

## Maternal plasma and amniotic fluid coenzyme Q10 levels in preterm and term gestations: a pilot study

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### Abstract

**Objective** To measure maternal plasma and amniotic fluid coenzyme Q10 (CoQ10) levels in preterm and term gestations.

**Study design** This pilot study comprised a convenience sample of 72 women admitted for labor with singleton live gestations and intact membranes (preterm  $n = 27$  and term  $n = 45$ ).

**Results** Median [interquartile range] maternal plasma CoQ10 levels did not differ among the studied women (preterm, 0.47 [0.12] vs. term, 0.47 [0.23] mmol/L,  $p = 0.90$ ). Overall CoQ10 amniotic fluid levels were nearly tenfold lower than those found in maternal plasma, with a significant lower level observed among those delivering preterm (0.050 [0.05] vs. 0.062 [0.04] mmol/L,  $p = 0.007$ ). Multiple linear regression analysis controlling for several covariates

determined a significant correlation between amniotic fluid CoQ10 levels and neonatal gestational age.

**Conclusion** This is the first study to assess CoQ10 levels in amniotic fluid during pregnancy in which levels were significantly lower among those delivering preterm. More research is warranted in this regard.

**Keywords** Amniotic fluid · Antioxidant · Coenzyme Q10 · Preterm delivery · Ubiquinone

### Introduction

Coenzyme Q10 (CoQ10), also known as ubiquinone, is a natural antioxidant present in the blood and many tissues and fluids [1, 2]. This small lipid molecule participates in cell energetic interchanges due to its hydrophobic features that allow free diffusion within the membrane. Acceptance of two electrons or release of two protons allows, respectively, its reduction to the ubiquinol form or ubiquinol's oxidation back to the ubiquinone form. Thus, the CoQ10 system has a pivotal role within the mitochondrial membrane as a lipid-soluble carrier of both electrons and protons through the redox cycle [3–5]. Clinical studies have demonstrated the efficacy of CoQ10 as a therapeutical adjuvant in reproduction, inflammation-related states, and cardiovascular and neurodegenerative diseases [5–9].

Preeclampsia, preterm birth and adolescent pregnancies are among the most challenging problems in modern obstetrics [10–12]. CoQ10 may play a significant antioxidant role at the placental site in normal or pathological pregnancies [13, 14]. Teran et al. [8] in a randomized double-blind placebo-controlled trial found that oral CoQ10 supplementation in pregnancy (20 weeks on) significantly reduced the risk of developing preeclampsia. Maternal and

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fetal compartments have distinct physiological roles in the peripartum defense against free radical production [15]. Reactive oxygen species have been linked to the pathogenesis of the preterm birth, and antioxidants proposed as potential therapeutical agents for ill neonates [16]. Despite this, data reporting on CoQ10 plasmatic status during pregnancy is limited and lacking in the case of amniotic fluid. Bearing this in mind, the present pilot study aimed at measuring maternal plasma and amniotic fluid CoQ10 levels in preterm and term gestations.

## Materials and methods

### Study design and participants

This pilot study was carried out from March 2008 to June 2008 at the Isidro Ayora Obstetrics and Gynecology Hospital, Quito, Ecuador (located at 2,800-m altitude). A convenience sample was obtained among women admitted for labor with singleton live gestations and intact membranes (preterm and term). Women with ruptured membranes, meconium staining, chronic diseases or any other obstetrical pathological conditions were excluded. Preterm birth was defined as that occurring between 21 and 36 weeks 6 days [17].

A 5-cc sample of amniotic fluid was obtained through direct sterile syringe puncture of the amniotic membranes prior to delivery (vaginal or cesarean section). Puncture was performed directly over protruded membranes during hysterotomy (cesarean section) or vaginally through sterile speculum application. Obtained samples were maintained on ice until centrifugation at 1,000g for 15 min at 4°C (Hettich Rotina 46R Zentrifugen, Germany). The supernatant was then transferred into polypropylene vials for CoQ10 extraction (<2 h). Extracted samples were then frozen at -40°C until assay.

