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Coenzyme Q in pregnant women and rats with intrahepatic cholestasis

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Keywords
bile acids – coenzyme Q10 – coenzyme Q9 – oxidative stress – pregnancy cholestasis

Abbreviations
ALP, alkaline phosphatase; ALT, alanine-aminotransferase; AST, aspartate-aminotransferase; BA, bile acids; CA, cholic acid; CDCA, chenodeoxycholic acid; CoQ, coenzyme Q; CoQ10, coenzyme Q 10; CoQ9, coenzyme Q 9; DCA, deoxycholic acid; EE, ethinyl estradiol; ICP, intrahepatic cholestasis of pregnancy; LCA, lithocholic acid; MDA, malondialdehyde; ROS, reactive oxygen species; TBARS, 2-thiobarbituric acid reactive substances; TSBA, total serum bile acids; UDCA, ursodeoxycholic acid; γ-GT, γ-glutamyltranspeptidase.

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Abstract

Background & Aims: Intrahepatic cholestasis of pregnancy is a high-risk liver disease given the eventual deleterious consequences that may occur in the foetus. It is accepted that the abnormal accumulation of hydrophobic bile acids in maternal serum are responsible for the disease development. Hydrophobic bile acids induce oxidative stress and apoptosis leading to the damage of the hepatic parenchyma and eventually extrahepatic tissues. As coenzyme Q (CoQ) is considered an early marker of oxidative stress in this study, we sought to assess CoQ levels, bile acid profile and oxidative stress status in intrahepatic cholestasis. *Methods:* CoQ, vitamin E and malondialdehyde were measured in plasma and/or tissues by HPLC-UV method whereas serum bile acids by capillary electrophoresis in rats with ethinyl estradiol-induced cholestasis and women with pregnancy cholestasis. *Results:* CoQ and vitamin E plasma levels were diminished in both rats and women with intrahepatic cholestasis. Furthermore, reduced CoQ was also found in muscle and brain of cholestatic rats but no changes were observed in heart or liver. In addition, a positive correlation between CoQ and ursodeoxycholic/lithocholic acid ratio was found in intrahepatic cholestasis suggesting that increased plasma lithocholic acid may be intimately related to CoQ depletion in blood and tissues. *Conclusion:* Significant CoQ and vitamin E depletion occur in both animals and humans with intrahepatic cholestasis likely as the result of increased hydrophobic bile acids known to produce significant oxidative stress. Present findings further suggest that antioxidant supplementation complementary to traditional treatment may improve cholestasis outcome.

Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-specific liver disorder usually benign to the mother but it may have serious consequences for the foetus, such as premature deliveries, foetal distress and perinatal mortality (1, 2). ICP is a high-risk hepatic disease, so an early and accurate diagnosis is crucial to find an appropriate medical treatment to improve foetal outcome. At present, the use of ursodeoxycholic acid (UDCA) is considered the treatment of choice (3).

ICP is characterized by the accumulation of bile acids (BA) in maternal serum which are likely responsible for the development of the disease because of their cytotoxic effects (4). Augmented hydrophobic BA induces oxidative stress and apoptosis leading to damage hepatic parenchyma and, eventually, extrahepatic tissues. If this event takes place during gestation, the health of the foetus may be a challenge (5). Oxidative stress results from an imbalance between increased free radical production and impairment of free radical scavenging. Coenzyme Q (CoQ) (2, 3-dimethoxy-5-ethyl-6-multiprenyl-1,4-benzoquinone) is a redox-active, lipophilic substance integrated in the mitochondrial respiratory chain which acts

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as an electron carrier for the production of cellular energy (6). In addition, it is recognized as a primary regenerating antioxidant playing an intrinsic role against oxidative damage (7). Recently, it has been hypothesized that CoQ interacts earlier than vitamin E in the antioxidant system and its depletion would be an earlier marker of oxidative damage (8). Moreover, CoQ effectively supports the antioxidant activity of vitamin E (up to now considered the most important antioxidant) by reducing its oxidated form (9).

The predominant analogue of CoQ in humans is coenzyme Q10 (CoQ10), which contains 10 isoprenoid units in the tail, whereas the predominant form in rodents is coenzyme Q9 (CoQ9) which has nine isoprenoid units in the tail.

