Measurement of the ratio between the reduced and oxidized forms of coenzyme Q_{10} in human plasma as a possible marker of oxidative stress

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Abstract It has been postulated that lipid peroxidation plays a crucial role in the pathogenesis of atherosclerosis. As $CoQ_{10}H_2$ (reduced form of coenzyme Q_{10}) is easily oxidized to CoQ_{10} (oxidized form of coenzyme Q_{10}), it has been proposed that the $CoQ_{10}H_2/CoQ_{10}$ ratio may be used as a possible marker of in vivo oxidative stress. However, sample preparation has an important effect on the redox status of coenzyme Q_{10} due to the extreme sensitivity of $CoQ_{10}H_2$ towards oxidation. We now report a rapid, simple isocratic HPLC procedure for the determination of $CoQ_{10}H_2$ and CoQ_{10} in plasma isopropanol extracts, and we used this method to investigate conditions by which the $CoQ_{10}H_2/CoQ_{10}$ ratio can be reliably measured. Our results indicate that $CoQ_{10}H_2$ is unstable in whole blood, plasma, and isopropanol extracts; subsequently the $CoQ_{10}H_2/CoQ_{10}$ ratio changes considerably soon after a blood sample has been obtained. The time period since blood sampling and HPLC analysis, as well as the sample pretreatment procedure, are two factors that have a profound effect on the pre-analytical variation in the determination of the $CoQ_{10}H_2/CoQ_{10}$ ratio. If these two factors are properly controlled, the $CoQ_{10}H_2/CoQ_{10}$ ratio may be a sensitive and practical way to measure in vivo oxidative stress. Furthermore, this indicator is independent from plasma total cholesterol concentrations, implying that groups who differ with respect to cholesterol levels may be compared directly.—**Lagendijk, J., J. B. Ubbink, and W. J. H. Vermaak.** Measurement of the ratio between the reduced and oxidized forms of coenzyme Q_{10} in human plasma as a possible marker of oxidative stress. *J. Lipid Res.* 1996. **37:** 67–75.

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Coenzyme Q_{10} is well known for its role as electron carrier in the lipid phase of the mitochondrial membrane. The reversible oxidation and reduction of coenzyme Q_{10} is the basis for its function as carrier of electrons between flavoproteins and cytochromes (1). The low potential required for oxidation or reduction of this compound makes it possible to fulfil its pivotal role in the electron transport chain. Coenzyme Q_{10} is therefore essential for ATP production and is thus present in all human tissues (1).

In the circulation, coenzyme Q_{10} is mainly carried by lipoproteins (2), where it is predominantly present in the reduced form of ubiquinol ($CoQ_{10}H_2$). $CoQ_{10}H_2$ in low density lipoprotein (LDL) is, however, easily oxidized to ubiquinone (CoQ_{10}). In fact, $CoQ_{10}H_2$ is the first antioxidant to be depleted when LDL is subjected to oxidative stress in vitro (3). It has therefore been postulated that $CoQ_{10}H_2$ prevents the initiation and/or the propagation of lipid peroxidation in plasma lipoproteins and biological membranes (4). This antioxidant function of $CoQ_{10}H_2$ may be important to protect LDL from lipid peroxidation, a process which is increasingly linked to atherogenesis and vascular disease progression (5).

As $CoQ_{10}H_2$ may be easily oxidized, it has been proposed that the $CoQ_{10}H_2/CoQ_{10}$ ratio may be used as a possible marker of in vivo oxidative stress to which circulating LDL has been exposed (6, 7). Dudman, Wilcken, and Stocker (7) measured $CoQ_{10}H_2/CoQ_{10}$ ratios in the plasma as well as isolated HDL and LDL of patients with cystathionine β-synthetase deficiency (n = 8) and normal subjects (n = 14) and used this ratio as marker of oxidative stress. Bowry, Stanley, and Stocker (6) investigated which lipoprotein was the main carrier of hydroperoxides in human plasma and also suggested that the redox status of coenzyme Q_{10} may be a useful early marker for the detection of oxidative LDL modification. Above-mentioned methods exploit the sensitivity of $CoQ_{10}H_2$ towards oxidation to define an in vivo marker of oxidative stress. In practice, however, the

Abbreviations: $CoQ_{10}H_2$, ubiquinol; CoQ_{10}, ubiquinone; TQ_{10}, total coenzyme Q_{10}; TC, total cholesterol.

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lability of $CoQ_{10}H_2$ is a potential disadvantage because of its susceptibility to pre-analytical variation. It is important to ensure that a measured $CoQ_{10}H_2/CoQ_{10}$ ratio reflects in vivo oxidative stress, and that it is not merely a function of the analytical procedure.

Although various methods (8–11) have been described for the determination of total coenzyme Q_{10} (TQ_{10}: the sum of CoQ_{10} and $CoQ_{10}H_2$), only a few authors have reported analytical techniques for the simultaneous determination of CoQ_{10} and $CoQ_{10}H_2$ (12–15). To ensure reliable $CoQ_{10}H_2$ analysis, long extraction procedures should be avoided as it may increase the risk for a significant amount of $CoQ_{10}H_2$ to be oxidized to CoQ_{10}. In the above-mentioned methods, coenzyme Q_{10} was extracted from plasma using a mixture of ethanol and hexane, whereafter the hexane layer was evaporated to dryness and the residue was redissolved in a suitable alcohol before HPLC analysis (12–15). Recently, Edlund (16) developed a rapid procedure for the extraction of CoQ_{10} and $CoQ_{10}H_2$ from plasma, followed by HPLC with electrochemical detection. In order to prevent artefactual $CoQ_{10}H_2$ oxidation, sample preparation was kept to a minimum and consisted only of isopropanol-mediated protein precipitation from an aliquot of plasma. To prevent a coulometric overload, Edlund (16) used a sophisticated dual column system that ensured that only the highly lipophilic components were channelled through the coulometric cells. This HPLC method resulted in reliable $CoQ_{10}H_2$ analysis but is too tedious for clinical trial work, where the analyses of large numbers of samples may be required.

