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Ubiquinol-10 ameliorates mitochondrial encephalopathy associated with CoQ deficiency

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ABSTRACT

Coenzyme Q10 (CoQ_{10}) deficiency (MIM 607426) causes a mitochondrial syndrome with variability in the clinical presentations. Patients with CoQ_{10} deficiency show inconsistent responses to oral ubiquinone-10 supplementation, with the highest percentage of unsuccessful results in patients with neurological symptoms (encephalopathy, cerebellar ataxia or multisystemic disease). Failure in the ubiquinone-10 treatment may be the result of its poor absorption and bioavailability, which may be improved by using different pharmacological formulations. In a mouse model ($Coq9^{X/X}$) of mitochondrial encephalopathy due to CoQ deficiency, we have evaluated oral supplementation with water-soluble formulations of reduced (ubiquinol-10) and oxidized (ubiquinone-10) forms of CoQ_{10}. Our results show that CoQ_{10} was increased in all tissues after supplementation with ubiquinone-10 or ubiquinol-10, with the tissue levels of CoQ_{10} with ubiquinol-10 being higher than with ubiquinone-10. Moreover, only ubiquinol-10 was able to increase the levels of CoQ_{10} in mitochondria from cerebrum of $Coq9^{X/X}$ mice. Consequently, ubiquinol-10 was more efficient than ubiquinone-10 in increasing the animal body weight and CoQ-dependent respiratory chain complex activities, and reducing the vacuolization, astrogliosis and oxidative damage in diencephalon, septum–striatum and, to a lesser extent, in brainstem. These results suggest that water-soluble formulations of ubiquinol-10 may improve the efficacy of CoQ_{10} therapy in primary and secondary CoQ_{10} deficiencies, other mitochondrial diseases and neurodegenerative diseases.

1. Introduction

Coenzyme Q_{10} (CoQ) is a lipophilic molecule that is involved in the mitochondrial ATP synthesis because of its function as an electron between mitochondrial complexes I and II, as well as ETF:Q oxidoreductase, and mitochondrial complex III [1]. Moreover, CoQ_{10} functions as an antioxidant, which protects the cells both directly by preventing the oxidation of biomolecules and indirectly by regenerating other antioxidants such as vitamins C and E [1]. Due to these properties, oral supplementation with CoQ_{10} (in its stable oxidized form, ubiquinone-10) has been proposed for the treatment of diseases involving mitochondrial dysfunction and/or oxidative stress, i.e. primary mitochondrial disorders, Parkinson's Disease, Alzheimer's Disease, Amyotrophic Lateral Sclerosis, Huntington's Disease or heart failure [2–6]. Moreover, oral ubiquinone-10 supplementation is the main choice in the treatment of primary (MIM 607426) and secondary CoQ_{10} deficiencies [7].

Despite the good expectation that ubiquinone-10 therapy has presented, the studies in different diseases, both at preclinical and clinical levels, have shown contradictory results. Specially, ubiquinone-10 seems to be less effective in improving the neurological symptoms and, in some cases, higher doses are needed to appreciate some clinical improvement [7]. The mild or completely lack of response to ubiquinone-10 therapy has been attributed to its low absorption and bioavailability that limit the increase of CoQ_{10} in cell mitochondria, where it is biologically active [8]. This limitation is even more important in the brain because the exogenous ubiquinone-10 must be able to cross the blood brain barrier. Thus, different strategies have been investigated to increase the absorption and bioavailability of the exogenous CoQ_{10}. In this regard, water-soluble formulations of ubiquinone-10 seem to increase its bioavailability. Different studies have shown that concentration of CoQ_{10} in plasma after administration of water-soluble formulations of ubiquinone-10 is higher than that after supplementation with ubiquinone-10 administered as powder water-insoluble formulations [8]. Moreover, the plasma levels of CoQ_{10} are also higher

Abbreviations: 8-OHdG, 8-hydroxyguanosine; BN-PAGE, Blue Native Poly-Acrylamide Gel Electrophoresis; CoQ, Coenzyme Q; CoQ_9, Coenzyme Q9; CoQ_{10}, Coenzyme Q10; DMQ_9, Demethoxyubiquinone 9; ETF, Electron-transfer flavoprotein; GFAP, Glial fibrillary acid protein; H&E, Hematoxylin and eosin; HPLC, High-performance liquid chromatography; TUJ1, Tubulin beta III

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when CoQ_{10} is administered as ubiquinol-10, the reduced form of CoQ_{10}, than when it is administered as ubiquinone-10 [8].

To evaluate whether water soluble formulations of ubiquinone-10, as well as the use of the reduced form, ubiquinol-10, may increase the efficacy of CoQ_{10} therapy in the nervous system, in this study we have used water-soluble formulations of ubiquinone-10 and ubiquinol-10 to treat a mouse model of mitochondrial encephalopathy and CoQ deficiency due to *Coq9* mutations (*Coq9*$^{X/X}$) [9].

2. Materials and methods

2.1. Mice use and experimental treatment

Generation and characterization of *Coq9*$^{X/X}$ mice (C57BL/6 genetic background) were previously reported [9]. All experiments were performed according to a protocol approved by the Institutional Animal Care and Use Committee of the University of Granada (procedures CEEA 2009-254 and 2010-275) and were in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (CETS # 123) and the Spanish laws (32/2007 and R.D. 1201/2005). Mice were housed in the Animal Facility of the University of Granada under a specific pathogen free zone with lights on at 7:00 AM and off at 7:00 PM, and with unlimited access to water and rodent chow. Mice were sacrificed using CO_2 narcosis followed by cervical dislocation at 3 months of age.

The treatment consisted of administering ubiquinone-10 or ubiquinol-10 in the drinking water in a dose of 240 mg/kg bw/day. The treatment started at 1 month of age and the mice were sacrificed at 3 months of age. Ubiquinone-10 and ubiquinol-10 were provided by Kaneka Corporation (Japan) in a water-soluble formulation that contains dextrin, Arabic gum and ascorbic acid. A control group with vehicle at the same dose was also studied. The drinking water was changed twice a week.

2.2. Mitochondrial isolation

Cerebrum was homogenized in a glass-Teflon homogenizer in a proportion 1:5, w/v, in the homogenization medium A (0.32 M sucrose, 1 mM EDTA, 10 mM Tris–HCl [pH 7.4]) plus 0.2% fatty acid–free bovine serum albumin. Homogenate was centrifuged at 1000 *g* for 5 min at 4 °C to remove nuclei and debris. Mitochondria were collected from supernatants after centrifuging at 14,400 *g* for 2 min at 4 °C. The mitochondrial pellet was suspended in the corresponding buffer and an aliquot of each sample was used for protein determination [9].

