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Evidence for the participation of the stimulated sympathetic nervous system in the regulation of carnitine blood levels of soccer players during a game

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Abstract

Catecholamines and carnitine blood levels are closely implicated with training. The aim of the study was to investigate the effect of sympathetic nervous system stimulation on carnitine and its fraction levels during training. Blood was obtained from 14 soccer players pregame, at intermission, and postgame. Catecholamines were measured with high-performance liquid chromatography methods; muscle enzymes creatine kinase and lactate dehydrogenase as well as lactate, pyruvate, and total antioxidant status with commercial kits; and carnitine and fraction levels with tandem mass spectrometry. Total antioxidant status (2.97 ± 0.13 vs 0.96 ± 0.10 mmol/L, $P < .01$) as well as free carnitine levels (20.47 ± 4.0 vs 12.30 ± 2.8 μmol/L, $P < .001$) were remarkably decreased especially postgame. Total acylcarnitines (5.20 ± 1.8 vs 9.42 ± 3.0 μmol/L, $P < .001$) and especially total very long-chain acylcarnitines (0.80 ± 0.01 vs 1.85 ± 0.03 μmol/L, $P < .001$) as well as catecholamine levels (adrenaline: 230 ± 31 vs 890 ± 110 pmol/L, $P < .01$; noradrenaline: 1.53 ± 0.41 vs 3.7 ± 0.6 nmol/L, $P < .01$) were significantly increased in players postgame. A statistically significant inverse correlation was found between adrenaline and free carnitine ($r = -0.51$, $P < .01$); and a positive correlation was found between adrenaline, total acylcarnitines ($r = 0.58$, $P < .01$), and total long-chain acylcarnitine ($r = 0.49$, $P < .01$). The significant positive correlation of adrenaline levels with total acylcarnitine and total long-chain acylcarnitine blood levels in athletes as well as the inverse correlation with free carnitine levels may indicate participation of the stimulated sympathetic nervous system in the regulation of some carnitine fraction levels during exercise.

1. Introduction

L-Carnitine (L-C) is a naturally occurring quaternary ammonium compound, endogenous in all mammalian species, and is a vital cofactor for the mitochondrial oxidation of fatty acid (FA). L-Carnitine exists in the body primarily in its unesterified form, but also as a number of esters (acylcarnitines) including acetyl-L-carnitine. Humans take in carnitine primarily from diet (75%), with the remaining 25% coming from endogenous synthesis. Another major role for the carnitine is the transfer of acetyl–coenzyme A (CoA) from the mitochondria into the cytoplasm, thereby providing acetyl groups in the synthesis of the neurotransmitter acetylcholine [1].

In keeping with polar characteristics, L-C does not bind to plasma proteins (factor unbound–1) and enters erythrocytes very slowly. The uptake of L-C into most tissues of the body, including liver, kidney, skeletal and cardiac muscle, neurons, and epididymal tissue, involves carrier-mediated transport systems, which maintain high tissue-to-plasma concentration ratio [2]. The transport of L-C from the plasma into the neurons is mediated by a Na+ and energy-dependent process and may involve the organic cation transporter [3,4].

Studies in humans have also demonstrated that a decrease on muscle free carnitine concentration occurs during short-duration, high-intensity exercise and at the onset of exhaustive submaximal exercise and is matched by an almost equivalent increase in acetylcarnitine [5,6].

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It is well known that regular physical exercise and sport performance are beneficial. However, it has been documented that physical exercise augments the production of reactive oxygen species in exercising muscle [7]. This is apparently contradictory to the beneficial effects of exercise. Studies have also shown that depletion and supplementation of antioxidants in the diet have a protective effect on exercise-induced oxidative stress [8]. Anaerobic exercise has been associated with a substantial lactic acidosis in both blood and muscle [1] and also with a major increase in plasma catecholamine levels [8,9].

