Original Communication

High coenzyme Q10 plasma levels improve stress and damage markers in professional soccer players during competition

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Abstract: Ubiquinol, the reduced form of Coenzyme Q_{10} (CoQ_{10}), is a key factor in bioenergetics and antioxidant protection. During competition, professional soccer players suffer from considerable physical stress causing high risk of muscle damage. For athletes, supplementation with several antioxidants, including CoQ_{10}, is widely recommended to avoid oxidative stress and muscle damage. We performed an observational study of plasma parameters associated with CoQ_{10} levels in professional soccer players of the Spanish First League team Athletic Club de Bilbao over two consecutive seasons (n = 24–25) in order determine their relationship with damage, stress and performance during competition. We analyzed three different moments of the competition: preterm, initial phase and mid phase. Metabolites and factors related with stress (testosterone/cortisol) and muscle damage (creatine kinase) were determined. Physical activity during matches was analyzed over the 2015/16 season in those players participating in complete matches. In the mid phase of competition, CoQ_{10} levels were higher in 2015/16 (906.8 ± 307.9 vs. 584.3 ± 196.3 pmol/mL, p = 0.0006) High levels of CoQ_{10} in the hardest phase of competition were associated with a reduction in the levels of the muscle-damage marker creatine kinase (Pearsons' correlation coefficient (r) = − 0.460, p = 0.00168) and a trend for the stress marker cortisol (r = −0.252, p = 0.150). Plasma ubiquinol was also associated with better kidney function (r = −0.287, p = 0.0443 for uric acid). Furthermore, high CoQ_{10} levels were associated with higher muscle performance during matches. Our results suggest that high levels of plasma CoQ_{10} can prevent muscle damage, improve kidney function and are associated with higher performance in professional soccer players during competition.

Keywords: Coenzyme Q_{10}, creatine kinase, muscle damage, muscle performance, soccer, kidney function, cortisol

Introduction

The soccer season is a hard and strenuous sport competition. In Spain, the combination of League, Spanish Soccer Cup and European Championships require that two matches be played per week during the hardest part of the season. This leads to stress in musculoskeletal, nervous, immune and metabolic systems in professional soccer players [1]. Participation in a single soccer match can produce acute and residual fatigue, characterized by the decline in physical performance over the following hours and days [2]. Muscle damage, determined by the levels of intramuscular enzymes such as creatine kinase (CK) and inflammatory markers, increases after matches [2–4]. During intense competition periods, accumulation of muscle damage can severely affect aerobic endurance [5].

A wide range of nutritional supplements are used in sports nutrition [6], some of them have been reported to avert or prevent exercise-induced muscle damage [7]. Many antioxidant supplements are used in sport in the belief that they will reduce muscle damage. However, to date acute intake of some of them is likely to be beneficial although chronic intakes of most of them can have harmful effect on performance [8]. Other compounds such as branched-chain amino acids seems to decrease delayed onset muscle soreness when supplemented before exercise

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[9]. Coenzyme Q_{10} (CoQ_{10}) is a lipid-soluble antioxidant that prevents lipid peroxidation in cell membranes and plasma lipoproteins [10, 11]. Near total CoQ_{10} in blood plasma is in the reduced form (ubiquinol), and its levels have been inversely associated with cholesterol oxidation in both active and sedentary people [12, 13]. CoQ_{10} is also an essential factor in the prevention of oxidative damage in cell membranes [10, 14, 15]. In addition to its widely known activity as an antioxidant, the main activity of CoQ_{10} in cells is as an essential component of the electron transport chain transferring electrons from complexes I and II to complex III, to finally produce ATP synthesis through oxidative phosphorylation [16]. For this reason, supplementation with CoQ_{10} has been associated with the improvement of many mitochondrial related dysfunctions including muscle capacity [17].

CoQ_{10} has been used in many sport studies to determine its effect on the prevention of oxidative stress or inflammation. These studies differ in the dose and time of supplementation, in the characteristics of the CoQ_{10} formulation and the type of physical test. Several studies showed little or no improvement [18, 19], others showed improvement in prevention of oxidative stress and maintenance of physical capacity [20–22], while others reported impairment of the capacity of participants [23, 24]. Many of these studies included non-professional healthy participants, essentially men. In most cases, the effect of a short-term supplementation was determined after only a single bout of physical activity or exercise. The most important problem of many of these studies is that, in general, authors do not determine the incorporation of CoQ_{10} into blood plasma and thus, they do not know if the compound reaches enough levels in the organism [18, 23–25]. We know that the bioavailability of CoQ_{10} varies enormously among formulations depending on the redox nature of the compound and the characteristics of the vehicle [26]. Therefore, these differences can severely affect studies with CoQ_{10} supplementation in athletes.

We hypothesized that intermittent exercise exerted during soccer matches would benefit from higher concentrations of CoQ_{10} by preventing skeletal muscle damage and stress. Therefore, the aim of this study was to determine if plasma CoQ_{10} levels in professional soccer players are associated with muscle damage, stress, physiological parameters and performance over two consecutive seasons of competition. For this reason, we determined the levels of CoQ_{10} in plasma of professional soccer players in three different moments of two correlative seasons. Our study did not affect the nutritional rules of the medical and nutritional team of the Athletic Club de Bilbao or alter the normal development of the preparation of participants. Our results can help to design further studies in order to establish when chronic supplementation with CoQ_{10} can be useful for professional athletes during competition.

Materials and methods

Overview

This is an observational study about the relationship of plasma levels of CoQ_{10} during soccer competition and other physiological markers. Participants from the Athletic Club de Bilbao were followed over the 2014/15 and 2015/16 soccer seasons. Most of the players remained in the study in both seasons due to the low turnover of players in this soccer team. Researchers did not interfere in the particular indications of the nutritional team of the Athletic Club de Bilbao on the participants in the study and received and analyzed the samples in a double blind system.

