*n*	Mean	95 % CI	*n*	Mean	95 % CI	
Lag-phase	m	28	92[a]	82, 103	29	157[b]	140, 175	35	128[b]	115, 142	25	89·1[a]	84, 95	29	92[a]	83, 103	0·001
	f	24	91[w]	81, 102	27	181[z*]	170, 193	28	139[y]	126, 153	26	96[wx]	89, 103	28	110[x*]	103, 117	0·001

a,b,c Mean values for m groups within a row were significantly different (Scheffé's post-hoc test, $P<0.05$).
w,x,y,z Mean values for f groups within a row were significantly different (Scheffé's post-hoc test, $P<0.05$).
*Mean values for f groups were significantly different from those of m groups (Student's *t* test): $^{*}P<0.05$).
† For details of subjects and procedures, see Table 1 and p. 23.
‡ Geometric mean and 95 % CI back-transformed from mean and 95 % CI of '$\log_{10}$ transformed' skewed data.

differences between sexes for most of the variables (Table 2). Between centres, α-tocopherol concentrations of LDL were low in Dutch volunteers. In males, the results reached significance compared with UK only, and in females they were significantly lower compared with all centres except IE. β-+γ-tocopherol were significantly higher in Dutch compared with all other participating centres both for males and females ($P<0.001$). In females, total tocopherol concentration was not significantly different between centres, but for male volunteers, total tocopherol was significantly different between ES and UK.

LDL β-carotene concentrations were significantly different between ES and FR with ES showing the lowest β-carotene and FR the highest (Table 2). The values for the other three centres (IE, NL, UK) were not significantly different from each other. LDL lycopene was not significantly different between any centres. LDL lutein was significantly higher for FR (almost twofold compared with NL and threefold compared with UK and IE) compared with all centres (Table 2, $P<0.001$) in the order FR>ES > NL>IE and UK. When the total LDL carotenoid concentrations (sum of β-carotene, lutein and lycopene) were compared, no differences emerged for males. In the case of females, FR showed significantly higher total carotenoid concentration compared with all other centres (Table 2, $P<0.001$). The remaining four centres were not significantly different.

LDL concentration of fatty acids

Some differences between regions in LDL concentrations of absolute saturated, monounsaturated, polyunsaturated and total unsaturated fatty acids were observed. In male volunteers, LDL saturated fatty acid content was not significantly different among the volunteers from FR, UK and IE, but only subjects from FR and UK showed a significantly higher concentration than those from NL and ES (Table 3, $P<0.001$, one way ANOVA). In females, differences were not significant. There were minor differences in the mono and polyunsaturated FA content of LDL and in general LDL from UK volunteers showed highest unsaturated FA concentrations, though not significantly different from other centres (except for ES males). The unsaturated:saturated fatty acid ratio was significantly different only between male volunteers from UK and FR.

LDL lag-phase comparisons

Ex vivo resistance of LDL to Cu-initiated oxidation was highest in UK (both male and female) compared with the other four centres (Table 4). The order of LDL's resistance to oxidation was UK>IE>ES>FR>NL. Lag phases were not significantly different between the two Irish populations (UK and IE). No significant correlation was observed between the LDL lag-phase and other variables, i.e. fatty acid, carotenoid and tocopherol content of LDL for either the combined or separate data for countries. There were no correlations between lag phase with lipid-standardized LDL tocopherol and carotenoid levels. LDL composition did not correlate with the CVD mortality rates reported by Sans *et al.* (1997). However, when the LDL lag-phase from

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five centres was compared with incidence rates of CVD reported by Sans *et al.* (1997) a significant positive correlation was observed both for males and females (r 0·905 and 0·915 respectively, $P<0·05$).

