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Coenzyme Q10, carotenoid, tocopherol, and retinol levels in cord plasma from multiethnic subjects in Hawaii

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Abstract

Coenzyme Q10 (Q10), carotenoids, tocopherols, and retinol are the major circulating lipid-phase micronutrients (LPM) known to help mitigate oxidative damage and prevent chronic diseases. However, the functions of these compounds in newborns are little understood. This is due, in part, to the paucity of studies reporting their concentrations in this population.

We measured Q10, carotenoids, tocopherols, and retinol in cord plasma from 100 multiethnic subjects living in Hawaii using HPLC with diode array and electrochemical detection. Appropriate internal standards were used including, for the first time, custom designed oxidized (UN10) and reduced (UL10) Q10 analogues. These compounds reflected the oxidation of UL10 to UN10 that occurred during sample processing and analysis and thus permitted accurate adjustments of natively circulating Q10 levels.

All LPM measured were much lower in cord than in peripheral plasma. Cord plasma levels of total carotenoids, tocopherols, and retinol were approximately 10-fold, 3- to 5-fold and 1.5- to 3-fold lower than those in children or women. Cord plasma levels of total Q10 (TQ10; median, 113 ng/mL) were approximately 2-fold or 7- to 9-fold lower than peripheral plasma levels of neonates or children and adults, respectively. In contrast, the UN10/TQ10 ratio was substantially higher in cord (24%) than in peripheral plasma of children (3 – 4%) or adults (9%). Among the 5 ethnic groups in our cohort, no differences were observed in the levels of UN10, UL10, or TQ10. However, significant differences in many of the LPM were observed between ethnicities. More research is needed to explain these phenomena.

Keywords

coenzyme Q10; carotenoids; tocopherols; retinol; cord plasma

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Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

Introduction

Coenzyme Q (Q) compounds are lipid-phase micronutrients (LPM) with very strong antioxidant effects that occur in humans predominantly by cellular biosynthesis but also, albeit little, by dietary exposure [1]. These compounds function in cells as electron/proton carriers during cellular respiration [2–4]. In humans, both Q9 and Q10 homologues exist with the latter dominating in plasma, by a factor of more than 40 [5]. Ubiquinol-10 (UL10), the chemically reduced form of Q10, is considered a free radical scavenger that protects cells against oxidative injury and stress [4] and minimizes damage to low-density lipoproteins (LDL) *in vitro* by dehydrogenating to ubiquinone-10 (UN10) [6–15]. Thus, the UL10/UN10 ratio has been postulated as a useful biomarker of oxidative damage such as LDL modification [15–17]. Relatively low UL10/UN10 ratios have been reported in individuals with liver, coronary, pulmonary, and neurodegenerative diseases [18–21], while decreased TQ10 levels have been linked with cardiovascular diseases, ataxia, and statin use [22–25].

Approximately 95% of circulating total Q10 (TQ10) occurs natively as UL10 [26–28]. However, UL10 oxidizes easily and rapidly to UN10 during processing and analysis of blood which requires the inclusion of internal standards adjusting for these changes [26]. The UN10/UL10 (or UN10/TQ10) ratio has been recommended as a sensitive measure of oxidative stress [29], whereas TQ10 may represent general physiological events such as cell death, as dying cells would leak Q10 into the circulation [7,19,30,31].

Carotenoids, tocopherols, and retinol are LPM shown to function as important antioxidants via chain-breaking or other reactions that help to mitigate oxidative stress and/or prevent oxidative damage [15,32–37]. In addition, epidemiological and clinical evidence has consistently supported a central role for these LPM in protecting against a wide array of chronic conditions, particularly when exposure happens through a healthy diet [reviewed in 38, 39–42]. Many mechanisms have been revealed including involvement in processes of cell growth and regulation, maintenance of the immune system, reproduction, and gene expression to name a few [34,43,44]. LPM are present in various concentrations throughout the life cycle; however, the reported levels so far are available only from small cohorts, few ethnicities, and/or from few analytes [45,46].

The literature on the functions of LPM is encyclopedic; however, the role of these compounds in newborns is little understood [46]. This is due, in part, to the paucity of studies reporting their concentrations in this population. The few studies reporting levels of carotenoids and tocopherols in cord plasma have observed approximately 90% lower levels than in maternal plasma with high correlations between the two matrices (except for lycopene and α -tocopherol); however, these data are reported from a small sample size ($n = 10$) of mother-newborn pairs [46].

The aim of this study was to determine the levels of LPM (carotenoids, tocopherols, retinol, UL10 and UN10) in cord plasma from a relatively large cohort of altogether 100 individuals of varying ethnicities. A particular focus was to accurately measure natively circulating

levels of UL10 and UN10 by considering artificial oxidation during sample handling, processing, extraction, and analysis. In addition, we aimed to compare the levels of LPM among different ethnicities and between cord and peripheral plasma collected from subjects at different ages.