We now report a rapid, simple isocratic HPLC procedure for the determination of $CoQ_{10}H_2$ and CoQ_{10} in plasma isopropanol extracts. We used this method to investigate conditions by which the $CoQ_{10}H_2/CoQ_{10}$ ratio can be reliably measured. Our results indicate that $CoQ_{10}H_2$ is very labile and highly susceptible to oxidation. We found that the $CoQ_{10}H_2/CoQ_{10}$ ratio may only be used as an indicator of in vivo oxidative stress if a rapid extraction and analysis procedure is implemented. Delays or additional steps in the extraction procedure result in a lowered $CoQ_{10}H_2/CoQ_{10}$ ratio, which then only becomes a function of the total analysis time.

MATERIALS AND METHODS

Materials

Oxidized coenzyme Q_{10} was obtained from Sigma (St. Louis, MO), and n-propanol was supplied by Baxter (Muskegon, MI). Methanol was purchased from Millipore (Bedford, MA). All other reagents were supplied by Merck (Darmstadt, Germany).

Determination of $CoQ_{10}H_2$ and CoQ_{10}

Sample preparation. Venous blood with EDTA as anticoagulant was collected and plasma was immediately separated from red blood cells by centrifugation (1600 *g;* 10 min). Coenzyme Q_{10} was extracted from plasma according to the method of Edlund (16). In brief, to 300 µl of plasma or standard coenzyme Q_{10} solution, 1 ml of n-propanol was added; after vigorous mixing the mixture was left to stand for 3 min, followed by remixing and centrifugation at 2500 *g* for 5 min. A supernatant volume of 80 µl was immediately injected for HPLC analysis of CoQ_{10} and $CoQ_{10}H_2$.

HPLC analysis. A Spectra-Physics (Fremont, CA) P1000 pump coupled to a Waters Model U6K Universal Liquid Chromatography Injector valve, was fitted with a Phase Sep S 5 ODS 2 (5 cm × 4.6 mm, Norwalk, CT) reversed-phase analytical column. To protect the analytical column, a Whatman reversed-phase guard column was fitted between the analytical column and the injector valve. The column eluate was monitored by a Coulochem II electrochemical detector (ESA, Bedford, MA) serially fitted with a Model 5021 conditioning cell and a Model 5010 analytical cell. This implies that three working electrodes were connected in series; the first electrode was set at a potential of +500 mV, while the second and third electrodes were set at potentials of -500 mV and +300 mV, respectively. Using the electrochemical detector in the oxidation–reduction–oxidation mode as described by Edlund (16) conferred a high specificity to the detection system and only those compounds able to undergo reversible oxidation at the low potentials used were detected at the third electrode. To protect the coulometric cells, one carbon filter was placed just before the injector valve and another carbon filter was fitted between the analytical column and the conditioning cell. A pneumatically activated 2-way valve was fitted between the column and the Model 5021 conditioning cell. In valve position 1, the mobile phase flowed through the coulometric cells back into the mobile phase reservoir, while valve position 2 short-circuited the mobile phase flow past the coulometric cells directly to a waste container. The mobile phase, (32.5 mM perchloric acid and 57 mM sodium perchloric in methanol with 20% n-propanol (v/v)) was essentially the same as used by Edlund (16) and the flow rate was 1 ml/min. The electrochemical detector was coupled to a Hewlett-Packard interface and a HP SX-16 computer (HP 3365 Series II chemstation) was used for data acquisition.

Standard solutions and reproducibility. A CoQ_{10} stock standard solution of 30 µmol/L was prepared in ethanol and the molar absorption coefficient (ε: 13207 $cm^{-1}mol^{-1}L$) was determined at 275 nm. Fresh stock standard solutions were prepared daily and the exact concentrations were determined by UV absorbance at 275 nm.

The stock solution was diluted to standards containing 150, 600, and 900 nM (in methanol), which were used to calibrate the analysis. Only CoQ_{10} was used for calibration. As detection was achieved by three working electrodes operated in an oxidation–reduction–oxidation mode, both the reduced and oxidized forms of coenzyme Q_{10} were detected as $CoQ_{10}H_2$ at the third electrode. For calibration, peak area was plotted against standard concentration. The electrochemical detector response was linear up to a concentration of at least 3200 nM. For reproducibility experiments, fresh EDTA blood was drawn, plasma was immediately separated from red blood cells, pooled and frozen at -75°C in 500-µl aliquots. The within-day precision was determined by analyzing 10 aliquots on the same day.

