



ORIGINAL ARTICLE

Coenzymes Q₉ and Q₁₀: Contents in Foods and Dietary Intake

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Coenzyme Q₉ and Q₁₀ contents in 35 food items were determined and coenzyme Q intake of Finns was estimated. The analytical method employed direct solvent extraction or saponification before extraction and quantification using high-pressure liquid chromatography (HPLC) equipped with diode array detection. Intakes of coenzymes Q₉ and Q₁₀ were estimated using the determined values and food consumption data from a national dietary survey. Contents of coenzymes Q₁₀ and Q₉ in foods varied from 157.9 µg/g to below the detection limit and from 8.5 µg/g to below the detection limit, respectively. Average intakes of coenzyme Q₁₀ were 5.4 mg/day (men) and 3.8 mg/day (women) while daily intakes of coenzyme Q₉ were 0.6 mg (men) and 0.4 mg (women). Coenzyme Q₁₀ was primarily obtained from meat, poultry, fish and rapeseed oil. Cereals were the major source of coenzyme Q₉. © 2001 Academic Press

Key Words: coenzyme Q₁₀; coenzyme Q₉; ubiquinone; intake; contents in foods.

INTRODUCTION

Coenzyme Q (ubiquinone) is a lipid soluble, endogenous hydroxybenzoquinone compound found in the majority of aerobic organisms (Lütke-Brinkhaus *et al.*, 1984; Lang *et al.*, 1986; Battino *et al.*, 1990) in two redox forms, and varying length of the isoprenoid side chain (6–10 isoprenoid units). The predominant form of coenzyme Q in humans and most animals is coenzyme Q₁₀, containing 10 isoprenoid units in the side chain (Lester and Crane, 1959).

It is well known that coenzyme Q plays an important role as an essential electron carrier in the mitochondrial respiratory chain. In addition, it has been shown that coenzyme Q (mainly in the reduced form) can act as an antioxidant, protecting numerous cellular membranes and plasma lipoproteins from free radical-induced damage (e.g., Beyer, 1990; Frei *et al.*, 1990; Ernster and Dallner, 1995). Exogenous coenzyme Q may also have beneficial effects on human health: i.e., coenzyme Q present in food or taken as a dietary supplement appears to act primarily by elevating the coenzyme Q level in blood, where it may function as an antioxidant (e.g., Mohr *et al.*, 1992; Weber *et al.*, 1994; Ernster and Dallner, 1995).

Despite the presumption that exogenous coenzyme Q may have beneficial effects on human health, only few studies have assessed contents of coenzyme Q in foods (Page *et al.*, 1959; Lester and Crane, 1959; Kraszner-Berndorfer and Kovats, 1972;

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Kamei *et al.*, 1986; Weber *et al.*, 1997). In particular, Page *et al.* (1959) and Lester and Crane (1959) did not obtain sufficient sensitivity and selectivity for the determination of coenzyme Q. Subsequent development of high-pressure liquid chromatography (HPLC) techniques has enabled the reliable determination of coenzyme Q in foods. In addition to scant information on its contents in foods, there is little data on average dietary intake of coenzyme Q in any population. To our knowledge, only one study (based on dietary survey data) has been published (Weber *et al.*, 1997).

The present study sought to determine coenzyme Q₉ and Q₁₀ contents in different food groups and estimate the average dietary intake of these compounds in Finland using both the data generated and a recently published dietary survey of Finnish adults (National Public Health Institute, 1998).

MATERIALS AND METHODS

Sampling

Milk products and meat samples were purchased from major dairies and slaughterhouses, respectively, as well as from retail stores. Bread samples were purchased from major bakeries. Vegetables were collected from large-scale producers, wholesalers, retail stores and a local marketplace, fruit samples from the wholesalers and retail stores, and berry samples from producers, wholesalers, and a local marketplace. Egg, fish and rapeseed oil samples were purchased from retail stores. The number of subsamples most frequently exceeded 10. In the laboratory, samples were pooled, homogenized and stored at -18°C until analysis. Bread, vegetable, fruit and berry samples were also lyophilized before analysis.

Standards

Coenzyme Q₉ and Q₁₀ standards were obtained from Sigma Chemicals Co., U.S.A. Coenzyme Q₉ and Q₁₀ stock solutions were prepared by dissolving 10 mg of each in 100 ml of ethanol (99%). Concentrations of the standard solutions were confirmed by measuring absorbance at a wavelength of 275 nm, and by reference to known coefficients ($E_{1\text{ cm}}^{1\%}$ 185 for coenzyme Q₉ and 165 for coenzyme Q₁₀; Hatefi, 1963). Working standard solutions (five levels of concentration) were in the range *c.* 2.5–55 µg/mL.

