

**Haematological, inflammatory and immunological responses in elite judo athletes
maintaining high training loads during Ramadan**

Abstract

During Ramadan, Muslims abstain from food and fluid intake from dawn to sunset for one month. These behavioural changes that accompany Ramadan may impact upon Muslim athletes who continue to train intensely. The aim of the present study was to evaluate the effect of Ramadan intermittent fasting (RIF) on the haematological, inflammatory, and immunological measures in elite judo athletes maintaining their usual high training loads. Haematological markers of inflammation, hormones, and immune status were studied in 15 elite male judo athletes before, during, and after Ramadan. The RIF produced small but significant changes in inflammatory, hormonal, and immunological profiles in judo athletes. Serum C-reactive protein increased from $2.93 \pm 0.26 \text{ mg}\cdot\text{l}^{-1}$ pre-Ramadan to $4.60 \pm 0.51 \text{ mg}\cdot\text{l}^{-1}$ at the end of Ramadan. Haptoglobin and antitrypsin also significantly increased at different phases during Ramadan, while homocysteine and pre-albumin remained relatively unchanged. Albumin decreased slightly by mid-Ramadan then recovered. Immunoglobulin A increased from $1.87 \pm 0.56 \text{ g}\cdot\text{l}^{-1}$ before Ramadan to $2.49 \pm 0.75 \text{ g}\cdot\text{l}^{-1}$ at the end, and remained high three weeks after Ramadan. There were no changes in the leucocyte cell counts throughout the study. The mean blood level of thyroid stimulating hormone, and free thyroxine significantly increased during RIF. Most of these changes were within the normal ranges. These results suggest that athletes who continue to train intensely during Ramadan are liable to experience a myriad of small fluctuations in hormones, immunoglobulins, antioxidants, and inflammatory responses.

Key Words: intensive training, nutrition, intermittent fasting, elite athletes

Introduction

During the lunar month of Ramadan, healthy Muslim adults refrain from eating, drinking, and smoking during the daytime. Several studies have shown that the Ramadan intermittent fast (RIF) can alter the eating and sleeping patterns (for a review see Reilly and Waterhouse 2007; Waterhouse et al. 2008). These behavioural patterns may alter the physiological responses to exercise that may influence the athletes' responses to training and subsequent exercise performance. At present there is relatively little information regarding the effect of the RIF on the physiological responses in well-trained athletes who maintain high training loads throughout Ramadan (Bouhlel et al. 2006; Chaouachi et al. 2008; Chaouachi et al. 2009; Maughan et al. 2008; Zerguini et al. 2008).

Both exercise and diet can modulate the human immune and inflammatory states (Kelley 2001; Meksawan et al. 2004). For example, intense exercise has been shown to increase inflammatory response markers and depress the immune function (Kasapis and Thompson 2005; Mackinnon 2000; Nieman 1997) whilst regular moderate intensity exercise can reduce inflammation (Albert et al. 2004; Ford 2002; Kasapis and Thompson 2005; Rauramaa et al. 2004) and improve immunocompetence (Mackinnon 2000). The negative effects of intense activity may be reduced by carbohydrate ingestion during exercise (Scharhag et al. 2006) or by increasing total dietary fat intake (Venkatraman et al. 2000). Unfortunately, for Muslim athletes, however, the intake of carbohydrates during exercise is prohibited if the training is completed during the daytime. Therefore, the combination of intense training with altered carbohydrate intake may place fasting Muslim athletes at greater risk of overreaching or overtraining during Ramadan (Halson et al. 2004; Smith 2000).

It is not clear if the RIF significantly alters the physiological responses of athletes who continue to train with high load during Ramadan. Several studies have shown that the metabolic response to exercise is changed during the RIF in well-trained athletes who train intensely (Bouhleb et al. 2006; Bouhleb et al. 2008; Chaouachi et al. 2008). However, other investigators have reported no changes in biochemical, nutritional, subjective well-being, and performance variables in young male football players who followed RIF in a controlled training camp environment (Maughan et al. 2008; Zerguini et al. 2008). At present however, it is unknown if these behaviour modifications during the RIF may influence other physiological parameters such as the inflammatory, hormonal, and/or immune systems.

Since athletes who participate in the RIF are unable to ingest carbohydrate during and immediately following daytime exercise, they may undergo altered haematological, inflammatory, and/or immunological responses to training. At present there are few published studies describing the effects of the RIF on the haematological, inflammatory, and immunological responses in elite athletes undertaking a high training load (Bouhleb et al. 2006; Chaouachi et al. 2008; Maughan et al. 2008; Zerguini et al. 2008). Therefore, the aim of this study was to investigate the effect of Ramadan on the haematological, inflammatory, and immunological markers of high-level judo athletes who maintained high training loads.

Materials and methods

Subjects

Fifteen healthy male elite judo athletes from the Tunisian national team who were maintaining their training schedule, volunteered to participate in the study spanning from October 2005 to December 2005 (Chaouachi et al. 2009). The mean and standard deviation (SD) of their age, body mass, and body mass index (BMI) were 18 ± 1 years, 68.1 ± 8.2 kg and 22.4 ± 1.8 kg·m²,

respectively. All participants had a minimum of 8 years of competitive experience in regional and national tournaments, and ten of them had competed internationally. All subjects who underwent Ramadan fasting, were non-smokers, and did not consume alcohol. Each subject signed a written consent prior to participation after being informed orally and in writing about the experimental procedures and of the possible risks and benefits of the study. The National Research Ethics Committee approved the experimental protocol.

