Does exercising at a certain time-of-day affect athletes' skeletal muscle damage markers? A systematic review and meta-analysis

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Abstract: The objective of the study was to examine time of day effects of exercise on athletes' skeletal muscle damage markers. The skeletal muscle damage marker enzymes like creatine kinase, lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase are the markers of the functional status of muscle tissue and vary widely with pathological and physiological conditions. 2212 potential citations were identified through PubMed, MEDLINE, SPORTDiscus, EMBASE and Google Scholar searches. Based on the eligibility criteria, 10 studies were included for analysis. Heterogeneity test (I²), Effect Size (ES) and Standardized Mean Differences (Std. MD) for time of day results were calculated at 95% CI and 5% alpha-level for each study. The protocol is registered in PROSPERO with registration number (PROSPERO 2018: CRD42018112116). Using a random effect model, the overall pooled time of day effect of exercise were, SMD = -1.38 (95%CI, -2.69, -0.95; P < 0.001) and (I² = 76%) for lactate dehydrogenase, SMD = -1.24 (95%CI, -2.43, -0.46; P < 0.004) and (I² = 74%) for creatine kinase, SMD = -1.89 (95%CI, -2.30, -1.30; P < 0.001) and (I² = 22%) for aspartate aminotransferase, SMD = -0.46 (95%CI, -1.94, -0.98; P < 0.001) and (I² = 41%) for white blood cells (WBC). Our study partly confirms the diurnal variations of skeletal muscle damage markers and recommends conducting further meta-analysis to investigate the concomitant effect of exercise and time-of-day variations in relation to hormonal, core temperature and oxidative responses. The results obtained from our study may help athletes, active individuals, team physicians and coaches to consider these markers and protect the physicians from misinterpreting abnormal values when evaluating the training level of their athletes.

PUBLIC INTEREST STATEMENT

The conducted study is interdisciplinary and worthy of a broad audience with differing and multidisciplinary expertise. To our knowledge, previous studies on diurnal variation in skeletal muscle damage markers do not pooled study results. In this paper, we reported pooled meta-analysis for each parameter independently. The results obtained from our study may help athletes, active individuals, team physicians and coaches to consider these markers and protect the physicians from misinterpreting abnormal values when evaluating the training level of their athletes.
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Subjects: Bioscience; Health and Social Care; Medicine, Dentistry, Nursing & Allied Health

Keywords: athletes; exercises; skeletal muscle damage markers; time of day variations

1. Introduction
Health and fitness biomarkers related to athletics performance possess diurnal variations (Chtourou, Hammouda, Aloui, & Souissi, 2013a; Teo, Newton, & McGuigan, 2011). In responses to exercise, these variations are reflected by differences between responses in the morning (AM) and in the afternoon (PM) times of day (Enoka, 1995). The existence of diurnal variations is recognized by the scheduling of athletic training sessions at different times of the day (Kentiba, George, et al., 2019) which influences performances (Chtourou, Hammouda, Aloui, & Souissi, 2013b; Kentiba, Mondal, Mathivanan, & George, 2018; Kentiba, Berhe, et al., 2019). Currently, it is well established that, creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are markers of skeletal muscle injury and fatigue markers during exercise (Ammar et al., 2015; Brancaccio et al., 2010; Yalcin, Alpaslan, Sedat, Melek, & Oguzk, 2003) and white blood cells (WBC) are general inflammatory markers (Hammouda, Chtourou, et al., 2012a; Yalcin et al., 2003). Moreover, the serum level of skeletal muscle enzymes is a marker of the functional status of muscle tissue and varies widely in both the pathological and physiological conditions (Hammouda, Chtourou, et al., 2012b).

