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Plasma ubiquinol-10 is decreased in patients with hyperlipidaemia

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Abstract

Ubiquinol-10, the reduced form of ubiquinone-10 (coenzyme Q_{10}), is a potent lipophilic antioxidant present in nearly all human tissues. The exceptional oxidative lability of ubiquinol-10 implies that it may represent a sensitive index of oxidative stress. The present study was undertaken to assess the hypothesis that the level of ubiquinol-10 in human plasma can discriminate between healthy subjects and patients who are expected to be subjected to an increased oxidative stress in vivo. Using a newly developed method, we measured plasma ubiquinol-10 in 38 hyperlipidaemic patients with and without further complications, such as coronary heart disease, hypertension, or liver disease, and in 30 healthy subjects. The oxidizability of plasma samples obtained from hyperlipidaemic patients was found to be increased in comparison with control subjects, suggesting that the patients were subjected to a higher oxidative stress in vivo than the controls. Plasma ubiquinol-10, expressed as a percentage of total ubiquinol-10 + ubiquinone-10 or normalized to plasma lipids, was lower in the patients than in controls ($P = 0.001$ and 0.008, respectively). The proportion of ubiquinol-10 decreased in the order young controls > aged controls > hyperlipidaemic patients without complications > hyperlipidaemic patients with complications ($P = 0.003$). A negative correlation was found between the proportion of ubiquinol-10 and plasma triglycerides. The hyperlipidaemic patients with hypertension had a lower proportion of ubiquinol-10 than subjects without. When the study population was divided into smokers and non-smokers, plasma ubiquinol-10 was found to be reduced amongst smokers, independently of whether it was expressed as a percentage of total ubiquinol-10 + ubiquinone-10 ($P = 0.006$) or normalized to plasma lipids ($P = 0.009$). These data suggest that the level of ubiquinol-10 in human plasma may represent a sensitive index of oxidative stress in vivo especially indicative of early oxidative damage. Measuring plasma ubiquinol-10 can be proposed as a practical approach to assess oxidative stress in humans. © 1997 Elsevier Science Ireland Ltd.

Keywords: Ubiquinol-10; Lipid peroxidation; Hyperlipidaemia; Coronary heart disease; Atherosclerosis; Smoking; Hypertension; Liver disease

1. Introduction

Ubiquinone-10, also known as coenzyme Q_{10}, is most recognized for its role in energy production by mitochondria, where it functions as an essential proton–electron carrier in the lipid phase of the inner mitochondrial membrane [1]. It is less well known then ubiquinol-10, the reduced and most common form of ubiquinone-10 in vivo, is a potent lipophilic antioxidant for protection of lipids in different biological and model systems [2,3]. The human body contains about 1.6 g ubiquinone-10 which is present in nearly all tissues [4]. Ubiquinol-10 represents more than 80% of the total ubiquinol-10 + ubiquinone-10 pool in human plasma, intestine and liver [5–7] and is accordingly a principal antioxidant in plasma lipoproteins [8–10] and hepatocytes [11].

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Table 1
Clinical characteristics of control subjects and hyperlipidaemic patients

	Young controls ($n=19$)	Aged controls ($n=11$)	HL patients without complications ($n=25$)	HL patients with complications ($n=13$)
Age	28.8 ± 5.5	49.5 ± 7.1^{a}	48.1 ± 10.1^{a}	49.5 ± 8.8^{a}
Body mass index (kg/m^2)	21.1 ± 1.9	22.2 ± 4.0	24.3 ± 2.5^{b}	$26.9 \pm 3.6^{a,d}$
Sex (M/F)	9/10	5/6	13/12	10/3
Smoking (Y/N)	4/15	3/8	9/16	8/5[c]
Alcohol (Y/N)	0/19	2/9	3/22	2/11
Family history (Y/N)	6/13	1/10	5/20	4/9
Total plasma cholesterol (mg/dl)	170 ± 30	190 ± 22	$266 \pm 67^{a,d}$	244 ± 51^{a}
Plasma triglycerides (mg/dl)	75 ± 34	99 ± 38	253 ± 263	$424 \pm 466^{b,f}$
Plasma HDL cholesterol (mg/dl)	55 ± 14	54 ± 19	45 ± 16	$33 \pm 12^{b,e}$

HL, hyperlipidaemic.
[a] $P<0.001$, [b] $P<0.01$, [c] $P<0.05$ vs. young controls, [d] $P<0.001$, [e] $P<0.01$, [f] $P<0.05$ vs. aged controls.