Methods

Chemicals and reagents

Butylated hydroxytoluene (BHT), bis-tris-propane (BTP), dimethylamino pyridine, dodecanoic anhydride, lithium acetate dehydrate, sodium carbonate, and ubiquinone-10 (UN10) were purchased from Sigma Aldrich (St. Louis, MO). Tocol was purchased from Matreya, Inc. (Pleasant Gap, Pa.). Ethanol (EtOH) (100%) was obtained from Pharmco (Brookfield, CT). Acetonitrile (MeCN), butanol (BuOH), dichloromethane (DCM), glacial acetic acid (AA), hexane, hydrochloric acid (HCL), isopropanol (IPA), methanol (MeOH), potassium hydroxide (KOH), sodium acetate trihydrate, and sodium borohydride (NaBH₄) were of HPLC grade and were purchased from Fisher (Pittsburgh, PA). Hexane and MeOH were kept refrigerated before and during extractions. Ultrapure deionized water (H₂O) was used throughout.

Subjects

This study was part of a prospective investigation on antenatal seafood exposure and neurodevelopmental outcomes in infants. Healthy pregnant women admitted for delivery to Kapi 'olani Medical Center for Women and Children (KMCWC) in Honolulu, Hawaii (island of Oahu) between June 2010 and March 2011 agreed to donate cord blood after assessment for study eligibility [47]. Patients were considered eligible for this study if they were between 18 and 45 years, pregnant with a singleton live fetus and at a gestational age of 37 weeks. A total of 107 cord blood samples were collected after cord clamping, but only 100 samples were used in the final analysis after excluding mothers who could not provide consent or whose infants were not able to be examined prior to discharge. Mothers completed a questionnaire assessing personal and demographic data that included the following: income and education level, age, number of years lived in Hawaii, place of birth, and ethnicity. Routine newborn examinations were performed immediately after birth and information on infant weight, APGAR scores, head circumference, length, gestational age, sex and date of birth were obtained. In addition, a Ballard scale and a Neonatal Intensive Care Unit Network Neurobehavioral Scale examinations were performed. Exclusion criteria included reported tobacco, alcohol, or illicit drug use; English illiteracy, pregnancy with multiple gestation, women who wanted to take home their placentas, women with diabetes, hypertension or other chronic diseases; and women pregnant with a fetus with growth restriction or any known congenital anomalies. Study participants self-reported 17 ethnicities, which were condensed into 5 ethnic groups: Caucasian (white, non-Hispanic), African American (black, non-Hispanic), Hispanic (includes Spanish), Asians (Japanese, Chinese, Korean, Filipino, other Asian), Pacific Islander (Hawaiian, Samoan, Tongan, Micronesian, Chamorro, Cook Islander), and other (Indian, American Indian, unknown). Consolidation resulted in a total of 6 groups and allowed for better inter-ethnic evaluations. Participants were assigned to one of the 6 groups if they possessed a combined 50% or more

ethnic makeup present in that group. The Institutional Review Board (IRB) at KMCWC and the Western IRB approved this study, and all participants signed a consent form.

For comparisons against cord plasma values, 1 – 3 mL of EDTA or Li-heparinized blood was obtained from 17 young children (aged 0.25 – 6 years) and analyzed for LPM. These children were part of a study to determine biomarkers of exposure from computed tomography (CT) [48]. Only pre-CT (non-exposed) plasma samples were used for this study. The Western IRB approved this study, and the parents of the participants signed a consent form.

Also for comparison purposes, 10 – 30 mL of blood was collected into green-top vacutainers from healthy adult volunteers (both genders, aged 23 – 63 years) who are part of ongoing proficiency testing at the University of Hawaii Cancer Center. The University of Hawaii Committee on Human Subjects approved this study, and all participants signed a consent form.

Umbilical cord blood collection and processing

After delivery, 15 – 20 mL of whole blood from the umbilical cord was collected into standard EDTA tubes and processed within 2 h of collection. Blood was centrifuged at $1050 \times g$ for 20 min at 8°C to obtain plasma, which was then aliquoted into microfuge tubes. For aliquots designated for Q10 analyses, study personnel at KMCWC were provided with micro-centrifuge vials containing a consistent and defined mixed amount of dried butylated UL10 (Bu-UL10) and butylated UN10 (Bu-UN10) internal standards (syntheses see below) that was kept at -80°C until immediate use; conditions known to preserve these standards [26]. The Bu-UL10 and Bu-UN10 internal standards were redissolved in 200 μL chilled MeOH, and 200 μL of freshly prepared plasma was added immediately followed by thorough mixing and storage at -80°C . The amounts of added internal standards matched the expected analyte levels using the applied detection techniques.

The blood collected from the children and adult volunteers was processed immediately after collection in a similar manner as cord blood by centrifugation of whole blood at $1050 \times g$ for 20 min at 8°C with final storage at -80°C .

Extraction and analyses of UL10 and UN10 in cord plasma

Quantitation of cord plasma UL10 and UN10 was performed using our recently introduced HPLC method with slight modifications [26]. Briefly, 200 μL thawed plasma spiked with Bu-UL10 and Bu-UN10 internal standards were extracted with 200 μL chilled hexane immediately after thawing by vortexing for 1.5 min followed by centrifugation at $5042 \times g$ for 5 min at 5°C . Thereafter, 2 μL of the hexane layer was injected into an HPLC system (Model Surveyor; ThermoFisher, San Jose, CA) consisting of a Kinetex C18 column (150 $\times$ 3.0 mm, 2.6 μm ; 100A; Phenomenex, Torrance, CA) preceded by a filter cartridge (21 mm, 0.2 μm ; Thermo Scientific, Bellefonte, PA).

