

# Changes in cell free DNA during a college soccer season

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**Objectives:** This study investigated chronic changes in cell free DNA (cf-DNA) throughout a collegiate soccer season. The relationship between cf-DNA, C-reactive protein (CRP), creatine kinase (CK), testosterone (T), cortisol (C), testosterone-cortisol ratio (T:C), body mass and body composition were also examined.

**Design:** Longitudinal study design with repeated measures and group comparisons.

**Methods:** Twenty three NCAA Division I male soccer players were divided into two groups. Starters were placed in Group 1 (G1) and non-starters were placed in Group 2 (G2). cf-DNA, CRP, CK, T, C, T:C, body mass and body composition were taken three times, corresponding to pre-season, approximately mid-season and immediately after the concluding the season.

**Results:** In G1, cf-DNA, CRP, CK, cf-DNA % $\Delta$ , CRP % $\Delta$  and, CK % $\Delta$  were all statistically higher at T2 and T3 than T1. In G2, CRP % $\Delta$  was statistically higher at T2 than T1. In G2, cf-DNA % $\Delta$ , CRP % $\Delta$  and CK % $\Delta$  were higher at T2 and T3 than T1.

**Conclusions:** This suggests that cf-DNA may be a useful marker that can reflect accumulated soccer training and competitive stressors.

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**Key words:** cf-DNA ■ RPE ■ C-reactive protein ■ creatine kinase ■ testosterone ■ cortisol ■ sport

## INTRODUCTION

Monitoring acute and chronic responses to training in athletes may be enhanced by the use of biochemical markers in the monitoring process.<sup>1</sup> When considering a biochemical marker for use in a long term athlete monitoring program (LTAMP), the manner in which the marker responds to chronic changes in training and in relation to additional stressors is of importance. In order to avoid interruptions in training while a biochemical marker returns to baseline, the time course of a biochemical marker response after a single bout of exercise should be considered. If the concentration of a biochemical marker remains elevated above its pre-exercise baseline for multiple days after a single exercise session, it may be challenging to conclude whether these changes in concentration reflect chronic changes or acute responses. For use in a LTAMP it may be useful to employ a single or combination of biochemical markers which change chronically with training stressors, but also return to the current baseline within hours instead of days.

Plasma cell-free DNA (cf-DNA) has been employed as a marker of tissue damage and inflammation for a range of conditions. For example, elevated cf-DNA concentrations have been associated with the severity of a variety of chronic conditions including but not limited to cancer<sup>2</sup>, cardiovascular disease<sup>3</sup>, pre-eclampsia<sup>4</sup>, sepsis<sup>5</sup>, sleep apnea<sup>6</sup> and aging<sup>7</sup>. cf-DNA

concentrations have also been shown to be related to the degree of injury after a traumatic events such as myocardial infarction<sup>8</sup>, strokes<sup>9</sup>, blunt trauma<sup>10</sup> and burn victims<sup>8</sup>. However, few investigations of cf-DNA, as it relates to exercise and/or training, have been conducted. Recent studies of cf-DNA have explored the acute response of cf-DNA after a single bout of exercise<sup>12-17</sup>, as well as chronic changes in cf-DNA concentrations with resistance training<sup>18</sup>. These initial inquiries of post-exercise cf-DNA concentrations and training have suggested that cf-DNA may provide benefits over other markers of tissue damage and inflammation such as creatine kinase (CK) and C-reactive protein (CRP), for inclusion in a LTAMP.

Considering the research addressing the acute and chronic response of cf-DNA to exercise, inquiries into the acute response of cf-DNA have demonstrated that cf-DNA peaks immediately after exercise and returns to its pre-exercise baseline approximately 2-4 hours post-exercise.<sup>13,14,16,19,20</sup> The characteristic timecourse of cf-DNA after a single exercise session may offer benefits over common markers of tissue damage and inflammation. For example, during an investigation employing a protocol of exhaustive treadmill running, C-reactive protein (CRP), uric acid (UA) and creatine kinase (CK) concentrations increased until the final blood draw 24 hours post-exercise. In the same study, cf-DNA concentrations peaked immediately