Although the importance of CoQ in mitochondrial function is well established, the significance of CoQ deficiency has recently achieved clinical relevance. In this sense, CoQ determination has acquired clinical significance as a biomarker, particularly for metabolic and oxidative stress abnormalities (6, 10). Human CoQ deficiency can be classified as primary or secondary resulting in different heterogeneous diseases. Primary CoQ deficiency seems to be relative rare and has been associated with mutations of diverse genes involved in its biosynthesis (11, 12). Growing evidence supports that secondary or acquired CoQ deficiency is more common. Low plasma CoQ has been reported in different diseases including muscular, neurodegenerative, cardiovascular and reproductive diseases as well as cancer (13–16), among others. However, none of these reports demonstrated CoQ deficiency by measuring it in tissues (17). Moreover, although clinically important, to our knowledge, CoQ status in ICP has not been so far reported.

In this study, we sought to assess CoQ levels, BA profile and oxidant stress status in rats treated with ethinyl estradiol (EE) as well as women with ICP.

Materials and methods

Animals

Female Sprague–Dawley strain rats (School of Pharmacy and Biochemistry, University of Buenos Aires) weighing between 200 and 230 g were used in the experiments. Animals were housed in steel cages and maintained at a temperature between 22 and 24°C in a controlled room with a 12 h light/dark cycle (light from 7:00 to 9:00). All experiments were performed following the recommendations of the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publication N85-23, 1985, revised 1996). All experimental protocols were approved by the Animal Care Committee of the School of Pharmacy and Biochemistry, University of Buenos Aires.

Intrahepatic cholestasis in rats was induced by daily subcutaneous injection of 5 mg/kg EE dissolved in propylene glycol for five consecutive days (Sigma-Aldrich, St Louis, MI, USA). ICP is the clinical correlate of this animal model of cholestasis (18, 19). Control animals were injected with the same volume of vehicle. Rats were sacrificed by decapitation, a blood sample obtained and the whole heart, brain, left hepatic lobule and skeletal muscle (quadriceps) were excised. Tissues and blood samples were stored at −80°C until assayed.

In order to evaluate whether EE *per se* affected CoQ, a set of animals were sacrificed 24 h following the first EE injection.

Patients

This study was conducted in women with normal pregnancy and with ICP under gestational control at the national hospital Jose de San Martin, associated with the University of Buenos Aires, Argentina.

This study complied with the Declaration of Helsinki and it was approved by the Institutional Review Board and the Bioethical Committee of our Institution. Written consent from all patients was obtained.

Thirty-seven healthy pregnant women and 19 ICP patients (both groups in the third trimester of pregnancy) were included in the study.

Diagnosis of ICP was based on the presence of pruritus with elevation of total serum BA (TSBA) higher than 11 μmol/L and/or at least one of the two aminotransferases [alanine-aminotransferase (ALT) or aspartate-aminotransferase (AST)] higher than the upper normal limit (40 and 31 UI/L, respectively), and/or elevation of γ- glutamyltranspeptidase (γ-GT) above 36 UI/L during the second half of an otherwise uneventful pregnancy, and the absence of infection by hepatitis A virus, hepatitis B virus and hepatitis C virus, autoimmune diseases, alcohol intake, HIV, skin diseases or biliary obstruction.

Pruritus was arbitrarily measured in an ordinary scale: grade 1 intermittent, nocturnal, and slight; grade 2, continuous diurnal and nocturnal, from slight to moderate; grade 3 severe and grade 4 severe but also accompanied by insomnia or itching lesions. The patients were not treated with UDCA at the time of the study.

Blood samples were obtained after a fasting period of 10 h, and frozen at −80°C until assayed to avoid CoQ degradation (20).

Analytical procedures

Coenzyme Q determination in plasma and tissues. Tissue samples were weighed and homogenized with 3.2 ml cold 1-propanol on ice bath. The mixture was then vortex-mixed for 1 min, sonicated and transferred to a polypropylene tube, ultracentrifuged at 9000*g* for 10 min.

Heparinized plasma (100 μl) was supplemented with 200 μl cold 1-propanol, centrifuged and the organic layer was evaporated to dryness under a stream of nitrogen. The dry residue was dissolved in 50 μl ethanol.

Assessment of CoQ in plasma and tissues was performed by an optimized micro HPLC-UV method as previously detailed (21, 22). The final chromatographic conditions were: 30°C column temperature, the isocratic mobile phase consisted of methanol 100% and the flow rate was set at 0.4 ml/min. UV-detection was performed at 275 nm with an injection volume of 10 μl. Separation was achieved using an XTerra C18 analytical microcolumn (50 × 2.1 mm i.d., 3.5 μm particle size) with a guard column XTerra C18 (Waters corp., Milford, MA, USA).