Catecholamine regulation of adipose tissue triacylglycerol (TG) hydrolysis is well studied [10,11]; the hormonal regulation of skeletal muscle TG hydrolysis is also reported. Elevated adrenaline (A) and noradrenaline (NA) levels cause TG breakdown in adipose tissue through adrenergic stimulation of hormone-sensitive lipase (HSL) [12,13].

Skeletal muscle lipolysis is differently regulated than adipose tissue lipolysis. In skeletal muscle, only the $\beta 2$ subtype is of importance for regulation of catecholamine-induced lipolysis [14]. In addition, no effect of atrial natriuretic peptide on in vivo skeletal muscles was reported [15]. As in adipose tissue, HSL has been demonstrated in human skeletal muscle. This represents HSL myocytes and not HSL originating from interlaced adipocytes [16]. Hormone-sensitive lipase expression is higher in muscles containing predominantly type 1 and type 2 fibers, and its expression correlates with muscle TG concentration and oxidative capacity [17].

Obviously, exercise is closely implicated with the stimulated sympathetic system and consequently with increased catecholamine blood levels, lipolysis, and free radical production as well as free carnitine reduction. Therefore, we aimed to investigate the effect of the raised catecholamine levels in soccer players during their game on the above-mentioned biochemical parameters and especially on carnitine and its fraction blood levels.

2. Methods

The study was approved by the Greek Ethics Committee of Athens University as amended in 1989. All players signed a consent form.

2.1. Participants

Fourteen (N = 14) 20-year–old soccer players (mean age, 19 ± 1.5 years; mean height, 172 ± 6 cm; mean weight, 70 ± 6.5 kg) who were actively participating in the Greek Soccer Championships volunteered to participate in the study. They had at least 2 years of training that consisted of two to three 60-minute sessions per week for 9 months of the year. A 7 a-side championship game took place in a 60-m × 40-m pitch. Of the 7 players of each team, 2 were forwards, 2 were mild helders, 2 were defenders, and 1 was a goalkeeper. To ensure similar technical ability between the sides, opposing teams were chosen with a similar league position to the "experimental" team.

2.2. Procedures

Permission was obtained from the referees. Clinical and biochemical blood parameters were taken at the beginning (3-4 minutes pregame), at intermission, and at the end of the meeting (3-4 minutes postgame). Blood pressure, both systolic and diastolic, was determined with an electronic (Nova-test, Darmstedt, Germany) instrument. Heart rate (beats per minute) was simultaneously measured [18].

Blood was obtained from each player for the measurement of lactate; pyruvate; catecholamines A, NA, and dopamine (DA); total antioxidant status (TAS); muscle enzymes creatine kinase (CK) and lactate dehydrogenase (LDH) levels; as well as carnitine levels and its fraction levels on Guthrie cards at the mentioned times (pregame, at intermission, and postgame).

Duplicate 25-μL capillary blood samples were collected from the left thumb 2 to 3 minutes after the test for lactate and pyruvate evaluation. In addition, 2.5 mL of blood and plasma was obtained into EDTA tubes for catecholamine and TAS measurements, respectively.

2.3. Measures

2.3.1. Lactate and pyruvate evaluation

Samples for lactate and pyruvate estimations were centrifuged at 1000*g* for 10 minutes and analyzed enzymatically. For the measurement of blood lactate and pyruvate concentrations, commercially available kits were used: Lactate Pap No. 61192 Biomerieux (Biomerieux, Marcy, L'Etoile, France) and pyruvate Roche Pyr 124982 (Roche Diagnostics, Munich, Germany). Coefficients of variations (CVs) for these analyses were 2.2% and 2.0%, respectively.

