Relationship between biomarkers of muscle damage and redox status in response to a weightlifting training session: effect of time-of-day

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The aims of the present study were to: (1) investigate the effect of a weightlifting training session and time-of-day (TOD) upon biological parameters (i.e., oral temperature, hematological, C-reactive protein (CRP), and oxidative stress) and (2) assess their possible link with muscle damage responses. Nine weightlifters (21 ± 0.5 years) performed, in a randomized order, three Olympic-Weightlifting sessions (i.e., at 08:00, 14:00, and 18:00). Blood samples were collected at rest, 3 min and 48 h after each training session. Between pre- and post-training session, ANOVA showed significant increases in oxidative stress markers at the three TODs ($p < 0.01$) and significant increases for creatine kinase (CK) and lactate dehydrogenase (LDH) only at 08:00 and 18:00 ($p < 0.05$). At rest, the results showed a significant diurnal variation for the majority of the selected parameters except for malondialdehyde (MDA), total bilirubin, and CRP with higher values observed at 18:00 ($p < 0.05$). After the training session, given the higher rate of increase during the morning session, these diurnal variations persisted for temperature and WBC ($p < 0.01$) and were suppressed for CK, LDH, uric acid (UA), catalase, and glutathione peroxidase. The main significant correlations ($p < 0.001$) were observed between: (1) CK and MDA ($r = 0.6$) and CK and UA ($r = 0.66$ and $r = 0.82$) during the morning and evening training sessions; (2) CK and CRP only during the morning session ($r = 0.5$); and (3) CRP and WBC during the three training sessions ($r = 0.8$). In conclusion, the present findings: (1) confirm that the muscle damage responses could be induced by a high level of oxidative stress and (2) suggest to avoid scheduling training sessions in the morning given the higher muscle damage, inflammatory, and oxidative responses at this TOD.

Keywords: lipid peroxidation, creatine kinase, lactate dehydrogenase, strength exercises, diurnal variation

Introduction

Creatine kinase (CK) and lactate dehydrogenase (LDH) levels have previously been monitored in untrained and trained subjects, who underwent resistance exercise sessions, to assess muscle damage responses (10, 29). These parameters as well as the white blood cells (WBCs)

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and their subpopulations have also been identified as indicators of muscle fatigue and overtraining (35, 45). It has been shown that rugby sevens match (41) or high-intensity maximal tasks such as intermittent running, sprinting, jumping, resistance exercises (33, 45), or Wingate test (16) resulted in transient leukocytosis and increase in biochemical markers of muscular damage for up to 10 days after exercise (10). Particularly, after a single weightlifting session (3) or 1 week of intensive weightlifting training (25, 33), a profound increase (i.e., up to 2 days and 1 week, respectively) of the above-mentioned parameters have been reported. On the other hand, resting WBC, CK, and LDH have been shown to be affected by circadian rhythms in weightlifters (3) and soccer players (16) with acrophases generally observed at 17:00 close to the peak values of the oral temperature (36). In addition, a transient leukocytosis and increase in muscle damage have been reported after high-intensity exercise (e.g., repeated sprint and Wingate test) with higher post-exercise values observed at 17:00 compared to 07:00 (16–18). However, after a weightlifting training session, an alteration of the diurnal variation of WBC, CK, and LDH has been reported (3). Despite the important number of studies in this field, the exact mechanisms underlying these fluctuations are, yet, not well known (17, 18).

It has been shown that the diurnal variation of CK, WBC, and neutrophils (NE) variations was significantly correlated to the changes in malondialdehyde (MDA) levels (1, 25). Indeed, exercise-induced lipid peroxidation – as a part of normal metabolism that may cause myocellular damage (37) – could be in the origin of hematological and muscle damage circadian rhythm and could even alter the whole circadian system (11). In addition, it has also been suggested that increases in total bilirubin (TBIL) and uric acid (UA) levels are thought to reflect the imbalances in protein metabolic homeostasis and increased protein catabolism associated with tissue damage (i.e., CK and LDH increases) (32). Moreover, the previous studies have confirmed that there is a causal link between the diurnal variation of resting WBC, CK, and LDH and the circadian rhythm of core temperature in soccer players (16). From the evidence of similarity in the time of peak between temperature, muscle damage, and MDA levels (20), Kanabrocki et al. (22) demonstrated a correlation between increased oxidative damage and increased rate of anabolic–catabolic events (e.g., oral temperature). Furthermore, a causal relationship has been reported between inflammatory responses and generalized muscle damage after strenuous exercise (42).

In view of the above considerations, it seems that the origin of hematological and muscle damage alterations in response to physical exercises or to different time-of-day (TOD) has not been clarified yet. Divergences between studies could be due to the participants' training levels, temporal specificity of training, and the lack of studies which investigated all the parameters under the same condition. Therefore, the aims of the present study were to: (1) investigate the diurnal variation of oral temperature, hematological and inflammatory parameters, and oxidative stress markers before and after weightlifting training sessions and (2) assess the possible relationship of these parameters with the circadian rhythm of muscle damage.

We hypothesize that intense weightlifting exercises could exert a strong enough influence to alter the circadian profile of the different parameters studied and that a causal relationship could be established between markers of muscle damage and oxidative stress responses.

Materials and Methods

Experimental design
For the purpose of the present study, the participants randomly performed snatch, clean and jerk, and the squat exercise during weightlifting training sessions scheduled at different TODs
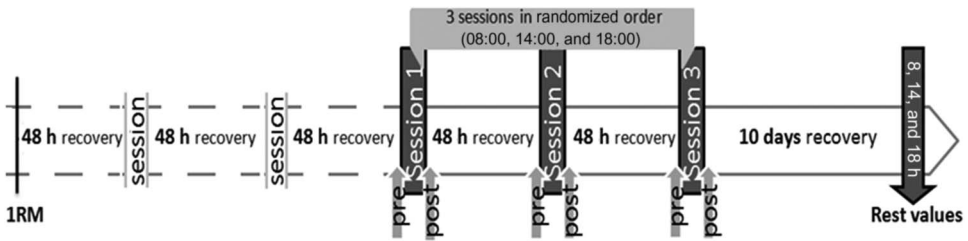
Fig. 1. Experimental design.
NB: the pre-session values correspond to the values of 48 h of recovery

(i.e., in the morning between 08:00 and 09:45, in the afternoon between 14:00 and 15:45, and in the evening between 18:00 and 19:45) with at least 48 h of recovery period after each session (Fig. 1). Olympic exercises and training hours were chosen according to the peak and the minimum of circadian rhythm of anaerobic performances and with regard to the findings reported in the literature (2, 3, 16).

