Ubiquinol Effects on Antiphospholipid Syndrome Prothrombotic Profile

A Randomized, Placebo-Controlled Trial

Carlos Pérez-Sánchez,* María Ángeles Aguirre,* Patricia Ruiz-Limón, María Carmen Ábalos-Aguilera, Yolanda Jiménez-Gómez, Iván Arias-de la Rosa, Antonio Rodriguez-Ariza, Lucía Fernández-del Río, José Antonio González-Reyes, Pedro Segui, Eduardo Collantes-Estévez, Nuria Barbarroja, Francisco Velasco, Savino Sciascia, Irene Cecchi, María José Cuadrado, José Manuel Villalba,† Chary López-Pedrera†

Objective—Antiphospholipid syndrome (APS) leukocytes exhibit an oxidative perturbation, directly linked to alterations in mitochondrial dynamics and metabolism. This disturbance is related to the patients' prothrombotic status and can be prevented by in vitro treatment with coenzyme Q10. Our aim was to investigate short-term effects of in vivo ubiquinol (reduced coenzyme Q_{10} [Q_{red}]) supplementation on markers related to inflammation and thrombosis in APS through a prospective, randomized, crossover, placebo-controlled trial.

Approach and Results—Thirty-six patients with APS were randomized to receive Q_{red} (200 mg/d) or placebo for 1 month. Thirty-three patients with APS completed the intervention, which increased plasma coenzyme Q_{10}. Q_{red} improved endothelial function and decreased monocyte expression of prothrombotic and proinflammatory mediators, inhibited phosphorylation of thrombosis-related protein kinases, and decreased peroxides and percentage of monocytes with depolarized mitochondria; mitochondrial size was increased, and mitochondrial biogenesis–related genes were upregulated. Q_{red} ameliorated extruded neutrophil extracellular traps in neutrophils and downregulated peroxides, intracellular elastase, and myeloperoxidase. Nanostring microRNA profiling revealed 20 microRNAs reduced in APS monocytes, and 16 of them, with a preponderance of cardiovascular disease–related target mRNAs, were upregulated. Monocytes gene profiling showed differential expression of 29 atherosclerosis-related genes, 23 of them changed by Q_{red}. Interaction networks of genes and microRNAs were identified. Correlation studies demonstrated co-ordinated effects of Q_{red} on thrombosis and endothelial function–associated molecules.

Conclusions—Our results highlight the potential of Q_{red} to modulate the overexpression of inflammatory and thrombotic risk markers in APS. Because of the absence of clinically significant side effects and its potential therapeutic benefits, Q_{red} might act as safe adjunct to standard therapies in APS.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT02218476

Visual Overview—An online visual overview is available for this article. **(*Arterioscler Thromb Vasc Biol*. 2017;37: 1923-1932. DOI: 10.1161/ATVBAHA.117.309225.)**

Key Words: antiphospholipid syndrome ■ extracellular traps ■ inflammation ■ oxidative stress ■ thrombosis ■ ubiquinol

The antiphospholipid syndrome (APS) is a clinical disorder characterized by thrombosis and pregnancy morbidity associated with the persistent presence of antiphospholipid antibodies (aPL), including anticardiolipin antibodies, anti-β2GPI (beta2-glycoprotein I), and lupus anticoagulant. Extensive analyses have demonstrated that anticardiolipin antibodies production is associated with plasma and immune cells oxidation, endothelial dysfunction, and vascular disease.[1]

See accompanying editorial on page 1801

Received on: February 16, 2017; final version accepted on: June 26, 2017.

From the Instituto Maimónides de Investigación Biomédica de Córdoba, Córdoba, Spain (C.P.-S., M.Á.A., P.R.-L., M.C.A.-A., Y.J.-G., I.A.-d.l.R., A.R.A., P.S., E.C.-E., N.B., F.V., C.L.-P.); Unidad de Gestión Clínica Reumatología (M.Á.A., E.C.-E., C.L.-P.), Unidad de Gestión Clínica Radiología (P.S.), and Unidad de Gestión Clínica Hematología (F.V.), Hospital Universitario Reina Sofía, Córdoba, Spain; Departamento de Biología Celular, Fisiología e Inmunología, Campus de Excelencia Internacional Agroalimentario, ceiA3 (L.F.-d.R., J.A.G.-R., J.M.V.) and Departamento de Medicina (Medicina, Dermatología y Otorrinolaringología) (E.C.-E.), Universidad de Córdoba, Córdoba, Spain; Lupus Research Unit, Hospital St Thomas, London, United Kingdom (M.J.C.); and Center of Research of Immunopathology and Rare Diseases—Coordinating Center of Piemonte and Valle d'Aosta Network for Rare Diseases, University of Turin, Italy (S.S., I.C.).

*C.P.-S. and M.Á.A. shared first authorship.

†J.M.V. and C.L.-P. shared last authorship.

The online-only Data Supplement is available with this article at http://atvb.ahajournals.org/lookup/suppl/doi:10.1161/ATVBAHA.117.309225/-/DC1.

Correspondence to Chary López-Pedrera, MSc, PhD, Instituto Maimónides de Investigación Biomédica de Córdoba/Reina Sofia University Hospital/ University of Córdoba, Avda, Menéndez Pidal s/n, E-14004 Córdoba, Spain. E-mail rosario.lopez.exts@juntadeandalucia.es

Nonstandard Abbreviations and Acronyms	
aPL	antiphospholipid antibodies
APS	antiphospholipid syndrome
CoQ_{10}	coenzyme Q_{10}
CVD	cardiovascular disease
NETs	neutrophil extracellular traps
NO	nitric oxide
Q_{red}	reduced coenzyme Q_{10}
TF	tissue factor

Thus, aPLs promote oxidative perturbations and mitochondrial dysfunction that trigger increased expression of prothrombotic factors and generate an inflammatory cascade with increased expression of several cytokines, chemokines, and mediators of endothelial dysfunction.[2,3] Furthermore, aPLs cross-react with oxidized low-density lipoproteins, thus accelerating their influx into macrophages, and promoting monocyte activation and atherosclerosis development.[4]

Current therapeutic options for the treatment of thrombotic APS remain confined to long-term oral anticoagulation with vitamin K antagonists, which have several drawbacks, including an increased risk of bleeding and recurrent thrombosis with subtherapeutic international normalized ratio. Because more insight is gained about the pathophysiology of the disease and the involved receptors and intracellular pathways, targeted treatment modalities have been proposed as possible alternatives to the current treatment options. Thus, in the past few years, several potential new therapeutic approaches to APS have emerged, including combination of antiaggregants, statins, and direct thrombin inhibitors, among others.[5–7]

Several studies have detached the beneficial effects of coenzyme Q_{10} (CoQ_{10}) supplementation on cardiovascular disease (CVD).[8–10] CoQ_{10} is a member of the mitochondrial respiratory chain that plays an important role in cellular metabolism, participating as an electron carrier in both mitochondrial and extramitochondrial membranes and protecting membrane and lipoproteins from protein oxidation and lipid peroxidation. CoQ_{10} provides membrane-stabilizing properties and acts as an antioxidant with cell-protective effects, including inhibition of low-density lipoprotein oxidation, and thus the progression of atherosclerosis. Furthermore, CoQ_{10} decreases the production of proinflammatory cytokines, as well as blood viscosity, demonstrated to be helpful in patients with heart failure and coronary artery disease.[11]

We have recently investigated the role of oxidative stress and mitochondrial dysfunction in the prothrombotic status of patients with APS induced by aPLs and the beneficial effects of supplementing cells in vitro with CoQ_{10}.[12] This study demonstrated the presence of an oxidative perturbation in APS patient leukocytes, which was directly related to an inflammatory and prothrombotic status, and relied on alterations in mitochondrial dynamics and metabolism, which were prevented and reverted by in vitro treatment with CoQ_{10}.

