Ubiquinol supplementation modulates energy metabolism and bone turnover during high intensity exercise

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Bone and energy metabolism are profoundly influenced by exercise. The objective of this study was to determine for the first time whether a short-term supplementation with ubiquinol could have a modulating effect on bone turnover and energy metabolism associated with strenuous exercise. The participants (n = 100 healthy and well-trained firemen) were randomly divided into two groups: ubiquinol group (ubiquinol (200 mg day⁻¹)) and control group (placebo) for two weeks. The protocol consisted of conducting two identical strenuous exercise tests with a rest period between tests of 24 h. Blood samples were collected before supplementation (basal value) (T1), after supplementation (T2), after the first physical exercise test (T3), after 24 h of rest (T4), and after the second physical exercise test (T5). Parathyroid hormone (PTH), osteocalcin (OC), osteoprotegerin (OPG), osteopontin (OPN), sclerostin (SOST), alkaline phosphatase (AP), adrenocorticotropin (ACTH), insulin, leptin, adrenaline, noradrenaline and peroxisome proliferator activated receptor-γ coactivator-1α (PGC-1α) were determined. Our protocol increased ACTH, SOST, PTH and OC levels, while it decreased OPN. This protocol also increased adrenaline, noradrenaline and PGC-1α, and decreased insulin. After ubiquinol supplementation, PTH, OC, OPG, alkaline phosphatase, leptin, insulin, noradrenaline and PGC-1α levels increased in the supplemented group compared to the control group after the exercise protocol. Strenuous exercise has a clear effect on energy metabolism and bone turnover. These effects are modulated by ubiquinol supplementation, which especially increases the biomarkers of bone formation during strenuous exercise. In addition, ubiquinol has a beneficial effect on the mobilization of energy sources, fact that it could represent an ergogenic and physiological advantage for skeletal muscles.

1. Introduction

Numerous reports have revealed that moderate physical activity enhances the function of various organs and tissues, and it is associated with a reduced threat of cardiovascular disease, cancer and diabetes, and in general, with a lower risk of all causes of mortality. Bone and energy metabolism are profoundly influenced by exercise. The objective of this study was to determine for the first time whether a short-term supplementation with ubiquinol could have a modulating effect on bone turnover and energy metabolism associated with strenuous exercise. The participants (n = 100 healthy and well-trained firemen) were randomly divided into two groups: ubiquinol group (ubiquinol (200 mg day⁻¹)) and control group (placebo) for two weeks. The protocol consisted of conducting two identical strenuous exercise tests with a rest period between tests of 24 h. Blood samples were collected before supplementation (basal value) (T1), after supplementation (T2), after the first physical exercise test (T3), after 24 h of rest (T4), and after the second physical exercise test (T5). Parathyroid hormone (PTH), osteocalcin (OC), osteoprotegerin (OPG), osteopontin (OPN), sclerostin (SOST), alkaline phosphatase (AP), adrenocorticotropin (ACTH), insulin, leptin, adrenaline, noradrenaline and peroxisome proliferator activated receptor-γ coactivator-1α (PGC-1α) were determined. Our protocol increased ACTH, SOST, PTH and OC levels, while it decreased OPN. This protocol also increased adrenaline, noradrenaline and PGC-1α, and decreased insulin. After ubiquinol supplementation, PTH, OC, OPG, alkaline phosphatase, leptin, insulin, noradrenaline and PGC-1α levels increased in the supplemented group compared to the control group after the exercise protocol. Strenuous exercise has a clear effect on energy metabolism and bone turnover. These effects are modulated by ubiquinol supplementation, which especially increases the biomarkers of bone formation during strenuous exercise. In addition, ubiquinol has a beneficial effect on the mobilization of energy sources, fact that it could represent an ergogenic and physiological advantage for skeletal muscles.

Physical activity can act on bone remodeling directly and indirectly through various metabolic pathways. For this reason, it is important to consider that bone metabolism cannot be studied independently of other physiological processes that are also affected by exercise, such as energy metabolism. Thus, bone regulation is part of a complex system involving mechanisms of central origin (nervous system), systemic (endocrine) and local.