The high antioxidative efficiency of ubiquinol-10 is closely related to its extreme sensitivity to oxidation. Ubiquinol-10 is easily oxidized to ubiquinone-10 both in vivo and in vitro [5–11]. Exceptional oxidative lability of ubiquinol-10 implies that in vivo it is expected to be oxidized before other natural antioxidants, as is the case in vitro during lipoprotein [8–10] and hepatocyte [11] oxidation. Lipid peroxidation is strongly implicated as playing an important role in the development of various pathologies, such as atherosclerosis, cancer or Alzheimer's disease [12]. Oxidation of plasma lipoproteins (where most of the blood ubiquinol-10 is located [4]) appears to represent a crucial step in atherogenesis [13] and is also likely to occur in other diseases linked to increased free radical production [14]. Taken together, these data suggest that measurement of ubiquinol-10 in human plasma might represent a sensitive index of oxidative stress in vivo.

We have recently developed a method to measure ubiquinol-10 in human plasma and lipoproteins [15] and applied it to characterize the antioxidative activity of ubiquinol-10 in human low density lipoprotein [10,16,17]. In the present study we used this method to assess the hypothesis that the level of ubiquinol-10 in human plasma can discriminate between healthy subjects and patients who are expected to be subjected to an increased oxidative stress in vivo. We compared plasma ubiquinol-10 in hyperlipidaemic patients with and without coronary heart disease, hypertension and liver disease, and in age-matched control subjects. Several factors potentially important for oxidative stress in vivo, such as current smoking [18], increased alcohol intake and positive family history of atherosclerosis, were also considered in both the patient and control groups.

2. Methods

2.1. Subjects

Seventy hyperlipidaemic patients were recruited from the medical clinic of the University Hospital Eppendorf, Hamburg, Germany. The inclusion criteria were total plasma cholesterol higher than 220 mg/dl (5.69 mmol/l) or total plasma triglycerides higher than 200 mg/dl. Patients were excluded from the study if they were on antioxidant or lipid-lowering medication or had diabetes, pancreatitis or renal failure. Of the 38 remaining patients, 16 had hypercholesterolaemia, seven had hypertriglyceridaemia and 15 had mixed hyperlipidaemia. Twenty-five patients included in the study had no further complications and formed a hyperlipidaemic group (mean age 48.1 years, range 29–70, Table 1). Thirteen patients had such complications, specificallyq coronary heart disease (seven patients), hypertension (eight patients, five of whom also had coronary heart disease) and liver disease (three patients). These patients were analyzed either as a single group (mean age 49.5 years, range 30–61) or as separate groups according to their disease. The presence of coronary heart disease was proven angiographically. Hypertension was diagnosed as a systolic blood pressure higher than 140 mmHg or diastolic higher than 90 mmHg on three different occasions. Liver disease was diagnosed as a double elevation of liver enzymes in blood or as a sonographically proven fatty liver.

Fifty apparently healthy subjects were recruited from the hospital staff as controls. When subjects taking any medication or vitamin supplements or having hyperlipidaemia were excluded, 30 control subjects remained. To match the hyperlipidaemic and control groups, aged controls (11 subjects, between 40 and 61 years old, mean age 49.5 years) were analyzed as a separate group (Table 1). Other control subjects formed a group of

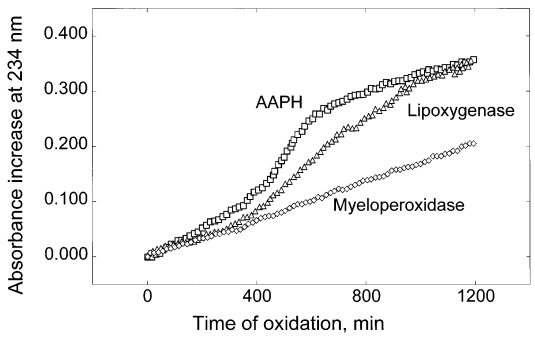

Fig. 1. Typical time-courses of the oxidation of plasma samples measured as an increase in sample absorbance at 234 nm after addition of the oxidant. Heparin plasma (20 μl) was diluted 150-fold with PBS containing 0.16 M NaCl and oxidized at 37°C by 25 U/ml lipoxygenase (△), 0.5 U/ml myeloperoxidase + 1 mM H_2O_2 (◇) or 330 μM AAPH (□). The initial oxidation rate was 7.02, 5.84 and 10.41 nM conjugated dienes/min for the oxidation by lipoxygenase, myeloperoxidase and AAPH, respectively.

young controls (19 subjects between 22 and 38 years old, mean age 28.8 years).