Those results prompted us to evaluate the in vivo effects of ubiquinol supplementation on the expression of parameters related to inflammation and thrombosis in patients with APS through the implementation of a prospective, randomized, double-masked, placebo-controlled study that was supported by Kaneka Corporation (Osaka, Japan). The cellular and molecular mechanisms involved have been further characterized.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement. Design and study treatment of the trial: Prospective, randomized, double-masked crossover, placebo-controlled study. Thirty-six patients with APS were randomized to receive reduced coenzyme Q_{10} (Q_{red}; 200 mg/d) or placebo for 1 month. Both the Q_{red} and the placebo capsules were specially produced by the same company (Kaneka Corporation) and were identical in weight and external aspects. Treatments were given for 1 month according to a randomized and crossover design (with wash-out periods of 4 weeks) as described in Figure I in the online-only Data Supplement. During this treatment, patients were not withdrawn from their therapy (composed of anticoagulants/antiaggregants [ie, acenocoumarol or aspirin] and antimalarials [ie, hydroxychloroquine], and immunosuppressants, and statins, and corticosteroids [median dose of 7.7±3.1 mg]; Table II in the online-only Data Supplement). Clinical and laboratory parameters of the patients with APS included in the Q_{red} protocol are displayed in the Table. Routine and disease-specific clinical and laboratory data were recorded in 4 monthly visits.

Results

Thirty-three of the 36 patients completed the intervention, which increased significantly plasma CoQ_{10} levels (3.5±0.6 pmol/μL after Q_{red} versus 1.0±0.10 pmol/μL before Q_{red}; $P<0.05$; Figure II in the online-only Data Supplement). Demographics, clinical manifestations, and treatments other than Q_{red} were equally distributed between the 2 randomized groups. After multivariate analysis, no demographics, clinical manifestations, and treatment rather than Q_{red} was statistically proven to act as a confounding variable.

Q_{red} Supplementation Improved Endothelial Activity

Endothelial function improved notably as shown by the amelioration in the highest perfusion value after occlusion was released in ubiquinol-treated patients, expressed as the difference between peak flow and rest flow values or the hyperemic area (Figure 1A–1C). Accordingly, significantly reduced plasma levels of vascular cell adhesion molecule 1 were demonstrated in patients with APS after ubiquinol treatment (Figure 1D).

To specifically evaluate the effect of Q_{red} on endothelial dysfunction in these patients, we performed in vitro studies where human umbilical vein endothelial cells were treated with serum obtained from healthy donors or patients with APS before and after Q_{red} treatment. Q_{red} improved the endothelial dysfunction induced by serum from patients with APS before Q_{red} supplementation as demonstrated by the downregulation of reactive oxygen species production (Figure 1E), adhesion molecules (ie, vascular cell adhesion molecule 1; Figure VB–VE in the online-only Data Supplement), and inflammatory mediators (ie, interleukin 8 and vascular endothelial growth factor; Figure 1F). In addition, the expression (at both, gene and protein levels) and activity of the prothrombotic receptor tissue factor (TF) were significantly reduced (Figure 1F; Figure IVA–IVC in the online-only Data Supplement). We further observed a fall near to basal levels in the expression of nitric oxide (NO; Figure VA in the online-only Data Supplement) and NO-producing enzymes (ie, inducible NO synthase and endothelial NO synthase) overexpressed by effect of serum from patients with APS before Q_{red} treatment (Figure 1F).

Table. Clinical and Laboratory Parameters of the Patients With APS Recruited to the Study

	Patients With APS (n=36)
Clinical parameters	
Females/males (n)	27/9
Age, y (mean±SD)	51.89±10.56
Patients with APS (n)	23 (64%)
Patients with APS+SLE (n)	13 (36%)
Thrombosis (n)	30 (83%)
Arterial thrombosis (n)	23 (77%)
Venous thrombosis (n)	9 (30%)
Recurrences (n)	15 (50%)
Pregnancy morbidity (n)	16 (44%)
Preeclampsia (n)	4 (25%)
Fetal loss (n)	12 (75%)
Diabetes mellitus (n)	3 (8%)
Increased CIMT (n)	16 (44%)
Smoking (n)	11 (31%)
Hypertension (n)	11 (31%)
BMI (mean±SD)	28.6±5.73
LA positivity (n)	29 (67%)
aPL positivity (n)	13 (36%)
aCL IgG (GPL, mean±SD)	19.5±33.70
aCL IgM (MPL, mean±SD)	45.10±132.00
Anti-β2GPI-IgG (SGU, mean±SD)	52.40±129.00
Anti-β2GPI-IgM (SGU, mean±SD)	8.30±12.60
Treatment	
Antiplatelet (ASA/clopidogel; n)	14 (39%)
Anticoagulant (warfarin/acenocoumarol; n)	21 (58%)
Hydroxychloroquine (n)	12 (33%)
Statins (n)	13 (36%)
Corticosteroids (n)	12 (33%)
Immunosuppressants (n)	2 (6%)
Laboratory parameters	
Total cholesterol, mg/dL (mean±SD)	190.46±30.20
Cholesterol HDL, mg/dL (mean±SD)	50.00±12.48
Cholesterol LDL, mg/dL (mean±SD)	115.03±30.36
Triglycerides, mg/dL (mean±SD)	122.37±81.86
C reactive protein, mg/dL (mean±SD)	4.36±4.69
Apolipoprotein A, g/L (mean±SD)	151.31±25.71
Apolipoprotein B, g/L (mean±SD)	80.31±20.37

Except where otherwise indicated, values are the number of subjects. β2GPI indicates beta2-glycoprotein I; aCL, anticardiolipin antibodies; aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; BMI, body mass index; CIMT, carotid intimae media thickness; GPL, G phospholipid units; HDL, high-density lipoprotein; LA, lupus anticoagulant; LDL, low-density lipoprotein; MPL, M phospholipid units; SGU, standard IgG beta2-GPI IgG units; and SLE, systemic lupus erythematosus.

Concomitantly, a significant reduction in the activity of p38 MAPK (p38 mitogen-activated protein kinase), the key intracellular pathway regulating the expression of such molecules, was observed (Figure 1G).

Q_{red} Reduced Oxidative Status and Mitochondrial Dysfunction

Q_{red} supplementation produced a reduction in both the levels of peroxides and the percentage of monocytes with altered mitochondrial membrane potential ($\Delta\Psi m$), as well as a significant increase in glutathione (Figure 2A–2C). In neutrophils, a significant inhibition in peroxide production was observed after Q_{red} treatment, along with a trend in the upregulation of glutathione (Figure III in the online-only Data Supplement).

At plasma level, we also found a significant increase in total plasma antioxidant capacity, along with a reduction in oxidized low-density lipoprotein levels (Figure 2D and 2E).

Q_{red} Treatment Promoted Significant Changes in Mitochondrial Ultrastructure and Biogenesis

Electron microscopy analyses indicated that ubiquinol treatment promoted an increase in monocytes' mitochondrial size in terms of both area and calculated volume of mitochondrial profiles appearing in cell sections. Interestingly, Q_{red} produced a significant increase of mitochondrial volume density (ie, the fraction of cell volume occupied by mitochondria), and a trend to increased mitochondrial numeric density (ie, the mean number of mitochondrial profiles per cell volume unit) was observed as well (Figure 3A and 3B).

In line with this observation, genes participating in mitochondrial biogenesis, including sirtuin 1, peroxisome proliferator-activated receptor γ coactivator 1α, peroxisome proliferation-activated receptor α, nuclear respiratory factor 2α, and nuclear respiratory factor 2β, were significantly increased in monocytes from Q_{red}-treated patients with APS (Figure 3C).

Q_{red} Supplementation Improved the Prothrombotic and Inflammatory Status of Patients With APS

Q_{red} supplementation significantly improved the atherosclerosis-related gene profile in monocytes from patients with APS, which was profoundly altered in these autoimmune subjects in relation to healthy donors. Thus, 29 atherosclerosis-related genes were found altered in patients with APS, and 23 of them were significantly reversed by effect of Q_{red}, including numerous chemokines, interleukins, adhesion molecules, lipoproteins, metalloproteinases, and growth factors (Figure 4A and 4B).

Q_{red} treatment also decreased interleukin 6 and interleukin 8 protein expression levels in monocytes (Figure 4D and 4E) along with TF expression (Figure 4C) and activity (Figure IVD in the online-only Data Supplement). Several other proinflammatory mediators (previously reported to be significantly increased in monocytes from patients with APS)[12] were found further reduced in these cells by effect of Q_{red} treatment, including vascular endothelial growth factor, macrophage inflammatory protein 1α, interleukin-1β, interleukin 6, and tumor necrosis factor α (Figure 4F). No differences among primary patients with APS and patients with APS+systemic lupus erythematosus were noticed.