2.3. Quantification of CoQ_9 and CoQ_{10} levels in plasma, mice tissues and cerebrum mitochondria

CoQ_9 and CoQ_{10} from mice tissues were extracted by mixing tissue extracts with 1-propanol. After 2 min vortex, the solution was centrifuged at 11,300 *g* for 5 min. The resultant supernatant contained the lipid extract [9]. CoQ_9 and CoQ_{10} from plasma and cerebrum mitochondria were extracted in a hexane:ethanol mixture [10]. The lipid extract was injected in a HPLC system (Gilson, WI, USA) and the lipid components were separated by a reverse phase Symmetry C18 3.5 μm, 4.6 × 150 mm column (Waters, Spain), using a mobile phase consisting of methanol, ethanol, 2-propanol, acetic acid (500:500:15:15) and 50 mM sodium acetate at a flow rate of 0.9 ml/min. The electrochemical detector consisted of an ESA Coulochem III with the following setting: guard cell (upstream of the injector) at +900 mV, conditioning cell at −600 mV (downstream of the column), followed by the analytical cell at +350 mV [9]. CoQ_9 and CoQ_{10} concentrations were estimated by comparison of the peak areas with those of standard solutions of known concentrations. The results were expressed in ng CoQ/mg prot.

2.4. CoQ-dependent respiratory chain activities

CoQ dependent respiratory chain activities were measured in submitochondrial particles. To prepare submitochondrial particles, each mitochondrial pellet (100 μg prots) was suspended and sonicated in 100 μl of 0.1 M potassium phosphate buffer, pH 7.5. Complex I + III activity was measured at 30 °C in the presence of 0.5 mM potassium cyanide, 0.2 mM NADH and 0.1 mM cytochrome c, as the rotenone-sensitive reduction of cytochrome c at 550 nm [9,11]. The results were expressed in nmol reduced cyt c/min/mg prot. Complex II + III activity was measured at 30 °C in the presence of 0.5 mM KCN, 0.3 mM succinate and 0.01 mM rotenone. The reaction was initiated by addition of 0.1 mM cytochrome c and decrease in absorbance was monitored at 550 nm. The results were expressed in nmol reduced cyt c/min/mg prot [11].

2.5. Blue native gel electrophoresis and immunoblotting for the evaluation of mitochondrial supercomplex pattern

Blue native gel electrophoresis (BNGE) was performed on mitochondrial fraction from cerebrum. The mitochondrial pellets were suspended in 140 μl in the homogenization medium A. An aliquot of each sample was used for protein determination. The remaining samples were then centrifuged at 17,000 *g* for 3 min at 4 °C. Mitochondrial pellets were suspended in an appropriate volume of buffer B (1 M 6-amiohexanoic acid, 50 mM Bis-Tris–HCl [pH 7.0]) to be at 10 mg/ml, and the membrane proteins were solubilized by the addition of digitonin (4 g/g) and incubated for 5 min in ice. After 30 min centrifugation at 13,000 *g*, the supernatant was collected, and 3 μl of 5% Brilliant Blue G dye prepared in 1 M 6-amiohexanoic acid was added [9]. Mitochondrial proteins (100 μg) were then applied and ran on a 3%–13% gradient native gel using electrophoresis system mini-PROTEAN Tetra Cell (Bio-rad). Western blot was performed using a mini Trans-blot Cell onto PVDF membranes and probes with specific antibodies against complex I, anti-NUDFA9 (Abcam, ab14713), complex III, anti-ubiquinol-cytochrome c reductase Core Protein I (Abcam, ab110252) and Vdac1 (Abcam, ab14734) [9,12].

2.6. Mitochondrial complex I in-gel catalytic activity assay

Mitochondrial membrane proteins (100 μg) were applied and ran on a 3%–13% first-dimension gradient BNGE gel as described elsewhere [13]. The assay buffer contained 10 mg of NTB and 0.14 mM NADH added to 10 ml of 100 mM Tris/HCl, pH 7.4. After about 30 min the reaction was stopped using 5 mM Tris/HCl, pH 7.4 and scanned for densitometric quantitation.

2.7. Histology and Immunohistochemistry

Mice tissues were formalin-fixed and paraffin-embedded. Multiple sections (4 μm thickness) were deparaffinized with xylene and stained with hematoxylin and eosin (H&E). Immunohistochemistry was carried out in the same sections, using the following primary antibodies: anti-glial fibrillary acidic protein (GFAP) (Millipore, MAB360), anti-Neuronal Class III β-tubulin (TUJ1) (Covance, MMS-435P) and anti-8-hydroxy-2′-deoxyguanosine (8-OHdG) (QED Bioscience, 12501). Dako Animal Research Kit for mouse primary antibodies (Dako Diagnóstico S.A., Spain) was used for the qualitative identification of antigens by light microscopy. Sections were examined at 40–400 magnifications with an OLYMPUS CX41 microscope, and the images were scanned under equal light conditions with the CELL A computer program [9].

2.8. Statistical analysis

All statistical analyses were performed using the GraphPad scientific software. Data are expressed as the mean ± SD of seven–ten experiments per group. A one-way ANOVA with a Tukey post hoc test was used to

Table 1
Concentration of CoQ_9 and CoQ_{10} in plasma of 3 months old mice after two months of treatment.

Experimental group	Plasma CoQ_9 (μM)	Plasma CoQ_{10} (μM)
Coq9$^{+/+}$	0.27 ± 0.03	UND
Coq9$^{X/X}$ + V	0.09 ± 0.01 **	UND
Coq9$^{X/X}$ + Q_{10}	0.07 ± 0.01 **	1.36 ± 0.67
Coq9$^{X/X}$ + $Q_{10}H_2$	0.05 ± 0.02 **	2.06 ± 0.65

Data are expressed as mean ± SD of seven animals per group. V = vehicle; Q_{10} = ubiquinone-10; $Q_{10}H_2$ = ubiquinol-10. UND = undetectable.
** $P < 0.01$ versus *Coq9*$^{+/+}$.

compare the differences between groups. A *P*-value of 0.05 was considered to be statistically significant.