Several factors potentially important for oxidative stress in vivo were considered in our study in both the patient and control groups. They included current smoking [18], increased alcohol intake and positive family history. Increased alcohol intake was defined as a systematic daily consumption of 50 g alcohol or more. Positive family history of atherosclerosis was defined as, at least one case of heart attack or stroke among first-degree relatives before 55 years of age or more than one such case independent of the relatives age. There was no significant difference in sex, number of smokers, alcohol consumption habits and family history between the hyperlipidaemic patients and control groups involved in our study, except that the number of smokers was significantly higher in a group of hyperlipidaemic patients with complications than in young controls (Table 1). The study protocol was approved by the human studies committee of Hamburg, and informed consent was obtained from all subjects.

Table 2
Susceptibility of plasma from control subjects and hyperlipidaemic patients to in vitro oxidation

Oxidant	Initial plasma oxidation rate, nM conjugated dienes/min		*P*-value
	Aged controls	Hyperlipidaemic patients	
Lipoxygenase	4.33 ± 1.85	6.88 ± 4.97	0.050
Myeloperoxidase	4.64 ± 2.38	5.53 ± 4.42	ns[a]
AAPH	5.09 ± 4.98	10.09 ± 6.38	0.012

Heparin plasma was diluted 150-fold with PBS containing 0.16 M NaCl and oxidized at 37°C by 25 U/ml lipoxygenase, 0.5 U/ml myeloperoxidase + 1 mM H_2O_2 or 330 μM AAPH.
[a] ns, Not significant.

2.2. Analytical measurements

After an overnight fast, blood was taken in ethylenediaminetetraacetic acid (EDTA) or heparin-containing tubes (Sarstedt, Nümbrecht, Germany). To obtain plasma, the blood was immediately centrifuged at 4°C for 10 min. Total cholesterol, high density lipoprotein (HDL) cholesterol and triglycerides were determined in EDTA-plasma samples by commercially available enzymatic tests. Ubiquinol-10, ubiquinone-10 and α-tocopherol were quantified in the same samples by reversed-phase high-performance liquid chromatography with electrochemical detection as described elsewhere [15,19]. Ubiquinol-9, ubiquinone-7 and δ-tocopherol were used as internal standards.

To independently assess the level of oxidative stress, to which the subjects were subjected in vivo, the susceptibility of their plasma to the in vitro induced oxidation was measured as described elsewhere [17,20]. Heparin plasma samples were diluted 150-fold with phosphate-buffered saline (PBS), pH 7.4, containing 0.16 M NaCl and oxidized by soybean lipoxygenase (25 U/ml), human myeloperoxidase (0.5 U/ml) + H_2O_2 (1 mM) or 2,2′-azobis-(2-amidinopropane) hydrochloride (AAPH; 330 μM). The oxidation was performed in a spectrophotometrical cuvette at 37°C and monitored as a change in sample absorbance at 234 nm. This parameter is known to reflect the level of conjugated dienes in the samples and has been shown to correlate with other indices of plasma lipid peroxidation and to be indicative of the oxidation of the lipid moiety of plasma lipoproteins [17,20,21]. The absorbance was measured every 5 min over a period of 20 h.

2.3. Statistical analysis

Between-group differences in continuous variables were analyzed by a two-way analysis of variance using the subject's age as a covariate. Differences in dichotomous variables were analysed by Fisher's exact test. Spearman's rank correlation coefficients were calculated to evaluate relationships between variables. To assess independent relations with plasma ubiquinol-10, it was dichotomized into two groups, below or above the median value and used as a dependent variable in the logistic regression analysis. All results are presented as mean ± S.D.

