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Source	<i>Chronobiology International</i> , 29(8), 1139-1150. http://dx.doi.org/10.3109/07420528.2012.708375
Published by	Taylor & Francis (Routledge)

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Submitted: 28 November 2011; Returned for 1st revision: 11 January 2012;

Accepted: 24 June 2012

Conducting an acute intense interval exercise session during the Ramadan fasting month: What is the optimal time of the day?

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Running title: Time-of-day to exercise during Ramadan

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ABSTRACT

This study examines the effects of Ramadan fasting on performance during an intense exercise session performed at three different times of day, i.e., 08:00, 18:00, and 21:00 h. The purpose was to determine the optimal time of day to perform an acute high-intensity interval exercise during the Ramadan fasting month. After familiarization, nine trained athletes performed six 30-s Wingate Anaerobic Test (WAnT) cycle bouts followed by a time-to-exhaustion (T_{exh}) cycle on six separate randomized and counterbalanced occasions. The three time-of-day non-fasting (Control, CON) exercise sessions were performed before the Ramadan month, and the three corresponding time-of-day Ramadan fasting (RAM) exercise sessions were performed during the Ramadan month. Note that the 21:00 h session during Ramadan month was conducted in the non-fasted state after the breaking of the day's fast. Total work (TW) completed during the six WAnT bouts was significantly lower during RAM compared to CON for the 08:00 and 18:00 h ($p < .017$; effect size [d] = .55 [small] and .39 [small], respectively) sessions, but not for the 21:00 h ($p = .03$, $d = .18$ [trivial]) session. The T_{exh} cycle duration was significantly shorter during RAM than CON in the 18:00 ($p < .017$, $d = .93$ [moderate]) session, but not in the 08:00 ($p = .03$, $d = .57$ [small]) and 21:00 h ($p = .96$, $d = .02$ [trivial]) sessions. In conclusion, Ramadan fasting had a small to moderate, negative impact on quality of performance during an acute high-intensity exercise session, particularly during the period of the daytime fast. The optimal time to conduct an acute high-intensity exercise session during the Ramadan fasting month is in the evening, after the breaking of the day's fast. (Author correspondence: abdul_rashid_aziz@ssc.gov.sg)

Keywords: Wingate Test, Exercise, Time-of-day, Circadian rhythm, Time-to-exhaustion, Aerobic, Anaerobic

INTRODUCTION

Those of the Islamic faith are obliged to fast daily during the holy month of Ramadan. During this period of 30 d, Muslims do not eat or drink (i.e., total abstinence from food and fluids) from pre-dawn until dusk. The duration of the day's fast is influenced by the seasonal conditions and geographical location; thus, it may vary from between 11 to 18 h. However, for countries in the equatorial region where the present investigation was conducted, the duration of the daily fast remains fairly consistent, with the daily fasting times falling between 05:30 to 19:15 h. Traditionally, Muslims usually consume their main meals at two daily sittings, between ~04:30 to 05:30 h before the commencement of the day fast (i.e., *sahur* meal), and at ~19:15 h for the breaking of the day fast (i.e., *iftar* meal). Thus, during the Ramadan month, Muslim athletes face the problem of acute lack of food and fluid during the daylight hours as well as poor sleep as a consequence of altered bed times that could negatively impact exercise performance (Aziz & Png, 2008; Chaouachi et al., 2009). Although some findings indicate the contrary (Aziz et al., 2010a; Kirkendall et al., 2008), many studies have demonstrated that Ramadan fasting has a negative impact on various aspects of exercise performance (Aziz et al., 2010b; Bigard et al., 1998; Brisswalter et al., 2011; Chtourou et al., 2012; Hamouda et al., 2012; Meckel et al., 2008; Zerguini et al., 2007).

The calendar of international sporting events does not take into account the practices of Ramadan fasting, and Muslim athletes may have to train through the Ramadan month. Thus, one of the many strategies adopted by coaches and athletes to try to minimize the impact of Ramadan fasting includes training or exercising at specific times of the day (Rai, 2003; Roy et al., 2011). For example, exercising in the early morning hours, between 08:00 – 10:00 h, seems ideal because athletes would have consumed the *sahur* meal merely 3-4 h earlier. However, the inability to consume food and fluid after an exercise session until the

breaking of the day's fast, which is at least 6-8 h later, could result in impaired recovery that may hamper training-induced adaptations (Ivy, 2004). Also, the thought of going on for the next 6-8 h without food and fluid after intense, exhaustive exercise might cause the fasted athletes to conserve their efforts during the morning training session (Waterhouse, 2010). Another suggestion is to exercise in the late afternoon, just prior to the breaking of the day's fast (i.e., between ~17:00 to 18:00 h), a time where human muscular performance tends to peak, at least in the non-fasted state (Atkinson & Reilly, 1996). However, the fasted-athlete would have been without nutrition and fluids for the last 10-12 h prior to the exercise session, which could result in poorer quality of performance during the session (Zerguini et al., 2007). Indeed, there is evidence that the same bout of physical exercise at this time of day places greater physical and mental stress on the body and is perceived to be more physically demanding in the fasted vs. the non-fasted state (Chtourou et al., 2012; Waterhouse et al., 2009). In contrast to exercise during the daytime, others have argued that during the Ramadan month, training sessions should ideally be conducted in the evening, several hours after the athlete has broken the day's fast i.e., after the *iftar* meal, when nutrition support is available without restriction before, during, and after exercise (Mujika et al., 2010; Waterhouse, 2010). This may, however, be logistically challenging when considering the impact of gastrointestinal tract distress on exercise if the exercise session is scheduled too close to the *iftar* meal (Reilly & Waterhouse, 2007), or too late so as to impact on sleep, which potentially could affect exercise performance the following day (Souissi et al., 2011; Waterhouse, 2010).

There have only been very few studies investigating the impact of Ramadan fasting on exercise performance at different times of the day. Souissi et al. (2007) examined the anaerobic performance of diurnally active fasted Muslims at 07:00, 17:00, and 21:00 h during

repeated short sprints (each of 6 s duration) and a 30-s Wingate anaerobic cycle test. The investigators observed significant decline in peak and mean power in both tests during Ramadan in the 17:00 and 21:00-h sessions, but not in the 08:00-h session (Souissi et al., 2007). On the contrary, Kordi and colleagues (2011) observed no significant impact of Ramadan fasting on jumping and agility performance in trained athletes, undertaken either in the late afternoon (60 min pre-*iftar*) or in the evening (3 h post-*iftar*). In another study, work done during repeated short bouts of maximal cycle sprints and time-to-exhaustion during the Yo-Yo Intermittent Recovery Test decreased in the late afternoon (17:00 h) compared to the morning (07:00 h) during Ramadan (Hamouda et al., 2012). Similar findings were observed during Wingate cycle exercise in young trained football players (Chtourou et al., 2012). Thus, the findings of the impact of Ramadan fasting on exercise performance undertaken at different times of the day are mixed (Reilly & Waterhouse, 2007) and in need of further investigation. Therefore, the aims of the present study were to assess the quality of exercise performance during an acute high-intensity exercise session in trained athletes during the Ramadan month, performed at three different times of the day: (i) in the early morning at 08:00 h, (ii) in the late afternoon at 18:00 h, and (iii) in the evening at 21:00 h, after the breaking of the day's fast. These three time-of-day sessions were chosen because they represent the typical period of the day when fasted Muslim athletes would normally exercise or train during the Ramadan month, in particular, within the context and locality where the study was conducted. The primary objective was to determine the best time of day to conduct an acute intense interval exercise session during the Ramadan fasting month.

MATERIAL and METHODS

Subjects

Nine healthy, male Muslim athletes (age: 18.9 ± 1.1 yrs; body mass: 71 ± 10 kg; stature: 171 ± 4 cm) participated in the study. The volunteers were national representatives from the martial arts sport of pencak silat (Aziz et al., 2002) with 2-6 yrs of international competitive experience. All participants were well-accustomed to high-intensity interval exercise with running and sports-specific drills as their primary mode of exercise, but none routinely included cycle ergometry in their training. They had also performed the annual Ramadan fasting ritual for the last 6-9 yrs. At the time of the study, the athletes were training 3-4 times/wk, for 60-90 min/session. These sessions were typically scheduled either in the late afternoon or evening period and consisted of sport-specific technical drills and sparring matches with none of the participants involved in any systematic fitness training program. Ethical approval from the Singapore Sports Council human research ethics committee was granted for the study. Athletes provided written informed consent after all procedures were explained to them in detail. Athletes <18 yrs of age provided informed assent and also had their parents' consent to participate in the study. The experimental protocols were conducted in accordance with international ethical standards and guidelines (Portaluppi et al., 2010).