Plasma vitamin E determination. Heparinized plasma (50 μl) was supplemented with 100 μl cold 1-propanol, centrifuged and the organic layer was evaporated to dryness under a stream of nitrogen. The dry residue was dissolved in 100 μl methanol.

The analysis of vitamin E in plasma was performed by an HPLC-UV method with some modifications (23). The final chromatographic conditions were: 25°C column temperature, the isocratic mobile phase consisted of methanol: water (94:6) and the flow rate was set at 0.4 ml/min. UV-detection was performed at 296 nm with an injection volume of 10 μl. Separation was achieved using a BDS Hypersil C18, Thermo Scientific analytical microcolumn (100 × 2.1 mm i.d., 2.4 μm particle size) with a guard column.

Malondialdehyde (MDA) determination in tissues and plasma. Aliquots of tissue homogenates (75–300 μg protein) and 250 μl of plasma samples was added with 0.2% (w/v) butylhidroxytoluene in ethanol and 2.8% (w/v) trichloroacetic acid and the resulting suspension centrifuged at 4000*g* for 10 min. The supernatant was mixed with 0.6% (w/v) thiobarbituric acid, heated for 45 min at 90°C, and then centrifuged at 4000*g* for 10 min at 4°C.

MDA was determined in plasma and tissues by the 2-thiobarbituric acid reactive substances (TBARS) method by isocratic reverse phase HPLC with minor modifications (24). The analytical column was a Microsorb C18, Agilent (15 × 4.6 mm i.d., 5 μm particle size) and a C18 guard column. The mobile phase was 50 mM phosphate buffer pH 7.00: Methanol (65:35). The flow rate was 1.0 ml/min, the injection volume was 100 μl and the detection wavelength was set at 532 nm.

TSBA and BA profile in serum. TSBA and their individual profile: cholic acid (CA), chenodeoxycholic acid, deoxycholic acid (DCA), LCA and UDCA, in their free, glycine and taurine derivative forms were assessed by capillary electrophoresis. A detailed description of the analytical method was previously reported (25).

Liver function tests

ALT, AST, alkaline phosphatase (ALP), γ-GT enzymatic activity and total and conjugated bilirubin concentration were performed by standard routine automated techniques (Roche Cobas C501 Chemistry Analyzer; Roche Diagnostics, Holliston, MA, USA). Normal range for total bilirubin is up to 1.0 mg/dl, whereas for direct bilirubin up to 0.3 mg/dl. Normal ranges for AST, ALT, ALP, γGT and cholesterol are 12–31, 12–40, 90–240, 5–36 UI/L and <200 mg/dl respectively.

Statistical analysis

Shapiro–Wilkś *W*-test of normality was performed. Kruskal–Wallis non-parametric analysis followed by the Mann–Whitney *U*-test was used. Differences between groups were analysed by Student's *t*-test or non-parametrical tests, according to the distribution. Pearson's *r* coefficient was calculated for correlations. Levels of significance were established at $P < 0.05$.

Results

Animals

EE-treated rats showed significantly reduced bile flow as compared with control rats (Table 1). In addition, cholestatic animals also showed increased TSBA (Fig. 1). The increase in LCA (Fig. 1B) was more pronounced than that of TSBA (Fig. 1A).

Figure 2 shows the levels of CoQ9 (Fig. 2A) and CoQ10 (Fig. 2B) in blood and tissues of control and cholestatic (EE-treated) rats. The plasma concentration of CoQ9 in control rats was more than three-fold higher than that of CoQ10 in accordance with previous findings further supporting that the predominant CoQ homologue in rodents is CoQ9 (26). In cholestatic rats, CoQ10 plasma levels showed a decreasing trend but it was not statistically significant. However, plasmatic CoQ9 concentration was markedly decreased in EE-treated rats (Fig. 2A).

In all tissues studied (skeletal muscle, brain, heart and liver) of control rats, the concentration of CoQ9 was approximately two- to 12-fold higher than that of CoQ10. The concentration of total CoQ (CoQ9 + CoQ10) varied greatly with tissues being the rank order as follows: heart>skeletal muscle>brain>-liver.

