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Regulation of hepatic coenzyme Q biosynthesis by dietary omega-3 polyunsaturated fatty acids

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ABSTRACT

Dietary fats are important for human health, yet it is not fully understood how different fats affect various health problems. Although polyunsaturated fatty acids (PUFAs) are generally considered as highly oxidizable, those of the n-3 series can ameliorate the risk of many age-related disorders. Coenzyme Q (CoQ) is both an essential component of the mitochondrial electron transport chain and the only lipid-soluble antioxidant that animal cells can synthesize. Previous work has documented the protective antioxidant properties of CoQ against the autoxidation products of PUFAs. Here, we have explored *in vitro* and *in vivo* models to better understand the regulation of CoQ biosynthesis by dietary fats. In mouse liver, PUFAs increased CoQ content, and PUFAs of the n-3 series increased preferentially CoQ_{10}. This response was recapitulated in hepatic cells cultured in the presence of lipid emulsions, where we additionally demonstrated a role for n-3 PUFAs as regulators of CoQ biosynthesis *via* the upregulation of several COQ proteins and farnesyl pyrophosphate levels. In both models, n-3 PUFAs altered the mitochondrial network without changing the overall mitochondrial mass. Furthermore, in cellular systems, n-3 PUFAs favored the synthesis of CoQ_{10} over CoQ_9, thus altering the ratio between CoQ isoforms through a mechanism that involved downregulation of farnesyl diphosphate synthase activity. This effect was recapitulated by both siRNA silencing and by pharmacological inhibition of farnesyl diphosphate synthase with zoledronic acid. We highlight here the ability of n-3 PUFAs to regulate CoQ biosynthesis, CoQ content, and the ratio between its isoforms, which might be relevant to better understand the health benefits associated with this type of fat. Additionally, we identify for the first time zoledronic acid as a drug that inhibits CoQ biosynthesis, which must be also considered with respect to its biological effects on patients.

1. Introduction

Coenzyme Q (CoQ) is an essential redox-active lipid that can be found in all biological membranes of eukaryotes and in plasma lipoproteins [1]. Structurally, it is formed by a fully substituted benzoquinone ring attached to a polyisoprenoid tail whose length is species-specific. The number of isoprene units (n) in the tail denotes different CoQ isoforms: CoQ_6 in *Saccharomyces cerevisiae*, CoQ_8 in *Escherichia coli*, CoQ_9 and CoQ_{10} in mice and rats, and mainly CoQ_{10} in humans [1,2]. Homeostatic values of both CoQ content and the ratio between its isoforms are distinctive among different tissues and organs and so are believed to be crucial for the proper function of each tissue [3–5]. Although CoQ participates in several aspect of the cellular metabolism, its best characterized functions are as electron transporter in OXPHOS at the inner mitochondrial membrane, and as a cellular antioxidant in all membranes and serum lipoproteins where it resides [6]. In humans, age- and disease-related CoQ deficiency is usually related to cardiovascular and metabolic disorders. The current treatment for these deficiencies is to restore an adequate CoQ content *via* oral supplementation, but its success is limited due to its low bioavailability [7]. It is crucial to fully understand the regulation of CoQ metabolism within the cell, which can provide alternative strategies to enhance endogenous CoQ content.

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Abbreviations

ADCK3	aarF domain containing kinase 3
C(I/II/III/IV/V)	complex (I/II/III/IV/V) of the mitochondrial oxidative phosphorylation system
CLAP	Chymostatin, Leupeptin, Antipain and Pepstatin cocktail
CoQ_n	coenzyme Q containing a polyisoprenyl tail of *n* isoprene units
CR	calorie restriction
DMAPP	dimethylallyl diphosphate
DTT	dithiothreitol
ECAR	extracellular acidification rate
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N'-tetraacetic acid)
FBS	fetal bovine serum
FDPS	farnesyl diphosphate synthase
FIS1	mitochondrial fission protein 1
FPP	farnesyl pyrophosphate
GGDPS	geranyl-geranyl diphosphate synthase
GPP	geranyl pyrophosphate
HMG-CoA	3-Hydroxy-3-methylglutaryl-CoA
HPLC	high performance liquid chromatography
IDI	Isopentenyl-diphosphate isomerase
IMM	inner mitochondrial membrane
IPP	isopentenyl diphosphate
MFN(1–2)	Mitofusin (1–2)
MS	mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide;
MUFA(s)	monounsaturated fatty acid(s)
NBP(s)	nitrogen-containing bisphosphonate(s)
NRF1	Nuclear Respiratory Factor 1
OCR	oxygen consumption rate
OXPHOS	mitochondrial oxidative phosphorylation system
PBS	phosphate buffered saline;
PDSS1-2	decaprenyl-diphosphate synthase subunit 1 and 2
PFA	*para*formaldehyde
PMSF	phenylmethylsulfonyl fluoride;
PUFA(s)	polyunsaturated fatty acid(s)
Rap1	ras-related protein 1
SFA(s)	saturated fatty acid(s)
siRNAs	small interfering RNAs
SREBPs	sterol regulatory element-binding proteins
TFAM	Mitochondrial transcription factor A
VDAC	Voltage-Dependent Anion Channel
Vv	mitochondrial volume density
ZOL,	zoledronic acid

Previous studies have described CoQ antioxidant capacity as protective against the oxidative stress produced by polyunsaturated fatty acids (PUFAs) [8–11]. Moreover, a growing body of evidence shows that dietary fats can influence CoQ content [12–14]. Previous work in our laboratory with mice fed calorie restricted diets showed that the content of both CoQ_9 and CoQ_{10} in skeletal muscle were increased in animals fed soybean oil- or fish oil-enriched diets as compared with those fed diets enriched in lard [4,5]. Aged rats fed a diet supplemented with fish oil showed increased CoQ content in heart as compared to animals fed a diet supplemented with sunflower oil [15]. However, despite the existing relationship between CoQ and dietary fats, the specific mechanism of CoQ regulation by dietary fat remains to be established.

To gain novel insight into this mechanism, we have determined how different fat sources influence the content of CoQ *in vivo* and *in vitro*. Overall, our results show that n-3 PUFAs can augment hepatic CoQ biosynthesis, CoQ content, and decrease the ratio between CoQ_9 and CoQ_{10} isoforms. Since PUFAs are known to inhibit several steps of the mevalonate pathway, we examined the levels of farnesyl pyrophosphate and the upregulation of COQ proteins, particularly COQ7. We show that the downregulation of farnesyl diphosphate synthase (FDPS), a key enzyme in the mevalonate pathway, increases the ratio of CoQ_9 to CoQ_{10}, which can be recapitulated by both siRNA silencing of the *FPDS* gene, and by pharmacological inhibition of FPDS with zoledronic acid (ZOL), a drug that we identify here for the first time as an inhibitor of CoQ biosynthesis.

2. Materials and methods

2.1. Animal model, dietary interventions, and collection of tissue samples

The Bioethics and Biosafety Committee of the University of Cordoba (Spain) approved all animal procedures, which were in accordance with current EU regulations (authorization code: 20/04/2016/053). Male C57BL/6 mice were purchased from Charles River Laboratories (Barcelona, Spain) and housed in sterile filter-capped cages in a fully controlled environment with 12 h light/dark cycle. Prior to the interventions, mice were fed a standard chow diet. Three months old mice were randomly divided into four dietary groups and fed during four months with semi-purified diets based on the AIN93 M formulation that differed exclusively in the main lipid source as follows: lard (41.5% SFAs and 50.7% MUFAs), olive oil (80.5% MUFAs), fish oil (37% n-3 PUFAs) and soybean oil (55.3% n-6 PUFAs). The exact composition of the experimental diets, as well as their fatty acid content are described in Supplementary Tables S1 and S2 (note that lard, olive oil, and fish oil diets were supplemented with the adequate amounts of soybean oil to meet linoleic acid requirements [16]). To ensure there was no CoQ present in the diets, some pellets were broken down and pooled together, lipids were extracted, and CoQ analyzed (see method below). Chromatograms confirmed the total absence of CoQ in all the diets used (not shown).

Once the intervention period was completed, mice were fasted for 24 h and anesthetized with isoflurane. Blood was then withdrawn by cardiac puncture to allow the measurement of several biochemical parameters, and mice were finally euthanized by cervical dislocation. Organs were quickly dissected and weighed. Tissue samples were processed immediately for electron microscopy (see below) and the remaining tissue was frozen in liquid nitrogen with a cryopreserving buffer (25 mM Tris–HCl pH 7.6, 0.21 M mannitol, 0.07 M sucrose, 20% DMSO), and then stored at −80 °C until use for biochemical determinations. When required, a thin slice of liver was homogenized in 1 mL of homogenization buffer (5 mM Tris–HCl pH 7.4, 0.225 M mannitol, 0.075 M sucrose, 0.5 mM EGTA, 10 mM EDTA, 1 mM PMSF, 1 mM DTT and 20 μg/μL chymostatin, leupeptin, antipain, and pepstatin A (CLAP)) using an electric tissue disrupter (Ultra-Turrax T25, IKA, Staufen, Germany) during 30 s at 4 °C. Afterwards, the samples were sonicated for 30 s. Liver homogenates were stored at −80 °C until subsequent use.

