BioFactors 32 (2008) 185–190
IOS Press

Coenzyme Q_{10} levels in women with preeclampsia living at different altitudes

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Abstract. *Background:* Preeclampsia is a common disorder of pregnancy exhibiting abnormal plasma and placental coenzyme Q_{10} (CoQ_{10}) levels when compared to normal pregnancies.

Objective: To evaluate CoQ_{10} levels both in plasma and placenta among normal pregnant ($n = 60$) and preeclamptic ($n = 63$) primigravid women and determine the effect of high or low altitude residency.

Study design: CoQ_{10} was determined using High Performance Liquid Chromatography (HPLC) technique and group comparisons were performed.

Results: Preeclamptic women living at high altitude displayed significantly lower CoQ_{10} plasma levels (0.64 ± 0.23 vs. 0.82 ± 0.46 μmol/L, $p = 0.05$). No differences were found in CoQ_{10} plasma levels among women living at sea level. Interestingly, plasma CoQ_{10} levels at low altitude in normal pregnancies were significantly lower than high altitude normal pregnancies. Compared to normal pregnancies, preeclamptic women displayed higher placental CoQ_{10} content, which was only significant among those living at sea level (0.120 ± 0.07 vs. 0.076 ± 0.04 ng/mg protein, $p < 0.005$). Normal pregnant women living at high altitude displayed higher placental CoQ_{10} content when compared to those residing at sea level ($p < 0.0005$).

Conclusion: Women suffering from preeclampsia (high or low altitude) display high placental CoQ_{10} content, with significant low plasma CoQ_{10} levels among those residing in high altitude. More research is warranted to establish the cause-effect relationship between CoQ_{10}levels and preeclampsia.

Keywords: Coenzyme Q_{10}, preeclampsia, pregnancy, placenta, altitude

1. Introduction

Preeclampsia is a common (∼7% of all pregnancies) disorder of human pregnancy in which the normal hemodynamic response to pregnancy is compromised. It remains a leading cause of maternal morbidity and mortality, associated with a significant increase in perinatal mortality [3].

Clinical symptoms of preeclampsia arise from secondary systemic circulatory disturbances; there is evidence suggesting that its diverse manifestations, including altered vascular reactivity, vasospasm and discrete pathology in many organic systems, are derived from pathological changes within the maternal vascular endothelium [5]. Those changes, in addition to increased hypoxic conditions and oxidative stress, cause maternal symptoms of hypertension, proteinuria, clotting and liver dysfunction.

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A key event in the development of preeclampsia is the reduction of placental perfusion in the early stages of pregnancy, which is due to defective trophoblastic invasion of uterine spiral-artery-vessel walls, therefore leading to poor development of the immature placenta and its maternal blood supply. This reduced placental perfusion leads to generalized dysfunction of the maternal vascular endothelium by mechanisms that still remain to be elucidated [18]. However, among patients with preeclampsia, there is an observed increase in the rate of lipid peroxidation, increased lipid availability, and decrease of several antioxidants such as alpha tocopherol, ascorbate, beta carotene and selenium [12]. Moreover, a significant decrease in plasma coenzyme Q_{10} (CoQ_{10}) in women with preeclampsia has recently been reported [11,14].

Since CoQ_{10} is a part of the non-enzymatic defense system against oxygen species (antioxidative function) [7], playing a key role in mitochondrial complexes I and III reaction mechanisms (electron transport) [1], it seems to be logical to hypothesize a role for CoQ_{10} at the placental level. In this sense, we previously reported lower plasma and higher placental CoQ_{10} content among preeclamptic women living in Quito, Ecuador (2,800 m above sea level) when compared to normal pregnancies [15]. On the other hand, it has recently been demonstrated that the number of uteroplacental vessels in low altitude placentas are significantly decreased compared to placentas at high altitude [16], a finding also reported in preeclampsia in which there were more uteroplacental arteries in placental bed biopsies from preeclamptic vs. uncomplicated pregnancies [13]. However, in normal pregnancies at sea level, significantly more arteries were remodeled per placenta than in normal pregnancies at high altitude [17]. Thus, the hypothesis that uteroplacental arterial remodeling would be reduced in high altitude pregnancy was supported, and the findings, as predicted, are intermediate between what is observed in preeclampsia and what is normal in healthy sea-level pregnancies [17]. Consistent with previous reports, uteroplacental arterial remodeling was not an 'all or none' phenomena at either high or low altitude [9], rather the proportion of arteries remodeled was reduced at high vs. moderate altitude.