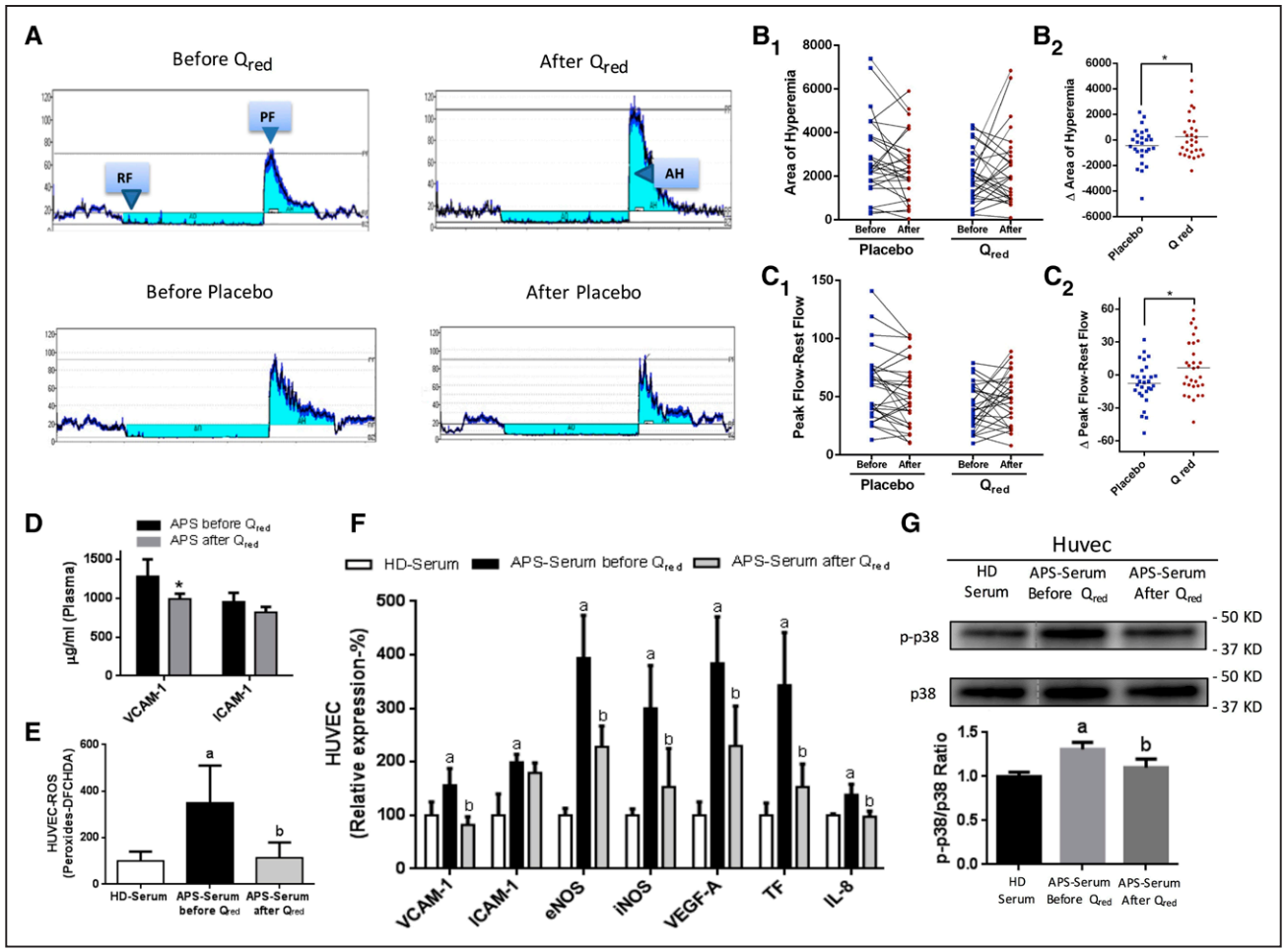

Figure 1. Effects of reduced coenzyme Q_{10} (Q_{red}) supplementation on the endothelial activity of patients with antiphospholipid syndrome (APS). **A**, Microvascular function, measured by Laser Doppler linear Periflux 5010 and performed before and after Q_{red} or placebo treatment. Scatter plots showing individual values are displayed, in parallel with beeswarm plot showing individual changes in all the patients with APS included in the study in the area of hyperemia (**B_1** and **B_2**) and peak flow minus rest flow (**C_1** and **C_2**) after release of blood flow occlusion. **D**, Vascular cell adhesion molecule 1 (VCAM-1) and intercellular cell adhesion molecule 1 (ICAM-1) levels measured in plasma from patients with APS before and after Q_{red} treatment by FlowCytomix immunoassay. Data are presented as mean±SD. *Significant differences (at $P<0.05$) vs patients with APS before Q_{red} treatment. **E**, Human umbilical vein endothelial cells (HUVECs) were treated with serum obtained from healthy donors (HD) and patients with APS before and after Q_{red} treatment. Peroxide production determined by addition of the fluorescent probe DCFHDA (dichloro-dihydro-fluorescein diacetate) and flow cytometry analysis. **F**, Quantitative reverse transcription polymerase chain reaction was performed on a panel of genes, including adhesion molecules, inflammatory and thrombotic mediators, and nitric oxide–producing enzymes. Bar graphs show the mean±SEM of 5 independent experiments. **G**, Modulation of p38 MAPK (p38 mitogen-activated protein kinase) activity in HUVEC treated with serum obtained from HD and patients with APS before and after Q_{red} treatment. Results of a representative Western blot are shown. Graph represents results from 5 separate experiments. Lanes were run on the same gel under the same experimental conditions but were noncontiguous. Cropping lines are used in the figure. (a) Indicates significant differences vs HUVEC+HD serum ($P<0.05$); (b) indicates significant differences vs HUVEC+APS serum before Q_{red} treatment. IL indicates interleukin; iNOS, inducible nitric oxide synthase; ROS, reactive oxygen species; TF, tissue factor; and VEGF, vascular endothelial growth factor.

A gene network showing the inter-relationship between all the Q_{red}-modulated genes in patients with APS (performed by using the Ingenuity Pathways Analysis software) is displayed in Figure 4G.

Q_{red} Prevents the Activation of Several Intracellular Kinases Involved in Thrombosis and Inflammation on Monocytes From Patients With APS

Among the 18 intracellular kinases evaluated in the protein array, Q_{red} supplementation markedly reduced the phosphorylation of 7 protein kinases in APS monocytes, including Akt-Ser, Akt-Thr, AMPKa (AMP-activated protein kinase), ERK1/2 (extracellular signal-regulated kinases), p38 MAPK, S6 ribosomal, and STAT3, all of them previously reported to be directly involved in the immune-mediated chronic inflammation and thrombotic status present in these autoimmune patients (Figure VIA–VIC in the online-only Data Supplement).

Q_{red} Supplementation Prevents NETosis in Patients With APS

Given the relevant contribution of neutrophils to the maintenance of the oxidative status in patients with APS and the proven presence of aPL-induced NETosis in those patients,[12–15] we further evaluated the regulation of their activity by effect of Q_{red} through the analysis of NETosis. Q_{red} supplementation promoted a significant reduction in the percentage of extruded neutrophil extracellular traps (NETs), evaluated by fluorescence microscopy