Stability of blood samples. The stability of CoQ_{10}, $CoQ_{10}H_2$, and TQ_{10} was investigated in whole blood, plasma, and the n-propanol extract as follows. On 4 consecutive days, different volunteers each donated a fresh blood sample with EDTA as anticoagulant. On the first day, whole blood was kept on ice and 1-ml aliquots were withdrawn at hourly intervals; the plasma was separated from blood cells and analyzed immediately for CoQ_{10}, $CoQ_{10}H_2$, and TQ_{10}. On the second and third days, plasma was immediately separated from blood cells and the plasma was either kept on ice (2nd day) or at room temperature (3rd day). Aliquots were again withdrawn at hourly intervals and analyzed as described above. On the fourth day, an n-propanol extract was prepared from a 1.0-ml plasma sample and the extract was analyzed at hourly intervals. A plasma sample was also divided in aliquots (n = 20), stored at -75°C and analyzed during regular intervals over a 13-month period.

Comparison with an alternative extraction procedure. Fresh plasma samples from nine volunteers were obtained and kept frozen at -75°C. Each plasma sample was thawed separately and analyzed for $CoQ_{10}H_2$ and CoQ_{10} as described above. These plasma samples were also analyzed using the method of Lang and Packer (13). In short, the latter procedure was as follows: to 100 µl of plasma, 400 µl of water was added. This was mixed with 500 µl of a sodium dodecyl sulfate (SDS, 0.02 M) solution. One ml of ethanol was added and the mixture was vortexed for 30 sec. Thereafter, 1 ml of hexane was added and vigorously vortex-mixed for 2 min. To separate the layers, the mixture was centrifuged for 5 min at 2500 *g*. From the hexane layer, 80 µl was evaporated to dryness under a stream of nitrogen gas and the residue was redissolved in 300 µl methanol–ethanol 1:1 mixture. Eighty µl of this solution was used for HPLC analysis.

Determination of plasma cholesterol concentrations

Serum total cholesterol concentrations were determined by a multi-channel, continuous flow analyzer ("SMAC", Technicon Instrument, Tarrytown, NY). The cholesterol analytical method is based on automated enzymatic analyses according to Technicon method no SU4-0092B88.

Studies in humans

Blood samples from 130 apparently healthy young male police recruits stationed at the Pretoria Training College were analyzed for plasma CoQ_{10}, $CoQ_{10}H_2$, and serum total cholesterol levels. The group of young men consisted of 69 black males aged 18–31 [mean(SD) = 21.7(3.27)] years and 61 white males aged 18–27 [mean(SD) = 19.4(1.62)] years. Another 81 blood samples were obtained from apparently healthy white males aged 19–62 [mean(SD) = 38.9(11.9)] years living in the Pretoria region, and were analyzed for plasma $CoQ_{10}H_2$ and CoQ_{10}. The blood samples were immediately cooled on ice, plasma was removed by low speed centrifugation and immediately thereafter frozen on dry ice. The time between blood sampling and freezing of the plasma in dry ice was not more than 15 min. The samples were transferred to the laboratory in dry ice and upon arrival stored in a -75°C freezer. Plasma samples were thawed one at a time for coenzyme Q_{10} analysis as described above.

Statistics

The $CoQ_{10}H_2$, CoQ_{10}, and TQ_{10} concentrations between smokers (n = 61) and non-smokers (n = 81) in white males were compared with the Student's *t* test. Pearson's coefficient of variation was used to establish correlation between variables.

RESULTS

Figure 1A illustrates a chromatogram obtained after analysis of an isopropanol extract from plasma. The mobile phase flowed through the coulometric cells during the whole analysis period (postcolumn 2-way valve in position 1). An unknown number of unidentified electroactive components eluted from the column within the first 4 min, thus partially overlapping with the $CoQ_{10}H_2$ peak. This cluster of unknown components eluting early from the column was expected, because *i)* very little sample clean-up was performed before HPLC analysis, and *ii)* a strong mobile phase was used, implying that biomolecules more hydrophillic than $CoQ_{10}H_2$ will not be retained on the column but will elute together with or just after the solvent front. To improve the chromatograms and to protect the coulometric cells from the potentially damaging effect of the very high loads of electroactive components in the first minutes after sample injection, the postcolumn valve was