Chemicals used in this study were from Merck, Barcelona, Spain except when indicated.

Participants

Participants in this study were professional players of the Athletic Club Bilbao team competing in the first division of the Spanish soccer league, in the 2014/15 and 2015/16 seasons. Inclusion criteria were to participate as player of the professional first team of the club and to sign the informed consent. Regarding the medical routine checking procedure at the Athletic de Bilbao team, height, weight, body mass index (BMI), and fat percentage were determined. Fat percentage was determined by six skinfold method (over triceps, subscapular, suprailiac, abdominal, thigh and leg) (Table 1). We did not find significant differences in the physiognomic parameters of the participants between both seasons. Players followed the nutritional program during the competition depending on the normal evolution of the season and according to the instructions of the nutrition service of the Club in a blind procedure for the researchers.

All procedures were performed according to the ethical rules approved by the Pablo de Olavide University ethical committee (ethical code 2013/00141) and the Athletic de Bilbao Football Club medical team and following the World Medical Association Declaration of Helsinki ethical principles for medical research involving human subjects. The head of the medical department (J.A. Lekue) and the nutritionist (T. Viar) supervised the routine medical and nutritional procedures with professional players of the Athletic Club de Bilbao. Players were informed and signed an informed consent to participate in this study.

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Table 1. Characteristics of the participants in this study

	Seasons		
Parameter	2014/15	2015/16	P value
Age (years)	25.8 ± 4.4	27.0 ± 3.8	0.320
Weight (kg)	76.7 ± 6.6	77.0 ± 6.4	0.843
Height (m)	1.81 ± 0.06	1.83 ± 0.05	0.396
BMI (kg/m^2)	23.3 ± 1.2	23.1 ± 1.3	0.543
Fat (%)	7.19 ± 1.03	7.09 ± 0.75	0.684
N	24	25	

Data represent the mean ± SD. Statistical significance between the participants during each season is indicated (unpaired Student's t was performed). BMI: Body Mass Index.

Blood extraction for biochemical determinations in plasma

Periphery blood sampling was performed following the routine medical procedures of the Athletic Club de Bilbao supervised by its medical team. Samples were always taken after O/N fasting (at least 8 hours) between 8 and 9 in the morning two days after the last match or training and after a resting period of one day. Antecubital blood was taken by venipuncture 48 h after the last match at three different moments of the competition: pre-season (July), beginning of the competition (around September) and mid-season (between March-April). Blood samples were collected in tubes containing EDTA or lithium heparin and immediately centrifuged at 3000 × g for 10 min at room temperature. Plasma was collected and stored at −80 °C until determinations. Plasma samples were analyzed by Axpe Clinical Laboratory and the Laboratory of Cell Physiopathology and Bioenergetics at the Pablo de Olavide University. All samples were labelled with a code hiding the identity of the players to the researchers.

CoQ_{10} determination in plasma.

Plasma CoQ_{10} was extracted and determined from 100 µl plasma using 100 pmol CoQ_6 as internal control [27]. Sodium dodecyl sulphate (1%) was added to the sample and vortexed immediately for 1 min. Double the volume of the sample of ethanol:isopropanol (95:5) was added and mixed again for 1 min. After that, 600 µL hexane were added to the mixture and vortexed again for 1 min. After centrifugation at 1000 × g at 4 °C, the upper organic phase was removed and stored. Organic extraction was repeated twice and all the upper organic phases mixed. CoQ_{10}-containing organic phase was dried with speed-vac at 35 °C. Dried lipid extract was dissolved in 60 µL ethanol and injected in duplicate in HPLC with a 20 µL loop.

Lipid components were separated by a HPLC system Beckman 166–126 (Beckman Coulter, Brea, California, USA) equipped with a 15-cm Kromasil C-18 column (Sigma-Aldrich, Barcelona, Spain) maintained at 40 °C in a flux of 1 mL/min of mobile phase of 65:35 methanol/2-propanol and 1.42 mM lithium perchlorate. Total levels of CoQ_{10} were detected by an electrochemical detector and expressed as nmol/L.

Blood metabolites analysis

Samples were analyzed using routine techniques for blood determinations in AXPE clinical laboratory (AXPE, Bilbao, Spain). Determinations of cholesterol, glucose, GPT, GOT and CK were carried out by spectrophotometry in a Cobas c501 auto-analyzer (Roche Diagnostics, Basel, Switzerland) using reagents from the same manufacturer: CHOL2 Cholesterol oxidase, esterase, peroxidase; GLUC3 Hexokinase; ALTL IFCC; ASTL IFCC, CKL Test UV. Determinations of Testosterone and Cortisol were performed by chemiluminescence in a COBAS e601 immunoanalyzer (Roche Diagnostics, Basel, Switzerland) using reagents from the same manufacturer: TESTO II, CORTISOL II. Furthermore, samples used for CoQ determinations were also analyzed for cholesterol (Chol Reflotron: 10745065) and HDL-cholesterol (HDL Reflotron: 11208756) using the Reflotron plus 2008 (Roche Diagnostics, Basel, Switzerland) system, checked and optimized following the indications of the manufacturer. Triglycerides were determined using a specific kit (QCA S.A. Ref: 992320, Amposta, Spain). The presence of LDL-cholesterol was determined following the Friedewald formula [28]. Creatine kinase (CK) levels were re-tested in samples received in CABD by using the Reflotron system (CK Reflotron: 11126695202, Basel, Switzerland).

Physical activity determination during matches

To determine the capacity of the players in matches during competition, Athletic de Bilbao soccer team provided data obtained by video analysis using the MediaCoach system (Mediapro, Barcelona, Spain). Analysis of data was performed only from those players who played complete matches during the 2015/16 season.