Participants
Nine male elite weightlifters [age: 21 ± 0.5 years, body mass: 80 ± 9.5 kg, and height: 175 ± 8.1 cm (mean $\pm$ SD)] were volunteered to participate in the present study. They were recruited on the basis of: (1) at least five sessions of training per week (between 15:30 and 17:30) with 90–120 min per session and (2) more than three years of experience in Olympic weightlifting. After receiving a thorough explanation of the possible risks and discomforts associated with the experimental procedures, the participants provided a written informed consent to take part in the experiment. The study was conducted according to the Declaration of Helsinki. The protocol and the consent form were fully approved by the review board "Local committee of the Laboratory of Biochemistry, CHU Habib Bourguiba, Sfax, Tunisia" before the commencement of the assessments. Answers to the Dosseville et al. (13), the questionnaire categorized the subjects as "moderately morning type" ($n = 1$) or "neither type" ($n = 8$).

Procedures
One week before the start of the experimental period, the heaviest weight lifted in a single repetition (1-RM) was assessed for each participant in each movement. After an ascending warm-up (i.e., first set with 8–10 repetitions at 50%, second set with 3–5 repetitions at 75%, and third set with 1–3 repetitions at 90% of the athlete's estimated maximum), 1-RM was determinated in three trials with 5 min of recovery time in-between (31). As suggested by Kraemer and Fry (24), they emphasize that on-going encouragement and communication with the athletes during this testing is crucial to obtain the best performance. In addition, the estimated 1-RM was verified in the following 2 days. Then, three training sessions in a randomized order with a recovery period of at least 48 h were performed in the: morning (08:00–09:45), afternoon (14:00–15:45), and evening (18:00–19:45). Upon arrival for their first test session, each participant's body mass (Tanita, Tokyo, Japan) and height were recorded. Moreover, before and after each training session, oral temperature was measured with a calibrated digital clinical thermometer (Omron, Paris, France; accuracy: 0.05 °C) inserted sublingually for at least 3 min the subjects being seated in a resting position for at least 15 min. Before the morning test session, participants were allowed to drink only

one glass of water to avoid the effects of post-prandial thermogenesis (9). Moreover, as recommended by Bougard et al. (9), they were requested to ingest a standardized meal at least 4 h before the afternoon and evening test sessions and they were not allowed to eat anything else until the end of the testing sessions.

Training session

Each training session includes three Olympic-Weightlifting exercises: snatch, clean and jerk, and the squat (2, 3, 26) with five sets for each exercise (i.e., two sets at 85% of 1-RM with three repetitions per set and three sets at 90% of 1-RM with two repetitions per set) with a passive recovery period of 5 min in-between (6). Each session was preceded by a 10-min warm-up, wherein the participants performed three to five repetitions with increasing loads (i.e., from 40% to 80% of the 1-RM).

Dietary records

To assess the adequacy of nutrient intake, a consecutive dietary record over 7 days was completed. Participants were asked to continue their usual dietary habits during the period of dietary recording and to be as accurate as possible in recording the amounts and types of food and fluid consumed. Each individual's diet was calculated using the Bilnut 4 software package (SCDA Nutrisoft, Cerelles, France) and the food composition tables published by the Tunisian National Institute of Statistics in 1978. Estimated nutrient intakes were referred to reference dietary intakes for physically active people (5, 30). The daily nutrient data showed that total calorie, macronutrient, and micronutrient intakes are situated in the interval of the reference dietary intakes for healthy Tunisian adults.

Blood sampling and analysis

Fasting blood samples were collected from a forearm vein at rest, 3 min and 48 h after each training session. At rest and after 48 h, blood samples were collected after 5 min of being seated. Samples were placed in an ice bath and centrifuged immediately at 2,500 r/min ($\times$ g) and 4 °C for 10 min. Aliquots of the resulting plasma were stored at –80 °C until analyzed.

All samples were analyzed in the same assay run to eliminate inter-assay variance. All assays were performed in duplicate in the same laboratory with the simultaneous use of a control serum from Randox. WBC was generally performed within 3 h (using total blood) in a multichannel automated blood cell analyzer Beckman Coulter Gen system-2 (Coulter T540). CK, LDH, UA, TBIL, and C-reactive protein (CRP) were determined spectrophotometrically using Architect Ci 4100 d'ABOTT, respectively, by N-acetyl-L-cysteine, oxidation of lactate on pyruvate, uricase, diazonium, and immunoturbidimetric methods. The intra-assay coefficient of variation for these parameters were 1.3%, 0.2%, 0.5%, 0.5%, and 1.16%, respectively. Catalase (CAT) activity was measured following the decrease in H_2O_2 concentration. To check the activity, a kinetic curve had to be measured during 30 s at $\lambda = 240$ nm using a molar extinction coefficient 43.6 $cm^{-1} M^{-1}$ (43) to know the amount of H_2O_2 eliminated. To determine glutathione peroxidase (GPX) activity, the continuous decrease in NADPH concentration was measured, while GSH levels were maintained, following the Flohe and Gunzler (14) methods. The molar extinction coefficient used for the calculations is $\varepsilon = 6.22 \times 10^3$ $cm^{-1} M^{-1}$. MDA was measured as reported by Wong et al. (44). Plasma proteins are precipitated with methanol and removed from the reaction mixture by centrifugation. The protein-free extract is fractionated by HPLC on a column of octadecyl silica gel to separate the MDA–thiobarbituric acid (TBA) adduct from interfering chromogens. The MDA–TBA

adduct is eluted from the column with methanol/phosphate buffer and quantified spectro-photometrically at $\lambda = 532$ nm. Plasma lipoperoxide concentrations are computed by reference to a calibration curve prepared by assays of tetraethoxypropane.