Thus, it can be hypothesized that CoQ_{10} content differs not only in preeclampsia, but also depends on women's altitude of residency. The aim of the present study was to evaluate the CoQ_{10} levels both in plasma and placenta among normal pregnant and preeclamptic women residing either at high or low altitude.

2. Patients and methods

This study was carried out after approval of the Bioethics Committee of the Biomedical Center, Universidad Central, Quito, Ecuador, in which normal pregnant ($n = 60$) and preeclamptic ($n = 63$) primigravid women admitted to the "Hospital Gineco Obstétrico Isidro Ayora" in Quito, Ecuador and the "Hospital Gineco Obstétrico Enrique C. Sotomayor" in Guayaquil, Ecuador, were recruited after signing informed consent of participation. Out of a total of 123 pregnant women, 63 resided in Quito (2,800 m altitude) and 60 in Guayaquil (sea level). Women with a medical history of cardiovascular or gynecological problems, and on any medication (especially non steroidal anti-inflammatory drugs) were excluded from the study. Women with preeclampsia registered blood pressures over 140/90 mmHg on at least two occasions 6 hours apart, and proteinuria greater than $++$ as assessed by dipstick ($>$300 mg/dL) on two occasions 4 to 24 hours apart. Blood pressure was measured as previously described [8]. For the measurement of CoQ_{10}, a blood sample (10 ml) was obtained from each woman at the antecubital venous puncture site, and immediately transferred into a polypropylene vial containing 3.15% sodium citrate (1:9, v/v). These samples were centrifuged at room temperature and 500 ul plasma aliquots were frozen at – 40°C. After delivery of the placenta, a sample (2–3 g) from the maternal side was

obtained (any infracted area was avoided), washed twice with saline solution to remove the excess of blood, and transferred into a vial containing Krebs buffer solution. Measurement of CoQ_{10} content in placenta was carried out with approximately 100 mg of freeze-clamped tissue accurately weighed in the frozen state and subsequently homogenized with 2 ml of Krebs buffer in a glass homogenizer with a manual pestle, as described elsewhere [6]. Briefly, prior to homogenization, 50 ul of ethanolic BHT (2,6-di-tert-butyl-p-cresol; Sigma Aldrich, MO, USA) was added to prevent lipid autoxidation without reducing the ubiquinones. After addition of 1 ml of 0.1 M aqueous sodium dodecyl sulphate (SDS; Sigma-Aldrich, MO, USA) and a brief mixing by homogenization, the sample was transferred to a 10 ml test tube, fitted with a Teflon-lined screw cap. The homogenizer was rinsed with 2 ml of reagent alcohol, which was combined with the homogenate. The mixture was vortexed for 30 s, and 2 ml of hexane (Merck, Darmstadt, Germany) was added, and the tightly screwed test tube was vigorously vortexed for 2 min. It was then centrifuged for 5 min at 1,000 g to separate the layers. One milliliter of the hexane layer was transferred to a small vial and dried under nitrogen. The residue was redissolved in methanol/[ethanol/isopropanol 95/5, v/v] (1:1 v/v). Samples were analyzed immediately and kept on ice and covered with aluminum foil to prevent photodegradation of ubiquinones. CoQ_{10} was measured in a high performance liquid chromatography (HPLC) system (Perkin-Elmer, CN, USA) equipped with a Lichrosorb® RP18 (5 um, 125 $\times$ 4 mm; Phenomenex, CA, USA) column and with a guard column (Merck, Darmstadt, Germany), as previously described [14]. Samples were measured in duplicates and the mean value was used for statistical analysis.

Data was analyzed using GraphPad InStat version 3.01 for Windows (GraphPad Software, CA) and presented as mean $\pm$ standard deviations (S.D). Comparison of continuous data was performed with unpaired student T test. A p value of $\leqslant$0.05 was considered as statistically significant.