Previous studies concerning time-of-day effects on skeletal muscle injury markers presents inconclusive results (Chtourou et al., 2013b). Studies conducted on active male participants showed that at the beginning of the test performance and injury marker values are different between morning (AM) and evening (PM) trials (Lericollais, Gauthier, Bessot, & Davenne, 2011; Racinais, Perrey, Denis, & Bishop, 2010), whereas, at the end of the trials there is no difference between AM and PM time results (Lericollais et al., 2011). Consequently, some investigations did not show any time-of-day effect, most studies reported higher muscle injury markers and decrease in muscle performance during short-term maximal exercises in the evening compared with the morning (Chtourou et al., 2013b; Lericollais, Gauthier, Bessot, Sesboüé, & Davenne, 2009). Other studies reported that WBC peaks at the PM time of day (Ammar et al., 2015; Boussetta, Abedelmalek, Aloui, & Souissi, 2017; Erdemir, 2013; Hammouda, Chtourou, et al., 2012a; Hammouda, Chtourou, et al., 2012b; 2011; Shahidi, Jalal, Alhosseini, Mohammad, & Panah, 2012; Souissi, Chtourou, & Chaouachi, 2012). However, further studies by Kilic and Aldemir (2005) and Ahmadizad and Bassami (2010) shown different results and others also shown no peak at all (Ahmadizad & Bassami, 2010) for some markers. Notably, estimates have ranged quite widely and there is no consensus on estimates of phase.

By considering the above issues and the literature on the time of day effect of exercise on skeletal muscle damage markers, the present meta-analysis aimed to investigate the time of day variations of exercise on LDH, CK, AST, ALT and WBC of athletes.

2. Materials and methods
2.1. Literature search
A systematic search was conducted in accordance with Cochrane Handbook Guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher, Liberati, Tetzlaff, & Altman, 2009; Panic, Leoncini, de Belvis, Ricciardi, & Boccia, 2013) from 18 June 2018, to 18 September 2018, on the databases PubMed, MEDLINE, SPORTDiscus, EMBASE, Google Scholar, and grey literature search. The following key words relevant to time of day were used in search process (“diurnal variations”, “AM/PM training”, “AM training”, “PM training”, “morning training”, “evening training”), (“hematological markers”, “biochemical markers”, “biomarkers” “complete blood count”) and (“athletes”, “sports person”, “runners”, “long distance runners”, “college athletes”). To combine the words, Boolean operator “OR” and “AND” were used. Hand search of the
reference lists of the retrieved studies to identify studies not found by the search in the electronic engines mentioned above was made. To ensure literature saturation, the reference lists of included studies or relevant reviews identified through the search was scanned. We also made searching the authors’ personal files to make sure that all relevant material has been captured and finally lists of the included articles were shared among the meta analysis team. The protocol is registered in PROSPERO with the registration number (PROSPERO 2018: CRD42018112116).

2.2. Inclusion criteria
Controlled trials that are conducted on male athletes included in the meta-analysis. All the included studies compared the outcomes in the AM and PM times of the day. Studies were included when at least one of the following outcome parameters has been reported; LDH, CK, AST, ALT and WBC. Only studies in the English language and conducted on athlete participants with aerobic exercise interventions were included.

2.3. Exclusion criteria
Studies that are descriptive like study protocols, review and theoretical articles, articles on non-healthy, non-athlete, female, obese subjects and articles that did not reported at least one of the six outcomes before 2005 were excluded.

2.4. Selection process
Three reviewers individually screened the titles, abstracts, and keywords to identify eligibility and assessed the methodological quality of the included studies and recorded the findings. The reviewers were blinded to the names of the authors, the institution where the study had been conducted, and the journal. Any disagreement was discussed with another (fourth) reviewer.

2.5. Data extraction
Results were combined based on the following five outcome indicators; creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and white blood cells (WBC). The following data were extracted from studies included in the meta-analysis for the outcome indicators; author name, publication year, sample size, characteristics of the population studied (age and geographical location) were recorded. For continuous variables, means and standard deviations were used.