Statistical analysis

All statistical tests were processed using STATISTICA 10.0 Software (Stat-Soft, Maison-Alfort, France). Following normality confirmation using the Shapiro–Wilks W-test, paired simple t-test was applied to analyze the effect of each training session (i.e., morning, afternoon, and evening) on the biochemical parameters. To analyze the diurnal variation of the same parameters at rest, immediately and 48 h after the training sessions, one-way ANOVA [three levels (TOD)] was used for each period. *Post hoc* least significant difference (LSD) tests were conducted when significant main effects were found. Effect sizes were calculated as partial eta-squared (η_p^2) for the ANOVA analysis and as Cohen's d for the paired sample t-test to assess the practical significance of the findings. Pearson correlation was used to assess the relationship between the studied parameters. Significance was set at $p < 0.05$.

Results

The immediate biochemical responses (i.e., 3 min) to the different training sessions were shown in Tables I–III. As these tables indicate, significant increases in the rate of lipid peroxidation and the antioxidant activities were shown after the morning (Table I), afternoon (Table II), and evening (Table III) training sessions compared to the pre-training values ($p < 0.001$ for MDA, UA, CAT, and GPX and $p < 0.01$, $p < 0.05$, and $p < 0.01$ for TBIL at 08:00, 14:00, and 18:00, respectively). Concerning markers of muscle damage, significant increases were shown for the CK only after the morning and evening training sessions ($p < 0.05$) and for LDH after the evening training session ($p < 0.01$). For CRP, a significant increase was shown after the morning session only ($p < 0.01$). However, for WBC and core temperature, no significant changes were recorded after the different training sessions. Significant correlations were shown between CK and MDA and CK and UA in the morning and evening ($p < 0.01$, $r = 0.6$ for CK-MDA and $p < 0.01$, $r = 0.66$ and $p < 0.001$, $r = 0.82$ for CK-UA), between CK and GPX in the morning and afternoon sessions ($p < 0.05$, $r = 0.54$ and $p < 0.01$, $r = 0.6$, respectively), between CK and CAT in the evening training session ($p < 0.001$, $r = 0.73$), and between CK and WBC in the morning training session ($p < 0.05$, $r = -0.5$). Regarding the inflammatory markers, CRP was highly correlated ($p < 0.001$) with WBC in the different training sessions ($r = 0.76$, $r = 0.77$, and $r = 0.83$, respectively, at 08:00, 14:00, and 18:00). Similarly, WBC was correlated ($p < 0.05$) to core temperature during the morning and evening sessions ($r = 0.6$ and $r = -0.5$, respectively).

Table IV shows the rest values of the biological markers recorded at 08:00, 14:00, and 16:00. A significant diurnal variation of core temperature, markers of muscle damage, and the majority of antioxidant parameters was observed with: (1) higher values recorded in the evening compared to the morning for temperature, WBC, CK, LDH, UA, CAT, and GPX ($p < 0.05$, $p < 0.01$, $p < 0.01$, $p < 0.001$, $p < 0.05$, $p < 0.001$, and $p < 0.001$, respectively); (2) there were higher values in the afternoon compared to the morning for temperature, LDH, UA, CAT, and GPX ($p < 0.05$, $p < 0.001$, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively); and (3) higher values in the evening compared to the afternoon for the WBC ($p < 0.01$). In this table, CK was correlated ($p < 0.05$) to MDA, UA, GPX ($r = 0.53$, $r = 0.52$,

Table I. Correlation between markers of muscle's damage and markers of redox status in response to the morning training session

Variables	Effect of the morning training session				Correlation with:			
	Before	3 min after	Rate of increase	p values	Temper	WBC	CK	LDH
Temperature (°C)	35.9 ± 0.19	36.3 ± 0.13	$p > 0.05$	$t = -2.25, p > 0.05, d = 0.65$	1	$r = 0.60$	$p > 0.05$	$p > 0.05$
Muscle damage markers								
CK (UI/l)	716.2 ± 235.7	$1,104 \pm 400^*$	$59.9 \pm 7.71\%$	$t = -2.57, p < 0.05, d = 0.36$	$p > 0.05$	$r = -0.50$	1	$r = 0.57$
LDH (U/l)	202.8 ± 17.5	244 ± 16.2	$p > 0.05$	$t = -1.86, p > 0.05, d = 0.74$	$p > 0.05$	$p > 0.05$	$r = 0.57$	1
Lipid peroxidation and antioxidants								
MDA (µmol/l)	1.83 ± 0.21	$2.46 \pm 0.16^*$	$34.5 \pm 4.5\%$	$t = -3.82, p < 0.001, d = 0.41$	$r = 0.53$	$p > 0.05$	$r = 0.60$	$p > 0.05$
UA (µmol/l)	321.4 ± 19.6	$419.2 \pm 23.5^*$	$30.4 \pm 2.3\%$	$t = -13.1, p < 0.001, d = 0.60$	$p > 0.05$	$p > 0.05$	$r = 0.66$	$p > 0.05$
TBIL (µmol/l)	12.3 ± 1.88	$16.02 \pm 2.28^*$	$32.1 \pm 5.6\%$	$t = -5.13, p < 0.01, d = 0.62$	$p > 0.05$	$r = 0.50$	$p > 0.05$	$p > 0.05$
CAT (units)	$15.9 \pm 1,51$	$20.1 \pm 1.38^*$	$25.7 \pm 3.9\%$	$t = -14.7, p < 0.001, d = 0.42$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
GPX (units)	0.89 ± 0.08	$1.11 \pm 0.07^*$	$25.9 \pm 4.9\%$	$t = -7.36, p < 0.001, d = 0.48$	$p > 0.05$	$p > 0.05$	$r = 0.54$	$p > 0.05$
Leucocyte and inflammatory marker								
WBC (10^3/µl)	6.83 ± 0.57	6.89 ± 0.68	$p > 0.05$	$t = -0.25, p > 0.05, d = 0.03$	$r = 0.60$	1	$r = -0.50$	$p > 0.05$
CRP (mg/l)	0.53 ± 0.4	$0.67 \pm 0.17^*$	$29 \pm 10\%$	$t = -3.59, p < 0.01, d = 0.29$	$r = 0.60$	$r = 0.76$	$r = 0.50$	$p > 0.05$

