The mitochondrial-targeted antioxidant, MitoQ, increases liver mitochondrial cardiolipin content in obesogenic diet-fed rats

Gilles Fouret, Evanthia Tolika, Jérôme Lecomte, Béatrice Bonafos, Manar Aoun, Michael P. Murphy, Carla Ferreri, Chryssostomos Chatgilialoglu, Eric Dubreucq, Charles Coudray, Christine Feillet-Coudray

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A B S T R A C T

Cardiolipin (CL), a unique mitochondrial phospholipid, plays a key role in several processes of mitochondrial bioenergetics as well as in mitochondrial membrane stability and dynamics. The present study was designed to determine the effect of MitoQ, a mitochondrial-targeted antioxidant, on the content of liver mitochondrial membrane phospholipids, in particular CL and its fatty acid composition in obesogenic diet-fed rats. To do this, twenty-four 6 week old male Sprague Dawley rats were randomized into three groups of 8 animals and fed for 8 weeks with either a control diet, a high fat diet (HF), or a HF diet with MitoQ (HF + MitoQ). Phospholipid classes and fatty acid composition were assayed by chromatographic methods in liver and liver mitochondria. Mitochondrial bioenergetic function was also evaluated. While MitoQ had no or slight effects on total liver fatty acid composition and phospholipid classes and their fatty acid composition, it had major effects on liver mitochondrial phospholipids and mitochondrial function. Indeed, MitoQ both increased CL synthase gene expression and CL content of liver mitochondria and increased 18:2n-6 (linoleic acid) content of mitochondrial phospholipids by comparison to the HF diet. Moreover, mitochondrial CL content was positively correlated to mitochondrial membrane fluidity, membrane potential and respiration, as well as to ATP synthase activity, while it was negatively correlated to mitochondrial ROS production. These findings suggest that MitoQ may decrease pathogenic alterations to CL content and profiles, thereby preserving mitochondrial function and attenuating the development of some of the features of metabolic syndrome in obesogenic diet-fed rats.

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1. Introduction

Metabolic syndrome comprises pathological conditions that include visceral adiposity, insulin resistance (IR) and hepatic steatosis which favor the development of type 2 diabetes and cardiovascular diseases [1]. The liver plays a major role in substrate metabolism and is a primary target of insulin action, so it is at the crossroads of metabolism and disease. Liver mitochondria are central to energy metabolism, converting the energy stored in nutrients into ATP through the action of oxidative phosphorylation. Mitochondria are also a major source of cellular reactive oxygen species (ROS), which arise as byproducts of the mitochondrial respiratory chain complexes. It is generally accepted that the development of metabolic syndrome is accompanied with mitochondrial dysfunction and increased mitochondrial ROS production [2].

Phospholipids play multiple roles in cellular and organelle membranes including establishing a permeability barrier, providing the matrix for the assembly and function of a wide variety of catalyzed reactions, acting as donors in the synthesis of macromolecules and actively influencing the functional properties of membrane-associated processes [3]. Prominent among mitochondrial phospholipids is cardiolipin (CL) which is predominantly localized in the mitochondrial inner membrane, at the heart of mitochondrial metabolism. Cardiolipin is known to affect mitochondrial bioenergetics and contributes to mitochondrial membrane stability and dynamics [4]. In fact, significant changes in CL content/profile may alter membrane fluidity, organization and functioning of the mitochondrial respiratory chain complexes and their interactions, and may be associated with increased mitochondrial production of ROS [5]. Therefore, alterations to CL content or its fatty
acid composition would be expected to modulate mitochondrial function in health and disease [6,7].

MitoQ is a mitochondrial-targeted antioxidant that is directed to the matrix surface of the mitochondrial inner membrane where it is particularly effective against lipid peroxidation [8,9]. The effects of MitoQ on the phospholipid content and the fatty acid composition of the mitochondrial membranes are unknown. Here, we assessed the hypothesis that a redox-dependent modulation of CL content or fatty acid metabolism could contribute to the development of metabolic syndrome [10] and that MitoQ might slow down this process [11]. To do this, we determined the effect of MitoQ on the phospholipid content and their fatty acid composition of liver membranes and mitochondrial membranes from obeseogenic diet-fed rats, with a particular focus on CL content. In addition, we also investigated how the modulation of mitochondrial CL content and fatty acid composition impacted the function of liver mitochondria.