3. Results

As expected, hyperlipidaemic patients had higher total plasma cholesterol and triglyceride levels, higher body mass index and lower plasma HDL cholesterol than control subjects (Table 1).

Table 3
Ubiquinol-10 in plasma from control subjects and hyperlipidaemic patients

Units	Young controls	Aged controls	HL patients without complications	HL patients with complications
μM	0.66 ± 0.41	0.77 ± 0.35	0.87 ± 0.34	1.14 ± 0.61^{b}
% of total ubiquinol-10 + ubiquinone-10	85.7 ± 7.8	83.1 ± 8.3	78.6 ± 10.4	72.9 ± 11.0^{a}
pmol/(mg total cholesterol + triglycerides	285 ± 180	281 ± 156	179 ± 61^{b}	183 ± 54

HL, hyperlipidaemic.
[a] $P < 0.01$, [b] $P < 0.05$ vs. young controls.

When plasma samples from the patients and controls were diluted 150-fold and oxidized, their absorbance at 234 nm was consistently found to increase in the presence of each of the oxidants used (Fig. 1). The absorbance increase in the samples was characterized by the absence of any discernible lag-phase. Such oxidation kinetics were in accordance with previously reported data [17,22] and are known to reflect mild oxidative conditions used to oxidize plasma lipoproteins (such as those employed in our study). Samples oxidized by AAPH or lipoxygenase typically revealed biphasic oxidation kinetics characterized by an initial increase in the sample absorbance followed by further acceleration of the oxidation (Fig. 1). Samples oxidized by myeloperoxidase normally exhibited a monotonic increase in their absorbance at 234 nm. The oxidation curves obtained were therefore, described using a single parameter, i.e. the mean oxidation rate within the first monotonic phase of the oxidation, which was interpreted as the initial rate of plasma oxidation because this phase began immediately after the addition of the oxidants to the plasma.

When the initial oxidation rates were compared between the subject groups studied, they were found to be higher in the hyperlipidaemic patients than in healthy controls when lipoxygenase or AAPH was used to oxidize plasma (Table 2). The difference in the initial oxidation rate between the patients and controls was less pronounced when the samples were oxidized by myeloperoxidase (Table 2). When patients with hypercholesterolaemia, hypertriglyceridaemia and mixed hyperlipidaemia were analyzed as separate groups, hypercholesterolaemic patients had a significantly higher plasma oxidation rate by lipoxygenase (7.60 ± 5.91 nM conjugated dienes/min, $P = 0.05$) and patients with mixed hyperlipidaemia had a significantly higher plasma oxidation rate by AAPH (11.83 ± 5.15 nM conjugated dienes/min, $P = 0.004$) than control subjects.

Similar to the total plasma lipids, the absolute amount of ubiquinol-10 expressed as μmol/l was higher in the plasma of hyperlipidaemic patients than control subjects (Table 3). The absolute plasma level of ubiquinol-10 paralleled that of the total lipids and was highest in a group of hyperlipidaemic patients with complications and lowest in young controls. The absolute plasma level of α-tocopherol revealed a similar between-group distribution (Table 4). A significant positive correlation was found between the plasma content of ubiquinol-10 and triglycerides ($r = 0.37$, $P = 0.002$).

This relationship was reversed, when ubiquinol-10 was expressed as a percentage of total plasma ubiquinol-10 + ubiquinone-10. The proportion of ubiquinol-10 was highest in a group of young controls and lowest in a group of hyperlipidaemic patients with complications (Table 3). The proportion of ubiquinol-10 decreased in the order young controls > aged controls > hyperlipidaemic patients without complications > hyperlipidaemic patients with complications ($P = 0.003$; Table 3). A significant negative correlation was found between plasma ubiquinol-10 expressed as a percentage of total plasma ubiquinol-10 + ubiquinone-10 and plasma triglycerides ($r = -0.37$, $P = 0.002$). Ubiquinol-10 expressed as a percentage of total ubiquinol-10 + ubiquinone-10 also correlated negatively with the initial rate of plasma oxidation by lipoxygenase ($r = -0.27$, $P = 0.03$). A similar correlation calculated for the plasma oxidation rate by myeloperoxidase and AAPH did not reach significance (data not shown). When all hyperlipidaemic patients and all control subjects were combined into two groups, the proportion of ubiquinol-10 was significantly lower in the patients than in the controls (76.7 ± 5.3 versus $84.7 \pm 9.3\%$, $P = 0.001$). When patients with hypercholesterolaemia, hypertriglyceridaemia and mixed hyperlipidaemia were analyzed as separate groups, only hypertriglyceridaemic patients had a significantly lower proportion of ubiquinol-10 ($70.0 \pm 11.2\%$, $P = 0.003$) than control subjects.