When CoQ was examined in cholestatic rats, no changes in heart and liver CoQ9 and CoQ10 were observed whereas both CoQ homologues were signifi-

Table 1. Bile flow of control and EE-treated rats. Bile flow is expressed as μl/min/100 g body weight (BW). Results are expressed as means ± SEM

Time (min)	Control (μl/min/100 g BW)	EE-treated (μl/min/100 g BW)
5	4.970 ± 0.303	2.460 ± 0.1521***
10	4.493 ± 0.544	1.803 ± 0.163***
15	4.463 ± 0.211	1.673 ± 0.202***
20	4.478 ± 0.132	1.425 ± 0.251***

***$P < 0.001$ vs. control.

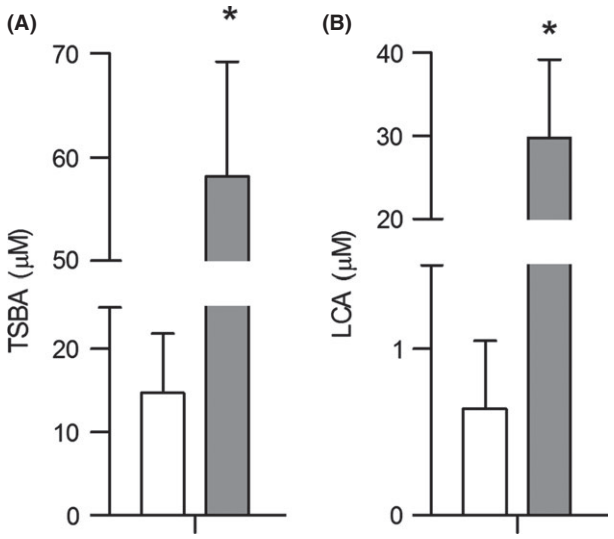

Fig. 1. Total bile acids (TSBA) and lithocholic acid (LCA) in rats with intrahepatic cholestasis. Cholestasis was induced in rats by ethinyl estradiol administration and TSBA (A) and LCA (B) were determined in serum as detailed in Materials and methods. Results are expressed as means ± SEM. □, Control; ■, cholestatic rats. *$P < 0.05$ vs. control; n: 5–9.

cantly decreased in skeletal muscle and brain as compared to control rats (Fig. 2A and B).

CoQ homologues in plasma and tissues were similar in control and rats sacrificed at 24 h showing that EE *per se* does not affect CoQ content.

Furthermore, plasma levels of vitamin E were also significantly diminished in cholestatic rats (Fig. 2C). However, TBARS showed no statistical differences in tissues or plasmas between the two groups (Table 2).

In plasma, CoQ9 correlated negatively with LCA in EE-treated rats (Fig. 3A) and positively with vitamin E (Fig. 3B) and UDCA/LCA ratio (Fig. 3C).

Patients

The clinical data and biochemical markers of the patients studied are shown in Table 3. The age and gestational age of women with normal pregancy or ICP were comparable at the time of the study. Nine of the 19 patients had pruritus grade 1 or 2 at the time of the query and extraction of blood. Results showed that women with ICP had aminotransferases, ALP and γGT higher than normal pregnant women. The analysis of BA showed that women with ICP had significantly higher levels of TSBA, LCA, DCA and free bile acids than clinically healthy pregnant women, whereas UDCA concentration remained unchanged (Table 4).

In accordance with the results obtained in animals, CoQ10 was significantly diminished in ICP patients with respect to normal pregnant women (Fig. 4A).

Vitamin E was also markedly decreased in ICP patients as seen in cholestatic rats (Fig. 4B). Furthermore, positive correlation was observed between CoQ10 and vitamin E ($r = 0.9890$, $P < 0.05$).

Discussion

Reactive oxygen species (ROS) are highly reactive compounds that induce structural and functional alterations that may eventually cause cell death. Therefore, the study of antioxidant systems raises great interest because of their potential beneficial and therapeutic effects.

CoQ is an endogenous antioxidant compound considered an early marker of oxidative damage (8). CoQ10 levels are elevated during human pregnancy, particularly in the third trimester. The high plasmatic level in full-term pregnant women may represent a CoQ10 storage to protect mothers and neonates from oxidative stress caused by higher energetic requirements at delivery time (9). Hence, a CoQ10 decrease in pregnant women may turn dangerous for the foetus.

ICP is a high- risk pregnancy disease characterized by a hydrophobic BA increase in plasma (27). BA disrupt cell membranes through their detergent action on lipid components and promote the generation of ROS that react with lipids, proteins and nucleic acids eventually leading to hepatocyte apoptosis. Additionally, they can activate Kupffer cells to generate ROS that may contribute to hepatocyte insult (28–30). BA cytotoxicity is associated with the most hydrophobic species, being LCA the most hydrophobic (5, 31) and UDCA the most hydrophilic (32). Growing evidence supports that the increase in serum BA is responsible for the enhanced oxidative stress that occurs in ICP (33–35).

