Short-term ubiquinol supplementation reduces oxidative stress associated with strenuous exercise in healthy adults: A randomized trial

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Abstract

Studies about Coenzyme Q\(_{10}\) (CoQ\(_{10}\)) supplementation on strenuous exercise are scarce, especially those related with oxidative stress associated with physical activity and virtually nonexistent with the reduced form, Ubiquinol. The objective of this study was to determine, for the first time, whether a short-term supplementation with Ubiquinol can prevent oxidative stress associated to strenuous exercise. The participants (n = 100 healthy and well trained, but not on an elite level) were classified in two groups: Ubiquinol (experimental group), and placebo group (control). The protocol consisted of conducting two identical strenuous exercise tests with a rest period between tests of 24 h. Blood and urine samples were collected from the participants before supplementation (basal value) (T1), after supplementation (2 weeks) (T2), after first physical exercise test (T3), after 24 h of rest (T4), and after second physical exercise test (T5). The increase observed in the lactate, isoprostanes, DNA damage, and hydroperoxide levels reveals the severity of the oxidative damage induced by the exercise. There was a reduction in the isoprostanes, 8-OHdG, oxidized LDL, and hydroperoxides in the supplemented Ubiquinol group, an increase in total antioxidant status, fat soluble antioxidant (both plasma and membrane), and CAT activity. Also, NO in the Ubiquinol-supplemented group was maintained within a narrow range. Oxidative stress induced by strenuous exercise is accumulative and increases transiently in subsequent sessions of physical activity. A short-term supplementation (2 weeks) with Ubiquinol (200 mg/day) before strenuous exercise, decreases oxidative stress and increases plasma NO, fact that could improve endothelial function, energetic substrate supply, and muscle recovery after strenuous exercise. © 2016 BioFactors, 00(00):000000, 2016

Keywords: high intensity (strenuous) exercise; Ubiquinol; oxidative damage

1. Introduction

It is well known that regular, moderate and planned exercise has many benefits in trained people [1,2]. Exercise reduces the risk of heart disease, hypertension, heart failure, depression, diabetes, reduced threat of mortality, less risk of cancer, and reduced rates of metabolic syndrome [1]. However, with strenuous exercise these benefits are diminished because this type of physical activity induces fatigue resulting in decreased performance, alterations of several hormonal processes,
increased susceptibility to infection, and an increased free radical output [3,4].

Given the importance of oxidative stress and muscle damage associated with high intensity exercise [3–5], it would be interesting to assess the effect of oral supplementation with an antioxidant substance capable of diminishing muscle dysfunction and free radical generation associated with this performance [5].

Coenzyme Q10 (CoQ10) could be a suitable supplement during strenuous exercise. This compound is an obligatory component of the mitochondrial electron transport chain [6,7] and has been used for many years as a dietary supplement intended to promote good health, made possible through its role in energy production, as well as its antioxidant and anti-inflammatory properties [7,8]. Ubiquinol is especially important in those organs with high metabolic activity and/or bioenergetic requirements, such as heart and skeletal muscle, which are highly dependent on adequate supply of CoQ10 [6]. For these reasons, numerous studies have been focused on CoQ10 supplementation during exercise [7,9,10]. However, the oxidative stress induced during strenuous exercise has yielded conflicting results. Previously, beneficial effects from CoQ10 supplementation has been reported as it reduced the degree of oxidative stress, maintained cell integrity [11], and countered effects that could be considered negative such as an increase in malondialdehyde [12], and absence of effects [13,14].

The reasons for these contradictory results could be the different doses, different formulations affecting bioavailability, variations in the type of exercise, and/or different periods of supplementation used. Although another possible explanation is that, in most studies, the oxidized form of CoQ10 has been used, and this form features poor absorption after oral ingestion [15]. Recently, Ubiquinol (the reduced form of CoQ10) has received more attention because several studies have shown that is more bioavailable and demonstrates greater efficacy at lower doses than oxidized CoQ10 since it is already in its active form and does not have to be converted by the body [16,17]. The newly developed Ubiquinol form of CoQ10 (Kaneka Ubiquinol) has been reported to have excellent bioavailability [16] and is considered a safe compound, without any side effects, legally authorized, and nondoping [14].