When normalized to total plasma cholesterol and triglycerides, ubiquinol-10 was also significantly lower in hyperlipidaemic patients than in control subjects (180 ± 49 versus 283 ± 169 pmol/mg total cholesterol + triglycerides, $P = 0.008$; Fig. 2). Of patients with different forms of hyperlipidaemia, both the patients with hypercholesterolaemia and mixed hyperlipidaemia revealed significantly lower levels of lipid-normalized

Table 4
α-Tocopherol in plasma from control subjects and hyperlipidaemic patients

Units	Young controls	Aged controls	HL patients without complications	HL patients with complications
μM	24.2 ± 12.2	22.0 ± 5.2	27.4 ± 8.6	$39.9 \pm 24.8^{b,c,d}$
$n$mol/(mg total cholesterol + triglycerides)	10.25 ± 4.84	7.78 ± 2.02	5.99 ± 2.49^{a}	6.30 ± 1.62^{b}

HL, hyperlipidaemic.
[a] $P<0.001$, [b] $P<0.01$ vs. young controls, [c] $P<0.01$ vs. aged controls, [d] $P<0.05$ vs. hyperlipidaemic patients without complications.

ubiquinol-10 than control subjects (162 ± 43 and 173 ± 62 versus 283 ± 169 pmol/mg total cholesterol + triglycerides, $P = 0.01$ and 0.03, respectively). When hyperlipidaemic patients were subdivided according to their complications and controls according to their age, lipid-normalized ubiquinol-10 was significantly lower in a group of hyperlipidaemic patients without complications than in young controls (Table 3). Lipid-normalized α-tocopherol was also found to be lower in the hyperlipidaemic patients than in control subjects (Table 4). When the group of hyperlipidaemic patients with complications was subdivided into those with coronary heart disease, hypertension or liver disease, the patients with hypertension revealed a significantly lower proportion of ubiquinol-10 than subjects without this pathology (Table 5). The patients with liver disease also had a slightly decreased proportion of ubiquinol-10 but this difference did not reach significance. Similar differences were also not significant, when ubiquinol-10 was normalized to total plasma cholesterol and triglycerides (data not shown). When the study population was divided into smokers and non-smokers, plasma ubiquinol-10 was found to be significantly reduced in the smokers' group. This conclusion did not depend on whether ubiquinol-10 was expressed as a percentage of total ubiquinol-10 + ubiquinone-10 (Table 5) or normalized to plasma lipids (171 ± 69 versus 255 ± 146 pmol/mg total cholesterol + triglycerides, $P = 0.009$). Similar comparisons between subjects with different alcohol intake and family history revealed slightly decreased plasma ubiquinol-10 levels in those with increased alcohol intake and a positive family history, although the corresponding differences did not reach significance (Table 5). There was no difference in plasma ubiquinol-10 between males and females. When similar comparisons were performed only within the group of hyperlipidaemic patients, the differences between subjects with different alcohol intake and family history reached significance, whereas the differences between subjects with different smoking habits or different blood pressure became insignificant (Table 6). No significant difference between any subgroups in lipid-normalized α-tocopherol was found (data not shown).

When a logistic regression analysis on the plasma ubiquinol-10 category (below or above the median value expressed as a percentage of total ubiquinol-10 + ubiquinone-10), including the categorical variables, smoking, increased alcohol intake, positive family history, presence of hyperlipidaemia, coronary heart disease, hypertension and liver disease and continuous variables, age and body mass index, was performed, only smoking ($P = 0.013$) and the presence of hyperlipidaemia ($P = 0.044$) entered the final regression equation.