2.3.2. TAS measurement

Total antioxidant status was measured in plasma of players before and after the game as previously reported by Miller et al [19]. The TAS calculation estimates the additive antioxidant action of known as well as unknown antioxidants. Because of the different ways of action among the several components of the antioxidative mechanism, TAS measurement can be regarded as more effective [19]. Plasma was frozen for up to 14 days before analysis. 2,2′-Azino-di-(3-ethylbenzthiazoline sulfonate) (ABTS) was incubated with peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation ABTS+, which had a relatively stable blue-green color measured spectrophotometrically at 600 nm. Antioxidants in the added sample cause suppression of the above color production to a degree proportional to their concentration. The assay range was 0 to 2.5 mmol/L. Samples with concentrations greater than 2.5 mmol/L were diluted with 0.9% NaCl and reassayed. The present method calculates both the radical scavenging effect and the effect on the rate of ABTS+ oxidation (free radical production).

Table 1
Clinical characteristics in soccer players (N = 14) pregame, at intermission, and postgame

	Pregame	Intermission	Postgame
Systolic blood pressure (mm Hg)	122 ± 8[a]	160 ± 15[b]	17812[c]
Diastolic blood pressure (mm Hg)	70 ± 5[a]	85 ± 5[b]	905 [c]
Cardiac rate (beats/min)	67 ± 8[a]	98 ± 16[b]	116 ± 10[c]

Values are expressed as mean ± SD. Statistics: a/b, a/c, b/c; $P < .001$.

Intraassay and interassay variations were 3.4% and 3.9%, respectively.

2.3.3. Muscle enzyme evaluation

Serum LDH and CK were determined by an enzymatic method at 405 nm using commercial kits (Hyman Geselschaft fur Biochemie and Diagnostica, Taunustein, Germany). The CVs for LDH and CK were 2.5% and 3.0%, respectively.

2.3.4. Catecholamine evaluation

Plasma catecholamines A, NA, and DA levels were measured by a sensitive high-performance liquid chromatography with a reversed-phase ion-pair chromatography and electrochemical detection with an ESA Coulochem (Model 1050A; Hewlett-Packard, Cleveland, OH). The CV for A, NA, and DA was 2.4%, 2.6%, and 2.3%; and their lower detection limit was 5 pmol/L, 0.06 nmol/L, and 8 pmol/L, respectively [20].

2.3.5. Carnitine and fractions evaluation

All samples (blood spots) were collected on a Schleicher and Schuell (Dassel, Germany) grade 903 filter paper (Guthrie cards) and analyzed by a previously described electrospray mass spectrum/mass spectrum method [21,22] with slight modifications.

This filter paper meets the Center for Disease Central Laboratory standards and the Centers for Disease Control and Prevention specifications, and carnitine values are validated with the Centers for Disease Control and Prevention regularly. We used a Waters (Manchester, UK) LC Quattro Tandem MS (mass spectrum) with 2795 liquid handling system. Briefly, one 3-mm–diameter dot per sample was punched out from a 10-mm dried blood spot into a vial of a 96-well microtiter plate. The spots were eluted for 20 minutes in 100 μL of 3 N HCl with butanol added. The microtiter plates were sealed and incubated at 65°C in a forced-air oven for 15 minutes. After removal of the seal, excess HCl-butanol evaporated to dryness in a freeze-dryer. The derived samples were reconstituted with 100 μL acetonitrile/water (1:1 vol/vol) containing 0.025% formic acid. The tandem mass spectrometer with an ion spray source was used for analysis. Acylcarnitines were detected by looking for the precursor ions of carnitine concentrations and were summarized to obtain levels for short-chain (SCA), medium-chain (MCA), long-chain (LCA), and total acylcarnitines as described before. Inter- and intraassay variations for carnitines were 1.8% and 2.2%, respectively; and lower detection limit was 0.02 μmol/L.

2.4. Statistical analyses

SPSS 13.0 statistical package (SPSS, Chicago, IL) was used for statistical analysis. Data were analyzed with analysis of variance for repeated measurements followed by Tukey test, when applicable. Pearson test was used for the coefficient correlations evaluation. P less than .05 was considered statistically significant. Data are expressed as mean ± SD.