In this study, a validated animal model of ICP which shows similar biochemical hepatic alterations as observed in ICP patients was used in the study (36, 37). Rats with intrahepatic cholestasis induced by EE (38) showed a significant decrease in CoQ9 and vitamin E in plasma that correlated well with an increase in LCA levels. Oxidative damage has traditionally been assessed using TBARS assay although different studies support that TBARS may not be sensitive enough to evaluate oxidative stress (39). In our study, TBARS levels were similar in cholestatic and non-cholestatic rats. In accordance, Aboutwereat and co-workers found that TBARS levels were similar in control and patients with primary biliary cirrhosis although the assessment of other markers support increased oxidative stress (39). In fact, oxidative stress is a feature of all types of cholestasis as supported by different studies (40–43). Although TBARS levels were similar in cholestatic and control rats, CoQ and vitamin E were significantly reduced supporting increased oxidative stress. Present results further support the hypothesis that CoQ is an early marker of oxidative stress.

Plasma CoQ more likely reflects the degree of tissue metabolic request. However, tissue CoQ is strictly related to the balance existing between biosynthetic and dietary supply on one side and energetic consumption

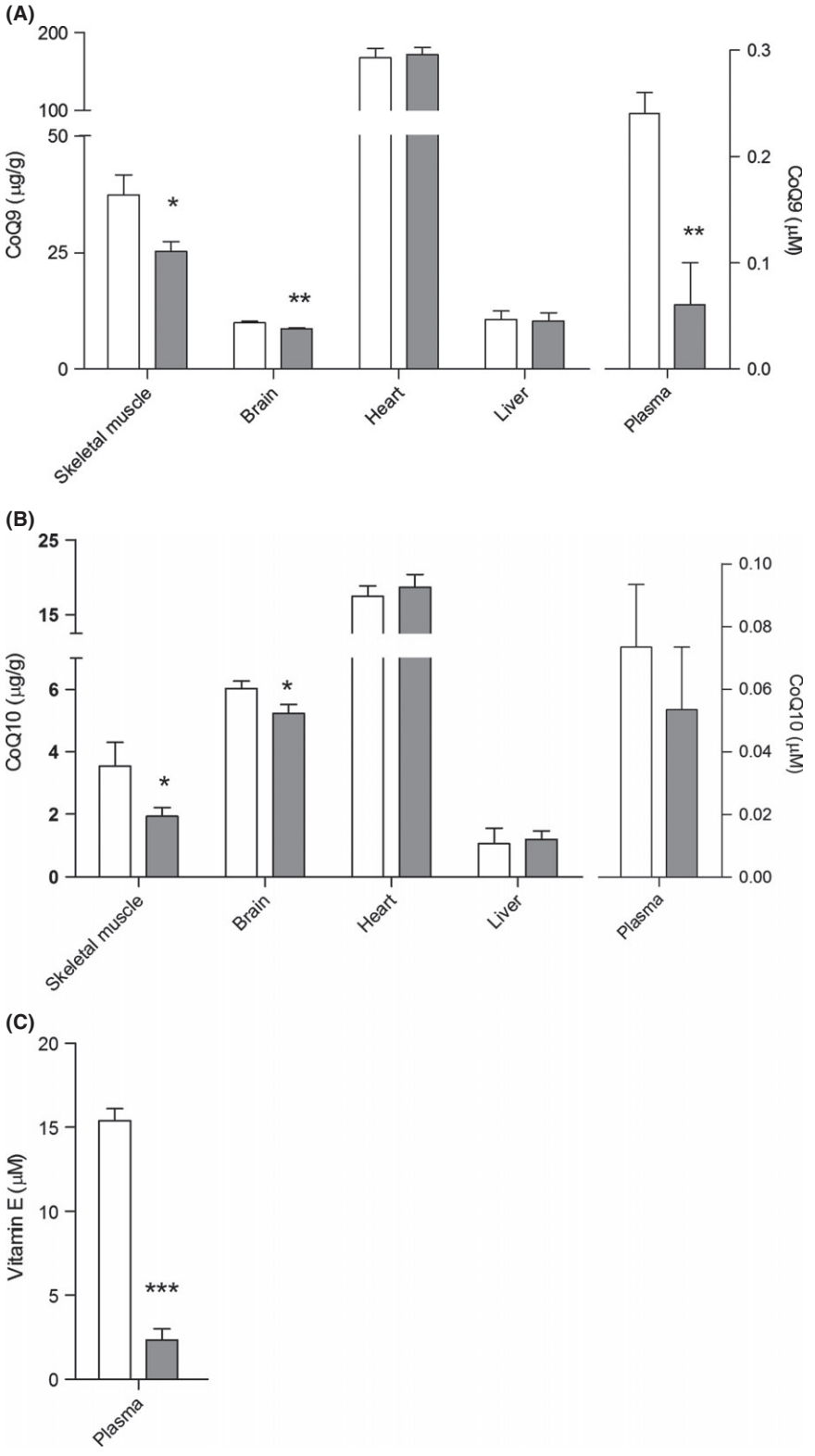

Fig. 2. Coenzyme Q homologues and vitamin E levels in rats with intrahepatic cholestasis. Rats with cholestasis induced by ethinyl estradiol were assessed for coenzyme Q9 (CoQ9) (A) and coenzyme Q10 (CoQ10) (B) in plasma and tissues and vitamin E in plasma (C) by HPLC as detailed in Materials and methods. □, Control; ■, cholestatic rats. Results are expressed as means ± SEM. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ vs. control. n: 5–9.

Table 2. TBARS levels in rats with intrahepatic cholestasis

	TBARS	
	Control (n = 5)	EE-treated (n = 9)
Plasma (μM)	1.19 ± 0.16	0.86 ± 0.10
Skeletal muscle (pmol/μg protein)	0.62 ± 0.43	0.90 ± 0.42
Brain (pmol/μg protein)	1.06 ± 0.27	0.77 ± 0.07
Heart (pmol/μg protein)	12.6 ± 1.5	11.7 ± 0.8
Liver (pmol/μg protein)	0.44 ± 0.08	0.49 ± 0.07