3. Results

3.1. CoQ_{10} levels in plasma and tissues of *Coq9*$^{X/X}$ after 2 months of treatment

We previously reported that *Coq9*$^{X/X}$ mice showed a significant decrease in both CoQ_9 (the major form of ubiquinone in rodents) and CoQ_{10} levels at 3 months of age compared with the age-mated *Coq9*$^{+/+}$ mice in all examined tissues (cerebrum, cerebellum, heart, kidney, hind legs skeletal muscle and liver) [9]. Two months of ubiquinone-10 or ubiquinol-10 therapies increased plasma levels of CoQ_{10} in *Coq9*$^{X/X}$ mice, while levels of CoQ_9 did not change in the same animals (Table 1). Treatment with vehicle did not produce any effects in the plasma CoQ_{10} levels. In tissues, a significant increase of CoQ_{10} after ubiquinone-10 treatment was only detected in liver and muscle. On the contrary, CoQ_{10} levels were significantly increased in the cerebrum, cerebellum, heart, kidney, liver and hind leg skeletal muscle of *Coq9*$^{X/X}$ mice treated with ubiquinol-10 (Fig. 1). The increase of CoQ_{10} levels after ubiquinone-10 or ubiquinol-10 treatments was in parallel to a decrease in the CoQ_9/CoQ_{10} ratio (Fig. S1), which indicates that these therapies did not affect the CoQ_9 levels (Fig. S2). Compared to the vehicle group, the highest increase of CoQ_{10} after ubiquinone-10 or ubiquinol-10 treatments was found in liver and muscle, followed by heart, kidney, cerebrum and cerebellum. Only muscle and heart of *Coq9*$^{X/X}$ mice treated with ubiquinol-10 reached similar CoQ_{10} levels than that of *Coq9*$^{+/+}$ mice, while liver accumulated huge amounts of CoQ_{10} after ubiquinone-10 or ubiquinol-10 treatment (Fig. 1).

3.2. CoQ levels in cerebral mitochondria of *Coq9*$^{X/X}$ after 2 months of treatment

Because *Coq9*$^{X/X}$ mice develop mitochondrial encephalopathy [9], we evaluated the effects of the therapies on mitochondrial CoQ levels and mitochondrial respiratory chain function in cerebrum of the mutant mice. Mitochondrial CoQ_{10} levels were significantly increased only after ubiquinol-10 treatment in *Coq9*$^{X/X}$ mice, while vehicle or ubiquinone-10 supplementation did not increase the mitochondrial CoQ_{10} levels (Fig. 2A). Considering the total mitochondrial CoQ pool (CoQ_9 + CoQ_{10}), ubiquinol-10 treatment increased mitochondrial CoQ levels (Fig. 2B) and decreased the CoQ_9/CoQ_{10} ratio after ubiquinol-10 treatment (Fig. 2C).

3.3. CoQ-dependent mitochondrial respiratory chain activities and supercomplex pattern in cerebral mitochondria of *Coq9*$^{X/X}$ after 2 months of treatment

Cerebral mitochondria of *Coq9*$^{X/X}$ mice treated with vehicle showed a significant decrease of CI + III and CII + III activities compared to those of *Coq9*$^{+/+}$ mice (Fig. 2D and E). Similarly to the *Pdss2*$^{kd/kd}$ mice, the decrease in CI + III activity was higher than the decrease in CII + III activity [14]. The increase of CoQ_{10} levels in cerebral mitochondria of

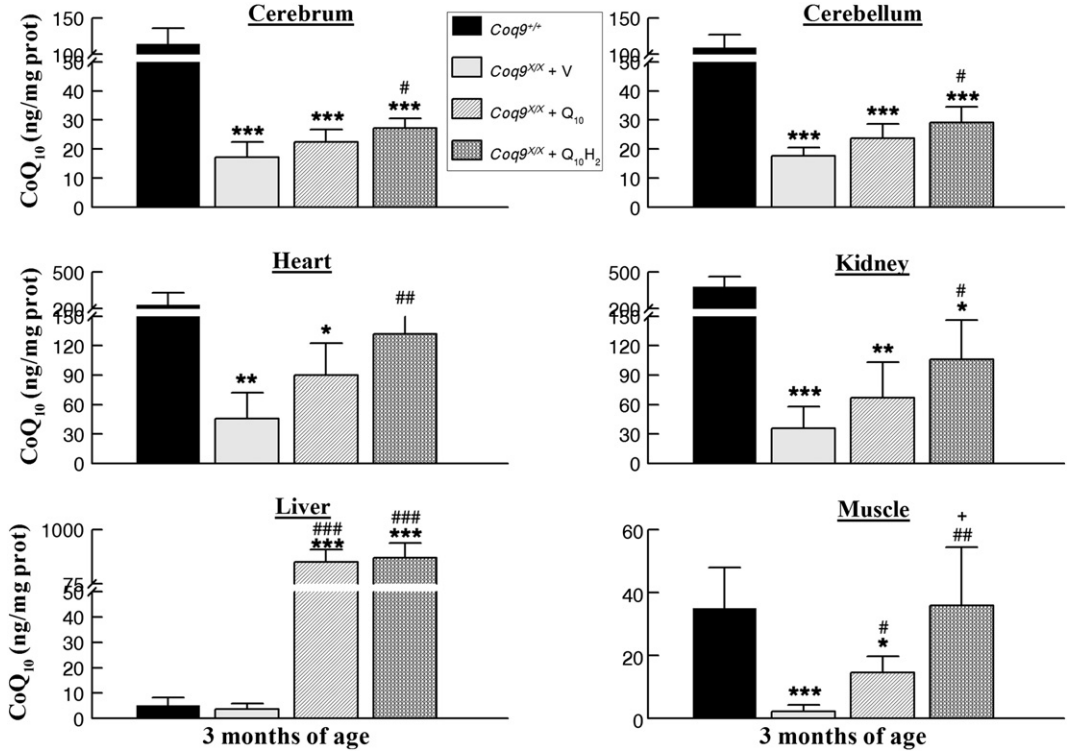

Fig. 1. Ubiquinone-10 and ubiquinol-10 increase tissue levels of CoQ_{10} in *Coq9*$^{X/X}$ mice. CoQ_{10} levels in tissue homogenates from *Coq9*$^{+/+}$ (N = 10), *Coq9*$^{X/X}$ + V (N = 10), *Coq9*$^{X/X}$ + Q_{10} (N = 10) and *Coq9*$^{X/X}$ + $Q_{10}H_2$ (N = 10) mice after 2 months of treatment. Data are expressed as mean ± SD. V = vehicle; Q_{10} = ubiquinone-10; $Q_{10}H_2$ = ubiquinol-10. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.005$ versus *Coq9*$^{+/+}$; #$P < 0.05$, and ##$P < 0.01$ versus *Coq9*$^{X/X}$ + V; +$P < 0.05$ versus *Coq9*$^{X/X}$ + Q_{10}.