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after exercise and returned to the pre-exercise baseline within two hours after the treadmill protocol.<sup>19</sup> Akin to the post-endurance exercise response, the concentration of cf-DNA follows a similar time course after a single bout of resistance training.<sup>13</sup> Prior to this study, the authors are aware of only a single investigation addressing cf-DNA concentrations in response to chronic changes in exercise workload.<sup>18</sup> Fatouros and Destouni (2006) demonstrated that cf-DNA may be a valuable biochemical marker of overtraining, reflecting changes in resistance training volume load. We are unaware of any literature addressing the efficacy of cf-DNA as a monitoring variable in soccer or other team sport. The mechanisms by which cf-DNA is released into circulation resulting from exercise is not entirely understood.<sup>21</sup> It has been proposed that different mechanisms are responsible for acute and chronic changes in cf-DNA concentration related to exercise.<sup>22</sup> The immediate increase in cf-DNA concentration during and after exercise is likely a result of releasing neutrophil extracellular traps or other rapid processes.<sup>21-23</sup> Changes in cf-DNA concentration associated with disease, chronic increases in exercise workload, and overtraining, seem to be a product of elevated necrosis or apoptosis causing a steady and gradual release of DNA into circulation.<sup>22</sup> This suggests that cf-DNA may serve as a marker of acute and chronic tissue damage and inflammation.

In addition to cf-DNA, the time course of CRP and CK post-exercise are of particular interest to this investigation. CRP and CK are common markers of inflammation and tissue damage and have been shown to remain elevated for a number of days after a single exercise session.<sup>24,25</sup> Athletes seldom refrain from sport-related training for multiple days which may not allow markers such as CRP and CK to return to baseline. The fact that CRP and CK remain elevated for several days after a single bout of exercise is potentially problematic for use in a LTAMP.

Testosterone and cortisol have also been the focus of numerous investigations. Long periods of high intensity training and/or high volumes of training have been shown to cause decreases in serum testosterone as well as decreases in the testosterone to cortisol ratio (T:C). Decreases in serum testosterone and T:C are associated with fatigue and decreases in maximal strength.<sup>26-28</sup> It has also been suggested that T:C is an indicator of anabolic-catabolic balance.<sup>29,30</sup> Despite testosterone, cortisol, and T:C being several of the most common biochemical markers used in training studies in an attempt to monitor an athlete's response to training stimuli, the literature offers mixed conclusions about whether these are viable markers of an athlete's training status. For instance, testosterone, cortisol and T:C ratio in endurance athletes have not shown to respond consistently to increased or decreased training load or when overreached and overtrained.<sup>31-35</sup> There are also inconsistencies in resistance trained individuals.<sup>36</sup>

The purpose of this study was to investigate changes in cf-DNA and other commonly used biochemical markers throughout a collegiate soccer season in order to examine the utility of cf-DNA as a potential variable to be included in a LTAMP for NCAA Division I collegiate soccer.

## METHODS

### Subjects

Twenty-three NCAA Division I male collegiate soccer players (age  $21.2 \pm 1.4$  years, height  $180.1 \pm 7.1$  cm, body mass  $79.9 \pm 8.6$  kg) volunteered for this study. Athletes were divided into two groups based upon whether they started during Fall season soccer games. Subjects of Group 1 (G1,  $n = 13$ , age  $21.7 \pm 1.5$  years, height  $178.8 \pm 6.9$  cm, body mass  $77.9 \pm 6.5$  kg) started games during the Fall season and subjects of Group 2 (G2,  $n = 10$ , age  $20.6 \pm 1.0$  years, height  $181.8 \pm 7.4$  cm, body mass  $82.5 \pm 10.5$  kg) did not start in games during the Fall season. Session rating of perceived exertion (sRPE, minutes played  $\times$  RPE) was also used to verify differences in regular season game time training loads between G1 and G2.<sup>37</sup> This ensured that all athletes in a single group participated in similar levels and volumes of training.

Written informed consent was obtained from each subject. The University's Institutional Review Board approved this study.

### Procedures

**Testing Sessions.** Anthropometric and biochemical measurements were performed three times during the 15-week investigation. These three testing sessions corresponded to pre-season (T1, August 10), approximately mid-season (T2, September 12) and immediately following the conclusion of the season (T3, November 13).

