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Cell Free DNA as a Marker of Training Status in Weightlifters

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Cell free DNA as a marker of training status in weightlifters

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ABSTRACT: The purpose of this investigation was to elucidate the changes in cf-DNA as it relates to fluctuations in resistance training workloads and intensities. The relationship between cell free DNA (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. Eight weightlifters (5 males and 3 females, age = 25 ± 3.5 yr, body mass = 88.3 ± 22.7 kg, height = 173.8 ±8.4 cm) volunteered to participate in this study. Venous blood samples, body mass and body composition were taken six times, each corresponding to the end of a training phase. CK ($p = 0.018$, $\eta^2 = 0.409$) and CK %Δ ($p < 0.001$, $\eta^2 = 0.594$) were the only biochemical variables to reach statistical significance at any point. A number of statistically significant correlations were found among variables. VLD4wk was related to CK %Δ ($r = 0.86$), VLD4wk %Δ was related CK %Δ ($r = 0.86$) and TID1wk was related to CRP ($r = 0.83$). cf-DNA %Δ was correlated with CRP and CRP %Δ ($r = 0.83$ and 0.86, respectively). CRP and CRP %Δ were correlated with BF % ($r = 0.94$ and 0.92, respectively). CK and CK %Δ were both related to T:C ($r = 0.94$ and 0.89, respectively) and T:C %Δ ($r = 0.87$ and 0.86, respectively). The correlation between cf-DNA and CRP suggests that cf-DNA may be a valuable indicator of inflammation in weightlifters.

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INTRODUCTION

Biochemical markers can serve a valuable purpose when monitoring an athlete's acute and chronic responses to training and the accumulative effect of non-training related stressors. However, a paucity of research has addressed the use of biochemical markers as a routine component of a long-term athlete monitoring program (LTAMP). It is important that performance and other related testing (i.e. LTAMP) interfere with the training process as little as possible. Physiological markers of a LTAMP should be sensitive to acute and chronic training challenges and other stressors. The acute response or time course in which a marker responds should also be considered. If concentrations of a biochemical marker remain elevated or continue to increase above baseline for more than 48 hours after a single bout of exercise, it is difficult to determine whether changes in marker concentration reflect variables such as tissue damage or inflammation resulting from the latest training session, or these changes reflect chronic or accumulated stress.

The existence of nucleic acids (DNA and RNA) in plasma was initially described by Mandel and Metais in 1948 [1]. The presence of nucleic acids in blood then went largely uninvestigated until 1977 when it was determined that cancer patients had elevated levels of serum DNA [2]. Plasma cell-free DNA (cf-DNA) has since been recognized as a marker of tissue damage and inflammation for a variety of conditions. Conditions such as cardiovascular disease [3], sepsis [4] and cancer [5] have been associated with elevated cf-DNA concentrations. The degree or severity of injury associated with traumatic events including but not limited to blunt trauma and burn victims, have also been shown to be reflected in cf-DNA concentrations [6,7].

In recent years there have been several investigations of cf-DNA regarding its response to exercise and its potential use as an indicator of tissue damage, inflammation and overtraining [8-13]. While not specifically discussed in any previous investigation, several of these initial inquiries have demonstrated that the chronic and acute response of cf-DNA to exercise may offer benefits over other common markers of tissue damage and inflammation for inclusion as an athlete assessment in a LTAMP.

The literature is still scant in addressing the acute and chronic response of cf-DNA to exercise. However, those investigations addressing the acute response have clearly shown that cf-DNA typically peaks immediately after exercise and returns to baseline within approximately 2-4 hours post exercise [14,9,10,15,13]. Transitory increases in plasma cf-DNA concentrations after a single bout of exercise may offer advantages over some more commonly reported markers of tissue damage and inflammation. For example, in a study using an exhaustive treadmill running protocol, C-reactive protein (CRP), uric acid (UA) and creatine kinase (CK) concentrations continued to increase 24 hr after exercise. However, cf-DNA peaked immediately after exercise and returned to the pre-exercise baseline within 2 hours post exercise [15]. The time course in which cf-DNA concentrations respond after resistance training have been similar to that of endurance exercise where cf-DNA concentrations peak immediately post exercise and return to baseline within 2 hrs [9]. The time course of CRP and CK responses post exercise, which are of specific interest for the purpose of this investigation, has been shown to remain elevated for a number of days after a single bout of exercise [16,17]. Because athletes should rarely abstain from training or exercise for several days to allow markers such as CK and CRP to return to baseline, the acute response of cf-DNA may be more appropriate for use in a LTAMP. Fatouros et al. investigated chronic changes in cf-DNA during a 12-week resistance training study and demonstrated that cf-DNA may be a useful marker of overtraining as changes in cf-DNA reflected fluctuations in resistance training volume load or workloads [12]. Statistically significant increases in cf-DNA concentrations have also been seen throughout a men's collegiate soccer season [18].