3. Results

Characteristics of women and neonates included in the study are presented in Table 1. Placental and neonatal weight at delivery from women with preeclampsia living in high altitude were found to be significantly lower. Preeclamptic women living at high altitude showed significantly lower CoQ_{10} plasma levels (0.64 $\pm$ 0.23 vs. 0.82 $\pm$ 0.46 μmol/L, $p = 0.05$). Contrarily, there were no differences in plasma CoQ10 levels among women living at sea level (normal: 0.50 $\pm$ 0.18 vs. preeclampsia: 0.51 $\pm$ 0.19 μmol/L, $p = NS$). Interestingly, plasma levels of CoQ_{10} in normal pregnant women living at sea level were significantly lower that those in normal pregnant women living at high altitude ($p = 0.001$) and comparable to those found in preeclampsia at high altitude ($p = NS$, Fig. 1). Placental CoQ_{10} content was found to be significantly higher among preeclamptic women living at sea level compared to those with normal pregnancies (0.120 $\pm$ 0.07 vs. 0.076 $\pm$ 0.04 ng/mg protein, $p < 0.005$). The same trend was found among women living at high altitude although this was not found to be significant (0.159 $\pm$ 0.13 vs. 0.135 $\pm$ 0.07 ng/mg protein, $p = NS$). Although placental CoQ_{10} content was found to be significantly higher among normal pregnant women at high altitude compared to those residing at sea level ($p < 0.0005$), no differences were found among preeclamptic women, either at high altitude or sea level (Fig. 2).

4. Discussion

Despite the limitations of this study (cross-sectional design and small sample size) it is the first to determine CoQ_{10} levels, both in plasma and placenta, simultaneously in pregnant women residing either

Table 1
Characteristics of gravids and neonates included in the study

Parameter	Normal	PE	Quito		Guayaquil	
	$n = 60$	$n = 63$	Normal $n = 30$	PE $n = 33$	Normal $n = 30$	PE $n = 33$
Maternal age (years)	20.7 ± 4.4[a]	20.6 ± 4.4	20.6 ± 4.6	20.6 ± 4.4	20.7 ± 4.2	20.6 ± 4.5
Systolic blood pressure (mmHg)	110.2 ± 9.3	144.9 ± 11[b]	110.7 ± 6.8	143.6 ± 11.4[b]	109.7 ± 11.3	146.3 ± 10.7[b]
Diastolic blood pressure (mmHg)	96.8 ± 10.5	72.1 ± 6.6[b]	73 ± 7	98.9 ± 7[b]	71.3 ± 6.2	94.5 ± 9.7[b]
Neonatal gestational age at delivery (weeks)	38.5 ± 1.6	38.6 ± 1.3	38.7 ± 1.1	38.2 ± 1.9	38.6 ± 1.5	38.8 ± 1
Neonatal weight at delivery (grs)	2,980.8 ± 597.8	2,908.8 ± 454.4	3,097 ± 364.7	2,757.8 ± 429.1[b]	2,864 ± 752.2	3,073 ± 429.1[c]
Placental weight (grs)	576.4 ± 98.5	556 ± 130.8	580.1 ± 98.5	503.5 ± 120.1[b]	572.6 ± 100	614 ±118[c]

[a]Mean ± standard deviation; [b]$p < 0.05$ compared to normal gestations (non paired t student's test); PE: preeclampsia; c-Q_{10}: co-enzyme Q_{10}; [c]$p < 0.05$ compared to PE or normal gestations from Quito.

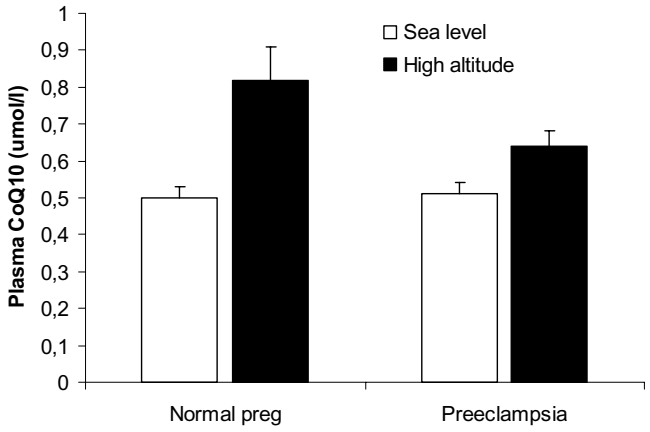

Fig. 1. Coenzyme Q_{10} plasma levels in normal pregnancies and during preeclampsia according to their residency (sea level or high altitude).