**Anthropometrics.** A stadiometer (Detecto, Webb City, MO) was used to measure subject height to the nearest 0.1 cm. Body mass was measured by digital scale and body composition was assessed by the same investigator via seven-site skinfold testing (right side, ICC = 0.95) using Lange Skinfold Calipers (Cambridge Scientific Industries, Cambridge, MA) and the Siri equation. Body mass was measured three times (T1-T3) and body composition was measured twice (T1 and T3) during the investigation. The absence of body composition measurements at T2 was a coaching decision.

**Biochemistry.** Venous blood draws were collected during all three time periods (T1-T3). All blood draws were performed in a fasted state between the hours of 6:00 - 7:30 am and took place 48 hours after the last training session or competition. All subjects were monitored closely by sport science staff which instructed and verified that all subjects were fasted and had not participated in physical activity for 48 hours prior to blood draws. Biochemical markers were quantified as concentrations in plasma or serum as well as percent change (% $\Delta$ ).

**C-reactive Protein.** Serum CRP concentrations were measured using a solid phase sandwich-type ELISA.<sup>38</sup> A polyclonal rabbit anti-human CRP Ab (1/1000 dilution in TBS; Sigma-Aldrich, St. Louis, MO) was used as the capture Ab and a monoclonal affinity purified anti-CRP Ab HD2.4 (0.5  $\mu$ g/ml) was used as the reporter. Standard curves were constructed with purified native CRP (1.56-100 ng/ml) diluted in TBS, 0.1% gelatin and 0.02% Tween 20. The secondary Ab used was Horseradish peroxidase-conjugated goat anti-mouse IgG H+L (Thermo Fisher Scientific, Rockford, IL). Color was developed with the HRP substrate kit (Bio-Rad, Hercules, CA)

and measured at 405 nm with a VersaMax™ plate reader (Molecular Devices, Menlo Park, CA). All samples were read in duplicate. The intra-assay and inter-assay coefficient of variation were 7.1% and 12.3% respectively.

**Testosterone (T) and Cortisol (C).** Serum total T and C concentrations were measured using commercially available chemiluminescence immunoassay on IMMULITE® 2000 immunoassay system (Siemens, Los Angeles, CA, USA). T and C concentrations were also used to calculate the ratio of testosterone to cortisol (T:C). The intra-assay and inter-assay coefficient of variation for T were 5.8% and 6.9%, respectively. The intra-assay and inter-assay coefficient of variation for C were 7.4% and 9.8% respectively.

**Creatine Kinase.** Serum CK concentrations were measured using the AU480 Chemistry System (Beckman Coulter, Krefeld, Germany) with a CK-Nac reagent kit (Olympus Diagnostica GmbH, Clare, Ireland). The intra-assay and inter-assay coefficient of variation were 1.1% and 1.8% respectively.

**Cell-free DNA.** DNA was isolated from plasma using the NucleoSpin® Plasma XS Kit (Macherey-Nagel GmbH & Co. KG, Germany). Standard curves were constructed (0-100 ng/ml) with DNA stock solution. All samples were measured in duplicate. DNA was quantified by measuring the fluorescence of the DNA-PicoGreen® complex using Quant-iT PicoGreen® dsDNA Kit (Molecular Probes, Eugene, OR) and measured at 405 nm with a Modulus® Microplate Reader (Turner BioSystems, Sunnyvale, CA). The intra-assay and inter-assay coefficient of variation were 5.2% and 14.0% respectively.

**Training Load Calculation.** Training load was determined with sRPE values by multiplying the athletes' reported RPE by the duration in minutes of the training session or competition.<sup>37,39,40</sup> Each athlete reported RPE values immediately after all training sessions and competitions. sRPE values were categorized as soccer training, weight training, soccer games, and other sRPE. Other sRPE consisted of activities that did not fit within the aforementioned categories and all sRPE data were reported as weekly means.

### Statistical Analysis

Data were analyzed with SPSS (V20, IBM, Armonk, NY, USA) and expressed as means, standard deviations (SD) and 95% confidence intervals. Two-way ANOVA with repeated measures was used to analyze within and between group data. Post-hoc tests were performed using a Bonferroni correction. A Greenhouse-Geisser adjustment was employed when the assumption of sphericity was violated according to Mauchly's test of sphericity ( $P < 0.05$ ). Pearson product-moment correlation coefficients were performed on all biochemical and anthropometric variables. The level of statistical significance was set at  $p \leq 0.05$ . Percent changes for all biochemical markers are also calculated.