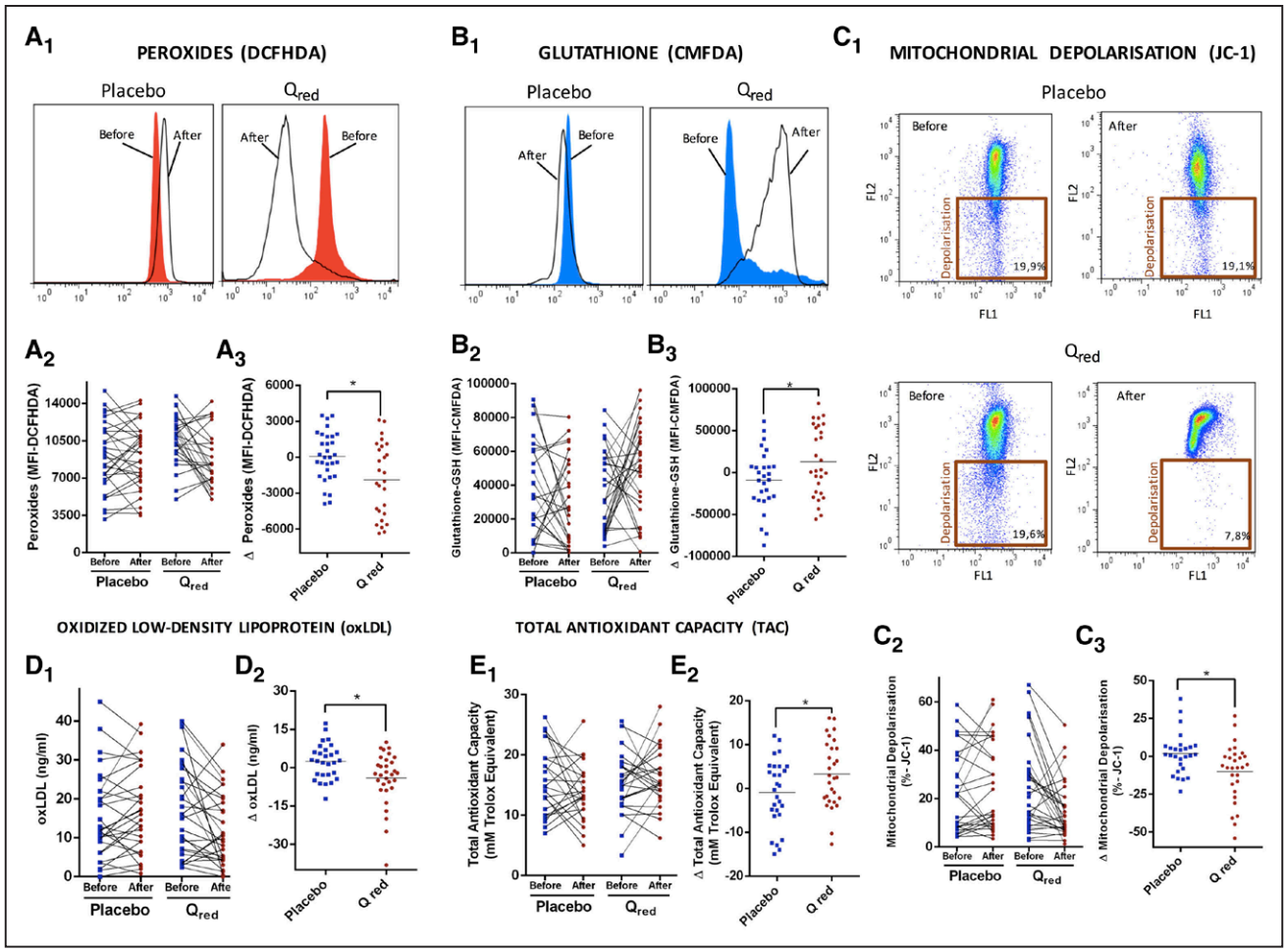

Figure 2. Effects of reduced coenzyme Q_{10} (Q_{red}) supplementation on plasma and cellular oxidative stress in patients with antiphospholipid syndrome (APS). **A**, Peroxide production in monocytes of patients with APS before and after Q_{red}/placebo treatments, determined by addition of the fluorescent probe DCFHDA (dichloro-dihydro-fluorescein diacetate) to the isolated cells and flow cytometry analysis. Histogram plot (**A_1**) and scatter plot (**A_2**) showing individual values (mean fluorescence intensity) are displayed, in parallel with beeswarm plot (**A_3**) showing individual changes of all the patients included in the study. **B**, Intracellular glutathione levels of monocytes of patients with APS before and after Q_{red}/placebo treatments, determined by addition of the fluorescent probe CMF-DA (5-chloromethylfluorescein diacetate) and measurement as described for (**A**). Histogram plot (**B_1**) and scatter plot (**B_2**) showing individual values (MFI) are displayed, in parallel with beeswarm plot (**B_3**) showing individual changes of all the patients included in the study. **C**, Proportion of circulating monocytes with depolarized mitochondria, determined with the JC-1 MitoScreen assay. Representative dot plots (**C_1**) of isolated cells from patients before and after Q_{red}/placebo treatments are shown together with the scatter plot (**C_2**) and beeswarm plot (**C_3**) showing the changes of all the patients included in the study. **D**, Oxidized low-density lipopreotein (oxLDL) in plasma. Scatter plot (**D_1**) and beeswarm plot (**D_2**) showing individual values and the changes of oxLDL levels in plasma from patients with APS after treatment with Q_{red}/placebo. **E**, Total antioxidant capacity in plasma. Scatter plot (**E_1**) and beeswarm plot (**E_2**) showing individual values and the changes of total antioxidant capacity in plasma from patients with APS after treatment with Q_{red}/placebo. *Significant differences (at $P<0.05$) vs placebo.

(Figure VIIA and VIIB in the online-only Data Supplement), along with the downregulation of intracellular neutrophil elastase and myeloperoxidase expression levels (Figure VIIC and VIID in the online-only Data Supplement). No changes in the number of neutrophils after either placebo or Q_{red} treatment were noticed (Table IV in the online-only Data Supplement).

Effects of Q_{red} Are Modulated by Underlying Epigenetic Mechanisms, Such as Regulation of MicroRNAs Associated With Atherothrombosis Development

Differential expression of microRNAs in monocytes of patients with APS before and after Q_{red} treatment was determined using Nanostring microRNA arrays and subsequent validation by real-time reverse transcription polymerase chain reaction. Comparing to controls, 21 microRNAs were found significantly altered in patients with APS, 18 of them downregulated and 3 of them upregulated (Figure VIIIA in the online-only Data Supplement). Functional classification of those microRNAs, using the Ingenuity Pathways Analysis software, showed a preponderance of target mRNAs involved in reproductive system disease, inflammatory response, and connective tissue disorders. Among them, 16 were significantly reversed by effect of Q_{red} treatment (Figure VIIIA and VIIIB in the online-only Data Supplement). Functional classification of the most significantly altered microRNAs after Q_{red} supplementation revealed a prevalence of target mRNAs involved in CVD and thrombosis, including those related to inflammation, lipid metabolism, mitochondrial dysfunction, and oxidative stress (Figure IX in the online-only Data Supplement).