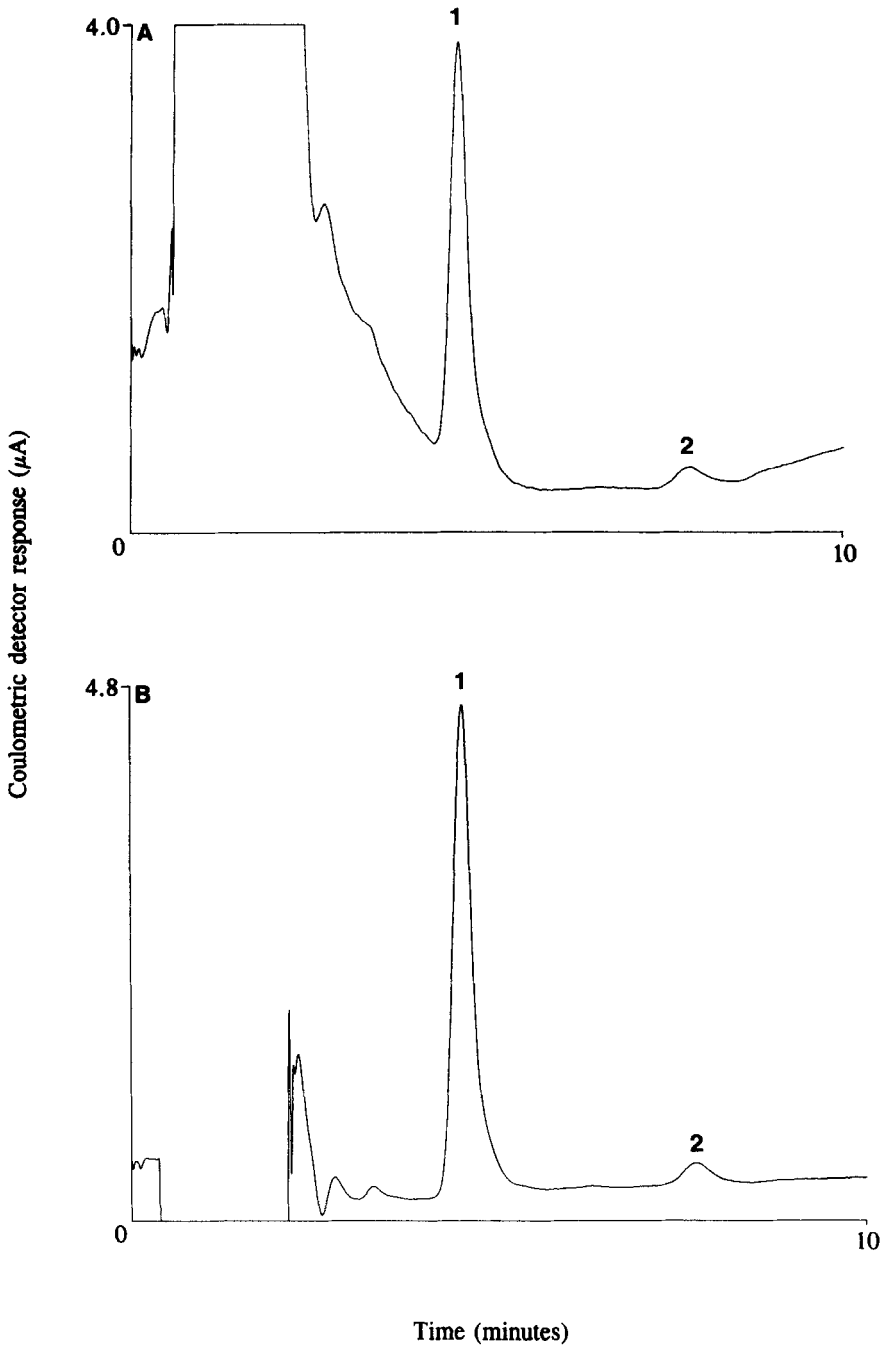

Fig. 1. HPLC determination of plasma $CoQ_{10}H_2$ and CoQ_{10} with electrochemical detection. A: Postcolumn valve in position 1. B: Postcolumn valve in position 2 for the first 2 min of analysis. Peaks: 1, $CoQ_{10}H_2$; 2, CoQ_{10}.

switched to position 2 with each sample injection. The unidentified components eluting within the first 2 min after sample injection were thus diverted from the analytical cells to the waste container. After 2 min, the pneumatically activated valve was switched to position 1, channelling mobile phase flow through the coulometric cells for detection of the compounds of interest. Because the coulometric detector was used at a relatively low sensitivity setting, the baseline perturbation associated with the disruption of mobile phase flow was minimal and the baseline stabilized before $CoQ_{10}H_2$ was eluted from the column (Fig. 1B). Comparing Figs. 1A and B shows clearly that using the postcolumn valve as described above, removed background interference