White blood cells (WBCs), creatine kinase (CK), lactate dehydrogenase (LDH), malondialdehyde (MDA), uric acid (UA), total bilirubin (TBIL), catalase (CAT), glutathion peroxydase (GPX), C-reactive protein (CRP), and temperature (temper) represented as mean ± SD. Significant differences between pre- and post-training session are represented by "*". Significance set at $p < 0.05$

Table II. Correlation between markers of muscle's damage and markers of redox status in response to the afternoon training session

Variables	Effect of the afternoon training session				Correlation with:			
	Before	3 min after	Rate of increase	p values	Temper	WBC	CK	LDH
Temperature (°C)	36.6 ± 0.12	36.8 ± 0.11	$p > 0.05$	$t = -2.25, p > 0.05, d = 0.59$	1	$p > 0.05$	$p > 0.05$	$p > 0.05$
Muscle damage markers								
CK (UI/l)	478.4 ± 142.6	854.1 ± 216.2	$p > 0.05$	$t = -2.21, p > 0.05, d = 0.65$	$p > 0.05$	$p > 0.05$	1	$p > 0.05$
LDH (U/l)	237 ± 8.51	279 ± 16.37	$p > 0.05$	$t = -2.09, p > 0.05, d = 0.47$	$p > 0.05$	$r = 0.50$	$p > 0.05$	1
Lipid peroxidation and antioxidants								
MDA (µmol/l)	1.90 ± 0.16	$2.54 \pm 0.15^*$	$33.3 \pm 8.33\%$	$t = -5.89, p < 0.001, d = 0.56$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
UA (µmol/l)	321.3 ± 23.1	$427.1 \pm 30.7^*$	$33 \pm 4.18\%$	$t = -7.85, p < 0.001, d = 0.54$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$r = 0.52$
TBIL (µmol/l)	10.46 ± 1.97	$12.48 \pm 2.24^*$	$14.9 \pm 6.17\%$	$t = -2.42, p < 0.05, d = 0.25$	$r = 0.58$	$p > 0.05$	$p > 0.05$	$p > 0.05$
CAT (units)	18.2 ± 1.39	$21.1 \pm 1.26^*$	$16 \pm 3.23\%$	$t = -8.28, p < 0.001, d = 0.73$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
GPX (units)	1.07 ± 0.04	$1.23 \pm 0.03^*$	$14.8 \pm 1.78\%$	$t = -10.8, p < 0.001, d = 0.62$	$p > 0.05$	$p > 0.05$	$r = 0.60$	$p > 0.05$
Leucocyte and inflammatory marker								
WBC (10^3/µl)	8.21 ± 0.86	9.14 ± 0.91	$p > 0.05$	$t = -1.74, p > 0.05, d = 0.33$	$p > 0.05$	1	$p > 0.05$	$r = 0.50$
CRP (mg/l)	0.82 ± 0.23	1.04 ± 0.39	$p > 0.05$	$t = -0.97, p > 0.05, d = 0.23$	$p > 0.05$	$r = 0.77$	$p > 0.05$	$p > 0.05$

White blood cells (WBCs), creatine kinase (CK), lactate dehydrogenase (LDH), malondialdehyde (MDA), uric acid (UA), total bilirubin (TBIL), catalase (CAT), glutathion peroxydase (GPX), C-reactive protein (CRP), and temperature (temper) represented as mean $\pm$ SD. Significant differences between pre- and post-training session are represented by "*". Significance set at $p < 0.05$

Table III. Correlation between markers of muscle's damage and markers of redox status in response to the evening training session

Variables	Effect of the evening training session				Correlation with:			
	Before	3 min after	Rate of increase	p values	Temper	WBC	CK	LDH
Temperature (°C)	36.9±0.06	36.7±0.12	$p > 0.05$	$t = 2.06, p > 0.05, d = 0.68$	1	$r = 0.50$	$p > 0.05$	$p > 0.05$
Muscle damage markers								
CK (UI/l)	530.7±142.1	764.4±190.7*	29.89±3.08%	$t = -3.09, p < 0.05, d = 0.41$	$p > 0.05$	$p > 0.05$	1	$r = 0.80$
LDH (U/l)	205±14.48	250±13.3*	26.25±3.7%	$t = -6.94, p < 0.01, d = 0.49$	$p > 0.05$	$p > 0.05$	$r = 0.80$	1
Lipid peroxidation and antioxidants								
MDA (µmol/l)	1.93±0.24	2.37±0.24*	22.46±4.1%	$t = -10.3, p < 0.001, d = 0.60$	$p > 0.05$	$p > 0.05$	$r = 0.60$	$p > 0.05$
UA (µmol/l)	347.4±29.2	426.3±30.9*	22.7±2.79%	$t = -11.2, p < 0.001, d = 0.47$	$p > 0.05$	$p > 0.05$	$r = 0.82$	$p > 0.05$
TBIL (µmol/l)	8.38±1.84	9.91±1.68*	18.3±4.95%	$t = -4.29, p < 0.01, d = 0.29$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
CAT (units)	19.3±1.74	21.9±1.57*	13.55±4.09%	$t = -5.31, p < 0.001, d = 0.52$	$p > 0.05$	$p > 0.05$	$r = 0.73$	$r = 0.61$
GPX (units)	1.12±0.10	1.27±0.09*	13.33±2.04%	$t = -7.26, p < 0.001, d = 0.52$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Leucocyte and inflammatory marker								
WBC (10^3/µl)	8.66±0.82	9.21±0.88	$p > 0.05$	$t = -1.07, p > 0.05, d = 0.20$	$r = 0.50$	1	$p > 0.05$	$p > 0.05$
CRP (mg/l)	0.61±0.22	0.81±0.33	$p > 0.05$	$t = -1.8, p > 0.05, d = 0.24$	$r = -0.50$	$r = 0.83$	$p > 0.05$	$p > 0.05$