Experimental procedures

The study was undertaken to coincide with the actual month of Ramadan when the duration of daily fasting was from ~05:30 to ~19:15 h (a total of ~13.7 h). Familiarization with all test procedures was undertaken in the weeks prior to the start of the study. Two familiarization sessions to the exercise protocols were conducted prior to the commencement of the data collection period. One of the two sessions was conducted in the morning at ~08:00 h to accustom the subjects to the intense exercise at that time of the day (Edwards et al., 2005). The other familiarization session was conducted either in the afternoon or evening. The three different time-of-day sessions (i.e., 08:00, 18:00, and 21:00 h) for each subject were

conducted in a counter-balanced crossover design; thus, subjects acted as their-own controls. Athletes performed the three different time-of-day, non-fasting exercise sessions (i.e., Control, CON) 2 wks prior to the Ramadan month and the three corresponding time-of-day, Ramadan fasting exercise sessions (RAM) during the last 2 wks of the Ramadan month (Figure 1). It must be highlighted that the 21:00 h exercise session during the Ramadan month was conducted after the breaking of the day's fast, and athletes performed the session in the non-fasted state. Each time-of-day exercise session for each of athlete was conducted as an individual trial separated by at least 3 d, but not more than 5 d, to reduce accumulative fatigue effects. Athletes were instructed to maintain their habitual physical activity during the period of the study but to avoid any vigorous activity the 24 h before their trial.

The RAM exercise sessions were conducted during the last 2 wks of the Ramadan month since previous studies have shown the fasted exercise performance of individuals tend to gradually improve or stabilize toward the latter part of the Ramadan month because fasted subjects learn to adapt and become more accustomed to Ramadan fasting and its associated perturbations (Roy et al., 2011; Souissi et al., 2007). An additional exercise session, consisting only of the six Wingate bouts, was further conducted between the CON and RAM periods to reduce the impact of mode-specific detraining phenomena (Figure 1) (Hamouda et al., 2012; Meckel et al., 2008; Mujika et al., 2010). This session was conducted at the athletes' convenience, choice of day and time, and the data during this session were omitted from the final analysis.

The acute exercise session

The exercise session consisted of two components, including six repeated 30-s Wingate Anaerobic Test (WAnT) cycle bouts (with 4 min of recovery between each bout), followed

by 6 min passive recovery before performing a time-to-exhaustion (T_{exh}) cycle, lasting between 8 to 20 min. Total duration of the session was about ~75 min, including a standardized 10-min warm-up (Figure 2). The exercise protocol was designed to simulate a typical high-intensity interval workout session that involves both the aerobic and anaerobic component of an athlete's energy systems.

All exercises were performed on friction-loaded cycle ergometers (834C, Monark Exercise AB, Vansbro, Sweden) equipped with a computerized data collection system (Opto-Sensor System, SMI, St. Cloud, USA). Each athlete was assigned to the same cycle ergometer throughout the entire study. During the test, seat height was adjusted for each athlete's leg length, and the feet were secured with toe-straps. For the WAnT cycle bouts, resistance was set at .075 kg relative to each of the individual athlete's body mass at the commencement of the study, and this resistance was kept the same for all the athlete's trials. Prior to exercise, athletes performed a standardized warm-up of 6 min at 50 W followed by dynamic stretching that focused on the muscles of the lower limbs. The athletes then had two ~3-4-s maximal sprints followed by 3 min of passive rest before the actual exercise trial. For each of the WAnT bouts, athletes cycled maximally for 30 s, in accordance with previously described methods (Inbar et al., 1996). Briefly, a rolling start was allowed and at the end of the count of 3 s, the full resistance was imposed when the athlete would be cycling at his highest cadence. Verbal encouragement was provided to spur the athlete to maximal effort throughout the 30-s duration. During recovery between bouts, the athlete continued to cycle slowly at minimal resistance (without any load applied) for ~30 s, then dismounted the ergometer and had a passive rest for the remaining recovery time. Cycle data were averaged every 1 s, and the sum of the workload (TW) completed for all the six WAnT cycle bouts was computed in both absolute (kJ) and relative to body mass ($\text{J}\cdot\text{kg}^{-1}$) terms.

After the last (sixth) WAnT bout, the athlete continued with 2 min of recovery cycling and then dismounted the ergometer for another 4 min of passive recovery. The athlete then re-mounted the ergometer to perform the T_{exh} cycle component of the exercise session. Resistance for the T_{exh} cycle was set at 4% of the individuals' body mass (Tan et al., 2006), and this resistance was again kept constant for all the athlete's trials. Again, the athlete was allowed a rolling start for 30 s before the resistance was imposed and the timing for the T_{exh} cycle started. Instructions were given to maintain cycling cadence at 60-80 $\text{rev}\cdot\text{min}^{-1}$ (revolution/min) throughout, and termination was confirmed when the athlete was unable to maintain a pedal rate of $>40 \text{ rev}\cdot\text{min}^{-1}$ for five continuous seconds, even after strong verbal encouragement.

No results or any measures of exercise responses were revealed to the athletes until the completion of the entire study. All sessions were conducted in an air-conditioned laboratory setting, with air temperature and relative humidity between 22 to 25°C and 55 to 65%, respectively. No food or fluid was allowed to be consumed during the exercise sessions, even during the CON trials. The total work (TW) during the six WAnT cycle bouts and duration of the T_{exh} cycle exercise was used as the criterion measure of the athletes' quality of exercise performance for the entire exercise session.

Exercise response variables

Upon arrival to the laboratory, the athlete emptied his bladder for collection of a urine sample. A digital refractometer (PR series, Atago, Tokyo, Japan) was used to determine the athlete's pre-exercise hydration status via the specific gravity of the urine sample (U_{sg}). The athlete's pre-exercise nude body mass was taken on a weighing scale with an accuracy of ± 50 g (Spider 2-150-P, Mettler Toledo, Albstadt, Germany). Each athlete then completed several

questionnaires on his mood and sleep patterns. A capillary blood sample was taken to determine blood lactate (Accutrend, Boehringer Mannheim, Germany) and blood glucose (Advantage, Roche Diagnostics Corp., IN, USA) concentration at pre- and post-exercise. Heart rate (HR) was monitored (S610, Polar Electro, Finland) every 5 s throughout the session, and data were averaged across the duration of the exercise, excluding rest periods. At the end of the entire training session, the athlete towed dry and his post-exercise nude body mass was taken, and the athlete's sweat rate and body mass changes for the session were then calculated. Athletes also provided their session ratings of perceived exertion (RPE), using the Borg's 1-10 ratio scale (Foster et al., 2001) that was administered ~20 min after the completion of the session.

Profile of mood state and sleep

Prior to exercise, the athletes completed the validated Brunel Mood State (BRUMS) profile (Terry et al., 1999) and Epworth Sleepiness Scale (EpSS; Johns, 1991). The BRUMS comprised 24 items to which the athletes were required to indicate how they felt "right now". There are six mood subscales state in the BRUMS, consisting of anger, confusion, depression, fatigue, tension, and vigor. Raw scores for each mood state were summed and converted to standard *T*-scores to describe the iceberg mood state phenomenon (Terry et al., 1999). The EpSS aims to quantify the individual's levels of daytime sleepiness. Athletes answered how likely they are to doze in eight different typical daily situations on a 4-point scale. Rating scores were summed, and excessive daytime sleepiness was defined as an EpSS score of >10 (Johns, 1991).