2. Materials and methods

2.1. Animals and diets

Twenty four 6 week old male Sprague Dawley rats (Charles River, L’Arbresle, France) were housed, 2 per cage, under conditions of constant temperature (20–22 °C), humidity (45–50%) and a standard dark cycle (20.00–08.00 h). The rats were randomized into three groups of 8 animals each and fed for 8 weeks one of the following semi-purified diets: 1) control diet with 4% lipid as soybean oil, 2) high fat diet (HF), and 3) HF diet with MitoQ (HF + MitoQ). The macronutrient composition of these three diets is given in the Supplementary Table 1. The HF diet contains 35% lipids (4% soybean oil + 31% lard). The lipid fraction of the control diet was composed of 14% saturated fatty acids (SFA), 26% monounsaturated fatty acids (MUFA) and 60% polyunsaturated fatty acids (PUFA), and that of the HF diet was composed of 40% SFA, 42% MUFA and 18% PUFA (Supplementary Table 2). MitoQ was used at the dose 0.86 μg MitoQ/kg diet. Rats were given free access to tap water and food. Rat body weight and food consumption were determined weekly. Our institution guidelines for the care and use of laboratory animals were observed and all experimental procedures were approved by the local ethical committee in Montpellier, France (Reference CEEA-LR-12002). MitoQ was provided by Prof. R.A.J. Smith from the University of Otago in New Zealand.

2.2. Oral glucose tolerance test

The oral glucose tolerance test (OGTT) was completed four to five days before rat sacrifice and performed as previously described [11]. Briefly, 16-hours fasting rats received by gavage 2 g glucose/kg body weight. Blood was sampled from the tail vein of conscious rats immediately prior to the gavage, and 20, 40, 60, 90, 120 and 180 min afterward and blood glucose was measured using glucose strips and a glucometer. The AUC values are expressed as mg glucose·dl⁻¹·min⁻¹.

2.3. Sampling and routine biochemical analysis

Four to five days after the OGTT, 16 hours-fasted rats were anesthetized with pentobarbital (40 mg/kg b.w.) (Ceva Santé Animale, Libourne, Fr) and blood was drawn from the abdominal artery with a heparinized syringe and distributed into dry and heparinized tubes. Blood tubes were centrifuged at 1000 g for 10 min at 4 °C, plasma or serum was collected and stored at −80 °C until analysis. Liver was removed, rinsed with 0.9% NaCl and cut into 2 parts: one part was frozen in nitrogen and kept at −80 °C until analysis, and one part was used for the immediate isolation of fresh mitochondria.

Plasma glucose, serum total cholesterol, triglycerides and free fatty acid levels were measured by enzymatic techniques (Konelab, Thermo Electron Corp., Vantaa, Finland). Plasma insulin and leptin were quantified with ELISA kits (Merck Millipore, Darmstadt, Allemagne). Insulin resistance (IR) was evaluated by the homeostasis model of IR (HOMA-IR) formula [11].

2.4. Liver mitochondrial isolation

Liver mitochondria were isolated from fresh liver by the differential centrifugation technique [12]. Briefly, about 3 g of liver was homogenized in 10 volumes of sucrose buffer (sucrose 0.25 M, Tris 10 mM, EDTA 0.5 mM, pH 7.5) and centrifuged at 900 g for 10 min at +4 °C. The supernatant was then centrifuged at 10,000 g for 10 min at +4 °C. The pellet was suspended in 3 mL of sucrose buffer. One aliquot of the mitochondrial suspension was used for mitochondrial function measurement [11] and one aliquot was frozen at −80 °C for lipid measurement. Protein content was determined according to Bradford with bovine serum albumin as the standard [13].

2.5. Liver TAG, FFA and cholesterol analysis

Liver samples were homogenized in NaCl (9 g/L) and Triton X-100 (0.1%) and free fatty acids, triglycerides and total cholesterol levels were quantified on the liver homogenate by enzymatic methods using Wako-NEFA-C kit (Oxoid, Dardilly, France) and Cholesterol RTU kit and triglycerides PAP kit (Biomérieux, Lyon, France).

2.6. Liver phospholipid separation and analysis

Liver samples and liver mitochondrial suspensions were extracted by a mixture of chloroform/methanol 2:1 (v/v) according to Folch et al. [14] in the presence of 50 mg/L of butylated hydroxytoluene. Phosphorus was quantified on Folch extracts of both liver samples and mitochondrial suspensions in order to determine total phospholipid quantity as previously described [15].

The application of Folch extracts of both liver samples and mitochondrial suspensions on silica gel 60 HPTLC plates (250 μm, 20 × 10 cm, Merck, Germany) pretreated with 2.3% w/v boric acid in ethanol (100%), was automatically performed on a 4 mm band width using a CAMAG ATS4 apparatus (Muttenz, Switzerland). The development was performed with methanol/acetic acid/pentane/chloroform (15/10/30/45, by volume), which allowed the separation of phospholipids and neutral lipids on a 60 mm total migration distance. The scanning of the plates was carried out using a CAMAG TLC scanner 3 (Muttenz, Switzerland), operating in the reflectance mode. The plates were scanned at 715 nm after dipping in a solution of Blue Spray (Sigma, France) (1/2/3 v/v/v, Blue Spray/H2SO4 4.2 M/acetone) and heating for 3 min at 55 °C. The different classes of phospholipids (SM, LPC, PC, PI, PS, PE, PG, PA, CL) were identified by comparing their retention factor (Rf) to authentic standards and quantified using calibration curves of the same standards.