At the time of the study, all Judokas trained and lived together at the Tunisian High School Sports Centre, Tunis, where the best national level athletes are accommodated in training camps. All the Judokas were regularly exercising to maintain their physical performance and maintain body mass by undertaking their usual training loads, supervised by their coaches, and were preparing for the up-coming national and international competitions. Both the training volume and intensity were relatively high; the weekly training program included 9 training sessions averaging a total of 17 hours (6 days/week and ~ 2 hours/day). Their regular judo training consisted mainly of a repetitive series of short and intense exercises involving various components within a judo session; judo specific interval training, technique and/or situation specific drills such as Uchikomi (the same technique practiced repeatedly, such as throw down, push down and hook down) and Randori (free-style sparring) (Pulkkinen 2001). Some additional cardiovascular and resistance training were incorporated within these sessions. Training heart rate was measured for 10 of the judokas using a portable monitor (Polar Electro, Kempele, Finland) during a typical week of training in the Ramadan. The mean heart rate of the judokas during training was 131 ± 16 beats \cdot min⁻¹ with a mean peak heart rate of 180 ± 12 beats \cdot min⁻¹. These values are comparable to the mean and peak heart rates reported during specific judo training sessions in previous studies (Houvenaeghela et al. 2005; Yaegaki et al. 2007), which reflect the high exercise intensity of the overall training regimen.

Experimental design

The aim of this study was to determine if the biological markers of inflammatory, and immunological status would be altered during RIF in elite judo athletes who were undertaking their usual high training load. The global training load was estimated using a subjective feeling assessment based on the post-training rating of perceived exertion (RPE) of each session and the length of time spent on training on each session (Chaouachi et al. 2008; Foster et al. 2001). The mean weekly training-load at baseline, during, and after Ramadan were 2095, 2102, and 2119 AU (arbitrary units), respectively (Chaouachi et al. 2008; Chaouachi et al. 2009). The mean weekly training loads in this study are similar to inseason training loads reported for professional Italian soccer players, professional Australian football and rugby league players and professional Tunisian soccer players (Coutts et al. 2008). However, the loads reported are less than overreached rugby league players (Coutts et al. 2007a; Coutts et al. 2007b) and overreached triathletes (Coutts et al. 2007c; Coutts et al. 2007d; Rietjens et al. 2005).

To assess the population validity of the session-RPE method in this study, association between session-RPE and training heart rate (HR) was examined. The session-RPE responses were compared to an objective HR-based method, described by Edwards (1993), assumed as the criterion validity (Impellizzeri et al. 2004). The HR and subjective perception of effort as session-RPE were assessed in 10 players (see above for details). Significant relationships were observed between individual session-RPE and all individual HR-based Training Load (TL) (r values from 0.76 to 0.86; $P < 0.001$). Additionally a significant correlation was observed between collective session-RPE and collective Edwards' training load ($r = 0.88$; $p < 0.002$).

Subjects reported to the laboratory on five separate occasions. The first test session (T1) was performed 4 or 5 days before Ramadan. The second (T2) was performed in the first week (7th

day, D7), the third (T3) was performed in the middle of Ramadan (days 15-16) and the fourth (T4) was performed at the end (days 28-29). The fifth test session (T5) was performed three weeks following Ramadan. At each phase of the study, fasting venous blood samples were collected in the morning, between 8:00 and 10:00 from an antecubital vein. During Ramadan, for the pre-blood sampling days, subjects ate dinner no later than 1:00 to ensure that there was at least 9 hours of fasting before the morning sampling (Chaouachi et al. 2008). A 5 ml blood sample was collected into a plain vacutainer and 2 ml of blood was immediately transferred into tubes containing EDTA anticoagulant. This aliquot of blood was used for determining cell counts, the remainder of the blood in the plain tube was allowed to stand at room temperature to clot. After clotting, serum was prepared by centrifugation at 1500 g for 10 min at 4°C, collected and stored at – 20°C until subsequent analysis.

Subjects were instructed to avoid the consumption of caffeine and intense physical activity in the 24 h prior to sampling. To avoid potential confounding effects of prior exercise on blood circulating biochemical and haematological parameters, coaches were asked to ensure that their judokas were given only light training within the 24-h period before they undertook the laboratory measurements (Leiper et al. 2008). During the study, subjects were asked to abstain from eating and drinking according to the religious recommendations. The hours for the fasting period in this study were from 04:57 to 18:09 h at the beginning of Ramadan and from 05:19 to 17:33 h at the end of Ramadan.

Biological measures

A range of hormones (leptin, thyroid stimulating hormone (TSH) and free thyroxine (FT4), and cortisol), inflammatory markers (pre-albumin, albumin, transferrin, antitrypsin, haptoglobin, homocystine, C-reactive protein (CRP)), immune function markers (immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), total leucocytes, neutrophils, lymphocytes,

and monocytes) and antioxidant markers (vitamin A, vitamin E) were assayed by the staff of an ISO Quality Certified biochemistry laboratory.