Isocratic elution was performed using a mobile phase of 85:15 A/B (v/v) where A: 9.4 mM lithium acetate dihydrate in MeOH/MeCN/H₂O/AA (70/30/1/0.05; v/v), and B: DCM, at a flow rate of 0.5 mL/min. Detection was carried out using UV monitoring at 275 and 290 nm

followed by coulometric detection using a model 5020 guard cell (ESA, Chelmsford, MA) at + 700 mV and a model 5011A analytical cell (ESA) at -700 mV on Channel 1 and + 800 mV on Channel 2. Concentrations of UL10 and UN10 were determined from external calibration curves after adjusting UL10 and UN10 for recovery of Bu-UL10 and Bu-UN10, respectively (syntheses of internal standards described below). TQ10 levels were calculated by summation of UL10 and UN10 levels. Inter- and intra-assay variability of UL10 and UN10 was 8% (820 nM) and 11% (110 nM), respectively.

Extraction and analyses of carotenoids, tocopherols, and retinol in cord and peripheral plasma

Measurements of carotenoids, tocopherols, and retinol were performed using our well-established HPLC assay applying photodiode array detection with minor modifications [49]. Briefly, 300 μ L freshly thawed plasma was mixed with 300 μ L EtOH containing BHT and spiked with the internal standards tocol or δ -tocopheryl laurate (for tocopherols) [26], retinyl laurate (for retinol) [26], and n-butyl- β -apo-8'-carotenoate (for carotenoids) [49]. Samples were then extracted with 1.5 mL hexane by vortexing for 1 min. The hexane layer was evaporated in HPLC amber vials at room temperature under a stream of N₂ followed by reconstitution in 150- μ L mobile phase (see below). Twenty microliters of the reconstituted extract was injected into an RP-HPLC system (Model Surveyor; ThermoFisher, San Jose, CA) consisting of a Spherex C18 analytical column (150 $\times$ 32 mm, 3 μ m) coupled to a Spherex C18 pre-column (4 $\times$ 3.0 mm, 10 μ m, both columns from Phenomenex; Torrance, CA).

Isocratic elution was performed using a mobile phase consisting of 1.14 mM BHT in MeOH/DCM/MeCN/aq BTP (65/25/10/2; v/v) at a flow rate of 0.3 mL/min. All extraction and handling procedures were carried out under yellow light to avoid analyte degradation. Concentrations of carotenoids, tocopherols, and retinol were determined from external calibration curves after adjusting for recovery of the appropriate internal standard.

Extraction and analysis of TQ10 in peripheral plasma

Measurements of plasma TQ10 from our children and adult volunteers were performed using the leftover extracts from the analysis of carotenoids, tocopherols, and retinol described above after applying precolumn electrochemical oxidation and post-column UV detection as UN10 [26]. Coulometric precolumn oxidation used a guard cell model 5020 at + 0.8 V (ESA), and separations were performed on a Gemini C18 pre-column (4.0 $\times$ 2.0 mm; 3 μ m) directly connected to a Gemini C18 analytical column (150 $\times$ 2.0 mm; 3 μ m; both columns from Phenomenex, Torrance, CA).

Isocratic elution was performed using a mobile phase consisting of 50 mM sodium acetate trihydrate in MeOH/Hexane/AA/IPA (69.5:27.5:1.5:1.5; v/v). Peak areas obtained by detection at 275 nm were used for quantitation applying external calibration (range, 50 – 2000 nM; stock levels determined by absorbance readings [26]), and final values were adjusted for internal standard (δ -tocopheryl laurate) recovery. Inter- and intra-assay variability of TQ10 was found to vary between 5% and 7% at levels of 345 ng/mL (400 nM).

Validation

All HPLC assays were validated by participation in quality assurance programs for fat-soluble vitamins organized by the National Institute for Standards and Technology (NIST, Gaithersburg, MD).

Synthesis of internal standards for UL10 and UN10

Bu-UN10 was synthesized from a previous report with modifications [49]. Briefly, 0.1 g UN10 was dissolved in 1 mL hexane followed by addition of 4 mL BuOH and 200 μ L 1 M KOH. The mixture was stirred in a darkened container for 1 h at room temperature. An ultra-HPLC system (Model Accela UHPLC system, ThermoFisher, San Jose, CA, USA) monitored the reaction progress, which was deemed complete when all starting material disappeared (UN10: RT = 6.1 min) and a new product peak emerged (Bu-UN10: RT= 13.1 min). The reaction was quenched by the addition of 15 μ L AA, and the resulting mixture was extracted twice with 5 ml hexane. The combined hexane fractions were washed once with 5 ml H₂O and then dried under N₂. Using HPLC, the Bu-UN10 yield obtained was approximately 80%.