However, despite the advantages exhibited by this new Ubiquinol CoQ10 form, studies on oral supplementation with Ubiquinol in strenuous exercises are very scarce and virtually not existent in relation to oxidative stress associated with this type of exercise [10]. Therefore, the aim of this study is to assess the effect of a short-term supplementation (2 weeks) with Ubiquinol (200 mg/day) on the oxidative stress induced by strenuous exercise.

2. Materials and Methods

2.1. Subjects

This is a randomized, double blind, and placebo-controlled trial. The experimental subjects taking part in this study were 100 healthy and well trained, but not on an elite level, firemen of the Fire Department of the City of Granada. Participants completed a medical and health history and physical activity questionnaire (IPAQ-SF) [18] prior to enrolment. Subjects characteristics are shown in Table 1. The firemen were randomly divided into two groups: Ubiquinol group (experimental group) (n = 50), and placebo group (control group) (n = 50). The Ubiquinol group was supplemented with an oral dose of 200 mg/day of Ubiquinol during 2 weeks, administering two brown liquid filled hard gelatin capsules of 100 mg/day whereas the subjects assigned to the control group took placebo using the same dose regimen. The capsules of Kaneka Ubiquinol (Kaneka Corporation, Osaka, Japan) contained 100 mg of ubiquinol solubilized in a matrix of canola oil, diglycerol monoolate, beeswax, and soy lecithin. The placebo capsules were comprised of the same constituents without Ubiquinol.

The study was approved by the Commission of Ethics in Human Research of the University of Granada (ref. 804). The study has been registered in ClinicalTrials.gov, with number NCT01940627. Informed consent was obtained from all subjects with written consent to participate in this study. The flowchart for participant enrolment and dropouts is shown in Fig. 1.

To avoid an important confounder in this type of trials we performed a four days diet journal including one day of the weekend to assess the nutritional status of the participants. The information obtained in this survey was evaluated by nutritional software (Nutriber, v1.1.1.5.r5, FUNIBER, Spain). Nutritional characteristics of the experimental subjects are summarized in Table 2.

2.2. Intense Physical Exercise Performance Programme

After 2 weeks period of Ubiquinol or placebo supplementation, subjects performed the strenuous exercise protocol to induce muscle damage. Prior to the starting of each test, subjects performed a warm-up that was divided into two phases: general activation phase and specific phase. The protocol consisted of conducting two identical strenuous exercise tests with a rest period between tests of 24 h. Both strenuous exercise tests consisted of performing a circuit composed of 10 bodybuilding
exercises (1. athletic press; 2. chest press in Smith Machine; 3. seated oar; 4. shoulders press; 5. femoral biceps flexion; 6. chest press in Smith Machine; 7. step with weight; 8. surveyor’s pole chest; 9. shove with weight; 10. quadriceps extension), which was executed twice with a rest period of 5 min between sets. The dynamics of each exercise was to perform 20 s of work maximizing the number of repetitions and maximizing the workload with 40 s of recovery between exercises. The minimum workload corresponded approximately to 60–70% of the Dynamic Maximum Force (DMF or 1RM). The effort performed was anaerobic-aerobic. Intensity of exercise and muscle aggression of this protocol was previously checked by measuring blood myoglobin and CK. The first exercise test induced an increase in CK (145.2 ± 125.9%) and myoglobin (402.7 ± 193.7%); the second exercise test also increased CK (193.0 ± 159.4%) and myoglobin (431.7 ± 245.4%). Similar increases have been observed in other strenuous exercise tests [19].

To establish the minimum magnitude of the load to be displaced for each subject, 1 week before strenuous exercise protocol a pretraining session was held with the subjects to conform the load individually in terms of two parameters in each
exercise: (a) Scale OMNI-RES [20] values of perceived exertion between 6 and 7, and (b) 10 repetitions.