2.7. Analysis of fatty acids in liver, liver phospholipids and liver mitochondrial phospholipids

To determine the fatty acid composition of the liver lipids, the Folch extracts of liver were used. To determine the fatty acid composition of liver phospholipids and liver mitochondrial phospholipids, the phospholipid fractions were separated from other neutral lipids, in the Folch extracts of both liver samples and mitochondrial suspensions, on TLC plates with hexane/diethyl ether/glacial acetic acid mixture (70:30:1, v/v/v). Plates were dried and then sprayed with dichlorofluorescein. Bands corresponding to phospholipids were
identified under UV illumination and collected by scraping the silica into a glass tube.

The fatty acids of total liver lipids, liver phospholipids and liver mitochondrial phospholipids were trans-esterified with an alkali mixture of KOH–methanol for 10 min at room temperature. The total fatty acid methyl ester pattern including the cis and trans PUFA was then analyzed by gas chromatography (Agilent Technologies, Milan, Italy) equipped with a DB-23 column and flame ionization detector. GC conditions have been described elsewhere [16]. Chromatograms were recorded, and peaks were integrated and identified by comparison with commercially available references. The FA composition of liver total lipids, liver phospholipids and mitochondrial phospholipids were compared in terms of the percentage content of various fatty acids.

2.8. Tissue desaturase indices and unsaturation index

Because it is not possible to directly measure the activity of the enzymes that catalyze the desaturation and elongation reactions in the fatty acid metabolic pathways, results in animals have supported the use of surrogate measures of desaturation activity such as use of desaturase indices [17]. These indices are based on the ratio of product to precursor of individual fatty acids. The desaturation indices were calculated as follows: Δ9, [16:1 n-7/16:0]; Δ6, [18:3 n-6/18:2 n-6]; and Δ5, [20:4 (n-6)/20:3 (n-6)]. The unsaturation index (UI) was calculated from the relative percentage of each type of mono- and polyunsaturated fatty acid (see Tables 2, 4 and 6) multiplied for the number of double bonds present in the molecule [18].

2.9. Mitochondrial characteristics and enzyme activities

The liver mitochondrial membrane fluidity, membrane potential and ROS production were assessed as previously described [12,19]. Mitochondrial respiration was determined by measuring mitochondrial oxygen consumption in a high resolution Oxygraph (Oroborus Oxysgraph, Austria) [12]. Citrate synthase activity and the activities of the mitochondrial respiratory complexes (complex I, complex II + III, complex IV and ATP synthase) in liver mitochondria were determined spectrophotometrically as described previously [18].

2.10. Reverse transcription q-PCR analyses

Reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) was used to measure target genes mRNA expression in liver. These genes include CDP-diacylglycerol synthase 1 and 2 (CDS1, CDS2), phosphatidylglycerophosphate synthase 1 (PGS1), cardiolipin synthase (CLS), lysocardiolipin acyltransferase 1 (LCLAT1), and tafazzin (TAZ). Ribosomal RNA (18S) was used as control. Total RNA was extracted with Trizol reagent (Invitrogen Life Technologies, Cergy Pontoise, France). Reverse transcription reaction was performed with 2 μg total RNA. cDNA was synthesized with the use of SuperScript II Reverse Transcriptase for first strand cDNA synthesis (Invitrogen Life Technologies, Cergy Pontoise, France) and oligo (dT) primers. The mRNA expressions of target genes were determined by RT-qPCR. RT-qPCR analysis was performed using IQ™ SYBR Green Supermix (Biorad, Hercules, CA, USA) with a MiniOpticon detection system (Biorad, Hercules, CA, USA). Results were normalized to the gene encoding 18S ribosomal RNA. The primer sequences used for real-time RT-qPCR are given in the Supplementary Table 3.

2.11. Statistical analysis

Data are expressed as means ± SD. Unpaired t-tests were performed to detect statistical significance between control diet and the HF diet or HF + MitoQ diet and between the HF diet and the HF + MitoQ diet. Correlations were performed with the Spearman method. The limit of statistical significance was set at p < 0.05. Statistical analyses were performed using the StatView program (SAS Institute, Cary, NC, USA).

3. Results

3.1. Characterization of the rat metabolic syndrome model

Weight gain at the end of the experiment was increased by feeding the HF diet compared to the control diet. Moreover, even though the weight of food consumed by rats on the HF diet was lower than for the control diet, the energy intake was increased (Table 1). With the HF + MitoQ diet, the weight gain was the same as for the control diet, while both dietary and energy intakes with HF + MitoQ diet were lower than with the HF diet.

While plasma glucose was not modified in the three groups, plasma insulin level and HOMA-IR index were decreased significantly with the HF + MitoQ diet compared to the control and HF diets (Table 1). Moreover, the AUC after OGTT and plasma leptin level were increased with the HF diet compared to the control diet, while their increases were largely attenuated with the HF + MitoQ diet.