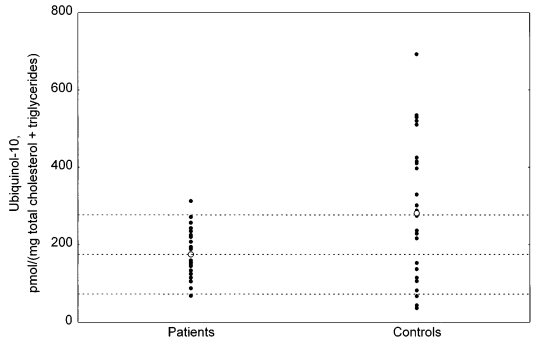

Fig. 2. Ubiquinol-10 in the plasma of hyperlipidaemic patients ($n = 38$) and control subjects ($n = 30$). Each filled dot represents a different subject. Opened dots represent median values for each group. Dotted lines correspond with the median value and interquartile range for the group of hyperlipidaemic patients.

4. Discussion

Plasma ubiquinol-10, expressed as a percentage of total ubiquinol-10 + ubiquinone-10 or normalized to plasma lipids, was found to be decreased in vivo under different pathological conditions. Ubiquinol-10 was significantly lower in hyperlipidaemic patients than in healthy controls. There was a clear trend in lowering the proportion of ubiquinol-10 in young to aged controls and then to hyperlipidaemic patients without and with complications. Hypertension and smoking were other factors related to a significant decrease in the plasma level of ubiquinol-10. Although smoking was associated with hyperlipidaemia in our study, these factors were found to be independently related to

Table 5
Influence of different factors on plasma ubiquinol-10 analysed in the whole study population

	Ubiquinol-10, % of total ubiquinol-10 + ubiquinone-10		*P*-value	Number of subjects
Coronary heart disease (Y/N)	77.5 ± 12.2	80.6 ± 12.0	ns[a]	7/61
Hypertension (Y/N)	70.5 ± 11.0	81.5 ± 9.7	0.006	8/60
Liver disease (Y/N)	69.4 ± 3.0	80.7 ± 10.4	ns[a]	3/65
Smoking (Y/N)	75.6 ± 11.1	82.8 ± 9.2	0.006	24/44
Alcohol (Y/N)	74.0 ± 13.4	81.0 ± 9.9	ns[a]	7/61
Family history (Y/N)	77.4 ± 13.6	81.1 ± 9.2	ns[a]	16/52
Sex (M/F)	80.0 ± 10.0	80.5 ± 11.1	ns[a]	37/31

[a]ns, Not significant.

plasma ubiquinol-10 in a multivariate logistic regression analysis. The presence of liver disease as well as increased alcohol intake and positive family history were also associated with a lower proportion of this antioxidant in human plasma.

Few data are currently available about plasma or tissue levels of ubiquinol-10 under pathological conditions. Stocker et al. were the first to hypothesize the usefulness of ubiquinol-10 as a promising indicator of oxidative damage in vivo [8,23]. However, they could not find any significant difference in the plasma levels of this antioxidant between patients with hyperhomocysteinemia and control subjects [23]. This has been interpreted as evidence for a non-oxidative character of hyperhomocysteinemia. In contrast, ubiquinol-10 has been found to be depleted following spinal cord impact trauma in rats [24]. More recently, a slightly, but not significantly, lower plasma level of ubiquinol-10 has been reported in male smokers compared to non-smokers [18]. A decrease in total plasma ubiquinol-10 + ubiquinone-10 has also been found in patients with ischaemic heart disease [25]. These findings are in agreement with the decrease in plasma ubiquinol-10 in smokers and in hyperlipidaemic patients with coronary heart disease found in our study.

Hyperlipidaemia has been repeatedly shown to be associated with increased oxidative stress. Hyperlipidaemic patients have decreased levels of lipid-normalized antioxidants in plasma and lipoproteins [26,27]. LDL isolated from such patients is more susceptible to oxidation than that from control subjects [28,29]. Increased oxidative stress in hyperlipidaemia is likely to be related to the delayed clearance and prolonged residence of plasma lipoproteins in the bloodstream in comparison with normolipidaemic subjects, which may result in the increased lipoprotein oxidation by arterial wall cells [30]. Insufficient protection of increased amounts of lipids by hydrophilic antioxidants and higher free radical production by leucocytes might also contribute to the increased lipoprotein oxidation in hyperlipidaemia [27]. In agreement with these findings, the plasma susceptibility to oxidation induced in vitro by lipoxygenase and AAPH was found to be increased in the hyperlipidaemic patients in comparison with control subjects also in our study. Antioxidants, oxidizable fatty acids and preformed lipid hydroperoxides seem to be the most important determinants of lipoprotein oxidizability [19,31]. Decreased content of ubiquinol-10 and α-tocopherol found in the patient plasma could therefore underlie its increased oxidizability.