Bu-UL10 was generated from Bu-UN10 by dissolving the latter in 1 mL hexane and 4 mL EtOH and by addition of 0.08 g NaBH₄. The mixture was stirred in the dark for 30 min at room temperature when the starting material disappeared (Bu-UN10: RT= 13.1 min) and a new product peak emerged (Bu-UL10: RT = 9.02 min). The resulting mixture was diluted with 10 mL hexane and washed twice with 8 mL H₂O. The pH of the combined H₂O layers was lowered to pH 5 – 6 using 1M HCL and then re-extracted with 2 mL hexane. The combined hexane layers were then dried under N₂, and the resulting crude Bu-UL10 product was purified using HPLC equipped with a semi-prep Spherex 5 C18 column (250 $\times$ 10 mm, Phenomenex, Torrance, CA, USA). The mobile phase contained 30% (v/v) DCM in MeOH and was set at a flow rate of 1 mL/min, and the reaction was monitored using UV at 295 nm. The desired Bu-UL10 product was eluted at 40 min and was immediately dried under N₂ to give the final product as white powder.

Bu-UL10 is not stable and easily converts to its oxidized form (Bu-UN10) when dissolved and exposed to oxygen, exactly like UL10 or UL9 regarding timing and extent of oxidation (data not shown) [26]. However, we confirmed that Bu-UL10 and Bu-UN10 internal standard are stable and thus maintain their ratio if kept undissolved at – 80°C

Statistical evaluation

Differences in LPM levels among ethnic groups were compared using ANOVA. *Student's* paired t-tests were used to analyze differences between paired ethnicities. Analyses were performed using Excel (Microsoft, Seattle, WA). A p-value of < 0.05 was considered significant.

Results

Our cohort of 100 mothers gave birth between June 2010 and March 2011. At the time of delivery, mothers were on average 28 years old (range, 18 – 44 years; Table I). All infants

were considered healthy according to physical examination findings and APGAR scores. Most of the participants (91%) were educated at a high school level or higher, and the average parity and gravida prior to this study was 1 and 3, respectively. The ethnic distribution of our participants was diverse with only half of the participants reporting to be of a single ethnicity while the other half were mixtures of two (22%), three (17%), four (8%), or five (3%) ethnicities in varying combinations and degrees. Other demographic characteristics of the study participants are summarized in Table I.

There were no significant differences in UL10, UN10, or TQ10 levels among the five ethnicities in our cohort. In contrast, significant differences in the levels of total trans lutein + zeaxanthin, total cis-lutein + zeaxanthin, cis beta-cryptoxanthin, alpha-carotene, total carotenoids and retinol were observed between these ethnicities as determined by ANOVA (Table II). The following pattern from highest to lowest LPM levels was observed: Asians > Caucasians > Hispanics African Americans Pacific Islanders Other. Using paired t-tests, we found that many of the aforementioned analytes were higher in Asians than in Pacific Islanders and that retinol was higher in Caucasians than in Pacific Islanders.

TQ10 levels of all cord plasma samples measured were uniformly much lower in cord plasma compared to those in peripheral plasma (Table III). The median TQ10 level in cord plasma was 113 ng/mL compared to mean levels of approximately 830 ng/mL in non-pregnant women from our volunteers and others (Table III). This indicated an increase in TQ10 level with aging. In contrast, the portion of UN10 relative to TQ10 (UN10/TQ10 ratio) in cord plasma appeared to decrease with age (except in cases of asphyxia, Table III). The median UN10/TQ10 ratio in our cord plasma samples was 24%, similar to that reported in venous cord plasma (26%) and peripheral plasma of neonates 1 – 5 days old (25 – 31%) [50] compared to UN10/TQ10 ratios in the peripheral plasma of infants 1– to 12 months old (9 – 19%), children and adolescents 0– to 17 years old (3 – 4%), and non-pregnant women 26– to 55 years old (9%) (Table III) [45,51].

Carotenoid, tocopherol, and retinol levels of all cord plasma samples were also lower than those in peripheral plasma (Table IV). Median cord plasma cryptoxanthin levels were 5 – 12 ng/mL and represented levels approximately of 6 – 24% of levels found in children or of 9 – 12% in non-pregnant women [52,53]. Likewise, median cord plasma levels of carotenes ranged 1 – 7 ng/mL and represented 5 – 11% and 4 – 6% of the levels in children and women, respectively. Lycopene levels were also only 5 – 8% of those found in children and women, but the levels of lutein + zeaxanthin were 38% in children and 8 – 13% in adults. Collectively, all carotenoids were approximately 10-fold higher in peripheral plasma of children or adults compared to those in cord plasma. Median cord plasma levels of beta + gamma tocopherol (which are measured together in C18-based RP-HPLC systems [54–56]) were 251 ng/mL compared to children (1180 ng/mL) and women (1048 ng/mL) levels, both of which were approximately 4-fold higher. Retinol levels in cord plasma were similar to but still lower than those in peripheral plasma of young children (209 – 276 ng/mL) and much lower than in women (663 – 679 ng/mL).

Plasma levels of UL10 and UN10 were determined after adjustment for custom designed Bu-UL10 and Bu-UN10, respectively. The latter were ideal internal standards since the Bu-

UL10/Bu-UN10 ratio changed in the very same fashion as the UL10/UN10 ratio. Similarly, plasma levels of carotenoids, tocopherols, and retinol were determined after adjustment for their respective well-established internal standards n-butyl- β -apo-8'-carotenoate (custom designed), tocol, and retinyl laurate (custom designed), respectively. This approach was validated by successful participation in the NIST quality assurance program for fat-soluble vitamins.