Plasma free fatty acid and total cholesterol levels were decreased with the HF diet and the plasma levels of free fatty acids and triglycerides were decreased with the HF + MitoQ diet, compared to the control diet (Table 1). In addition, as expected, liver weight and liver free fatty acids, triglycerides and total cholesterol levels were increased with both the HF and the HF + MitoQ diets compared to the control diet, reflecting overt liver steatosis (Table 1).

3.2. Liver fatty acid composition and desaturase indices

The main saturated fatty acids in liver were represented by palmitic acid (16:0) and stearic acid (18:0). If the palmitic acid level remained unchanged, the stearic acid level was significantly decreased with HF and HF + MitoQ diets compared to the control diet. So, the liver total SFA level was decreased with HF and HF + MitoQ diets compared to the control diet (Table 2). The liver oleic acid (18:1 n-9) level was significantly increased, while those of palmitoleic (16:1 n-7) and vaccenic acids (18:1 n-7) were decreased with HF and HF + MitoQ diets.
compared to the control diet. Thus, the total MUFA content was decreased (+25%) with HF and HF + MitoQ diets compared to the control diet. The levels of the two major PUFA: linoleic acid (18:2 n-6) and arachidonic acid (20:4 n-6) were respectively increased and decreased with HF and HF + MitoQ diets compared to the control diet. However, the level of total n-6 PUFA was decreased with HF and HF + MitoQ diets compared to the control diet. Therefore, the level of total n-6 PUFA was decreased with HF and HF + MitoQ diets compared to the control diet. The analysis of fatty acid composition of liver phospholipids showed that the palmitic acid (16:0) level was decreased while the stearic acid (18:0) level was increased with HF and HF + MitoQ diets. Overall, the levels of total SFA were slightly but significantly increased with HF and HF + MitoQ diets (+10%) compared to the control diet (Table 4).

The level of oleic acid (18:1 n-9) in the liver phospholipids was increased, while palmitoleic acid (16:1 n-7) and vaccenic acid (18:1 n-7) levels were decreased with HF and HF + MitoQ diets compared to the control diet. Moreover, the levels of palmitoleic and vaccenic acids were further decreased by the HF + MitoQ diet compared to the control diet. Finally, the level of total MUFA was only decreased with the HF + MitoQ diet compared to both control and HF diets.

The level of linoleic acid (18:2 n-6) in the liver phospholipids was increased, while the level of γ-linolenic acid (18:3 n-6) was decreased with HF and HF + MitoQ diets compared to the control diet, whereas the major PUFA, 20:4 n-6, remained unchanged. So, the levels of total n-6 PUFA and total n-3 PUFA increased in the three experimental groups. Regarding the n-3 PUFAs, when the HF and HF + MitoQ diets were compared to the control diet the levels of docosapentaenoic acid (22:5 n-3) and docosahexaenoic acid (22:6 n-3) were decreased, while that of 22:5 n-3 was increased, and the level of α-linolenic acid (18:3 n-3) remained unchanged. Finally, the level of n-3 PUFA was decreased with the HF and HF + MitoQ diets compared to the control diet, while the combined level of n-6 PUFA plus n-3 PUFA remained unchanged in the three experimental groups.

The level of trans linoleic acid (trans 18:2 n-6) decreased with the HF and HF + MitoQ diets, while that of trans oleic acid (trans 18:1 n-9) was only decreased with the HF + MitoQ diet compared to the control diet, and that of trans arachidonic acid (trans 20:4 n-6) remained unchanged. Finally, the overall level of trans fatty acids remained unchanged in the three experimental groups.

### 3.4. Fatty acid composition of liver phospholipids and desaturase indices

<table>
<thead>
<tr>
<th>Control diet</th>
<th>HF diet</th>
<th>HF + MitoQ diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total SFA</td>
<td>311 ± 2.1</td>
<td>281 ± 0.8*</td>
</tr>
<tr>
<td>Total MUFA</td>
<td>237 ± 1.5</td>
<td>23.2 ± 0.7</td>
</tr>
<tr>
<td>Total n-6 PUFA</td>
<td>7.40 ± 1.54</td>
<td>4.87 ± 0.57*</td>
</tr>
</tbody>
</table>
| Total n-3 PUFA | 19.4 ± 1.6 | 34.6 ± 2.6* | 345 ± 1.6 *
| Total n-7 PUFA | 4.32 ± 1.04 | 2.77 ± 0.22* | 2.63 ± 0.15* |

The analysis of fatty acid composition of liver phospholipids showed that the palmitic acid (16:0) level was decreased while the stearic acid (18:0) level was increased with HF and HF + MitoQ diets. Overall, the level of total SFA was slightly but significantly increased with HF and HF + MitoQ diets (+10%) compared to the control diet (Table 4).
According to the analysis of fatty acids from liver phospholipids, the indices of Δ9, Δ6, and Δ6 desaturase activity were dramatically decreased whereas the index of Δ5 desaturase activity was only non-significantly decreased with HF and HF + MitoQ diets compared to the control diet. Moreover, the index of Δ9 desaturase activity was further decreased with HF + MitoQ diet-fed rats compared to control diet. Unsaturation index (UI) was decreased with HF and HF + MitoQ diets compared to control diet. This is also in line with the marked decrease in desaturase activities and in total polyunsaturated fatty acids with HF + MitoQ diets compared to control diet.