A 10-cc blood sample was concomitantly obtained from each woman at the antecubital venous puncture site, immediately transferred into a polypropylene vial containing 3.15% sodium citrate (1:9, v/v) and then centrifuged at 4°C. The plasma obtained was decanted into 500- $\mu$ l aliquots and then stored at -40°C until biochemical analysis. During processing, both plasma and amniotic fluid samples were protected from light using aluminum foil to prevent photo-degradation of ubiquinones.

Maternal/neonatal information was registered on a data sheet elaborated for the purposes of the present study. Research protocol was approved by the Bioethics Committee of the Biomedical Center, Universidad Central, Quito, Ecuador. Participants were informed about the study and its objectives and written consent was obtained.

### Analyte assays

#### *CoQ10 measurement*

Plasma and amniotic fluid CoQ10 levels were measured in a high performance liquid chromatography (HPLC) system (Perkin-Elmer, Shelton, CT, USA) equipped with a Lichrosorb® RP18 column (5  $\mu$ m, 125  $\times$  4 mm; Phenomenex, Torrance, CA, USA) and a guard column (Merck, Darmstadt, Germany). Measurement of amniotic fluid CoQ10 levels was performed as a modification of the method previously described for plasma [18]. In brief, a 0.5 mL of previously processed amniotic fluid was mixed with 50  $\mu$ l of ethanol BHT solution (10 mg/mL) (Sigma-Aldrich, St. Louis, MO, USA), 0.2 mL of 0.1 M aqueous sodium dodecyl sulfate (Sigma-Aldrich) and 1.6 mL of CoQ9 solution (Fluka Biochemica, Buchs, Switzerland) in ethanol as an internal standard. The mixture was vortexed for 30 s, 2 mL of hexane (Merck, Darmstadt, Germany) was added, and the tightly screwed test tube was vigorously vortexed for 2 min. Subsequently, the mixture was centrifuged for 5 min at 1,000g to separate the layers. One milliliter of the hexane layer was transferred to a small vial and dried under nitrogen. The residue was dissolved again in methanol/ethanol/isopropanol 95/5, v/v) (1:1 v/v). The mobile phase was methanol/ethanol (30:70 v/v) previously filtered. The flow rate was 1 mL/min and the UV detector was set up at 275 nm. Detection limit of the assay was 0.1 mmol/L and the average recovery of the internal standard was 85%. The intra-assay error coefficient was 4.3% and the inter-assay error coefficient was 15.9%.

#### *Plasma total cholesterol*

Plasma total cholesterol was measured, as previously described [8], with a spectrophotometer (Eppendorf, Hamburg, Germany) and a Cholesterol Liquicolor test kit (Human GmbH, Wiesbaden, Germany). All biological processed samples were measured in duplicate and the mean value used for statistical analysis.

### Statistical analysis

Statistical analysis was performed using SPSS statistical package (Version 13.0 for Windows, SPSS, Chicago, IL, USA). Data are presented as medians, interquartile ranges (IQR) and percentages. The Kolmogorov–Smirnov test was used to determine the normality of data distribution. According to this, nonparametric continuous data were compared with Mann–Whitney’s test. Chi-square test was used to compare percentages. Spearman coefficients were calculated to determine correlations between CoQ10 levels (plasma and amniotic liquid) and various numeric variables

(bivariate analysis). Additionally, multiple linear regression analysis was performed adjusting for several confounding factors (maternal age, parity, marital status, mode of delivery, educational level and total plasma cholesterol levels). CoQ10 levels were log transformed prior to any linear regression analysis. A  $p$  value of  $<0.05$  was considered as statistically significant.

## Results

During the study period, a total of 75 women fulfilled inclusion criteria and were recruited,  $n = 30$  preterm (40%) and  $n = 45$  term (60%). Three preterm cases were excluded due to incomplete data or because the biological sample was not adequate for analysis. The general maternal data and biochemical analysis of plasma and amniotic liquid are depicted in Table 1. No differences were found among studied women except for maternal age, which was significantly higher among those delivering preterm. No women in the study had the habit of smoking.