2.2. Cellular model

Mouse liver hepatoma cells Hepa 1.6 were grown as described in Supplementary Material and Methods.

2.2.1. Treatments and cell viability assays

The lipid emulsions *Lipoplus* 20% (100 g/L medium-chain triglycerides, 80 g/L soybean oil and 20 g/L n-3 triacylglycerides; B. Braun Medical, Inc) and *ClinOleic* 20% (160 g/L olive oil and 40 g/L soybean

oil; Baxter Healthcare) were used in our cellular model as supplements of n-3 PUFAs and n-9 MUFAs, respectively. The drug ZOL (Santa Cruz Biotechnology) was used as a pharmacological inhibitor of FDPS. The most suitable conditions to perform treatments with lipid emulsions and ZOL were selected by MTT viability curves (see Supplementary Material and Methods). These previous determinations indicated the importance of controlling cell density and using fresh lipid emulsions that were prepared just before every experiment, since the use of emulsions that had been stored in vials at 4 °C led to a decrease of cell viability (not shown). Therefore, cells were seeded at a density of 15,000 cells/cm^2 and treated with fresh lipid emulsions at 7 μL/mL during 48 h for all our experiments (Fig. S1A). The selected concentration for ZOL was 20 μM since it was the highest concentration with no noticeable toxicity effects (Fig. S1B).

2.2.2. Silencing of FDPS using siRNA

Two commercial 27mer siRNA duplexes designed against mouse FDPS, as well as a universal scramble negative control (all obtained from OriGeneTM Technologies) were used to transfect Hepa1.6 cells. Transient transfections with FDPS siRNAs were performed using Lipofectamine 2000 (Thermo Fisher Scientific) according to the manufacturer's instructions. Approximately 250,000 cells were seeded in 9-cm^2 wells and cultured overnight until a 70–90% of confluence was reached. To perform the transfections, 6 μL of Lipofectamine and 20 mM of each siRNA were added to one mL Opti-MEM media (Thermo Fisher Scientific) per well. The culture medium was changed after 24 h and cells were incubated for another 24 h. Cells were then harvested using a cell scraper and processed for lipid measurements and western blotting. A specific antibody raised against FDPS was used to ascertain the effectiveness of the silencing by Western blot (see below).

2.2.3. Preparation of whole cell extracts from cells in culture

Whole cell lysates were prepared following the method described in Fernández-del-Río et al. [17].

2.3. Lipid extractions and analysis of CoQ content and CoQ biosynthesis

Lipid extractions from animal tissues and cultured cells were performed as described by Fernández-del-Río et al. [17]. CoQ content was measured by HPLC coupled to electrochemical detection, while CoQ biosynthesis was measured by HPLC-MS/MS, as described previously [17].

2.4. Protein electrophoresis and western blot

Protein concentrations were determined using the dye-binding method of Bradford as adapted by Stoscheck [18] for samples containing membranes. Polyacrylamide gel electrophoresis and Western blot were performed as described in Fernández-del-Río et al. [17] with an amount of 50 μg protein per lane. Membranes were incubated with the primary antibodies indicated in Table S3. In all cases, we used secondary antibodies conjugated with horseradish peroxidase for the immunodetection of the proteins by enhanced chemiluminescence (see Table S3). Luminescence was recorded using a ChemiDoc Imaging System (Bio--Rad) and images were analyzed using Image Lab™ Software (Bio-Rad). Each value obtained from quantification of stained bands (in arbitrary units) was normalized to the value of the corresponding lane stained with Ponceau S to correct for minor differences in protein loading between samples.

2.5. Ultrastructural analysis of mitochondria

Tissue processing for electron microscopy and mitochondrial analysis for ultrastructure and stereology were performed according to Khraiwesh et al. [19]. In the case of Hepa 1.6 cells, confluent cells from a 6-well plate were detached using Trypsin-EDTA for 2–3 min. The cell suspension was fixed with 2.5% glutaraldehyde in sodium cacodylate buffer (0.1 M, pH 7) and incubated for 1 h at 4 °C. Afterwards, cells were centrifuged, and the supernatant discarded. Fresh fixative solution was added to the pellet and the fixation was continued for 4–6 h at 4 °C. Subsequently, post-fixation, dehydration and embedding in Epoxy resin were carried out as described by Khraiwesh et al. [19]. The stained sections were viewed in a Jeol Jem 1400 Electron Microscope at the *Servicio Centralizado de Ayuda a la Investigación* (SCAI; University of Córdoba, Spain). Micrographs of whole hepatocytes and Hepa 1.6 cells were obtained at 6,000*X* magnification. On this material, a point counting method was applied to obtain the volume fraction of cell occupied by mitochondria (Volume density; Vv; see Ref. [19]). From the same material we also obtained micrographs at 15,000*X* to perform a planimetric analysis of mitochondrial area using ImageJ software (NIH; USA). The mean number of mitochondria per cell area (Na) was obtained using the same micrographs and software.

2.6. Determination of GPP and FPP levels

The method to determine GPP and FPP was adapted from Holstein et al. [20]. It uses the ability of farnesyl transferase to prenylate proteins with a consensus sequence CAAX in the carboxyl terminus, where C is cysteine, A is any aliphatic amino acid and X is the *C*-terminal amino acid, such as methionine, serine, cysteine, or alanine [21]. To isolate these metabolites, 10×10^6 cells, conveniently treated, were rinsed, scratched directly in Hank's balanced salt solution and pelleted by low-speed centrifugation. Cell pellets were resuspended in 1 mL of cold PBS and centrifuged at 100,000 g during 5 min at 4 °C. The PBS was aspirated and 0.5 mL of extraction solvent (isopropanol/75 mM ammonium hydroxide/acetone, in 1:1.5:5 proportion) was added. Samples were vortexed for 1 min and centrifuged at 10,000 g during 5 min at 4 °C. The extraction was repeated twice, and the combined supernatants were dried under nitrogen at 30 °C and stored at −80 °C. Then, an enzymatic reaction was performed to conjugate GPP and FPP with a dansyl-labeled peptide GCVLS (D*-GCVLS, BioNova cientifica s. l.) forming prenylated metabolites. For the reaction, the dried residue was dissolved in 40 μL of 50 mM Tris- HCl pH 7.5 containing 5 mM DTT, 5 mM $MgCl_2$, 10 μM $ZnCl_2$ and 1% octyl-β-D-glucopyranoside. The reaction starts when 0.25 nmoles of D*-GCVLS and 1 μL of farnesyl transferase (50 μg/uL, Jena Bioscience) were added and the mixture was incubated for 2 h at 38 °C. The reaction was terminated by the addition of 60 μL acetonitrile. Denatured proteins were removed by centrifugation at 100,000 g at 4 °C during 5 min and the supernatant was analyzed by HPLC coupled to fluorescence detection.

HPLC analysis was carried out on a Beckman Gold System (Beckman Coulter, USA) connected to a FP-2020 Plus intelligent fluorescent detector (Jasco Co., Tokyo, Japan). To perform the prenylated peptides (GPP-D*-GCVLS, FPP-D*-GCVLS) separation a C18 reverse phase analytical column (4.6 mm × 25 cm, Ultrasphere ODS, 5 μm particle) was used. Mobile phase was composed by solvent A (20 mM ammonium acetate in 40% acetonitrile) and solvent B (20 mM ammonium acetate in 90% acetonitrile) at a flow rate of 1.16 mL/min during all the analysis. Program starts with 26% solvent B for 2 min and then solvent B was brought to 60% through a linear gradient in 3 min. An isocratic 60% solvent B was run up to 15 min, then the solvent B was brought back to 26% by a linear gradient in 1 min. Geranyl- and farnesyl adducts were monitored by fluorescence at the excitation wavelength of 335 nm and the emission wavelength of 528 nm. Retention times for GPP-D*-GCVLS and FPP-D*-GCVLS peaks were approximately 9 and 16 min, respectively. Their area units were integrated and referred to GPP and FPP commercial standards, previously subjected to the same extraction and enzymatic reaction as the rest of the samples. Normalized values were obtained by referring to the amount of protein of each sample.

2.7. Statistics

Statistical analysis was performed using GraphPad Prism 8 (GraphPad Software Inc., San Diego, CA, USA). All the data shown are mean ± standard deviation (SD) from at least four biological replicates. Normality of data was verified using the Kolmogorov-Smirnov normality test. In case data did not pass the normality test due to a small sample size, equality of variances was considered to follow a normal distribution. Pairs of means were compared using either the *unpaired* parametric two-tailed Student's t-test or the *unpaired* non-parametric Mann-Whitney test, depending on data distribution. When more than three conditions were analyzed, means were compared using parametric one-way ANOVA with the Tukey post-test or non-parametric one-way ANOVA with Kruskal-Wallis correction and the Dunns post-test. Significant differences were expressed as: *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$), and ****($p < 0.0001$).

3. Results

3.1. Dietary PUFAs increase liver CoQ content and alter the mitochondrial network in mouse hepatocytes

To investigate the impact that supplementation with different dietary lipid sources exerts on CoQ content, CoQ_9 and CoQ_{10} were quantified in liver homogenates from mice that had been fed during four months with each of four different diets that differed in the primary lipid source: lard, soybean oil, olive oil, or fish oil (see composition details in Tables S1 and S2). Livers from mice fed the PUFA-based diets - either soybean oil- or fish oil - showed augmented levels of CoQ_9 and CoQ_{10}, as compared to lard- or olive oil-based diets (Fig. 1A–C). Notably, with the n-3 PUFA (fish oil) diet, CoQ_{10} increased more than CoQ_9, thus decreasing significantly the CoQ_9/CoQ_{10} ratio (Fig. 1D).