2.3. Blood and Urine Sampling
Blood samples were collected from the participants via venous catheter into heparinized tubes and urine samples were collected into sterilized tubes. Five blood samples and urine were taken: before supplementation (basal value) (T1), after supplementation (2 weeks) (T2), after first physical exercise test (T3), after 24 h of rest (T4), after second physical exercise test (T5). Blood was immediately centrifuged at 1,750 g for 10 min at 4°C in a Beckman GS-6R refrigerated centrifuge (Beckman, Fullerton, CA, USA) to separate plasma from red blood cell pellets. Erythrocyte cytosolic and membrane fractions were separated according to the method of Hanahan and Ekholm (1974).

2.4. Biochemical Parameters
Lactate was measured using a commercial kit (Spinreact, Barcelona, Spain) following the instructions of the manufacturer. Nitric Oxide (NO) was measured using a commercially available kit (DetectX® Nitric Oxide Colorimetric Detection Kit, Arbor Assays, Ann Arbor, MI, USA).

2.5. Oxidative Stress Parameters
Isoprostanes in urine were measured using a commercial kit Enzyme Immunoassay for Urinary Isoprostane (Oxford Biomedical Research, Oxford, England). This kit is a competitive enzyme-linked immunoassay (ELISA) for determining levels of 15-F2t-Isoprostane (the best characterized isoprostane) in urine samples.

8-Hydroxy-2’-deoxyguanosine (8-OHdG) was determined using a commercial kit (SOHdG Check, Japan Institute for the Control of Aging, Shizuoka, Japan). This competitive ELISA kit employs a monoclonal antibody specific for 8-OHdG quantification in plasma and urine samples. Results were read at 450 nm on a microplate reader (Bio-tek, Vermont, USA).

Plasma lipid peroxides content was assessed using a commercial kit (Oxystat, Biomedica Gruppe, Vienna, Austria). Oxystat is a colorimetric assay for the quantitative determination of peroxides in plasma, serum and other biological fluids.

Membrane lipid peroxides have been measured using a commercial kit (Pierce™ Quantitative Peroxide Assay Kits, Thermo Scientific, Rockford, USA) which is based on the rapid peroxide-mediated oxidation of Fe$^{2+}$ to Fe$^{3+}$ in acidic conditions.

Plasma and membrane protein carbonyl have been measured using a commercial kit (OxiSelect™ Protein Carbonyl ELISA Kit, Cells Biolabs, San Diego, USA). This is an enzyme immunoassay developed for rapid detection and quantitation of protein carbonyls.

Oxidized Plasma low density lipoproteins (LDL) were measured using a Mercodia Oxidized LDL ELISA (Merckodia AB, Uppsala, Sweden). The colorimetric endpoint of the reaction was read spectrophotometrically at 450 nm on a microplate reader (Bio-tek, Vermont, USA).

Total plasma antioxidant capacity in plasma samples was measured using a kit (OxiSelect™ Oxygen Radical Antioxidant Capacity (ORAC) Activity Assay, Cells Biolabs, San Diego, USA). The ORAC Activity Assay is based on the oxidation of a fluorescent probe by peroxyl radicals. The sample antioxidant capacity correlates to the fluorescence decay curve and is compared to an antioxidant standard curve of the water soluble vitamin E analog Trolox.

Determination of antioxidant enzymes, glutathione peroxidase (GPx), superoxide dismutase (SOD), and Catalase (CAT) in erythrocyte cytosol were measured as previously described Diaz-Castro et al. (2014) [21].

Fat soluble antioxidants in plasma (carotene, retinol, α-tocopherol, coenzyme Q9 and coenzyme Q10) and erythrocyte membrane (coenzyme Q10 and α-tocopherol) were determined using an ACQUITY UPLC H-Class detector coupled to a triple quadrupole Xevo TQ-S (Waters Corporation, Milford, USA). All the parameters studied were optimized individually using standard solutions from Sigma-Aldrich (minimum 98% purity, Grade HPLC) and quantified with standard curves. MassLynx 4.1. (Waters Corporation, Milford, USA) software was used to obtain all the data.