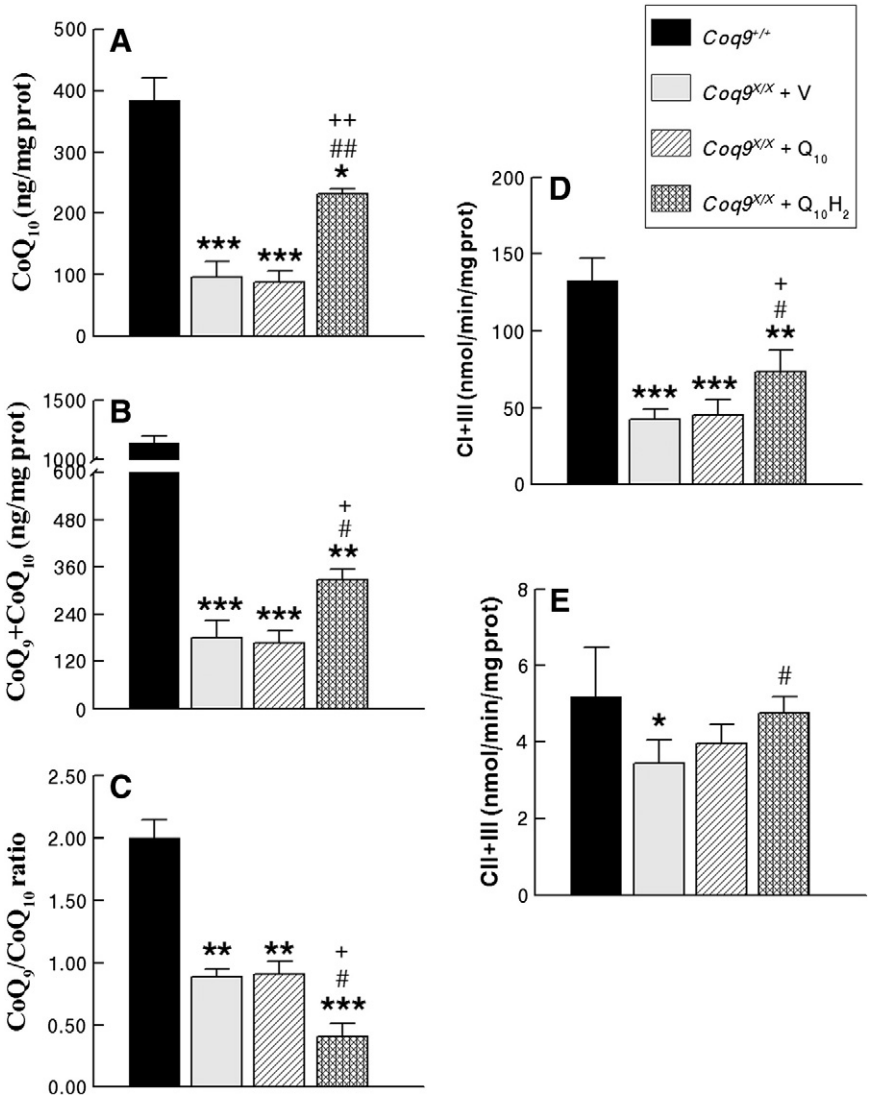

Fig. 2. Mitochondria from cerebrum of *Coq9*$^{X/X}$ mice show an increase of CoQ_{10} levels and CoQ-dependent respiratory chain activities after ubiquinol-10 treatment. (A) CoQ_{10} levels, (B) $CoQ_9 + CoQ_{10}$ levels and (C) CoQ_9/CoQ_{10} ratio in cerebrum mitochondria of *Coq9*$^{+/+}$ (N = 7), *Coq9*$^{X/X}$ + V (N = 7), *Coq9*$^{X/X}$ + Q_{10} (N = 7) and *Coq9*$^{X/X}$ + $Q_{10}H_2$ (N = 7) mice after 2 months of treatment. CoQ-dependent mitochondria respiratory chain activities represented by (D) CI + III and (E) CII + III. Data are expressed as mean ± SD. V = vehicle; Q_{10} = ubiquinone-10; $Q_{10}H_2$ = ubiquinol-10. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.005$ versus *Coq9*$^{+/+}$; #$P < 0.05$; ##$P < 0.01$ versus *Coq9*$^{X/X}$ + V; +$P < 0.05$, ++$P < 0.01$ versus *Coq9*$^{X/X}$ + Q_{10}.

Coq9$^{X/X}$ mice after ubiquinol-10 treatment induced a significant increase of CI + III activity (Fig. 2D) and the normalization of CII + III activity (Fig. 2E). On the contrary, vehicle or ubiquinone-10 treatments did not change the CoQ-dependent respiratory chain activities (Fig. 2D and E). The increase of the CoQ-dependent mitochondrial respiratory chain activities after ubiquinol-10 treatment was not due to an increase in the supercomplex I/III formation because the ratio supercomplex I/III/free complex III remained low in *Coq9*$^{X/X}$ mice after vehicle, ubiquinone-10 or ubiquinol-10 treatment compared to *Coq9*$^{+/+}$ mice (Fig. 3A, B and C). The ratio supercomplex I/III/free complex I (Fig. S3A), as well as complex I in gel activity, was similar in *Coq9*$^{+/+}$ mice and *Coq9*$^{X/X}$ mice (Fig. S3B), while the treatments did not produce any changes on these variables (Fig. S3).

3.4. Histopathological evaluation and oxidative damage in brain of *Coq9*$^{X/X}$ after 2 months of treatment

Coq9$^{X/X}$ mice show white matter vacuolization, severe reactive astrogliosis, reduction in neuronal dendrites and increased DNA oxidation, which were especially evident in diencephalon and brainstem [9]. The treatment with vehicle did not produce any change in these histopathological biomarkers because *Coq9*$^{X/X}$ animals similarly showed white matter vacuolization (Fig. 4C and D) and proliferation of astrocytes (Fig. 4K and L) in diencephalon, as well as increased DNA oxidation in diencephalon and septum–striatum (Fig. 5C, D, K and L) compared to *Coq9*$^{+/+}$ mice (Fig. 4A, B, I and J; Fig. 5A, B, I and J). Treatment with ubiquinone-10 did not reduce the vacuolization (Fig. 4E and F) and astrogliosis (Fig. 4M and N) in diencephalon of *Coq9*$^{X/X}$ mice, while the immunoreactivity against 8-OHdG was slightly decreased in both diencephalon (Fig. 5E and F) and septum–striatum (Fig. 5M and N). On the contrary, treatment with ubiquinol-10 was able to reduce the vacuolization (Fig. 4G and H) and astrogliosis (Fig. 4O and P) in diencephalon, as well as the DNA oxidation in both diencephalon (Fig. 5G and H) and septum–striatum (Fig. 5O and P). In brainstem, however, both ubiquinone-10 and ubiquinol-10 treatments were able to reduce the astrogliosis and DNA oxidation, as well as increase neuronal immunoreactivity (Figs. S4 and S5). Nevertheless, the vacuolization still persisted with both treatments (Figs. S4 and S5). The histology