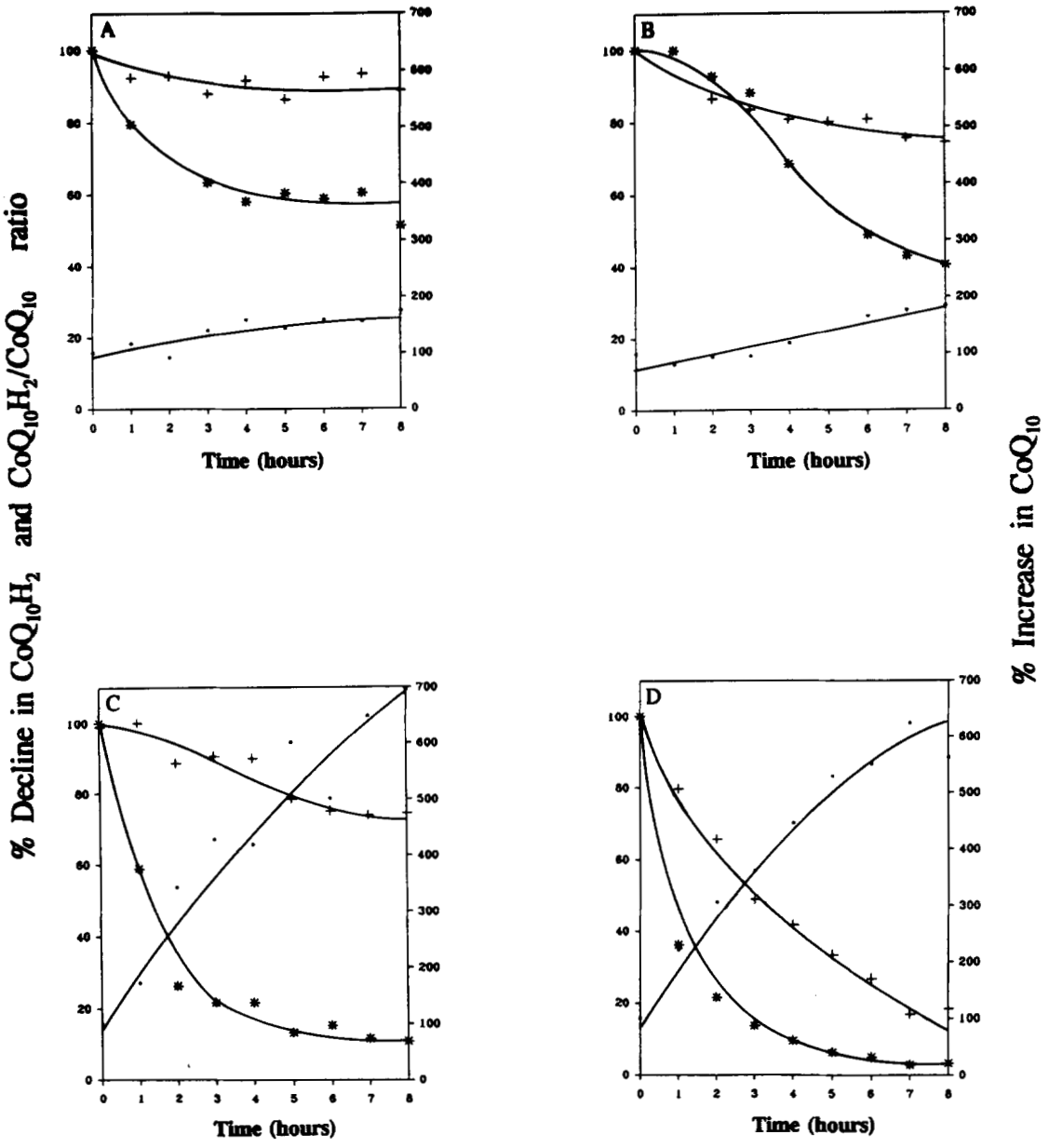

Fig. 2. Stability of $CoQ_{10}H_2$, CoQ_{10}, and $CoQ_{10}H_2/CoQ_{10}$ ratio in (A) whole blood kept on ice; (B) plasma kept on ice; (C) plasma at room temperature; and (D) n-propanol extract of plasma; (+), $CoQ_{10}H_2$; (■), CoQ_{10}; (*), $CoQ_{10}H_2/CoQ_{10}$ ratio.

which otherwise would have complicated reliable and reproducible measurement of $CoQ_{10}H_2$ and CoQ_{10}.

Our results indicate that $CoQ_{10}H_2$ is unstable in whole blood, plasma, and the n-propanol extract (**Fig. 2**). The decline in $CoQ_{10}H_2$ concentrations was accompanied by an increase in CoQ_{10} levels, indicating that the $CoQ_{10}H_2$ loss could be mainly explained by oxidation. Consequently, the $CoQ_{10}H_2/CoQ_{10}$ ratio changed substantially within an hour after the plasma sample had been obtained. The $CoQ_{10}H_2$ oxidation in whole blood continued at a slower rate when compared to plasma or the isopropanol extract (Fig. 2), which may be explained by possible competition between the reduction of CoQ_{10} by red blood cells (17) and the oxidation of $CoQ_{10}H_2$ by air. In contrast to the lability of $CoQ_{10}H_2$ at the conditions described above, this compound remained stable for at least 13 months when aliquots of a plasma sample were stored at -75°C (**Table 1**). Although there was some variation in plasma coenzyme Q_{10} levels over time, the ratio remained relatively constant.

The analytical performance of the method was satisfactory; the mean (SD) plasma CoQ_{10}, $CoQ_{10}H_2$, TQ_{10} concentrations and $CoQ_{10}H_2/CoQ_{10}$ ratio determined (n = 10) in a pooled plasma sample were 15.3 (1.2) nM;

TABLE 1. Stability of CoQ_{10}, $CoQ_{10}H_2$, $CoQ_{10}H_2/CoQ_{10}$ ratio, and TQ_{10} when plasma was stored at -75°C

Time of Storage	$CoQ_{10}H_2$	CoQ_{10}	$CoQ_{10}H_2/CoQ_{10}$ Ratio	TQ_{10}
Weeks	nM	nM		nM
0	843.6	106.3	7.8	941.0
2	930.3	93.8	9.9	1024.1
3	1110.3	117.5	9.4	1227.8
4	1020.5	125.5	8.1	1145.9
5	1164.6	114.3	10.2	1278.8
8	914.6	103.3	8.8	1017.9
43	1072.3	97.7	10.9	1170.0
48	1101.9	96.2	11.5	1198.2
49	1174.9	104.6	11.2	1279.5
50	1056.9	98.3	10.8	1155.3
Means(SD)	1038.1(106.8)	105.8(9.8)	9.9(1.2)	1143.8(109.0)
CV (%)	10.3	9.3	12.3	9.5

454 (15.2) nM; 470.1 (16.2) nM and 29.7 (1.6), respectively, with within-day coefficients of variation of 7.9% for CoQ_{10}, 3.4% for $CoQ_{10}H_2$, 3.5% for TQ_{10}, and 5.7% for the $CoQ_{10}H_2/CoQ_{10}$ ratio. When aliquots from a plasma sample were stored at -75°C and analyzed at regular intervals, the coefficients of variation were 10.3%, 9.3%, 9.5%, and 12.3% for $CoQ_{10}H_2$, CoQ_{10}, TQ_{10}, and $CoQ_{10}H_2/CoQ_{10}$ ratio, respectively (Table 1).