3.5. Mitochondrial phospholipid classes

The total phospholipid content in the liver mitochondria was the same for the three experimental diets (Table 5). The percent of PC decreased with HF and HF + MitoQ fed rats compared to control fed rats, whereas PE percent remained unchanged, thereby leading to a significant decrease in the PC/PE ratio. More interestingly, CL content was significantly increased with HF (+7%) and HF + MitoQ (+16%) rats compared to control rats. In addition, CL content was significantly increased in HF + MitoQ (+9%) rats compared to HF rats. The content of the other phospholipid classes PE, PI, PS and SM remained unchanged.

3.6. Fatty acid composition of liver mitochondrial phospholipids and desaturase indices

The fatty acid analysis of liver mitochondrial phospholipids showed that the palmitic acid (16:0) level was decreased and the stearic acid (18:0) level was increased with HF and HF + MitoQ diets compared to the control diet, so the total SFA content remained unchanged among the groups (Table 6). In contrast, the levels of both palmitoleic (16:1 n-7) and vaccenic acids (18:1 n-7) were decreased, but the level of oleic acid (18:1 n-9) was increased with HF and HF + MitoQ diets compared to the control diet. In addition, palmitoleic acid (16:1 n-7) level was further decreased with HF + MitoQ diet compared to HF diet. Overall, the content of total MitoQ was decreased with the HF and HF + MitoQ diets, compared to the control diet (Table 6).

The level of the PUFA linoleic acid (18:2 n-6) was significantly increased with HF + MitoQ diet compared to the control diet (+30%). Moreover, its level was 11% greater with the HF + MitoQ diet than with the HF diet, which was nearly significant (p = 0.0527). The γ-linolenic acid (18:3 n-6) level was significantly decreased with the HF and HF + MitoQ diets compared to the control diet, whereas that of the predominant PUFA arachidonic acid (20:4 n-6) remained unchanged. The major n-3 PUFA DPA, DHA (22:6 n-3) was significantly increased with HF and HF + MitoQ diets compared to the control diet, whereas the n-3/PUFA ratio decreased only with the HF diet while the n-6/PUFA ratio decreased and thus the (n-6)/(n-3) ratio was significantly increased with HF + MitoQ compared to the control diet (Table 6).

The total trans fatty acid content was decreased with the HF + MitoQ diet compared to the control diet, mainly because of a decrease in the trans-linoleic acid (18:2 n-6) content. The SFA/MUFA ratio was increased with HF + MitoQ compared to the control diet (+30%). Moreover, its level was further decreased with HF + MitoQ diet compared to the control diet. Overall, the content of total MitoQ was decreased with the HF and HF + MitoQ diets, compared to the control diet (Table 6).

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3.7. Liver mRNA expression of genes involved in CL synthesis and remodeling

The liver mRNA expression of two key enzymes in the CL remodeling pathway, namely CDP-diacylglycerol synthase (CDS1) and CL synthase (CLS), was increased with the HF and HF + MitoQ diets compared to
4. Discussion

We have previously observed that a high-fat diet in rats induced major features of the metabolic syndrome, and that MitoQ intake ameliorated the associated weight gain and glucose intolerance [11]. We speculated that the protective effect of MitoQ might be due to modulation of both redox signaling process and lipid metabolism. It is likely that the presence of MitoQ adsorbed to the matrix surface of the mitochondrial inner membrane might modulate cellular and mitochondrial membrane phospholipids, in particular CL, and their fatty acid composition, and that this in turn may contribute to the modulation of overall mitochondrial membrane fluidity, membrane potential and ROS production in vitro were unchanged by feeding the regimes used here, but that the high fat diet increased mitochondrial membrane fluidity and potential [11]. To extend these results, we have reported here positive correlations between liver mitochondrial CL content and between mitochondrial membrane fluidity, membrane potential and respiration (Fig. 3A, B, C), and a negative correlation between liver mitochondrial CL content and ROS production (Fig. 3D). Interestingly, mitochondrial membrane potential and fluidity were positively correlated with the linoleic acid content of mitochondrial phospholipids (r(ho) = 0.538, p = 0.0098 and r(ho) = 0.603, p = 0.0047 respectively) (not shown), which is potentially interesting knowing that linoleic acid is the major fatty acid of liver mitochondrial CL.

3.8. Enzymatic activities of mitochondrial respiratory chain complexes and correlations with CL content and fatty acid composition

The enzymatic activities of liver mitochondrial citrate synthase, complex I, complex II and complex IV remained unchanged in the three experimental groups. However, the enzymatic activities of complex II + III and of ATP synthase were significantly increased with HF + MitoQ diet compared to HF diet and to control diet, respectively (Table 7).