White blood cells (WBCs), creatine kinase (CK), lactate dehydrogenase (LDH), malondialdehyde (MDA), uric acid (UA), total bilirubin (TBIL), catalase (CAT), glutathion peroxydase (GPX), C-reactive protein (CRP), and temperature (temper) represented as mean ± SD. Significant differences between pre- and post-training session are represented by "*". Significance set at $p < 0.05$

Table IV. Correlation between markers of muscle's damage and markers of redox status in rest values at different TODs

Variables	Rest values			Difference in rate (%)			ANOVA	Correlation with:			
	Morning	Afternoon	Evening	Morning and evening	Morning and afternoon	Afternoon and evening		Temper	WBC	CK	LDH
Temperature (°C)	36.02 ± 0.16^{BC}	36.53 ± 0.13^{A}	36.51 ± 0.14^{A}	$1.4 \pm 0.8\%$	$1.4 \pm 0.6\%$	$p > 0.05$	$F = 3.9, p < 0.05, \eta_p^2 = 0.33$	1	$p > 0.5$	$p > 0.5$	$r = 0.60$
Muscle damage markers											
CK (UI/l)	164.2 ± 33.2^{C}	254.7 ± 63.1	326.7 ± 75.3^{A}	$114.4 \pm 43.3\%$	$p > 0.05$	$p > 0.05$	$F = 5.8, p < 0.05, \eta_p^2 = 0.42$	$p > 0.5$	$r = 0.60$	1	$r = 0.52$
LDH (U/l)	141.2 ± 5.3^{BC}	205.3 ± 13.8^{A}	212.9 ± 12.2^{A}	$52.03 \pm 9.7\%$	$45.7 \pm 8.73\%$	$p > 0.05$	$F = 15.7, p < 0.001, \eta_p^2 = 0.66$	$r = 0.60$	$r = 0.50$	$r = 0.52$	1
Lipid peroxidation and antioxidants											
MDA (µmol/l)	1.64 ± 0.20	1.79 ± 0.20	1.85 ± 0.22	$p > 0.05$	$p > 0.05$	$p > 0.05$	$F = 0.29, p > 0.05, \eta_p^2 = 0.03$	$p > 0.5$	$p > 0.5$	$r = 0.53$	$p > 0.5$
UA (µmol/l)	311.3 ± 18.8^{BC}	346.5 ± 24.5^{A}	348.2 ± 21.3^{A}	$13.2 \pm 5.4\%$	$12.3 \pm 4.46\%$	$p > 0.05$	$F = 4.52, p < 0.05, \eta_p^2 = 0.36$	$p > 0.5$	$p > 0.5$	$r = 0.52$	$r = 0.47$
TBIL (µmol/l)	10.1 ± 1.18	9.86 ± 1.73	9.81 ± 1.88	$p > 0.05$	$p > 0.05$	$p > 0.05$	$F = 0.03, p > 0.05, \eta_p^2 = 0.01$	$r = -0.42$	$p > 0.5$	$p > 0.5$	$p > 0.5$
CAT (units)	13.7 ± 1.14^{BC}	18.1 ± 1.19^{A}	19.2 ± 1.87^{A}	$50.0 \pm 22.6\%$	$39.1 \pm 12.5\%$	$p > 0.05$	$F = 3.36, p < 0.05, \eta_p^2 = 0.31$	$p > 0.5$	$p > 0.5$	$p > 0.5$	$r = 0.40$
GPX (units)	0.78 ± 0.08^{BC}	1.02 ± 0.04^{A}	1.08 ± 0.10^{A}	$50.5 \pm 19.8\%$	$43.8 \pm 15.1\%$	$p > 0.05$	$F = 4.03, p < 0.05, \eta_p^2 = 0.34$	$p > 0.5$	$p > 0.5$	$r = 0.40$	$p > 0.5$
Leucocyte and inflammatory marker											
WBC ($10^3/\mu l$)	6.99 ± 0.59^{C}	7.11 ± 0.47^{C}	8.36 ± 0.71^{B}	$20.29 \pm 5.9\%$	$p > 0.05$	$16.9 \pm 4.98\%$	$F = 9.4, p < 0.01, \eta_p^2 = 0.54$	$p > 0.5$	1	$r = 0.60$	$r = 0.55$
CRP (mg/l)	0.40 ± 0.08	0.49 ± 0.15	0.58 ± 0.16	$p > 0.05$	$p > 0.05$	$p > 0.05$	$F = 0.72, p > 0.05, \eta_p^2 = 0.08$	$p > 0.5$	$r = 0.60$	$p > 0.5$	$p > 0.5$

White blood cells (WBCs), creatine kinase (CK), lactate dehydrogenase (LDH), malondialdehyde (MDA), uric acid (UA), total bilirubin (TBIL), catalase (CAT), glutathion peroxydase (GPX), C-reactive protein (CRP), and temperature (temper) represented as mean ± SD. Significant differences between times of day (TODs) are represented by "A," "B," and "C" to compare 08:00, 14:00, and 18:00, respectively, with $p < 0.05$

and $r = 0.4$, respectively) and to WBC ($p < 0.01$, $r = 0.6$). LDH was correlated to WBC, UA, and CAT ($p < 0.01$, $r = 0.55$ for WBC; $p < 0.05$, $r = 0.47$ and $r = 0.4$, respectively, for UA and CAT). CRP was correlated to WBC ($p < 0.01$, $r = 0.6$) and core temperature was correlated ($p < 0.05$) to LDH and TBIL ($r = 0.60$ and $r = -0.42$, respectively).

The diurnal variations of the different parameters studied (i.e., temperature, markers of muscle damage, and markers of redox status and CRP) immediately and 48 h after the training sessions were shown in Tables V and VI, respectively. Immediately after the training sessions (Table V), the results showed a significant TOD effect on core temperature, WBC, and TBIL values ($F = 6.7$, $p < 0.01$, $\eta_p^2 = 0.45$, $F = 7.1$, $p < 0.01$, $\eta_p^2 = 0.47$, and $F = 6.1$, $p < 0.05$, $\eta_p^2 = 0.43$) with higher values registered in the evening and afternoon compared to the morning for core temperature and WBC ($p < 0.05$ and $p < 0.01$, respectively). However, lower values were registered immediately after the evening compared to the morning for TBIL ($p < 0.01$). As indicated in this table, significant correlations between CK and UA ($p < 0.05$, $r = 0.42$), between CRP and WBC ($p < 0.001$, $r = 0.7$), and between CRP and LDH ($p < 0.05$, $r = 0.43$) were shown. After 48 h of recovery (Table VI), the results showed a significant diurnal variation only for core temperature and WBC with higher values recorded in the evening and afternoon compared to the morning ($p < 0.001$). However, the diurnal variation was removed for the remaining parameters. Significant correlations were observed between CK and UA ($p < 0.05$, $r = 0.53$), between CRP and WBC ($p < 0.001$, $r = 0.73$), between WBC and core temperature ($p < 0.05$, $r = 0.46$), and between WBC and GPX ($p < 0.05$, $r = 0.4$).