Dietary intake

To control for subjects' diet across the three time-of-day exercise sessions as well as across

both the CON and RAM trials, athletes were provided with pre-packed cooked meals (Fus'zin Palate Catering Pte Ltd, Singapore) for the 24-h period prior to all exercise sessions. Each of the 24-h meal plans provided $7.0 \text{ g}\cdot\text{kg}^{-1}$ body mass of carbohydrate (CHO; $497 \pm 73 \text{ g}$) and $1.5 \text{ g}\cdot\text{kg}^{-1}$ body mass of protein ($107 \pm 6 \text{ g/d}$), plus 4.0 L of fluid (consisting of sports drinks, fruit juice, and bottled water). Athletes were provided with specific instructions by a certified nutritionist on the distribution of the specific foods and fluids to be consumed over the 24 h prior to each of the exercise session (Table 1). Although meals and fluids were taken at different times of the day during the CON and RAM periods, the amount consumed in the 24 h prior to each exercise trial was the same. Athletes also used a diary to record the types and quantity of food and fluid consumed in the 24 h prior to testing, with this subsequently verified by the same nutritionist to ensure consistency across CON and RAM trials.

Statistical analysis

The Statistical Programme for Social Sciences 15.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. All data are reported as mean \pm SD. The level of statistical significance for primary effects was set at $p < .05$. All variables of interest were analyzed by a repeated-measures, two-factor analysis of variance (ANOVA), with condition (i.e., CON and RAM) and time-of-day of exercise (i.e., 08:00, 18:00, and 21:00 h) as the between-condition and within-group factors, respectively. The assumption of sphericity was checked by Mauchly's test of sphericity and where violations occurred, the Greenhouse-Geisser Epsilon was applied. If a significant main effect was found, post-hoc paired t -test was used to detect where the differences occurred with a Bonferroni correction for multiple comparisons, $p = .05/3 = .017$. To illustrate the magnitude (meaningfulness) of differences in the variables of interest between the CON and RAM conditions, effect sizes (d) calculations ($[\text{CON minus RAM}]/\text{pooled standard deviations}$) were utilized. A modified scale by Hopkins

(2002) was used for interpretation of d ; where trivial: $<.2$; small: $.2$ -. $.6$; moderate: $.6$ - 1.2 ; large: 1.2 - 2.0 ; and very large: > 2.0 .

RESULTS

Total work (TW) during WAnT cycle bouts and time to exhaustion (T_{exh}) cycle exercise

All athletes successfully completed the 30 d of Ramadan fasting. There was significant main effect of condition for absolute TW (in kJ) ($F_{1,8} = 40.34$, $p = .001$) and interactions ($F_{2,16} = 4.26$, $p = .03$). No significant main effects of time-of-day for TW were observed ($F_{1,20,9.56} = 2.40$, $p = .15$). Post-hoc analysis indicated significant differences in TW between CON and RAM for the 08:00 h (CON: 88.2 ± 10.3 vs. RAM: 82.1 ± 11.5 kJ, $p < .001$; $d = .55$, small) and 18:00 h (86.7 ± 12.5 vs. 81.9 ± 12.3 kJ, $p = .003$; $d = .39$, small) sessions. There was no statistically significant difference in TW between CON and RAM for the 21:00 h (87.5 ± 12.1 vs. 85.3 ± 13.5 kJ, $p = .03$; $d = .18$, trivial) session (Figure 3a). For the T_{exh} cycle, there was significant main effect of condition ($F_{1,8} = 7.60$, $p = .03$) and interaction ($F_{2,16} = 4.02$, $p = .04$). There was no significant main effect of time-of-day in T_{exh} cycle ($F_{2,16} = .15$, $p = .86$). Post-hoc analysis indicated there were statistically significant differences in T_{exh} cycle between CON and RAM only for the 18:00 h (CON: 14.6 ± 7.5 vs. RAM: 7.9 ± 5.6 min, $p < 0.001$; $d = .93$, moderate) session but not for the 08:00 h (14.2 ± 8.3 vs. 9.7 ± 7.1 min, $p = .03$; $d = .57$, small) and 21:00 h (11.7 ± 7.3 vs. 11.6 ± 7.6 min, $p = .96$; $d = .02$, trivial) sessions (Figure 3b). Figure 4 provides a summary of both the TW and T_{exh} cycle results during the RAM exercise sessions relative to CON.

Body mass (Table 2)

There was significant main effect of condition ($F_{1,8} = 9.75$, $p = .014$), time-of-day ($F_{1,95,15.61} = 6.39$, $p = .01$), as well as interaction effects for pre-exercise body mass ($F_{1,26,10.04} = 6.79$, $p =$

.02). A subsequent post-hoc test showed body mass during CON was significantly higher compared to RAM for the 18:00 h session ($p = .001$). In addition, pre-exercise body mass at 18:00 h during RAM was the lowest among all exercise sessions.

Hydration status and exercise responses (Table 2)

There was main effect of condition for pre-exercise U_{sg} ($F_{1,8} = 6.58$, $p = .03$), but no other main or interaction effects (all F ratios, $p > .05$). Post-hoc analysis indicated significantly lower pre-exercise U_{sg} in athletes during CON than RAM for the 18:00 h ($p = .013$) but not the 08:00 ($p = .21$) or 21:00 h ($p = .07$) sessions.

Pre-exercise blood glucose concentration between CON and RAM was not significantly different ($F_{1,8} = 4.52$, $p = .07$). There was, however, significant main effects of time-of-day for pre-exercise blood glucose ($F_{2,16} = 9.83$, $p = .002$), but the interaction effect was not statistically significant ($F_{2,16} = 3.42$, $p = .06$). For the post-exercise blood glucose concentration, there was significant main effect for condition ($F_{1,8} = 13.33$, $p = .006$) only. Post-hoc analysis showed the post-exercise blood glucose concentration was significantly lower during CON vs. RAM for the 18:00 h session only ($p = .003$).

For pre-exercise blood lactate concentration, there were significant main effects of condition ($F_{1,8} = 92.63$, $p = .001$) and interaction ($F_{2,16} = 14.38$, $p = .001$), with no main time effect ($F_{2,16} = 2.27$, $p = .14$). Post-hoc analysis showed the pre-exercise blood lactate concentration was significantly higher during CON vs. RAM for the 08:00 ($p = .001$) and 18:00 h sessions ($p = .005$) but not for the 21:00 h session ($p = .20$). The post-exercise blood lactate concentration showed no significant main effects of condition or interactions (all F ratios, $p > .05$), but main effect of time was observed ($F_{2,16} = 4.95$, $p = .02$).

Mean HR during the WAnT cycle bouts showed significant main effect for time ($F_{2,16} = 9.69$, $p = .002$), with no significant main effect of condition or interaction (all other F ratios, $p > .05$). Post-hoc analysis showed HR significantly increased from the start of the first bout until termination of the sixth WAnT bout. Mean HR in the T_{exh} exercise showed no main effect for condition, time, or interaction (all F ratios, $p > .05$). A significant main effect for condition was evident for the variable of session RPE ($F_{1,8} = 7.21$, $p = .03$). No other time-of-day or interaction effects were observed for session RPE (all F ratios, $p > .05$). Post-hoc analysis indicated differences in the session RPE between CON and RAM trials did not reach statistical significance (all $p > .017$). For the % body mass change during exercise, there was significant main effect for condition ($F_{1,8} = 5.79$, $p = .04$), with no other significant main or interaction effects (all F ratios, $p > .05$). The post-hoc analyses of all the variables of interest with significant main effects are shown in Table 2.

Profile of mood state and daytime sleepiness

There were no significant main effects either for condition, time, or interaction in all the six mood subscales of BRUMS (all F ratios, $p > .05$; Figure 5). There were also no significant main effects for condition, time, or interactions for the EpSS scores (all F ratios, $p > .05$; Figure 6).

DISCUSSION

While many coaches have claimed that exercising at specific times of the day (e.g., early morning, early evening ,or late evening) as the best times to train (e.g., Mujika et al., 2010; Waterhouse, 2010; Wilson et al., 2009), there has been little investigation to our knowledge that directly examines the efficacy of exercise scheduling during the Ramadan fasting month. Thus, the aim of the present study was to determine the optimal time -- 08:00, 18:00, or 21:00

h -- to perform high-intensity exercise during the Ramadan fasting month. The study's results revealed that relative to CON sessions, high-intensity exercise performances during RAM was clearly superior for the 21:00 h session as compared to the other two time-of-day sessions, with the worst performance observed for the 18:00 h session (Figure 4). Thus, assuming the effectiveness of exercise training depends on the amount of work being performed during the session (Waterhouse, 2010), the main finding of the present study is that exercising at 21:00 h is the optimal time-of-day to perform high-intensity exercise during the Ramadan month in well-trained Muslim athletes.