Statistical analysis

Statistical analysis was performed using SigmaPlot 12.5 (Systat Software Inc, Chicago, USA) and GraphPrism 6.01 (GraphPad Software, San Diego, USA) software. Data is indicated as the mean ± SD. Comparison between two groups was performed by using the Two-tailed unpaired

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Student's t test applying the Shapiro-Wilk normality test and the F-test for equality of variances. Analysis of more than two groups was performed by One-way ANOVA test accompanied by a Bonferroni post-hoc analysis applying the Kolmogorov-Smirnov normality test and the Brown–Forsythe test for equality of variance. Pearson's correlation analysis was used to determine the relationship between the two parameters and a two-tailed p value was considered. Statistical significance was determined with $p <$ or equal to 0.05.

Results

Levels of CoQ_{10} in plasma vary during the season

In order to study the evolution of CoQ_{10} in plasma during competition, we determined different blood parameters including CoQ_{10} during pre-season (July), initial phase of the season (September) and mid-season (March–April) in the 2014/15 and 2015/16 (Table 2) seasons.

During the first season, we observed high levels of CoQ_{10} in plasma at pre-season although these levels decreased in the initial phase of the competition (Table 2). This difference can be attributed to dietary conditions during holidays and they returned to normal levels at the beginning of the competition. Levels of all the parameters fall to the normal range in a young and active population. Plasma CoQ_{10} levels increased at midseason, when competition is harder. As CoQ_{10} in plasma is transported by cholesterol particles, the relative levels of CoQ_{10} to cholesterol and its different forms in blood were also higher during mid-phase of the competition.

To analyze muscle damage, we determined the levels of creatine kinase (CK) in plasma. We observed a significant increase at mid-season in relationship with the values obtained at the initial phases of the season. These higher values can be associated with a higher overload during the hardest part of the season. In the case of other markers of muscle damage such as plasma transaminase levels, a significant increase was also found with GOT during mid-phase of the season in comparison with the initial phases. No significant increase was found with other transaminases such as GPT or GGT (Table 2).

The same analysis was performed during the second season (2015–16). We did not observe differences in parameters related with cholesterol and TGs in plasma throughout the season (Table 2). In this season, mean levels of CoQ_{10} were normal at the initial phase of the competition and showed a significant increase during mid-phase in comparison with the 2014/15 season. CoQ_{10} increase was also significant when its levels were related with cholesterol and TGs (Table 2).

In contrast to 2014/15 season, during 2015/16 season, markers of muscle damage showed a low increase at mid-phase but without reaching significant differences vs. the initial phases of the competition. The same occurred with GOT, that showed levels similar to those found during the previous phase of the competition. Levels of cortisol in this season decreased at mid-phase of the competition in comparison with the previous and initial phases.

Comparison of CoQ_{10} levels during mid-season

In order to determine the importance of CoQ_{10} during the hardest phase of the competition, we compared the levels of CoQ_{10}, CK and cortisol during mild-phase in both seasons (Figure 1). Levels of CoQ_{10} in plasma were significantly higher at mid-phase of the second season (Figure 1A). Interestingly, during this phase, levels of CK (Figure 1B) and cortisol (Figure 1C) were lower in comparison with the 2014/15 season, indicating a decrease of both muscle damage and stress in parallel with the increase of plasma CoQ_{10} in the players.

High levels of CoQ_{10} in plasma are associated with lower muscle damage

In order to determine if there is a relationship between CoQ_{10} levels in plasma and the degree of muscle damage or stress during the mid-phase of the season, we distributed the population into two groups depending on the level of CoQ_{10} in plasma according to the median of distribution of CoQ_{10} in plasma in humans (Figure 2). Players showing levels of CoQ_{10} below the median of the participants, showed significantly higher levels of CK in plasma than those with higher levels of CoQ_{10} (Figure 2A). The same relationship was found in the case of cortisol levels but without reaching a significant relationship (Figure 2B). Low levels of CoQ_{10} in plasma showed a clear and significant negative correlation with high muscle damage during the more intense phase of soccer competition (Figure 2C). In relationship with cortisol levels, a trend towards a negative relationship was also found, but in this case, the correlation was not significant (Figure 2D).

Higher levels of CoQ_{10} in plasma affected kidney function during 2015/16 season

During the 2015/16 season, we found that levels of uric acid, urea and creatinine, markers of kidney function, decreased during mid phase of the championship. This decrease was significant in the case of uric acid and creatinine (Figure 3). In this season, CoQ_{10} levels of the

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Table 2. Blood parameters during 2014/15 and 2015/16 seasons