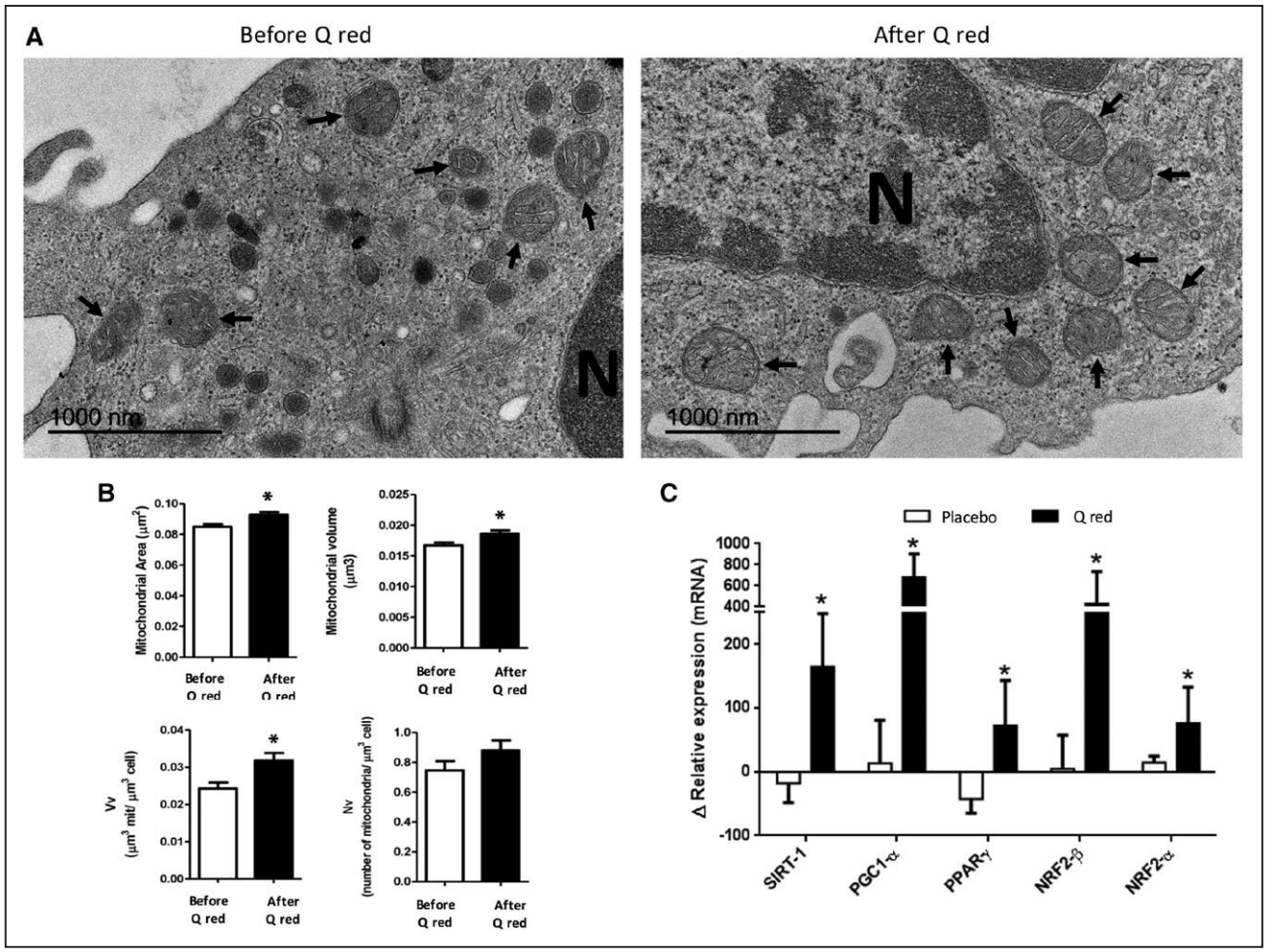

Figure 3. Mitochondrial ultrastructure and biogenesis changes promoted by effect of reduced coenzyme Q_{10} (Qr_{ed}) treatment in monocytes from patients with antiphospholipid syndrome (APS). Monocytes purified from patients with APS before and after Q_{red} treatment were fixed with aldehydes, post-fixed with osmium tetroxide, dehydrated, and embedded in epoxy resin. Thin sections were cut and stained with uranyl acetate and lead citrate for observation with an electron microscope. **A**, Representative micrographs of cells treated as described. Arrows indicate individual mitochondria and N indicate nucleus of monocyte. **B**, Planimetric and stereological analysis showing mitochondrial area, mitochondrial volume and circularity, volume density (Vv), and numeric density (nv) of mitochondria after Q_{red} treatment on 10 patients. *Significant differences (at $P<0.05$) vs patients before treatment. **C**, Waterfall plot showing the changes in mRNA levels of a set of genes involved in mitochondrial biogenesis. *Significant differences (at $P<0.05$) vs placebo. NRF indicates nuclear respiratory factor; PGC-1α, PPAR coactivator 1α; PPARα, peroxisome proliferation-activated receptor α; and SIRT1, sirtuin 1.

Moreover, interaction networks of those microRNAs and the above identified genes related to atherosclerosis and thrombosis were also identified so that the integrated analysis of validated microRNAs and the altered genes identified in the polymerase chain reaction array of atherosclerosis, using the software Ingenuity Pathways Analysis, demonstrated the presence of a complex network on which several Q_{red}-upregulated microRNAs seemed to control simultaneously the expression of various Q_{red}-downregulated genes. Complete interaction networks are shown in Figure VIIIC in the online-only Data Supplement.

Correlation Studies Showed Co-Ordinated Q_{red} Effects on Prothrombotic Mediators and Epigenetic Modulators

Correlation studies demonstrated that the reduced expression of several prothrombotic and inflammatory mediators in monocytes was inversely related to the increase in plasma CoQ_{10} levels found after Q_{red} treatment (Figure 5A–5F). Moreover, the rises in plasma CoQ_{10} levels were also linked to the changes promoted on genes related to mitochondrial biogenesis (Figure 5G–5J). Of note, changes operated on genes linked to mitochondrial biogenesis were found further associated with changes promoted on the levels of microRNAs, inflammatory mediators, and oxidative stress markers (Table I in the online-only Data Supplement).

Discussion

The overall results of this clinical trial demonstrated that Q_{red} supplementation significantly reduced the expression of several prothrombotic parameters in patients with APS. In previous studies,[12–14] we demonstrated that several mediators of autoimmunity, thrombosis, inflammation, and endothelial dysfunction orchestrate the pathophysiology of atherothrombosis in APS and systemic lupus erythematosus. Moreover, a redox-sensitive pathway, in which mitochondrial alterations perform a relevant

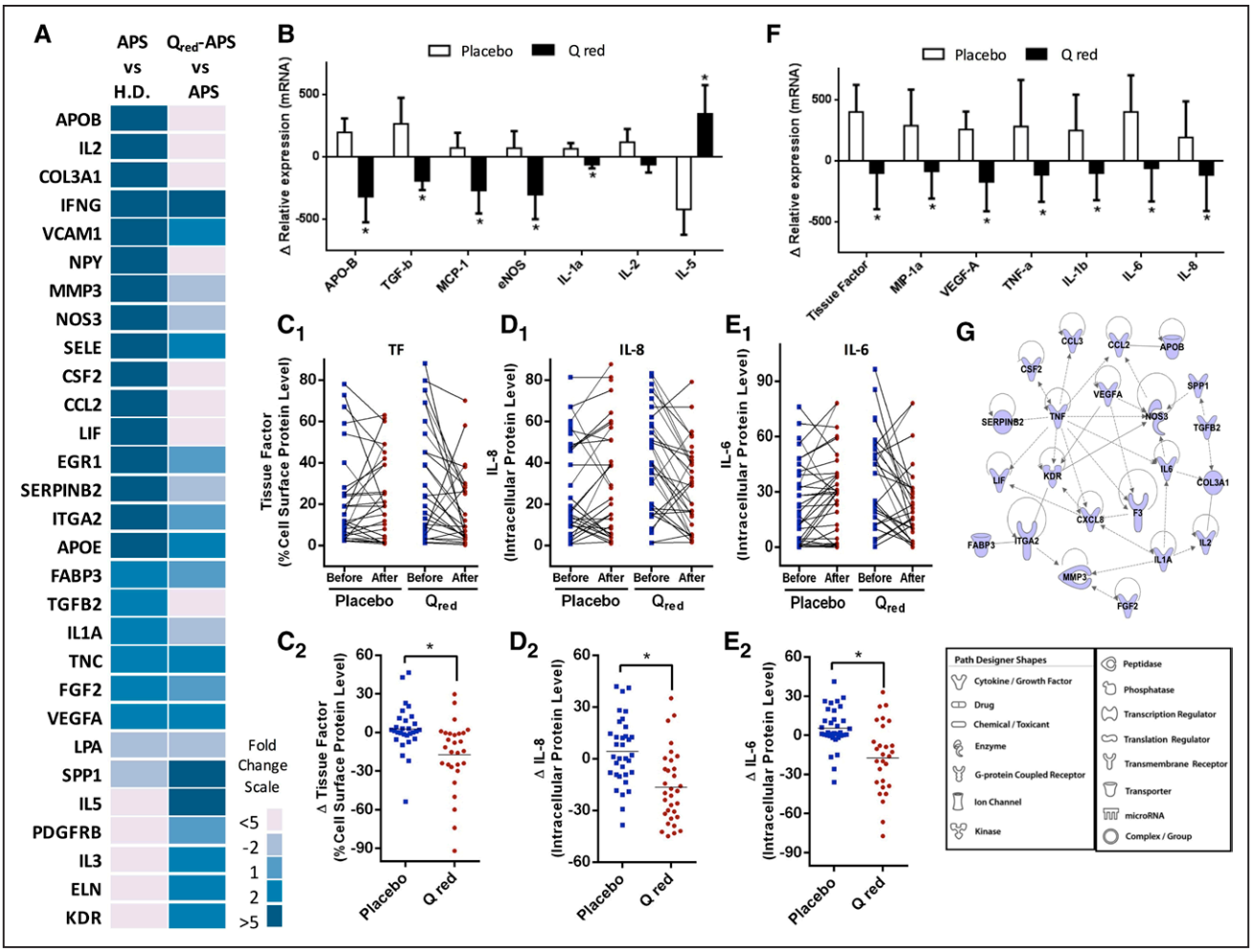

Figure 4. Changes in the prothrombotic and inflammatory status of patients with antiphospholipid syndrome (APS) promoted by reduced coenzyme Q_{10} (Q_{red}) treatment. **A**, Heat map depicting the expression data for the genes identified by the human atherosclerosis profile array as differentially expressed in monocytes between healthy donors (H.D.) and patients with APS and between patients with APS before and after Q_{red} treatment. **B**, Waterfall plot showing the changes ±SD in mRNA levels of a set of genes identified in the array and validated in the whole cohort of patients with APS included in the study. Scatter plot (**C_1**, **D_1**, and **E_1**) showing individual values are displayed, in parallel with beeswarm plot (**C_2**, **D_2**, and **E_2**) showing individual changes of all the patients included in the study in protein levels of monocyte tissue factor (TF), interleukin (IL)-8, and interleukin 6. **F**, Waterfall plots showing the changes ±SD in mRNA levels of a set of genes related to inflammation and thrombosis in monocytes from patients with APS after treatment with Q_{red}/placebo. **G**, Gene networks showing inter-relationship between differentially expressed genes after Q_{red} treatment, using Ingenuity Pathways Analysis software. Direct interactions appear in the network diagram as a solid line, whereas indirect interactions appear as a dashed line. *Significant differences (at $P<0.05$) vs placebo.

role, seems to elicit those pathological processes. Thus, specific treatments that prevent that co-ordinated deregulation, without promoting negative side effects, would be desirable.