The present study's results differ from those of the only other similar research (Souissi et al., 2007) that examined exercise performance of fasted individuals at three different times of the day. In that study, university-aged students performed both repeated bouts of 6-s cycle sprints and a 30-s WAnT cycle bout at 08:00, 17:00, and 21:00 h, in the Ramadan and non-Ramadan month. The study showed significant declines in peak and mean power in both cycle performances during Ramadan for the 17:00 and 21:00 h sessions, but not in the 08:00 h session (Souissi et al., 2007). We have no explanation for the contrasting findings of the Souissi et al. study versus ours. However, there are several stark differences in the methods of the two studies, including exercise protocols and volume (Souissi et al.: repeated maximal 6-s efforts + one WAnT bout vs. present study: six WAnT bouts + cycle to exhaustion), subjects' training status (Souissi et al.: physical education university students vs. present study: well-trained athletes), and other factors, such as daytime sleepiness (Souissi et al.: not measured vs. present study: assessed using the EpSS) and pre-exercise dietary intake (Souissi et al.: subjects completed 3-d food diary vs. present study: pre-prepared standardized food and fluid intake for 24 h) could have resulted in the conflicting findings (Chouauchi et al., 2009).

It is not surprising that 21:00 h seems to be the ‘best’ time-of-day to exercise during Ramadan, because the RAM exercise session was performed under favorable circumstances that were comparably similar to the corresponding time-of-day CON session. In the RAM 21:00 h session, the fasted athletes were able to refuel and rehydrate from the consumption of their pre-prepared *iftar* meals between 19:05 –19:20 h. They further had ~90 min of passive rest before undertaking the evening exercise session. The athletes’ pre-exercise blood glucose concentration levels were within the normal range (Table 2). Further, no athlete reported or complained of stomach cramps or upsets. Nonetheless, there are several concerns with exercising at this time of day. First, the practical opportunities to undertake exercise during the evening hours are significantly reduced. Second, the disruptive effects of late evening exercise on subsequent sleep cannot be discounted; indeed, there is evidence that prior intense exercise can result in delay of onset and/or poor quality of sleep (Reilly & Waterhouse, 2007; Waterhouse, 2010). This effect could further be exacerbated over the following day, since fasted athletes need to wake-up early before dawn to consume the *sahur* meal. In retrospect, it is not unexpected that among the three time-of-day sessions the athletes’ exercise performance at the 18:00 h session was the worst. Exercising at this time of the day is least ideal, since athletes have been without food and fluid for >10 h and were probably experiencing higher levels of fatigue and/or feelings of lethargy. Previous research has also indicated that an exercise bout performed at this time of day is relatively more physically and mentally demanding on fasted subjects compared to the same exercise in the non-fasted state (Chtourou et al., 2012; Waterhouse et al., 2009).

While the main aim of the present study was to determine the optimal time to train during the Ramadan month, it is, however, justified to speculate about the possible reasons for the lower quality of the athletes’ exercise performance during the daytime as compared to

the evening session in Ramadan month. First, it is likely that substrate and fluid play contributing roles, since the largest margins of differences in exercise performance between RAM and CON were observed in the fasted state with the 18:00 h session when the athletes consumed their previous meal some 10-11 h earlier. For the 08:00 and 21:00 h sessions during RAM, athletes would have been in a relatively food and fluid-filled state, because they would have just consumed their meals (i.e., *sahur* meal for 08:00 h and *iftar* meal for 21:00 h sessions, between 3 and 1.5 h, respectively, earlier). Indeed, consuming a normal mixed meal 3 h prior to exercise has been shown, via the muscle biopsy technique, to further increase the available endogenous muscle glycogen levels by 15-30% (Chryssanthopoulos et al., 2004; Wee et al., 2005): thus, it is unlikely that the muscles in the fasted athletes would have been muscle-glycogen deprived at these two sessions (i.e., 08:00 and 21:00 h). In addition, the relatively short duration of the actual work time of <30 min, albeit highly intense, of the present study's exercise protocol would clearly be deemed as not glycogen limiting (Burke, 2010). Endogenous muscle glycogen concentration was not measured in the present study, but the comparably similar post-exercise blood glucose and lactate concentration levels in RAM and CON provide indirect support to the view that endogenous glycogen was still available within the athletes in RAM at the end of all their exercise sessions (Hargreaves, 1997). In regards to fluid availability, fasted athletes were clearly in a less than optimal hydration state (as determined via the subjects' pre-exercise body mass and U_{sg}) during RAM as compared to CON (Table 2). For example, at the 18:00 h session, body mass was lower by a mean of ~1.5 kg (equivalent to ~2.0% of CON body mass). Given the additional average mass lost during exercise of ~.7% and assuming all these losses are due to fluid imbalance, it is likely that the subjects were further dehydrated during the RAM trial, at a level sufficient to adversely impact power generation capability and muscular endurance during high-intensity exercise (Judelson et al., 2007).

Perhaps, the poorer performance during RAM was less related to metabolic factors but was more associated with central fatigue. The central fatigue syndrome during exercise has been explained by reduction in electric signals to the peripheral motor pathways resulting in lower power output, and this phenomenon is always characterized by increase in subjects' RPE (Nybo & Secher, 2004). Although there was no significant difference between CON and RAM for each of the time-of-day sessions, there was an overall significantly higher post-exercise session RPE (i.e., main effects) for RAM compared to the CON condition, supporting this as a plausible occurrence. Indeed, during Ramadan fasted individuals typically sleep late and wake-up very early before the break of dawn to consume their main meal before commencing the day's fast (e.g., Wilson et al., 2009). Loss of 1-2 h sleep/d tends to accumulate over days, which could result in chronic daytime sleepiness, with impact upon the individual's daytime fatigue, lethargy, and mood. The present study indicated that fasted subjects experienced excessive daytime sleepiness relative to CON (mean EpSS scores in Ramadan fasted subjects for all three time-of-day sessions were >10, which is the threshold for excessive daytime sleepiness; Figure 6). The pre-exercise subjective sensations could further be exacerbated during exercise leading to poorer desire or motivation to sustain high-intensity or maximal efforts (Roky et al., 2004). Indeed, previous studies have shown that trained fasted Muslim athletes felt less ready to train during the daytime throughout Ramadan (Leiper et al., 2008; Zerguini et al., 2007), although this was not observed in all cases (e.g., Aziz et al., 2011). Thus, the effects of sleepiness and mood disturbances affecting intrinsic motivation during exercise could have contributed to the impaired daytime exercise performance during Ramadan, and should not totally be discounted in the present study (Reilly & Waterhouse, 2007).

It was previously suggested that the drastic shift or alterations in the fasted

individual's body natural day-to-day circadian rhythm (e.g., Bogdan et al., 2001) was the main contributor to the observed negative impact of Ramadan fasting on exercise performance (Waterhouse, 2010). For example, previous studies have shown that Ramadan fasting modified the diurnal pattern of exercise performance (consisting of repeated 6-s cycle sprints, 30-s all-out cycle, and Yo-Yo endurance running tests) by lowering the athletes' performance in the evening during the Ramadan month when compared to the same performance in the evening during the non-Ramadan month (Chtourou et al., 2012; Hamouda et al., 2012). This, however, does not seem to be the case in the present study. There were no significant differences in total work in the WAnT bouts and duration of T_{exh} cycle between the three different times-of-day exercise sessions, when these performance variables were assessed within both the CON and RAM periods, independently. Furthermore, Rowland and colleagues (2011) recently reported absence of significant differences in cardiac and metabolic responses during progressive maximal cycle exercise between the morning (08:00 – 11:00 h) and evening (16:00 – 19:00 h) sessions, albeit in normally fed adolescent males. Although previous Ramadan fasting studies have shown diurnal variation in exercise performance, these same studies showed the effects of diurnal variation tended to disappear when the same exercise was performed during the later part of the Ramadan month (Hamouda et al., 2012; Kirkendall et al., 2008; Kordi et al., 2011; Souissi et al., 2007). Further, the well-trained status of subjects as well as the very intense nature of the present study's exercise protocols could have also masked and/or blunted the diurnal effects (Reilly & Garrett, 1998).