3. Results

As shown in Table 1, statistically significant elevations ($P < .001$) of blood pressure, especially systolic, and heart rate were observed in the players at intermission and especially postgame.

As presented in Table 2, remarkably increased lactate, pyruvate, muscle enzymes CK and LDH levels, as well as catecholamine levels were measured in the blood of soccer players at intermission and postgame. In addition, statistically significant reduction of TAS blood levels was determined at the same times of study.

As illustrated in Table 3, total carnitine levels were just statistically elevated. Total acylcarnitine, total SCA (tSCA), and total LCA (tLCA) levels were also significantly elevated, whereas free carnitine levels were statistically reduced at intermission and especially postgame.

As presented in Table 4, SCA (C2 and C3) levels were significantly decreased, MCA levels remained unaltered, whereas LCA (C14, C16, C18) blood levels were significantly increased at intermission and postgame.

In addition, significantly positive coefficient correlations were found between A and total acylcarnitine levels and tLCA ($r = 0.58$, $P < .01$ and $r = 0.49$, $P < .01$, respectively), whereas negative correlations were determined between A and free carnitine blood levels ($r = -0.51$ $P < .01$) in the soccer group (Figs. 1 and 2). No correlations were found between NA or DA levels with carnitine levels.

4. Discussion

As expected, blood pressures as well as the heart rate were almost 2-fold increased in all the studied teams at

Table 2
Biochemical parameters in soccer players (N = 14) pregame, at intermission, and postgame

	Pregame	Intermission	Postgame
Lactate (mmol/L)	1.92 ± 0.02[a]	5.22 ± 0.63[b]	6.80 ± 0.88 [c]
Pyruvate (μmol/L)	82.30 ± 2.30[a]	222.73 ± 22.30[b]	260.00 ± 9.00[c]
CK (U/L)	86.0 ± 7.4[a]	116.0 ± 10.0[b]	286.0 ± 12.0[c]
LDH (U/L)	387 ± 13[a]	427 ± 85[b]	688 ± 88[c]
A (pmol/L)	230 ± 31[a]	630 ± 96[b]	890 ± 110[c]
NA (nmol/L)	1.53 ± 0.41[a]	2.70 ± 0.60[b]	3.70 ± 0.80[c]
DA (pmol/L)	55 ± 39[a]	130 ± 56[b]	56 ± 49[c]
TAS (mmol/L)	2.97 ± 0.13[a]	1.47 ± 0.12[b]	0.96 ± 0.10[c]

Values are expressed as mean ± SD. Statistics: a/b, a/c, b/c; $P < .01$.

Table 3
Total carnitine, free carnitine, total acylcarnitines, tSCA, total MCA, and tLCA blood levels in soccer players pregame, at intermission, and postgame

Carnitine (μmol/L)	Pregame	Intermission	Postgame
TC	21.9 ± 5.5[a]	23.8 ± 4.2[b]	24.8 ± 3.0[c]
FC	20.47 ± 4.00[a]	16.40 ± 3.00[b]	12.30 ± 2.80[c]
tAC	5.20 ± 1.80[c]	7.20 ± 1.80[b]	9.42 ± 3.00[a]
tSCA	3.80 ± 0.50[c]	5.00 ± 1.80[b]	7.15 ± 2.00[a]
tMCA	0.42 ± 0.01[a]	0.38 ± 0.01[a]	0.40 ± 0.01[a]
tLCA	0.60 ± 0.01[a]	1.00 ± 0.02[b]	1.85 ± 0.30[c]
tAC/FC	0.42 ± 0.20[a]	0.44 ± 0.20[a]	0.46 ± 0.20[a]
FC/TC	0.70 ± 0.03[a]	0.69 ± 0.30[a]	0.68 ± 0.30[a]

Values are expressed as mean ± SD. TC indicates total carnitine; FC, free carnitine; tAC, total acylcarnitines; tMCA, total MCA.
Statistics:
TC: a/b, a/c; $P < .05$.
FC: a/b, a/c, b/c; $P < .001$.
tAC: a/b, a/c, b/c; $P < .001$.
tSCA: a/b, a/c, b/c; $P < .001$.
tMCA: a/a; $P > .05$.
tLCA: a/b, a/c, b/c; $P < .001$.
tAC/FC: a/a; $P > .05$.
FC/TC: a/a; $P > .05$.

intermission and posttraining as compared with those determined pretraining, probably because of the high catecholamine levels measured at the mentioned times of study.