2.6. Statistical Analysis
All data are presented as the mean ± SD. All variables were tested to see if they followed the criteria of normality and homogeneity of variance using the Kolmogorov–Smirnoff’s and Levene’s tests, respectively. To compare general characteristics of the subjects in both experimental groups, unpaired Student’s t test was used. To assess the effect of the

### TABLE 2

**Nutritional intake analyses for the ubiquinol and control groups**

<table>
<thead>
<tr>
<th></th>
<th>Ubiquinol</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (Kcal/day)</td>
<td>2959.9 ± 568.1</td>
<td>2843.7 ± 485.2</td>
</tr>
<tr>
<td>Protein intake (g/day)</td>
<td>132.9 ± 28.5</td>
<td>128.6 ± 32.2</td>
</tr>
<tr>
<td>Fat intake (g/day)</td>
<td>106.2 ± 22.0</td>
<td>102.5 ± 22.7</td>
</tr>
<tr>
<td>Carbohydrate intake (g/day)</td>
<td>368.2 ± 97.7</td>
<td>351.6 ± 75.0</td>
</tr>
<tr>
<td>Cholesterol intake (mg/day)</td>
<td>295.1 ± 86.6</td>
<td>277.2 ± 111.2</td>
</tr>
<tr>
<td>Fiber intake (g/day)</td>
<td>27.8 ± 9.5</td>
<td>27.6 ± 10.9</td>
</tr>
<tr>
<td>Vitamin C (mg/day)</td>
<td>165.0 ± 71.8</td>
<td>144.4 ± 54.1</td>
</tr>
<tr>
<td>Retinol (µg/day)</td>
<td>1253.4 ± 483.2</td>
<td>1101.6 ± 402.2</td>
</tr>
<tr>
<td>Vitamin E (mg/day)</td>
<td>13.7 ± 5.2</td>
<td>12.8 ± 5.9</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Statistically significant differences between groups (P < 0.05).
supplementation and the evolution in the time of each variable studied in each experimental group a general linear model of variance for repeated measures with an adjustment by means of Bonferroni's test has been performed. Bonferroni’s test allowed us to know intra- and intersubject differences (effect of time in each group and supplementation in each period, respectively) in a very robust way in terms of power. A value of \( P < 0.05 \) was considered significant. For data analysis, we used the SPSS version 20.0 (SPSS Statistics for Windows, 20.0.0. SPSS, Inc., Chicago, IL, USA).

3. Results

No statistically significant differences between both groups were found for weight, age, height, and BMI (Table 1). The analysis of 4 days diet record revealed no differences between groups with respect to the intake of macro and micronutrients (Table 2). No statistically significant differences were recorded between groups for the short form of the International Physical Activity Questionnaire (Table 3). The dropout percentage was similar in both groups (24% after finishing the first test and 32% after finishing the second exercise test) and neither differences were observed between dropout reasons in both groups (Fig. 1).

Intense physical activity increased lactate levels in both days of the physical activity test (T3 and T5) though without differences between both groups (Ubiquinol and placebo) (Fig. 2). With regard to NO, higher values were recorded after the first physical activity test in both groups (T3) with regard to T1. Nevertheless, in the control group, NO diminished progressively from this sample to the next ones, exhibiting a significant decrease in T5 compared with T3. In contrast, the supplemented group maintained elevated levels exhibiting significant differences between the control and supplemented group in T5 (Fig. 2).