The influence of placebo effects due to the inability to blind the subjects to the intervention of Ramadan fasting in the present study cannot be ruled out. It could be argued that in the present study the performance of the athletes at 08:00 and 18:00 h was more

“psychologically-influenced”, since they had not consumed any food and fluid in the hours (for a continuous duration of ~3 and ~10 h, respectively) prior to performing the daytime exercise sessions as compared to the 21:00 h session (when subjects had their *iftar* or breaking of the day’s fast meal 1.5 h earlier). However, it must be highlighted that the indirect markers of exercise responses in the present study, such as mean exercise HR during the WAnT bouts and T_{exh} cycle exercise, post-exercise blood lactate, and RPE, were not significantly different between the CON and RAM trials with predominantly trivial to small effect sizes (Table 2). Overall, it is most likely that the detrimental effects of Ramadan fasting on exercise performance during the daytime are due to the combined effects of metabolic and central drive factors, rather than to a single factor, alone (Chaouachi et al., 2009). However, further studies are needed to confirm this assertion.

While the present investigation has shown that acute high-intensity exercise performance is impaired in the fasted state, the present study’s results cannot be inferred to similar interpretation of the chronic impact of Ramadan fasting on training adaptations over the 4 wks of daily intermittent fasting. Endurance training in the fasted state may lead to greater muscular oxidative capacity than training in the non-fasted state (van Proeyen et al., 2011), although performances, per se, may not be impacted (Hawley & Burke, 2010). It was argued further that high-intensity endurance training in the fasted state (or more precisely, in the low endogenous CHO or prolonged post-absorptive state of <10 h) induces greater metabolic perturbations within the working muscles that further enhanced the oxidative markers of endurance training adaptations as compared to exercising in the normal or superior level of endogenous CHO state (Hawley & Burke, 2010; van Proeyen et al., 2011). Thus, high-intensity endurance training in the Ramadan fasted state, on this basis, is perhaps defensible. This view gained further credence with the recent finding that adaptations to

chronic high-intensity training (consisting of repeated 30-s bouts of maximal cycle efforts three times per week) across the Ramadan month were equivalent between Ramadan fasted and non-fasted individuals (Aziz et al., 2012).

There are several limitations to the present study. First, the inability to 'blind' athletes to either the CON or RAM intervention may have impacted on motivation to perform the tests maximally (Beedie & Foad, 2009). Nonetheless, measures, such as exercise HR, post-exercise blood lactate, and session RPE, were equivalent between conditions, which indicated equivalent exercise efforts across the entire study period. Second, to eliminate any order effect post-Ramadan period tests should have been undertaken. Also, the results of this study are clearly limited to high-power anaerobic work and may not be generalized to other types of exercise, such as resistance, agility, or speed. Last, it could be argued that sport-specific training three to four times per week could have impacted on cycle ergometer performance. However, given the non-specific nature of pencak silat training to cycle ergometer performance, the impact on performance is likely negligible (Aziz et al., 2002; Tanaka, 1994).

In conclusion, the results of the present study indicate that in well-trained athletes Ramadan fasting had a small to moderate negative impact on the quality of performance during acute high-intensity interval exercise when performed during the daytime period in the fasted state. The reason for this is not clear, but it is most likely due to the combined effects of lack of food, fluid, and sleep, as well as reduced central drive related to metabolic factors or poor intrinsic motivation. Exercise performance undertaken in the evening, after the breaking of the day's fast, was not affected.

PRACTICAL IMPLICATIONS

Many coaches and athletes are still uncertain as to the best time to schedule high-intensity exercise sessions during the Ramadan month (Mujika et al., 2010; Waterhouse, 2010). The present study findings indicate that, based on the amount of work being performed as a criterion measure of exercise performance, the optimal time of the day to conduct an acute intense interval exercise session during the Ramadan fasting month is in the evening i.e., 1.5-h post-absorptive of the breaking of the day's fast. If training for fitness during the daytime is still paramount, these data suggest that exercising in the morning is relatively better than exercising in the late afternoon.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the participation and cooperation of the athletes and coaches of the Singapore Silat Federation. Part of this study was presented at the 1st International Consensus Meeting on Ramadan and Football, at Aspetar in Doha, Qatar, from 25th to 26th November 2011.

DECLARATION of INTEREST

No external sources of funding were used to assist this study. The authors have no conflicts of interest that are directly relevant to the content of this study.

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TABLES and FIGURES CAPTION

Table 1. Meal distribution patterns of the 24-h pre-packed food and fluid prior to each exercise session in the Control (CON) and Ramadan (RAM) period during the study.

Table 2. Physiological responses before, during, and post-exercise during the Ramadan (RAM) and Control (CON) sessions at the three different times of day, i.e., 08:00, 18:00, and 21:00 h.

Figure 1. Schematic representation of study timeline.

Figure 2. Schematic representation of the study's acute exercise session. WAnT = Wingate Anaerobic test; U_{sg} = urine specific gravity; BRUMS = Brunel Mood State; EpSS = Epworth Sleepiness Scores; RPE = ratings of perceived exertion.

Figure 3. The (a) total work during six 30-s Wingate Anaerobic test (WAnT) cycle bouts, and (b) time to exhaustion cycle (T_{exh}) between Control (CON) and Ramadan (RAM) for the three times of the day, i.e., 08:00, 18:00, and 21:00 h, exercise sessions ($n = 9$). Post-hoc analyses showed significant differences between CON and RAM, $p < .017^{**}$ (after Bonferroni adjustment); d = magnitude of effect sizes.

Figure 4. Summary of Ramadan (RAM) period exercise performance, i.e., total work during six 30-s Wingate Anaerobic test (WAnT) cycle bouts and time to exhaustion (T_{exh}) cycle, relative to the Control (CON) period exercise performance at the three times of the day, i.e., 08:00, 18:00, and 21:00 h, sessions ($n = 9$). RAM exercise performance values (shaded bar) are expressed as a percentage of the corresponding time-of-day CON performance (open bar)

and assuming CON values are 100%. RAM performance values (in %) relative to the same corresponding time-of-day CON session performance are indicated at the top of each time-of-day bar.

Figure 5. Brunel Mood State (BRUMS) in the Control (CON) and Ramadan (RAM) for the three time-of-day exercise sessions: a) 08:00 h, b) 18:00 h, and c) 21:00 h (n = 9). No statistical significant main effect was observed between CON and RAM. A = anger, C = confusion, D = depression, F = fatigue, T = tension, and V = vigor.

Figure 6. Epworth Sleepiness Scale (EpSS) scores between Control (CON) and Ramadan (RAM) for the three time-of-day exercise sessions: a) 08:00 h, b) 18:00 h, and c) 21:00 h (n = 9). No statistical significant main effect was observed between CON and RAM. *d* = magnitude of effect sizes.

Table 1. Meal distribution patterns of the 24-h pre-packed food and fluid prior to each exercise session in the Control (CON) and Ramadan (RAM) period during the study.

	Distribution pattern of foods and fluids over the 24 h period prior to:		
	Exercise session at 08:00 h	Exercise session at 18:00 h	Exercise session at 21:00 h
CONTROL period	<u>Day Prior</u> M1 @ 13:00 h M2 @ 19:15 h	<u>Day Prior</u> M1 @ 19:15 h	<u>Day Prior</u> -
	<u>Test Day</u> M3 @ 06:00 h	<u>Test Day</u> M2 @ 07:00 h M3 @ 13:00 h	<u>Test Day</u> M1 @ 07:00 h M2 @ 13:00 h M3 @ 19:15 h
RAMADAN period	<u>Day Prior</u> M1 @ 05:00 h (<i>sahur</i>) M2 @ 19:15 h (<i>iftar</i>)	<u>Day Prior</u> M1 @ 19:15 h (<i>iftar</i>) M2 @ 23:00 h	<u>Day Prior</u> M1 @ 19:15 h (<i>iftar</i>)
	<u>Test Day</u> M3 @ 05:00 h (<i>sahur</i>)	<u>Test Day</u> M3 @ 05:00 h (<i>sahur</i>)	<u>Test Day</u> M2 @ 05:00 h (<i>sahur</i>) M3 @ 19:15 h (<i>iftar</i>)

M1 = Meal 1; M2 = Meal 2; M3 = Meal 3; *sahur* = meal sitting at the start of day's fast; *iftar* = meal sitting at the end of the day's fast. Note: Subjects were instructed to consume their three meals at the above specific times; however actual meal-sitting times varied (range from ~30 – 60 min during the CON and from ~10-15 min during the RAM periods).