## RESULTS

Tables 1 and 2 present the means, SD, 95% CI, within group differences and between group differences for G1 and G2, respectively. Of particular importance to this investigation

**Table 1** G1 anthropometric and biochemical changes

	T1 (pre-season)	T2 (mid-season)	T3 (post-season)
<b>Body Mass (kg)</b>	77.9 (6.5, 73.9-81.9)	78.1 (6.6, 74.1-82.1) <sup>c</sup>	77.3 (6.4, 73.5-81.2) <sup>b</sup>
<b>BF %</b>	9.5 (3.2, 7.8-11.2)	-	9.5 (3.2, 7.8-11.2)
<b>cfDNA (ng/ml)</b>	5.1 (3.5, 2.9-7.2) <sup>b*,c</sup>	9.1 (4.2, 6.6-11.6) <sup>a*,c</sup>	8.7 (4.6, 5.9-11.5) <sup>a</sup>
<b>CRP (ng/ml)</b>	491.2 (472.4, 205.7-776.7) <sup>b,c</sup>	1165.3 (884.3, 630.9-1699.7) <sup>a</sup>	1179.1 (629.8, 798.5-1559.7) <sup>b,f</sup>
<b>T (ng/dl)</b>	364.9 (134.1, 283.9-446.0)	406.8 (163.1, 308.2-505.3) <sup>c</sup>	322.2 (127.5, 245.2-399.3) <sup>b</sup>
<b>C (µg/dl)</b>	13.3 (4.0, 11.0-15.7) <sup>b,c</sup>	16.8 (3.0, 15.0-18.7) <sup>a,c*</sup>	7.8 (4.4, 5.2-10.5) <sup>a,b*</sup>
<b>T:C</b>	.0287 (.0099, .0228-.0347) <sup>c</sup>	.0243 (.0084, .0193-.0293) <sup>c</sup>	.0541 (.0318, .0349-.0733) <sup>a,c</sup>
<b>CK (U/L)</b>	125.5 (65.4, 86.0-165.1) <sup>b,c</sup>	296.2 (219.4, 163.6-428.8) <sup>a</sup>	270.8 (141.9, 185.0-356.5) <sup>a,f</sup>
<b>cfDNA %Δ</b>	100 <sup>b,c</sup>	213.8 (117.2, 143.0-284.6) <sup>a</sup>	222.9 (134.4, 141.7-304.1) <sup>a</sup>
<b>CRP %Δ</b>	100 <sup>b*,c</sup>	334.9 (253.5, 181.7-488.1) <sup>a</sup>	378.5 (264.0, 219.0-538.1) <sup>a*</sup>
<b>T %Δ</b>	100	116.4 (47.7, 87.5-145.2)	92.8 (35.1, 71.6-114.1)
<b>C %Δ</b>	100 <sup>b</sup>	135.1 (41.3, 110.1-160.1) <sup>c*</sup>	65.8 (44.5, 38.9-92.7) <sup>a,b*</sup>
<b>T:C %Δ</b>	100	89.7 (33.3, 69.6-109.8) <sup>c</sup>	206.6 (150.4, 115.8-297.5) <sup>b</sup>
<b>CK %Δ</b>	100 <sup>b*,c*</sup>	243.4 (132.2, 163.4-323.4) <sup>a*,c</sup>	244.1 (126.5, ) <sup>a*,f*</sup>

Values above are displayed as mean (SD, 95% CI).