2.6. Data analysis
Since outcome variables are continuous in nature, Heterogeneity test, (I^2), Effect Size (ES) and Standardized Mean Differences (SMD) for AM and PM results were calculated for each study at (95% CI). Statistical significance was set at p < 0.05 for all analyses. After calculating test of heterogeneity, we used a random effects meta-analysis and all calculations were made with (RevMan 5.3) software. The results were presented with the Forest plot, which showed the strength of the evidence; with the left-hand column lists the names and year of the studies; the right-hand column shows the measure of effect (expressed as SMD with 95% CI).

2.7. Risk of bias assessment
Risk of bias within studies was evaluated according to the criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions (Littel & Jacqueline Corcoran, 2008), which consists of the following seven fields; sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective outcome reporting and “other issues”. Each field was considered for each of the included studies and assigned either a low, high or unclear risk of bias. Based on this, studies with attrition bias include (Ammar et al., 2015; Boussetta et al., 2017; Hammouda, Chtourou, et al., 2012b; Mohammadnejad et al., 2012; Hammouda et al., 2012; 2013; 2011; Souissi et al., 2012), detection bias (Boussetta et al., 2017; Hammouda, Chtourou, et al., 2012a) and with other unknown bias (Souissi et al., 2012).
3. Results

The search strategy identified a total of 2212 citations. After removal of duplicates, 2210 were screened by title and 2091 studies were excluded. Then, 119 studies were included for full-text review. Of these, a further 109 were excluded leaving 9 studies for inclusion in the review and meta-analysis. The reasons for excluding articles are shown in [Figure 1]. Six studies reported diurnal effects on LDH, four studies on CK, AST and ALT, seven studies on WBC. A summary of study characteristics is given in (Table 1).

3.1. Lactate dehydrogenase (LDH)

Six studies were included in the meta-analysis to estimate the pooled diurnal variations on lactate dehydrogenase (LDH) with 144 participants. Out of them, five studies (Ammar et al., 2015; Boussetta et al., 2017; Hammouda et al., 2013; 2011; Souissi et al., 2012) reported significant diurnal variations between AM and PM exercising groups and the rest (Hammouda, Chtourou, et al., 2012b) reported non-significant difference between the two exercising groups. The different result reported in a study by Hammouda et al., (2012) might be related to the training level of the participants. Using random effect model, the overall pooled effect of exercise on different time
| Author & year            | Study design | Country | Sample | Participants characteristics and exercise type | Time of day | Biomarkers | Acrophases (Max.Value) |
|-------------------------|--------------|---------|--------|-----------------------------------------------|-------------|------------|------------------------|
| (Ammar et al., 2015)    | Controlled trial | Tunisia | 9      | Aerobic Exercise Male 21 ± 0.5 AM and PM WBC LDH CK AST ALT | AM and PM | WBC Sig. Sig. Sig. NS PM | PM AM |
| (Boussetta et al., 2017) | Controlled trial | Tunisia | 11     | Aerobic Exercise Male 21.8 AM and PM WBC LDH CK | AM and PM | WBC Sig. Sig. Sig. NS PM | PM PM |
| (Erdemir, 2013)         | Controlled trial | Turkey  | 12     | Aerobic Exercise Male 20 AM and PM WBC | AM and PM | WBC Sig. | PM |
| (Mohammadnejad et al., 2012) | Controlled trial | Iran    | 20     | Aerobic Exercise Male 20.8 ± 0.99 (AM) 21.0 ± 0.73 (PM) AM and PM WBC | AM and PM | WBC NS | PM |
| (Hammouda et al., 2011) | Controlled trial | Tunisia | 12     | Aerobic Exercise Male 17.4 ± 0.4 AM and PM WBC LDH AST ALT | AM and PM | WBC Sig. Sig. Sig. NS PM | PM PM |
| (Hammouda et al., 2012a)| Controlled trial | Tunisia | 15     | Aerobic Exercise Male 17.3 ± 0.5 AM and PM WBC LDH CK AST ALT | AM and PM | WBC Sig. NS NS Sig. NS PM | PM PM |
| (Shahidi et al., 2012)  | Controlled trial | Iran    | 21     | Aerobic Exercise Male 20.9 ± 0.99 (AM) 21.0 ± 0.63 (PM) AM and PM WBC | AM and PM | WBC Sig. | PM |