Because CoQ biosynthesis occurs within discrete mitochondria domains and plays important functional roles in mitochondrial metabolism [1,6,22,23], we studied putative changes in mitochondrial mass to investigate if this factor could explain the observed CoQ increase. Analysis by Western blot of outer mitochondrial membrane (VDAC), inner mitochondrial membrane (OXPHOS complexes), biogenesis (TFAM, NRF1), fusion (mitofusins 1 and 2, MFN1-2), and fission (FIS1) markers showed slight variations between the four diets but, overall, they did not point towards an increase in mitochondrial mass (Fig. S2). To confirm this phenotype, we also studied mitochondrial ultrastructure in hepatocyte micrographs using electron microscopy (Fig. 2). Since this is a very laborious and time-consuming technique, we focused on the comparison of fish oil-*vs*. olive oil-based diets, as examples of n-3 PUFA and n-9 MUFA-enriched diets, respectively. There were no changes in volume density (Vv, Fig. 2A), confirming that overall mitochondrial mass remained unchanged between olive and fish oil diets. However, in hepatocytes of mice fed with the fish oil-based diet, the mitochondrial sectional area was significantly smaller (Fig. 2B), and the number of mitochondria per cell unit area (Na; Fig. 2C) increased in comparison with the olive oil-based diet. These changes were also evident by direct observation of the electron microscopy images (Fig. 2D–G). Our results prove that a fish oil-enriched diet can produce changes in the size and number of mitochondria without altering the overall mitochondrial mass, indicating that the observed increase in CoQ content is not mitochondrial mass dependent.

Increased biosynthesis could account for the elevated CoQ content observed in liver from mice fed a PUFA-enriched diet. CoQ biosynthesis has been extensively investigated in yeast and bacteria, while studies in animal tissues are limited [1,2]. Due to the complexity of the metabolism of this lipid, we decided to use a complementary cell culture model in which mouse hepatoma Hepa 1.6 cells were grown in presence of two lipid emulsions: *Lipoplus* (based on n-3 PUFAs) or *ClinOleic* (based on n-9 MUFAs), mimicking fish oil- and olive oil-enriched diets respectively. As depicted in Fig. 3A–C, an elevated CoQ content (CoQ_9, CoQ_{10}, and total CoQ) in response to PUFAs, but not to MUFAs, was recapitulated *in vitro*. Likewise, the CoQ_9/CoQ_{10} ratio was significantly decreased in response to n-3 PUFAs (Fig. 3D), matching our previous observations in mouse liver (Fig. 1).

To evaluate whether the increased CoQ content in cells treated with *Lipoplus* could be related with a modification of CoQ biosynthesis, we measured newly synthesized CoQ by supplementing cells with $^{13}C_6$-labeled 4-hydroxybenzoic acid ($^{13}C_6$–4 HB), the most common precursor of CoQ biosynthesis [2]. Using HPLC-MS/MS we were able to differentiate between pre-existing (^{12}C-CoQ) *versus* newly synthesized CoQ ($^{13}C_6$-CoQ). We observed that under all the experimental conditions the majority of CoQ was $^{13}C_6$-labeled, indicating an active biosynthesis during the 48 h of treatment (Fig. 3H). Biosynthesis of CoQ_9 and CoQ_{10} increased significantly in the presence of *Lipoplus* as compared to *ClinOleic* treatment or to control cultures without emulsion (Fig. 3*E*–G), demonstrating that the increase of CoQ mediated by dietary n-3 PUFAs is due to the upregulation of CoQ biosynthesis. We noticed that the sole addition of $^{13}C_6$–4 HB to cell cultures induced an increase in the $^{13}C_6$-CoQ_9/$^{13}C_6$-CoQ_{10} ratio when compared with the non-labeled CoQ isoforms (Fig. 3H). We speculate that the increase in ring precursors availability potentiates the biosynthesis of both the predominant isoform CoQ_9 as well as CoQ_{10}, most likely due to a limitation of maximal

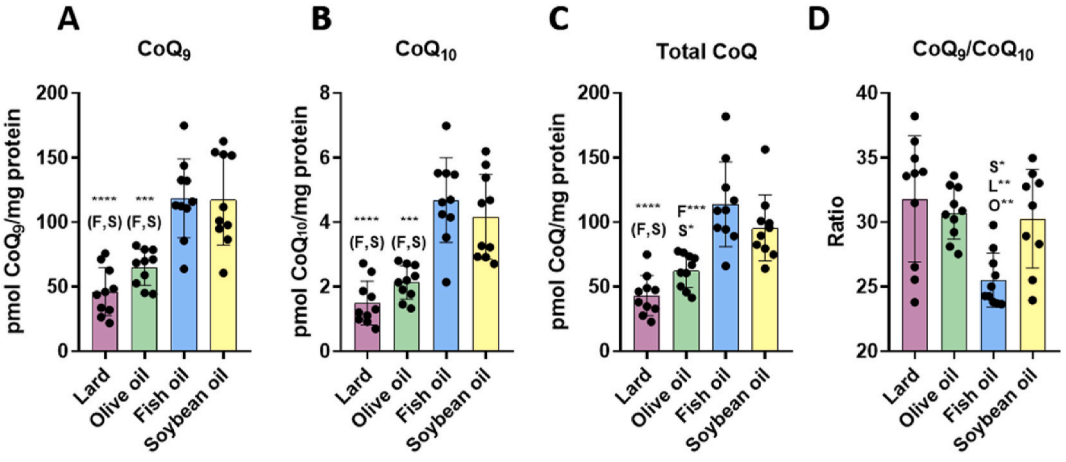

Fig. 1. Diets based on PUFAs increase CoQ content but only a n-3 PUFA diet decreases CoQ_9/CoQ_{10} ratio in mouse liver. **(A–C)** Livers from mice fed a soybean oil and fish oil-enriched diet for four months showed higher content of CoQ_9, CoQ_{10}, and total CoQ. **(D)** Only the fish-oil based diet altered the CoQ_9/CoQ_{10} ratio. Data represent mean ± SD of at least nine animals. Letters on top of columns represent significant differences. "L" stands for *Lard*, "O" for *Olive oil*, "F" for *Fish oil*, and "S" for *Soybean oil*. Statistically significant differences are represented as * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$). and **** ($p < 0.0001$).