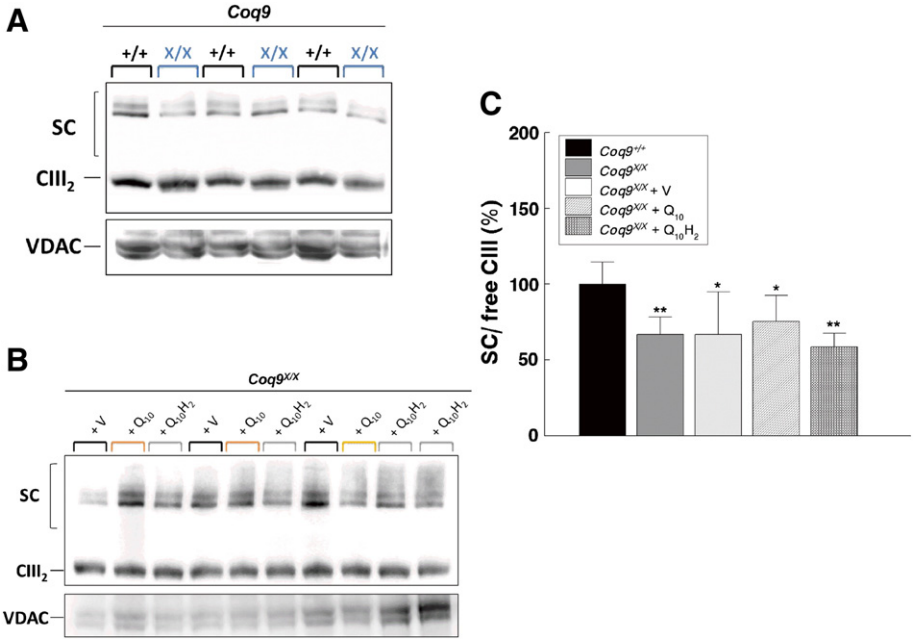

Fig. 3. The experimental treatments did not correct the decrease of SC/free CIII observed in cerebral mitochondria of *Coq9*$^{X/X}$ mice. Blue-native gel electrophoresis (BNGE) followed by immunoblotting analysis of mitochondrial supercomplexes from 3 months old (A) *Coq9*$^{+/+}$ (N = 7) and *Coq9*$^{X/X}$ (N = 7) mice; and (B) *Coq9*$^{X/X}$ + V (N = 7), *Coq9*$^{X/X}$ + Q_{10} (N = 7) and *Coq9*$^{X/X}$ + $Q_{10}H_2$ (N = 7). Antibody against ubiquinol-cytochrome c reductase core protein I was used to detect complex III. Antibody against Vdac1 was used as mitochondrial loading control. (C) Densitometry analysis of supercomplexes (SC) and free complex III, expressed as the SC/free CI ratio and considering the value of *Coq9*$^{+/+}$ as 100%. Data are expressed as mean ± SD. V = vehicle; Q_{10} = ubiquinone-10; $Q_{10}H_2$ = ubiquinol-10. *$P < 0.05$; **$P < 0.01$ versus *Coq9*$^{+/+}$.

structure of kidneys, muscle and heart was similar in all experimental groups (Fig. S6).

3.5. Consequences of the treatments in the animal weight

Coq9$^{X/X}$ mice show a reduction in the body weight between the age of 1 and 5 months [9]. Oral supplementation with vehicle or ubiquinone-10 did not produce any significant effect in body weight. On the contrary, ubiquinol-10 treatment significantly increased the body weight in both male (Fig. 6A) and female (Fig. 6B) *Coq9*$^{X/X}$ mice after two months of treatment, compared to *Coq9*$^{X/X}$ mice treated with vehicle (Movie S1).

4. Discussion

Therapy based on oral supplementation with ubiquinone-10 has shown contradictory results in the treatment of primary and secondary CoQ_{10} deficiencies, mitochondrial diseases and other neurological diseases like Parkinson's Disease, Alzheimer's Disease, Amyotrophic Lateral Sclerosis or Huntington's Disease [2–7]. These controversial results may be due to the poor absorption and bioavailability of ubiquinone-10 [7,10,15]. In this study, we demonstrate that a water-soluble formulation of ubiquinol-10, the reduced form of CoQ_{10}, was more effective than that of ubiquinone-10 in increasing the levels of CoQ_{10} in tissue homogenates and cerebral mitochondria, resulting in an increase of CoQ-dependent respiratory chain activities in the cerebrum of a CoQ deficient mouse model with mitochondrial encephalopathy (*Coq9*$^{X/X}$ mice). As a consequence, ubiquinol-10 was more efficient than ubiquinone-10 in reducing the vacuolization, astrogliosis and oxidative damage in *Coq9*$^{X/X}$ mice, thus increasing the animal body weight.