		Groups			
Parameter	Season	Previous	Initial	Mid	P value
Cholesterol (mg/dL)	2014/15	156.7 ± 30.8	148.6 ± 22.7	160.6 ± 26.8	0.3411
	2015/16	182.5 ± 32.5	175.2 ± 47.6	165.5 ± 47.6	0.4678
HDL-chol (mg/dL)	2014/15	54.1 ± 12.1	46.9 ± 12.9	51.3 ± 10.4	0.1751
	2015/16	66.7 ± 11.9	59.2 ± 11.8	63.9 ± 15.3	0.1783
LDL-chol (mg/dL)	2014/15	84.1 ± 29.2	82.9 ± 23.19	90.5 ± 25.8	0.5861
	2015/16	103.6 ± 31.7	106.4 ± 30.4	95.7 ± 31.17	0.5226
Ratio LDL-chol/HDL-chol	2014/15	1.66 ± 0.71	1.90 ± 0.68	1.86 ± 0.68	0.5092
	2015/16	1.62 ± 0.54	1.88 ± 0.65	1.62 ± 0.69	0.3083
TGs (mg/dL)	2014/15	92.6 ± 22.9	93.6 ± 31.4	94.0 ± 32.6	0.9895
	2015/16	72.4 ± 27.4	85.9 ± 33.1	77.7 ± 22.1	0.2968
Glucose (mg/dL)	2014/15	89.3 ± 5.3	89.4 ± 5.7	85.2 ± 6.9	0.0682
	2015/16	88.2 ± 7.0	86.8 ± 7.1	88.3 ± 6.7	0.7188
Urea (mg/dL)	2014/15	33.8 ± 6.2	34.8 ± 5.4	36.9 ± 7.1	0.3222
	2015/16	39.9 ± 8.8	39.3 ± 5.8	36.5 ± 6.5	0.2877
Uric acid (mg/dL)	2014/15	5.1 ± 0.8	4.5 ± 0.8	4.6 ± 0.8	0.0744
	2015/16	5.3 ± 0.9	5.0 ± 0.9	4.3 ± 0.7 [a,b]	0.0012
Creatinine (mg/dL)	2014/15	1.22 ± 0.71	1.07 ± 0.14	1.06 ± 0.12	0.4081
	2015/16	1.09 ± 0.13	1.17 ± 0.13	0.99 ± 0.10 [a,b]	<0.0001
CoQ_{10} (nmol/L)	2014/15	817.7 ± 223.8	495.40 ± 163.8 [a]	584.3 ± 196.3 [a]	0.0001
	2015/16	646.1 ± 160.2	636.1 ± 203.3	906.8 ± 307.9 [a,b]	0.0002
CoQ_{10}/Chol (nmol/mmol)	2014/15	204.7 ± 51.2	130.8 ± 41.5[a]	146.8 ± 97.4 [a]	0.0002
	2015/16	122.2 ± 19.7	122.6 ± 33.3	190.3 ± 79.5 [a,b]	<0.0001
CoQ_{10}/HDL-chol (nmol/mmol)	2014/15	605.5 ± 184.7	434.8 ± 207.6 [a]	446.4 ± 156.6 [a]	0.0089
	2015/16	445.8 ± 138.5	426.0 ± 148.7	520.1 ± 217.7	0.185
CoQ_{10}/LDL-chol (nmol/mmol)	2014/15	424.9 ± 204.8	270.4 ± 198.7 [a]	287.5 ± 166.9	0.0293
	2015/16	194.6 ± 38.9	211.8 ± 92.4	405.2 ± 283.6 [b]	<0.0001
CoQ_{10}/TGs (nmol/mmol)	2014/15	790.9 ± 206.4	472.9 ± 97.7 [a]	639.6 ± 360.6	0.0018
	2015/16	822.7 ± 248.2	732.1 ± 291.2 [a]	1518.0 ± 629.3 [a,b]	<0.0001
GPT (U/L)	2014/15	21.4 ± 5.9	22.0 ± 7.9	27.1 ± 11.7	0.1004
	2015/16	26.3 ± 10.9	20.0 ± 5.4	26.6 ± 8.4 [a,b]	0.0205
GOT (U/L)	2014/15	27.6 ± 8.4	25.5 ± 7.2	34.6 ± 11.3 [a,b]	0.0101
	2015/16	30.4 ± 10.9	22.0 ± 3.8 [a]	32.8 ± 7.9 [b]	0.0001
GGT (U/L)	2014/15	18.3 ± 6.2	17.7 ± 5.0	17.0 ± 5.5	0.7906
	2015/16	21.8 ± 8.6	19.3 ± 6.8	21.5 ± 8.1	0.5362
CK (U/L)	2014/15	82.3 ± 52.8	96.4 ± 75.3	365.2 ± 292.8 [a,b]	<0.0001
	2015/16	171.3 ± 146.8	112.0 ± 60.7	149.9 ± 87.5	0.1401
Testosterone (ng/ml)	2014/15	7.42 ± 1.52	6.91 ± 1.46	7.46 ± 1.27	0.4152
	2015/16	7.51 ± 2.10	6.52 ± 1.22	6.25 ± 1.58	0.047
Cortisol (μg/dL)	2014/15	24.8 ± 4.6	21.0 ± 5.4 [a]	22.80 ± 3.2	0.0438
	2015/16	23.0 ± 4.5	22.4 ± 2.8	15.5 ± 2.2 [a,b]	<0.0001

Data represent the mean ± SD. Statistical analysis was performed by using one way ANOVA analysis followed by Bonferroni post-hoc analysis by a Bonferroni post-hoc analysis applying the Kolmogorov-Smirnov normality test and the Brown–Forsythe test for equality of variance. Significant differences in each group are indicated; [a], significant differences vs. previous conditions, $p < 0.05$; [b], significant differences vs. initial conditions, $p < 0.05$. N for 2014/15 season = 24, and for 2015/16 season = 25. CK: creatine kinase; Chol: cholesterol; CoQ_{10}: Coenzyme Q_{10}; GGT: Gamma-glutamyltransferase; GPT: glutamate pyruvate transaminase; GOT: glutamic oxaloacetic transaminase; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; TGs: triglycerides.

participants were significantly higher at mid-phase of the championship (Figure 1). Thus, we sought to determine if there was a correlation between CoQ_{10} levels and these kidney-related parameters in plasma. We found a significant negative correlation with uric acid and a trend with urea and creatinine levels suggesting a positive effect of CoQ_{10} on kidney function in athletes under continuous training and during competition.