A higher level of urinary 15-F2t-Isoprostanes was observed in T3 and T5 (after strenuous exercise), though in the supplemented group it was only significant in the T5, whereas in the group control there were differences with regard to the T3 and T5. In sample T4, major values were also observed, though it was only significant in the control group. Between the groups, we found differences in T2, T3 and T4 (Fig. 3). The physical tests increased urinary 8-OHdG at T4 with respect to T3 and T5 in the control group. A significant difference between groups was observed in the sample T4 after the 24 h of rest and before the second physical test with a major content in 8-OHdG in the control group (Fig. 3).

Lower levels of plasma 8-OHdG were observed in both groups in sample T3 after the first physical test. In the significant decrease in T5 compared with T3. In contrast, the supplemented group maintained elevated levels exhibiting significant differences between the control and supplemented group in T5 (Fig. 2).

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Lower levels of plasma 8-OHdG were observed in both groups in sample T3 after the first physical test. In the

**TABLE 3** Results of the short last 7 days self-administration of IPAQ (International Physical Activity Questionary)

<table>
<thead>
<tr>
<th></th>
<th>Ubiquinol (days/week)</th>
<th>Control (days/week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vigorous physical activity</td>
<td>3.5 ± 1.5</td>
<td>3.3 ± 1.3</td>
</tr>
<tr>
<td>Vigorous physical activity (min/day)</td>
<td>100.8 ± 47.0</td>
<td>98.0 ± 43.2</td>
</tr>
<tr>
<td>Moderate physical activity (days/week)</td>
<td>3.6 ± 1.8</td>
<td>3.2 ± 1.7</td>
</tr>
<tr>
<td>Moderate physical activity (min/day)</td>
<td>88.6 ± 50.1</td>
<td>75.0 ± 33.3</td>
</tr>
<tr>
<td>Walking (days/week)</td>
<td>4.3 ± 1.3</td>
<td>4.0 ± 1.5</td>
</tr>
<tr>
<td>Walking (min/day)</td>
<td>66.4 ± 44.9</td>
<td>52.7 ± 28.4</td>
</tr>
<tr>
<td>Sitting (min/day)</td>
<td>193.6 ± 83.2</td>
<td>190.2 ± 77.5</td>
</tr>
</tbody>
</table>

Values are means ± SD.

*Statistically significant differences between groups (\( P < 0.05 \)).

![FIG 2](image-url) Effect of exercise and ubiquinol supplementation on plasma lactate (A) and on nitric oxide (B) levels. Results are expressed as mean ± SD. *Statistically significant differences between groups (\( P < 0.05 \)). T1: before supplementation (basal value); T2: after supplementation (2 weeks) and before the first physical test; T3: after first physical exercise test; T4: after 24 h of rest and before the second physical test; T5: after second physical exercise test. Different letters in every group indicates significant differences due to the time [Control (A, B, C, D, E); Ubiquinol (a, b, c, d, e)] (\( P < 0.05 \)).
supplemented group, a lower value is observed in samples T4 and T5, though it is only significant in sample T5 (Table 4). With regard to plasma hydroperoxides, in both experimental groups the same trend was observed, the physical tests increased the hydroperoxides level in both groups with significant differences in samples T3, T4, and T5 with regard to the rest period. Between experimental groups, the only difference observed was in sample T4 with a lower value in the supplemented group (Table 4).

With regard to membrane lipid peroxides, no differences were observed between groups, although there was a higher content in samples T4 and T5 (Table 4). Though in membrane protein carbonyl groups, no significant differences were observed between experimental groups, we found differences in the different samples. In the control group, differences were observed between samples T4 and T5 (just before and after the second physical test) and the values found in the samples T1, T2, and T3. In the supplemented group, just sample T4 showed significant differences with regard to the rest of samples (Table 4).

In the control group, a maximum antioxidant capacity was observed in sample T3 just after performing the first physical test with significant differences with regard to T1. A decrease was also observed in the following samples, T4 and T5 with significant differences in comparison with sample T3. In the supplemented group, a higher antioxidant capacity was observed in samples T2, T3, and T4 in comparison with T1 without the previously commented decrease in the control group. Between experimental groups, significant differences were shown in T2, T3, and T4 with higher values in the supplemented group (Fig. 4).