Ubiquinone-10 in its pure form is a powder product that is insoluble in water and has partial solubility in lipids and organic solutions, and therefore it is poorly absorbed. The uptake of ubiquinone-10 is very low in brain because of the blood brain barrier. Moreover, the limitation of the exogenous ubiquinone-10 to reach mitochondria is one of the major problems for the CoQ_{10} therapy because external ubiquinone-10 is distributed mainly in lysosomes and only a small amount, if any, is found in mitochondria [10,15]. To try to increase the absorption of ubiquinone-10, different ubiquinone-10 formulations have been manufactured and are currently available on the market. These formulations include powder-based compressed tablets, chewable tablets, powder-filled hard-shell capsules, softgels containing an oil suspension and water-soluble formulations in softgel or liquid forms [8]. The latter forms are based in the ability of dextrins to increase the solubility of poorly water-soluble compounds with no toxic effects [16]. Our study shows that water-soluble formulation of ubiquinone-10 in a dose of 240 mg/kg bw/day, which is equivalent to 30 mg/kg bw/day in humans [17,18] according to the body surface area [19], is able to increase CoQ_{10} levels in plasma of *Coq9*$^{X/X}$ mice. The increase of plasma CoQ_{10} concentration in *Coq9*$^{X/X}$ mice was reflected in an increase of CoQ_{10} levels in tissues of treated mice. The lowest increase of CoQ_{10} in cerebrum and cerebellum may be due to the blood brain barrier. The highest increase of CoQ_{10} in the liver may be explained by its mechanism of absorption in the gastrointestinal system. After gastric emptying, CoQ_{10} is absorbed along with other lipids as chylomicron particles in the small intestine, and transported via lymph vessels to blood circulatory system and then taken up by the liver cells. In the liver, CoQ_{10} is incorporated with lipoproteins and released into the blood, which is used as a transport vehicle to deliver CoQ_{10} in other tissues [20,21]. In another study, using ubiquinone-10 in oil suspension (LiQsorb, Tishcon) in doses of 200 and 400 mg/kg bw/day for 3–4 months, the authors did not find any increase of CoQ_{10} in kidneys of a mouse model of CoQ deficiency due to *Pdss2* mutation (*Pdss2*$^{kd/kd}$) [22]. Similarly, the water-soluble formulation of ubiquinone-10 used in our study did not show a significant increase in the levels of CoQ_{10} in kidney, suggesting that the vehicle does not affect the absorption of ubiquinone-10 at tissue level.

Our study also shows that ubiquinol-10 has better absorption, bioavailability and tissue uptake than ubiquinone-10 [23,24]. Importantly, the increase of CoQ_{10} after ubiquinol-10 treatment is not limited to tissue levels because CoQ_{10} is also increased in mitochondria from the cerebrum of *Coq9*$^{X/X}$ mice. As a consequence, ubiquinol-10 supplementation was able to increase the CoQ-dependent mitochondrial respiratory chain

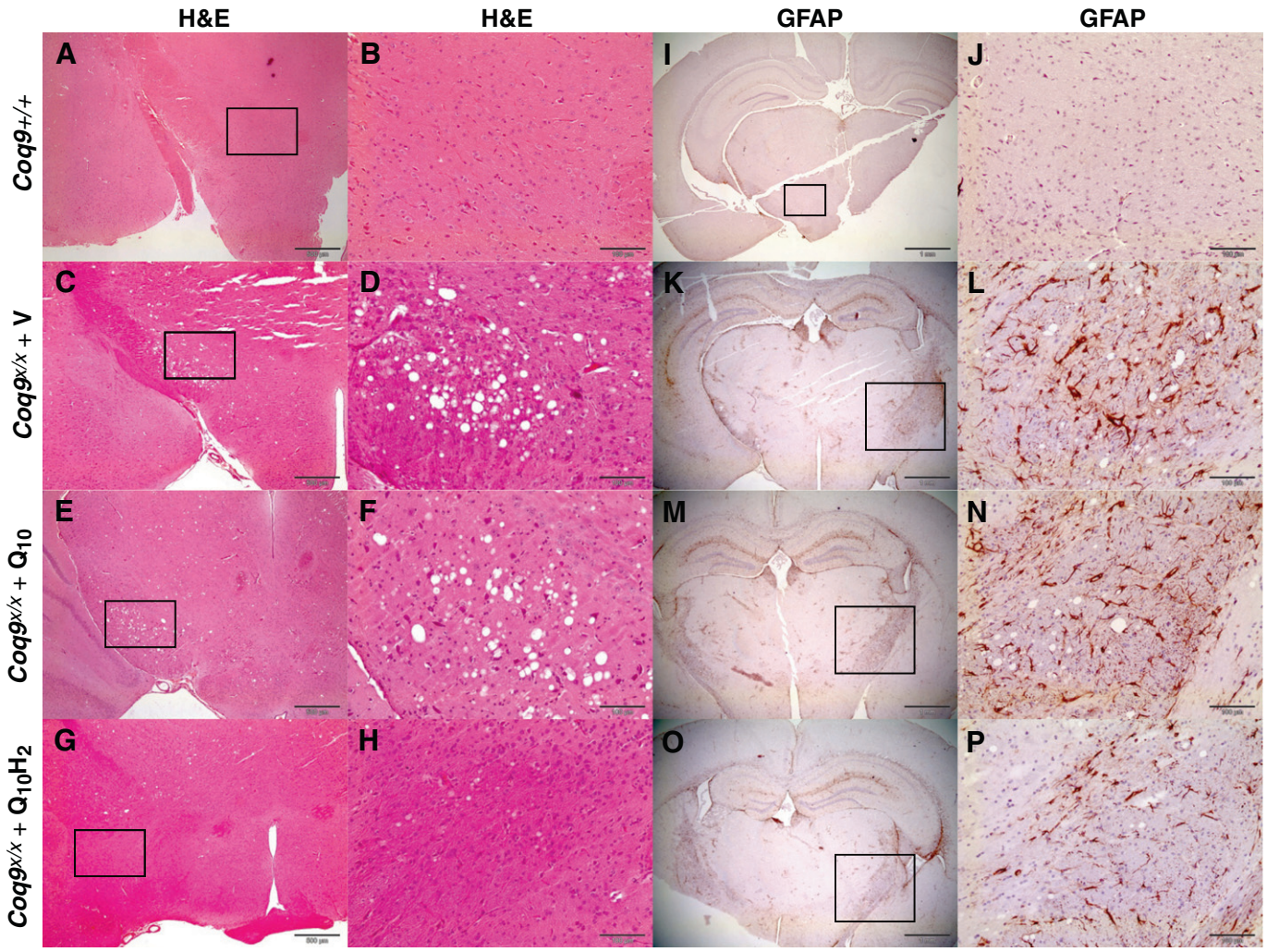

Fig. 4. (A–F) Structural changes and astrocyte distribution in diencephalon of *Coq9*$^{X/X}$ mice after two months of treatments. Hematoxylin and eosin (H&E) stains of diencephalon from (A–B) *Coq9*$^{+/+}$, (C–D) *Coq9*$^{X/X}$ + V (N = 3), (E–F) *Coq9*$^{X/X}$ + Q_{10} (N = 3) and (G–H) *Coq9*$^{X/X}$ + $Q_{10}H_2$ (N = 3) mice after 2 months of treatment. (G–L) Anti-glial fibrillary acid protein (anti-GFAP) antibody staining of diencephalon from (I–J) *Coq9*$^{+/+}$, (K–L) *Coq9*$^{X/X}$ + V (N = 3), (M–N) *Coq9*$^{X/X}$ + Q_{10} (N = 3) and (O–P) *Coq9*$^{X/X}$ + $Q_{10}H_2$ (N = 3) mice after 2 months of treatment. V = vehicle; Q_{10} = ubiquinone-10; $Q_{10}H_2$ = ubiquinol-10. (A, C, E, G), scale bars, 500 μm; (I, K, M, O), scale bars, 1 mm; (B, D, F, H) and (J, L, N, P), scale bars, 100 μm.