Table 2. Physiological responses before, during, and post-exercise during the Ramadan (RAM) and Control (CON) sessions at the three different times of day, i.e., 08:00, 18:00, and 21:00 h.

	CON	RAM	<i>P</i> value between CON and RAM trial	Effect sizes (<i>d</i>), interpretation
<u>Body mass (kg) [taken on arrival at laboratory]</u>				
08:00 h session	71.7 ± 10.5	71.4 ± 11.6	0.529	0.03, trivial
18:00 h session	71.8 ± 10.4	70.3 ± 10.8	< 0.001 [†]	0.03, trivial
21:00 h session	71.1 ± 10.7	71.3 ± 11.3	0.024	0.1, trivial
<u>Pre-exercise U_{sg} (arbitrary unit, AU)</u>				
08:00 h session	1.011 ± 0.009	1.019 ± 0.011	0.213	0.7, moderate
18:00 h session	1.014 ± 0.008	1.023 ± 0.003	0.013 [†]	1.2, large
21:00 h session	1.012 ± 0.010	1.022 ± 0.007	0.070	1.0, moderate
<u>Pre-exercise Blood glucose (mmol·l⁻¹)</u>				
08:00 h session	5.5 ± 0.8	5.3 ± 0.5	0.353	0.3, small
18:00 h session	5.2 ± 1.2	4.3 ± 0.3	0.032	0.6, small
21:00 h session	5.4 ± 0.8	4.7 ± 0.5	0.137	0.3, small
<u>Post-exercise Blood glucose (mmol·l⁻¹)</u>				
08:00 h session	4.9 ± 0.4	5.5 ± 0.9	0.031	0.9, moderate
18:00 h session	4.8 ± 1.0	5.7 ± 1.1	0.003 [†]	0.8, moderate
21:00 h session	4.9 ± 0.7	5.2 ± 1.0	0.278	0.3, small
<u>Pre-exercise Blood lactate (mmol·l⁻¹)</u>				
08:00 h session	2.5 ± 0.3	1.5 ± 0.4	< 0.001 [†]	1.5, large
18:00 h session	2.2 ± 0.4	1.6 ± 0.3	0.005 [†]	1.2, large
21:00 h session	1.9 ± 0.3	2.1 ± 0.4	0.202	0.7, moderate
<u>Post-exercise Blood lactate (mmol·l⁻¹)</u>				
08:00 h session	7.5 ± 2.5	9.4 ± 2.5	0.023	0.7, moderate
18:00 h session	9.5 ± 3.1	10.4 ± 2.9	0.209	0.3, small
21:00 h session	9.9 ± 3.1	10.2 ± 3.1	0.729	0.1, trivial

Mean HR during WAnT bouts ($\text{b} \cdot \text{min}^{-1}$)

08:00 h session	161 ± 8	162 ± 11	0.508	0.1, trivial
18:00 h session	165 ± 8	166 ± 9	0.746	0.1, trivial
21:00 h session	166 ± 10	168 ± 10	0.221	0.2, small

Table 2, continue

Mean HR during T_{exh} cycle ($\text{b} \cdot \text{min}^{-1}$)

08:00 h session	154 ± 12	149 ± 9	0.150	0.5, small
18:00 h session	157 ± 8	154 ± 7	0.030	0.4, small
21:00 h session	156 ± 10	153 ± 12	0.465	0.3, small

Post-exercise RPE (1-10 scale)

08:00 h session	6.9 ± 1.3	7.7 ± 1.0	0.065	0.7, moderate
18:00 h session	7.7 ± 1.1	8.0 ± 1.2	0.195	0.3, small
21:00 h session	7.0 ± 1.5	7.7 ± 1.3	0.169	0.3, small

Volume of sweat loss during exercise session (ml)

08:00 h session	642 ± 264	536 ± 237	0.006^{\dagger}	0.4, small
18:00 h session	617 ± 174	480 ± 124	0.005^{\dagger}	0.9, moderate
21:00 h session	674 ± 421	654 ± 372	0.864	0.1, trivial

Change in body mass over the exercise session (%)

08:00 h session	0.9 ± 0.2	0.7 ± 0.2	0.004^{\dagger}	0.6, small
18:00 h session	0.9 ± 0.2	0.7 ± 0.1	0.006^{\dagger}	1.0, large
21:00 h session	0.9 ± 0.5	0.9 ± 0.3	0.866	0.1, trivial

[†]statistically significant difference between CON and RAM at the same time of day session, $P < 0.017$ (after Bonferroni adjustment). U_{sg} = urine specific gravity; WAnT = Wingate anaerobic test; T_{exh} = time-to-exhaustion; RPE = ratings of perceived exertion; HR = heart rate. Note: For the 21:00 h session during the RAM month, the athletes were exercising in the non-fasted state.

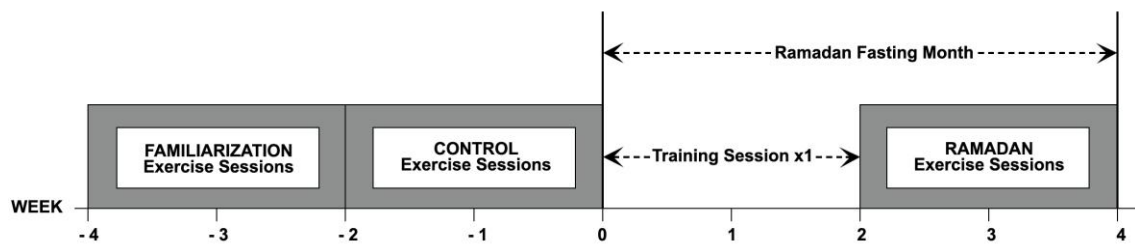


Figure 1. Schematic representation of study timeline.

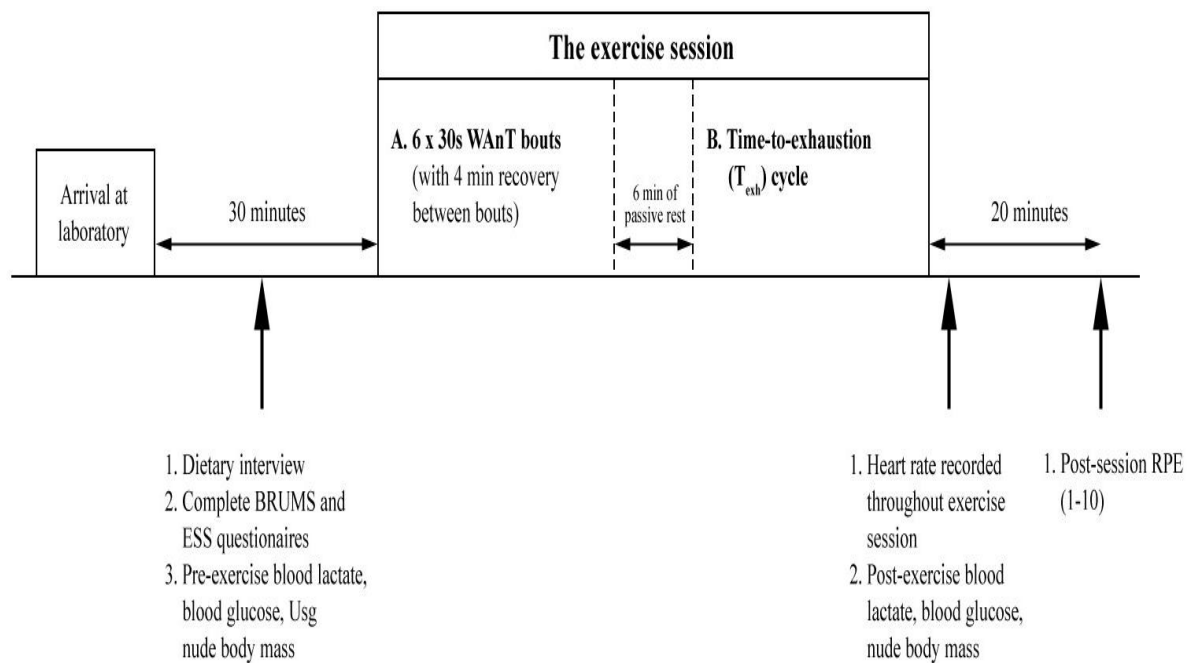


Figure 2. Schematic representation of the study's acute exercise session. WAnT = Wingate Anaerobic test; U_{sg} = urine specific gravity; BRUMS = Brunel Mood State; EpSS = Epworth Sleepiness Scores; RPE = ratings of perceived exertion.