Serum leptin concentrations were measured by radioimmunity methods (AxSYM System, Abbott Laboratories, Abbottpark, IL, USA) using the appropriate kit (Linco Research, St. Charles, USA). Thyroid stimulating hormone (TSH) and free thyroxine (FT4) were assessed by the respective immunoenzymatic method with an Axsym analyser (Abbott Laboratories, Abott Park, IL, USA). Serum homocysteine was assessed by a fluorescent polarizing immunoassay method using commercial kits on an Abott-Axsym analyser (Abbott Laboratories, Abbott Park, IL, USA). Serum inflammatory (pre-albumin, transferrin, alpha 1-antitrypsin, haptoglobin, C-reactive protein (CRP); immunoglobulins, urea and creatinine concentrations were determined on a Konelab 20i automated analyzer (Thermo Electron Corp, Oy, Vantaa, Finland) using the respective kits (Konelab, Vataa, Finland). Total haptoglobin, leukocyte, neutrophil and lymphocyte counts were measured using an automated analyser (an automatic cell counter) (MS9-Melet Schloesing Laboratoires, Osny, France) according to the manufacturer instructions. Finally, vitamin A and E were assessed by high-pressure liquid chromatography (HPLC) as described by Driskell et al. (1982).

Total score of fatigue

To assess changes in fatigue, each athlete answered an abbreviated-questionnaire (8-items) pertaining to fatigue (Chatard et al. 2003). This questionnaire has been demonstrated to be as effective as other physiological (heart rate variability (Atlaoui et al. 2007; Chatard et al. 2003), biochemical/immunological and hormonal tests (i.e. 24-h urinary cortisone levels, 24-h urinary cortisol and 24-h urinary cortisol/cortisone ratio and adrenaline/noradrenaline ratio (Atlaoui et al. 2004; Atlaoui et al. 2007)) for assessing fatigue. The total fatigue score (TFS) requires

participants had to rate their perceptions of training, sleep, leg-pain, infection, concentration, efficacy, anxiety, irritability, and general stress using a seven point likert scale ranging from very, very good (1 point) to very, very bad (7 points). The responses to the questions were summed to obtain the total score of fatigue (TSF). A lower TSF represented a good perception of well-being and a higher score represented an increased perception of fatigue. We have previously reported the changes in TSF for this group (Chaouachi et al. 2009).

Statistical analyses

All data are expressed as mean \pm SD. Friedman's two-way ANOVA for repeated measures was used to determine the differences between the phases of the study, with each subject serving as his own control. When a significant χ^2 value was achieved, appropriate Wilcoxon post-hoc test procedures were used to locate the difference between T1 (pre-Ramadan measures) and T2-T5; global α -level control was enforced with the Bonferroni principle. A global 0.05 alpha level was thus distributed evenly among the k individual comparisons to be done, so that each comparison was tested against a 0.05/k probability level. Pearson product moment correlation coefficients with linear regression analysis were calculated to determine whether there was a significant relationship between session-RPE and the HR-based training loads. Statistical analysis was performed using the SPSS/PC Statistical Package for the Social Science (version 13).

Results

Biological measures

The results showed that serum levels of albumin significantly decreased in the middle of Ramadan and that pre-albumin significantly decreased during the post-Ramadan period. The blood level of transferrin did not vary during Ramadan but significantly increased three weeks post-Ramadan (table I).

Blood CRP levels significantly increased in the middle (25.3%) and at the end (57.0%) of Ramadan, and returned to baseline value post-Ramadan. Alpha-1 antitrypsin increased at the start of Ramadan, returned to baseline levels in the middle, then significantly rose again at the end of Ramadan, where it remained for at least three weeks after Ramadan. Haptoglobin also significantly increased at the beginning and the middle of Ramadan but returned to the baseline values by the end of Ramadan (table I). The increase in these markers during the RIF did not reach the upper limit of the normal range (table I). There was no statistical difference in mean levels of homocysteine in comparison with the baseline value.

Serum levels of IgA and IgG increased significantly during Ramadan and had not returned to the baseline levels three weeks after Ramadan. The serum IgG concentrations, which fluctuated during Ramadan, slightly exceeded the normal range for healthy individuals in the post-Ramadan period. The mean serum level of IgM was elevated at the beginning of the fasting period, decreased in the middle and had returned to baseline levels by the end of the RIF. Circulating leucocyte numbers remained essentially unchanged throughout the study period, and all appeared to be within the normal range for healthy individuals.

INSERT TABLE I ABOUT HERE

The serum level of TSH had significantly increased by the end of Ramadan, but returned to baseline levels in the three weeks after Ramadan. The circulating concentration of FT4 increased slightly in the early part of Ramadan, but then returned to baseline levels. Serum levels of cortisol significantly decreased during the RIF and remained below baseline levels for at least

three weeks after Ramadan. The circulating leptin concentration did not appear to vary during the study (table II).

Serum vitamin A levels had increased significantly by the end of Ramadan and they tended to rise further in the immediate post-Ramadan period. Three weeks after Ramadan mean circulating levels of vitamin A were slightly higher than the normal range. Serum vitamin E concentrations were lower at the end of Ramadan than at baseline, but had recovered within three weeks following Ramadan (table II).

INSERT TABLE II ABOUT HERE

Total score of fatigue

The TSF (mean \pm SD) was significantly increased in the middle (T3 - 19 \pm 5) and at the end of Ramadan (T4 - 16 \pm 4) in comparison with the control period (T1- 12 \pm 3) (p = 0.001). The TSF returned to baseline levels by the third week after Ramadan (T5- 12 \pm 2; (Chaouachi et al. 2009)).