Discussion

The beneficial effects of circulating carotenoids, vitamin E, retinol, and coenzyme Q10 are encyclopedic. Carotenoids are thought to play a preventable role against certain diseases. There is some evidence reporting probable associations between higher intakes of dietary carotenoids and/or high serum concentrations of some carotenoids and lower incidence of cardiovascular disease, stroke, and cancer [57–59]. In addition, studies have suggested that carotenoids may be involved in regulating immune function and preventing atherosclerosis [60,61]. Vitamin E has been well recognized for its health benefits including its antioxidant properties and preventative role against coronary heart disease and cancer [62–68] in addition to its involvement in regulating cell proliferation in vascular smooth muscle, regulating gene expression, and modulating signal transduction [69,70]. Compounds containing vitamin A have been implicated in numerous biological functions such as growth, reproduction and development, vision, epithelial differentiation, and immune function [71,72]. Q10 has been shown to function as an electron/proton carrier during cellular respiration and as an intra-cellular antioxidant [2–4]. UL10 is considered a free radical scavenger that protects low-density lipoproteins against oxidative stress *in vitro* [15] and acts to reduce lipid peroxy or tocopheroxy radicals [14] and also minimizes oxidative stress by dehydrogenating UN10 [6–11,13]. Thus, the UL10/UN10 ratio has been postulated as an *in vivo* marker of oxidative stress [15–17], whereas TQ10 may represent general physiological events such as cell death, as dying cells would leak Q10 into the circulation [7,19,30,31].

In this study, we measured levels of these LPM in cord plasma from 100 multiethnic individuals residing in Hawaii to establish and confirm exposure of these agents to the newborn and fetus. The ethnic distribution of our study participants was almost identical to that present in the state of Hawaii according to the 2010 census – Asian, 43% vs. 44%; Pacific Islander, 25% vs. 26%; and Caucasian, 19% vs. 25% (available at www.census.gov). Other demographic characteristics of the study participants summarized in Table I do not necessarily reflect the average situation in the state of Hawaii.

The cord plasma UL10, UN10, and TQ10 levels between the 5 ethnicities were not significantly different nor were the UN10/TQ10 ratios. Median cord plasma levels of retinol differed between all 5 ethnic groups, whereas only some carotenoids (trans and cis lutein/zeaxanthin, cis beta-cryptoxanthin, alpha-carotene, total carotenoids) and none of the tocopherols showed differences. The Asians and Caucasian ethnic groups had generally higher levels of carotenoids than the Pacific Islander and Others groups. These differences were significant between Asians and Pacific Islanders for trans lutein/zeaxanthin, total carotenoids and retinol, and between Caucasians and Pacific Islanders for retinol (Table II).

Our study participants were unique in that not only were there several ethnicities present but also several ethnic mixtures. We do not know of any other study with such mixed ethnic diversity, and thus, the reasons for the differences between ethnicities may be multifactorial.

Studies have shown education to be positively associated with fruit and vegetable consumption [73,74], for which plasma carotenoid concentrations are biomarkers [75]. In our population, a small population (9%) did not graduate high school. Likewise, socioeconomic status [76] has been shown to be related to poor diet quality, that is, low intake of fruit and vegetables (sources of carotenoids) and 25% of our population indicated an average household income < \$ 24,999, well below the median Hawaii household income of ~\$67,000 (2007–2011, US Census Bureau for Hawaii - <http://quickfacts.census.gov/qfd/states/15000.html>) These factors may have played a role in the differences in ethnic LPM concentrations in addition to differences in dietary patterns which could have affected the bioavailability and/or bioconversions of some carotenoids [77]. Furthermore, marital status [78] and acculturation [79] may also have played a role as the blending of different ethnicities and cultures by marriage may have influenced the adoption of different lifestyle patterns and/or dietary behaviors that, in turn, may influenced concentration levels of LPM; however, information on such factors was not collected.

Our cord plasma samples being a mixture of arterial and venous cord plasma showed median TQ10 levels of 113 ng/mL and was in excellent agreement with levels found exclusively in venous cord plasma (mean, 112 ng/mL) [50], arterial (median, 154 ng/mL), and venous cord serum (median, 156 ng/mL) [80] or in arterial cord plasma levels from mothers who gave birth vaginally (mean, 150 ng/mL) [81]. However, cord plasma TQ10 levels from mothers who had given birth by cesarean section were reported to be lower when receiving general anesthesia (mean, 70 ng/mL) and higher when receiving spinal anesthesia (mean, 240 ng/mL), respectively (Table III) [81].

Peripheral TQ10 plasma levels in neonates/children and women were approximately 2-fold and 7 – 9 fold higher, respectively, compared to cord plasma levels (Table III). This parallels recent findings from Matsuzaki and others who found ca. 4-fold and 9-fold higher TQ10 levels in the peripheral serum of postpartum mothers and non-pregnant women, respectively, compared to those in cord serum [80]. Given that Q10 is derived mainly from endogenous synthesis via the mevalonate pathway, a similar route for cholesterol synthesis [4], the lower levels of TQ10 in cord blood may be due to the lower concentrations of circulating lipoproteins and cholesterol compared to adults [82].