With regard to fat-soluble vitamins (Table 5), as expected, CoQ10 was higher in the supplemented group in both plasma and erythrocyte membrane. We also recorded higher values of plasma CoQ9 in T2 and T4. Vitamin E showed higher plasma concentrations in T2 and T5 in the supplemented group, meanwhile in the control group, a decrease (in both plasma membrane) was observed only in T5.

With regard to cytosolic CAT activity, differences between groups were observed in sample T2 (just after finishing the supplementation) with a higher value in the supplemented group. The evolution of CAT activity during the physical tests showed an increase after the first physical test in both groups. Nevertheless, in the control group after finishing the second physical test a decrease in the CAT activity was observed in the control group with regard to sample T3, whereas this trend was not observed in the supplemented group (Fig. 5). In both groups a similar trend was observed for SOD cytosolic activity with a decrease of the activity of this enzyme in sample T3 (just after the first physical test) and an increase in T5 (just after the second physical test) and no differences were found between groups (Fig. 5). No differences in GPx were recorded due to the supplementation and time. In contrast, the GPx activity decreased in T3 (just after the first physical test), T4, and T5 (just after the second physical test) compared with the basal values (T1) in the control group (Fig. 5).

4. Discussion

There is some controversy about the value of using antioxidant supplements in sports [5]. However, there is strong evidence that free radicals linked to strenuous exercise can cause
Antioxidants for people involved with athletic activity may reduce the muscle damage induced by free radicals during sports activity, increase endurance, and achieve performance improvements [3,4]. CoQ10 could be suitable for this muscle-protective supplementation because it has properties related to bioenergetic and antioxidant activity, it is intimately involved in energy production, prevents peroxidative damage to membrane phospholipids and reduces free radical-induced oxidative damage to proteins and mitochondrial DNA [7,8]. However, very few studies of supplementation with this compound are available investigating its effects during strenuous physical exercise, especially in the area of oxidative stress and virtually nonexistent when referring to supplementation with the reduced Ubiquinol form of this molecule, [7,9,10].

Both groups of firemen studied are homogeneous in terms of weight, height, and age with similar nutrition. With regard to the physical activity carried out in this study, the results obtained from the evaluation of the Short International Physical Activity Questionnaire (IPAQ-short), the most common and most practical approaches used for evaluating physical activity status in large study populations [18], reveals a high physical activity level.

<table>
<thead>
<tr>
<th>TABLE 4</th>
<th>Effect of exercise and ubiquinol supplementation on different oxidative damage biomarkers in plasma and membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasmatic</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T1</td>
</tr>
<tr>
<td>8OHdG (ng/mL)</td>
<td></td>
</tr>
<tr>
<td>Ubiquinol</td>
<td>$41.0 \pm 15.2^a$</td>
</tr>
<tr>
<td>Control</td>
<td>$42.9 \pm 12.6^A$</td>
</tr>
<tr>
<td>Lipid peroxides ($\mu$mol/L)</td>
<td></td>
</tr>
<tr>
<td>Ubiquinol</td>
<td>$6.2 \pm 2.6^ac$</td>
</tr>
<tr>
<td>Control</td>
<td>$6.3 \pm 2.0^A$</td>
</tr>
<tr>
<td>LD Lipid oxidized (UL)</td>
<td></td>
</tr>
<tr>
<td>Ubiquinol</td>
<td>$31.5 \pm 9.9^a$</td>
</tr>
<tr>
<td>Control</td>
<td>$30.2 \pm 6.7^A$</td>
</tr>
<tr>
<td>Carbonyl (nmol/mg)</td>
<td></td>
</tr>
<tr>
<td>Ubiquinol</td>
<td>$0.9 \pm 0.3^a$</td>
</tr>
<tr>
<td>Control</td>
<td>$0.9 \pm 0.2^A$</td>
</tr>
<tr>
<td><strong>Membrane</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T1</td>
</tr>
<tr>
<td>Lipid peroxides ($\mu$mol/L)</td>
<td></td>
</tr>
<tr>
<td>Ubiquinol</td>
<td>$15.3 \pm 2.7^a$</td>
</tr>
<tr>
<td>Control</td>
<td>$16.0 \pm 3.0^A$</td>
</tr>
<tr>
<td>Carbonyl (nmol/mg)</td>
<td></td>
</tr>
<tr>
<td>Ubiquinol</td>
<td>$1.6 \pm 0.2^a$</td>
</tr>
<tr>
<td>Control</td>
<td>$1.5 \pm 0.2^A$</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD.