Moreover, the enzymatic activities of complex II and ATP synthase were positively correlated with the CL content of liver phospholipids (Fig. 2A, B), while the activities of complexes I, II + III and IV were not correlated (not shown). The enzymatic activity of complex IV was positively correlated with total PUFA (Fig. 2C) and with n-6 PUFA content (not shown, r(ho) = 0.406; p = 0.0945) and negatively correlated with SFA content of mitochondrial phospholipids (Fig. 2D).

3.9. Correlation of mitochondrial membrane fluidity, membrane potential, ROS production and respiration with CL content and fatty acid composition

We recently published that liver mitochondrial respiration and ROS production in vitro were unchanged by feeding the regimes used here, but that the high fat diet increased mitochondrial membrane fluidity and potential [11]. To extend these results, we have reported here positive correlations between liver mitochondrial CL content and between mitochondrial membrane fluidity, membrane potential and respiration (Fig. 3A, B, C), and a negative correlation between liver mitochondrial CL content and ROS production (Fig. 3D). Interestingly, mitochondrial membrane potential and fluidity were positively correlated with the linoleic acid content of mitochondrial phospholipids (r(ho) = 0.538, p = 0.0098 and r(ho) = 0.603, p = 0.0047 respectively) (not shown), which is potentially interesting knowing that linoleic acid is the major fatty acid of liver mitochondrial CL.

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4. Discussion

We have previously observed that a high-fat diet in rats induced major features of the metabolic syndrome, and that MitoQ intake ameliorated the associated weight gain and glucose intolerance [11]. We speculated that the protective effect of MitoQ might be due to modulation of both redox signaling process and lipid metabolism. It is likely that the presence of MitoQ adsorbed to the matrix surface of the mitochondrial inner membrane might modulate cellular and mitochondrial membrane phospholipids, in particular CL, and their fatty acid composition, and that this in turn may contribute to the modulation of overall mitochondrial membrane fluidity, membrane potential, ROS production and respiration with CL content and fatty acid composition.
However, nothing is known about the possible interaction/impact of the mitochondrial-targeting antioxidant, MitoQ, with these membrane lipid parameters. Our results show that MitoQ intake was accompanied by slight but significant decrease in food and energy intakes (about −10%) compared to the HF group. The food consumption was rather stable during the whole experimental period and thus the cause of this food intake decrease did not seem due to food aversion. Indeed, previous animal MitoQ supplementation studies did not show any significant decrease in food intake although MitoQ was administered for longer periods and at higher dosages in mice or rats fed chow/control diets[20–22]. While the positive effects observed in the MitoQ administered rats may be due in part to its effect on food intake, these are likely to play a minor role in the effects we see here. This is because the decrease in food consumption we see in the MitoQ group (~10%) is less than 25%–30% decrease compared to ad libitum caloric/dietary intake, which is known to decrease body weight and to have beneficial effects on some metabolic syndrome features and aging[23,24]. Therefore, it is unlikely that the observed beneficial effects of MitoQ can be explained by the relatively slight decrease in food or energy intake in this study, although a modest contribution cannot be excluded. Thus, it is likely that MitoQ impacts other important physiological mechanisms, in particular through possibly direct effects on both lipid metabolism and mitochondrial activity and consequent changes in ROS production as discussed below.

4.1. MitoQ had no effect on total liver fatty acid composition

In this study, the liver fatty acid composition in rats fed the HF diet was significantly altered. The HF diet intake decreased SFA and PUFA percent and increased MUFA percent and MitoQ did not affect significantly these modifications. The decrease in SFA level with the HF diet was surprising because the SFA percent was 2.5 fold higher in the fatty acids present in the HF diet fed to the rats, compared to the control diet. SFAs are the first product of de novo lipogenesis and are known to exert lipotoxic effects, in particular palmitic acid, by promoting liver fat accumulation, redox imbalance and ROS formation[25]. It is possible that an increase in β-oxidation represents an adaptive mechanism to limit free fatty acid lipotoxicity with the HF diet[26] and may explain the lower liver SFA percent found in our experiment.. In accordance with this hypothesis, we observed that Δ9-desaturase activity, as measured by the 16:1n-7/16:0 ratio, was significantly decreased with the HF diet. The Δ9 desaturase or stearoyl-CoA desaturase (SCD1) is the rate limiting enzyme catalyzing the synthesis of MUFA from SFA [27]. Consistent with this hypothesis, SCD1 (stearoyl-CoA desaturase-1) gene expression was strongly down-regulated in a dietary model of liver steatosis, resulting in enhanced delivery of SFA to mitochondria and fatty acid oxidation[10]. On the other hand, the

![Fig. 2. Spearman correlation between CL content in liver mitochondrial PL and mitochondrial ATP synthase activity (A) and mitochondrial complex II activity (B), and between mitochondrial COX activity and PUFA (C) and SFA content in liver mitochondrial PL (D).](image-url)
enrichment of the liver in MUFA may be favorable as MUFA are less toxic than SFA and induce significant triglycerides formation, preventing the accumulation of toxic lipid intermediates. Moreover, as previously observed [28], we found that the HF diet decreased the trans fatty acid content compared to the control diet, perhaps indicating that endogenously formed trans lipids could be rapidly replaced by the abundant natural fats in the diet. MitoQ, however, had no significant effect on either liver fatty acid percent or trans fatty acids percent in the overall liver lipids.