Biochemistry explains acidosis by the production of lactic acid. The free H+ can be buffered by bicarbonate, causing the nonmetabolic production of carbon dioxide. In turn, the developing acidosis and the raised blood carbon dioxide content stimulate an increased rate of ventilation, causing the temporal relationship between the lactate and ventilatory thresholds [23]. In this study, lactate as well as pyruvate blood levels were remarkably increased in groups during training.

In addition, TAS levels, which reflect the total antioxidant capacity but not specific oxidants, were found to be significantly decreased in the players during exercise (Table 2), as reported by other authors [23].

With regard to carnitine, it is reported that carnitine has to cross the cell membrane against a level gradient by an active transport system. The mechanisms that create and maintain these gradients are not thoroughly known [23,24]. Such regulatory factors include changes in blood volume, carnitine uptake and release in the muscle, and acylation of carnitine outside the contracting muscle. Although carnitine in the muscles of players pre- vs postexercise was not measured for ethical reasons, a limitation of this study, we may suggest that a sudden depletion of carnitine from the blood due to its shunt into the exercising muscles may take place, as found by other authors [25-27].

To assess sympathoadrenal activity, we measured venous catecholamine levels. Arterial samples reflect better the overall sympathetic activity including spillover from heart and kidney, whereas venous samples for a larger part reflect muscle sympathetic activity [28]. A release form muscle sympathetic nerve contributes to approximately 50% of peripheral venous A and NA [29]. Thus, if sympathetic activity in muscle tissues is the most important determinant, venous measurement of catecholamine levels would have been preferable [30], as we did.

Regulation of carbohydrate and fat utilization by skeletal muscle at rest and during exercise showed the so-called glucose-FA cycle to explain the reciprocal relationship between carbohydrate and fat metabolism. The suggested mechanisms were based on the premise that an increase in FA availability would result in increased fat metabolism [31]. Briefly, accumulation of acetyl-CoA would result in inhibition of pyruvate dehydrogenase; and accumulation of glucose-6-phosphate would reduce hexokinase activity [32]. Potential sites of regulation are the transport of FA into the sarcoplasm and the lipolysis of intramuscular TG by HSL; the latter is reported to be activated by catecholamines, especially by A [17]. Obviously, the high levels of A and NA in the studied athletes at intermission and especially postgame may contribute to the activation of HSL, resulting in the regulation of sarcoplasm lipolysis of intramuscular TG. Furthermore, the increased FA should be transported across the mitochondrial membrane via carnitine [33]. In addition, there are several potential regulators of fat oxidation: malonyl-CoA concentration, which is formed from acetyl-CoA, catalyzed by the enzyme acetyl-CoA-carboxylase, which in turn will inhibit muscle carnitine

Table 4
Short-chain acylcarnitine, MCA, and LCA in soccer players pregame, at intermission, and postgame