Cholestasis was induced in rats by ethinyl estradiol (EE) administrations as detailed in Materials and methods. Results are expressed as means ± SEM.

on the other (9). Therefore, CoQ homologues (CoQ9 and CoQ10) were assessed in different tissues from cholestatic rats. We evaluated CoQ in skeletal muscle, brain, heart and liver given that CoQ serves as an important antioxidant in these tissues and its depletion is particularly associated with central nervous system, heart and muscle diseases. In accordance, skeletal muscle and brain from cholestatic rats showed reduced levels of the coenzyme whereas no differences were observed in the liver or heart. Given that CoQ is considered the first line of defence in response to oxidative stress (8), tissue distribution of CoQ in cholestatic rats suggests that the brain and skeletal muscle seem to be more susceptible to the oxidative insult induced by cholestasis.

It is interesting to mention that EE-treated rats showed decreased spontaneous locomotor activity as compared to control rats likely attributed to the progressive muscle weakness and abnormal fatigue normally found in CoQ10 deficiency (14).

CoQ levels in the liver were similar in control and cholestatic rats and this finding may be related to the slight liver damage observed in this pathology. Furthermore, it may be related to a hepatic paradox previously described in animal models of cholestasis including EE-cholestasis. Despite increased hepatic BA, the activity of HMG-CoA reductase and 7alpha hydroxylase is increased (44–47). These authors explained this paradox by showing that increased hydrophilic BA in cholestatic livers do not result in enzyme inhibition. Since CoQ is synthesized via HMG-CoA reductase, it seems likely that the decrease in CoQ caused by oxidative stress in ICP would be compensated by an increase in its synthesis. This finding may contribute to explain the similar CoQ9 and CoQ10 levels found in the liver.

During parturition, normal oxidative stress occurs; hence, a defective response to an oxidant stimulus could be a possible factor in human early pregnancy failure. Compagnoni *et al.* (9) suggested that, in normality, nature 'knowing' the risk of oxidative stress deriving from the impact of neonate with the extrauterine environment, prepares the mother with and adequate storage of