Similar to TQ10, measured levels of carotenoids, tocopherols, and retinol were also lower in cord plasma compared to those in peripheral plasma. This may be due to the generation of free radicals induced during labor that cause oxidative stress. Pregnancy is a physiological state vulnerable to oxidative stress, especially when maternal-fetal circulation is being established [83]. Lower cord versus peripheral blood values could also be due to oxidative damage induced by the stress of childbirth in which case the antioxidant activity of the mother would increase in order to offset the ensuing stresses of pregnancy [84]. This would deplete the amount of antioxidants circulated to the neonate. Alternatively, oxidative stress during childbirth could be higher in the neonate circulation than in the mother's in which

case the neonate's ability to scavenge free radicals would increase and, in turn, would exhaust antioxidant reserves in the fetal compartment [85,86]. The lower levels of most analytes in cord versus peripheral plasma could be due to placenta filtration, a common and important process necessary for the processing of xenobiotics and drugs.

Either of these scenarios are plausible and may help to explain the low cord blood levels of LPM. Higher oxidative stress in the neonate has been shown for Q10 compounds when relatively more oxidized than reduced Q10 was in the arterial (nutrient poor) plasma versus venous (nutrient rich, coming from the mother) plasma [80]. However, in that study, the total concentration of Q10 was also lower in cord than in maternal peripheral plasma, which indicates that losses occur during the transfer through placenta (filter effect).

While maternal blood was not collected in our study, which impedes us from making direct maternal – cord comparisons, previous reports have indicated increased levels of serum malondialdehyde (a biomarker for oxidative stress – [87]) in women with uncomplicated pregnancies [88] while high concentrations of f2-isoprostanes (a biomarker of lipid peroxidation) [89] have been found in new-borns [90]. These studies suggest that oxidative stress during childbirth is a physiologically taxing event for both mother and newborn.

The collection of maternal blood is a very challenging task given the critical and stressful event of childbirth, and provided the information state above, we recognize that this needs to be included in future confirmatory studies.

A particular focus of our study was to accurately measure natively circulating levels of UL10 and UN10. The accurate measurement of UL10 and UN10 requires the use of appropriate internal standards that reflect not only absolute losses but also a surrogate for the inevitable fast oxidation of UL10 to UN10 that occurs during sample processing and analysis [26]. We confirmed by timed experiments that the rate of oxidation of the ethylated and butylated Q10 internal standard analogues were exactly the same as the native Q10 compounds. Previous studies applying exclusively ubiquinones (e.g. UN6, UN7, UN9) [7,91,92] or their alkoxy analogues [49,93] most likely were not able to appropriately account for the oxidative conversions and, therefore, were likely not able to accurately determine native levels of UL10 and UN10. In this study, we applied for the first time our custom synthesized ethylated or butylated analogues of UL10 and UN10, and showed that these were ideal internal standards. When replacing the methoxy groups of UL10 and UN10 by ethoxy or butyloxy groups, we observed identical oxidation rates (i.e., Bu-UL10/Bu-UN10 ratio) as the Q10 analytes (UL10/UN10). Our internal standards, therefore, allowed adjustments of not only the absolute losses of UL10 and UN10 that occurred during sample processing and analysis but also the oxidative conversions occurring during these steps. For these reasons, the concentrations measured in this study accurately reflect the native circulating levels of both UL10 and UN10 [26].

A weakness of this study was that maternal peripheral blood samples were not collected and thus direct maternal – cord comparisons could not be made. However, we could compare levels of cord plasma analytes with those of peripheral plasma of our current and recent studies in healthy young children and pre- and postmenopausal women. These studies used

identical techniques to the current study including the same internal standards, which lends great confidence in the performed comparisons (Table III and IV). In addition, validation was supported by agreement of values with those of other published studies.

In summary, our results were in agreement with previous studies reporting much lower LPM levels in cord plasma compared to those in peripheral plasma. Differences in levels between ethnicities were small or none. UL10 and UN10 were reliably measured with the use of appropriate analogues as internal standards which was found to mimic the redox behavior perfectly. TQ10 levels in cord plasma were approximately 10-fold lower than those in peripheral plasma of children or adults, but only 3-fold lower than in peripheral plasma of neonates. In contrast, the portion of oxidized Q10 relative to total Q10 was substantially higher in cord compared to that in peripheral plasma. This may be due to the higher oxidative stress during transplacental transfer and/or fetal circulation. More research is required to explain these observations.

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Abbreviations

AA	(Glacial) Acetic Acid
BHT	butylated hydroxytoluene
BuOH	Butanol
CT	Computer tomography
DCM	Dichloromethane
EtOH	Ethanol
H₂O	18 megaohm ultrapure water
HCL	Hydrochloric acid
IPA	Isopropanol
KMCWC	Kapi 'olani Medical Center for Women and Children
KOH	Potassium hydroxide
LPM	Lipid-phase micronutrients
MeCN	Methyl cyanide (Acetonitrile)
MeOH	Methanol
NaBH₄	Sodium borohydride
N₂	Nitrogen

Q	Coenzyme Q
Q10	Coenzyme Q10
TQ10	Total Q10 (2-propanol)
UL10	Ubiquinol-10
UN10	Ubiquinone-10

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Table I

Demographic characteristics of participants.