As coenzyme Q_{10} is transported in the circulation by lipoproteins and is synthesized by the mevalonate pathway which is also used for cholesterol biosynthesis, we investigated the relation between coenzyme Q_{10} and plasma total cholesterol concentrations. **Figure 3A** depicts the correlation between plasma $CoQ_{10}H_2$ and total cholesterol concentrations. $CoQ_{10}H_2$ concentrations correlated significantly ($r = 0.43$; $P < 0.0001$) with plasma cholesterol concentrations. Similar results were observed for CoQ_{10} (Fig. 3B) and TQ_{10} concentrations (Fig. 3C), which also correlated significantly with plasma total cholesterol levels ($r = 0.32$; $P = 0.002$ and $r = 0.44$; $P < 0.0001$, respectively). In contrast, the ratio ($CoQ_{10}H_2/CoQ_{10}$) is not significantly correlated with plasma total cholesterol levels (Fig. 3D), indicating that this ratio is independent from circulating cholesterol (and lipoprotein) concentrations.

Table 2 summarizes $CoQ_{10}H_2/CoQ_{10}$ ratios obtained with Edlund's (16) rapid extraction procedure (method 1) and with the extraction procedure of Lang and Packer (13) (method 2). Comparing the two methods, considerably lower plasma $CoQ_{10}H_2$ and higher CoQ_{10} concentrations were found when the longer extraction procedure of Lang and Packer was used (13). This resulted in $CoQ_{10}H_2/CoQ_{10}$ ratios that were more than 3-fold lower than those obtained with Edlund's extraction procedure (16). **Table 3** compares plasma coenzyme Q_{10} levels of white male smokers and non-smokers. There were no significant differences in the plasma concentrations of $CoQ_{10}H_2$, CoQ_{10}, TQ_{10}, or the $CoQ_{10}H_2/CoQ_{10}$ ratio, between the two groups.

DISCUSSION

The antioxidant properties of $CoQ_{10}H_2$ have been studied in vitro (3). It is postulated that $CoQ_{10}H_2$ prevents the initiation and/or the propagation of lipid peroxidation in plasma lipoproteins and biological membranes (4). Stocker, Bowry, and Frei (3) investigated the in vitro protection conveyed by various lipid-soluble antioxidants including $CoQ_{10}H_2$, lycopene, β-carotene, and α-tocopherol when exposed to peroxyl radicals. During this exposure, rapid and complete oxidation of $CoQ_{10}H_2$ was followed by depletion of the other antioxidants, which indicated that $CoQ_{10}H_2$ was the first antioxidant to be affected when LDL was exposed to free radical production. The study done by Bowry et al. (6) revealed that LDL particles were effectively protected from peroxyl radical-mediated oxidation until $CoQ_{10}H_2$ present in LDL was consumed.

As $CoQ_{10}H_2$ is the first antioxidant to be oxidized when lipoproteins are subjected to oxidative stress, it has been postulated that the $CoQ_{10}H_2/CoQ_{10}$ ratio may be a sensitive in vivo marker of oxidative stress (3, 6). However, the sensitivity of $CoQ_{10}H_2$ towards oxidation complicates reliable quantitation of this compound. If the $CoQ_{10}H_2/CoQ_{10}$ ratio is to be utilized as indicator of in vivo oxidative stress, then it is of paramount importance to minimize $CoQ_{10}H_2$ oxidation during the analytical procedure. We therefore studied conditions that are suitable for $CoQ_{10}H_2$ analysis.

Our results indicate that $CoQ_{10}H_2$ is unstable in whole blood, plasma, and isopropanol extracts; subsequently the $CoQ_{10}H_2/CoQ_{10}$ ratio changes considerably within an hour after the blood sample has been obtained, This implies that $CoQ_{10}H_2$ should only be determined in very fresh blood samples. To ensure reliable results, we limit the time span from when a blood specimen has been obtained until HPLC analysis to 15 min. The lability of $CoQ_{10}H_2$ limits the practical application of this method because only 8–10 samples can be analyzed per day. When plasma is kept frozen at -75°C, $CoQ_{10}H_2$ is stable for at least 13 months and the $CoQ_{10}H_2/CoQ_{10}$ ratio does not change significantly during this period. This implies that when blood samples are obtained from several patients at the same time, the plasma samples may be safely stored for later analysis.