Bone remodeling occurs in a constant way, which implies a high energy demand, and therefore, as we have mentioned earlier, there exists a strong physiological link between bone tissue, energy metabolism and sympathetic nervous system. This physiological link includes hormones from adipose tissue (such as leptin) and from bone tissue (osteocalcin), which have effects on the metabolism of glucose, fatty acids, insulin secretion and adiponectin, among others. In fact, bone tissue apart from being a support tissue, has an important endocrine role regulating energy metabolism and other meta-
bolic pathways affecting levels of glucose, levels of appetite, muscle function, among others.\textsuperscript{7,8}

Another important aspect to consider during the intense physical activity is the high output of free radicals,\textsuperscript{9} which is linked to both bone turnover and energy metabolism.\textsuperscript{3} Oxidative stress can negatively affect bone turnover by increasing bone resorption through increasing activity and proliferation of osteoclasts\textsuperscript{10,11} and it has been observed that dietary supplements with antioxidant capacity have shown positive effects on bone homeostatic alterations associated with oxidative stress.\textsuperscript{12}

However, although the benefits of moderate physical activity are evident, high intense exercise does not have these benefits on body mass density and may cause negative effects.\textsuperscript{13} For this reason, elite athletes and other populations, who continuously practice high intensity exercises, are a group that must pay special attention to bone health, because of the possible long-term appearance of bone pathologies such as osteopenia or osteoporosis, and also for the short-term microfractures.\textsuperscript{4} One way to reduce this bone damage would be by modifying training protocols, but this could lead to a decrease in physical performance, something not desirable for this group, although it can be improved through the supplementation with certain nutrients.\textsuperscript{4} One of such nutrients that acquires more interest every day is coenzyme CoQ10 (CoQ10), which has shown properties related to energy metabolism and antioxidant activity.\textsuperscript{14} In addition, supplementation with this molecule has shown positive effects during strenuous exercises,\textsuperscript{14} as well as on bone turnover,\textsuperscript{15,16} although the latter studies were performed \textit{in vitro} or using animal models.

The data available in the scientific literature have provided a direct link between physical performance and blood CoQ10 levels.\textsuperscript{17} However, most of these studies are focused mainly in the exercise performance and radical-scavenging activity of CoQ10 during low intensity exercise.\textsuperscript{17} Virtually, there are no studies showing the effect of supplementation with CoQ10 during strenuous physical exercise on the modulation of energy metabolism and bone turnover, especially when using the reduced form of CoQ10, the ubiquinol, which has shown greater bioavailability and efficacy than the oxidized form.\textsuperscript{18} Taking into account all the abovementioned facts, this study has been designed to assess for the first time whether oral ubiquinol short-term supplementation prior to the performance of a high intensity exercise has a positive role in bone and energy metabolism, assessing the effects of this agent during strenuous exercise.

2. Materials and methods

2.1. Subjects

This study is a randomized, double-blind and placebo-controlled trial. 100 firemen of the Fire Department of the City of Granada (healthy and well-trained) took part in the study. Participants completed a medical and health history and physical activity questionnaire (IPAQ-SF)\textsuperscript{19} prior to enrolment. All of them were non-smokers, did not take any nutritional supplements and did not present febrile/inflammatory clinical symptoms, chronic diseases, bone pathologies, did not use immunosuppressive or nephrotoxic drugs, and did not use energy, protein and/or antioxidant supplements. The firemen were randomly divided into two groups: ubiquinol group (ubiquinol) (\(n = 50\)) and placebo group (control) (\(n = 50\)). The ubiquinol group was supplemented with an oral dose of 200 mg day\textsuperscript{-1} of ubiquinol for 2 weeks (in the morning, under fasting conditions with a glass of water), which was administered in 2 brown liquid-filled hard gelatin capsules of 100 mg each, and the control group took placebo using the same scheme. The capsules Kaneka QH ubiquinol (Kaneka Corporation, Osaka, Japan) contained ubiquinol composed of canola oil, diglycerol monooleate, beeswax and soy lecithin. The placebo capsules contained the same composition without ubiquinol and were also supplied by Kaneka (Kaneka Corporation, Osaka, Japan).