The clinical benefits of CoQ_{10} supplementation in prevention and treatment of CVDs have been observed in numerous clinical trials.[8–10] More recently, positive effects of CoQ_{10} on biomarkers of oxidative stress and cardiac function in hemodialysis patients have been further demonstrated.[16] Besides, a recent trial showed that daily intake of 100 mg CoQ_{10} supplements among patients with metabolic syndrome for 8 weeks had beneficial effects on serum insulin levels, HOMA-IR (homeostasis model assessment of insulin resistance), HOMA-B (homeostasis model assessment of insulin resistance-beta), and plasma total plasma antioxidant capacity concentrations.[17] Thus, there is promising evidence of a beneficial effect of CoQ_{10} when given alone or in addition to standard therapies on various inflammatory and prothrombotic diseases, including CVD, renal diseases, and metabolic syndrome, but less extensive evidence exists in autoimmune-associated CVD.

In our hands, first, a direct anticoagulant effect of ubiquinol was established, as demonstrated by the significant inhibition, promoted on APS monocytes, of the expression and activity of TF, the main inductor of the coagulation in vivo previously shown to be directly involved in the pathogenesis of thrombotic complications in primary patients with APS.[18] In addition, parallel changes in the expression of several cytokines, chemokines, cell surface receptors, endothelial cell regulators, and markers related to oxidative stress in those patients after Q_{red} treatment were found intimately connected.

CVD prevention by Q_{red} is mainly dependent on their beneficial effects on vascular redox signaling.[19] Accordingly, various landmarks linked to oxidative stress in patients with

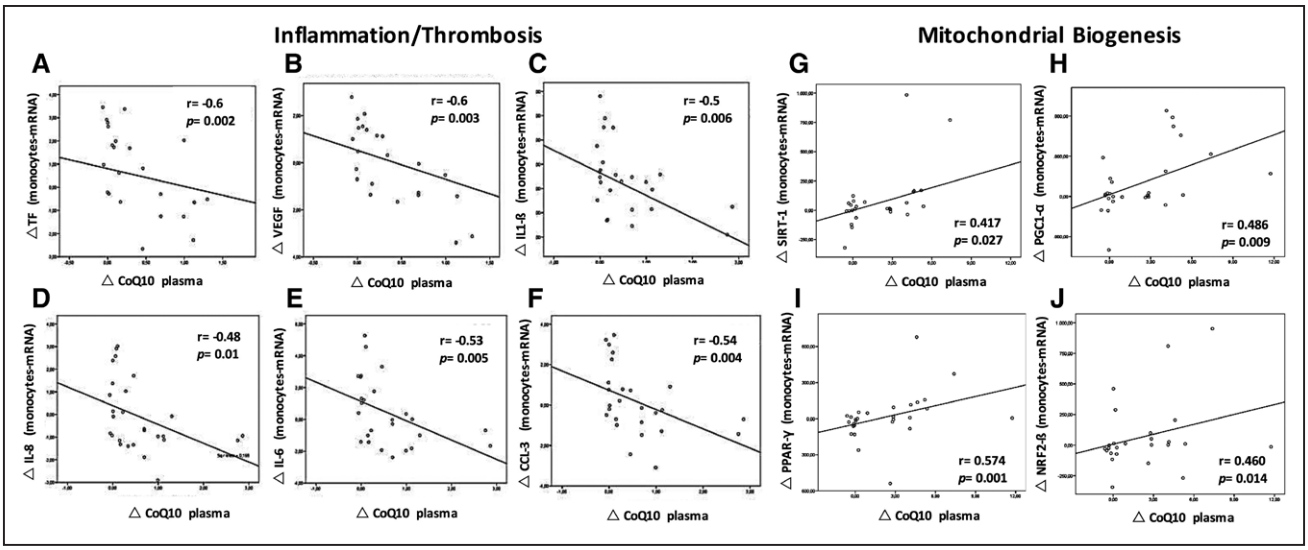

Figure 5. Correlation studies showed co-ordinated reduced coenzyme Q_{10} (Q_{red}) effects on atherothrombotic mediators and epigenetic modulators. **A–F**, Negative correlation between increased plasma coenzyme Q_{10} (CoQ_{10}) levels and reduced mRNA expression in monocytes of tissue factor (TF), vascular endothelial growth factor (VEGF), interleukin 1β (IL-1β), interleukin 8 (IL-8), interleukin 6 (IL-6), and chemokine (C-C motif) ligand 3 (CCL3), or macrophage inflammatory protein 1-α (MIP1-α). **G–J**, Positive correlation between increased plasma CoQ10 levels and increased mRNA expression levels in monocytes of the biogenesis-related genes sirtuin 1 (SIRT1), PPAR coactivator 1α (PGC-1α), peroxisome proliferation-activated receptor γ (PPARγ), and nuclear respiratory factor-β (NRFβ).

APS exhibited altered expression and activity in response to in vivo Q_{red} treatment. Mitochondrial studies further demonstrated a higher abundance of low-potential mitochondria in monocytes, with a lower reactive oxygen species production. We also showed that Q_{red} treatment on patients with APS upregulated genes related to mitochondrial biogenesis, a highly regulated process operating through peroxisome proliferator-activated receptor γ coactivator 1α–dependent nuclear respiratory factors.[20,21] The improvement on inflammatory and oxidative stress profiles, together with an increase of mitochondrial biogenesis, suggests that alterations in APS monocytes phenotype are highly likely a direct effect of Q_{red} although complementary effects of global improvements in serology activity cannot be ruled out. Our results are congruent with the beneficial effects observed in patients with type 2 diabetes mellitus treated with thiazolidinediones or resveratrol and in patients with systemic lupus erythematosus treated with fluvastatin, which stimulate mitochondrial biogenesis and reduce mitochondrial dysfunction.[14,22,23] Thus, the stimulation of mitochondrial biogenesis through pharmacological interventions seems as promising in attenuating the clinical prothrombotic status associated with APS.

The effects of Q_{red} on endothelial dysfunction have not been previously explored. Thus, our study shows for the first time a significant improvement of the microvascular function in patients with APS, with a significant increase in the peak flow and the area of reactive hyperemia after temporary occlusion of blood flow. Accordingly, levels of a cell adhesion molecule, such as vascular cell adhesion molecule 1, were found diminished in plasma of patients with APS after treatment with Q_{red}. Elevated levels of this molecule have been shown to be associated with an increased thrombotic risk in APS by both in vitro and in vivo studies, which jointly demonstrated that aPLs promoted key intracellular events that contributed to the thrombotic complications of APS by controlling the endothelial adhesive phenotype.[24–26]

Thus, these results evidence the positive effects of Q_{red} on endothelial dysfunction, which might be considered in the prevention of cardiovascular events. Our results were further supported by in vitro studies, on which treatment of endothelial cells with serum from patients with APS treated with Q_{red} downregulated the expression of peroxides, adhesion molecules, and inflammatory mediators. A significant antithrombotic effect was further demonstrated in endothelial cells through the inhibition of TF expression and activity. Furthermore, Q_{red} reestablished normal levels of both, NO and enzymes related to the NO activity induced by APS patients' serum, driving an improvement on endothelial function. All in all, our study supports the efficacy of Q_{red} in restoring endothelial function and inhibiting the expression of markers related to inflammation, thrombosis, and cell adhesion in patients with APS.