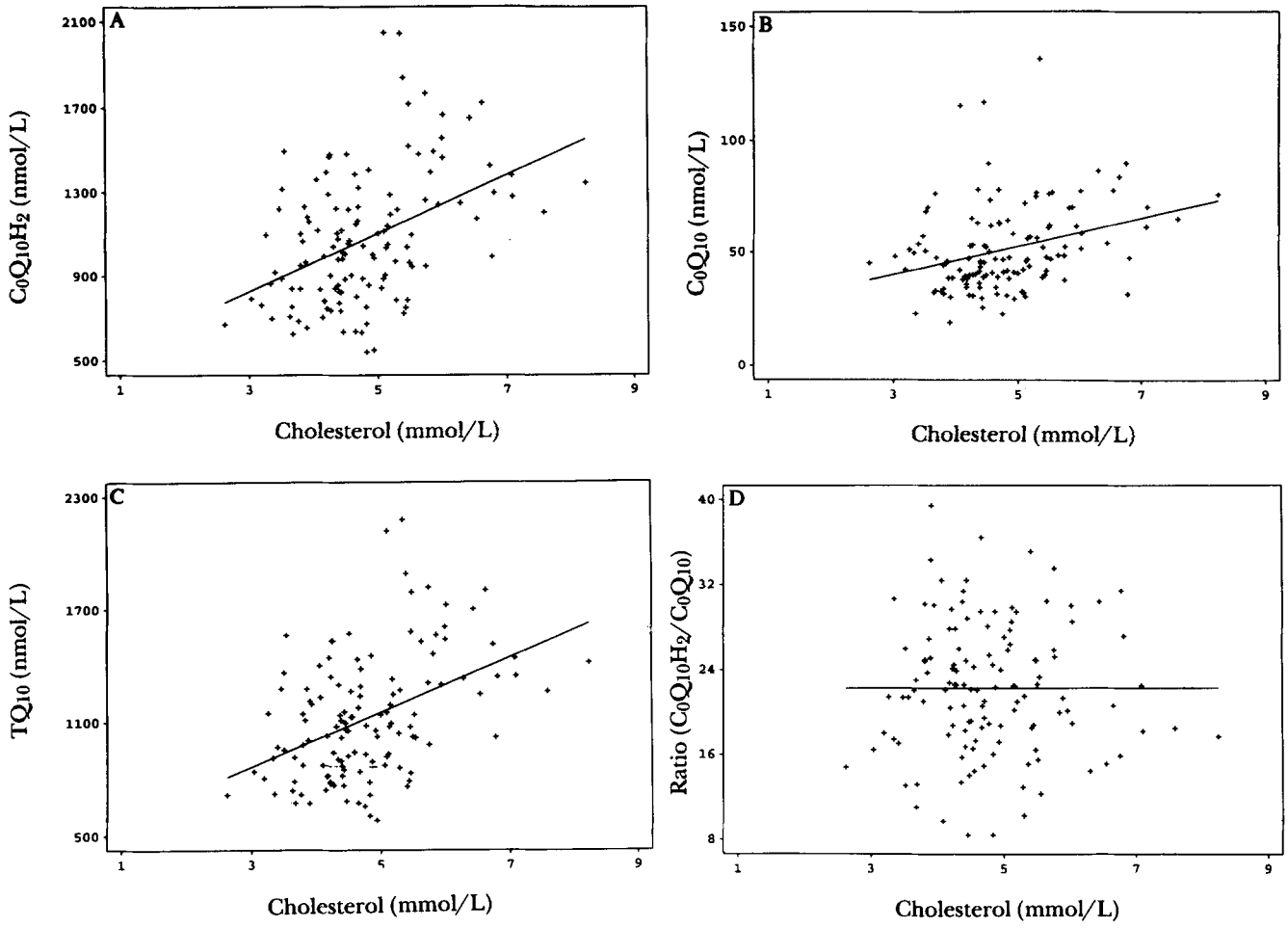

Fig. 3. Linear regression between plasma cholesterol and (A) $CoQ_{10}H_2$, (B) CoQ_{10}, (C) TQ_{10}, and (D) $CoQ_{10}H_2/CoQ_{10}$ ratio as measured in 130 apparently healthy men. Regression equations for A: y = 138.2x + 411.9; correlation coefficient (r) = 0.43 ($P < 0.0001$); B: y = 6.3x + 21.14; r = 0.32 ($P = 0.002$); C: y = 144.5x + 433.02; r = 0.44 ($P < 0.0001$); and D: y = 0.003x + 22.2; r = 0.

The lability of $CoQ_{10}H_2$ dictates that a rapid HPLC procedure should be used and that sample clean-up procedures should be kept to a minimum. The HPLC method of Edlund (16) to determine $CoQ_{10}H_2$ and CoQ_{10} complies with above-mentioned requirements. Sample clean-up consists only of isopropanol extraction of the plasma sample, which is then directly analyzed by HPLC and coulometric detection. Normally, coulometric detection requires careful sample clean-up to protect the analytical cells, but if clean-up procedures have to be limited, other approaches are required to optimize and protect the electrochemical detector. Edlund (16) used a sophisticated dual-column system where CoQ_{10}, $CoQ_{10}H_2$, and other lipophilic compounds were first retained on a short precolumn that was eluted by a relatively polar mobile phase. More polar compounds were eluted from the column to a waste container. After removal of the polar compounds, the retained components including CoQ_{10}, $CoQ_{10}H_2$, cholesterol, and vitamin E were transferred to the analytical column by using a stronger mobile phase. The coulometric cells were therefore only exposed to the relatively nonpolar constituents of the isopropanol extract. The disadvantage of this method is that the system is complicated, requiring two HPLC pumps as well as two further valves in addition to the injection valve and several valve switching steps per analysis.

As the coulometric detector can be used at a low sensitivity for the determination of $CoQ_{10}H_2$ and CoQ_{10} in human plasma, we found that a postcolumn two-way valve could be utilized to divert earlier eluting compounds from the analytical column directly to waste. Approximately 2 min before $CoQ_{10}H_2$ is eluted from the column, the valve is switched to allow the mobile phase to flow through the analytical cells. The analytical cells are therefore protected from a coulometric overload as the bulk of relatively polar compounds is directly eluted in the waste container. This isocratic HPLC pro-

TABLE 2. A comparison of two extraction methods to determine coenzyme Q_{10}

	Method 1			Method 2		
	Concentration (nM)			Concentration (nM)		
Sample No.	$CoQ_{10}H_2$	CoQ_{10}	Ratio	$CoQ_{10}H_2$	CoQ_{10}	Ratio
1	1382.1	81.0	17.0	962.5	164.8	5.8
2	480.8	30.2	15.9	238.7	61.9	3.9
3	1848.1	106.4	17.4	949.0	163.7	5.8
4	1353.6	80.6	16.8	731.5	121.8	6.0
5	976.0	56.7	17.2	592.1	85.5	6.9
6	1525.2	116.2	13.1	776.0	147.6	5.3
7	1402.5	75.0	18.7	748.7	147.8	5.1
8	1257.5	59.7	21.1	806.1	124.3	6.5
9	1281.7	86.0	14.9	660.8	317.8	21.
Mean	1269.4	77.1	16.7	718.4	148.4	5.3

Method 1 is the rapid extraction method of Edlund (16), while method 2 is the longer extraction method described by Lang and Packer (13).

cedure is much simpler than that reported by Edlund and requires only one extra valve in addition to the injection valve.