activities, while the oxidized form did not have this effect at mitochondrial level. However, CI + III activity in cerebrum of *Coq9*$^{X/X}$ mice treated with ubiquinol-10 was still half of the activity in *Coq9*$^{+/+}$ mice. This result may be explained by the fact that ubiquinol-10 treatment was not able to normalize the supercomplex/free CIII ratio (Fig. 3A) [9], and this may be justified by two possibilities: a) the accumulation of 5-demethoxyubiquinone-9 in *Coq9*$^{X/X}$ mice, which was not corrected by the treatments, could partially inhibit the transfer of electrons from CI to CoQ, as it has been reported in *Caenorhabditis elegans* mutant *clk-1* (analog to *Coq7* in human and mouse) [25]; and b) the increase of total CoQ (CoQ_9 + CoQ_{10}) after ubiquinol-10 treatment did not reach the required total CoQ levels in the Q binding sites of CI and CIII. Humans and mice have two CoQ forms, CoQ_9 and CoQ_{10}, which differ with each other in the length size of the polyprenyl tail. While the reason to synthesize two CoQ forms is not clear and the same functions are attributed indistinctly to the two forms, an adequate CoQ_9/CoQ_{10} ratio may be necessary for an optimal performance of mitochondrial bioenergetics, including a physiological proportion of CIII free and CIII bound to supercomplexes. In fact, each tissue has a particular value on CoQ_9/CoQ_{10} ratio, with cerebrum and cerebellum being the tissues with lowest CoQ_9/CoQ_{10} ratio in mouse (highest in human) [26]. This fact points out that CoQ_9/CoQ_{10} ratio seems to be tightly regulated in a tissue specific way. In *Coq9*$^{X/X}$ mice, a decrease in CoQ_9/CoQ_{10} ratio was detected in cerebrum and cerebellum homogenates, as well as in isolated mitochondria from cerebrum (Figs. S1 and 2C), and the ratio was even lower after ubiquinol-10 treatment (Figs. S1 and 2C). Future therapeutic strategies focused in increasing the endogenous CoQ_9 and CoQ_{10} biosynthesis could contribute to understand the importance of CoQ_9 in mitochondrial bioenergetics.

In addition to its bioenergetics role, CoQ_{10} is one of the most important endogenous antioxidants in the cell [1]. CoQ_{10} deficiency is accompanied with an increased production of reactive oxygen species (ROS) and oxidative damage to biomolecules, which leads to an increased cell death in vitro [27,28] and in vivo [14]. *Coq9*$^{X/X}$ mice also show an increase of 8-OHdG in diencephalon, septum–striatum and brainstem, [9] (Figs. 5 and 4s). Both ubiquinone-10 and ubiquinol-10 reduced the immunostaining against 8-OHdG, but the reduction was higher with ubiquinol-10. This result may reflect the higher uptake of ubiquinol-10 compared to ubiquinone-10, its higher antioxidant capacity and/or an effect in reducing the leak of electrons through the mitochondrial respiratory chain.

Following the biochemical changes after the treatments, supplementation with ubiquinol-10 reduced the vacuolization and astrogliosis in diencephalon, septum–striatum and, to a lesser extent, in brainstem of *Coq9*$^{X/X}$ mice. The lower efficiency of ubiquinol-10 treatment in reducing the histopathological changes in brainstem of *Coq9*$^{X/X}$ mice compared to diencephalon and septum–striatum may be related to an early and irreversible damage in this area, which is particularly susceptible in Leigh