SCA (μmol/L)	Pregame	Intermission	Postgame
C2	9.46 ± 2.00[a]	8.01 ± 1.05[b]	7.01 ± 0.05[c]
C3	1.22 ± 0.40[a]	0.92 ± 0.02[b]	0.58 ± 0.02[c]
C4	0.22 ± 0.01[a]	0.11 ± 0.01[b]	0.01 ± 0.01[c]
C5:1	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
C5	0.24 ± 0.01	0.21 ± 0.01	0.21 ± 0.02
MCA (μmol/L)			
C6	0.067 ± 0.02	0.064 ± 0.02	0.060 ± 0.02
C8	0.09 ± 0.01	0.08 ± 0.02	0.07 ± 0.02
C8:1	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
C10	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
C10:1	0.18 ± 0.01	0.16 ± 0.01	0.14 ± 0.01
C12	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
LCA (μmol/L)			
C14	0.18 ± 0.02[a]	0.29 ± 0.02[b]	0.40 ± 0.02[c]
C14:1	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.02
C16	0.51 ± 0.01[a]	0.78 ± 0.01[b]	0.98 ± 0.01[c]
C16:1	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
C 18	0.30 ± 0.03[a]	0.60 ± 0.03[b]	0.81 ± 0.01[c]
C18:1	0.83 ± 0.01	0.82 ± 0.02	0.80 ± 0.03
C18 OH	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
C16 OH	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
C16:1 OH	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
C18:1 OH	0.26 ± 0.01	0.02 ± 0.01	0.02 ± 0.01

Values are expressed as mean ± SD. Only the statistically significant values are presented.
Statistics:
a/b, a/c; $P < .001$.
b/c; $P < .01$.