Higher levels of CoQ_{10} in plasma can be associated with higher physical performance

Physical performance of the players during the Spanish Soccer League (2015/16 season) was also determined. We studied the distance covered and mean speed in those players who participated the whole time in each match for all the

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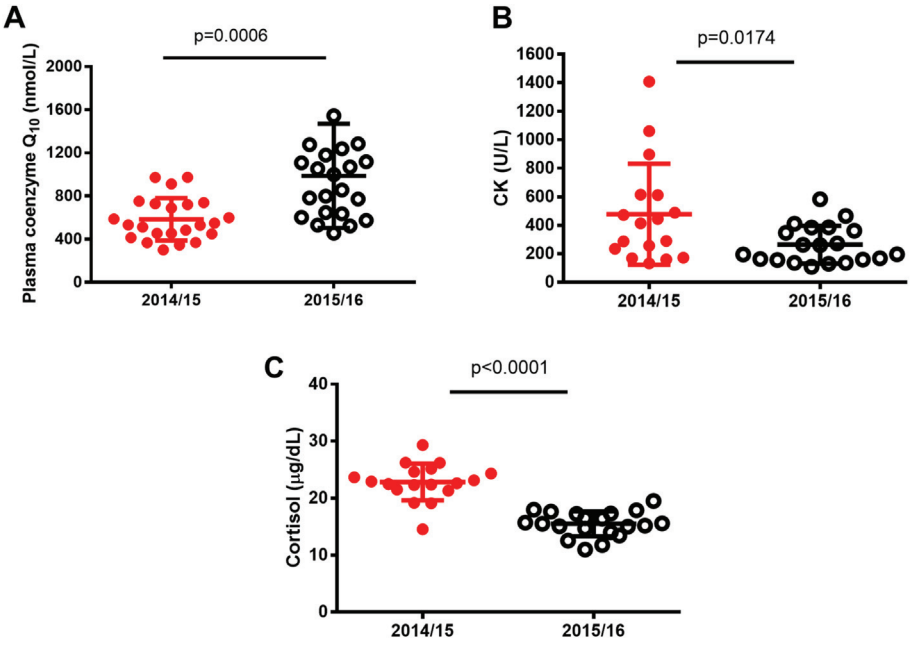

Figure 1. Plasma levels of samples at mid-competition during 2014/15 and 2015/16 seasons. A) CoQ_{10} levels in nmol/L. B) CK levels in U/L. C) Cortisol levels in µg/dL. Lines represent the mean ± SD of each group. Statistical differences are indicated. Non-paired student t test was performed; two-tailed significance is indicated in each case.

38 matches of the competition and correlated them with the mean level of CoQ_{10} in plasma during this season. In our analysis, the participation of the goalkeepers was not taken into consideration since they covered less distance (around 40% of a field player) with less mean speed due to their function in the match.

Although without reaching significant differences, players showing higher levels of CoQ_{10} showed a tendency to maintain higher performance during the whole match but especially during the first part (Figure 4). Both the mean distance and mean speed during the whole match (Figures 4A and 4B) and the first part (Figures 4C and 4D) were slightly higher in people showing high CoQ_{10} levels in plasma. However, this apparent improvement was lost during the second part of the matches (Figures 4E and 4F).

Discussion

The aim of this study was to determine if plasma CoQ_{10} levels in professional soccer players are associated with muscle damage, stress, physiological parameters and performance over two consecutive soccer seasons. We show here that plasma CoQ_{10} levels negatively correlated with muscle damage, cortisol and kidney function markers, indicating the benefit of maintaining high levels of CoQ_{10} in plasma in professional soccer players during competition. At the same time, we also found a trend to improve physical performance during matches.

CoQ is a key factor in oxidative phosphorylation and in antioxidant protection of cell membranes and plasma lipids. All cells have the capacity to synthesize CoQ but plasma CoQ levels in mammals can be associated with nutritional habits [29]. Biosynthesis in mammals involve a highly regulated multiprotein complex that has not been fully elucidated [30, 31], and its biosynthesis is impaired by genetic defects causing severe diseases that mainly involve skeletal muscle function [32]. Likely, CoQ absorption from plasma and distribution into cells and tissues is limited, which explains the biosynthesis requirement of each cell for proper survival [33]. However, human conditions like CoQ_{10} deficiency syndrome and less severe deficiency such as aging require CoQ_{10} treatment to improve tissue and organ functions [17].

Continuous practice of exercise seems to reduce CoQ_{10} plasma levels in young people [13] and people with higher aerobic capacity show lower circulating levels of antioxidants, including CoQ_{10} [34]. Moreover, ubiquinol, the active form of CoQ_{10}, in plasma is depleted during extenuating exercise, indicating its functional activity as an antioxidant [35].

It is widely known that exhaustive exercise causes oxidative stress and structural damage in muscle cells [36]. We can speculate that participation in the stressful season of soccer can produce accumulative damage in muscles and that higher CoQ_{10} in plasma can protect against this damage. Interestingly, despite the importance of CoQ_{10} in mitochondrial functions and muscle capacity in several pathologies [17], its use as a nutritional supplement in sports