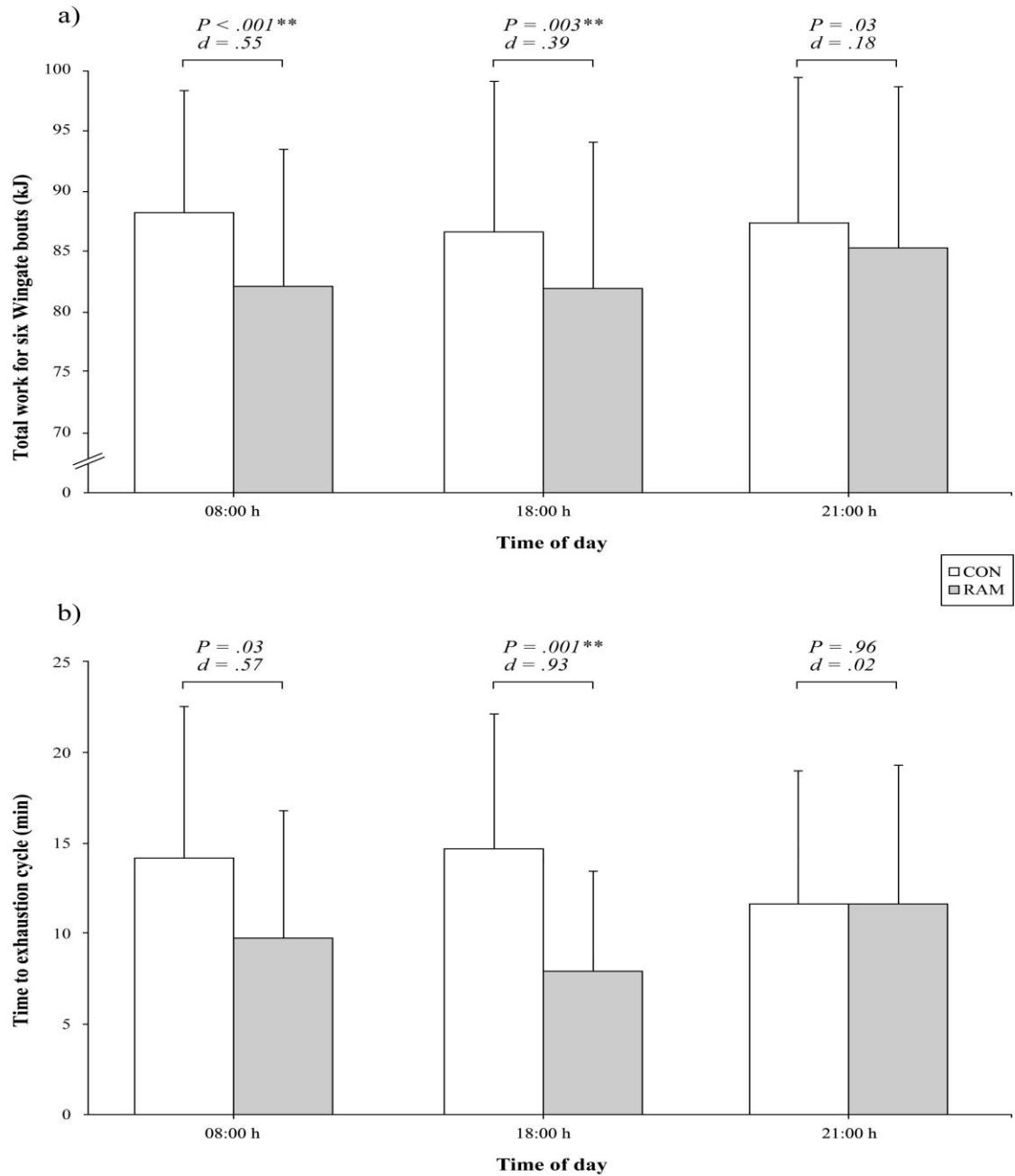


Figure 3. The (a) total work during six 30-s Wingate Anaerobic test (WAnT) cycle bouts, and (b) time to exhaustion cycle (T_{exh}) between Control (CON) and Ramadan (RAM) for the three times of the day, i.e., 08:00, 18:00, and 21:00 h, exercise sessions ($n = 9$). Post-hoc analyses showed significant differences between CON and RAM, $p < .017^{**}$ (after Bonferroni adjustment); d = magnitude of effect sizes.

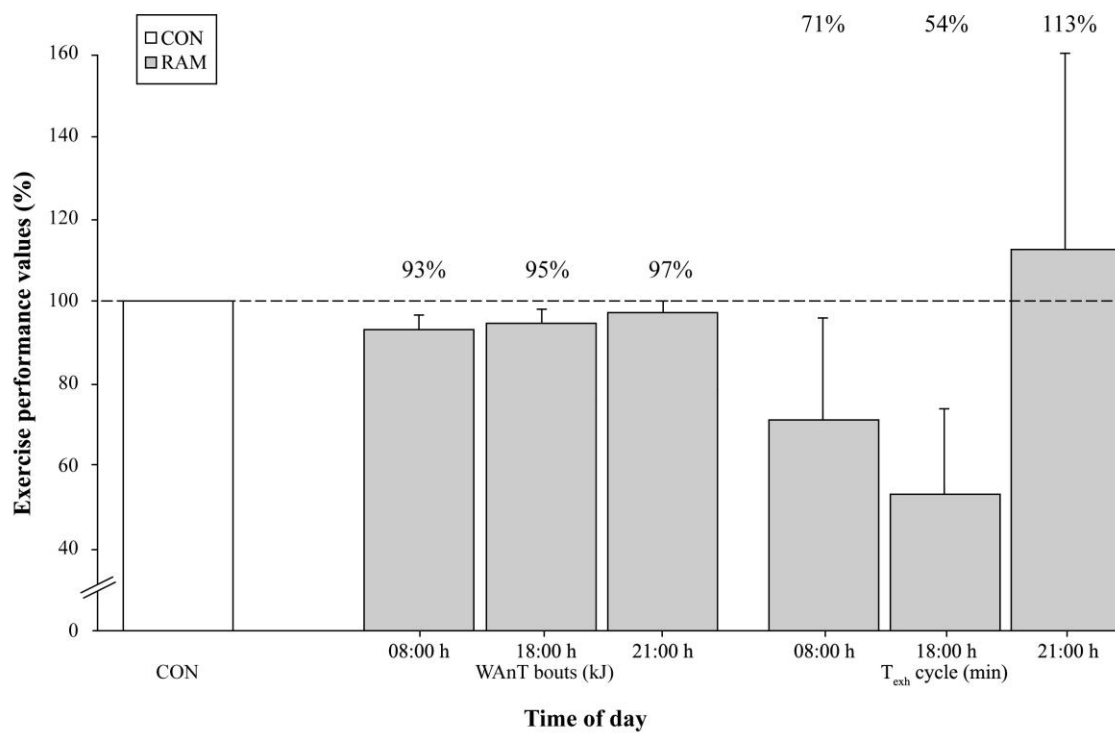


Figure 4. Summary of Ramadan (RAM) period exercise performance, i.e., total work during six 30-s Wingate Anaerobic test (WAnT) cycle bouts and time to exhaustion (T_{exh}) cycle, relative to the Control (CON) period exercise performance at the three times of the day, i.e., 08:00, 18:00, and 21:00 h, sessions ($n = 9$). RAM exercise performance values (shaded bar) are expressed as a percentage of the corresponding time-of-day CON performance (open bar) and assuming CON values are 100%. RAM performance values (in %) relative to the same corresponding time-of-day CON session performance are indicated at the top of each time-of-day bar.