All participants signed the informed consent. The study was approved by the Commission of Ethics in Human Research of the University of Granada (ref. 804) and it has been registered in ClinicalTrials.gov, with the number NCT01940627. To avoid an important confounder in this type of trials, we collected four-day diet records, including one day of the weekend to know the nutritional condition of the participants. The information obtained in this survey was evaluated by nutrition software (Nutriber, v1.1.1.5.r5, FUNIBER, Spain).

2.2. Intense physical exercise performance program

Characteristics, intensity and muscle aggression of this protocol were previously checked by measuring blood myoglobin, lactate and CK.\textsuperscript{9} After a 2-week period of ubiquinol or placebo supplementation, subjects performed the strenuous exercise protocol. Prior to the starting of each test, subjects performed a warm-up. The protocol consisted of conducting two identical strenuous exercise tests, with a rest period of 24 hours between the tests. Both strenuous exercise tests involved performing a circuit composed of 10 resistance exercises (1, athletic press; 2, chest press in Smith machine; 3, seated oar; 4, shoulder press; 5, femoral bicep flexion; 6, chest press in Smith machine; 7, step with weight; 8, surveyor’s pole chest; 9, push with weight; 10, quadriceps extension), with a minimum workload of approximately to 60–70% of the dynamic maximum force (DMF or 1RM).\textsuperscript{9} In order to establish the minimum magnitude of the load to be displaced for each subject, one week before the strenuous exercise protocol, a session of pre-training was held with the subjects to conform the load individually in terms of two parameters in each exercise: (a) scale OMNI-RES\textsuperscript{20} values of perceived exertion between 6 and 7, and (b) 10 repetitions.

2.3. Blood sampling

Five blood samples were collected from the participants via venous catheter into heparinized tubes before and immediately after the physical test. Five blood samples were taken: before supplementation (basal value) (T1), after supplementation (2 weeks) and immediately before the first physical exer-
cise test (T2), after the first physical exercise test (T3), after 24 h of rest and immediately before the second physical exercise test (T4), and after the second physical exercise test (T5). Blood was immediately centrifuged at 1750g 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. Plasma samples were immediately frozen and stored at −80 °C until analysis.

2.4. Bone turnover parameters
Luminex xMAP technology-based Milliplex Map Kits were used. Human Bone Panel 1A (Millipore, USA, cat. no. HBOBMAG-51K) was used for adrenocorticotropic hormone (ACTH), parathyroid hormone (PTH), osteocalcin (OC), osteopontin (OPN), osteoprotegerin (OPG), sclerotin (SOST), leptin and insulin determination. Preparation of samples, reagents and standards were carried out following the manufacturer's instructions. The standard curve pattern was performed by successive dilutions of the stock concentration for each biomarker. A standard curve was obtained with the following concentrations: OPG, 30 000 pg ml−1; insulin, 250 000 pg ml−1; leptin, 200 000 pg ml−1; OC, 600 000 pg ml−1; OPN, 400 000 pg ml−1; PTH, 20 000 pg ml−1. Equipment conditions were set following the manufacturer's instructions: events: 50 microsphere; sample size: 50 µl; gate settings: 8000–15 000; reporter gain: default (low PMT); time out: 60 seconds; bead set: OPG/17; OC/19; leptin/22; PTH/31; insulin/82.

2.5. Alkaline phosphatase (AP)
AP was measured using a BS-200 Chemistry Analyzer (Shenzhen, China), using a Pointe Scientific Alkaline Phosphatase liquid reagent (Pointe Scientific, MI, USA), by measuring the rate of hydrolysis of various phosphate esters under specified conditions.

2.6. Peroxisome proliferator activated receptor-γ coactivator-1α (PGC-1α)
Peroxisome proliferator activated receptor-γ coactivator-1α (PGC-1α) was measured using a commercial kit (Cloud Clone Corp., TX, USA). The microtiter plate provided is pre-coated with an antibody specific to PGC-1α. Standards or samples were added to the appropriate microtiter plate wells. Next, avidin conjugated to horseradish peroxidase (HRP) was added to each microplate well and incubated. The enzyme–substrate reaction was terminated by the addition of sulphuric acid solution and the color change was monitored spectrophotometrically (Thermo Spectronic, Rochester, USA) at 450 nm.