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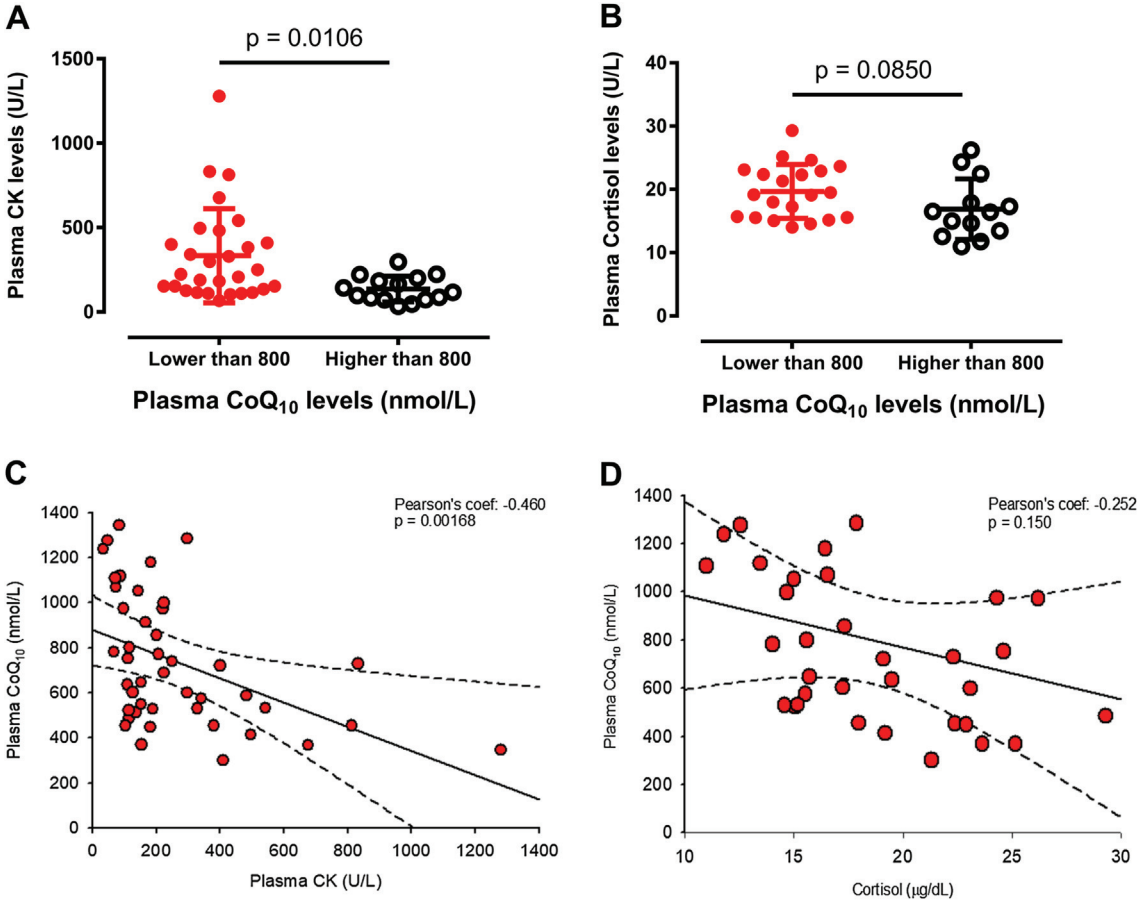

Figure 2. Comparison of the levels of plasma creatine kinase (CK) and cortisol with the amount of CoQ_{10} in plasma during mid-phase of the competition. Lines represent the mean ± SD of each group. Participants were divided in two groups depending on the median of CoQ_{10} at mid phase of the competition during the 2014/15 and 2015/16 seasons. A) Levels of plasma CK; B) levels of Cortisol. Correlation between plasma CoQ levels and CK levels (C) and Cortisol (D) at mid-phase of the competition during both seasons. Pearson's coefficient (solid line) and 99% confidence interval (dashed lines) and statistical differences are indicated. Non-paired student t test was performed; two-tailed significance is indicated in each case.

is under controversy. Most of the studies on the effect of supplementation with CoQ_{10} in muscle damage and capacity have been carried out in animal models [37–40]. In rats, supplementation with CoQ_{10} prevents the increase in CK in plasma after physical activity in an effect associated with the stabilization of muscle membranes [38]. CoQ_{10} also prevents the release of CK and lactate dehydrogenase (LDH) after strenuous exercise [37]. These results can be explained by the significant increase of CoQ_{10} found in muscles of these animals after supplementation [37].

The importance of CoQ_{10} in muscle activity and its effect on physical capacity in CoQ_{10}-deficient diseases in humans is clear [10, 41]. However, the number of studies on the CoQ_{10}-dependent protective effect developed in healthy humans is very low. These studies showed a high variability in the results since the dose of CoQ_{10}, the duration of the supplementation and the type and extent of exercise differ enormously. Probably this is the main cause that explains why in several studies, no effect of CoQ_{10} on muscle damage or performance was found [25, 42]. In some cases supplementation with CoQ_{10} has been associated with low physical performance [23] or even with cellular damage after extenuating exercise [24]. In other cases, the effect of supplementation with CoQ_{10} on physical capacity, muscle activity and muscle damage was null [19, 21], or very low [25, 43, 44]. Most of the studies performed on humans are based on short-term supplementation with different concentrations of CoQ_{10}, and study their effect after only a single bout of exercise, one match, or a series of exercises. Furthermore, many of the studies do not determine the actual levels of CoQ_{10} in plasma in order to control if supplementation was successful [18, 23, 24, 42]. Most interestingly, no study has followed the evolution of CoQ_{10} levels in professional athletes along two consecutive seasons of competition in order to determine their behavior in comparison with physiological markers.