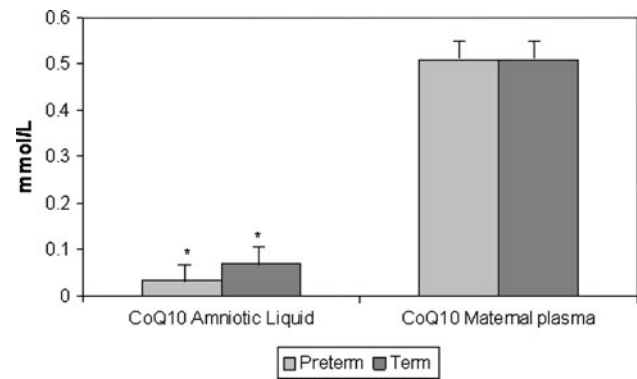
Median [interquartile range] maternal plasma CoQ10 levels did not differ among the studied women (preterm 0.47 [0.12] vs. term 0.47 [0.23] mmol/L,  $p = 0.90$ ), even when compared according to the mode of delivery. Overall, CoQ10 amniotic fluid levels were nearly tenfold lower than those found in maternal plasma. Women delivering preterm displayed lower CoQ10 levels (0.050 [0.05] vs. 0.062 [0.04] mmol/L,  $p = 0.007$ ) (Table 1, Fig. 1), with no differences observed for total plasma cholesterol levels. Upon

**Table 1** Maternal demographics, delivery characteristics and analyte (CoQ10 and total cholesterol) concentrations

Parameter	Preterm ( $n = 27$ )	Term ( $n = 45$ )	$p$ value*
Maternal age (years)	26 [8]	21 [8]	0.02
Parity	2.0 [3]	1.0 [2]	0.11
Nonmarried status	15 (55.6%)	27 (60%)	0.71
Low schooling ( $<12$ years)	19 (70.4%)	29 (64.4%)	0.60
Vaginal route of delivery	13 (48.1%)	29 (64.4%)	0.17
Gestational age (weeks)	33.0 [4.1]	40 [1.4]	0.0001
Amniotic fluid CoQ10 (mmol/L)	0.050 [0.05]	0.062 [0.04]	0.007
Plasma CoQ10 (mmol/L)	0.47 [0.12]	0.47 [0.23]	0.90
Plasma total cholesterol (mg/dL)	242.6 [50.9]	217.2 [73.38]	0.06

Values are presented as medians [interquartile range] or percentages (%)

\*  $p$  value as determined with the Mann–Whitney test or chi-square calculation



**Fig. 1** CoQ10 levels (amniotic fluid and plasma) according to gestation outcome (\* $p = 0.007$ )

bivariate Spearman analysis, amniotic fluid CoQ10 levels significantly correlated with neonatal gestational age ( $r^2 = 0.73$ ,  $p = 0.004$ ). This was confirmed after multiple linear regression analysis controlling for several covariates.

## Discussion

Free radicals, antioxidants and co-factors participate in health maintenance, aging and several age-related diseases. Oxidative stress is balanced by antioxidant systems [2, 13, 19]. Studies assessing plasma CoQ10 status during pregnancy are scarce. Noia et al. [20] have reported that maternal serum CoQ10 levels increase with gestational age, with an additional third trimester increase in relation to uterine contractile activity. In another study, it was found that after adjusting for age, lipid markers and smoking habit, serum CoQ10 levels significantly correlated with maternal weight and fat mass gain (second to third trimester) and infant birth weight [21]. Lower plasma [18, 22–24] and increased placental and cord blood CoQ10 levels [22] have been reported in pregnancies complicated with preeclampsia. One study also found that maternal interleukin-18 levels positively correlated with the reduced form of CoQ10 [25].

Compagnoni et al. [26] reported that serum CoQ10 levels were higher in mothers and neonates after vaginal or elective cesarean delivery with spinal anesthesia as compared to cesarean sections with general anesthesia, suggesting that mode of delivery may affect CoQ10 levels. Contrary to this, our study found that plasma CoQ10 levels did not differ among laboring women even after stratifying for mode of delivery.