The precise mechanisms responsible for the presence of cf-DNA in plasma are not entirely understood. While it is beyond the scope of this paper to review in detail the potential origins of cf-DNA in plasma as a result of exercise, the mechanisms are likely time-dependent [19]. Oxidative damage and apoptotic processes have been proposed as mechanisms for increased cf-DNA concentrations immediately after a single bout of exercise [8,9,19,20,21]. However, emerging theories of cf-DNA kinetics suggest that oxidative damage and apoptosis may not be responsible for peak cf-DNA concentrations immediately or shortly after an acute bout of exercise. Rather, oxidative damage and apoptosis may play a primary role in chronic cf-DNA concentrations or during long duration events such as marathons, where peak cf-DNA concentrations immediately post exercise may more likely result from mechanisms such as NETosis and trap formation, among others, which can increase plasma DNA concentrations rapidly [19].

This study served to investigate the merits of cf-DNA as a monitoring tool in a LTAMP. The purpose of this investigation was to elucidate the chronic changes in cf-DNA as it relates to fluctuations in resistance training workload and intensities, while also examining the relationship between cf-DNA and markers of inflammation, muscle tissue damage (CRP, CK), anabolic/catabolic status (testosterone, cortisol), body mass and body composition. This study also aimed to build upon the limited body of literature addressing chronic changes in cf-DNA as it relates to resistance training.

MATERIALS AND METHODS

Athletes

Eight weightlifters (5 males and 3 females, age = 25 ± 3.5 yr, body mass = 88.4 ± 22.7 kg, height = 173.8 ±8.4 cm) volunteered to participate in this study. Seven of the eight weightlifters were national class weightlifters and all had been participating in a LTAMP. Written informed consent was obtained from each subject. The University's Institutional Review Board approved this study. This study was performed in accordance with the ethical standards of the Helsinki Declaration.

Anthropometrics

A stadiometer (Detecto, Webb City, MO) was used to measure subject height. Body mass was measured by digital scale and body composition was assessed by plethysmography (BodPod, Concord, CA). Body mass and body fat percentage (BF %) measures were completed six times throughout the study on the same days/mornings as venous blood draws described below.

Biochemistry

Venous blood draws were collected 6 times (T1-T6), each corresponding to the end of a training phase. Testing sessions were constrained by the competition schedule and coaching/training considerations. All blood draws were completed 48 hours after the last training session. Blood draws were performed between at the same time each morning to minimize diurnal variation of testosterone and cortisol. All subjects were monitored closely by sport science staff which instructed and verified that all subjects were fasted overnight and had not participated in physical activity for 48 hours prior to blood draws. Hydration status was also assessed prior to each blood draw and athletes were encouraged to begin drinking water upon waking. Hydration was measured via urinary specific gravity (USG) with a PAL-10S Refractometer (Atago, Tokyo, Japan). A USG ≥ 1.020 signifies dehydration (25), but no subject USG measurements were ≥ 1.020 at any of the 6 measurement times. Biochemical markers are quantified as concentrations in plasma or serum as well as percent change (%Δ).

C-reactive Protein

Serum CRP concentrations were measured using a solid phase sandwich-type ELISA [22]. 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 μg/ml) was used as the reporter. The construction of standard curves was completed using purified native CRP (1.56-100 ng/ml) diluted in TBS, 0.1% gelatin and 0.02% Tween 20. Horseradish peroxidase-conjugated goat anti-mouse IgG H+L (Thermo Fisher

Scientific, Rockford, IL) was used as the secondary Ab. The HRP substrate kit (Bio-Rad, Hercules, CA) was used to develop color and was measured at 405 nm with a VersaMax™ plate reader (Molecular Devices, Menlo Park, CA). All samples were read in duplicate. The intra and inter-assay coefficient of variation were 7.1% and 12.3% respectively.