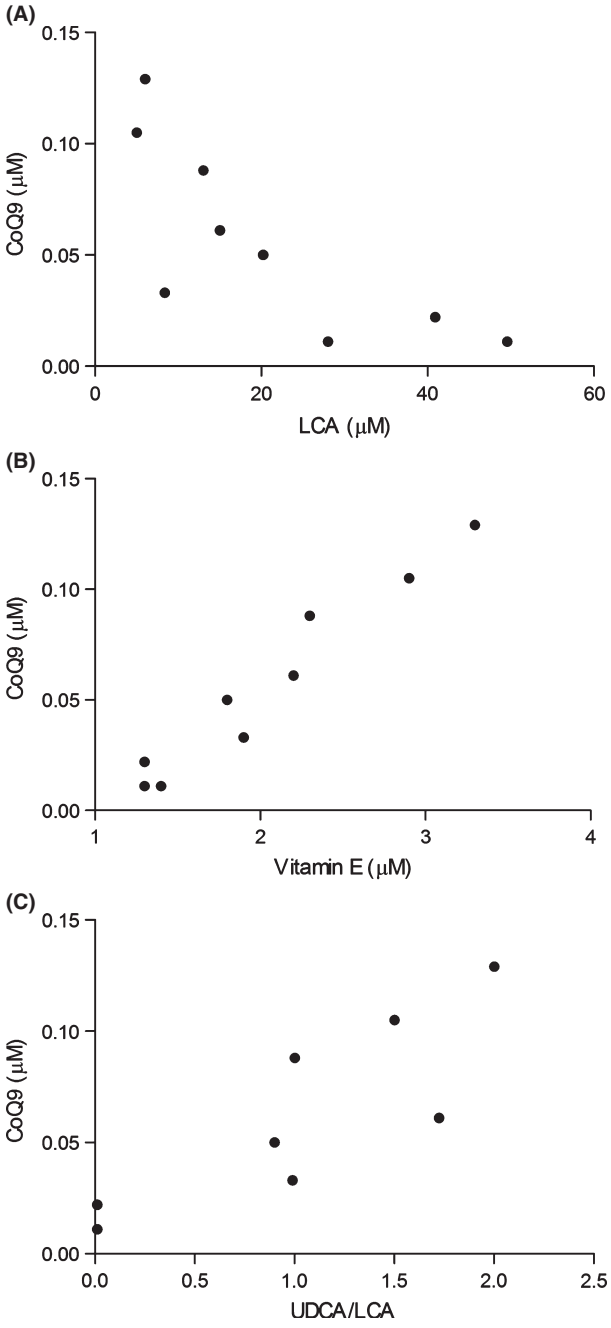

Fig. 3. Correlation of different markers in rats with intrahepatic cholestasis. Correlation between coenzyme Q9 (CoQ9) and lithocholic acid (LCA) ($r = -0.8298$, $P < 0.05$) (A), CoQ9 and vitamin E ($r = 0.9745$, $P < 0.01$) (B), and CoQ9 and ursodeoxycholic/lithocholic acid ratio (UDCA/LCA) ($r = 0.8615$, $P < 0.05$) (C). Cholestasis was induced by ethinyl estradiol as detailed in Materials and methods.

antioxidant system as CoQ10. However towards the end of gestation, there is an increase in oxygen tension causing ROS production and therefore distinct enzymatic antioxidant defences have evolved to quench the resultant radicals. When ROS generation exceeds the capacity

Table 3. Clinical and biochemical markers in normal pregnant women and patients with intrahepatic cholestasis of pregnancy (ICP)

	Normal pregnant women (n = 37)	ICP patients (n = 19)
Age in years (range)	17–41	18–42
Personal or familial history of ICP (n)	0	1
Gestational age (weeks)[a]	36.3 ± 0.7	33.9 ± 1.0
Presence of pruritus	0	9
Total bilirubin (mg/dl)	0.30 ± 0.02	0.35 ± 0.04
Direct bilirubin (mg/dl)	0.20 ± 0.03	0.25 ± 0.05
AST (UI/L)	19.3 ± 1.2	40.5 ± 10.4*
ALT (UI/L)	8.94 ± 5.63	44.7 ± 15.2**
ALP (UI/L)	309 ± 15	483 ± 57*
γGT (UI/L)	10.7 ± 0.9	24.0 ± 4.3*
Cholesterol (mg/dl)	246 ± 10	229 ± 17

Results are expressed as means ± SEM.
[a]At the time of the study.
*P < 0.05, **P < 0.01 vs. control.

Table 4. Total serum bile acids (TSBA) and bile acid profile in the serum of women with normal pregnancy and with intrahepatic cholestasis of pregnancy (ICP)

	Normal pregnant women (n = 37)	ICP patients (n = 19)
Bile acid		
TSBA (μmol/L)	6.70 ± 0.69	37.7 ± 5.9***
LCA (μmol/L)	0.33 ± 0.06	5.98 ± 1.61***
DCA (μmol/L)	1.39 ± 0.68	6.37 ± 2.69**
UDCA (μmol/L)	3.74 ± 0.81	3.26 ± 0.87
Free (μmol/L)	1.90 ± 0.40	20.5 ± 5.6***
UDCA/LCA	13.2 ± 3.3	2.50 ± 1.30***

Results are expressed as means ± SEM.
**P < 0.01, ***P < 0.001 vs. control.

of the antioxidant defences, oxidative stress results and indiscriminate damage to proteins, lipids and DNA occurs leading to eventual cell death (48). Therefore, decreased tissue CoQ content in the mother may represent a risk factor for the newborn. Besides, neonates, and especially preterm infants, are at higher risk for oxidative stress at birth and are very susceptible to oxidative damage by ROS because the extrauterine environment is richer in oxygen than the intrauterine environment. This problem is aggravated by the low efficiency of natural antioxidant systems in the newborn that could be worsened even more if the antioxidant capacity of the mother is deficient (9, 49).