When the $CoQ_{10}H_2/CoQ_{10}$ ratios obtained with our method are compared to previously published results, it becomes clear that other investigators may have been unaware of the extreme sensitivity of $CoQ_{10}H_2$ towards oxidation. Dudman et al. (7) reported a mean (SD) $CoQ_{10}H_2/CoQ_{10}$ ratio of 1.6 (1.3) in 14 normal subjects, which is considerably lower than our mean ratio of 25.3 (7.9) in 142 white men. Bowry et al. (6) reported similar low $CoQ_{10}H_2/CoQ_{10}$ ratios. We investigated whether the low $CoQ_{10}H_2/CoQ_{10}$ ratios reported by Dudman et al. and Bowry et al. could have been an artefact due to excessive $CoQ_{10}H_2$ oxidation during the extraction procedure. Both investigators mentioned above used the simplified extraction procedure of Lang et al. (13) to extract coenzyme Q_{10} from plasma. Table 2 indicates that the $CoQ_{10}H_2/CoQ_{10}$ ratio declined more than 3-fold when the extraction procedure of Lang et al. (13) was compared with Edlund's extraction method (16). The reason for the changed ratio was a substantial decline in $CoQ_{10}H_2$ concentrations and increased CoQ_{10} concentrations when the extraction procedure of Lang et al. was used. This strongly suggests artefactual $CoQ_{10}H_2$ oxidation during the extraction procedure; possibly the evaporation to dryness of the hexane extract from plasma contributed substantially towards $CoQ_{10}H_2$ oxidation. It is also of interest to note that the decrease in $CoQ_{10}H_2$ was not matched by an equimolar increase in CoQ_{10}, suggesting partial degradation of $CoQ_{10}H_2$ to other, nondetectable products. It may therefore be concluded that sample preparation may have a profound effect on the redox status of coenzyme Q_{10} and that utmost care is required to ensure reliable estimates of the $CoQ_{10}H_2/CoQ_{10}$ ratio.

Lipid peroxidation has been suggested to play a crucial role in the pathogenesis of atherosclerosis. Elevated circulating malondialdehyde concentrations have been demonstrated in patients with myocardial infarction (18); however, the significance of this finding is unclear because no circulating lipid hydroperoxides could be demonstrated in human plasma using very sensitive analytical techniques such as HPLC with chemiluminescence detection (19, 20). The sensitivity of $CoQ_{10}H_2$ towards oxidation suggests that an altered $CoQ_{10}H_2/CoQ_{10}$ ratio may be the most sensitive indicator of elevated in vivo oxidative stress, provided that artefactual $CoQ_{10}H_2$ oxidation is minimized in the analytical procedure. Although blood sampling requirements for reliable $CoQ_{10}H_2$ analyses are demanding, we believe that the $CoQ_{10}H_2/CoQ_{10}$ ratio may be a practical way to measure in vivo oxidative stress. This indicator is independent from total circulating cholesterol concentrations and may therefore be used to compare

TABLE 3. Comparison of plasma coenzyme Q_{10} levels in white male smokers and non-smokers

Variable	Smokers (n=61)	Non-smokers (n=81)
Age (yr)	31.1 (10.4)	32.0 (12.5)
$CoQ_{10}H_2$ (nM)	1114.8 (355.4)	1178.1 (345.2)
CoQ_{10} (nM)	50.0 (21.8)	49.1 (19.1)
TQ_{10} (nM)	1164.8 (366.0)	1227.3 (355.8)
$CoQ_{10}H_2/CoQ_{10}$	24.4 (7.7)	26.0 (8.1)

Values given as means (± SD). There were no significant differences between smokers and non-smokers.

groups who differ significantly with respect to plasma cholesterol concentrations.

We used the $CoQ_{10}H_2/CoQ_{10}$ ratio as a indicator of oxidative stress in smokers and non-smokers. Although this ratio was lower in the group of smokers, the difference was not statistically significant, indicating that the redox status of coenzyme Q_{10} was not significantly affected by habitual smoking. It should be emphasized, however, that our observations have not been corrected for possible confounders such as the number of cigarettes smoked per day or the time interval between the last cigarette smoked and phlebotomy. Nevertheless, our results may indicate that circulating LDL is well protected against peroxidation and is not affected by higher levels of oxidative stress associated with smoking. Presumably, the antioxidant defence mechanisms that include enzymes (e.g., glutathione peroxidase) as well as vitamins (e.g., vitamin C) are sufficient to protect circulating LDL against oxidative stress induced by smoking. Considering that lipid hydroperoxides also seem to be absent in plasma (19, 20), the question is raised whether oxidative modification of LDL occurs at all in circulation. As an altered $CoQ_{10}H_2/CoQ_{10}$ ratio is the first sign of LDL exposure to oxidative stress, this indicator should be used in future investigations to establish the role of oxidative modification of LDL in disease.

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