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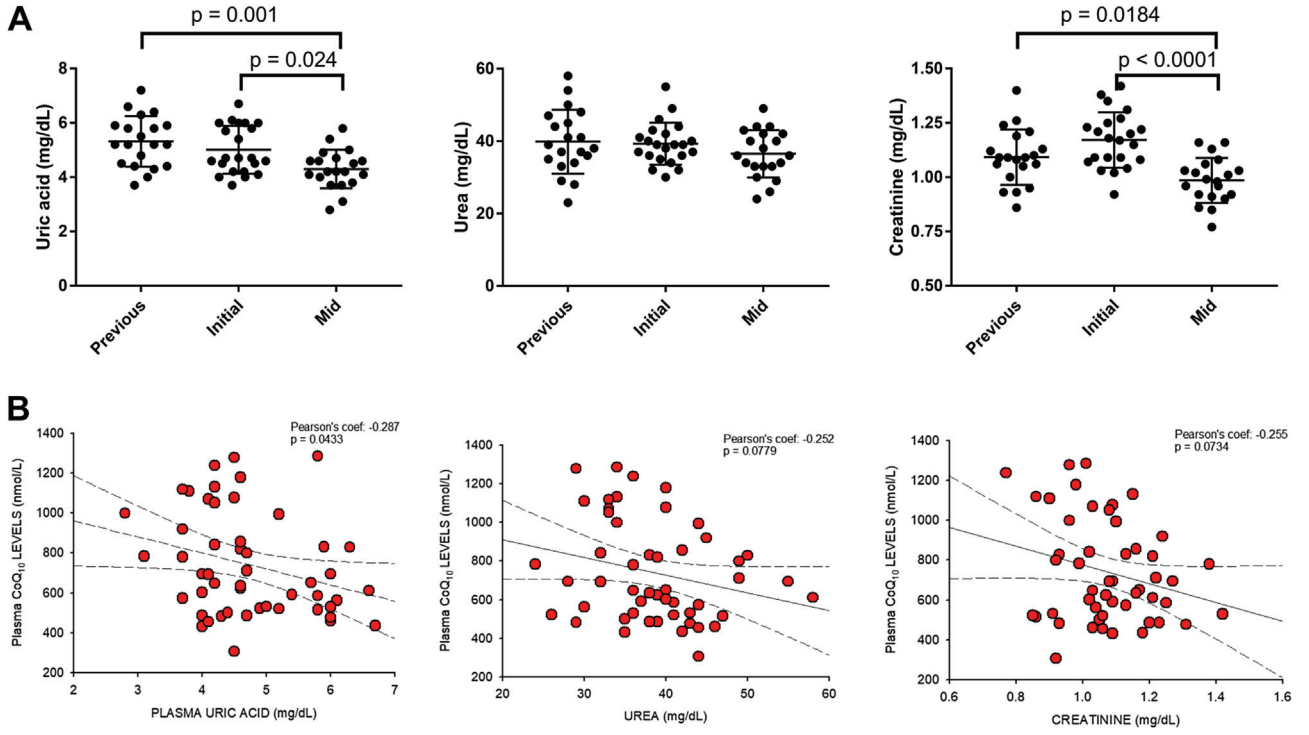

Figure 3. Comparison of the levels of plasma uric acid, urea and creatinine with CoQ_{10} during the 2015/16 season. A) Uric acid, urea and creatinine levels (mg/dL). B) Statistical differences (ANOVA analysis) are indicated. B) Correlation between plasma CoQ_{10} levels and uric acid, urea and creatinine levels during the 2015/16 season. Lines represent the mean ± SD of each group. Pearson's coefficient (solid line) and 95% confidence interval (dashed lines) and statistical differences are indicated.

Nutritional supplementation with CoQ_{10} does not guarantee its incorporation into plasma. For unknown reasons, some people do not incorporate supplemented CoQ_{10} [30]. Furthermore, bioavailability depends to a large degree on the carrier lipids used in the preparation of CoQ_{10} [26, 45]. Probably, this is the reason why in many of the studies performed on athletes, the amount of CoQ_{10}, used as a supplement, was not enough to increase plasma concentrations to such levels as to improve physical activity or protect against oxidation. Early studies in humans showed detrimental or null effect of CoQ_{10} supplementation in muscle damage and performance [46]. Moreover, the incorporation of CoQ_{10} into muscles after supplementation was not determined. It seems clear that low doses and short-term supplementation with CoQ_{10} can be insufficient to produce any increase in muscles explaining the null effect of CoQ_{10} on muscle capacity found in many of these studies [19].

In other studies, chronic supplementation with CoQ_{10} in young and healthy humans did not show significant effect on muscle CoQ_{10} levels [18] although, in agreement with our results, their physical capacity in repeated bouts of supramaximal exercise was improved, indicating a positive effect of CoQ_{10} in muscle performance [18]. A dose-dependent effect was found in 17 healthy volunteers supplemented with CoQ_{10}, in whom subjective fatigue sensation and physical performance were improved [47]. The increase of plasma CoQ_{10} levels was associated with lower fatigue and higher performance indexes [44, 48], indicating that a supplementation able to effectively increase ubiquinol levels in plasma can improve muscle capacity. Supplementation with 100 mg/day CoQ_{10} for 8 weeks in 14 athletes was enough to delay muscle exhaustion, which was reached at higher workloads in the CoQ_{10} group [49]. Furthermore, 300 mg/day ubiquinol for 6 weeks enhanced physical performance in healthy trained German Olympics athletes [50]. In swimmers, oral administration of CoQ_{10} for 14 days inhibited oxidative stress and plasma indices of muscle and liver damage [51] and even myocardial damage [52] during competition. These findings agree with our data that suggest higher CoQ_{10} levels in plasma can be associated with higher performance during soccer matches. Although not reaching statistical significance in our study, a trend is suggested (Figure 4). Distance and mean speed during the match are associated with aerobic physical conditions and this is associated with higher CoQ_{10} levels. This fact agrees with the positive relationship between CoQ_{10} levels in muscle and the percentage of type I fibers in muscle from healthy and active males [53] although in other studies, supplementation with CoQ_{10} in aged individuals was associated with the induction of type IIb fibers [54]. Interestingly, these studies suggest an age-dependent effect that must be studied in depth. Probably, CoQ_{10} levels in plasma