Discussion

The main findings from this study were that the combination of the RIF and a high training load in well-trained judo athletes increased fatigue levels and caused small alterations in many biochemical variables. Specifically, the blood levels of prealbumin and vitamin E were relatively stable throughout Ramadan and albumin concentration increase only at the mid-point of Ramadan. CRP, IgA, IgG, and antitrypsin levels were all increased at some phases of Ramadan. The circulating levels of cortisol were lower within the first week of the RIF and remained at these levels throughout the remainder of the study period. Although some of the measured acute phase protein and immunoglobulin levels altered during the RIF, immune cell numbers remained

relatively stable. These results suggest that athletes continuing to complete **high training loads** during Ramadan are likely to experience a myriad of small biochemical adjustments leading to alterations in the hormonal, immune and antioxidant systems. Despite this, there was not clear evidence to suggest a major increase in physiological stress or chronic systemic inflammation.

Judo is a dynamic, physically demanding combat sport that incorporates a variety of throwing and choking manoeuvres, which makes full use of the entire body. The brief and intense efforts sustained during judo fights may lead to tissue trauma and injury (Perrot et al. 2000). A limitation of this study is that we did not directly measure tissue damage in the judokas. However, there are studies that have shown that tissue damage occurs during intensive training similar to that completed in this study (Mochida et al. 2007; Umeda et al. 2008). It is possible that this training-related tissue trauma might be involved in the changes in the small changes observed in the inflammatory and immune measures (Smith 2004).

Previous research has shown that tissue trauma that occurs during intense physical training can lead to systemic inflammation and fatigue (Smith 2000). During the initial stages of the inflammation response, the liver produces large quantities of acute phase proteins (e.g. C-reactive protein, haptoglobin, alpha 1-antitrypsin, pre-albumin, and transferrin) as the primary mechanism for regulating the inflammatory process. Associated with the increase in the positive acute phase proteins, is a concomitant decrease in negative acute phase proteins (e.g. albumin) (Smith 2000). In accordance with this previous research, the present results showed that several of the positive acute phase proteins (i.e. CRP, alpha-1 antitrypsin and haptoglobin) increased during RIF. Additionally, there was a significant concomitant decrease in the albumin levels in the middle of Ramadan. These results contrast with recent research reporting that the RIF has positive effects on the inflammatory status of the body in healthy sedentary volunteers (Aksungar et al. 2007).

The difference between these studies may be the influence of the intense judo training completed in this study. Collectively, the findings of the present study suggest that judo athletes who undertake high training loads during RIF might endure an increased systemic inflammatory response and fatigue levels; however, these changes do not appear to be at levels that have been implicated in situations of severe tissue trauma such as overtraining (Smith 2000).

On the basis of the measures from this study, however, it is not possible to clearly differentiate between the effects of the physical exercise and that from the restricted daytime water and food intake. Indeed, the changes in plasma volume in this group of judokas during Ramadan did not follow the same variations as the acute phase proteins and immunoglobulins (Chaouachi et al. 2008). Therefore, it is unlikely that the changes in plasma volume are responsible for the observations in the present study. Moreover, because the subjects were fasted for at least 9 h prior to being bled on each occasion throughout the study, they would have been acutely hypohydrated at the time of sampling. However, the anthropometric measurements taken in this study also indicate that the judokas were hypohydrated by about 200 ml and 570 ml at the mid and end of Ramadan, respectively (Chaouachi et al. 2008). These small losses in body water are also unlikely to noticeably affect the circulating concentrations of the measured proteins.

Chronic systemic inflammation has also been implicated in compromising the immune system (Smith 2004). Several authors have reported that the immune system of athletes can be compromised during periods of **intensified training** (Foster 1998; Halson et al. 2003) or when there is insufficient food and fluid intake (Venkatraman and Pendergast 2002). Several of the immune markers taken in this study followed a pattern similar to that of the CRP and other positive acute phase proteins. Both the IgA, and IgG levels were elevated during the Ramadan period with a higher value after the end of fasting compared with the beginning of the RIF.

However, these changes were **relatively small and** not mirrored by the IgM **concentrations**. The fact that the immunoglobulin isotypes in this study did not respond in a similar manner could be explained by a possible switching of immunoglobulin isotypes from IgM to IgG, which has previously been reported following ultra-endurance marathon (Coffman et al. 1993; McKune et al. 2005), with IgM changes occurring before that of IgG. These immune changes may be related to an inflammatory response that occurs with tissue trauma associated with the intense physical training and RIF. However, given the relatively small and inconsistent variations in the acute phase reactants in the judokas in the present study, it is unlikely that there was marked increase in tissue trauma associated with the RIF. It is nevertheless possible that the transient changes in the markers of inflammatory response seen in the present study may be the result of tissue trauma produced several days before the relevant blood sample was collected.

It is well known that the total leucocyte count increases in the few hours after maximal exercise (Nieman 1997). Specifically, neutrophils are active within the first 24 h after intense exercise and thereafter monocytes move-in to provide macrophage activity. However, in agreement with other research that showed no changes in leucocytes in college judokas during weight loss induced through energy restriction (Kowatari et al. 2001), the elite judo athletes in the present study also demonstrated no changes in the numbers of either the total of different types of leucocytes during RIF. In the present study, the leucocytes were not measured immediately after training, which may explain the absence of significant changes found in these variables. The present results are similar to other studies on overreaching in rugby league players that have shown that **numbers or percentages of** leucocytes are not significantly changed following intense training periods (Coutts et al. 2007b). Nonetheless, the present results suggest that the combination of a high training load with the RIF does not significantly affect blood leucocytes counts 24 h after exercise in elite judo athletes.