4.2. MitoQ had slight effects on liver phospholipids classes and their fatty acid composition

The distribution of phospholipid classes in liver homogenates from the control rats was in agreement with those previously published [29–31]. PC and PE are the two major phospholipid classes and represent more than 80% of the total liver phospholipids. CL, the specific mitochondrial phospholipid, represented about 5–6% of total phospholipids in the liver homogenate. The HF diet induced significant modifications in liver phospholipid class distribution but MitoQ did not affect this. With both high fat diets, the PC percent was decreased and the PE percent was increased compared to the control diet with a decrease in the PC/PE ratio. It is known that the PC/PE ratio is a key regulator of membrane integrity/fluidity [32] and a decrease in PC/PE ratio is implicated in the development of liver steatosis [33]. The observed increase in the liver CL content with the HF diet probably contributes to preserving the bioenergetic function of the mitochondria and compensates for the altered PC/PE ratio that affects membrane properties. In line with this, it was suggested that the liver can adapt to HF diet feeding by increasing the activity of the mitochondrial oxidative phosphorylation chain and its uncoupling, to dissipate the excess fatty acids and to reduce the production of ROS [26]. Moreover, it was also suggested in diabetic rats that mitochondria have the capacity to enhance the synthesis of CL or adjust phospholipid metabolism, in order to decrease susceptibility to induction of the mitochondrial permeability transition [37]. We have shown that the increased CL content in mitochondria from rats fed the HF diet results, at least partly, from an increased synthesis of CL as gene expression of two enzymes involved in CL synthesis (the CDS1 that is involved in the first step of CL synthesis and the CLS which leads to formation of nascent CL) was significantly increased compared to controls. With MitoQ, the CL content was increased compared to HF diet and CLS gene expression was also significantly

Fig. 3. Spearman correlation between CL content in liver mitochondrial PL and mitochondrial membrane fluidity (A), mitochondrial membrane potential (B), mitochondrial respiration (C) and mitochondrial ROS production (D).
increased. This is a potentially important impact of MitoQ on mitochondria dynamics and bioenergetics that has not been reported before, although we did not find significant variations in the CL content in HF + MitoQ diet vs HF diet in the whole liver lipid extract. This inconsistency may be due to the higher concentration of CL in the mitochondrial lipid extract that renders its measurement more reliable.

The mitochondrial CL content was positively correlated to in vitro measures of mitochondrial membrane fluidity and potential, as well as to respiration, while it was negatively correlated to mitochondrial ROS production. Moreover, ATP synthase activity was positively correlated to the CL content, as we have previously observed [18]. Thus, the increase of CL content with the HF diet may preserve mitochondrial bioenergetic function and MitoQ may help to reinforce the resistance of mitochondria to metabolic stress. Our results are in agreement with previous observations that CL has an influential effect on cellular respiration [38]. The up-regulating effect of MitoQ on CLS gene expression also reinforces the hypothesis that CLS has the unexpected role of modulating the physiology of mitochondrial membranes, increasing their resistance to metabolic stress [6] and enhancing mitochondrial bioenergetic efficiency [39].