Spontaneous preterm birth is a worldwide significant public health problem. Although inflammation and the generation of reactive oxygen and nitrogen species may play significant roles in diverse pathological situations, including preterm labor and stillbirths, the etiology of preterm birth remains unknown. Studies analyzing CoQ10 levels in

amniotic fluid are lacking in literature. In this sense, to the best of our knowledge, the present pilot study may indeed be the first to report amniotic fluid CoQ10 levels, compared with plasma status and gestational age. Overall, CoQ10 amniotic fluid levels were nearly tenfold lower than those found in maternal plasma. The relevance of this finding is yet to be determined in future studies. Most interesting was the finding that women delivering preterm displayed significantly lower amniotic fluid CoQ10 levels as compared to term deliveries. The significant coefficient correlation found between amniotic fluid CoQ10 levels and gestational age (bivariate and multivariate analysis) seems to support our finding. Lower amniotic fluid CoQ10 levels in preterm pregnancies could be related to immaturity (lower production) or a higher degree of consumption. Imbalance between oxidants and antioxidants has been reported in preterm infants during the first few hours of life [27]. In any case, the present data seem to support that amniotic fluid CoQ10 levels are plasma independent and have an unexplored role in the pathogenesis of preterm delivery related to the fetal compartment. In this regard, CoQ10 oral supplementation in early pregnancy presents as an attractive preterm birth preventive/intervention measure. Supporting this is the fact that CoQ10 supplementation significantly decreased preeclampsia risk [9]. More research is warranted to confirm our preliminary findings and support CoQ10 as a biological marker of preterm birth when assessed in amniotic fluid.

The possible relation between CoQ10 and cholesterol levels is controversial [28, 29]. CoQ10 levels tend to be lower in subject with high cholesterol levels as compared to healthy ones of the same age. In addition, statin treatment appears to deplete natural CoQ10 levels [5, 30]. Plasma total cholesterol and CoQ10 levels did not differ among women in the present study.

As for the limitations of this study, one can mention the type of sample (small and convenient). Some variables were not recorded (i.e., maternal and neonatal weight) important for a complete multiple linear regression analysis. Despite this, two facts are worthy of highlighting: (a) it is the first study to assess CoQ10 levels in amniotic fluid and (b) the first to report lower levels among gestations delivered preterm. More research is required to further explore the role of amniotic fluid CoQ10 levels in the pathogenesis of preterm birth, which could support any intervention measure.