(Continued)
| Author & year               | Study design | Country    | Sample | Participants characteristics and exercise type | Time of day | Biomarkers | Acrophases (Max.Value) |
|-----------------------------|--------------|------------|--------|-----------------------------------------------|-------------|------------|------------------------|
| (Souissi et al., 2012)      | Controlled trial | Tunisia    | 15     | Aerobic Exercise                               | AM and PM   | WBC        | PM                     |
|                             |              |            |        | Male                                           |             | LDH        | PM                     |
|                             |              |            |        | Mean age 17.3 ± 0.5                            |             | CK         | PM                     |
|                             |              |            |        |                                                |             | AST        | PM                     |
|                             |              |            |        |                                                |             | ALT        | PM                     |
| (Hammouda et al., 2013)     | Controlled trial | Tunisia    | 15     | Aerobic Exercise                               | AM and PM   | LDH        | PM                     |
|                             |              |            |        | Male                                           |             | WBC        | NS                     |
|                             |              |            |        | Mean age 17.3 ± 0.3                            |             | LDH        | NS                     |
|                             |              |            |        |                                                |             | CK         | NS                     |
|                             |              |            |        |                                                |             | AST        | NS                     |
|                             |              |            |        |                                                |             | ALT        | NS                     |

WBC = White Blood Cells, CK = Creatinine Kinase, LDH = Lactate Dehydrogenase, AST = Aspartate amino trans, ALT = alanine aminotransferase, Vo2Max = Volume of Maximum Oxygen Consumption, Sig. = Significant, NS = Not Significant, NR = Not Reported, AM = Antemeridian, PM = Postmeridian.
of day were, SMD = −1.82 (95% CI, −2.69, −0.95; P < 0.001) and heterogeneity (I² = 76%). Therefore, LDH results of AM time compared to PM time showed significant (p < 0.001) differences [Figure 2]. Higher PM training LDH scores pointing out a stronger tendency towards diurnal effect to the specific time of the day.

3.2. Creatine kinase (CK)
Four studies were included in the meta-analysis to estimate the pooled diurnal variations on creatine kinase (CK) with 90 participants. Out of them, three studies (Ammar et al., 2015; Boussetta et al., 2017; Souissi et al., 2012) reported significant diurnal variations between the AM and PM exercising groups and the rest (Hammouda, Chtourou, et al., 2012b) reported non-significant difference between the two exercising groups. Similarly, the different result reported in a study by Hammouda et al., (2012) might be related to the training level of the participants. Using a random effect model, the overall pooled effect of exercise on different time of day were, SMD = −1.44 (95% CI, −2.43, −0.46; P < 0.004) and heterogeneity (I² = 74%). Therefore, CK results of AM time compared to PM time showed significant (p < 0.004) differences [Figure 2].

3.3. Aspartate aminotransferase (AST)
Four studies were included in the meta-analysis to estimate the pooled diurnal variations on aspartate aminotransferase (AST) with 82 participants. All AST reported studies (Ammar et al., 2015; Boussetta et al., 2017; Hammouda, Chtourou, et al., 2012b; 2013; 2011; Souissi et al., 2012) indicated significant diurnal variations between AM and PM exercising groups. Using a random
3.4. Alanine aminotransferase (ALT)

Four studies were included in the meta-analysis to estimate the pooled diurnal variations on alanine aminotransferase (ALT) with 82 participants. Out of them, three studies (Ammar et al., 2015; Hammouda, Chtourou, et al., 2012; 2011) reported non-significant diurnal variations between AM and PM exercising groups and the rest (Souissi et al., 2012) reported significant difference among the groups. Using random effect model, the overall pooled effect of exercise on different time of day were, SMD = −0.73 (95% CI, −1.39, −0.07; P < 0.03) and heterogeneity (I² = 56%). Thus, ALT results of AM time compared to PM time showed significant (p < 0.03) differences [Figure 2].