\* significant at  $P \leq 0.01$  level

### Within group differences

<sup>a</sup> statistically significantly different than T1

<sup>b</sup> statistically significantly different than T2

<sup>c</sup> statistically significantly different than T3

### Between group differences

<sup>d</sup> statistically significantly different than T1

<sup>e</sup> statistically significantly different than T2

<sup>f</sup> statistically significantly different than T3

**Table 2** G2 anthropometric and biochemical changes

	T1 (pre-season)	T2 (mid-season)	T3 (post-season)
<b>Body Mass (kg)</b>	82.5 (10.5, 75.0-90.1)	82.1 (11.0, 74.2-90.0)	82.17 (11.1, 74.2-90.1)
<b>BF %</b>	10.1 (1.9, 8.9-11.3)	-	10.5 (2.1, 9.2-11.8)
<b>cfDNA (ng/ml)</b>	3.8 (1.7, 2.5-5.0)	5.5 (3.1, 3.3-7.7) <sup>c</sup>	8.9 (8.8, 2.7-15.3)
<b>CRP (ng/ml)</b>	246.6 (115.2, 164.2-329.1) <sup>b*</sup>	612.5 (339.5, 369.6-855.3) <sup>a*</sup>	621.4 (634.4, 167.6-1075.2) <sup>f</sup>
<b>T (ng/dL)</b>	334.3 (91.7, 268.7-399.9)	392.7 (123.8, 304.2-481.2)	306.0 (80.7, 248.3-363.8)
<b>C (µg/dL)</b>	13.7 (4.2, 10.7-16.7) <sup>c</sup>	17.4 (2.0, 15.9-18.8) <sup>c*</sup>	9.4 (5.1, 5.8-13.1) <sup>a,b*</sup>
<b>T:C</b>	.0273 (.0147, .0168-.0378)	.0228 (.0081, .0171-.0286)	.0416 (.0251, .0237-.0596)
<b>CK (U/L)</b>	118.3 (58.6, 76.4-160.2)	165.9 (113.5, 84.7-247.1)	130.4 (93.6, 63.4-197.4) <sup>f</sup>
<b>cfDNA %Δ</b>	100	165.3 (97.5, 95.5-235.0)	246.8 (195.2, 107.1-386.4)
<b>CRP %Δ</b>	100 <sup>b</sup>	305.6 (208.1, 156.7-454.5) <sup>a</sup>	263.9 (239.5, 92.6-435.2)
<b>T %Δ</b>	100	121.9 (42.0, 91.8-151.9)	92.9 (18.1, 80.0-105.9)
<b>C %Δ</b>	100 <sup>c</sup>	141.7 (57.9, 100.3-183.1) <sup>c*</sup>	69.8 (26.6, 50.8-88.9) <sup>a,b*</sup>
<b>T:C %Δ</b>	100	91.4 (32.6, 68.1-114.7)	164.0 (97.3, 94.4-233.6)
<b>CK %Δ</b>	100	138.9 (76.1, 84.5-193.3) <sup>c</sup>	114.8 (65.1, 68.3-161.4) <sup>f*</sup>

Values above are displayed as mean (SD, 95% CI). \* significant at P ≤ 0.01 level

**Within group differences**

- <sup>a</sup> statistically significantly different than T1
- <sup>b</sup> statistically significantly different than T2
- <sup>c</sup> statistically significantly different than T3

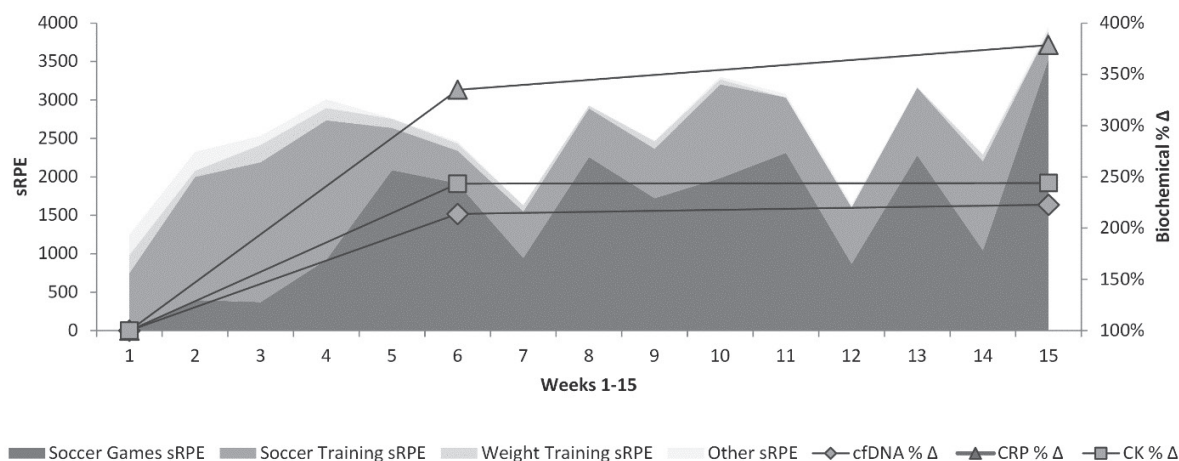
**Between group differences**

- <sup>d</sup> statistically significantly different than T1
- <sup>e</sup> statistically significantly different than T2
- <sup>f</sup> statistically significantly different than T3