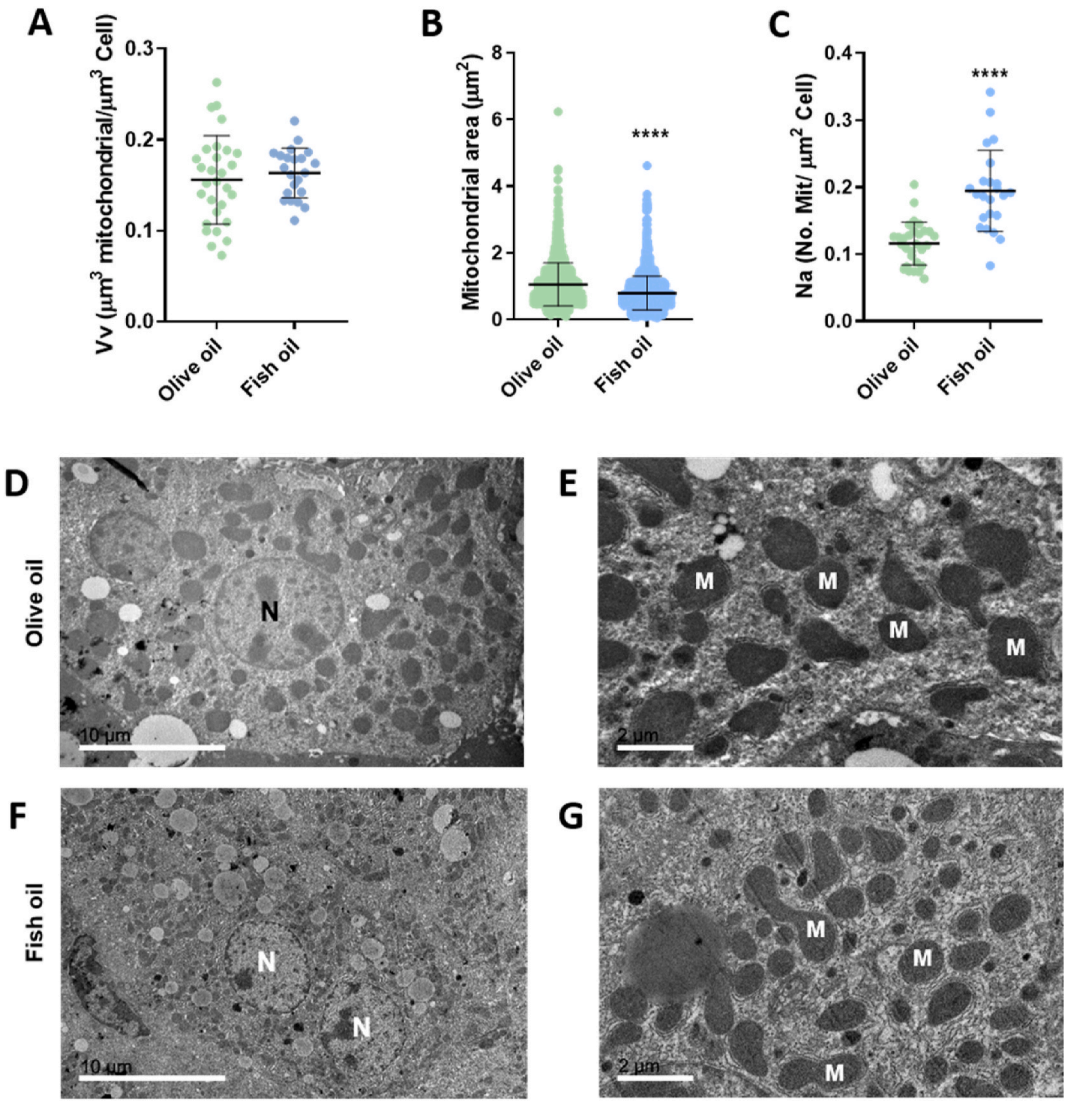

Fig. 2. A fish oil-enriched diet affects some mitochondrial parameters without changing mitochondrial mass. (A) Relative mitochondrial mass (Vv) remained constant between PUFA- and MUFA-enriched diets. **(B)** In a fish oil-enriched diet, mitochondria showed smaller area. **(C)** In a fish oil-enriched diet, more mitochondria were found per cell unit area (Na). **(D–E)** Representative electron microscopy images of an olive oil-enriched diet. **(F–G)** Representative electron microscopy images of a fish oil-enriched diet. In the images, "M" marks single mitochondria and "N" the nucleus. In D and F bar = 10 µm, in E and G bar = 2 µm. Data represent mean ± SD. Per group, images were taken from 6 independent samples and a minimum of 40 images were quantified. Statistically significant differences are represented as **** ($p < 0.0001$). n-3 PUFAs upregulate CoQ biosynthesis.

CoQ biosynthetic rate by endogenous ring precursors, as we also found previously in cultured kidney-derived cells [17]. Whereas, as expected, the ratio between pre-existent isoforms (^{12}C-CoQ$_9$/^{12}C-CoQ$_{10}$ ratio) was not altered by *Lipoplus*, this emulsion produced a significant decrease in the $^{13}C_6$-CoQ$_9$/$^{13}C_6$-CoQ$_{10}$ ratio in comparison with *ClinOleic* or with the emulsion-free controls (Fig. 3H), demonstrating that n-3 PUFAs potentiate CoQ$_{10}$ biosynthesis over that of CoQ$_9$ (1.4-fold increase in CoQ$_9$ *versus* 2.3-fold increase in CoQ$_{10}$).

Altered mitochondrial mass does not explain the increase in CoQ content in response to n-3 PUFAs.

Mitochondrial mass, as measured with Mitotracker, exhibited a small increase with *Lipoplus* (Fig. S3A), but this result was not confirmed by electron microscopy analysis (Figs. S3B–C). In these analyses, mitochondrial mass (represented as Vv) was unchanged with *Lipoplus* treatment, mimicking what it was observed in the animal model, although both mitochondrial and cellular volume were increased (Figs. S3B–C). The increment in the cell volume might be caused by the accumulation of lipids in the cytoplasm after the addition of lipid emulsions (Fig. S3D). A deeper characterization of the cellular model showed that some OXPHOS complexes subunits suffered subtle alterations in response to the lipid emulsions (Figs. S4A–B), but enzymatic activities of the complexes remained unaltered (Fig. S4C). Moreover, neither the oxygen consumption rate (OCR) nor the acidification rate (ECAR) were influenced by the emulsions (Fig. S4D). OCR values were also referred to the abundance of each complex of the electron transport chain as an estimate of activity turnover (Fig. S4E). Overall, these relative calculations indicated an increase of turnover in cells cultured in the presence of the olive oil-containing emulsion *ClinOleic* when compared both with emulsion-free controls and with the fish oil-containing emulsion *Lipoplus*, reinforcing the importance of dietary fat in the modulation of the activity turnover of mitochondrial complexes [24]. Additionally, we examined the levels of intracellular and mitochondrial ROS in response to lipid emulsions and, altogether, both *ClinOleic* and *Lipoplus* had a comparable effect increasing oxidative stress markers (Figs. S5A–C). A similar comparable effect was observed when measuring the level of protein carbonylation by Oxyblot (Fig. S5D), discounting an increase in oxidative stress as the cause for the observed elevated CoQ content induced by PUFAs.

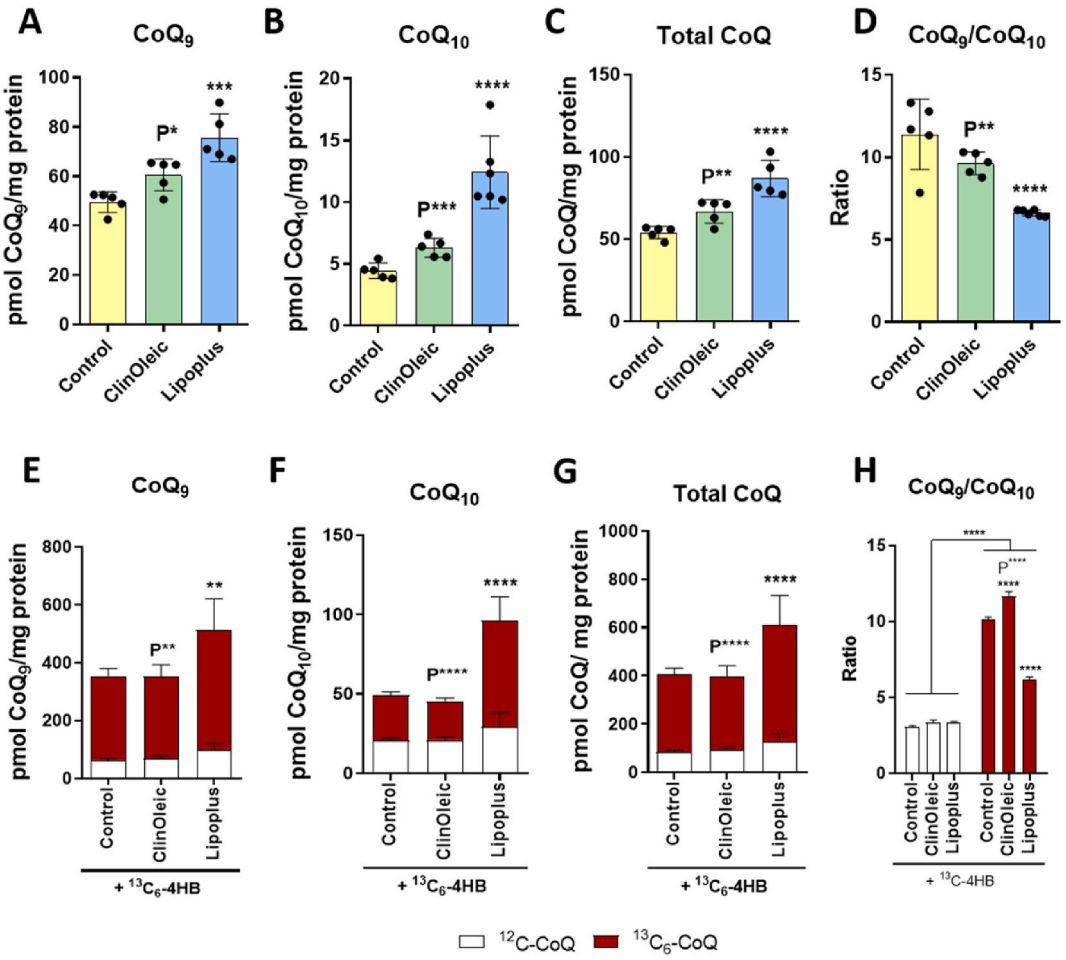

Fig. 3. N-3 PUFAs increase CoQ content in a cellular model by augmenting its biosynthesis. Hepa 1.6 cells were used in all experiments. **(A–C)** *Lipoplus* treatment increased CoQ_9, CoQ_{10}, and total CoQ content as compared to the control without emulsion and to the *ClinOleic* treatment. **(D)** Treatment with *Lipoplus* decreased the normal CoQ_9/CoQ_{10} ratio. **(*E*–G)** *Lipoplus* treatment increased CoQ_9, CoQ_{10}, and total CoQ biosynthesis as compared to the control without emulsion and to the *ClinOleic* treatment. **(H)** ^{13}C-CoQ_9/^{13}C-CoQ_{10} ratio decreases with *Lipoplus* as compared to the control or to the *ClinOleic* treatment. Data represent mean ± SD of 5 or more replicates. Differences to the corresponding control are represented as ** ($p < 0.01$), *** ($p < 0.001$), and **** ($p < 0.0001$). "P" refers to specific differences as compared to *Lipoplus*.

N-3 PUFAs downregulate FDPS, decrease cholesterol, and increase FPP and GPP levels.