Discussion

The main findings of the present study were: (1) no significant changes for core temperature and WBC after the training sessions at the different TOD was found; (2) redox markers (i.e., MDA, UA, TBIL, CAT, and GPX) increased from resting values to post-training with a higher rate of increases registered in the morning; and (3) significant post-training increases were recorded for markers of muscle damage in the morning and evening excepting CRP only at 08:00. Moreover, in response to the weightlifting training session, significant correlations were reported during the: (1) morning and evening between CK and MDA levels and (2) morning between CRP and MDA and CK and WBC. In resting state, a significant diurnal variation in core temperature, WBC, CK, LDH, UA, CAT, and GPX was registered with higher values at 18:00 compared to 08:00. Significant correlations were observed between: (1) CK and WBC, MDA, UA, and GPX and (2) between LDH and core temperature, WBC, UA, and CAT. Immediately and 48 h after training sessions, the diurnal variations persist only for core temperature and WBC and significant correlations were observed only between CK and UA and WBC and CRP.

Relationship between redox status and markers of muscle damage during weightlifting training sessions
The present results showed no significant differences between pre- and post-training for core temperature and WBC at the different TODs. However, markers of muscle damage increased after the training sessions at 08:00 and 18:00 with a higher rate of increase in the morning for CK ($59.9 \pm 7.71\%$ versus $29.89 \pm 3.08\%$). These findings are partially in line with the previous studies of Yalcin et al. (45) and Hammouda et al. (16, 17) who showed a significant

Table V. Correlation between markers of muscle's damage and markers of redox status immediately after the different training sessions

Variables	Post-training sessions values			Difference in rate (%)			ANOVA	Correlation with:			
	Morning	Afternoon	Evening	Morning and evening	Morning and afternoon	Afternoon and evening		Temper	WBC	CK	LDH
Temperature (°C)	36.3 ± 0.13^{BC}	36.8 ± 0.11^{A}	36.7 ± 0.12^{A}	$1.2 \pm 0.5\%$	$1.5 \pm 0.4\%$	$p > 0.05$	$F = 6.7, p < 0.01, \eta_p^2 = 0.45$	1	$p > 0.5$	$p > 0.5$	$p > 0.5$
Muscle damage markers											
WBC ($10^3/\mu l$)	6.89 ± 0.68^{BC}	9.14 ± 0.91^{A}	9.21 ± 0.88^{A}	$35.3 \pm 6.8\%$	$34.7 \pm 9.9\%$	$p > 0.05$	$F = 7.1, p < 0.01, \eta_p^2 = 0.47$	$p > 0.5$	1	$p > 0.5$	$p > 0.5$
CK (UI/l)	$1{,}104 \pm 400$	854.1 ± 216.2	764.4 ± 190.7	$p > 0.05$	$p > 0.05$	$p > 0.05$	$F = 0.66, p > 0.05, \eta_p^2 = 0.07$	$p > 0.5$	$p > 0.5$	1	$r = 0.55$
LDH (U/l)	244 ± 16.2	279 ± 16.4	250 ± 13.3	$p > 0.05$	$p > 0.05$	$p > 0.05$	$F = 2.1, p > 0.05, \eta_p^2 = 0.2$	$p > 0.5$	$p > 0.5$	$r = 0.55$	1
Lipid peroxidation and antioxidants											
MDA ($\mu mol/l$)	2.46 ± 0.16	2.54 ± 0.15	2.37 ± 0.24	$p > 0.05$	$p > 0.05$	$p > 0.05$	$F = 0.14, p > 0.05, \eta_p^2 = 0.02$	$p > 0.5$	$p > 0.5$	$p > 0.5$	$p > 0.5$
UA ($\mu mol/l$)	419.2 ± 23.5	427.1 ± 30.7	426.3 ± 30.9	$p > 0.05$	$p > 0.05$	$p > 0.05$	$F = 0.09, p > 0.05, \eta_p^2 = 0.01$	$p > 0.5$	$p > 0.5$	$r = 0.42$	$p > 0.5$
TBIL ($\mu mol/l$)	16.0 ± 2.28^{C}	12.5 ± 2.24	9.91 ± 1.68^{A}	$-33.1 \pm 9.3\%$	$p > 0.05$	$p > 0.05$	$F = 6.1, p < 0.05, \eta_p^2 = 0.43$	$p > 0.5$	$p > 0.5$	$p > 0.5$	$p > 0.5$
CAT (units)	20.1 ± 1.38	21.1 ± 1.26	21.9 ± 1.57	$p > 0.05$	$p > 0.05$	$p > 0.05$	$F = 0.42, p > 0.05, \eta_p^2 = 0.05$	$p > 0.5$	$p > 0.5$	$p > 0.5$	$p > 0.5$

(Continued)

Table V. Correlation between markers of muscle's damage and markers of redox status immediately after the different training sessions *(Continued)*