Interestingly, the plasma oxidizability by myeloperoxidase was not significantly different between patients and controls. This finding can reflect different mechanisms of lipoprotein oxidation by the oxidants used in our study. Lipoxygenase and AAPH are likely to oxidize lipoproteins via direct peroxidation of their lipids [22,32]. Lipoprotein oxidation by myeloperoxidase is known to occur via production of hypochlorite in the presence of chloride and hydrogen peroxide [33]. Hypochlorite more efficiently oxidizes the protein than the lipid moiety of LDL [33]. The absorbance increase at 234 nm used to characterize the plasma oxidizability in our study has been shown to reflect the oxidation of lipoprotein lipids [17,20,21]. The less pronounced difference between the patients and controls in the plasma oxidizability by myeloperoxidase than by lypoxygenase or AAPH may therefore be explained by the lower ability of myeloperoxidase to oxidize lipoprotein lipids in comparison with two other oxidants.

Similarly in hyperlipidaemia, other pathological conditions considered in our study are also known to be linked to increased oxidative stress. Aging is widely discussed as a process causally related to diminished antioxidant capacity in living organisms [34]. Oxidation of plasma lipoproteins is thought to represent a key step in the early development of atherosclerosis [13]. Oxidative mechanisms are also implicated in the pathology of hypertension (via increased lipoprotein influx and subsequent oxidation in the arterial wall) [14] and liver disease (via impaired antioxidant metabolism in the liver) [35] as well as in adverse effects found in smokers [36]. Taken together with these findings, our data strongly suggest that the level of ubiquinol-10 in human plasma reflects the level of oxidative stress in vivo.

Table 6
Influence of different factors on plasma ubiquinol-10 analysed in the group of hyperlipidaemic patients

	Ubiquinol-10, % of total ubiquinol-10+ubiquinone-10		*P*-value	Number of subjects
Coronary heart disease (Y/N)	77.5 ± 12.2	76.5 ± 10.7	ns[a]	7/31
Hypertension (Y/N)	70.5 ± 11.0	78.3 ± 9.7	ns[a]	8/30
Liver disease (Y/N)	69.4 ± 3.0	77.3 ± 10.4	ns[a]	3/35
Smoking (Y/N)	73.5 ± 11.3	79.3 ± 10.0	ns[a]	17/21
Alcohol (Y/N)	69.3 ± 12.5	77.8 ± 10.3	0.05	5/33
Family history (Y/N)	69.0 ± 12.3	79.1 ± 10.0	0.02	9/29
Sex (M/F)	76.6 ± 10.3	76.8 ± 12.0	ns[a]	23/15

[a]ns, Not significant.

Using plasma ubiquinol-10 as an indicator of oxidative stress offers several clear advantages over most of the common indices currently used for this purpose [37]. The diversity of the pathological conditions associated with a decreased level of ubiquinol-10 in our study, implies that this antioxidant may represent a general index of oxidative stress in humans. Exceptional oxidative lability of ubiquinol-10 makes it a sensitive indicator of very early stages of oxidative damage. Other natural lipophilic antioxidants, such as α-tocopherol or carotenoids, are known to be more slowly affected by oxidation in biological systems [8–11] and may therefore, not be indicative of early oxidative damage. In accordance with this, plasma α-tocopherol was less effective than ubiquinol-10 in discriminating between subject groups in our study. As an approach in obtaining information about early oxidation stages, the measurement of ubiquinol-10 is rather unique and can only be compared with a measurement of early products of lipid peroxidation, such as lipid hydroperoxides [37]. However, the latter method requires expensive and sophisticated techniques, needs relatively large plasma volume, is prone to interference and cannot be currently recommended for clinical use. In contrast, the chromatographical method applied to measure ubiquinol-10 in our study [15] is comparably simple, rapid, well reproducible and allows the use of very small plasma volumes (5–10 μl). Taken together, our data allow us to propose measuring the level of ubiquinol-10 in human plasma as a practical approach in assessing oxidative stress in humans.

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