Extraction

Extraction of samples other than rapeseed oil, pork liver, beef liver and Emmental cheese was performed using a solvent extraction method according to Ikenoya *et al.* (1981), Okamoto *et al.* (1988), Weber *et al.* (1997), and Mattila *et al.* (2000) with some modifications. A 2-g sample of meat, fish, bread, vegetable, fruit and berry was weighed into an extraction tube and homogenized in 5 mL 0.15 M NaCl (saline) with a Heidolph Diax 600 homogenizer (Heidolph, Germany). Sample size of orange juice, lingonberry juice, milk, and yogurt was 7 g and saline was not added to these samples. Eight milliliters of ethanol was added to the sample-saline mixtures and the 7-g samples followed by homogenization as above. After that, 20 mL *n*-hexane (HPLC-grade) was added into a tube and mixed vigorously for 10 min using a Heidolph Promax 2020 shaker (Heidolph, Germany). The tube was quickly centrifuged to separate the layers. Hexane layer was saved and the lower layer was re-extracted twice with 5 mL ethanol and 20 mL *n*-hexane. The combined *n*-hexane layer was evaporated with a Rotavapor (30–40°C) and the residue dissolved in 5 mL (reindeer, pork

heart, beef heart) or in 2 mL (other samples) 2-propanol and filtered with Lida PTFE membrane filter (0.2 μm) prior to the HPLC analysis.

Extraction of rapeseed oil, pork liver, beef liver and Emmental cheese was performed using a saponification method according to Salo-Väänänen *et al.* (2000) with some modifications. Two grams of pork liver, beef liver and Emmental cheese or 0.2 g of rapeseed oil was weighed into a 50-mL plastic tube. Two milliliters of 2% ascorbic acid (in water) and 5 mL methanol were added and the tube was homogenized using a Heidolph Diax 600 homogenizer (Heidolph, Germany). After homogenization 0.5 mL KOH solution (50 mL KOH + 50 mL H₂O) was added and the tube was capped and transferred into a boiling water bath for 10 min. The sample extract was vortexed after 5 min of boiling. After cooling the tube in a cold water bath 7 mL of 10% NaCl was added into the tube to avoid emulsion formation.

After saponification the coenzymes Q₉ and Q₁₀ were extracted using three 20 mL portions of *n*-hexane. Extraction was performed each time by shaking (2 min) with a Heidolph Promax 2020 shaker (Heidolph, Germany), centrifuging to separate hexane layer and collecting this layer into a 100 mL tube. The combined layer was washed with 20 mL of 5% NaCl. After washing, the organic layer was transferred to a 250 mL bottle and evaporated with a Rotavapor (30–40°C) to dryness. To remove the remaining water from the samples 5 mL of ethanol (99%) and 5 mL of *n*-hexane were added into the bottle and the solution was re-evaporated to dryness. The residue was dissolved in 2 mL of *n*-hexane-2-propanol solution (50% Hex: 50% IPA for rapeseed oil and 30% Hex: 70% IPA for the other samples) and the sample extract was filtered with Lida PTFE membrane filters (0.2 μm) prior to the HPLC analysis.

HPLC Analysis

The analytical HPLC system consisted of a Hewlett-Packard 1090 Series II High-Performance Liquid Chromatograph equipped with a diode array detector (Hewlett-Packard, U.S.A.) and a Vydac 201TP54 column (5 μm , 25 cm \times 4.6 mm, The Separations Group, U.S.A.) fitted with an ODS guard column. Instrumentation was controlled by the HP 3D Chem Station computer program Rev. A.06.01. The mobile phase consisted of methanol, 2-propanol and ethanol (70:15:15, all HPLC grade); the flow rate was 0.8 mL/min, and the injection volume 10 μL . The HPLC analysis was performed at room temperature. Coenzymes Q₉ and Q₁₀ were quantified by an external standard method, and the quantification was based on peak area. The wavelength used for identification and quantification of the coenzymes was 275 nm.