Testosterone and Cortisol

Serum total T and C concentrations were measured using a chemiluminescence immunoassay on IMMULITE® 2000 immunoassay system (Siemens, Los Angeles, CA, USA). The ration of testosterone to cortisol (T:C) was also calculated. The intra and inter-assay coefficient of variation for T were 5.8% and 6.9%, respectively. The intra 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 and inter-assay coefficient of variation were 1.1% and 1.8% respectively.

Cell-free DNA

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

Training Volume Load and Training Intensity

A 20-week training period was investigated, including several phases of resistance training. The loads and repetitions for all exercises

TABLE 1. Anthropometric and Training Changes.

	T1 (baseline)	T2	T3	T4	T5	T6
Body Mass	88.4 (22.7)	90.0 (23.7)	89.2 (22.8)	88.7 (22.1)	89.8 (22.5)	88.8 (22.4)
BF % c**,d**	18.8 (8.6)	18.6 (7.7)	18.9 (8.6)	18.8 (8.5)	19.9 (8.2)	19.4 (8.2)
VLD1wk (thousands)	5.2 (2.2) T2,T3,T5&T6***	23.2 (8.8) T1&T3-T5***	15.0 (3.6) T1 & T2***,T4**	8.2 (4.4) T2***,T3&T5**,T6***	14.9 (5.6) T1&T2***,T4**	19.2 (6.6) T1&T4***
VLD4wk (thousands) f*	5.2 (2.2) AT***	28.9 (8.1) AT***	22.2 (7.2) T1,T2&T4***,T5**	15.5 (4.8) T1-T3***,T6*	17.4 (5.8) T1&T2***,T3**	20.2 (6.2) T1&T2***,T4*
TID1wk c*	116.6 (59.2) T3**,T5&T6***	159.6 (61.6)	208.6 (74.0) T1**	136.7 (101.2) T5&T6**	233.9 (79.5) T1***,T4**	234.1 (73.9) T1***,T4**
TID4wk	116.6 (59.2) AT***	171.5 (49.3) T1&T3***,T5*,T6***	213.6 (53.9) T1&T2***	198.5 (61.7) T1&T6***	202.3 (64.0) T1***,T2**,T6**	237.3 (79.7) T1,T2&T4***,T5**
VLD1wk %Δ	100% T2,T3,T5&T6***	460.4% (72.3) T1&T3-T5***	319.2% (85.8) T1,T2&T4***	160.4% (46.4) T2,T3,T5&T6***	299.3% (62.3) T1,T2&T4***,T6*	390.1% (87.1) T1&T4***,T5*
VLD4wk %Δ f*	100% AT***	600.0% (135.0) AT***	452.5% (87.7) T1,T2&T4***,T5*	317.4% (65.6) T1-T3***,T6**	360.7% (89.2) T1&T2***,T3*	415.1% (90.2) T1&T2***,T4**
TID1wk %Δ	100% T3,T5,T6***	146.4% (27.7) T3*,T5&T6**	210.9% (79.3) T1***,T2*,T4***	119.5% (39.6) T3,T5&T6***	222.6% (57.4) T1***,T2**,T4***	226.5% (65.8) T1***,T2**,T4***
TID4wk %Δ	100% AT***	166.4% (45.4) T1***,T3**,T6***	212.9% (73.1) T1***,T2**	191.0% (49.4) T1***	194.1% (50.3) T1***	226.7% (61.0) T1&T2***

T1-T6 – statistically significant difference than the listed time
AT – statistically significant different than all other times
* p ≤.05, ** p ≤.01, *** p ≤.001
a – correlated with cf-DNA
b – correlated with cf-DNA %Δ
c – correlated with CRP
d – correlated with CRP %Δ
e – correlated with CK
f – correlated with CK %Δ
g – correlated with T
h – correlated with T %Δ
i – correlated with C
j – correlated with C %Δ
k – correlated with TC
l – correlated with TC %Δ
m – correlated with Body Mass
n – correlated with BF %
o – correlated with VLD1wk
p – correlated with VLD1wk %Δ
q – correlated with VLD4wk
r – correlated with VLD4wk %Δ
s – correlated with TID1wk
t – correlated with TID1wk %Δ
u – correlated with TID4wk
v – correlated with TID4wk %Δ

performed were recorded by each subject throughout the 20-week period. A V-scope 120 (Lipman Electronic Engineering Ltd, Ramat Hahayal, Israel) was used to measure the concentric displacement (CD) for each of the 24 exercises performed by all subjects over the course of this investigation. Prior to the 20-week training period, each subject completed three trials of all 24 exercises and mean CD was calculated for each exercise (ICC = 0.99).