It has been suggested that the effect of intensified training periods on the inflammatory and the immune function could be mediated by cortisol (DeRijk et al. 1997). In the present study the blood cortisol levels decreased rather than increased when tested during Ramadan. The main reason for this is likely to be the timing of the blood sampling, as the normal phase shift in cortisol secretion during Ramadan results in lower circulating levels in the morning than outwith the Ramadan period (al-Hadramy et al. 1988; Reilly and Waterhouse 2007). Other studies have reported that cortisol increased at 15:00 h and at night time compared with that collected at the same times while not following Ramadan (al-Hadramy et al. 1988; Fedail et al. 1982). Therefore it is possible that the cortisol levels of the judokas in this study may have increased later in the day. However, since all blood samples were collected at the same time of day after at least 9 h of fasting and several hours of sleep, comparisons at each stage of this study are possibly more pertinent. Indeed, the protocol used in the present study probably gives a better comparison between parameters as the measures were made at the same time of day following similar lifestyle and behavioural patterns (i.e. exercise, eating times and sleep). Despite this, the present study cannot clarify whether cortisol levels in the previous 24 h influenced the small changes that were observed in some of the immune responses. Future studies should correct biological variable for circadian rhythms to allow for appropriate examination of the relationships between cortisol, inflammatory and immune responses during RIF.

In accordance with previous research on healthy adults observing Ramadan, we discovered elevated TSH (Sajid et al. 1991) and FT4 (Fedail et al. 1982) during the RIF. However, both of these changes appeared to be transient and remained within the normal healthy range. It appears that these hormonal changes may be related to the metabolic adjustments made with the alteration of food and fluid intake along with the continued high training loads during the RIF.

Reduced vitamin E at the end of the RIF suggests that there may have been a training-induced down-regulation of the antioxidant system (Finaud et al. 2006b); however, dietary changes cannot be discounted. This has also been reported in team sport athletes and triathletes following periods of intensified training or competition schedules. A deficiency in vitamin E has previously been associated with increased oxidative stress, fatigue and reduced endurance performance (Coombes et al. 2002; Finaud et al. 2006a; Finaud et al. 2006b) and also with periods of intensified training and competition in elite rugby players (Finaud et al. 2006b). In contrast, the elevated Vitamin A during the RIF suggests an up-regulation in retinol for the judo athletes in this study. There is not a clear explanation for the increases in plasma vitamin A during the RIF in the present study; however, dietary changes cannot be discounted. Previous studies have shown that increased dietary vitamin A over a 35-day period increased plasma vitamin A levels in elite basketball players (Schroder et al. 2001).

The health and wellbeing of athletes is important in optimizing training and performance. Therefore, the stability of the immune system and inflammatory process is therefore crucial for elite athletes especially during periods of increased physical or psychological stress (Smith 2000). The differences between biochemical and immunological parameters in blood samples observed before and during Ramadan fasting must be carefully interpreted. Indeed, it is well established that small variations in circulating haematological and biochemical levels in athletes, that may not be considered clinically relevant and fall into normal values described for the general healthy population (Banfi et al. 2006), may still signal physiological stress. The changes in biochemical parameters in the present study show a trend towards an elevated inflammatory response but a stable immune system response. However, the present results do not provide clear

evidence to confirm that the judokas were showing signs of overreaching/overtraining or suffering chronic systemic inflammation.

In summary, the present results indicate that experienced Muslim athletes who continue to undertake high training loads during Ramadan are likely to experience slightly higher levels of fatigue and a cascade of small biochemical adjustments including hormonal, immunoglobulin and antioxidant system changes, and an elevated inflammatory response. It appears that that Muslim athletes who are experienced in training throughout Ramadan can adopt coping strategies that allow them to prepare for competition during a month of intermittent fasting (Zerguini et al. 2008). Coaches, sport scientists, and nutritionists of athletes who undergo Ramadan fasting should, however, be aware of the potential impact of the RIF on fatigue, the immune system and athletic performance, and ought to carefully periodize the training and monitor the dietary intake and fatigue levels of their athletes.

References

- Aksungar, F.B., Topkaya, A.E. and Akyildiz, M. 2007. Interleukin-6, C-reactive protein and biochemical parameters during prolonged intermittent fasting. *Ann. Nutr. Metab.* **(51)**: 88-95.
- al-Hadramy, M.S., Zawawi, T.H. and Abdelwahab, S.M. 1988. Altered cortisol levels in relation to Ramadan. *Eur. J. Clin. Nutr.* **(42)**: 359-62.
- Albert, M.A., Glynn, R.J. and Ridker, P.M. 2004. Effect of physical activity on serum C-reactive protein. *Am. J. Cardiol.* **(93)**: 221-5.
- Atlaoui, D., Duclos, M., Gouarne, C., Lacoste, L., Barale, F. and Chatard, J.C. 2004. The 24-h urinary cortisol/cortisone ratio for monitoring training in elite swimmers. *Med. Sci. Sports Exerc.* **(36)**: 218-24.
- Atlaoui, D., Pichot, V., Lacoste, L., Barale, F., Lacour, J.R. and Chatard, J.C. 2007. Heart rate variability, training variation and performance in elite swimmers. *Int. J. Sports Med.* **(28)**: 394-400.
- Banfi, G., Del Fabbro, M., Mauri, C., Corsi, M.M. and Melegati, G. 2006. Haematological parameters in elite rugby players during a competitive season. *Clin. Lab. Haematol.* **(28)**: 183-8.
- Bouhlef, E., Salhi, Z., Bouhlef, H., Mdella, S., Amamou, A., Zaouali, M., Mercier, J., Bigard, X., Tabka, Z., Zbidi, A. and Shephard, R.J. 2006. Effect of Ramadan fasting on fuel oxidation during exercise in trained male rugby players. *Diabetes Metab.* **(32)**: 617-24.