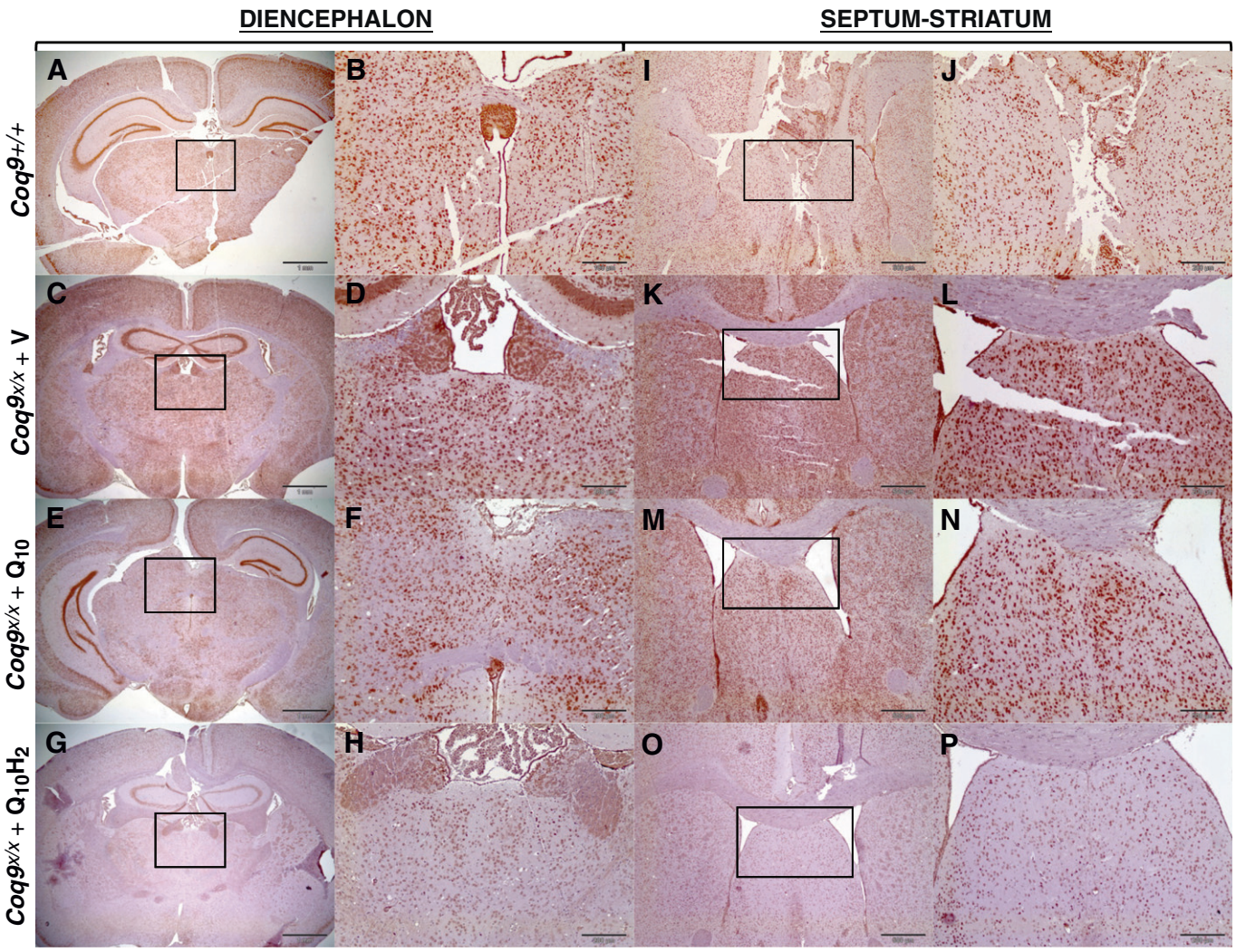

Fig. 5. DNA oxidation in diencephalon and septum–striatum of $Coq9^{X/X}$ mice after two months of treatments. Anti-8-hydroxy-2′-deoxyguanosine (anti-8OHdG) antibody staining of (A–H) diencephalon and (I–P) septum–striatum from (A, B, I, J) $Coq9^{+/+}$, (C, D, K, L) $Coq9^{X/X}$ + V (N = 3), (E, F, M, N) $Coq9^{X/X}$ + Q_{10} (N = 3) and (G, H, O, P) $Coq9^{X/X}$ + $Q_{10}H_2$ (N = 3) mice after 2 months of treatment. V = vehicle; Q_{10} = ubiquinone-10; $Q_{10}H_2$ = ubiquinol-10. (A, C, E, G), scale bars, 1 mm; (I, K, M, O), scale bars, 500 μm; (B, D, F, H) and (J, L, N, P), scale bars, 200 μm.

syndrome [29,30]. Notably, ubiquinol-10 was more powerful than ubiquinone-10 in reducing the histopathological changes in $Coq9^{X/X}$ mice, resulting in an increase in the body weight. These results are particularly important because patients with CoQ_{10} deficiency showed variable responses to ubiquinone-10 treatment and, in some cases, the treatment failed or did not show a clear response [31–40], which may be due to the reduced uptake of ubiquinone-10 [7]. Thus, our results suggest that ubiquinol-10 supplementation could improve the efficacy showed by ubiquinone-10 supplementation, which will be especially important in patients with encephalopathy or cerebellar ataxia associated to CoQ_{10} deficiency. In agreement with that, in a patient with CoQ_{10} deficiency, mental retardation, encephalomyopathy and dimorphic features due to a *COQ4* mutation, ubiquinol-10 in a dose of 15 mg/kg bw/day had the same efficiency than ubiquinone-10 in a dose of 30 mg/kg bw/day [18].

5. Conclusions

Our results demonstrate that dextrin-based water-soluble formulations of ubiquinol-10 have better absorption and uptake at tissue and mitochondrial levels, which results in an increase of CoQ-dependent respiratory chain activities, reduction in vacuolization, astrogliosis and oxidative damage in different brain areas, and an increase of body weight in a CoQ deficient mouse model with mitochondrial encephalopathy. This data suggest that water-soluble formulations of ubiquinol-10 should be preferentially used for CoQ_{10} therapy. However, ubiquinol-10 supplementation did not completely rescue the encephalopathic phenotype of the $Coq9^{X/X}$ mouse model. Considering that mice and humans produce both CoQ_9 and CoQ_{10}, future therapeutic strategies focused in increasing both CoQ_9 and CoQ_{10} levels could lead to obtain better results.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbadis.2014.02.008.

Competing interest statement

None of the authors have competing interests to declare.

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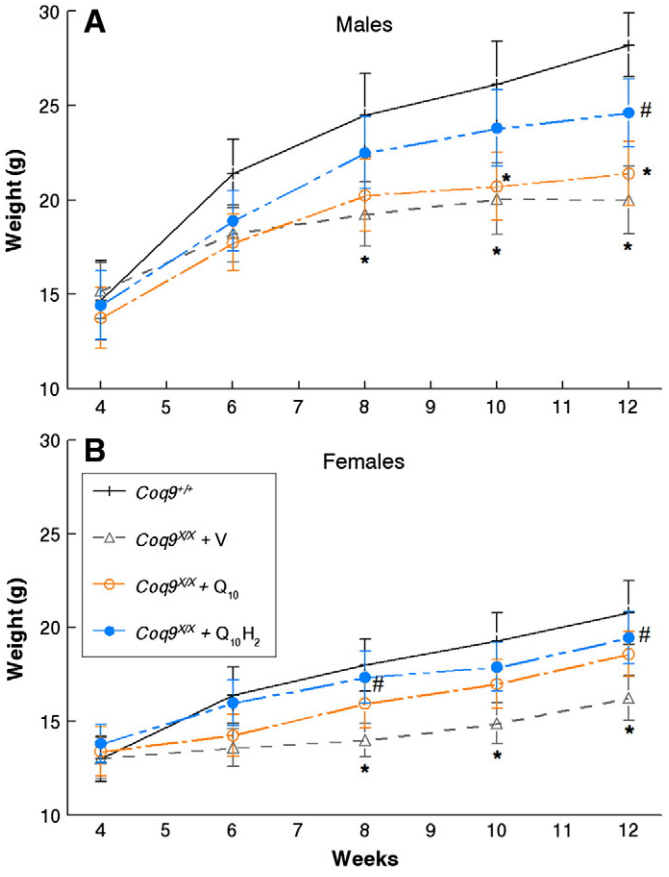

Fig. 6. Evolution of the animal body weight during two months of treatments. (A) Body weight of male and (B) female *Coq9*$^{+/+}$ (N = 10), *Coq9*$^{X/X}$ + V (N = 10), *Coq9*$^{X/X}$ + Q_{10} (N = 10) and *Coq9*$^{X/X}$ + $Q_{10}H_2$ (N = 10) mice. V = vehicle; Q_{10} = ubiquinone-10; $Q_{10}H_2$ = ubiquinol-10. *$P < 0.05$ versus *Coq9*$^{+/+}$; #$P < 0.05$ versus *Coq9*$^{X/X}$ + V.

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