The most common manner to calculate volume load (VL) and training intensity (TI) are VL = sets x reps x load and TI = VL / reps, respectively. VL provides a unit-less estimate of total work and TI provides a unit-less estimate of work per repetition [23,24]; however, neither of these measures considers barbell or dumbbell displacement. To provide a more accurate estimate of work, volume load with displacement (VLD) was calculated using VLD = sets x reps x load x CD. Similarly, in order to provide a more precise estimate of average work per repetition, training intensity with displacement (TID) was calculated using TID = VLD / reps.

VLD and TID weekly means were calculated for each four-week (VLD4wk, TID4wk) and one-week (VLD1wk, TID1wk) period prior to all venous blood draws. The only exception to this was T1. Because there were only 11 days recorded prior to the initial baseline blood draw, VLD and TID were reported as the total for the week prior to T1.

STATISTICS

Data were analyzed with JASP version 0.8 for Windows and expressed as means and standard deviations (SD). Data were analyzed using one-way ANOVA with repeated measures. Greenhouse-Geisser adjustment was employed when the assumption of sphericity was violated according to Mauchly's test of sphericity ($P < 0.05$). Post hoc tests were completed using the Bonferroni correction. Pearson product-moment correlation coefficients were performed on all mean biochemical and anthropometric variables. The level of significance was set at $p \leq 0.05$. Percent change for biochemical markers, VLD and TID was also calculated.

Table 2. Biochemical Changes.

	T1 (baseline)	T2	T3	T4	T5	T6
cf-DNA (ng/ml)	7.5 (1.8)	7.4 (4.4)	8.1 (3.1)	7.0 (3.16)	9.2 (6.8)	6.7 (2.8)
CRP (ng/ml) b*,n**,S*	740.4 (538.5)	865.7 (766.9)	963.0 (992.4)	723.0 (547.8)	1618.3 (2173.5)	1141.3 (1331.6)
CK (U/L) k**,l*	102.5 (75.2) T2**	189.4 (148.7) T1&T4**,T5&T6*	143.1 (94.8)	98.4 (79.92) T2**	106.8 (64.3) T2*	113.5 (80.2) T2*
T (ng/dL)	200.7 (151.6)	206.4 (161.7)	230.0 (172.2)	220.9 (164.0)	201.0 (152.2)	223.8 (179.4)
C (μg/dL)	16.8 (6.7)	17.4 (11.5)	17.2 (6.9)	18.2 (6.4)	18.2 (7.6)	17.7 (8.1)
T:C e**,f*	0.0160 (0.013)	0.0236 (0.0217)	0.0181 (0.0148)	0.0153 (0.0121)	0.0151 (0.0129)	0.0185 (0.0168)
cf-DNA %Δ c*,d*	100	98.4 (54.6)	111.9 (46.8)	97.1 (52.4)	132.0 (107.1)	95.9 (51.6)
CRP %Δ b*,c**,d**	100	115.6 (61.2)	124.0 (56.2)	108.8 (34.9)	186.4 (169.5)	134.4 (95.0)
CK %Δ k*,l*,q*,r*	100 T2***,T3*	189.0 (64.0) T1,T4,T5&T6***	150.5 (56.2) T1*	106.1 (48.2) T2***	118.2 (42.4) T2***	115.1 (23.3) T2***
T %Δ	100	94.8 (16.4)	114.7 (30.8)	114.0 (30.3)	102.4 (23.6)	105.9 (25.5)
C %Δ	100	96.6 (36.9)	107.2 (36.5)	113.7 (29.8)	109.8 (21.3)	105.3 (22.6)
T:C %Δ e*,f**	100	115.9 (56.2)	113.3 (32.9)	105.2 (38.5)	97.5 (36.1)	104.1 (32.5)