*Statistically significant differences between groups ($P < 0.05$). T1: before supplementation (basal value); T2: after supplementation (2 weeks) and before the first physical test; T3: after first physical exercise test; T4: after 24 h of rest and before the second physical test; T5: after second physical exercise test. Different letters in every group indicates significant differences due to the time (Control (A, B, C, D, E); Ubiquinol (a, b, c, d, e) ($P < 0.05$).
activity profile with a total PAscore > 3000 MET x min/week, according to the Physical Activity Classification Criteria (Papathanasiou et al., 2010).

The increase observed in the lactate levels in both days of the physical activity tests reveals the severity of the physical activity. These finding is in agreement with previous results reporting significant positive correlations found between maximal postexercise blood lactate concentration and excess post-exercise oxygen consumption after intense exercise [22]. A recent study suggest that the potential effect of blood lactate on excess postexercise oxygen consumption would be concentration dependent, indicating the degree of effort associated with the physical activity [23].

Another compound that increases during physical activity is Nitric Oxide (NO) [24]. Exercise increases shear stress, which induces endothelial-nitric oxide synthase (e-NOS) expression [24]. This increase has been also observed in the current study with higher values exhibited after the first physical activity test in both experimental groups compared with T1. Nevertheless, in the control group NO diminished progressively after the first physical test (T3) to the next ones, whereas in the supplemented group NO levels remained elevated, exhibiting significant differences between the control and supplemented group at the end of the study (T5). There are various benefits of increased NO during exercise including vasodilator action that helps both exercise performance and nutrient supply in muscle recovery, as well as improvement in the supply of substrates such as glucose, together facilitate the regulatory role in the immune system [24–26]. This study shows for the first time an effect of Ubiquinol on NO during intense physical activity in healthy adult subjects. One possible mechanism of action would be the induction of nitric oxide synthase [24] or the antioxidative effect of Ubiquinol since it is known that oxidative stress is one of the causes of NO inactivation and superoxide anion is one of the main molecular species responsible for it [24,25] avoiding nitrosative stress [11].

Measurement of isoprostanes is listed as one of the most widely recognized and trusted biomarkers for the study of oxidative stress or lipid peroxidation in vivo, especially during physical exercise [27,28]. In this sense, several studies have reported a significant increase in the levels of isoprostanes after strenuous exercise compared to baseline [11,27] findings in agreement with the increase observed in the current study revealing that the oxidative aggression due to the high intensity exercise is accumulative. Increasing level of isoprostanes in the control group after the first exercise test is clear, however, in the Ubiquinol group this increase was not observed with this parameter remaining similar to baseline. This effect on isoprostanes output has been observed in studies about ubiquinone supplementation [11], but never has been reported in studies with the reduced form, Ubiquinol. This result highlights the antioxidative effect of Ubiquinol which inhibits the expression of NADPH oxidase (one of the main sources of ROS) [29] and scavenges lipid peroxidation products during free radical reactions directly or mediating the regeneration of α-tocopherol, the active form of vitamin E, by reducing the tocopherol radical [7,8,11,30].