Method Reliability Tests

Linearity of detector response was tested for coenzymes Q₉ and Q₁₀ and found to be linear in the range of 12–500 ng per injection, the coefficients of correlation being 0.9998 for coenzyme Q₉ and 0.99995 for coenzyme Q₁₀. The accuracy of the method was controlled by recovery tests by spiking the studied compounds into the samples before extraction. Recovery tests at least using coenzyme Q₁₀ were made for all samples determined. Mean recovery for coenzyme Q₉ was $92 \pm 7.2\%$ ($n = 14$) and that for coenzyme Q₁₀ $93 \pm 9.0\%$ ($n = 38$). Repeatability tests were done by monitoring the coefficient of variation (cv.%) of the triplicated samples. Mean cv.% was $8 \pm 5.5\%$ ($n = 31$) for coenzyme Q₁₀ and $15 \pm 11.1\%$ ($n = 21$) for coenzyme Q₉. Detection limits for coenzymes Q₉ and Q₁₀ were 3 and 5 ng/injection, respectively. The identity and purity of the coenzyme peaks were confirmed with DAD system comparing the spectral data of the sample peaks with those obtained for the coenzyme standards.

Calculations of Coenzyme Q₉ and Q₁₀ Intakes in the Finnish Diet

The presently determined coenzyme Q₉ and Q₁₀ contents, as well as the data on the average food consumption by the Finnish men and women (National Public Health Institute, 1998) were used to calculate coenzyme Q₉ and Q₁₀ intakes.

TABLE 1
Contents of coenzymes Q₉ and Q₁₀ in foods (µg/g fresh wt.)

Food	CoQ ₉	CoQ ₁₀
Meat, poultry and egg		
Reindeer	8.5	157.9
Pork heart	3.1	126.8
Beef heart	n.d.	113.3
Beef liver	1.4	39.2
Pork liver	1.2	22.7
Beef	0.4	36.5
Pork ham	0.9	20.0
Chicken	0.4	14.0
Egg	n.d.	1.2
Dietary fats		
Rapeseed oil	n.d.	63.5
Fish		
Tuna, canned	0.3	15.9
Baltic herring	n.d.	15.9
Pollack, frozen	0.6	14.4
Rainbow trout	0.3	8.5
Cereals		
Crispbread, rye	4.7	n.d.
Bread (rye)	4.7	n.d.
Bread (wheat)	2.1	n.d.
Vegetables		
Pea	0.1	2.7
Cauliflower	0.04	2.7
Bean	0.06	1.8
Carrot	n.d.	1.7
Tomato	n.d.	0.9
Potato	n.d.	0.5
Fruits and berries		
Blackcurrant	0.8	3.4
Lingonberry	2.9	0.9
Strawberry	0.1	1.4
Orange	n.d.	1.4
Apple	0.2	1.3
Clementine	n.d.	0.9
Orange juice	n.d.	0.3
Lingonberry juice	0.08	n.d.
Dairy products		
Yogurt	n.d.	2.4
Cheese, Emmental	n.d.	1.3
Cheese, Edam	n.d.	1.2
Milk (1.5% fat)	n.d.	0.1

Note: n.d.: not detected.

RESULTS AND DISCUSSION

Coenzymes Q₉ and Q₁₀ have been extracted from food and biological tissues using either direct ethanol-*n*-hexane extraction (Ikenoya *et al.*, 1981; Lang *et al.*, 1986; Lang and Packer, 1987; Okamoto *et al.*, 1988; Weber *et al.*, 1997) or saponification before solvent extraction (Abe *et al.*, 1978; Kamei *et al.*, 1986). Direct ethanol-*n*-hexane extraction was employed for most of the samples in this study because it was simple to perform and efficient for tissue samples (Ikenoya *et al.*, 1981). In addition, according to Weber *et al.* (1997) it appeared to give comparable results with the saponification procedure. However, direct solvent extraction was not suitable for some samples (pork and beef liver, rapeseed oil and Emmental cheese) resulting in interfering compounds which made quantification difficult. These samples could be accurately quantified only by using the saponification and extraction procedures. No reduced coenzyme Q was detected after saponification or direct solvent extraction, thus ensuring conversion of all coenzyme Q into the oxidized form.

Both UV- and electrochemical detection can be employed in coenzyme Q analysis. Although the electrochemical detection was much more sensitive than the diode array detection, it lacked selectivity in some cases (Mattila *et al.*, 2000). Hence, in this study coenzymes Q₉ and Q₁₀ were quantified using diode array detection. The chromatographic conditions used effectively separated coenzymes Q₉ and Q₁₀ from each other and the matrix, allowing reliable quantification of these compounds.