Variables	Post-training sessions values			Difference in rate (%)			ANOVA	Correlation with:			
	Morning	Afternoon	Evening	Morning and evening	Morning and afternoon	Afternoon and evening		Temper	WBC	CK	LDH
GPX (units)	1.11 ± 0.07	1.23 ± 0.03	1.27 ± 0.09	$p > 0.05$	$p > 0.05$	$p > 0.05$	$F = 1.19, p > 0.05,$ $\eta_p^2 = 0.13$	$p > 0.5$	$p > 0.5$	$p > 0.5$	$p > 0.5$
Leucocyte and inflammatory marker											
WBC ($10^3/\mu l$)	6.89 ± 0.68^{BC}	9.14 ± 0.91^{A}	9.21 ± 0.88^{A}	$35.3 \pm 6.8\%$	$34.7 \pm 9.9\%$	$p > 0.05$	$F = 7.1, p < 0.01,$ $\eta_p^2 = 0.47$	$p > 0.5$	1	$p > 0.5$	$p > 0.5$
CRP (mg/l)	0.67 ± 0.17	1.04 ± 0.39	0.81 ± 0.33	$p > 0.05$	$p > 0.05$	$p > 0.05$	$F = 0.57, p > 0.05,$ $\eta_p^2 = 0.07$	$p > 0.5$	$r = 0.70$	$p > 0.5$	$r = 0.43$

White blood cells (WBCs), creatine kinase (CK), lactate dehydrogenase (LDH), malondialdehyde (MDA), uric acid (UA), total bilirubin (TBIL), catalase (CAT), glutathion peroxydase (GPX), C-reactive protein (CRP), and temperature (temper) represented as mean $\pm$ SD. Significant differences between times of day (TODs) are represented by "A," "B," and "C" to compare 08:00, 14:00, and 18:00, respectively, with $p < 0.05$

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Table VI. Correlation between markers of muscle's damage and markers of redox status 48 h after the different training sessions

Variables	48 h of recovery period values			Difference in rate (%)			ANOVA	Correlation with:			
	Morning	Afternoon	Evening	Morning and evening	Morning and afternoon	Afternoon and evening		Temper	WBC	CK	LDH
Temperature (°C)	35.9 ± 0.19^{BC}	36.6 ± 0.12^{A}	36.9 ± 0.06^{A}	$2.7 \pm 0.5\%$	$1.8 \pm 0.4\%$	$p > 0.05$	$F = 20.9, p < 0.001, \eta_p^2 = 0.72$	1	$r = 0.46$	$p > 0.5$	$p > 0.5$
Muscle damage markers											
CK (UI/l)	716.2 ± 235.7	478.4 ± 142.6	530.7 ± 142.1	$p > 0.05$	$p > 0.05$	$p > 0.05$	$F = 1.19, p > 0.05, \eta_p^2 = 0.13$	$p > 0.5$	$p > 0.5$	1	$p > 0.5$
LDH (U/l)	202.8 ± 17.5	237 ± 8.51	205 ± 14.48	$p > 0.05$	$p > 0.05$	$p > 0.05$	$F = 1.44, p > 0.05, \eta_p^2 = 0.15$	$p > 0.5$	$p > 0.5$	$p > 0.5$	1
Lipid peroxidation and antioxidants											
MDA (µmol/l)	1.83 ± 0.21	1.90 ± 0.16	1.93 ± 0.24	$p > 0.05$	$p > 0.05$	$p > 0.05$	$F = 0.07, p > 0.05, \eta_p^2 = 0.01$	$p > 0.5$	$p > 0.5$	$p > 0.5$	$p > 0.5$
UA (µmol/l)	321.4 ± 19.6	321.3 ± 23.1	347.4 ± 29.2	$p > 0.05$	$p > 0.05$	$p > 0.05$	$F = 1.83, p > 0.05, \eta_p^2 = 0.19$	$p > 0.5$	$p > 0.5$	$r = 0.53$	$p > 0.5$
TBIL (µmol/l)	12.3 ± 1.88	10.46 ± 1.97	8.38 ± 1.84	$p > 0.05$	$p > 0.05$	$p > 0.05$	$F = 1.99, p > 0.05, \eta_p^2 = 0.20$	$p > 0.5$	$p > 0.5$	$p > 0.5$	$p > 0.5$
CAT (units)	15.9 ± 1.51	18.2 ± 1.39	19.3 ± 1.74	$p > 0.05$	$p > 0.05$	$p > 0.05$	$F = 1.11, p > 0.05, \eta_p^2 = 0.12$	$p > 0.5$	$p > 0.5$	$p > 0.5$	$p > 0.5$
GPX (units)	0.89 ± 0.08^{C}	1.07 ± 0.04	1.12 ± 0.10^{A}	$34.1 \pm 16.8\%$	$p > 0.05$	$p > 0.05$	$F = 2.7, p > 0.05, \eta_p^2 = 0.25$	$p > 0.5$	$r = 0.40$	$p > 0.5$	$p > 0.5$

(Continued)

Table VI. Correlation between markers of muscle's damage and markers of redox status 48 h after the different training sessions *(Continued)*

Variables	48 h of recovery period values			Difference in rate (%)			ANOVA	Correlation with:			
	Morning	Afternoon	Evening	Morning and evening	Morning and afternoon	Afternoon and evening		Temper	WBC	CK	LDH
Leucocyte and inflammatory marker											
WBC ($10^3/\mu l$)	6.83 ± 0.57^C	8.21 ± 0.86	8.66 ± 0.82^A	$27.9 \pm 7.9\%$	$p > 0.05$	$p > 0.05$	$F = 2.6, p < 0.05,$ $\eta_p^2 = 0.25$	$r = 0.46$	1	$p > 0.5$	$p > 0.5$
CRP (mg/l)	0.53 ± 0.4	0.82 ± 0.23	0.61 ± 0.22	$p > 0.05$	$p > 0.05$	$p > 0.05$	$F = 0.8, p > 0.05,$ $\eta_p^2 = 0.09$	$p > 0.5$	$r = 0.73$	$p > 0.5$	$p > 0.5$

White blood cells (WBCs), creatine kinase (CK), lactate dehydrogenase (LDH), malondialdehyde (MDA), uric acid (UA), total bilirubin (TBIL), catalase (CAT), glutathion peroxydase (GPX), C-reactive protein (CRP), and temperature (temper) represented as mean ± SD. Significant differences between times of day (TODs) are represented by "A," "B," and "C" to compare 08:00, 14:00, and 18:00, respectively, with $p < 0.05$

increase in CK, LDH, and WBC whatever the TOD of physical exercises has been. The present study showed significant pre–post training increases in MDA, UA, TBIL, CAT, and GPX at different TODs with a higher rate of increase observed in the morning compared to the afternoon and the evening values (e.g., MDA: morning = $34.5 \pm 11.9\%$ versus evening = $22.46 \pm 4.1\%$). In agreement, the previous studies (4, 16, 21) reported the increases in lipid peroxidation (i.e., MDA) and antioxidant defenses (i.e., CAT, UA, and TBIL) after intense physical exercise. The present result showed a significant correlation between CK and MDA in the morning and evening. These findings confirm those of Liu et al. (25) and Akbulut et al. (1) after a resistance training session. Likewise, in line with the result of Nakajima et al. (28), the present study indicates a significant difference pre- to post-training in CRP only in the morning. The significant increase of CRP, CK, and MDA at this TOD and the significant correlation between CRP-WBC, CRP-CK, and CK-MDA, confirmed that inflammatory responses could be due to generalized muscle damage induced by a high level of oxidative stress (42).