T1-T6 – statistically significant difference than the listed time
AT – statistically significant different than all other times
* p ≤.05, ** p ≤.01, *** p ≤.001
a – correlated with cf-DNA
b – correlated with cf-DNA %Δ
c – correlated with CRP
d – correlated with CRP %Δ
e – correlated with CK
f – correlated with CK %Δ
g – correlated with T
h – correlated with T %Δ
i – correlated with C
j – correlated with C %Δ
k – correlated with TC
l – correlated with TC %Δ
m – correlated with Body Mass
n – correlated with BF %
o – correlated with VLD1wk
p – correlated with VLD1wk %Δ
q – correlated with VLD4wk
r – correlated with VLD4wk %Δ
s – correlated with TID1wk
t – correlated with TID1wk %Δ
u – correlated with TID4wk
v – correlated with TID4wk %Δ

RESULTS

There were no statistically significant differences for body mass or BF % at any test. Because of the weight class requirements of weightlifting and the well-trained status of these subjects, statistically significant changes in body mass or composition were not expected. CK ($p = 0.018$, $\eta^2 = 0.409$) and CK %Δ ($p < 0.001$, $\eta^2 = 0.594$) were the only biochemical variables to reach statistical significance at any point. VLD and TID were significantly different at numerous periods during the study. A number of statistically significant correlations were found among variables. VLD4wk was related to CK %Δ ($r = 0.86$), VLD4wk %Δ was related CK %Δ ($r = 0.86$) and TID1wk was related to CRP ($r = 0.83$). cf-DNA %Δ was correlated with CRP and CRP %Δ ($r = 0.83$ and 0.86, respectively). CRP and CRP %Δ were correlated with BF % ($r = 0.94$ and 0.92, respectively). CK and CK %Δ were both related to T:C ($r = 0.94$ and 0.89, respectively) and T:C %Δ ($r = 0.87$ and 0.86, respectively). All means, standard deviations, changes and correlations can be seen in Table 1 and Table 2.

DISCUSSION

The purpose of this investigation was to elucidate the chronic changes in cf-DNA as it relates to fluctuations in resistance training workload and intensities while also examining the relationship between cf-DNA and markers of inflammation, muscle tissue damage (CRP, CK), anabolic/catabolic status (testosterone, cortisol), body mass and body composition. To do this, the relationships between cf-DNA, changing resistance training workload/intensities and other biochemical markers often employed to quantify inflammation, tissue damage and anabolic/catabolic status of athletes were investigated. The current study is the first long-term investigation of cf-DNA and its relationship with training variables and biochemical markers in well-trained athletes. This study is also one of the few investigations to address volume load and training intensity with measures of displacement which should provide more accurate estimates of total and average workloads, respectively [24].

Training for weightlifting consists entirely or mostly of resistance training and calculating total training stress or workloads is straightforward. Weightlifting is unlike many sports which require athletes to participate in multiple training modalities. Furthermore, sports such as soccer and basketball require many changes of direction which can make it difficult to quantify total workloads and any link between biochemical markers and work completed in sport related activities. Considering the exploratory nature of this study, it was important that exercise workloads were easily calculated and that exercise stressors originated from a single exercise modality. Weightlifting allowed for a detailed investigation, with fewer training stressors with which to contend. It should also be mentioned that subjective measures of stress and recovery were not collected during this study. Subjective reports should be collected on a routine basis (several times a week), and blood draws would have been required more frequently than was feasible during this study in order to make comparisons between subjective reports and biochemical markers.

One-week and four-week VLD or TID means were calculated to investigate any correlation that may exist between VLD or TID and other biochemical and anthropometric variables. A training-load-recency effect is likely to have greater influence on biochemical markers and fatigue. While CK %Δ was related to VLD4wk ($r = 0.86$) and VLD4wk %Δ ($r = 0.86$), curiously, CK was not related to the most recent one-week measures of VLD or TID. Upon further visual inspection of VLD, TID and biochemical changes, CK does not appear to respond to VLD as its correlation with four-week mean VLD would suggest. To illustrate this point, Figure 1 shows daily VLD, cf-DNA