PUFAs have been described as inhibitors of several steps of the mevalonate pathway, decreasing cholesterol levels and inhibiting 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, sterol regulatory element-binding proteins (SREBPs), and FDPS [25,26]. Since CoQ is synthesized *via* a branch of this pathway [1], we decided to study this connection to understand how n-3 PUFAs regulate CoQ metabolism. In our animal model, we found that FPDS was dramatically decreased in the liver of mice that had been fed the fish oil-based diet as compared to both olive oil and lard diets, although olive oil produced a milder downregulation of FPDS in comparison with the lard-based diet (Fig. 4A). Cholesterol levels were decreased in plasma from mice fed based in olive oil, fish oil or soybean oil as compared with mice fed the lard-based diet (Fig. S6A). FDPS polypeptide (Fig. 4B) and cholesterol (Fig. S6B) were also decreased in Hepa 1.6 cells treated with *Lipoplus* or *ClinOleic* with respect to untreated cells. Farnesyl pyrophosphate (FPP), the isoprene intermediate metabolite produced by FDPS [20], and geranyl pyrophosphate (GPP) were also evaluated in our cellular model. Of note, both FPP and GPP were found significantly increased in cells treated with *Lipoplus*, but no changes were observed with *ClinOleic* (Fig. 4C and D).

Regulation of the CoQ branch enzymes in the mevalonate pathway by n-3 PUFAs.

The decrease of cholesterol levels agrees with the inhibitory effect that n-3 PUFAs exert on the mevalonate pathway, as previously described. However, if PUFAs are well-known inhibitors of this pathway, how can these fatty acids induce a higher CoQ content with increased biosynthesis of CoQ_{10} relative to CoQ_9? As a first attempt to answer this question, we studied the levels of several COQ proteins in our cellular model and observed that COQ2, COQ6, COQ7 and ADCK3 were significantly increased in response to *Lipoplus*, and COQ5 also exhibited a trend towards an increase, although COQ9 and COQ10B remained unchanged (Fig. 5C and Figs. S7A and B). Concerted upregulation of several COQ proteins might contribute to elevate CoQ levels, although additional factors could be also necessary to fully explain the higher CoQ content in response to n-3 PUFAs. The analysis of *COQ* transcripts and COQ proteins in mouse liver also suggests that additional regulatory factors are necessary (Figs. S7C–E). Nevertheless, hepatic levels of *COQ7* mRNA (Fig. 5A) and COQ7 polypeptide were found elevated with fish oil in comparison with lard (Fig. 5B).

Genetic inhibition of FPDS recapitulates a decrease of CoQ_9/CoQ_{10} ratio.

We focused again on the central stem of the mevalonate pathway by analysing whether the specific inhibition of FDPS could influence CoQ levels. FDPS was successfully downregulated using FDPS-directed siRNAs (named A and B) in control Hepa 1.6 cells (Fig. 6A), but its depletion did not affect the amounts of GPP (Fig. 4C) or FPP (Fig. 4D), the

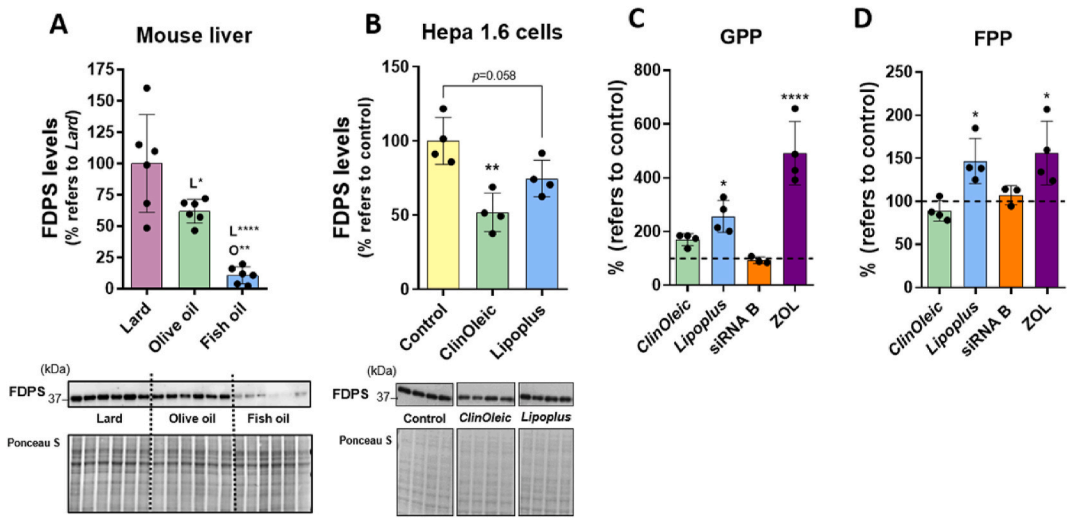

Fig. 4. Downregulation of FDPS influences GPP and FPP levels. (A) In mouse liver, a fish oil-enriched diet produced a strong decrease of FDPS protein levels. An olive oil-enriched diet had a similar effect although to a lesser extent. **(B)** FDPS levels in Hepa 1.6 cells decreased with *ClinOleic* and a downward trend was observed with *Lipoplus*. In A-B, arbitrary units depicted in the graphs relate directly to the immunoblots underneath. In all cases, intensity of the bands was normalized with the corresponding Ponceau S staining. Every group of samples was analyzed in the same blot but, in some cases, images were cropped to maintain a specific order. **(C)** GPP levels were increased with *Lipoplus* and ZOL treatments, but no effects were observed with *ClinOleic* or by genetic silencing of FPDS. **(D)** FPP levels increased with *Lipoplus* and ZOL, but no effects were observed with *ClinOleic* or by genetic silencing of FPDS. In D-E, control levels were considered 100% and represented as a dashed line. In all panels data represent mean ± SD of 4 or more replicates. Letters on top of columns represent significant differences. "L" stands for *Lard* and "O" for *Olive oil*. Differences with the corresponding control are represented as * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), and **** ($p < 0.0001$).

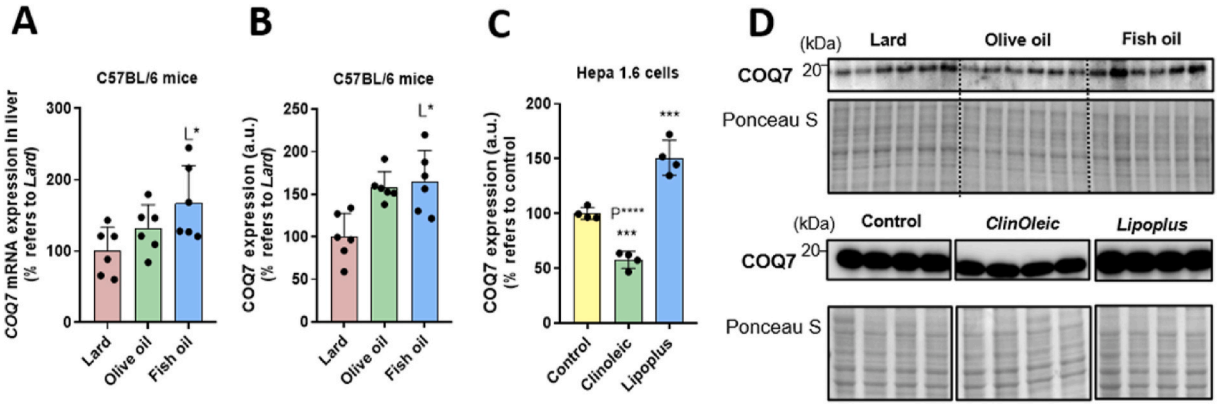

Fig. 5. COQ7 is upregulated by n-3 PUFAs. Steady-state levels of *COQ7* transcript **(A)** and COQ7 polypeptide **(B)** are augmented in liver from mice fed a fish oil-based diet in comparison with lard. **(C)** COQ7 polypeptide is increased by *Lipoplus* in Hepa 1.6 cells in comparison with both *ClinOleic*-treated cells and with control cells grown in the absence of lipid emulsion **(D)** Blots showing immunostaining of COQ7 in mouse liver homogenates and Hepa 1.6 cells. The corresponding Ponceau S staining is shown underneath each blot, to show equal protein loading. Each set of samples was analyzed in the same blot but, in some cases, images have been cropped to maintain a specific order. Data represent mean ± SD of 4 or 6 replicates. Differences to the corresponding control are represented as * ($p < 0.05$), *** ($p < 0.001$), and **** ($p < 0.0001$). "P" refers to specific differences comparing to Lipoplus. "L" refers to specific differences comparing to a lard-enriched diet.

metabolite produced by its catalytic activity. FDPS-directed siRNAs did not alter cholesterol (Fig. S6C), or CoQ_9 content (Fig. 6B). Since CoQ_9 accounts for the majority of ubiquinone in mouse liver cells, no differences were observed in total CoQ either (Fig. 6D). However, similarly to the effect of *Lipoplus* (Fig. 3D), both siRNAs led to significantly elevated levels of CoQ_{10} in comparison with the scrambled RNA (Fig. 6C), and the CoQ_9/CoQ_{10} ratio was dramatically decreased in FDPS-depleted cells (Fig. 6E).

Zoledronic acid decreases CoQ content and modifies CoQ isoforms ratio by inhibiting its biosynthesis and targeting multiple enzymes in the mevalonate pathway.