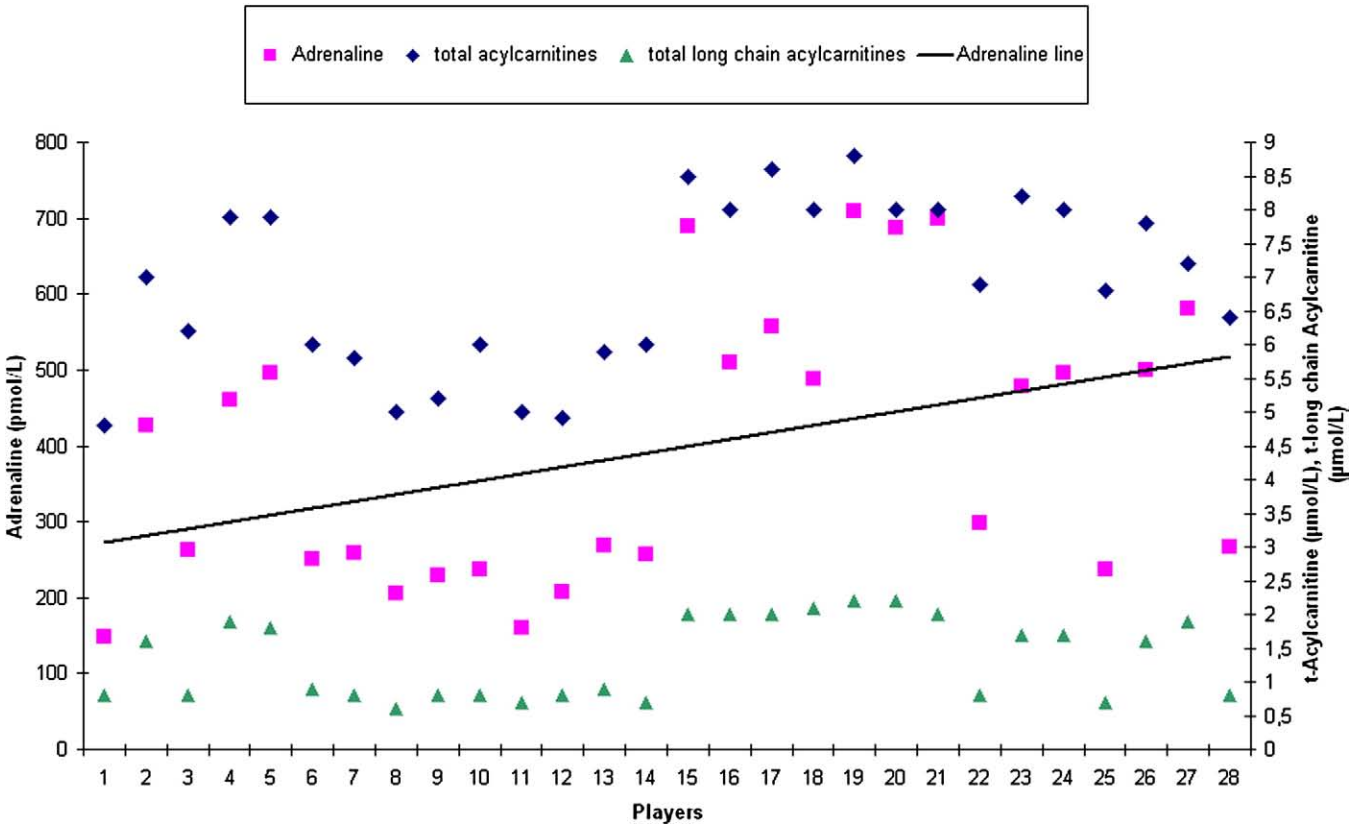

Fig. 1. Correlations between total acylcarnitine and tLCA levels with A plasma levels per soccer player at intermission (1-14) and postgame (15-28).