Contents of coenzymes Q₉ and Q₁₀ in different food items are shown in Table 1. Coenzyme Q₁₀ was found in almost all samples determined, and contents ranged from 157.9 µg/g (reindeer steak) to below the detection limit (cereals). As expected, meat and fish were the richest sources of coenzyme Q₁₀, because muscle tissue contains high levels of mitochondria. Especially, reindeer steak as well as beef and pork heart had very high contents of coenzyme Q₁₀ (113.3–157.9), while the contents in fish and other meat samples varied in the ranges 14.0–39.2 and 8.5–15.9 µg/g, respectively. Weber *et al.* (1997) and Kamei *et al.* (1986) reported similar results for meat and fish samples (Table 3) though Weber *et al.* (1997) performed analyses from processed samples and Kamei *et al.* (1981) used saponification before extraction for all samples. In addition to meat and fish also, rapeseed oil is a good source of coenzyme Q₁₀. The determined value of 63.5 µg/g was very close to the value reported by Kamei *et al.* (1986; 73.4 µg/g). Kamei *et al.* (1986) determined coenzymes Q₉ and Q₁₀ in 11 different vegetable oils and found highest contents of coenzyme Q₁₀ in soybean (92.3 µg/g) and

TABLE 2
Dietary intake of coenzymes Q₉ and Q₁₀ from different food groups

Food group	Consumption (g/day)		CoQ ₁₀ (mg/day)		CoQ ₉ (mg/day)	
	Men	Women	Men	Women	Men	Women
Meat	130	79	3.2	1.9	0.09	0.05
Eggs	22	16	0.03	0.02	0	0
Fish	32	25	0.44	0.35	0.01	0.01
Dairy products	537	405	0.35	0.37	0	0
Cereals	209	150	0	0	0.47	0.33
Fruits and berries	173	214	0.18	0.23	0.04	0.05
Vegetables	243	206	0.25	0.26	0	0
Vegetable fats	29	19	0.99	0.66	0	0
Total			5.4	3.8	0.6	0.4

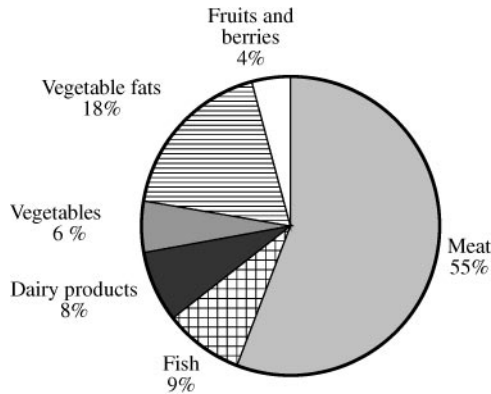
FIGURE 1. Coenzyme Q₁₀ intake from different food groups.

TABLE 3
Contents of coenzymes Q₉ and Q₁₀ in foods according to different studies

Food	Q ₉ µg/g, this study	Q ₉ µg/g other studies ^{1,2}	Q ₁₀ µg/g, this study	Q ₁₀ µg/g other studies ^{1,2}
Beef	0.4	2.6 (1), n.d. (2)	36.5	31 (1), 31.0 (2)
Chicken	0.4	0.81 (1), n.d. (2)	14.0	17 (1), 21 (2)
Pork	0.9	0.32 (1), n.d. (2)	20.0	7.7 (1), 24.3–41.1 (2)
Pork heart	3.1	3.9 (1)	126.8	203 (1)
Fish	n.d.–0.6	n.d. (1), n.d.–0.6 (2)	8.5–15.9	4.3–27 (1), 5.5–64.3 (2)
Rainbow trout	0.3	n.d. (1)	8.5	11 (1)
Egg	n.d.	0.4 (1), n.d. (2)	1.2	1.5 (1), 3.7 (2)
Bread (rye)	4.7	4.7 (1)	n.d.	< 0.17 (1)
Bread (wheat)	2.1	1.1 (1)	n.d.	< 0.09 (1)
Cauliflower	0.04	n.d.(1)	2.7	4.9 (1)
Potato	n.d.	n.d (1), n.d. (2)	0.5	0.52 (1), 1.0 (2)
Tomato	n.d.	n.d.(1)	0.9	0.19 (1)
Carrot	n.d.	n.d.(1), n.d. (2)	1.7	< 0.24 (1), 2.2 (2)
Orange	n.d.	0.44 (1)	1.4	2.2 (1)
Apple	0.2	n.d. (1)	1.3	1.1 (1)
Yogurt	n.d.	0.34 (1)	2.4	1.2 (1)
Cheese	n.d.	n.d. (1), n.d. (2)	1.3	< 0.16 (1), 2.1 (2)
Cow milk	n.d.	0.2 (2)	0.1	0.4 (2)
Rapeseed oil	n.d.	2.1 (2)	63.5	73.4 (2)

¹ Weber *et al.* (1997).² Kamei *et al.* (1986).

n.d.: not detected.

rapeseed oils. According to the present and earlier studies, vegetables, fruits, berries and dairy products have relatively low levels of coenzyme Q₁₀. In cereals the contents of this compound were below the detection limit.