**Conflict of interest** The authors declare no conflict of interest.

## References

- Mitchell P (2010) David Keilin's respiratory chain concept and its chemiosmotic. *Nobel lecture*. Nobel foundation. Available at: [http://nobelprize.org/nobel\\_prizes/chemistry/laureates/1978/mitchell-lecture.pdf](http://nobelprize.org/nobel_prizes/chemistry/laureates/1978/mitchell-lecture.pdf). Accessed 2 December 2010
- Navas P, Villalba JM, de Cabo R (2007) The importance of plasma membrane coenzyme Q in aging and stress responses. *Mitochondrion* 7(Suppl):S34–S40
- Crane FL (2001) Biochemical functions of coenzyme Q10. *J Am Coll Nutr* 20:591–598
- Bonakdar RA, Guarneri E (2005) Coenzyme Q10. *Am Fam Physician* 72:1065–1070
- Littarru GP, Tiano L (2010) Clinical aspects of coenzyme Q10: an update. *Nutrition* 26:250–254
- Horvath R, Schneiderat P, Schoser BG et al (2006) Coenzyme Q10 deficiency and isolated myopathy. *Neurology* 66:253–255
- Schmelzer C, Lindner I, Rimbach G, Niklowitz P, Menke T, Döring F (2008) Functions of coenzyme Q10 in inflammation and gene expression. *Biofactors* 32:179–183
- Terán E, Hernández I, Nieto B, Távora R, Ocampo JE, Calle A (2009) Coenzyme Q10 supplementation during pregnancy reduces the risk of pre-eclampsia. *Int J Gynaecol Obstet* 105:43–45
- Balercia G, Mancini A, Paggi F et al (2009) Coenzyme Q10 and male infertility. *J Endocrinol Invest* 32:626–632
- Danso KA, Opere-Addo HS (2010) Challenges associated with hypertensive disease during pregnancy in low-income countries. *Inter J Obstet Gynecol* 110:78–81
- Pérez-López FR, Pérez-Roncero G, López-Baena MT (2010) Problemas actuales y controversias concernientes a las adolescentes embarazadas. *Rev Ecuator Ginecol Obstet* 17:185–192
- Salazar-Pousada D, Arroyo D, Hidalgo L, Pérez-López FR, Chedraui P (2010) Depressive symptoms and resilience among pregnant adolescents: a case-control study. *Obstet Gynecol Inter*. Article ID: 952493. <http://www.hindawi.com/journals/ogi/2010/952493/> (Accessed 2 March 2011)
- Noia G, Romano D, De Santis M et al (1999) The antioxidants (coenzyme Q10) in materno-fetal physiopathology. *Minerva Ginecol* 51:385–391
- Roberts JM, Hubel CA (1999) Is oxidative stress the link in the two-stage model of preeclampsia? *Lancet* 354:788–789
- Buhimschi IA, Buhimschi CS, Pupkin M, Weiner CP (2003) Beneficial impact of term labor: nonenzymatic antioxidant reserve in the human fetus. *Am J Obstet Gynecol* 189:181–188
- Lee JW, Davis JM (2011) Future applications of antioxidants in premature infants. *Curr Opin Pediatr* (in press)
- Ross MG, Eden RD. Preterm labor. E-medicine obstetrics and gynecology. <http://emedicine.medscape.com/article/260998-overview> (Accessed 2 March 2011)
- Terán E, Racines-Orbe M, Vivero S, Escudero C, Molina G, Calle A (2003) Preeclampsia is associated with a decrease in plasma coenzyme Q10 levels. *Free Radic Biol Med* 35:1453–1456
- Rahman K (2007) Studies on free radicals, antioxidants, and co-factors. *Clin Interv Aging* 2:219–236
- Noia G, Littarru GP, De Santis M et al (1996) Coenzyme Q 10 in pregnancy. *Fetal Diagn Ther* 11:264–270
- Haruna M, Matsuzaki M, Ota E et al (2010) Positive correlation between maternal serum coenzyme Q10 levels and infant birth weight. *Biofactors* 36:312–318
- Terán E, Vivero S, Racines-Orbe M et al (2005) Coenzyme Q10 is increased in placenta and cord blood during preeclampsia. *Biofactors* 25:153–158
- Terán E, Chedraui P, Racines-Orbe M et al (2008) Coenzyme Q10 levels in women with preeclampsia living at different altitudes. *Biofactors* 32:185–190
- Palan PR, Shaban DW, Martino T, Mikhail MS (2004) Lipid-soluble antioxidants and pregnancy: maternal serum levels of coenzyme Q10, alpha-tocopherol and gamma-tocopherol in preeclampsia and normal pregnancy. *Gynecol Obstet Invest* 58:8–13
- Roland L, Gagné A, Bélanger MC, Boutet M, Julien P, Bilodeau JF (2010) Plasma interleukin-18 (IL-18) levels are correlated with

- antioxidant vitamin coenzyme Q(10) in preeclampsia. *Acta Obstet Gynecol Scand* 89:360–366
26. Compagnoni G, Lista G, Giuffrè B, Mosca F, Marini A (2004) Coenzyme Q10 levels in maternal plasma and cord blood: correlations with mode of delivery. *Biol Neonate* 86:104–107
  27. Ochoa JJ, Ramirez-Tortosa MC et al (2003) Oxidative stress in erythrocytes from premature and full-term infants during their first 72 h of life. *Free Radic Res* 37:317–322
  28. Chu CS, Kou HS, Lee CJ et al (2006) Effect of atorvastatin withdrawal on circulating coenzyme Q10 concentration in patients with hypercholesterolemia. *Biofactors* 28:177–184
  29. Ratnam DV, Chandraiah G, Meena AK, Ramarao P, Kumar MN (2009) The co-encapsulated antioxidant nanoparticles of ellagic acid and coenzyme Q10 ameliorates hyperlipidemia in high fat diet fed rats. *J Nanosci Nanotechnol* 9:6741–6746
  30. Littarru GP, Langsjoen P (2007) Coenzyme Q10 and statins: biochemical and clinical implications. *Mitochondrion* 7(Suppl):S168–S174