Characteristics	Mean± SD	Median	Range
Mother age, y	28.3 ±62	27.5	18.0 – 44.0
Baby birthweight, g	3411.6 ±464.3	3388.0	2345.0 – 4839.0
Baby Head circumference, cm	34.7 ±15	34.9	31.1 – 39.4
Baby Length, cm	51.5 ±21	51.4	45.7 – 57.2
Gravida	3.0 ±20	2.0	1.0 – 9.0
Parity	1.0 ±10	1.0	0.0 – 6.0
APGAR at 1 min, score	8.0 ±10	8.0	2.0 – 9.0
APGAR at 5 min, score	9.0 ±00	9.0	8.0 – 9.0
Mothers ' Ethnicity, n (% within subgroup)			
Single ethnicity	50 (100%)		
Caucasian (white, non-Hispanic)	6 (12%)		
Asian (Japanese, Chinese, Korean, Filipino, other Asians)	26 (52%)		
Pacific Islander (Hawaiian, Samoan, Tongan, Micronesian, Chamorro, Cook Islander)	15 (30%)		
Hispanic (includes Spanish)	3 (6%)		
Black (black, non-Hispanic)	0 (0%)		
Other (Indian, American Indian, unknown)	0 (0%)		
Mixed ethnicity(50%–< 100% of one ethnicity)	50 (100%)		
Caucasian (white, non-Hispanic)	13 (26%)		
Asian (Japanese, Chinese, Korean, Filipino, other Asians)	17 (34%)		
Pacific Islander (Hawaiian, Samoan, Tongan, Micronesian, Chamorro, Cook Islander)	10 (20%)		
Hispanic (includes Spanish)	0 (0%)		
Black (black, non-Hispanic)	2 (4%)		
Other (Indian, American Indian, unknown)	8 (16%)		
Two or more ethnicities	50 (100)		
Two ethnicities	22 (44%)		
Three ethnicities	17 (34%)		
Four ethnicities	8 (16%)		
Five ethnicities	3 (6%)		
Six ethnicities	0 (0%)		
Education, n			
Before high school	2		
Some high school	7		
Completed high school	39		
Some college	18		
BS/BA completed college	28		
Postgraduate college degree	6		
Income, n			
<\$24,999	25		
\$25,000-\$49,999	16		

Characteristics	Mean± SD	Median	Range
\$50,000-\$75,000	17		
>\$75,000	24		
not stated	18		
Baby gender, n			
Male	62		
Female	38		

n= number of subjects; also percentage since the total number is 100.

Table II

LPM levels in cord plasma according to ethnicity.

Analyte	n	ng/mL					p (overall)
		43	19	3	2	25	8
UL10		120 ± 72	94 ± 78	121 ± 75	176 ± 42	122 ± 67	142 ± 67
UN10		31 ± 16	27 ± 13	23 ± 10	57 *	32 ± 20	39 ± 32
Tq10		128 ± 93	119 ± 82	150 ± 84	204 *	152 ± 90	133 ± 117
UN10/TQ10		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
TottrLUT + ZEA		38 ± 17a	27 ± 17	21 ± 22	32 ± 15	24 ± 10 ^a	25 ± 8
TotcisLUT + ZK		17 ± 6 ^a	14 ± 8	11 ± 9	13 ± 6	13 ± 4 ^a	13 ± 3
αCRX		6 ± 2	5 ± 2	4 ± 2	4 ± 3	4 ± 2	4 ± 1
tr βCRX		15 ± 16	11 ± 21	11 ± 4	9 ± 8	9 ± 7	8 ± 4
cis βCRX		6 ± 3	4 ± 5	4 ± 2	3 ± 2	3 ± 2	3 ± 1
trLYC		11 ± 4	10 ± 5	8 ± 4	10 ± 7	11 ± 6	11 ± 5
5cisLYC		15 ± 6	13 ± 8	12 ± 4	13 ± 7	14 ± 8	13 ± 7
TotLYC		37 ± 13	32 ± 16	26 ± 12	31 ± 16	32 ± 18	34 ± 16
αCAR		3 ± 3	2 ± 3	2 ± 2	1 ± 1	2 ± 1	2 ± 1
tr βCAR		9 ± 22	6 ± 14	9 ± 1	7 ± 7	5 ± 7	4 ± 4
cis βCAR		1 ± 2	1 ± 2	1 ± 0	0 ± 1	1 ± 1	0 ± 0
TotCAROT		127 ± 58 ^a	90 ± 69	73 ± 47	88 ± 50	85 ± 32 ^a	84 ± 28
β-yTOC		263 ± 114	222 ± 116	182 ± 49	227 ± 9	267 ± 132	290 ± 90
αTOC		2322 ± 670	2165 ± 770	2110 ± 885	1720 ± 130	2047 ± 522	1887 ± 471
TotTOC		2566 ± 719	2408 ± 859	2291 ± 934	1946 ± 147	2264 ± 621	2131 ± 482
RET		224 ± 49 ^a	221 ± 51 ^b	206 ± 50	220 ± 64	168 ± 53 ^a	196 ± 67

Median values (ng/mL) ± standard deviation of this study (n = 100); p = difference between ethnic groups determined using ANOVA, in boldface when < 0.05, superscripts with the same letter are significantly different (p < 0.05) as determined using t-tests. Number of data points: Caucasians 19; African American 2; Hispanic 2; Asian; Pacific Island 25; other.