2.7. Adrenaline and noradrenaline
Adrenaline and noradrenaline were measured using a DRG Instruments Cat Combi ELISA (catecholamine combination) commercial kit (Marburg, Germany). After pipetting the enzyme solutions, incubating and making the subsequent washes, stop solutions were added to both plates and adrenaline and noradrenaline were monitored spectrophotometrically (Thermo Spectronic, Rochester, USA) at 450 nm, following the manufacturer's instructions.

2.8. Statistical evaluation
All data are presented as mean ± SEM. All variables were tested to see if they followed the criteria of normality and homogeneity of variance using the Kolmogorov–Smirnov and Levene tests, respectively. To compare the general characteristics of the subjects in both experimental groups, unpaired Student’s t-test was used. To assess the effect of the 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 was performed. Bonferroni’s test allowed us to study the intra- and inter-subject differences (effect of time in each group and supplementation in each period, respectively). A value of p < 0.05 was considered significant. For data analysis, we used SPSS version 20.0 (SPSS Statistics for Windows, 20.0.0., SPSS Inc., Chicago, IL, USA).

3. Results
No differences were observed in the general characteristics of the subjects (Table 1). Also, we have previously published that there are no significant differences between the groups neither for the results obtained in the nutritional questionnaire nor in the physical activity questionnaire, and both groups have been categorized as category 3, with the highest measurement threshold of the total physical activity of the questionnaire. The percentage of dropouts and the reasons for them were, also, similar in both groups, without significant differences being observed, as previously reported.9

Bone turnover biomarkers were influenced by exercise and ubiquinol. ACTH increased with exercise in T3 and T5 in both groups (p < 0.05) and no effect of ubiquinol was observed (Fig. 1A). PTH increased in the ubiquinol group compared to

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>BMI (kg m⁻²)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>RHR (beats per min)</th>
</tr>
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<tbody>
<tr>
<td>Ubiquinol</td>
<td>38.9 ± 1.4</td>
<td>175.4 ± 0.8</td>
<td>76.8 ± 1.5</td>
<td>25.0 ± 0.4</td>
<td>137.0 ± 2.2</td>
<td>81.4 ± 1.5</td>
</tr>
<tr>
<td>Control</td>
<td>38.2 ± 1.2</td>
<td>174.5 ± 1.2</td>
<td>76.3 ± 2.0</td>
<td>25.0 ± 0.5</td>
<td>134.1 ± 2.1</td>
<td>79.1 ± 1.9</td>
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<td></td>
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<td>57.1 ± 1.5</td>
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Data are expressed as mean ± SEM. Abbreviation: BMI (body mass index); DBP (diastolic blood pressure); RHR (resting heart rate); SBP (systolic blood pressure).
the control group in T2, T4 and T5 (p < 0.05). With regard to the evolution, in the ubiquinol group an increase was observed in T3 compared to T1 and T2 (p < 0.05) and in T5 compared to T1, T2, T3 and T4 (p < 0.05). In the control group, an increase was observed in T3 compared to T2 and T4 and in T5 compared to T1, T2 and T4 (p < 0.05) (Fig. 1B). Ubiquinol administration induced an increase in the OPG level, being statistically significant in T3 and T5 (p < 0.05). There were no differences due to the evolution of time in OPG levels in the ubiquinol and control groups (Fig. 1C). OPN decreased with exercise in T1, T2 and T4 in the control group and only in T5 in the ubiquinol group (p < 0.05). No differences were observed by the supplementation (Fig. 1D). With regard to SOST, an increase was observed in both groups in T3 with respect to T1, T2 and T4 (p < 0.05) (Fig. 1B). Ubiquinol showed no effect (Fig. 1E). OC increased in the ubiquinol group compared to the control group in T2 and T5 (p < 0.05). With regard to the evolution, in the ubiquinol group the highest value was observed in T3.
pared to T1 ($p < 0.05$), while in the control group in T3 an increase was observed compared to T1, T2, T4 and T5 ($p < 0.05$) (Fig. 1F). AP levels were higher in the ubiquinol group compared to the control group, being statistically significant in T2, T3 and T4 ($p < 0.05$). With regard to the evolution, no differences were observed (Fig. 1G).