- Bouhleb, E., Zaouali, M., Miled, A., Tabka, Z., Bigard, X. and Shephard, R. 2008. Ramadan fasting and the GH/IGF-1 axis of trained men during submaximal exercise. *Ann. Nutr. Metab.* (52): 261-6.
- Chaouachi, A., Chamari, K., Roky, R., Wong, P., Mbazza, A., Bartagi, Z. and Amri, M. 2008. Lipid profiles of Judo athletes during Ramadan. *Int. J. Sports Med.* (29): 282-8.
- Chaouachi, A., Coutts, A.J., Chamari, K., Wong, D.P., Roky, R., Chaouachi, M., Chtara, M., and Amri, M. 2009. Effect of Ramadan on athletic performance in judo-athletes. *J Strength Cond. Res.* in press.
- Chatard, J.C., Atlaoui, A., Pichot, V., Gourné, C., Duclos, M. and Guézennec, Y.C. 2003. Training follow up by questionnaire fatigue, hormones and heart rate variability measurements. *Science & Sports* (18): 302-304.
- Coffman, R.L., Leberman, D.A. and Rothman, P. 1993. Mechanism and regulation of immunoglobulin isotype switching. *Adv. Immunol.* (54): 229-70.
- Coombes, J.S., Rowell, B., Dodd, S.L., Demirel, H.A., Naito, H., Shanely, R.A. and Powers, S.K. 2002. Effects of vitamin E deficiency on fatigue and muscle contractile properties. *Eur. J Appl. Physiol.* (87): 272-7.
- Coutts, A.J., Chamari, K., Rampinini, E. and Impellizzeri, F.M. 2008. Contrôle et suivi de l'entraînement : périodisation et charges d'entraînement. *In De L'entraînement à la Performance en Football Edited by* (ed). pp. 242-263.
- Coutts, A.J., Reaburn, P., Piva, T.J. and Murphy, A. 2007a. Changes in selected biochemical, muscular strength, power, and endurance measures during deliberate overreaching and tapering in rugby league players. *Int. J. Sports Med.* (28): 116-124.
- Coutts, A.J., Reaburn, P., Piva, T.J. and Rowsell, G.J. 2007b. Monitoring for overreaching in rugby league players. *Eur. J. Appl. Physiol.* (99): 313-324.
- Coutts, A.J., Slattery, K.M. and Wallace, L.K. 2007c. Practical tests for monitoring fatigue and recovery in triathletes. *J. Sci. Med. Sport* (10): 372-381.
- Coutts, A.J., Wallace, L.K. and Slattery, K.M. 2007d. Monitoring changes in performance, physiology, biochemistry, and psychology during overreaching and recovery in triathletes. *Int. J. Sports Med.* (28): 125-134.
- DeRijk, R., Michelson, D., Karp, B., Petrides, J., Galliven, E., Deuster, P., Paciotti, G., Gold, P.W. and Sternberg, E.M. 1997. Exercise and circadian rhythm-induced variations in plasma cortisol differentially regulate interleukin-1 beta (IL-1 beta), IL-6, and tumor necrosis factor-alpha (TNF alpha) production in humans: high sensitivity of TNF alpha and resistance of IL-6. *J. Clin. Endocrinol. Metab.* (82): 2182-91.
- Driskell, W.J., Neese, J.W., Bryant, C.C. and Bashor, M.M. 1982. Measurement of vitamin A and vitamin E in human serum by high-performance liquid chromatography. *J. Chromatogr.* (231): 439-44.
- Edwards, S. 1993. High performance training and racing. *In The Heart Rate Monitor Book Edited by* (ed). Feet Fleet Press, Sacramento, CA, pp. 113-123.
- Fedail, S.S., Murphy, D., Salih, S.Y., Bolton, C.H. and Harvey, R.F. 1982. Changes in certain blood constituents during Ramadan. *Am. J. Clin. Nutr.* (36): 350-3.
- Finaud, J., Degoutte, F., Scislowski, V., Rouveix, M., Durand, D. and Filaire, E. 2006a. Competition and food restriction effects on oxidative stress in judo. *Int. J. Sports Med.* (27): 834-841.
- Finaud, J., Scislowski, V., Lac, G., Durand, D., Vidalin, H., Robert, A. and Filaire, E. 2006b. Antioxidant status and oxidative stress in professional rugby players: evolution throughout a season. *Int. J. Sports Med.* (27): 87-93.
- Ford, E.S. 2002. Does exercise reduce inflammation? Physical activity and C-reactive protein among U.S. adults. *Epidemiology* (13): 561-8.