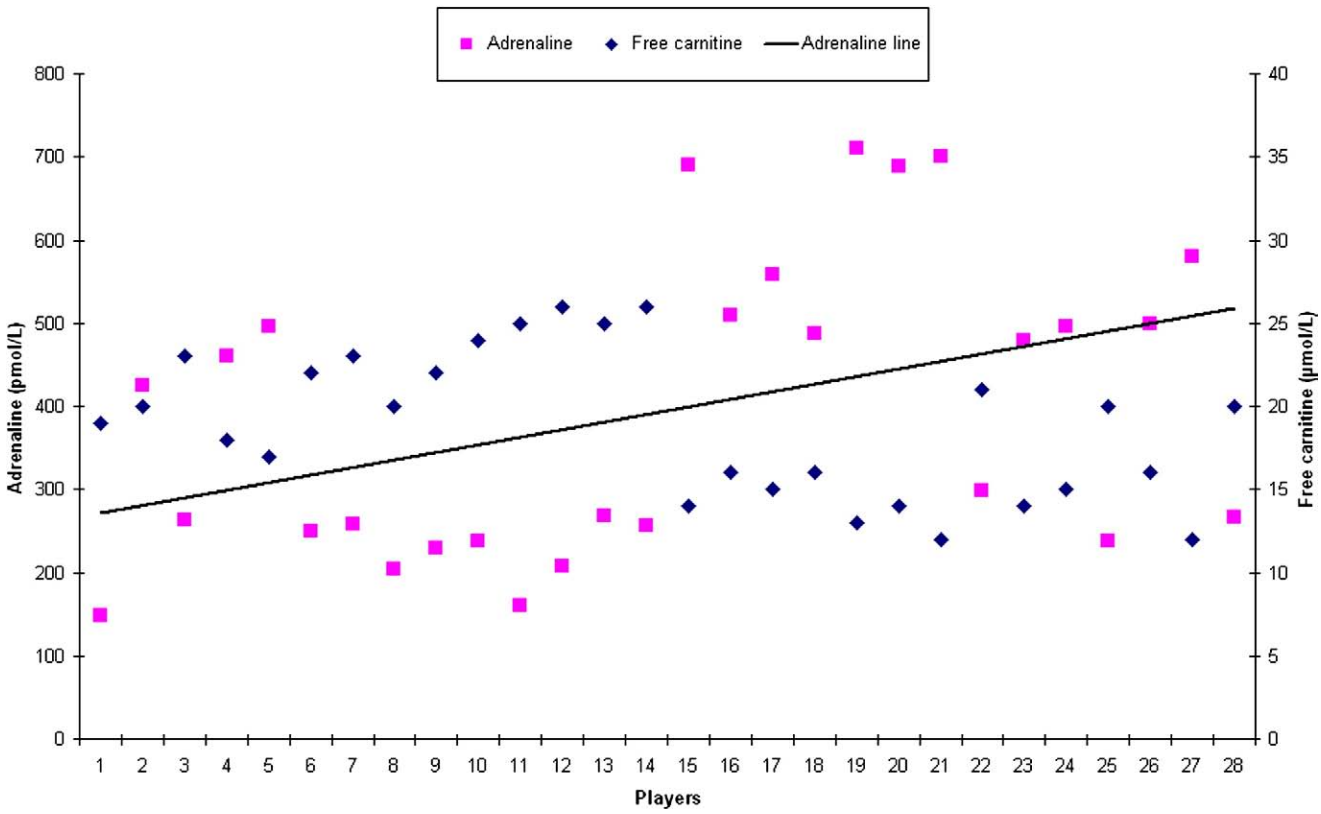

Fig. 2. Correlation between free carnitine and A blood levels per soccer player at intermission (1-14) and post game (15-28).

palmitoyltransferase 1 (CPT-1). Consequently, the increased catecholamine blood levels may be autooxidized [33,34], thus contributing to muscle CPT-1 inhibition. Another possible mechanism is accumulation of acetyl-CoA that will result in acetylation of the carnitine pool reducing the free carnitine concentration in the blood [35], as we found in athletes, especially postgame.

Plasma total carnitine tended to rise; free carnitine fell clearly as found in the soccer players at intermission and postgame. It was reported [33] that the observed rise of plasma acylcarnitine levels, as we also measured in the bloodstream of players especially postgame, was due to the release of carnitine from muscle during exercise.

Carlin et al [35] reported that cycle ergometry for 90 minutes at low intensity did not affect total carnitine in muscle, but there was a large elevation in acylcarnitine and fall in the free carnitine fractions as we present here. Plasma acylcarnitines progressively increased during exercise, which was largely because of the decrement in free carnitine and not net loss of carnitine from muscle [35]. Our results are in accordance with the above findings. Interestingly, it was reported [33] that, during exercise, as in other conditions of falling insulin levels (eg, fasting diabetes), changes in plasma carnitine could result from an exchange with hepatic carnitine pool. It was also suggested that much greater carnitine turnover occur between plasma and liver than between muscle and plasma [33]. Therefore, the evaluation of both carnitine and fraction levels as well as catecholamine levels in the soccer players' blood could mirror alterations that take place during the game.

Furthermore, catecholamines are known to be secreted from 2 main sources: the sympathetic nerves and adrenal glands. In addition, low activity of CPT-1 in the liver but not in the muscles in adrenalectomized rats has been reported [36]. Further study of the effect of the central sympathetic pathways revealed the same results on the enzyme as above. Incubation of the liver enzyme with catecholamines resulted in a complete recovery of its activity, showing that only CPT-1 in the liver was mediated by sympathetic system. From this point of view, we may suggest that the high catecholamine and especially A levels measured in the blood of players at intermission and postgame may be implicated with their liver CPT-1 stimulation resulting in lipolysis followed by oxidation as an additional effect of catecholamine autooxidation, contributing to the presented low TAS levels evaluated in the blood at the same times of study. Moreover, we may not exclude that the measured high blood concentration of catecholamines may stimulate hepatic CPT-1, causing an increased transport of the long-chain FAs mainly via acylcarnitines in the mitochondria and thus providing enough fuel for energy during the game.

These preliminary suggestions may be further supported by the strong positive correlations found between plasma A and total acylcarnitines as well as with tLCA levels (Fig. 1) evaluated in the studied soccer players. In contrast, A blood levels inversely correlated with free carnitine levels in the athletes (Fig. 2), showing that the observed decrease in free carnitine availability might be indirectly responsible for the reported decrease of muscle CPT-1 activity [37] leading to the reduction of fat oxidation by limiting long-chain FA entry into the mitochondria of the training muscles [26,38,39].

In conclusion, sympathetic nervous system stimulation may participate via A secretion in the regulation of carnitine and some fraction blood levels.

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