The anti-inflammatory effects of Q_{red} can be at least partially explained by its impact on proinflammatory signal transduction pathways. Thus, after treatment, various intracellular mediators intimately related to inflammation, thrombosis, and mitochondrial dysfunction displayed reduced activity. We and others previously showed that aPLs from patients with APS induce monocyte expression of TF through the simultaneous activation of nuclear factor-κB/Rel proteins via the p38 mitogen-activated protein kinase pathway, and of the MEK-1 (mitogen-activated protein kinase kinase 1)/ERK pathway, thus supporting a prothrombotic role for those transduction pathways.[27] Moreover, p38 MAPK and MEK-1/ERK signaling pathways, along with intracellular mediators such as AKT or STAT3, have been shown to regulate the expression of almost all the inflammatory molecules found deregulated in patients with APS, and even being activated by aPL-induced reactive oxygen species production.[12,28,29] Therefore, their modulation by effect of Q_{red} treatment underlies their role as

molecular pathways involved in the control of several prothrombotic mediators in APS.

Previous studies reported that neutrophils from patients with systemic lupus erythematosus are activated, producing increased levels of reactive oxygen species and leading to aberrant NET formation, which induced endothelial dysfunction and vascular damage, thus driving the development of premature atherosclerosis and CVD in these patients.[30,31] More recently, it has been demonstrated that the release of NETs by neutrophils, induced by aPLs, constitutes a new mechanism of thrombosis in APS.[15] Hence, we evaluated the effects of Q_{red} supplementation on NETs formation as a putative additional pathway for thrombosis prevention. Our study demonstrated, along with the inhibition in the production of peroxides, a direct effect of Q_{red} in inhibiting NETosis, thus suggesting a direct link among both processes. Because reactive oxidants and NET constituents have been shown to co-ordinately injure host tissue,[32] it is important that these pathways be tightly regulated. Thus, our data point at Q_{red} as a beneficial antioxidant compound that further prevents toxic side effects of NETs in inflammation and thrombosis in patients with APS.

MicroRNAs are small noncoding RNAs that play critical roles in the regulation of host genome expression at the post-transcriptional levels, having a relevant role in autoimmunity and CVD. In patients with APS, we previously identified 2 microRNAs as potential modulators of TF expression (ie, microRNA-19b and microRNA-20a).[33] More recently, several microRNAs, deregulated in monocytes from patients with APS , were demonstrated to modulate the prothrombotic status of those autoimmune patients.[34] With these premises, we investigated the modifications promoted by Q_{red} treatment in the profile of monocyte microRNAs and their relationship with the changes operated on the inflammatory and prothrombotic profile of patients with APS. The data obtained in the present study demonstrated, for the first time, the altered expression of several microRNAs by effect of Q_{red}. The functional classification of those microRNAs allowed us to demonstrate that the majority of them have as potential target molecules/proteins/transcription factors involved in inflammation and thrombotic processes. Therefore, the increased levels of those microRNAs after Q_{red} supplementation might be associated with a reduction in the prothrombotic profile of patients with APS. Accordingly, the integrated analysis of validated microRNAs and the altered genes identified in the polymerase chain reaction array of atherosclerosis demonstrated the presence of a complex network on which several Q_{red}-upregulated microRNAs seem to control simultaneously the expression of various Q_{red}-downregulated genes. Thus, we have identified novel and specific microRNA–mRNA regulatory networks, related to CVD in patients with APS and modified by effect of Q_{red}.

The present study has the advantage of a randomized crossover design, in which all the participants have experienced Q_{red} and placebo treatments, each individual acting as his/her own control and strengthening the fact that the effects observed are because of the influence of Q_{red}. In summary, our results support the short-term impact of Q_{red} supplementation in the reduced expression of several markers related to inflammation and thrombosis in patients with APS. Because of the notable absence of clinically significant side effects and its potential therapeutic benefits, we propose that Q_{red} might act as a safe adjunct to standard therapies in patients with APS.

Acknowledgments

We thank all patients for their participation in this clinical trial.

Sources of Funding

This study was supported by Kaneka Corporation and by grants from the Junta de Andalucia (CTS-7940), the Instituto de Salud Carlos III (refs. PI12/01511 and PI15/01333), cofinanciado por el Fondo Europeo de Desarrollo Regional de la Unión Europea Una manera de hacer Europa, Spain, and the Spanish Inflammatory and Rheumatic diseases Network (RIER), Instituto de Salud Carlos III (RD16/0012/0015). C. López-Pedrera was supported by a contract from the Spanish Junta de Andalucía.

Disclosures

None.

References

1. Giannakopoulos B, Krilis SA. The pathogenesis of the antiphospholipid syndrome. *N Engl J Med*. 2013;368:1033–1044. doi: 10.1056/NEJMra1112830.
2. Charakida M, Besler C, Batuca JR, Sangle S, Marques S, Sousa M, Wang G, Tousoulis D, Delgado Alves J, Loukogeorgakis SP, Mackworth-Young C, D'Cruz D, Luscher T, Landmesser U, Deanfield JE. Vascular abnormalities, paraoxonase activity, and dysfunctional HDL in primary antiphospholipid syndrome. *JAMA*. 2009;302:1210–1217. doi: 10.1001/jama.2009.1346.
3. Cugno M, Borghi MO, Lonati LM, Ghiadoni L, Gerosa M, Grossi C, De Angelis V, Magnaghi G, Tincani A, Mari D, Riboldi P, Meroni PL. Patients with antiphospholipid syndrome display endothelial perturbation. *J Autoimmun*. 2010;34:105–110. doi: 10.1016/j.jaut.2009.07.004.
4. Ames PR, Matsuura E, Batuca JR, Ciampa A, Lopez LL, Ferrara F, Iannaccone L, Alves JD. High-density lipoprotein inversely relates to its specific autoantibody favoring oxidation in thrombotic primary antiphospholipid syndrome. *Lupus*. 2010;19:711–716. doi: 10.1177/0961203309357765.
5. Lopez-Pedrera Ch, Aguirre MA, Ruiz-Limon P, Pérez-Sánchez C, Jimenez-Gomez Y, Barbarroja N, Cuadrado MJ. Immunotherapy in antiphospholipid syndrome. *Int Immunopharmacol*. 2015;27:200–208. doi: 10.1016/j.intimp.2015.06.006.
6. Ruiz-Irastorza G, Crowther M, Branch W, Khamashta MA. Antiphospholipid syndrome. *Lancet*. 2010;376:1498–1509. doi: 10.1016/S0140-6736(10)60709-X.
7. López-Pedrera C, Ruiz-Limón P, Aguirre MÁ, Barbarroja N, Pérez-Sánchez C, Buendía P, Rodriguez-García IC, Rodriguez-Ariza A, Collantes-Estevez E, Velasco F, Khasmahta M, Cuadrado MJ. Global effects of fluvastatin on the prothrombotic status of patients with antiphospholipid syndrome. *Ann Rheum Dis*. 2011;70:675–682. doi: 10.1136/ard.2010.135525.
8. Villalba JM, Parrado C, Santos-Gonzalez M, Alcain FJ. Therapeutic use of coenzyme Q10 and coenzyme Q10-related compounds and formulations. *Expert Opin Investig Drugs*. 2010;19:535–554. doi: 10.1517/13543781003727495.
9. Turunen M, Olsson J, Dallner G. Metabolism and function of coenzyme Q. *Biochim Biophys Acta*. 2004;1660:171–199.
10. Littarru GP, Tiano L. Clinical aspects of coenzyme Q10: an update. *Nutrition*. 2010;26:250–254. doi: 10.1016/j.nut.2009.08.008.
11. Garrido-Maraver J, Cordero MD, Oropesa-Avila M, Vega AF, de la Mata M, Pavon AD, Alcocer-Gomez E, Calero CP, Paz MV, Alanis M, de Lavera I, Cotan D, Sanchez-Alcazar JA. Clinical applications of coenzyme q10. *Front Biosci (Landmark Ed)*. 2014;19:619–633.
12. Perez-Sanchez C, Ruiz-Limon P, Aguirre MA, Bertolaccini ML, Khamashta MA, Rodriguez-Ariza A, Segui P, Collantes-Estevez E, Barbarroja N, Khraiwesh H, Gonzalez-Reyes JA, Villalba JM, Velasco F, Cuadrado MJ, Lopez-Pedrera C. Mitochondrial dysfunction in antiphospholipid syndrome: implications in the pathogenesis of the disease and effects of coenzyme Q(10) treatment. *Blood*. 2012;119:5859–5870. doi: 10.1182/blood-2011-12-400986.

13. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, Derksen RH, DE Groot PG, Koike T, Meroni PL, Reber G, Shoenfeld Y, Tincani A, Vlachoyiannopoulos PG, Krilis SA. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost*. 2006;4:295–306. doi: 10.1111/j.1538-7836.2006.01753.x.
14. Ruiz-Limon P, Barbarroja N, Perez-Sanchez C, Aguirre MA, Bertolaccini ML, Khamashta MA, Rodriguez-Ariza A, Almadén Y, Segui P, Khraiwesh H, Gonzalez-Reyes JA, Villalba JM, Collantes-Estevez E, Cuadrado MJ, Lopez-Pedrera C. Atherosclerosis and cardiovascular disease in systemic lupus erythematosus: effects of *in vivo* statin treatment. *Ann Rheum Dis*. 2015;74:1450–1458. doi: 10.1136/annrheumdis-2013-204351.
15. Yalavarthi S, Gould TJ, Rao AN, Mazza LF, Morris AE, Núñez-Álvarez C, Hernández-Ramírez D, Bockenstedt PL, Liaw PC, Cabral AR, Knight JS. Release of neutrophil extracellular traps by neutrophils stimulated with antiphospholipid antibodies: a newly identified mechanism of thrombosis in the antiphospholipid syndrome. *Arthritis Rheumatol*. 2015;67:2990–3003. doi: 10.1002/art.39247.
16. Rivara MB, Yeung CK, Robinson-Cohen C, Phillips BR, Ruzinski J, Rock D, Linke L, Shen DD, Ikizler TA, Himmelfarb J. Effect of coenzyme Q10 on biomarkers of oxidative stress and cardiac function in hemodialysis patients: the CoQ10 biomarker trial. *Am J Kidney Dis*. 2017;69:389–399. doi: 10.1053/j.ajkd.2016.08.041.
17. Raygan F, Rezavandi Z, Dadkhah Tehrani S, Farrokhian A, Asemi Z. The effects of coenzyme Q10 administration on glucose homeostasis parameters, lipid profiles, biomarkers of inflammation and oxidative stress in patients with metabolic syndrome. *Eur J Nutr*. 2016;55:2357–2364. doi: 10.1007/s00394-015-1042-7.
18. Cuadrado MJ, López-Pedrera C, Khamashta MA, Camps MT, Tinahones F, Torres A, Hughes GR, Velasco F. Thrombosis in primary antiphospholipid syndrome: a pivotal role for monocyte tissue factor expression. *Arthritis Rheum*. 1997;40:834–841. doi: 10.1002/1529-0131(199705)40:5<834::AID-ART8>3.0.CO;2-#.
19. Victor VM, Rocha M, Solá E, Bañuls C, Garcia-Malpartida K, Hernández-Mijares A. Oxidative stress, endothelial dysfunction and atherosclerosis. *Curr Pharm Des*. 2009;15:2988–3002.
20. Scarpulla RC. Nuclear activators and coactivators in mammalian mitochondrial biogenesis. *Biochim Biophys Acta*. 2002;1576:1–14.
21. Aquilano K, Vigilanza P, Baldelli S, Pagliei B, Rotilio G, Ciriolo MR. Peroxisome proliferator-activated receptor gamma co-activator 1alpha (PGC-1alpha) and sirtuin 1 (SIRT1) reside in mitochondria: possible direct function in mitochondrial biogenesis. *J Biol Chem*. 2010;285:21590–21599. doi: 10.1074/jbc.M109.070169.
22. Mensink M, Hesselink MK, Russell AP, Schaart G, Sels JP, Schrauwen P. Improved skeletal muscle oxidative enzyme activity and restoration of PGC-1 alpha and PPAR beta/delta gene expression upon rosiglitazone treatment in obese patients with type 2 diabetes mellitus. *Int J Obes (Lond)*. 2007;31:1302–1310. doi: 10.1038/sj.ijo.0803567.
23. Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, Auwerx J. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell*. 2006;127:1109–1122. doi: 10.1016/j.cell.2006.11.013.
24. Simoncini S, Sapet C, Camoin-Jau L, Bardin N, Harlé JR, Sampol J, Dignat-George F, Anfosso F. Role of reactive oxygen species and p38 MAPK in the induction of the pro-adhesive endothelial state mediated by IgG from patients with anti-phospholipid syndrome. *Int Immunol*. 2005;17:489–500. doi: 10.1093/intimm/dxh229.
25. Vega-Ostertag ME, Ferrara DE, Romay-Penabad Z, Liu X, Taylor WR, Colden-Stanfield M, Pierangeli SS. Role of p38 mitogen-activated protein kinase in antiphospholipid antibody-mediated thrombosis and endothelial cell activation. *J Thromb Haemost*. 2007;5:1828–1834. doi: 10.1111/j.1538-7836.2007.02680.x.
26. Ioannou Y, Romay-Penabad Z, Pericleous C, Giles I, Papalardo E, Vargas G, Shilagard T, Latchman DS, Isenberg DA, Rahman A, Pierangeli S. *In vivo* inhibition of antiphospholipid antibody-induced pathogenicity utilizing the antigenic target peptide domain I of beta2-glycoprotein I: proof of concept. *J Thromb Haemost*. 2009;7:833–842. doi: 10.1111/j.1538-7836.2009.03316.x.
27. López-Pedrera C, Buendía P, Cuadrado MJ, Siendones E, Aguirre MA, Barbarroja N, Montiel-Duarte C, Torres A, Khamashta M, Velasco F. Antiphospholipid antibodies from patients with the antiphospholipid syndrome induce monocyte tissue factor expression through the simultaneous activation of NF-kappaB/Rel proteins via the p38 mitogen-activated protein kinase pathway, and of the MEK-1/ERK pathway. *Arthritis Rheum*. 2006;54:301–311. doi: 10.1002/art.21549.
28. Dutzmann J, Daniel JM, Bauersachs J, Hilfiker-Kleiner D, Sedding DG. Emerging translational approaches to target STAT3 signalling and its impact on vascular disease. *Cardiovasc Res*. 2015;106:365–374. doi: 10.1093/cvr/cvv103.
29. Brown DI, Griendling KK. Regulation of signal transduction by reactive oxygen species in the cardiovascular system. *Circ Res*. 2015;116:531–549. doi: 10.1161/CIRCRESAHA.116.303584.
30. Carmona-Rivera C, Zhao W, Yalavarthi S, Kaplan MJ. Neutrophil extracellular traps induce endothelial dysfunction in systemic lupus erythematosus through the activation of matrix metalloproteinase-2. *Ann Rheum Dis*. 2015;74:1417–1424. doi: 10.1136/annrheumdis-2013-204837.
31. Smith CK, Vivekanandan-Giri A, Tang C, et al. Neutrophil extracellular trap-derived enzymes oxidize high-density lipoprotein: an additional proatherogenic mechanism in systemic lupus erythematosus. *Arthritis Rheumatol*. 2014;66:2532–2544. doi: 10.1002/art.38703.
32. Almyroudis NG, Grimm MJ, Davidson BA, Röhm M, Urban CF, Segal BH. NETosis and NADPH oxidase: at the intersection of host defense, inflammation, and injury. *Front Immunol*. 2013;4:45. doi: 10.3389/fimmu.2013.00045.
33. Teruel R, Pérez-Sánchez C, Corral J, Herranz MT, Pérez-Andreu V, Saiz E, García-Barberá N, Martínez-Martínez I, Roldán V, Vicente V, López-Pedrera C, Martínez C. Identification of miRNAs as potential modulators of tissue factor expression in patients with systemic lupus erythematosus and antiphospholipid syndrome. *J Thromb Haemost*. 2011;9:1985–1992. doi: 10.1111/j.1538-7836.2011.04451.x.
34. Pérez-Sánchez C, Aguirre MA, Ruiz-Limón P, Barbarroja N, Jiménez-Gómez Y, de la Rosa IA, Rodriguez-Ariza A, Collantes-Estévez E, Segui P, Velasco F, Cuadrado MJ, Teruel R, González-Conejero R, Martínez C, López-Pedrera Ch. Atherothrombosis-associated microRNAs in antiphospholipid syndrome and systemic lupus erythematosus patients. *Sci Rep*. 2016;6:31375. doi: 10.1038/srep31375.

Highlights

- This trial analyzed short-term effects of reduced coenzyme Q10 (Q_{red}) on thrombosis-related markers in antiphospholipid syndrome.
- Qred improved endothelial function and decreased prothrombotic mediators.
- Q_{red} decreased peroxides and damaged mitochondria in monocytes.
- Q_{red} modulated atherosclerosis-related genes and microRNAs.
- Thus, Q_{red} might act as a safe adjunct to standard therapies in patients with antiphospholipid syndrome.