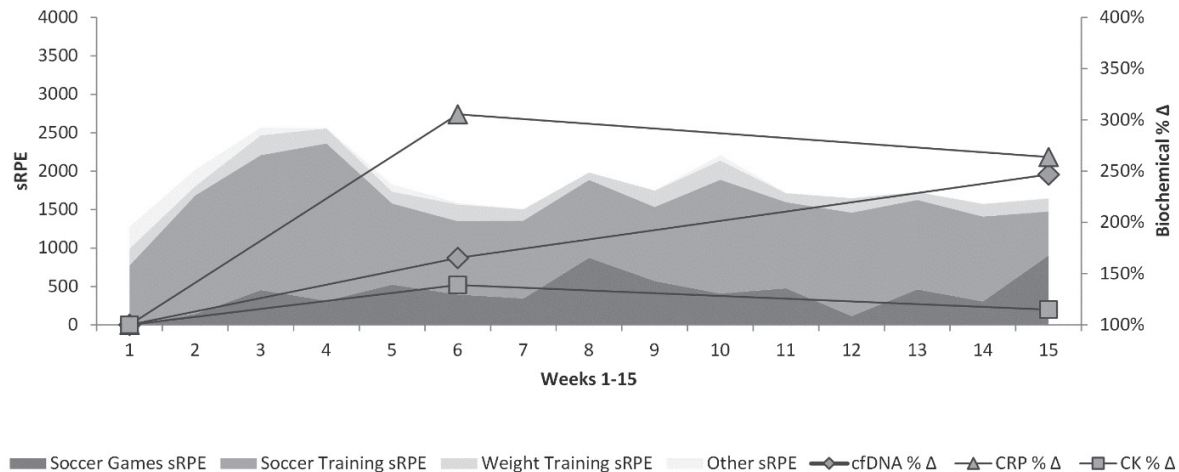
are markers of inflammation and tissue damage including cf-DNA, CRP and CK. In G1, cf-DNA (F(2,24) = 9.413, P = 0.001, observed power = 0.962), CRP (F(2,24) = 12.531, P = 0.001, observed power = 0.992), CK (F(2,24) = 7.528, P = 0.003, observed power = 0.913), cf-DNA %Δ (F(2,24) = 8.017, P = 0.002, observed power = 0.930), CRP %Δ (F(1,235, 14.816) = 12.126, P = 0.002, observed power = 0.936), CK %Δ (F(2,24) = 8.024, P = 0.002, observed power = 0.930) were all statistically higher than T1 at T2 and T3. In G2, CRP (F(2,18) = 4.234, P = 0.031, observed power = 0.665) and CRP %Δ (F(2,18) = 3.912, P = 0.039, observed power = 0.628) were significantly higher at T2 than T1. There were also sever-

al between-group differences where G1 was significantly higher than G2 at each instance; cf-DNA at T2, CRP at T3, CK at T3 and CK %Δ at T2 and T3. Figure 1A and 1B show changes in cf-DNA %Δ, CRP %Δ and CK %Δ for G1 and G2, respectively.

Based on data for T2 and T3, there was a statistically significant and negative correlation between cf-DNA %Δ and CRP %Δ (r = -0.40) in G1; there was also a statistical relationship between CRP %Δ and CK %Δ (r = 0.46). For G2, there was a statistically significant correlation between CRP %Δ and CK %Δ (r = 0.64).