UL10 = ubiquinol-10, UN10 = ubiquinone-10, TQ10 = total coenzyme Q10, UN10/TQ10 = ubiquinol-10/ubiquinone-10 ratio, TottrLUT + ZEA = total (trans lutein + trans zeaxanthin), TotcisLUT + ZEA = total (cis lutein + cis zeaxanthin), αCRX = alpha-cryptoxanthin, trβCRX = trans beta-cryptoxanthin, cisβCRX = cis beta-cryptoxanthin, trLYC = all trans lycopene, 5cisLYC = 5-cis lycopene, TotLYC = total (cis lutein + cis zeaxanthin), αCAR = alpha-carotene, trβCAR = trans beta-carotene, cisβCAR = cis beta-carotene, TotCAROT = total (trans lutein + trans zeaxanthin), β-yTOC = beta-y-carotene, αTOC = alpha-tocopherol, TotTOC = total (trans lutein + trans zeaxanthin), RET = retinol.

Table III

Coenzyme Q10 levels in cord and peripheral plasma of healthy subjects.

Analyte	Cord plasma				Peripheral plasma					
	Arterial + Venous		Arterial		Venous		Neonates ^d			
	VD ^a	CS ^g	CSs	VD	VD	VD	1-d old	3-d old	5-d old	Infants ^e
n	100	15 ^b	15 ^b	15 ^b	10 ^c	10 ^c	20	20	20	1- to 4m old
U/Q10 (ng/mL)	113 ± 78	70 ± 30 ^{1M}	240 ± 60 ^m	150 ± 60 ^m	158	153	242 ± 78 ^m	337 ± 164 ^m	294 ± 86 ^m	5- to 12m old
U/Q10/TQ10 (%)	24 ± 9	-	-	-	16	14	25 ± 12 ^m	29 ± 12 ^m	31 ± 11 ^m	9- to 17y old
										Children ^f
										0- to 7y old
										10- to 17y old
										18
										50
										25
										882
										916 ± 28 ^m
										761 ± 225 ^m
										830 ± 225 ^m
										869/813 ^m
										na/4 ^m
										9 + 3111
										3 ± 7 ^m
										4 ± 10 ^m
										9
										9
										25
										9- to 12m old
										17y old
										10- to 17y old
										26- to 52y olds
										29- to 55y olds ^b
										10/6

Mean values ± standard deviation except mean when marked with “m”, n = number of subjects other abbreviations shown in Table II.

^a Present study (arterial ± venous cord plasma).

^b [18].

^c [86].

^d [55].

^e [55].

^f [45].

^g [95].

^h Present study with ten and six adult volunteers (levels higher by a factor of 2 after Q10 supplementation with a slight increase in the UL10/TQ10 ratio).

Table IV

Carotenoid, tocopherol, and retinol levels in cord and peripheral plasma of healthy subjects.

Analyte *	Peripheral plasma				Cord blood relative to Reference			
	Cord plasma		Children		Women			
	a		b		c		b	c d ^m
n	100		17		381		29	
ng/mL								
TotrlUT + ZEA	30 ± 16		80 ± 42		230 ± 116		361 ± 99	38% 13% 8%
αCRX	5 ± 2		21 ± 8		43 ± 17		n/a	24% 12%
tr βCRX	12 ± 15		75 ± 86		120 ± 15)		187 ± 8	6% 5% 9%
TotLYC	12 ± 6		217 ± 240		220 ± 100		169 ± 68	6% 5% 8%
αCAR	2 ± 2		24 ± 32		45 ± 46		65 ± 41	8% 4% 5%
tr βCAR	7 ± 17		141 ± 73		149 ± 171		273 ± 217	5% 5% 5%
cis βCAR	0.9 ± 2		8 ± 7		15 ± 15		n/a	11% 6%
TotCAROT	99 ± 56		937 ± 473		1233 ± 568		1234 ± 405	11% 8% 9%
± + γ TOC	251 ± 116		1180 ± 533		1048 ± 717		n/a	21% 24%
αTOC	2158 ± 653		7146 ± 2334		9254 ± 4406		11554 ± 4583	30% 23% 20%
RET	209 ± 55		276 ± 88		663 ± 224		679 ± 76	76% 32% 31%

* Analyte abbreviations shown in Table II.

Median values (ng/mL) + standard deviation except when marked with “ m to indicate means; n= number of subjects.

^a Present study

^b Children age, 0.25–6 years.

^c Premenopausal women age, 35 – 52 years not undergoing intervention (control group) [52].

^d Women age, 18–70 years [53].

^{a–d} Measured with the same HPLC technique (see Methods).