Besides phospholipid classes, fatty acid composition of biological membranes also has a strong influence on their characteristics. In fact, free fatty acids not only are essential fuels for the organism, but also play also an essential role in the membrane composition and thus modulate the structural properties and functions of many membrane proteins. With the HF diet, SFA and PUFA contents were not modified in mitochondrial membrane while MUFA content was decreased. If high amounts of SFAs are incorporated into phospholipids in mitochondrial membrane bilayers, this can disturb organelle function as it significantly decreases membrane fluidity; therefore the fact that SFA content is not modified with the HF diet may be favorable to the mitochondrial function. The PUFA/SFA ratio was decreased with the HF diet; however, such a decrease may be advantageous for the mitochondrial membrane as it makes the mitochondria less sensitive to oxidative stress and lipid peroxidation [40]. In fact, the highly unsaturated nature of CL renders it very susceptible to oxidation by mitochondrial ROS while CL peroxidation may hamper mitochondrial efficiency and trigger mitochondrial dysfunction and apoptosis. With MitoQ, 18:2n-6 (linoleic acid) content was significantly increased compared to control diet even if total PUFA content was not modified, while HF diet was low in linoleic acid. The increase in linoleic acid content of mitochondrial phospholipids with MitoQ probably results from linoleic acid enrichment of CL. In fact, the two major phospholipids PC and PE of mitochondrial membrane are composed principally of 20:4n-6 and SFAs while 18:2n-6 only accounts for 10 and 5% in PC and PE, respectively [18]. Linoleic acid is the primary fatty acid constituent of CL as it represents up to 50% to 70% of CL acyl chains in the liver [18]. In order for CL to achieve its specific acyl composition, synthesized CL, or nascent/mature CL, undergoes acyl chain remodeling that implices principally tafazzin (TAZ) action [41]; CL remodeling increases the content of linoleic acid and leads to an increase in the “good” or mature CL. However, the gene expression of two enzymes involved in remodeling of CL, TAZ and LCLAT1, was not modified whatever the diet. It is possible that the remodeling of CL with MitoQ does not occur at the transcriptional level. It was also demonstrated that CLS has the ability to enhance tetra-18:2 molecular species content of CL thereby helping in preventing pathological alterations to cardiac mitochondrial lipidomic composition and bioenergetic function during diabetes [39], and the same mechanisms could be involved with MitoQ. Moreover, total trans fatty acids were significantly decreased in the MitoQ-fed rats, probably reflecting a positive local antioxidant effect of MitoQ preventing lipid peroxidation as this effect was not observed on total trans fatty acids or phospholipid trans fatty acid content. Particularly, CL, due to its high degree of unsaturation and its proximity to the mitochondrial respiratory chain, is a likely target of ROS and is prone to lipid peroxidation, and MitoQ may be effective in limiting its fatty acid peroxidation. Moreover, MitoQ intake prevented the decrease in the UI induced by the HF diet. Together with the increase in linoleic acid content and the decrease in trans fatty acid content, MitoQ may preserve membrane physico-chemical properties and mitochondrial function in the obesogenic diet-fed rats. The long chain PUFAs are involved not only in membrane fluidity but also in changing the bilayer thickness with consequential differential exposure of membrane active enzymes sites [42]. For example, the complex IV activity was positively correlated to PUFA content, thus MitoQ may favorably influence its functional properties. Δ9-desaturase activity was also decreased with MitoQ compared to HF maybe reflecting amelioration of mitochondrial function as this has been already suggested [10].

It is likely that these biochemical (fatty acids/CL amounts) and physiological (mitochondrial membrane potential and fluidity) modifications by HF and MitoQ intake have impacted mitochondrial function in particular ATP synthase activity. It is possible that the increase in complex II + III activity, in the MitoQ group vs HF group, could be due to preventing loss of the CoenzymeQ pool by MitoQ intake [43]. Such a hypothesis deserves to be assessed in a separate study. The biochemical/physiological modifications mentioned above increased the mitochondrial ATP synthase activity in the MitoQ group vs control group. HF diet plus MitoQ intake increased ATP synthase activity significantly. Indeed, it has been reported that specific phospholipid classes and/or their ratios, in particular CL, play a role in modulation of the activity of this enzyme [44]. Moreover, it is known that ATP synthase activity is modulated by membrane fluidity [45]. Finally, it was suggested by Degl’Esposito et al. [46] that ubiquinone can interact directly with the mitochondrial ATP synthase.

5. Conclusion

While accumulating evidence suggests a major role for mitochondrial dysfunction in the pathophysiology of fatty liver steatosis, it seems that mitochondria first adapt to an HF diet by remodeling mitochondrial membrane phospholipid content and fatty acid composition in order to preserve their function. However, this compensatory mechanism seems limited as ultimately decreased CL content and/or oxidative damage have been observed in a range of pathological conditions associated with mitochondrial dysfunction [47]. MitoQ may protect unsaturated fatty acid moieties from oxidative damage, thereby facilitating favorable phospholipid remodeling of the mitochondrial membrane, with a possible positive impact on mitochondrial function. Indeed, the increase in the liver mitochondrial CL content, that accompanied MitoQ intake, was associated with an amelioration in mitochondrial respiration and with a decrease in mitochondrial ROS production. This effect probably results from the increased activity of some oxidative phosphorylation complexes, in particular complex II + III and ATP synthase activity. Moreover, MitoQ favorably increased linoleic acid content in mitochondrial membranes and decreased total content of trans fatty acids. These changes are likely to underlie the positive effects on membrane fluidity and membrane potential, again preserving mitochondrial function. Therefore, MitoQ might protect against, or at least slow down, changes in CL profiles that may cause mitochondrial dysfunction and thereby contribute to the development of the metabolic syndrome. Whether MitoQ can restore CL profiles in other oxidative stress pathways, and thereby helps preserve bioenergetic efficiency and metabolic capacity, remains to be explored.

Abbreviations

AUC area under the curve
CL cardiolipline
CLS cardiolipin synthase
CDS CDP-diacylglycerol synthase
FFAs free fatty acids
HFD high fat diet
HF + MitoQ high fat + MitoQ diet
Antipodean Pharmaceuticals Inc. that develops and commercializes Dr. M Murphy who holds patents related to mitochondria-targeted antioxidant mitochondria-targeted antioxidant that decreases ethanol-dependent micro and macro hepatotoxicity, Hepatology 54 (2011) 153–163.


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