- Foster, C. 1998. Monitoring training in athletes with reference to overtraining syndrome. *Med. Sci. Sports Exerc.* **(30)**: 1164-8.
- Foster, C., Florhaug, J.A., Franklin, J., Gottschall, L., Hrovatin, L.A., Parker, S., Doleshal, P. and Dodge, C. 2001. A new approach to monitoring exercise training. *J Strength Cond. Res.* **(15)**: 109-115.
- Halson, S.L., Lancaster, G.I., Achten, J., Gleeson, M. and Jeukendrup, A.E. 2004. Effect of carbohydrate supplementation on performance and carbohydrate oxidation following intensified cycling training. *Journal of Applied Physiology* **(97)**: 1245–1253.
- Halson, S.L., Lancaster, G.I., Jeukendrup, A.E. and Gleeson, M. 2003. Immunological responses to overreaching in cyclists. *Med. Sci. Sports Exerc.* **(35)**: 854-61.
- Houvenaeghela, M., Bizzaric, C., Giallurachis, D. and Demelasb, J.M. 2005. Continuous recording of heart rate during specific exercises of judo *Science & Sports* **(20)**: 27-32.
- Impellizzeri, F.M., Rampinini, E., Coutts, A.J., Sassi, A. and Marcora, S.M. 2004. Use of RPE-based training load in soccer. *Med. Sci. Sports Exerc.* **(36)**: 1042-7.
- Kasapis, C. and Thompson, P.D. 2005. The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review. *J. Am. Coll. Cardiol.* **(45)**: 1563-9.
- Kelley, D.S. 2001. Modulation of human immune and inflammatory responses by dietary fatty acids. *Nutrition* **(17)**: 669-73.
- Kowatari, K., Umeda, T., Shimoyama, T., Nakaji, S., Yamamoto, Y. and Sugawara, K. 2001. Exercise training and energy restriction decrease neutrophil phagocytic activity in judoists. *Med. Sci. Sports Exerc.* **(33)**: 519-24.
- Leiper, J.B., Maughan, R.J., Kirkendall, D.T., Bartagi, Z., Zerguini, Y., Junge, A. and Dvorak, J. 2008. The F-MARC study on Ramadan and football: research design, population, and environmental conditions. *J. Sports Sci.* **(26 Suppl 3)**: S7-13.
- Mackinnon, L.T. 2000. Chronic exercise training effects on immune function. *Med. Sci. Sports Exerc.* **(32)**: S369-76.
- Maughan, R.J., Leiper, J.B., Bartagi, Z., Zrifi, R., Zerguini, Y. and Dvorak, J. 2008. Effect of Ramadan fasting on some biochemical and haematological parameters in Tunisian youth soccer players undertaking their usual training and competition schedule *J. Sports Sci.* **(26)**: 39-46.
- McKune, A.J., Smith, L.L., Semple, S.J. and Wadee, A.A. 2005. Influence of ultra-endurance exercise on immunoglobulin isotypes and subclasses. *Br. J. Sports Med.* **(39)**: 665-70.
- Meksawan, K., Venkatraman, J.T., Awad, A.B. and Pendergast, D.R. 2004. Effect of dietary fat intake and exercise on inflammatory mediators of the immune system in sedentary men and women. *J. Am. Coll. Nutr.* **(23)**: 331-40.
- Mochida, N., Umeda, T., Yamamoto, Y., Tanabe, M., Kojima, A., Sugawara, K. and Nakaji, S. 2007. The main neutrophil and neutrophil-related functions may compensate for each other following exercise—a finding from training in university judoists. *Luminescence* **(22)**: 20-28.
- Nieman, D.C. 1997. Immune response to heavy exertion. *J. Appl. Physiol.* **(82)**: 1385-94.
- Perrot, C., Mur, J.M., Mainard, D., Barrault, D. and Perrin, P.P. 2000. Influence of trauma induced by judo practice on postural control. *Scand. J. Med. Sci. Sports* **(10)**: 292-7.
- Pulkkinen, W.J. 2001. The sport science of elite judo athletes: a review and application for training. Pulkinetics Inc., Guelph, Ontario, Canada.
- Rauramaa, R., Halonen, P., Vaisanen, S.B., Lakka, T.A., Schmidt-Trucksass, A., Berg, A., Penttila, I.M., Rankinen, T. and Bouchard, C. 2004. Effects of aerobic physical exercise on inflammation and atherosclerosis in men: the DNASCO Study: a six-year randomized, controlled trial. *Ann. Intern. Med.* **(140)**: 1007-14.

- Reilly, T. and Waterhouse, J. 2007. Altered sleep-wake cycles and food intake: the Ramadan model. *Physiol. Behav.* **(90)**: 219-28.
- Rietjens, G.J., Kuipers, H., Adam, J.J., Saris, W.H., van Breda, E., van Hamont, D. and Keizer, H.A. 2005. Physiological, biochemical and psychological markers of strenuous training-induced fatigue. *Int. J. Sports Med.* **(26)**: 16-26.
- Sajid, K.M., Akhtar, M. and Malik, G.Q. 1991. Ramadan fasting and thyroid hormone profile. *Journal of the Pakistan Medical Association* **(41)**: 213-6.
- Scharhag, J., Meyer, T., Auracher, M., Gabriel, H.H. and Kindermann, W. 2006. Effects of graded carbohydrate supplementation on the immune response in cycling. *Med. Sci. Sports Exerc.* **(38)**: 286-92.
- Schroder, H., Navarro, E., Mora, J., Galiano, D. and Tramullas, A. 2001. Effects of alpha-tocopherol, beta-carotene and ascorbic acid on oxidative, hormonal and enzymatic exercise stress markers in habitual training activity of professional basketball players. *Eur. J. Nutr.* **(40)**: 178-84.
- Smith, L.L. 2000. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? *Med. Sci. Sports Exerc.* **(32)**: 317-31.
- Smith, L.L. 2004. Tissue trauma: the underlying cause of overtraining syndrome? *J Strength Cond. Res.* **(18)**: 185-93.
- Umeda, T., Saito, K., Matsuzaka, M., Nakaji, S., Totsuka, M., Okumura, T., Tsukamoto, T., Yaegaki, M., Kudoh, U. and Takahashi, I. 2008. Effects of a bout of traditional and original sumo training on neutrophil immune function in amateur university sumo wrestlers. *Luminescence* **(23)**: 115-20.
- Venkatraman, J.T., Leddy, J. and Pendergast, D. 2000. Dietary fats and immune status in athletes: clinical implications. *Med. Sci. Sports Exerc.* **(32)**: S389-95.
- Venkatraman, J.T. and Pendergast, D.R. 2002. Effect of dietary intake on immune function in athletes. *Sports Med.* **(32)**: 323-37.
- Waterhouse, J., Alkib, L. and Reilly, T. 2008. Effects of Ramadan upon fluid and food intake, fatigue, and physical, mental, and social activities: a comparison between the UK and Libya. *Chronobiol. Int.* **(25)**: 697-724.
- Yaegaki, M., Umeda, T., Takahashi, I., Matsuzaka, M., Sugawara, N., Shimaya, S., Tanabe, M., Kojima, A., Mochida, N., Yamamoto, Y. and Nakaji, S. 2007. Change in the capability of reactive oxygen species production by neutrophils following weight reduction in female judoists. *Br. J. Sports Med.* **(41)**: 322-7.
- Zerguini, Y., Dvorak, J., Maughan, R.J., Leiper, J.B., Bartagi, Z., Kirkendall, D.T., Al-Riyami, M. and Junge, A. 2008. Influence of Ramadan fasting on physiological and performance variables in football players: Summary of the F-MARC 2006 Ramadan fasting study *J. Sports Sci.* **(26)**: 3-6.