Coenzyme Q₉ levels in foods were low, ranging from 8.5 µg/g to below the detection limit (Table 1). Significant contents were found only in reindeer meat, pork heart and cereals. These findings were in agreement with previous studies (Weber *et al.*, 1997; Kamei *et al.*, 1986; Table 3). Interestingly, most fruits and berries contained mainly coenzyme Q₁₀ whereas coenzyme Q₉ was the major component in lingonberry.

Weber *et al.* (1997) studied the loss of coenzyme Q₁₀ during cooking, and found that cooking did not significantly destroy coenzyme Q₁₀. Hence, the composition data generated in the present study of raw foods could be applied for the intake estimations. As seen in Table 1, the diet contains considerable amounts of coenzyme Q₁₀, and, depending on eating habits, the intake from food was notable. For example, in Northern Finland, in some populations consumption of reindeer meat is enormous. Reindeer meat contains as much as *c.* 160 µg coenzyme Q₁₀/g, and a 100 g serving provides 16 mg coenzyme Q₁₀. This portion is the same amount as that provided by commercial dietary coenzyme Q₁₀ supplements (sizes 10 and 30 mg/day). However, optimal daily intake of coenzyme Q is unknown and no recommendations exist.

To estimate the average adult dietary intake of coenzyme Q, daily consumption of food groups by men and women was extrapolated from a report recently published by the National Public Health Institute (1998). The calculated mean daily intake of coenzyme Q₁₀ was 5.4 mg for men and 3.8 mg for women. As shown in Figure 1, meat was the most important source of coenzyme Q₁₀, representing 55% of the daily intake, while cereals, fish, vegetables, dairy products, fruits and berries provided less than 10%. Fish, despite its relatively high coenzyme Q₁₀ contents had a minor contribution due to its low consumption (Table 2). The relatively high contribution (18%) of vegetable fats is explained by the popularity of coenzyme Q₁₀-rich rapeseed oil which accounts for about 70% of the vegetable oil consumed by Finns. However, it should be noticed that part of the vegetable oil is used for frying during which very high temperatures are used. These high temperatures could be destructive for the coenzymes.

Coenzyme Q₉ intake was considerably lower than that of coenzyme Q₁₀ (Table 2). The calculated mean daily intake of coenzyme Q₉ was 0.6 mg for men and 0.4 mg for women. Cereals provided 76% of coenzyme Q₉ intake (Fig. 2) followed by meat contributing 13%.

The calculated mean daily intakes of coenzymes Q₁₀ and Q₉, and those from different food groups, were close to the Danish results (intake of coenzyme Q₁₀:

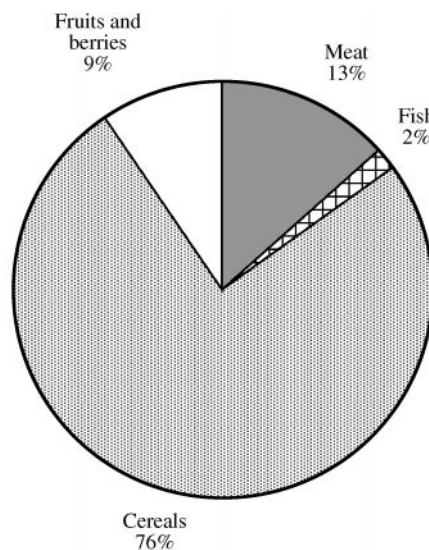


FIGURE 2. Coenzyme Q₉ intake from different food groups.

3–5 mg/day and coenzyme Q₉: 0–1 mg/day) reported by Weber *et al.* (1997). The only significant difference between the two studies was the contribution from edible fats, which was higher in Finland (18%) than in Denmark (6%). As discussed, this arises from the high consumption of rapeseed oil by Finns, but the intake from this food group could be somewhat overestimated in the present study. The high similarity of the intake results of Weber *et al.* (1997) and the present study is probably due to similar eating habits in Denmark and Finland (see Table 3 and Weber *et al.*, 1997). In addition, contents of coenzymes Q₉ and Q₁₀ in foods appear to be similar throughout the world (Denmark: Weber *et al.*, 1997; Japan: Kamei *et al.*, 1986; Finland: the present study).

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