Insulin was higher in the ubiquinol group compared to the control group in T4 ($p < 0.05$). With regard to the evolution, in the ubiquinol group the highest value was observed in T4 compared to T3 and T5 ($p < 0.05$), while in the control group an increase was observed in T2 compared to T3 and T5 ($p < 0.05$) (Fig. 2A). Leptin was always higher in the ubiquinol group compared to the control group, being statistically significant in T2, T3 and T4 ($p < 0.05$). With regard to the evolution, in both groups there were no statistically significant differences in the insulin levels (Fig. 2B).

Adrenaline levels do not show statistically significant differences between the groups. With regard to the evolution, the highest value in the ubiquinol group was observed in T3 compared to T1, T2 and T4 ($p < 0.05$). In the control group, the maximum value was also observed in T3 compared to the rest of the time line points ($p < 0.05$) (Fig. 3A). Noradrenaline increased in the ubiquinol group compared to the control group in T3 and T5 ($p < 0.05$). With regard to the evolution, in the ubiquinol group the highest level of noradrenaline was observed in T3 and T5 compared to T1, T2 and T4 ($p < 0.05$) (Fig. 3B).

PGC-1α increased in the ubiquinol group compared to the control group in T3, T4 and T5 ($p < 0.05$). With regard to the evolution, in the ubiquinol group the highest value of noradrenaline was observed in T3 and T5 compared to T1, T2 and T4 ($p < 0.05$) (Fig. 4A).

4. Discussion

According to the American College of Sports Medicine, moderate intensity, weight-bearing endurance activities are recommended to help preserve or increase bone mass in adults.21 It has been demonstrated that exercise may positively affect strength, density, cortical geometry and trabecular microarchitecture.22 The effect of physical activity on the process of bone remodeling can be direct (dependent on the type of physical activity, duration and especially load) and indirect through
PTH secretion. PTH is one of the main hormones involved in a decrease in serum total and ionized Ca, which is a trigger for bone resorption.\(^2\) Endocrine situations of high intensity or strenuous exercise, as can happen with elite athletes, can lead to a negative effect on bone tissue, both for possible long-term and short-term damages.\(^4\) In addition, when studying bone metabolism, it must be considered as part of a complex system that includes the mechanisms of central origin (SN), endemic (endocrine) and local.\(^3\) Before beginning to discuss the results obtained in this study, we would like to indicate that, previously, we have shown that the exercise protocol performed is of high intensity (increase in lactate of 290% after the first exercise test and 350% after the second exercise test) and induces muscle damage (increased myoglobin after the first and second exercise tests, 358% and 387% respectively, and increased CK-MM levels of 158% after the first exercise test and 358% and 387% respectively, and increased CK-MM levels of 158% after the first exercise test and 350% after the second exercise test) and induces muscle damage (increased myoglobin after the first and second exercise tests, 358% and 387% respectively, and increased CK-MM levels of 158% after the first exercise session and 196% after the second). These increases were similar to those observed in other strenuous exercise tests.\(^23\) We have, also, reported that our supplementation with ubiquinol effectively increases plasma CoQ10 up to five times higher levels.\(^9\)

The exercise protocol showed a clear effect on bone turnover, with a higher incidence in the biomarkers of formation, as has been indicated by other authors.\(^2\) Our exercise increased ACTH, PTH, osteocalcin and decreased OPN (related to a greater bone formation) and increased SOST (related to resorption). Also, our exercise protocol increased adrenaline, noradrenaline and PGC-1α, and decreased insulin, showing that all these results indicate a positive effect on the mobilization of glucose, fatty acids and energy metabolism.