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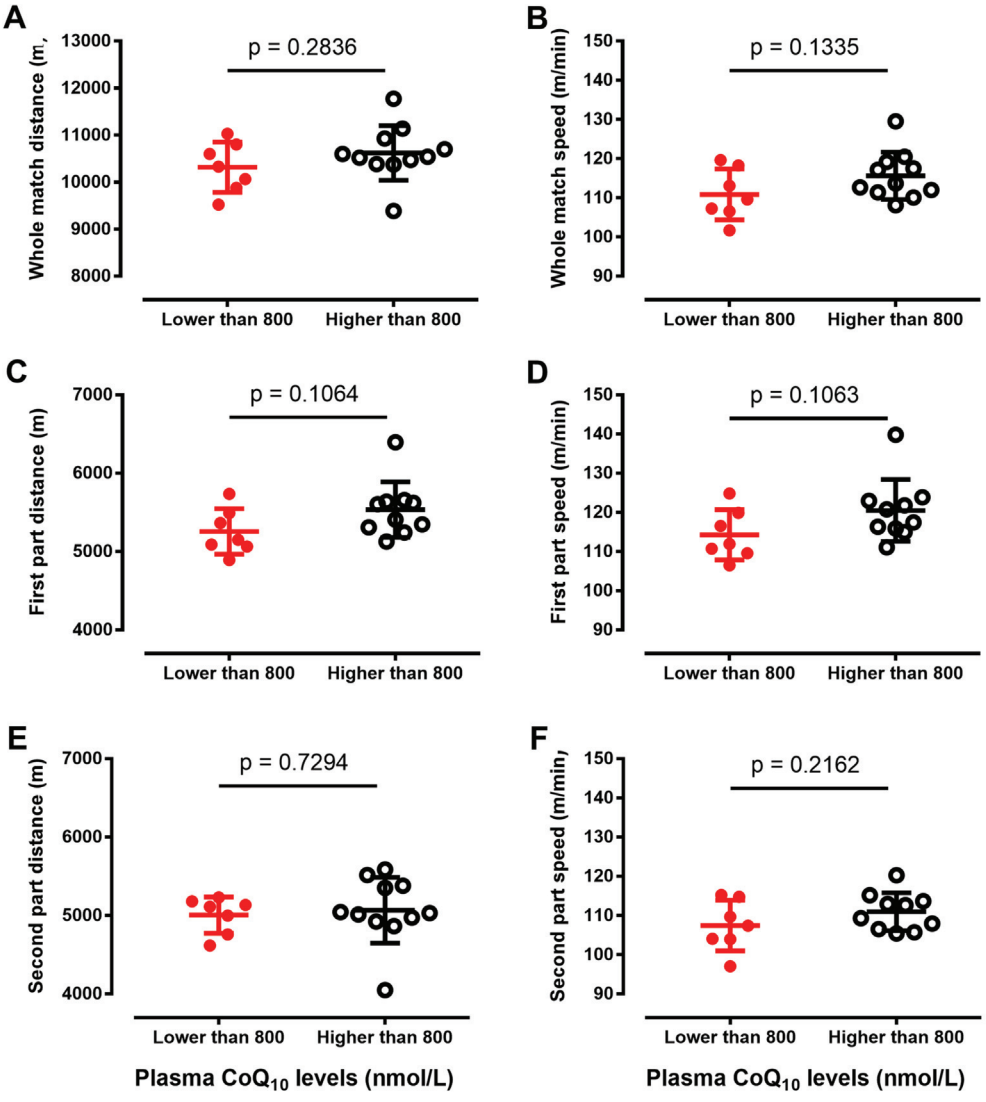

Figure 4. Physical performance along 2015/16 season. Determination of physical activity in the league matches during 2015/16 season in relationship with mean plasma CoQ_{10} levels. Players were divided in two groups depending on the levels of CoQ in plasma. A) Performance in mean distance covered during the whole match. B) Mean speed (m/min) during the whole match. C) Mean distance covered during the first part of the match. D) Mean speed during the first part of the match. E) Mean distance covered during the second part of the match. F) Mean speed during the second part of the match. Data are the mean ± SD of each parameters from players that participate during the whole match. Non-paired T-student was used as statistic method.

can influence the activation of genetic changes in muscle to adapt to physical activity in a different way in young and in old individuals [12, 54].

Endurance exercise can also produce kidney damage associated with higher oxidative stress and activation of neutrophils [55]. The practice of prolonged exercise has been associated with the increase in biomarkers related to kidney injury in young subjects in an effect associated with dehydration during exercise [56]. Furthermore, high intensity resistance training also increases the levels of biomarkers of acute kidney injury such as creatinine in plasma and other markers in urine in healthy young individuals [57]. Our results suggest that higher CoQ_{10} levels can also improve kidney efficiency during soccer competition reducing the levels of kidney damage markers in plasma. Our results agree with previous studies performed in long distance runners [22]. In the case of CoQ_{10} deficiency, nephropathies are a hallmark [17]. Thus, in these cases and other pathological conditions, supplementation with CoQ_{10} has been associated with the improvement of kidney function [17]. Further research is needed in order to demonstrate a protective effect of CoQ_{10} in kidney function during competition.

As a main strength of our research, this is, to our knowledge, the first study using professional soccer players over two complete seasons of competition. Our results indicate

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that maintaining high levels of CoQ_{10} in plasma during, at least, the hardest phase of the season, will help professional players to prevent muscle and kidney damage, reduce stress and promote higher physical capacity. The main weakness of this study is that it is an observational study and the researchers had no way of controlling diet and supplement consumption. Obviously, the control of professional athletes during competition is impossible in order to perform a typical clinical trial using placebo and supplemented groups for the duration of two complete soccer seasons. However, we consider this is an important occasion to study the evolution of CoQ_{10} levels in professional athletes suffering from the pressure of strong competition as in soccer.

In general, our results indicate that supplementation with CoQ_{10} can be important to prevent muscle damage during intense phases of competition, at least in soccer. Further analysis is needed, but our results allow us to speculate that CoQ_{10} can be safely used as a supplement in the prevention of accumulated damage in elite athletes and can reduce stress, probably improving recovery of functional capacity after muscle damage or even after retirement from the competition. It would be interesting to perform similar studies on other professional players of different sports such as basketball or handball. These studies could indicate if the CoQ_{10} relationship with aerobic performance is associated with the type of physical activity such as soccer, which has high aerobic performance or is general for other sports.

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