at sea level or at high altitude. In preeclamptic women residing at high altitude, there were lower CoQ_{10} plasma levels when compared to normal pregnancies. However, it might be important to emphasize that this difference was still present, although plasma levels in normal pregnant women residing at high altitude in the present report were slightly lower than those previously reported [15]. It is possible that gestational age at which samples were taken was responsible for this difference (unpublished data). In the present series, there were unexpected findings, not only the absence of differences in CoQ_{10} plasma levels between normal pregnant and preeclamptic women living at sea level; also for the relatively low CoQ_{10} plasma levels found in normal pregnant women residing at sea level. The latest results, that is in clear discrepancy with previous reports in pregnant women living close to the sea level [10,11], could not be interpreted without having the CoQ_{10} plasma levels in a group of Ecuadorian non-pregnant women residing at sea level, an additional control group not planned in this study. Contrarily to plasma levels, placental CoQ_{10} content appeared to be higher in preeclamptic women as compared to normal

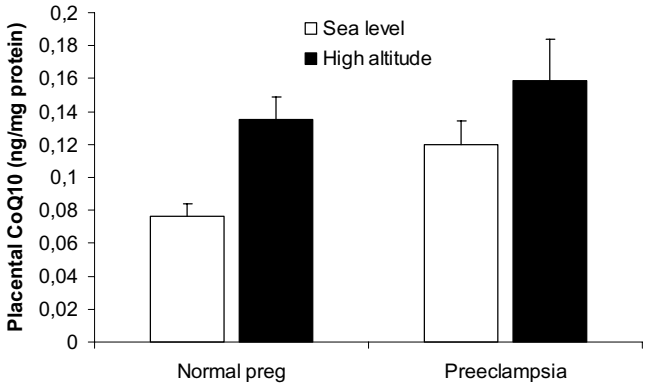

Fig. 2. Coenzyme Q_{10} placental content in normal pregnancies and during preeclampsia according to their residency (sea level and high altitude).

pregnancies, independently of the altitude of women's residency. This might be a surprising finding, as plasma concentrations of CoQ_{10} are significantly influenced by dietary uptake [19], while tissue levels of CoQ_{10} depend mainly on de novo synthesis [20]. Therefore, dietary CoQ_{10} concentrations could mask tissue deficiencies. On the other hand, it has been suggested that higher tissue concentrations of ubiquinone may be a result of a higher metabolic rate [2]. In this sense, higher placental CoQ_{10} contents were found among sea level residing Italian pregnant women with HELLP syndrome (a severe complication of preeclampsia) when compared to normal pregnancies, but plasma levels were not measured [4].

The observations in our study allow us to hypothesize that CoQ_{10} plasma levels are not directly related to placental CoQ_{10} content. Moreover, it is also plausible to assume that placental development is totally an energy dependent process and that CoQ_{10} could be an essential element in its physiological role. If this is true, then the next obvious questions to be considered should be: what occurs during preeclampsia? Are these high placental levels a late compensatory mechanism?

In conclusion it was determined that (1) during pregnancy there is no relationship between plasma CoQ_{10} levels and placental CoQ_{10} content and (2) women complicated with preeclampsia (high or low altitude) displayed high placental CoQ_{10} content, with significantly low plasma CoQ_{10} levels among those residing at high altitude. More research is warranted to establish the cause-effect relationship between CoQ_{10} levels and preeclampsia.

Acknowledgments

Financial support provided by "Secretaria Nacional de Ciencia y Tecnología – SENACYT", Ecuador through grant PIC-05-036.

References

[1] U. Brandt and J.G. Okun, Role of deprotonation events in ubihydroquinone: cytochrome c oxidoreductase from bovine heart and yeast mitochondria, *Biochemistry* **36** (1997), 11234–11240.

[2] L. Ernster, Ubiquinol as a biological antioxidant: a review, in: *Oxidative Processes and Antioxidants*, R. Paoletti, ed., Raven Press, Ltd., New York. 1994, pp. 185–198.