In relation to other indicators of oxidative damage studied, 8-OHdG (in plasma and urine), lipid peroxides (plasma and membranes), oxidized LDL, and carbonyl groups (plasma and membranes), reveal that strenuous exercise has induced oxidative damage to various macromolecules, especially lipids and DNA, findings which are in agreement with previous reports, although always influenced by the intensity and volume of exercise [11,31,32]. The relationship between these biomarkers and isoprostanes content is qualitative rather than quantitative, which has been highlighted in other studies and show the importance of isoprostanes as a preferred indicator of oxidative damage [27].

The effect of Ubiquinol on these biomarkers is positive showing a decrease especially in T4 and T5, reduction, which is important considering that oxidative stress associated with exercise is cumulative and therefore is higher at these points. Thus, in our study, we observed a positive effect of a short-term supplementation with Ubiquinol on the levels of 8-OHdG in T4 (urine) and T5 (plasma) and plasma peroxides in T4. Other studies have also shown an effect of Coenzyme Q10 on the oxidative damage to DNA and lipids in situation of strenuous exercise [10,11].

Our study demonstrates that intense exercise induces oxidative damage to LDLs, although not equally in both groups. Ubiquinol supplementation decreases LDL oxidation because we observed that in the control group this oxidative damage increased during the two sessions of physical exercise and even during the rest period, while in the supplemented group...
an increase was observed at the end of the second session. This protective effect in the oxidation of lipoproteins is important if we take into account their negative effects [33]. In this sense, Del Pozo-Cruz et al. [34] found a positive relationship between levels of coenzyme Q10 in plasma, physical activity in elderly and oxidation of lipids and LDL; however, to our knowledge, this is the first study demonstrating this protective effect of Ubiquinol supplementation in lipoproteins during intense physical exercise in healthy adults.

Finally, to better understand the protective mechanism of the Ubiquinol supplementation, we studied the effect on the antioxidant defense system. Ubiquinol supplementation has a clear effect on plasma total antioxidant capacity which in turn may result from its effect on plasma lipid-soluble antioxidants,
particularly tocopherol and coenzyme Q. In relation to the tocopherol, it has been observed that Ubiquinol avoids the reduction of this fat-soluble antioxidant in the last test of exercise, probably due to its ability to regenerate tocopherol into its active form [7,8]. The increase in plasma levels of CoQ10 is similar to that found in other studies of supplementation with Ubiquinol [16,17], although this is the first study to show an effect on the isoform Q9 in plasma and also increased nearly 1.4 times CoQ10 in erythrocyte membrane during strenuous exercise. Regarding the effect of Ubiquinol supplementation on cytosolic enzymatic antioxidants, we only observed an effect on the activity of CAT and in a lower extent in GPx. In other studies with the oxidized form (CoQ10), a similar effect was also observed on CAT activity, although no effect was reported on GPx [11].

5. Conclusion

Our results reveal the severity and cumulative character of the oxidative damage induced by our exercise protocols. There was a reduction in the urinary isoprostanes and 8-OHdG in the Ubiquinol-supplemented group after the first physical test, highlighting the antioxidative and protective effect of this compound during physical activity. NO diminished progressively in the control group, whereas in the supplemented group NO was maintained within a narrow range, and this could improve endothelial function, energetic substrates supply and muscle recovery after the physical tests. Ubiquinol supplementation also decreased oxidized LDL, revealing a protective effect of Ubiquinol in the oxidation of lipoproteins and also a positive effect on plasma total antioxidant capacity, which in turn may result from its effect on plasma lipid-soluble antioxidants, particularly tocopherol and coenzyme Q (showing a positive effect on the isoform Q9 in plasma and CoQ10 in erythrocyte membrane). Ubiquinol also showed an effect on the activity of CAT and in a lower extent in GPx. Therefore this study provides evidence that Ubiquinol is a safe compound, efficiently ameliorating oxidative stress, improving muscle substrate supply and recovery after strenuous exercise.

Conflict of Interests

Financial support for this work was provided by (Kaneka Corporation, Osaka, Japan). The investigators and the University of Granada have no direct or indirect interest in the tested product (Kaneka QH) or in Kaneka Corporation and therefore the authors declare no conflict of interest.

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