Our results showed that the inhibition of FDPS expression, either by PUFAs supplementation or by genetic silencing with specific siRNAs, targets key steps in CoQ biosynthesis. Thus, we took advantage of the existence of pharmacological agents known to inhibit this enzyme, to test if CoQ biosynthesis is altered in a similar fashion. ZOL, a well-known FDPS inhibitor [27,28], is a third-generation nitrogen-containing bisphosphonate (NBPs) widely used for medical applications, especially in bone-related diseases [29]. It has been reported that these drugs selectively bind and inhibit FDPS [30] producing a loss of post-translational isoprenylation of proteins, among other consequences. As expected, ZOL treatment did not modify FDPS levels in Hepa 1.6 cells (Fig. S8A). To validate the action of ZOL on Hepa1.6 cells, we quantified the accumulation of unprenylated Rap1A, described previously as a biochemical indicator of the pharmacological action of the NBPs [31]. Since Rap1A is specifically geranyl-geranylated, when using this marker, we are actually measuring the inhibition of the geranyl-geranyl diphosphate synthase (GGDPS) which is also sensitive to NBPs [32]. While total Rap1A levels remained unaltered (Fig. S8C), unprenylated Rap1A levels increased dramatically in Hepa 1.6 cells

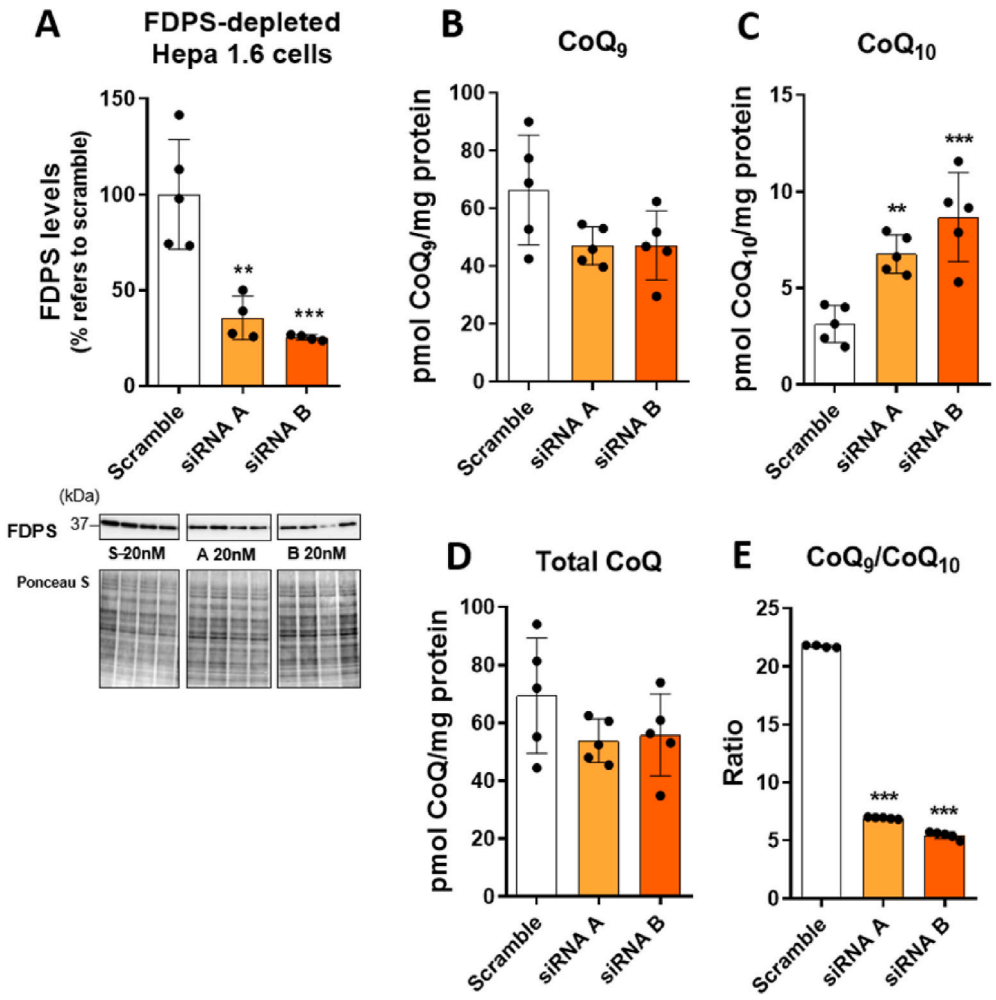

Fig. 6. Decreased expression of FDPS influences CoQ_{10} content, (A) FDPS protein levels were firmly decreased using FDPS-specific siRNAs in Hepa 1.6 cells **(B–D)** In FDPS-depleted Hepa 1.6 cells, CoQ_{10} content increased but no change was observed in CoQ_9 or total CoQ. **(E)** Depletion of FDPS significantly decreased the normal CoQ_9/CoQ_{10} ratio. In all cases, data represent mean ± SD of 3 or more replicates. Differences with the control are represented as ** ($p < 0.01$), and **** ($p < 0.0001$).

treated with ZOL (Fig. S8B), which validates the action of ZOL on Hepa 1.6 cells through abolishment of geranyl-geranylation.

We first measured GPP and FPP in Hepa 1.6 cells that had been treated with ZOL and found that, analogously to *Lipoplus* treatment, this drug produced a significant accumulation of both GPP (Fig. 5A) and FPP (Fig. 5B). We then studied the levels of CoQ isoforms and found that ZOL produced a general decrease in CoQ_9 and CoQ_{10} (Fig. 7A). However, the decrease in CoQ_{10} was much less pronounced than that of CoQ_9, resulting again in a significant decrease of the CoQ_9/CoQ_{10} ratio (Fig. 7A) that is fully in agreement with the changes observed by n-3 PUFAs treatment and by genetic silencing of FPDS (see above). Our results also showed that the general decrease of CoQ levels observed in cells treated with ZOL is consequence of a decreased biosynthesis concomitant with a strong deregulation of the ratio between CoQ isoforms (Fig. 7B). Indeed, Hepa 1.6 cells that had been treated with ZOL exhibited a decrease in $^{13}C_6$-CoQ_9, $^{13}C_6$-CoQ_{10} and total $^{13}C_6$-CoQ content, as well as a dramatic decrease of the $^{13}C_6$-CoQ_9/$^{13}C_6$-CoQ_{10} ratio. Again, the inhibition of CoQ biosynthesis was less pronounced for $^{13}C_6$-CoQ_{10} than for $^{13}C_6$-CoQ_9 (Fig. 7B) indicating that, although the biosynthesis of CoQ is generally depressed in the presence of ZOL, CoQ_{10} is the preferential isoform that is still synthesized under these conditions. Together, our results identify ZOL for the first time as a novel inhibitor of CoQ biosynthesis, able to severely impact CoQ content and the ratio between CoQ isoforms.

4. Discussion

Understanding the relationships between macronutrients and the different metabolic pathways is of outstanding importance for improving health. Here, we studied how different dietary fats influence the metabolism of CoQ, the only lipid antioxidant that is endogenously synthesized by essentially all aerobic cells [1]. Diets supplemented with lard, olive oil, fish oil, or soybean oil were analyzed, and only those containing high levels of PUFAs enriched CoQ in mouse liver. However, the n-3 PUFAs provided by fish oil, but not soybean oil, produced a preferential increase of CoQ_{10} over CoQ_9. Huertas et al. [12] proposed that the higher mitochondrial CoQ content detected in liver from rats fed a corn oil-based diet, compared with olive oil, might be related with the necessity of higher antioxidant defense in more unsaturated mitochondrial membranes. Several oxidative stress markers (as peroxides, intracellular and mitochondrial superoxide, and protein carbonylation) were indeed augmented in cells treated with lipid emulsions, but this effect was not associated specifically with fish oil, supporting that oxidative stress is unlikely to be the major factor determining CoQ upregulation. On the other hand, several lines of investigation have suggested an antioxidant rather than a prooxidant role for n-3 PUFAs [33], which also exert several health benefits [34–36]. CoQ is a potent antioxidant and a key player in cellular respiration [1], and CoQ_{10} supplements have revealed beneficial in several disease states [37,38]. Whether higher CoQ_{10} content is related with some of the health benefits linked to n-3