Physical activity and especially strenuous exercise can cause a decrease in serum total and ionized Ca, which is a trigger for PTH secretion.\(^24\) PTH is one of the main hormones involved in Ca metabolism,\(^25–27\) and it has been assigned for catabolic and anabolic actions in bone.\(^5\) A continuous or chronic increase in the output of this hormone, as observed in pathological situations, stimulates the processes of bone resorption (through induction of the receptor activator for nuclear factor κ B ligand [RANKL] and inhibition of OPG),\(^26,28,29\) while intermittent increases, such as those associated with physical exercise, show a clear anabolic effect for bone tissue.\(^28\) This positive effect on bone formation is due to various mechanisms, stimulates the differentiation and proliferation of osteoblasts, and stimulates OC levels and decreases SOST.\(^28,29\) In addition, that beneficial effect of PTH has been associated with an increase in blood flow to bone showing a local vasodilatory effect.\(^30\) This aspect is interesting, given the importance of an adequate blood supply for a correct bone formation and the fact of having observed vasodilation and angiogenesis of the osseous blood vessels during exercise.\(^30\) In the current study, ubiquinol supplementation induced an increase in PTH (higher than that observed by exercise alone), which presumably could improve its effect on bone. In addition, a previous study of our research group\(^9\) showed a vasodilatory effect of ubiquinol supplementation by increasing nitric oxide (NO), which would improve this beneficial effect on bone tissue.

The bone remodeling process requires a balance between resorption and formation, with OPG/RANK signaling being a key path in regulating this balance.\(^7\) Our exercise protocol showed no effect on OPG, although in other studies the results have been contradictory;\(^31\) however, an increase was observed with our ubiquinol supplementation, which could have a beneficial effect on bone formation.\(^7\)

Another very interesting effect of the ubiquinol supplementation in the current study was the effect on AP, showing higher values than the placebo group in T2, T3 and T4. This enzyme, in the absence of liver damage, is one of the most used biomarkers of bone metabolism,\(^2\) indicating osteoblastic activity. In relation to exercise, no effect on this biomarker was observed, although in the literature there is controversy about the effect of exercise on alkaline phosphatase.\(^2\)

Together with PTH, OPG and AP, OC has also been reported to indicate the newly synthesized bone.\(^23\) OC is a non-collagenous protein of osteoblast origin, highly represented in the bone extracellular matrix and actively involved in bone formation,\(^32\) and its levels have a correlation with the number of osteoblasts and bone formation, as well as with the proportion of new bone generation.\(^5\) Our results show an increase in OC associated with our exercise protocol and that this increase is greater in the ubiquinol group and these hormones reflect the bone formation rate, fact that it is related with significant increments in bone mass.\(^33\) Therefore, in the light of these results, we can affirm that ubiquinol supplementation during exercise could increase bone turnover rate.

However, as we have commented previously, we cannot study bone turnover independently of the other physiological processes, being one of the reasons of this fact, which is the endocrine effect of OC.\(^5,8\) It has been described that this hormone favors adaptation to exercise, featuring a critical role on energy metabolism, by acting on glucose and fat metabolism, stimulation of insulin (bone-pancreas loop),\(^8\) prolifer-
ation of pancreatic cells and induction of adiponectin,\textsuperscript{2,3,6} and also, in muscle capacity and cognitive capacity.\textsuperscript{6} This clear link between OC and energy metabolism has led to consider that the changes in the activity of osteocalcin during physical exercise, mainly respond to an adaptation to a greater energy need and thereby to the mobilization of substrates such as glucose and fatty acids.\textsuperscript{8} It is important to emphasize once again that the effect on OC associated with our exercise protocol is increased by ubiquinol supplementation.