Taking into account the importance of the consequences related to decreased antioxidant defense in ICP and in order to assess whether the results observed in animals were confirmed in patients and conclusively demonstrate CoQ status in ICP, we analysed plasmatic

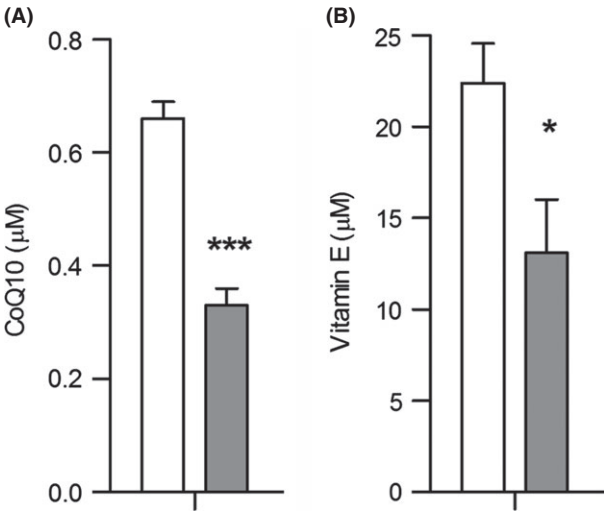

Fig. 4. Plasmatic levels of Coenzyme Q10 (CoQ10) and vitamin E in women with normal pregnancy and with intrahepatic cholestasis. CoQ10 (A) and vitamin E (B) in normal pregnant women (□) and patients with intrahepatic cholestasis of pregnancy (■). Results are expressed as means ± SEM. *P < 0.05 and ***P < 0.001 vs. control. n: 19–37.

CoQ10, vitamin E levels and serum BA profiles in women with normal pregnancy and with ICP.

In this study, the plasma concentration of CoQ10 found in normal pregnant women of our population was similar to those previously reported by other authors (50). However, we have found decreased CoQ10 levels in patients suffering ICP. This decrease was accompanied by reduced vitamin E plasmatic level. Various reports have addressed vitamin E levels in other cholestatic diseases but results are controversial. Levels of vitamin E were found to be decreased in primary biliary cirrhosis and sclerosing cholangitis (n = 105) (51) but other authors found no significant changes in plasma vitamin E levels in patients with primary biliary cirrhosis, although the number of patients included in the study was considerably lower (n = 41) (39). However, to our knowledge, this is the first study to report decreased CoQ10 and vitamin E levels in pregnancies complicated with cholestasis.

We have also found an increased hydrophobic serum BA profile in ICP patients as we have previously reported (27, 52). Reduced levels of CoQ10 may be attributed to the increase in serum BA which are thought to be responsible for the enhanced oxidative stress occurring in ICP (5, 33–35). An interesting finding was that UDCA/LCA ratio was diminished in ICP because of increased LCA with no changes in UDCA with respect to normal pregnancies. It has been well established that UDCA has protective and antioxidant properties whereas LCA is toxic (5, 33–35). Therefore, the decrease in the UCDA/LCA ratio in ICP together with reduced CoQ10 supports impaired antioxidant protection.

Decreased CoQ10 in IPC patients may reveal an imbalance in oxidative stress and antioxidant defences thus affecting energy production and increasing risk for the foetus. Although the relationship between CoQ10 and serum BA is not well established, it is possible that reduced CoQ10 levels result from increased production of hydrophobic BA and enhanced consumption of the coenzyme by free radicals. However, it is also possible that CoQ10 depletion may be caused by enhanced coenzyme extraction from blood because of increased cellular demand.

Although investigations on the effect of antioxidants in risk pregnancies are scarce, recognition of CoQ deficiency is highly important because it is possible that complementary supplementation with CoQ10 to patients with ICP may protect against the production of free radicals improving maternal and foetal outcomes.

In conclusion, present findings show that CoQ and vitamin E were diminished in plasma of both animals and patients with intrahepatic cholestasis. Furthermore, reduced CoQ was also found in muscle and brain of cholestatic rats. In addition, a positive correlation between CoQ and ursodeoxycholic/lithocholic acid ratio (UDCA/LCA) was found suggesting that increased plasma LCA may be intimately related to CoQ depletion in blood and tissues.

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