Discussion

The five European regions studied (Grenoble, FR; Coleraine, Northern Ireland, UK; Cork, IE; Zeist, NL, and Madrid, ES) lie on a north–south axis and the population in southern Europe has a lower mortality from cardiovascular disease than those from the northern and eastern parts (World Health Organization, 1993). In the present study, a similar age group (25–45 years) was compared between the countries and the homogeneity of inclusion criteria and centralization of the analysis would have minimized the analytical CV.

Minor differences were observed between regions for LDL α-tocopherol concentration. It is however, interesting to note that Dutch volunteers had significantly lower α-tocopherol and higher β- + γ-tocopherol concentration than other participating centres. Both α-and γ-tocopherol have been reported to compete for their incorporation in lipoprotein particles in the liver (Traber *et al.* 1989), and supplementation with γ-tocopherol-rich corn oil has been reported to decrease the plasma α-tocopherol (Elmadfa & Park, 1999). It is therefore not surprising that the population with high LDL content of α-tocopherol shows low β- + γ-tocopherol in the LDL and *vice versa*. However, in the present study, in Dutch subjects, we did not find any significant correlation between α- and β- + γ-tocopherol.

Previously, it has been shown that in supplemented subjects, changes in dietary intake of carotenoids leads to a change in their concentrations in plasma and LDL (Chopra *et al.* 2000). Therefore, plasma and LDL carotenoids may be considered as biomarkers of fruit and vegetable intake (Scott *et al.* 1994; Parker, 1997). In the present study, ES, the southernmost country (a 'Mediterranean' region perceived to have a fruit and vegetable consumption relatively greater than the northernmost regions) had the lowest LDL concentrations of β-carotene and lycopene. On comparison of the dietary intake and plasma carotenoid concentrations, Spanish volunteers were found to have a lowest carotenoid intake, 9·5 mg/d compared with 16·1 mg/d in the French and approximately 14 mg/d in subjects from the other three centres (UK, IE, NL; O'Neill *et al.* 2001). The plasma concentration of carotenoids in the Spanish subjects were also found to be low (Olmedilla *et al.* 2001). It is therefore not surprising that LDL concentration of carotenoids was low in the Spanish. Previously, it was reported that there are regional differences in the fruit and vegetable intakes within ES, however, the fruit and vegetable consumption in Madrid is reported to be similar to that consumed on average in ES (Instituto Nacional de Estadistica, 1994). Although the Spanish are perceived to be high consumers of fruits and vegetables, the results of the present study and our previous reports (O'Neill *et al.* 2001; Olmedilla *et al.* 2001) show that the volunteers from Madrid consumed less vegetables, and had lower plasma carotenoids and LDL than the subjects from other participating centres. It can be argued that the participants were not necessarily representatives of the overall population of their respective countries. However, since CI indicate a high precision in the estimation of means, one can extrapolate the results of the present study to populations with similar characteristics. It is interesting to note that a previous comparative study (Howard *et al.* 1996) between southern FR and Northern Ireland reported very similar results to those obtained in the present European Union study. The mean plasma lutein concentrations of adult French men and women reported by Howard *et al.* (1996) were double in subjects from Toulouse, FR, than matched group of Irish subjects from Belfast Northern Ireland, UK ($P<0·001$). In the present study, LDL lutein was also two to threefold higher in the subjects from Grenoble, FR, than those in Coleraine, UK. It is also interesting to note that the LDL carotenoid and tocopherol concentrations in both Irish populations (Coleraine (UK) and Cork (IE)) were not significantly different. Toulouse and Grenoble are on opposite sides of FR, and Cork and Coleraine lie at the opposite ends of IE. Therefore, at least for France and Ireland the results of the present study can be extrapolated to the respective population of those countries.