Another hormone that has been involved in this physiological link between bone turnover and energy metabolism and increased ubiquinol supplementation is leptin, an adipokine associated with appetite suppression regulation, energy homeostasis, immunology, and respiration, among other biological actions and that regulates energy metabolism by direct effects on central nervous system and skeletal muscle tissue.\textsuperscript{28} The relationship between leptin and bone tissue is complex and controversial. It seems to act through a direct peripheral pathway with stimulation on bone formation and growth (differentiation of osteoblasts, inhibition of apoptosis, increased mineralization and induced expression of OPG) and, also, through an indirect central pathway via hypothalamus and sympathetic nervous system that seems to reduce bone formation and have an effect on resorption.\textsuperscript{34} Therefore, although the effects of leptin on energy metabolism are clear, its influence on bone tissue is doubtful, indicating that it could be effective only in extreme situations.\textsuperscript{34}

Leptin also has a clear effect on insulin\textsuperscript{3,6} and enhances the expression of noradrenaline.\textsuperscript{34} The reduction of insulin, associated with sport,\textsuperscript{35} reduces gluconeogenesis and increases the bioavailability of glucose and fatty acids, an effect that is enhanced by the increase in adrenaline and noradrenaline.\textsuperscript{36,37} Thus, an increase in noradrenaline associated with physical exercise shows a clear effect on promoting glycogenesis and lipolysis,\textsuperscript{36} but also has an effect on bone metabolism, increasing PTH and favoring the bone resorption processes, thanks to the β2-adrenergic receptors existing in osteoblasts and osteoclasts.\textsuperscript{34} However, more recent studies report the existence of α receptors, involved in the formation processes,\textsuperscript{\textsuperscript{18,39}} thus showing a complex and controversial relationship with bone tissue that requires further research.

Finally, we have recorded an increase in PGC-1α associated with exercise with a higher increase in the ubiquinol group. PGC-1α is a major factor involved in the regulation of a diverse array of physiologic processes including adipogenesis, lipid metabolism, insulin sensitivity and inflammation, interacting with several nuclear receptors and transcription factors to activate the transcription of their target genes.\textsuperscript{40,41} Additionally, in skeletal muscle, PGC-1α has also been shown to regulate skeletal muscle fiber type switch (favoring the exchange of glycolytic fibers to oxidative fibers), glucose transport, lipid utilization and mitochondrial biogenesis and fusion.\textsuperscript{41–43} In relation to its effect on bone turnover, it has been observed that its loss in the mesenchymal cells results in an increased bone loss, due to a decrease in the formation processes and increased adipogenesis in the bone marrow,\textsuperscript{41} having a positive effect on the bone due to its effect on oxidative stress and inflammation.\textsuperscript{44} As we have commented, in the current study, the expression of PGC-1α increased after exercise and especially with ubiquinol supplementation, which could have substantial beneficial effects on improving glucose transport, fatty acid oxidation and the mitochondrial function of skeletal muscle,\textsuperscript{41,45} representing an ergogenic effect on the skeletal muscle. In addition, the increase in noradrenaline recorded after the exercise tests in the ubiquinol group indicates a stimulation of glucose uptake and clearance, enhancing the ability to shift to higher levels of muscular carbohydrate use,\textsuperscript{37} representing also a metabolic advantage for the myofibres, because the post-exercise hyperglycaemia induced by noradrenaline is necessary for muscle glycogen repletion.\textsuperscript{46}

5. Conclusion

To the best of our knowledge, this is the first study to show beneficial effects of ubiquinol supplementation on bone and energy metabolism during strenuous exercise, which could draw high interest in physical performance and muscle recovery during exercise (especially of high intensity). Ubiquinol supplementation is able to enhance bone turnover (especially bone formation) and block some specific detrimental effects, improving bone metabolism during strenuous exercise. In addition to the effects on bone, ubiquinol increased OC, PGC-1α, insulin, leptin and noradrenaline, which can exert positive effects on skeletal muscle fiber switch, glucose transport and lipid utilization in skeletal muscles, mitochondrial biogenesis and fusion and muscle glycogen repletion and recovery, and these could represent an ergogenic and physiological advantage for the skeletal muscles. Therefore, the knowledge gained from these findings reveals the benefit of ubiquinol supplementation in athletes performing strenuous exercise in order to improve bone turnover and energy metabolism during high intensity exercise.

Author contributions

JJO designed the research proposal and provided the funding. JDC, PJMR and JMF conducted the research and wrote the manuscript. IC and RG revised the data and the manuscript. All authors read and approved the final draft of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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References


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