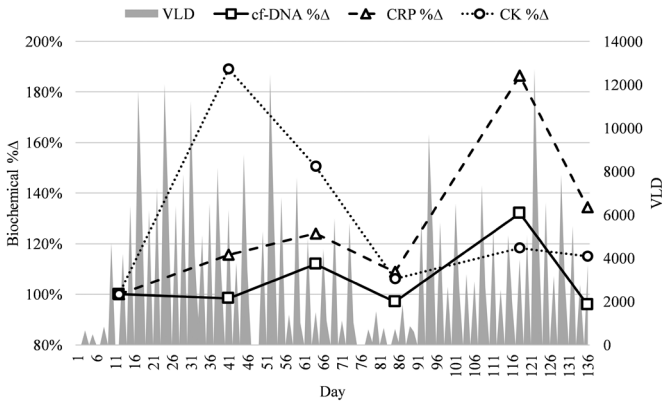

FIGURE 1. Daily VLD, cf-DNA %Δ, CRP %Δ and CK %Δ.

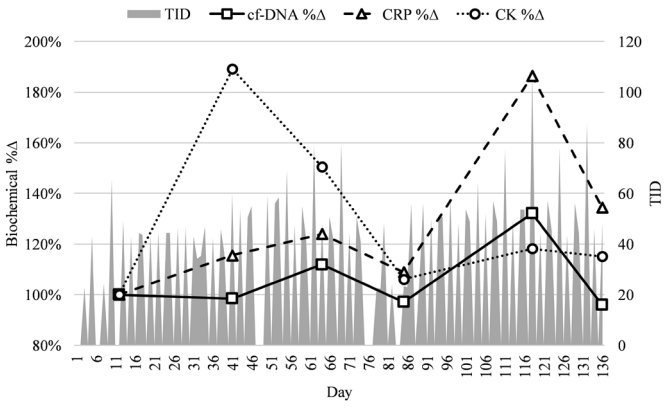

FIGURE 2. Daily TID, cf-DNA %Δ, CRP %Δ and CK %Δ.

Table 3. Group mean and individual subject cf-DNA %Δ and CRP %Δ.

		T1	T2	T3	T4	T5	T6
Subject 1	**cf-DNA %Δ**	100.0%	152.0%	81.2%	85.8%	104.2%	66.4%
	CRP %Δ	100.0%	174.1%	252.6%	81.5%	535.1%	336.5%
Subject 2	**cf-DNA %Δ**	100.0%	108.7%	106.8%	65.7%	41.7%	44.1%
	CRP %Δ	100.0%	112.4%	85.7%	66.3%	120.0%	109.3%
Subject 3	**cf-DNA %Δ**	100.0%	62.1%	65.2%	84.9%	146.1%	58.1%
	CRP %Δ	100.0%	140.5%	72.3%	159.1%	93.5%	94.2%
Subject 4	**cf-DNA %Δ**	100.0%	78.8%	156.3%	24.8%	110.5%	156.0%
	CRP %Δ	100.0%	62.4%	114.6%	116.9%	57.4%	36.2%
Subject 5	**cf-DNA %Δ**	100.0%	65.0%	63.8%	193.3%	382.7%	68.4%
	CRP %Δ	100.0%	56.4%	122.2%	121.7%	125.4%	88.0%
Subject 6	**cf-DNA %Δ**	100.0%	204.4%	200.8%	120.1%	123.4%	189.8%
	CRP %Δ	100.0%	86.0%	96.7%	62.4%	84.2%	76.7%
Subject 7	**cf-DNA %Δ**	100.0%	40.6%	110.6%	62.7%	51.5%	106.6%
	CRP %Δ	100.0%	227.2%	106.3%	136.7%	114.5%	129.6%
Subject 8	**cf-DNA %Δ**	100.0%	75.8%	110.4%	139.0%	96.1%	77.8%
	CRP %Δ	100.0%	65.7%	141.4%	126.0%	361.2%	204.5%
Group Mean	**cf-DNA %Δ**	100.0%	98.4% ± 54.6	111.9% ± 46.8	97.1% ± 52.4	132.0 ± 107.1	95.9% ± 51.6
	CRP %Δ	100.0%	115.6% ± 61.2	124.0% ± 56.2	108.8% ± 35.0	186.4% ± 170.0	134.4% ± 95.0