Saturated fat is believed to increase the risk of CVD through its hypercholesterolaemic effect (Mazier & Jones, 1997; Salter *et al.* 1998) and previously (Sans *et al.* 1997) in men, a twofold difference in the risk of CVD was reported for the participating countries of the present study. Nevertheless, in the present study, in male volunteers, only small differences were observed in LDL saturated fatty acid content with concentrations in ES being lower than those in FR, UK and IE but not significantly different from those in the NL. ES is a country with a low risk of CVD, but in addition to the low concentrations of saturated fat, the male volunteers in ES also had the lowest concentrations of polyunsaturated fatty acids. Polyunsaturated fatty acids are considered beneficial due to their lipid-lowering effects, especially on LDL cholesterol (Howard *et al.* 1995; Carmena *et al.* 1996; Mazier & Jones, 1997). However, polyunsaturated fats are more prone to oxidation than saturated fats and increased dietary intake of polyunsaturated fatty acids may increase the susceptibility to *ex vivo* LDL oxidation (Reaven *et al.* 1993, Louheranta *et al.* 1996) and hence the atherogenic properties of the LDL particle. In the present study, LDL fatty acids (both saturated and unsaturated) were highest in volunteers from the UK, and surprisingly the LDL from the UK were more resistant to oxidation than other participating centres and showed the highest lag phase. This suggests that LDL content of tocopherols, carotenoids, and fatty acids alone cannot predict susceptibility of LDL to oxidation. This is supported by the observations of Dieber-Rothender *et al.* (1991) who reported that in unsupplemented subjects there are no correlations between the LDL content of tocopherols and the resistance of LDL to oxidation. There were no significant relationships between the lipid-soluble antioxidant content or fatty acid composition, and *ex vivo* LDL resistance to Cu-induced oxidation. Neither the LDL polyunsaturated fatty acid nor the lipid-soluble antioxidant composition predicted the resistance of LDL to oxidation. Surprisingly, a significant positive correlation was observed between the LDL lag-times and incidence of CVD reported

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by Sans *et al.* (1997). At present, we have no obvious explanation for the observed correlations.

Results of the present study show no evidence that LDL from volunteers in regions with the lower rates of CVD have greater *ex vivo* resistance to Cu-induced LDL oxidation. French subjects are reported to be at a low risk of CVD compared with the Irish, yet, the UK and IE samples were more resistant to oxidation than those of French. The higher resistance of LDL from the Irish volunteers to Cu-induced oxidation appears to be at odds with their generally higher rates of early deaths from CVD (Sans *et al.* 1997) compared with French or Spanish subjects. The current study also indicates that a region perceived to have a high fruit and vegetable consumption i.e. ES, does not equate with having high LDL concentrations of lipid-soluble antioxidants. Only LDL lutein concentration showed a clear inverse relationship with the risk of CVD in the five European countries (Sans *et al.* 1997). Green vegetables are a rich source of lutein (International Agency for Research on Cancer, 1998) and high plasma LDL lutein concentration indicates high consumption of green vegetables in volunteers (Thurnham *et al.* 1998; Chopra *et al.* 2000). These observations further support the previous suggestions by Howard *et al.* (1996) that low blood hydroxy-carotenoids may be a good indicator of increased CVD risk. Concentrations of other carotenoids, tocopherols, fatty acids and LDL oxidizability did not follow the same pattern as described for the CVD risk between countries, as well as lutein. Due to the restrictions of the 'inclusion criteria' in the present study, it could be argued that volunteers were not representatives of the populations of their respective countries. However, as explained earlier, since the information obtained in this study for IE and FR agrees with previous studies undertaken in different regions of respective countries, we believe that at least for these two countries, volunteers can be considered representative of the rest of the population.

The results of the present study did not show any relationship between the fatty acid profile or the antioxidant content of LDL and *ex vivo* resistance of LDL to Cu-induced oxidation in healthy subjects from five areas in Europe with different CVD mortality rates. Furthermore, there were no clear inter-relationships between population-based CVD mortality data and the composition of LDL in the healthy subjects included in the present study. In conclusion, we believe that the fatty acid and antioxidant (tocopherols and carotenoids) composition of LDL alone and its resistance to *ex vivo* oxidation may not necessarily provide clues to the risk of CVD incidence.

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