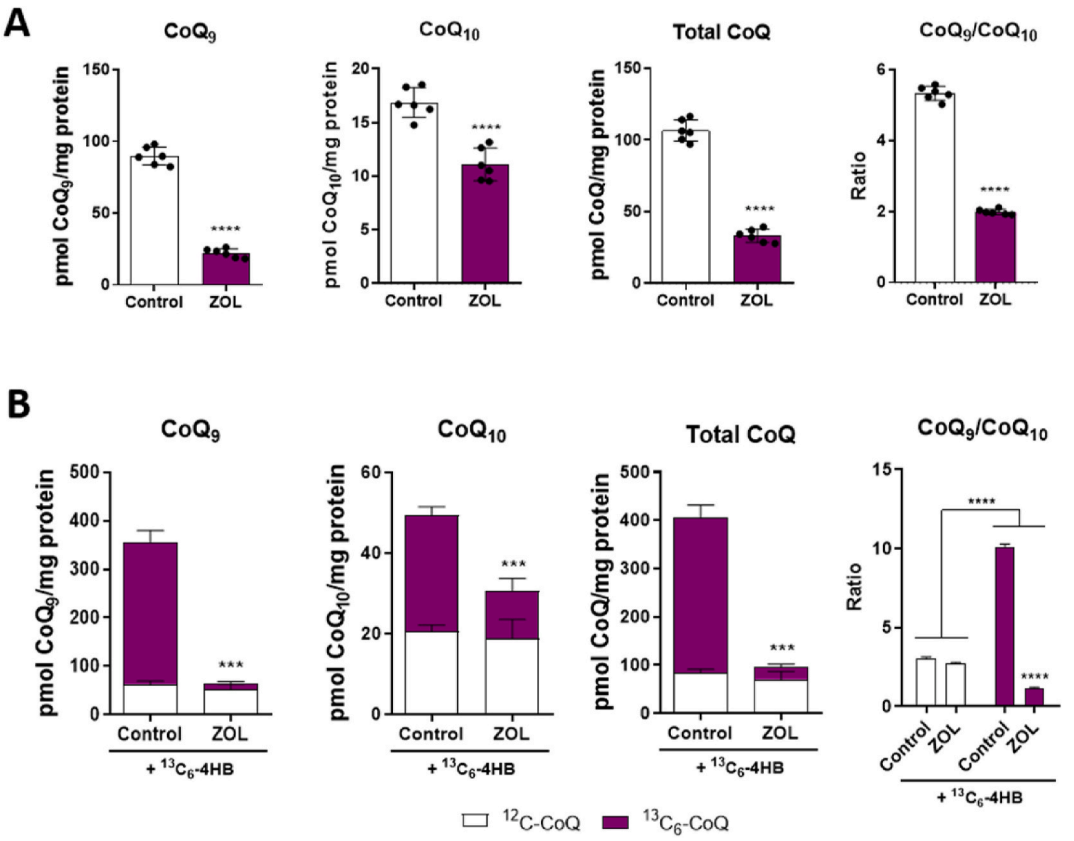

Fig. 7. Zoledronic acid downregulates CoQ content and CoQ biosynthesis and decreases the CoQ_9/CoQ_{10} ratio in Hepa 1.6 cells. (A) ZOL treatment decreased CoQ_9, CoQ_{10}, total CoQ, and the CoQ_9/CoQ_{10} ratio**. (B)** The biosynthesis of both CoQ isoforms and the $^{13}C_6$-CoQ_9/$^{13}C_6$-CoQ_{10} ratio were significantly decreased with ZOL. Data represent mean ± SD of 4 or more replicates. Differences to the corresponding control are represented as *** ($p < 0.001$), and **** ($p < 0.0001$).

PUFAs consumption will deserve further investigation.

N-3 PUFAs did not increase mitochondrial mass but altered the mitochondrial network, as more abundant but smaller mitochondria were observed in liver from mice fed a fish oil-based diet. Previous studies have reported the alteration of mitochondria by starvation, calorie restriction (CR) and different types of fatty acids [39]. A more fragmented mitochondrial network not only enables each mitochondrion to function independently, allowing cell quality control mechanisms to assess the performance of each individual organelle, but may be also involved in changes of fuel preference allowing the development of different subtypes of mitochondria [40]. Fragmented mitochondrial networks have also been reported in CR conditions [19,41], an intervention linked to multiple health benefits [42], as well as in cold adaptation and exercise [43,44]. Recently, it has been described that the CoQ-synthome, the multiprotein complex necessary for CoQ biosynthesis [1,2], localizes to discrete foci in the IMM known as "CoQ domains" [22,23]. The possibility exists that the increased number of mitochondria would lead to a higher number of CoQ domains and, therefore, to increased CoQ biosynthesis.

The connection between n-3 PUFAs and CoQ was confirmed in a cellular model, where mitochondrial mass also remained stable, and the respiratory function was not affected by n-3 PUFAs or MUFAs. It should be acknowledged however that our *in vivo* and *in vitro* models do differ in several aspects. The cellular model included a control without lipid emulsion, but a fat-free diet is linked to multiple health issues which precludes its utilization in mice [45]. Moreover, lipid emulsions based on saturated fatty acids are not available [46]. Additionally, due to the different durations of our interventions (four months in mice *versus* 48 h in cells), differences derived from the comparison of chronic *versus* acute effects might also exist. Despite these differences, the effects of n-3 PUFA on CoQ were successfully recapitulated in the cellular system, where n-3 PUFA potentiated endogenous CoQ biosynthesis partially by upregulating several COQ proteins including COQ2, COQ5, COQ6, COQ7 and ADCK3. The increase of COQ7 by n-3 PUFA is noteworthy because this protein has been recognized as a central component of the machinery which integrates endogenous and exogenous signals to regulate CoQ levels in response to altered environmental or metabolic conditions [4,5].

Interestingly, n-3 PUFAs enhanced the rate of CoQ_{10} biosynthesis to a greater extent than that of CoQ_9, which explains the decrease of the CoQ_9/CoQ_{10} ratio. Our results identify n-3 PUFAs as novel modulators that enhance the CoQ biosynthetic branch in addition to their multiple targets in the mevalonate pathway (Fig. 8), including cholesterol downregulation [26]. Bentinger and collaborators [47] reported a similar regulation of CoQ_{10} biosynthesis by lipid epoxides in cell cultures, which also inhibited cholesterol synthesis. Increased biosynthesis might also account for the higher CoQ content induced by fish oil in our mouse model, although CoQ biosynthesis was not measured *in vivo*. Moreover, in comparison with cultured cells, upregulation in COQ proteins in liver was much more limited to account fully for this phenomenon. Nevertheless, hepatic *COQ7* mRNA and COQ7 protein were upregulated by fish oil in comparison with lard, and these effects were not observed with olive oil. We then focused our investigations into the central stem of the mevalonate pathway to gain further insights into how these fatty acids target CoQ biosynthesis to modulate the CoQ_9/CoQ_{10} ratio and total CoQ content.

The CoQ_9/CoQ_{10} ratio varies between tissues and organs, although its value is highly conserved within a specific tissue [3] suggesting that the maintenance of a correct proportion between CoQ_9 and CoQ_{10} isoforms may be important for the physiology of each tissue [4,5]. Our results have linked unequivocally the differential regulation between CoQ_9 and CoQ_{10} isoforms to the activity of FDPS, and, to our knowledge,

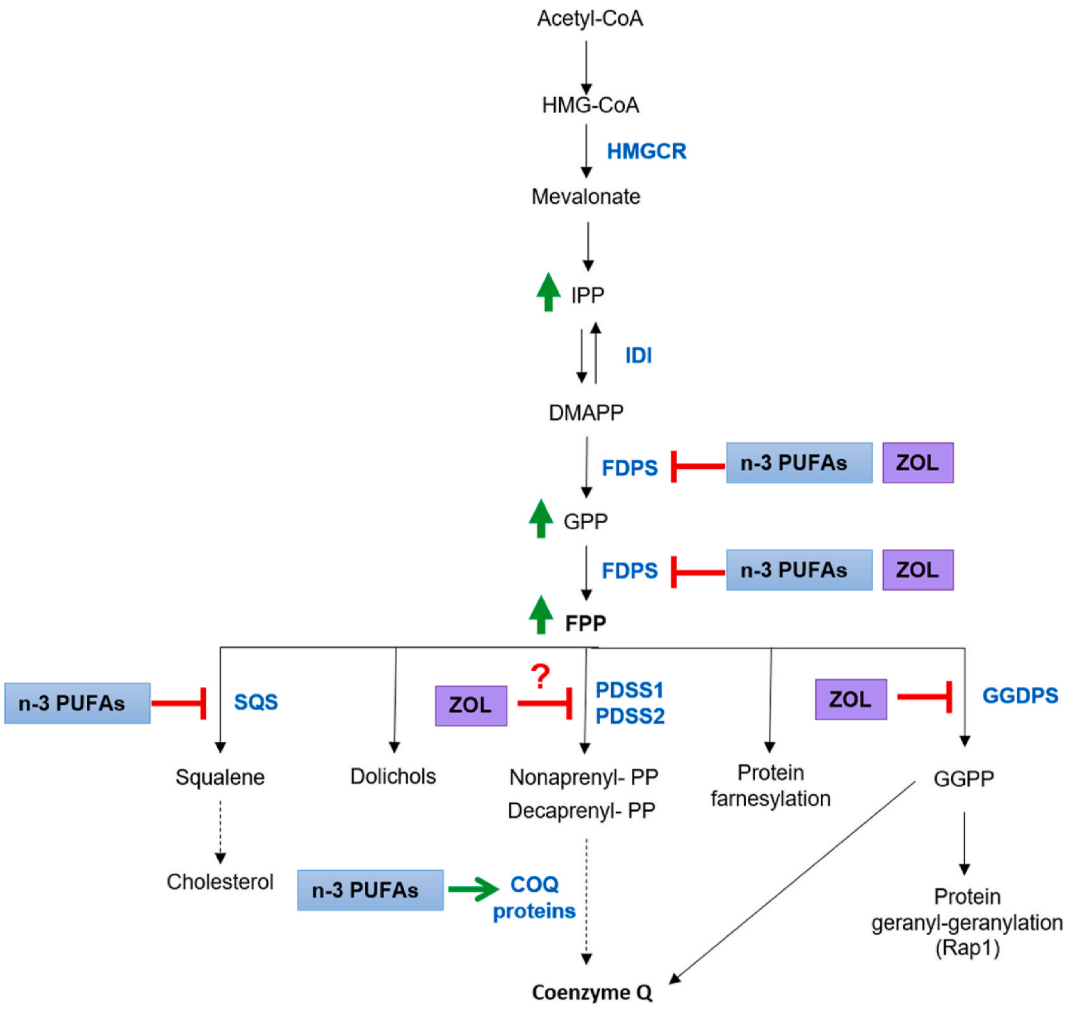

Fig. 8. Specific targets of PUFAs and ZOL in the mevalonate pathway. Overview of the different steps in the pathway that are targeted by PUFAs and ZOL according to the results described in this study. Abbreviations: FDPS, farnesyl diphosphate synthase; GGDPS, geranylgeranyl diphosphate synthase; HMGCR, HMG-CoA reductase; IDI, isopentenyl diphosphate isomerase; PDSS1/2, polyprenyl diphosphate synthase 1/2; SQS, squalene synthase.