**Figure 1A** Group 1 percent change for cf-DNA, CRP and CK



**Figure 1B** Group 2 percent change for cf-DNA, CRP and CK

## DISCUSSION

Despite no significant differences between T2 and T3 for cf-DNA %Δ, CRP %Δ and CK %Δ in G1, the relative changes in all three markers remained elevated and were statistically greater than T1 (see Table 1). Considering the previously discussed time course advantages cf-DNA offers over CRP and CK, this may suggest that cf-DNA is a useful marker to reflect accumulated soccer training and competitive stressors that should not require training interruptions in order to measure a current baseline value. In G2, cf-DNA %Δ, CRP %Δ and CK %Δ were all higher at T2 and T3 than T1, but T2 and T3 were not consistently statistically different than T1 as they were in G1; this is potentially a result of the lower sRPE values in G2 versus G1. Season total sRPE was statistically greater for G1 than G2 (G1 mean sRPE = 38,917, SD = 4763; G2 mean sRPE = 27632, SD = 3119;  $p = 0.001$ ). Weekly sRPE for G1 was statistically greater than G2 for all weeks other than weeks 1,2,3,7 and 12.

Unexpectedly, there was a moderate but negative correlation between cf-DNA %Δ and CRP %Δ ( $r = -0.40$ ) in G1. Previous literature has shown positive correlations between cf-DNA and CRP. Fatouros and Destouni (2006) completed a 12 week resistance training study in recreationally trained men. Plasma DNA and CK were measured five times during the 12 week study (baseline and T1-T4) and a correlation between plasma DNA and CK was found at two of the five measurement times T2 ( $r = 0.793$ ,  $P < 0.01$ ) and T3 ( $r = 0.744$ ,  $P < 0.01$ ). A correlation between cf-DNA and CRP ( $r = 0.36$ ,  $P < 0.001$ ) has also been demonstrated in intensive care unit patients.<sup>41</sup> Other research has found no correlation between cf-DNA and CRP.<sup>42,43</sup> Previous research in team sports such as soccer and rugby has shown that CRP and CK may not peak until 24-48 hours post competition, and may remain significantly elevated above baseline for several days.<sup>44-46</sup> To our knowledge this is the first study to perform repeated measurements of cf-DNA and CRP 48 hours after the last bout of exercise; the Fatouros and Detouni (2006) study performed blood draws 96 hours after the last bout or resistance training. While there is no clear explanation for this negative relationship between cf-DNA %Δ

and CRP %Δ in the current study, it is possible that CRP concentrations were near peak values 48 hours after the latest training session or competition, while cf-DNA concentrations had returned to the current baseline earlier as expected. This discrepancy warrants further research but may lend more evidence to the value cf-DNA as a marker of training-related stress and further underlines the importance of selecting a biochemical marker that does not require substantial training interruptions in order to return to baseline.

Despite the frequency which T, C and T:C are used in the literature as markers of fatigue and anabolic-catabolic balance<sup>27,28,47</sup>, this study did not find consistent changes in T, C, or T:C as workloads changed throughout the season, as reported by sRPE. There were also no relationships found between T, C or T:C and CRP, CK or cf-DNA. These findings reiterate the results of previous research that found no consistent changes in T, C and T:C with changing training loads<sup>31,32</sup> and suggests that practitioners should use care when interpreting T:C.

Future research should include more frequent blood draws in order to elucidate any relationship that may exist between cf-DNA and soccer training-related variables. Because non-training related stressors may influence the concentrations of biochemical markers, future research should also attempt to quantify non-training related stressors.

## CONCLUSION

Few studies have investigated changes in cf-DNA concentration as result of exercise and the majority of these studies have explored changes in cf-DNA concentrations during, immediately after and several hours after exercise. The literature addressing chronic changes in cf-DNA concentrations resulting from exercise and sport is limited. For coaches and sport scientists interested in using biochemical markers in a LTAMP, this research provides evidence that cf-DNA may be used as an alternative to, or in conjunction with, common markers of tissue damage and inflammation such as CRP and CK. The greatest advantage of using cf-DNA versus markers such as CRP and CK, may be the time course which cf-DNA responds after a bout of exercise or competition. While CRP

and CK may remain elevated for a number of days after a single bout of exercise, cf-DNA typically returns to pre-exercise levels within hours. As a result, the use of cf-DNA should not require interruptions in training in order to assess baseline concentrations. This allows coaches to be more confident that changes in the concentration cf-DNA reflect accumulated training stressors and not the last bout of training or competition.

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