3.5. White blood cells (WBC)

Lastly, concerning the skeletal muscle damage markers, seven studies were included in the meta-analysis to estimate the pooled diurnal variations on white blood cells (WBC) with 157 participants. Out of them, six studies (Ammar et al., 2015; Hammouda, Chtourou, et al., 2012; 2011; Mohammadnajad et al., 2012; Shahidi et al., 2012; Souissi et al., 2012) reported significant diurnal variations between AM and PM exercising groups and the rest (Erdemir, 2013) reported non-significant difference between the two exercising groups. Using a random effect model, the overall pooled effect of exercise on different time of day were, SMD = −1.46 (95% CI, −1.94, −0.98; P < 0.001) and heterogeneity (I² = 41%). Therefore, WBC results of AM time compared to PM time showed significant (p < 0.001) differences [Figure 2]. The higher PM training WBC scores pointing out a stronger tendency towards diurnal effect to the specific time of the day.

4. Discussion

The study performed a meta-analysis to investigate the diurnal effects of exercise on selected skeletal muscle damage markers (LDH, CK, AST, ALT and WBC). Overall, analysis indicated that the selected skeletal muscle damage markers possess diurnal variations with higher values in the PM time of the day. The pooled meta-analysis results reveal that the skeletal muscle injury makers like LDH, CK, AST, ALT and WBC possess diurnal variations. The maximum values in these markers were seen in the PM time of the day. Our meta-analysis results are in contrast to previous findings (Miles et al., 2008) which reported non-significant differences among AM compared to PM times of the day. Whereas, our result was in agreement with previous studies (Brancaccio et al., 2010; Gutenbrunner, 2000; Kanabrocki et al., 1990; Nathwani, Pais, Reynolds, & Kaplowitz, 2005). The difference between the AM and the PM time in responses to exercise might be attributed to an increased catecholamine activity since catecholamine follow very similar patterns in response to exercise (Deschenes, Sharma, Brittingham, Casa, & LE, 1998). The rise in these markers is also related to rise in muscle fatigue and reduction in performances, this is confirmed by (Hammouda, Chtourou, et al., 2012; 2011) where the higher muscle fatigue in the PM during short-term maximal exercise could be due to higher levels of biological markers of muscle injury and lower resting antioxidant status at this time-of-day. Consequently, the diurnal variations in muscle damage enzymes could be linked to the circadian rhythm of core body temperature (Haus, Lakotua, Swoyer, & Sackett-Lundeen, 1983; Rivera-Coll et al., 1993). In fact, the PM time elevation in body temperature would increase the activity of some enzymes such as phosphofructokinase and LDH (Dalton, McNaughton, & Davoren, 1997). Even if other studies reported the diurnal variation in core temperature as not necessarily the cause of the rhythm in muscle performance (Waterhouse et al., 2007). Others also reported that the skeletal muscle damage markers affecting exercise performances are higher in the evening than morning and afternoon and recommend afternoon training with less fatigue compared to the morning and the evening training sessions (Ammar et al., 2015). Therefore, the diurnal variation of skeletal muscle damage markers was confirmed, but the reason for the diurnal variations in these markers has remained unclear.
It is known that a meta-analysis of well-conducted controlled trials is the most rigorous way to show the best evidence by pooling results obtained from different studies (Kentiba, George, et al., 2019). The results obtained from our study may protect the physicians from misinterpreting of abnormal values when evaluating the training level of their athletes. It is also important for active individuals, athletes and coaches. The low number of studies included in some markers appears to be the major limitation of the present study. To maintain homogeneity the participants were male across the included studies. Thus, further studies should be conducted on female participants. In conclusion, our study partly confirms the diurnal variations of the selected skeletal muscle damage markers and recommended to conduct meta-analysis aiming to investigate the concomitant effect of exercise and time-of-day (morning-afternoon-evening) variations of these markers in relation to hormonal, core temperature and oxidative responses.

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