we are the first to demonstrate that the specific inhibition of FDPS decreases the CoQ_9/CoQ_{10} ratio. Bentinger et al. [47] found that lipid epoxides increased CoQ_9/CoQ_{10} ratio in human hepatic cells. Complementary experiments allowed them to propose that low levels of isopentenyl diphosphate (IPP), an early metabolite of the mevalonate pathway, favor the synthesis of CoQ_9 while high amounts of IPP favor the synthesis of CoQ_{10} [48]. As the enzyme that consumes IPP, inhibition of FDPS by any of the effectors used in our study (n-3 PUFA, siRNAs against FDPS, or pharmacological inhibition with ZOL) might lead to IPP accumulation, which could drive PDSS1-PDSS2, the enzymatic complex responsible for the synthesis of CoQ isoprenoid chain precursor in mammals [2], to produce preferably CoQ_{10} thus decreasing the CoQ_9/CoQ_{10} ratio. According to this model, ZOL has been previously demonstrated to increase IPP in cell lines [49]. However, the mechanisms by which FDPS is targeted by siRNAs, ZOL or n-3 PUFA are distinct. Whereas siRNAs acted through a dramatic depletion of FDPS polypeptide, ZOL acted through inhibition of FDPS enzyme activity, as demonstrated by the lack of changes in FDPS polypeptide and the abolishment of geranylgeranylation in cells treated with the drug. In mice, fish oil produced a dramatic downregulation of FDPS *in vivo*, similar to what it was observed with siRNAs *in vitro*, while the decrease with olive oil was more limited. It is possible that the remaining FDPS polypeptide is still sufficient to provide with enough metabolite intermediates and sustain CoQ biosynthesis in mice fed an olive oil-based diet, which would explain its lack of effect on CoQ levels and the CoQ_9/CoQ_{10} ratio *in vivo*. In cellular systems, both *Clinoleic* and *Lipoplus* had comparable effects on FDPS levels, but only *Lipoplus* significantly increased CoQ levels and decreased CoQ_9/CoQ_{10} ratio. These observations are compatible with the idea that enough FDPS is still produced in the presence of both lipid emulsions *in vitro*, but n-3 PUFA could additionally exert a direct effect on FDPS enzyme activity.

Previous research has demonstrated that ZOL treatment increases GPP [20] due to the displacement of this metabolite from the FDPS active site [50]. A similar effect might be also accomplished by high IPP levels. Isopentenyl-diphosphate isomerase converts IPP to its highly nucleophilic isomer dimethylallyl diphosphate (DMAPP) [51], and previous studies on FDPS kinetics have proposed that when DMAPP levels are high, the GPP formed in the first reaction catalyzed by FDPS can leave the catalytic site of the enzyme and can be replaced by DMAPP prior to the second step of the reaction. Only after the DMAPP concentration is reduced, the reaction proceeds towards FPP synthesis [28]. Thus, putative changes of GPP could give us insights into the mechanisms of action of the different effectors acting on FDPS. Neither *Clinoleic* nor siRNAs directed against FPDS produced significant changes on GPP, supporting that these two treatments are acting only at the level of gene expression, although the effect produced by siRNAs on FDPS levels was strong enough to decrease the CoQ_9/CoQ_{10} ratio, most likely *via* IPP increase (see above). In accordance with previous research [20], we corroborated that GPP was increased by ZOL. Interestingly, GPP was also increased by n-3 PUFA, supporting that these fatty acids are not

only targeting FDPS at the level of gene expression as reported [25], but also at the level of enzyme activity, which explains the differences between *ClinOleic* and *Lipoplus* in the regulation of the CoQ_9/CoQ_{10} ratio.

Of note, the various treatments that commonly targeted FDPS and decreased the CoQ_9/CoQ_{10} ratio had disparate outcomes in terms of total CoQ, which was increased (n-3 PUFA), decreased (ZOL), or exhibited no change (siRNAs). This indicates that CoQ abundance is controlled at additional points and can be biochemically dissociated from the regulation of the CoQ_9/CoQ_{10} ratio. The decrease of CoQ levels produced by ZOL agrees with previous observations [52,53] and, importantly, we report here for the first time that this effect is due to a profound impairment of CoQ biosynthesis. Investigating how the different effectors of the central stem of the mevalonate pathway affected FPP could help us to understand the regulation of total CoQ levels. As a major sink of metabolic intermediates, inhibition of the cholesterol branch of the mevalonate pathway at the level of squalene synthase by squalestatin-1 has been shown to increase the CoQ pool, and this effect has been explained by enhanced availability of FPP for the CoQ branch, which, in spite of the generally established assumptions, might be not saturated by FPP at least under some circumstances [54,55]. Lipid epoxides also enhanced CoQ biosynthesis at the level of gene expression and elevated its cellular content while simultaneously inhibiting cholesterol synthesis at the level of oxidosqualene cyclase, another enzyme in the cholesterol branch [47]. Alternatively, the possibility also exists that PDSS1 and PDSS2 could utilize the upstream metabolites IPP and DMAPP and thereby synthesize the polyprenyl diphosphate tails needed for CoQ synthesis. This interesting hypothesis remains for further research.

Neither *Clinoleic* nor FDPS-directed siRNAs modified FPP levels, which agrees with a lack of effect on total CoQ. Interestingly, both n-3 PUFA and ZOL increased FPP but affected total CoQ levels differentially. The increase of FPP by ZOL is also in agreement with a previous report [49]. Since this drug inhibits FDPS, the enzyme responsible for FPP generation and, at the same time, the synthesis of CoQ is severely reduced, we suggest that ZOL has targets in several branches of the mevalonate pathway, and elevation of FPP is a result of its decreased consumption. Guo et al. described NBPs as inhibitors of hexaprenyl diphosphate synthase from *Sulfolobus solfataricus* and octaprenyl diphosphate synthase from *E. coli* [56]. Since these long-chain prenyltransferases are potently inhibited by bisphosphonates, PDSS1-PDSS2 becomes a strong candidate to be an additional ZOL target in the inhibition of the CoQ branch. ZOL is frequently used to treat bone-related diseases [29] and some types of cancers [57]. Therefore, the impairment of CoQ biosynthesis by ZOL and its CoQ lowering effect should be considered to understand the pharmacological and side effects produced by the administration of this drug. In the case of n-3 PUFA, the increase of FPP fully agrees with their role depressing the cholesterol branch of the mevalonate pathway [26], leading to increased availability of this metabolite due to its lesser consumption. This effect, together with the upregulation of several COQ proteins, could account for the increase of CoQ, preferentially in its CoQ_{10} isoform (Fig. 8).

5. Conclusions

We present here novel evidence about the role of dietary (n-3 PUFA) and pharmacological (ZOL) interventions in the regulation of the biosynthesis of the endogenous antioxidant CoQ. Altogether, our results show that dietary fats differentially affect the CoQ system in mouse liver and in cultured cells. n-3 PUFAs have been revealed for the first time as effectors that activate CoQ biosynthesis, regulating the ratio between its isoforms and increasing CoQ levels. Regulation of the ratio between CoQ_9 and CoQ_{10} has been linked to FDPS activity, whereas modulation of FPP and the levels of some COQ proteins, particularly COQ7, are linked to the regulation of total CoQ levels. Finally, we have characterized for the first time ZOL as a drug that decreases CoQ levels by impairing CoQ biosynthesis.

AUTHORS'S contributions

JMV and MIB conceived and designed the project; SRL was responsible of raising and maintaining the colony of mice; LFR, SRL, JAGR, and EGC performed the experimental determinations and conducted the data analysis; JAGR, MIB, and CFC provided valuable advice; JMV and CFC provided the resources and the funding. LFR and SRL wrote the original manuscript draft; JMV, JAGR, MIB, CFC, SRL, and LFR edited and reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare no conflict of interest for any of the work presented in this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2021.102061.

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Compliance with ethical standards

All animals were cared for in accordance with the University of Córdoba policy for animal welfare, which complies current European, Spanish, and Andalusian regulations. This study was approved by the bioethics committee of the University of Córdoba and authorized by the Consejería de Agricultura, Pesca y Desarrollo Rural, Junta de Andalucía (authorization code: 20/04/2016/053).

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