[3] S.A. Friedman, R.N. Taylor and J.M. Roberts, Pathophysiology of pre-eclampsia, *Clin Perinatol* **4** (1991), 661–682.
[4] S.R. Giannubilo, A.L. Tranquilli, C. Santolini, F. Prinicipi, R. Mancinelli and G.P. Littarru, Placental CoQ_{10} levels in HELLP syndrome, *Biofactors* **25** (2005), 159–163.
[5] J.P. Granger, B.T. Alexander, M.T. Llinas, W.A. Bennett and R.A. Khalil, Pathophysiology of preeclampsia: linking placental ischemia/hypoxia with microvascular dysfunction, *Microcirculation* **9** (2002), 147–160.
[6] J.K. Lang, K. Gohil and L. Packer Simultaneous determination of tocopherols, ubiquinols, and ubiquinones in blood, plasma, tissue homogenates, and subcellular fractions, *Anal Biochem* **157** (1986), 106–116.
[7] G. Lenaz, M. Cavazzoni, M.L. Genova, M. D'Aurelio, M. Merlo Pich, F. Pallotti, G. Formiggini, M. Marchetti, G. Parenti Castelli and C. Bovina, Oxidative stress, antioxidant defences and aging, *Biofactors* **8** (1998), 195–204.
[8] P. Lopez-Jaramillo, M. Narvaez, R.M. Weigel and R. Yepez, Calcium supplementation reduces the risk of pregnancy-induced hypertension in an Andes population, *Br J Obstet Gynaecol* **96** (1989), 648–655.
[9] J.W. Meekins, R. Pijnenborg, M. Hanssens, I.R. McFadyen and A. van Asshe, A study of placental bed spiral arteries and trophoblast invasion in normal and severe pre- eclamptic pregnancies, *Br J Obstet Gynaecol* **101** (1994), 669–674.
[10] G. Noia, G.P. Littarru, M. De Santis, A. Oradei, C. Mactromarino, C. Trivellini and A. Caruso, Coenzyme Q10 in pregnancy, *Fetal Diagn Ther* **11** (1996), 264–270.
[11] P.R. Palan, D.W. Shaban, T. Martino and M.S. Mikhail, Lipid-soluble antioxidants and pregnancy: maternal serum levels of coenzyme Q_{10}, alpha-tocopherol and gamma- tocopherol in preeclampsia and normal pregnancy, *Gynecol Obstet Invest* **58** (2004), 8–13.
[12] J.A. Spinnato and J.C. Livingston, Prevention of preeclampsia with antioxidants: evidence from randomized trials, *Clin Obstet Gynecol* **48** (2005), 16–429.
[13] K.A. Starzyk, C.M. Salafia, J.C. Pezzullo, J.M. Lage, V. Parkash, L. Vercruysse, M. Hanssens and R. Pijnenborg, Quantitative differences in arterial morphometry define the placental bed in preeclampsia. *Human Pathol* **28** (1997), 353–358.
[14] E. Teran, M. Racines-Orbe, S. Vivero, C. Escudero, G. Molina and A. Calle, Preeclampsia is associated with a decrease in plasma coenzyme Q_{10} levels, *Free Radic Biol Med* **35** (2003), 1453–1456.
[15] E. Teran, S. Vivero, M. Racines-Orbe, A. Castellanos, G. Chuncha, G. Enriquez and W. Moya, Coenzyme Q_{10} is increased in placenta and cord blood during preeclampsia, *Biofactors* **25** (2005), 153–158.
[16] M.C. Tissot van Patot, J. Bendrick-Peart, V.E. Beckey, N. Serkova and L. Zwerdlinger Greater vascularity, lowered HIF-1/DNA binding, and elevated GSH as markers of adaptation to *in vivo* chronic hypoxia, *Am J Physiol Lung Cell Mol Physiol* **287** (2004), L525–L532.
[17] M. Tissot van Patot, A. Grilli, P. Chapman, E. Broad, W. Tyson, D.S. Heller, L. Zwerdlinger and S. Zamudio, Remodelling of uteroplacental arteries is decreased in high altitude placentae, *Placenta* **24** (2003), 326–335.
[18] M. Vatish, H.S. Randeva and D.K. Grammatopoulos, Hormonal regulation of placental nitric oxide and pathogenesis of pre-eclampsia, *Trends Mol Med* **12** (2006), 223–232.
[19] C. Weber, A. Bysted and G. Lamer, The coenzyme Q_{10} content of the average Danish diet, *Int J Vitam Nutr Res* **67** (1997), 123–193.
[20] Y. Zang, F. Aberg, E.-L. Appelkvist, G. Dallner and L. Ernster, Uptake of dietary coenzyme Q supplement is limited in rats, *J Nutr* **154** (1995), 446–534.