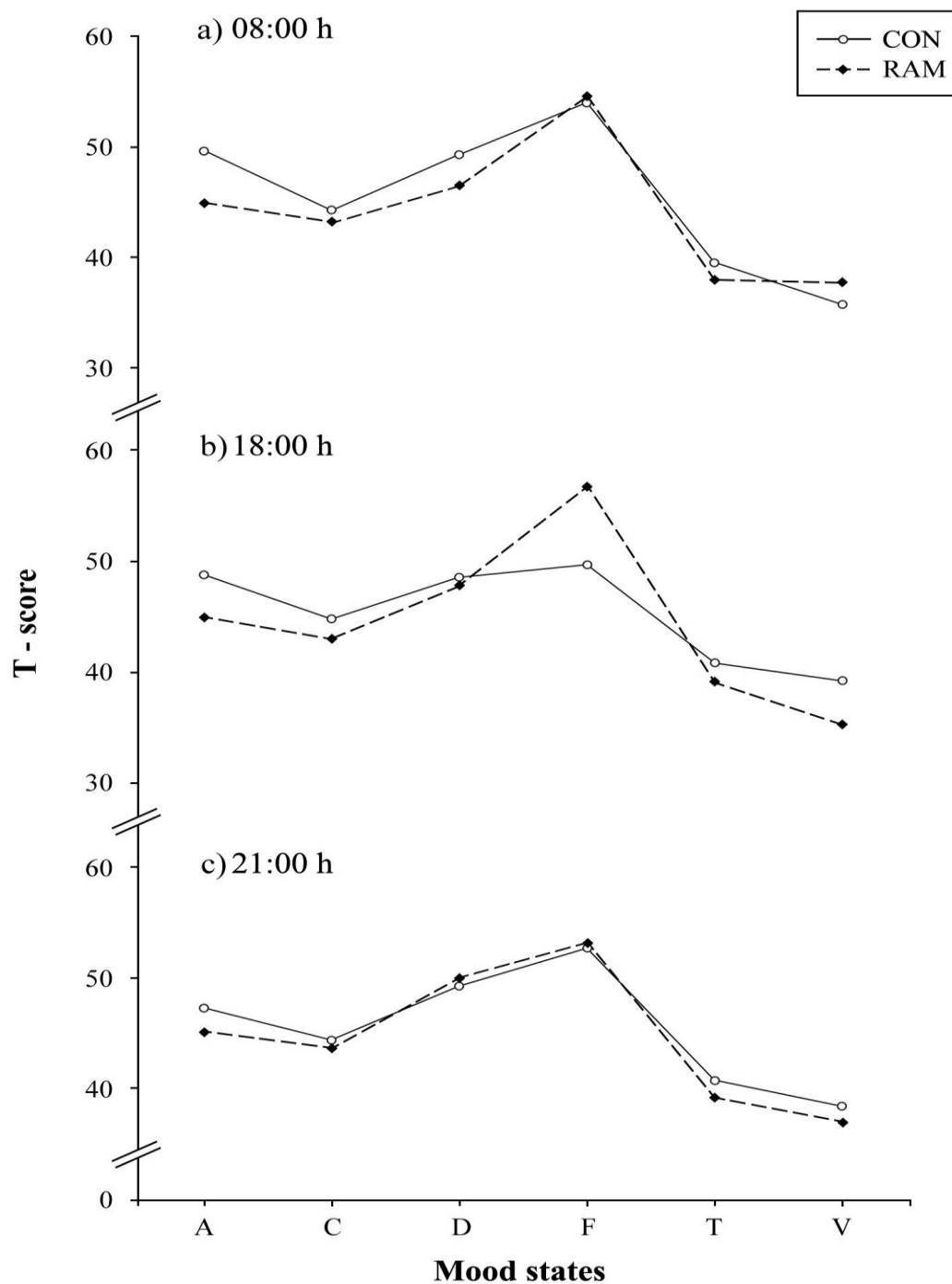


Figure 5. Brunel Mood State (BRUMS) in the Control (CON) and Ramadan (RAM) for the three time-of-day exercise sessions: a) 08:00 h, b) 18:00 h, and c) 21:00 h (n = 9). No statistical significant main effect was observed between CON and RAM. A = anger, C = confusion, D = depression, F = fatigue, T = tension, and V = vigor.

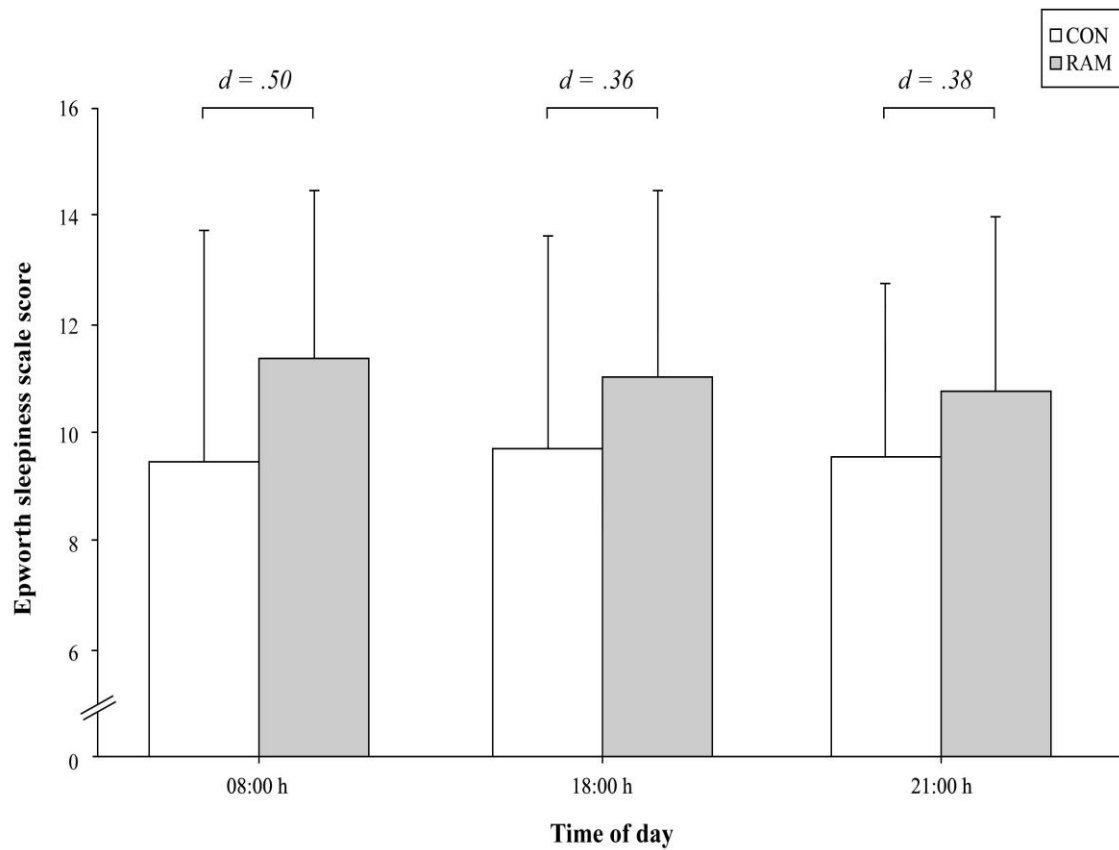


Figure 6. Epworth Sleepiness Scale (EpSS) scores between Control (CON) and Ramadan (RAM) for the three time-of-day exercise sessions: a) 08:00 h, b) 18:00 h, and c) 21:00 h ($n = 9$). No statistical significant main effect was observed between CON and RAM. d = magnitude of effect sizes.