Relationship between redox status and markers of muscle damage at rest and after training sessions

The present results showed that resting oral temperature, WBC, CK, and LDH were higher at 18:00 than 08:00. This confirms the results of the previous studies in which the authors found that the resting levels of WBC and biochemical markers of muscle damage were higher in the evening close to the acrophase of oral temperature (16–18, 39). These results confirmed the suggestion that circadian changes in core temperature would increase the activity of enzymes, such as phosphofructokinase and LDH (12). In this context, significant correlations between resting core temperature and LDH and between WBC and CK and LDH were registered. These findings confirmed that the presence of leucocytosis at a specific TOD could be due to the high concentrations of catecholamines (23) and to the general increase in muscle damage (34). The present study showed higher antioxidant activity in the evening and no diurnal variation for MDA. However, in healthy sedentary subjects, the previous studies (12, 15–20, 22, 23, 38, 45) showed a higher efficiency of the antioxidant system in the early morning (i.e., peak of CAT, UA, and TBIL recorded at 06:00) and a greater rate of lipid peroxidation in the early evening (i.e., peak of MDA occurred at 18:00). These divergences between elite weightlifter and healthy sedentary subjects could be due to the training level of the subjects and the effect of training at a specific TOD (i.e., participants of the present study regularly trained between 15:30 and 17:30). In this context, Bloomer et al. (7, 8), Subudhi et al. (40), and Ammar et al. (2) indicated that being experienced resistance trained could result in a lower MDA and muscle damage levels. On the other hand, the previous studies suggest a link between the diurnal variation of the oxidative stress and the muscle damage markers (11, 32). Cudney et al. (11) suggest that lipid peroxidation could be in the origin of hematological and muscle damage circadian rhythm and could even alter the whole circadian system. Petibois et al. (32) suggest that the increases in TBIL and UA are thought to reflect the imbalances in protein metabolic homeostasis that increased protein catabolism associated with tissue damage (i.e., increase in CK and LDH levels). The present results confirm the majorities of these suggestions and showed that resting: (1) CK was significantly correlated to MDA, UA, and GPX and (2) LDH was correlated to UA and CAT. Furthermore, given the similarity in the time of peak between core temperature and MDA (20), Kanabrocki et al. (22) suggest a correlation between increased oxidative damage and increased rate of anabolic–catabolic events (e.g., oral temperature). The present results are in disagreement

with this suggestion. Indeed, at resting state, core temperature was only negatively correlated to TBIL.

Immediately and 48 h after the training sessions, the present study showed that the diurnal variation persisted only for core temperature and WBC which is in line with the previous findings after the repeated sprint and Wingate exercise (16, 17). However, the present findings showed the suppression of TOD effect in markers of muscle damage and antioxidant activities immediately and 48 h after weightlifting training sessions. These results suggest that the effect of intensive weightlifting exercises dominates the TOD effect in marker of muscle damage and antioxidant activity. However, for core temperature and WBC, the TOD effect dominates the physical exercise's effect.

Concerning the relationship between redox status and markers of muscle damage, the present results showed that the majorities of resting significant correlations were suppressed immediately and 48 h after the resistance training session. Therefore, the authors suggest the existence of further underlying mechanisms [i.e., such as catecholamine Kanabrocki et al. (23)] which can be in the origin of muscle damage response to high-intensity weightlifting exercises. Concerning the CRP, our results showed the absence of diurnal variations at resting state and immediately and 48 h after the training sessions. These findings are in line with those of Miles et al. (27) who investigated the diurnal variation of CRP during eccentric exercises and showed the absence of significant daily fluctuation at both pre- and post-physical exercises. Moreover, the present study shows that CRP and WBC are correlated under different conditions (i.e., resting state, immediately and 48 h after the training sessions) and this suggests that WBC could also be a marker of inflammation during physical exercise.

Conclusion

In conclusion, the present study suggests a higher sensitivity to the resistance exercises for the lipid peroxidation and antioxidant activity compared to WBC and markers of muscle damage. However, core temperature and WBC were more sensible to TOD than the remaining parameters (i.e., lipid peroxidation, antioxidant activity, and markers of muscle damage and CRP). Indeed, immediately and 48 h after the training sessions, the present study showed that the diurnal variation already observed in resting state persist only for core temperature and WBC. This finding could be explained, as reported in the present study, by the fact that the resting circadian rhythm of enzyme activities (e.g., LDH) and CK and the diurnal fluctuation in core temperature, lipid peroxidation, and activities of some potent antioxidants are causally linked. However, the suppression of this relationship after the intense resistance exercises suggests the participation of further underlying mechanisms in the muscle damage process that needs further studies.

Practical Applications

Given the fact that the increase in the inflammatory markers and the higher rate of increase pre–post training session for the redox and the muscle damage markers were registered during the morning, this session seems to generate higher muscle damage, inflammatory, and oxidative responses. Therefore, weightlifting coaches should avoid scheduling their training sessions in the morning hours.

Limits

The majority of the present study subjects are used to participate essentially in afternoon/evening training schedules which could induce a temporal specificity adaptation upon biological measures. Therefore, further studies could examine the same protocol with subjects habitually trained in the morning hours.

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Conflict of interest

The authors report no conflict of interest. The authors are solely responsible for the content and writing of this paper.

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