%Δ, CRP %Δ and CK %Δ, while Figure 2 shows daily TID, cf-DNA %Δ, CRP %Δ and CK %Δ. In Figures 1 and 2, it can be seen that CK %Δ did not consistently respond to increases or decreases in VLD or TID. In contrast, both cf-DNA and CRP concentrations appear to rise and fall more synchronously with changes in VLD, as illustrated in Figure 1. cf-DNA and CRP may also be influenced by TID as seen in Figure 2. The literature pertaining to cf-DNA and resistance training is limited and has focused on training workload/volume-load and the resulting oxidative damage and inflammatory response to resistance training [19,12]. However, it has previously been suggested that the release of cf-DNA is multifactorial and does not appear to be related to a specific training factor such as VLD [19]. In order to better determine how these markers respond to recent versus accumulated training loads, more frequent blood draws, perhaps once or more a week, should be performed. Future research should also include measures of stress-related self-report data to better quantify training and non-training-related stressors.

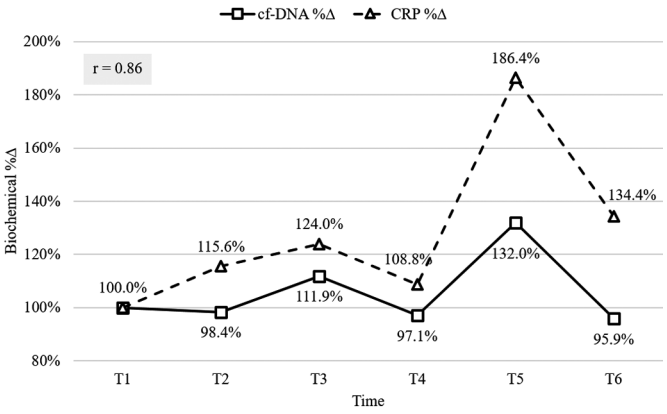

FIGURE 3. Relationship between mean cf-DNA %Δ and CRP %Δ.

Mean cf-DNA %Δ was statistically correlated with mean CRP %Δ ($r = 0.86$) over the course of the six tests. A correlation between cf-DNA and CRP has been reported [12,25]. This correlation between cf-DNA and CRP suggests that cf-DNA may be a valuable indicator of inflammation. CRP has been shown to be related to BF% in a variety of populations, including healthy non-obese individuals [26,27,28]. Practitioners should use caution when interpreting these results, as substantial individual differences may exist despite the correlation between mean values. Figure 3 illustrates the relationship between mean cf-DNA %Δ and CRP %Δ, while Table 3 presents cf-DNA %Δ and CRP %Δ for each subject.

Testosterone, cortisol, and T:C have been used in training studies to monitor an athlete's response to a training stimulus. Periods of high volume and high intensity training have been shown to cause

decreases in serum testosterone as well as decreases in T:C [29,30,31]. However, the literature offers mixed conclusions about whether T, C and T:C respond consistently to training volume and intensity. For example, among well-trained experienced athletes, short-term increases VL can be accompanied by increases in T and T:C [32] and endurance athletes have not responded consistently to increased or decreased training load, or when overreached or overtrained [33-36]. In this study, T, C or T:C were not statistically different at any time and were not related to measures of VLD or TID. With no discernible physiological rationale, T:C and T:C %Δ were both related to CK (r = 0.94 and r = 0.87, respectively) and CK %Δ (r = 0.89 and 0.86, respectively).

The small number of subjects, as well as the number of male and female subjects, were the major limitations of this investigation and likely contributed to the dearth of significant biochemical changes. Unfortunately, this is a common problem in sport science where small sample sizes are commonplace. Future research should monitor acute and chronic changes in cf-DNA concentrations more frequently, and in larger well trained populations.

CONCLUSIONS

A limited number of studies have explored changes in cf-DNA concentrations resulting from exercise, most of which have investigated changes in cf-DNA immediately or within hours after exercise. The present study is one of only several investigations to examine chronic changes in cf-DNA related to athlete training programs. This study suggests that cf-DNA may be a valuable marker of inflammation in weightlifters as indicated by the strong relationship between cf-DNA and CRP. It has been previously demonstrated that cf-DNA returns to pre-exercise concentrations hours after exercise. Considering that inflammatory markers such as CRP remain elevated for several days after a single bout of exercise, cf-DNA may provide advantages over CRP as a marker of inflammation in a LTAMP where an athlete's training program should rarely be interrupted for more than two consecutive days.

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