Table I. Blood levels (mean \pm SD) of inflammatory and immunological parameters before, during and after Ramadan.

Parameters	Units	Pre Ramadan T1	Start Ramadan T2	Mid Ramadan T3	End Ramadan T4	Following Ramadan T5	Normal Range
Acute Phase Proteins							
Pre-Albumin	g \cdot l $^{-1}$	0.27 \pm 0.05	0.27 \pm 0.07	0.26 \pm 0.05	0.29 \pm 0.05	0.20 \pm 0.08 *	0.2-0.4
Albumin	g \cdot l $^{-1}$	46.2 \pm 1.5	47.0 \pm 1.8	45.0 \pm 1.5 *	47.6 \pm 3.7	49.2 \pm 4.6	35-52
Transferrin	g \cdot l $^{-1}$	2.69 \pm 0.27	2.77 \pm 0.38	2.58 \pm 0.28	2.88 \pm 0.42	3.44 \pm 0.49 *	2.0-3.6
Alpha-1 Antitrypsin	g \cdot l $^{-1}$	1.36 \pm 0.10	1.55 \pm 0.21 †	1.38 \pm 0.14	1.49 \pm 0.20 *	1.69 \pm 0.19 †	0.9-2.0
Haptoglobin	g \cdot l $^{-1}$	0.77 \pm 0.48	1.09 \pm 0.6 *	1.15 \pm 0.38 *	1.01 \pm 0.46	0.88 \pm 0.44	0.3-2.0
Homocysteine	μ mol \cdot l $^{-1}$	10.72 \pm 1.78	11.77 \pm 2.49	11.56 \pm 2.03	11.8 \pm 3.09	11.37 \pm 1.46	8-12
C-reactive protein	mg \cdot l $^{-1}$	2.93 \pm 0.26	3.40 \pm 0.83	3.67 \pm 0.49 *	4.60 \pm 0.51 †	2.53 \pm 1.06	0-10
Immune Measures							
IgA	g \cdot l $^{-1}$	1.87 \pm 0.56	1.98 \pm 0.54 †	2.02 \pm 0.58 †	2.40 \pm 0.7 †	2.49 \pm 0.75 †	0.7-4
IgG	g \cdot l $^{-1}$	13.54 \pm 2.75	14.48 \pm 2.63 *	13.82 \pm 2.63	15.53 \pm 3.18 *	17.42 \pm 3.84 *	7-16
IgM	g \cdot l $^{-1}$	1.19 \pm 0.39	1.35 \pm 0.42 *	0.95 \pm 0.42 *	1.27 \pm 0.45	1.65 \pm 0.65	0.4-2.3
Total leucocytes	10 9 \cdot l $^{-1}$	6.29 \pm 1.70	6.51 \pm 1.81	6.06 \pm 1.23	6.87 \pm 1.85	6.32 \pm 1.46	4-10
Neutrophils	10 9 \cdot l $^{-1}$	3.46 \pm 1.46	3.86 \pm 1.65	3.19 \pm 0.94	3.87 \pm 1.47	3.48 \pm 1.17	2-6
Lymphocytes	10 9 \cdot l $^{-1}$	2.53 \pm 0.53	2.39 \pm 0.40	2.55 \pm 0.35	2.66 \pm 0.45	2.53 \pm 0.52	1.5-7.0
Monocytes	10 9 \cdot l $^{-1}$	0.20 \pm 0.09	0.16 \pm 0.05	0.19 \pm 0.05	0.21 \pm 0.11	0.19 \pm 0.09	0.1-1.5

Significantly different from the pre-Ramadan phase (p<0.05)*, (p<0.01) †

Table II. Mean (\pm SD) blood levels of hormonal and antioxidant markers.

Parameters	Units	Pre	Start	Mid	End	Following	Normal
		Ramadan T1	Ramadan T2	Ramadan T3	Ramadan T4	Ramadan T5	Range
Hormones							
Leptin	ng·ml ⁻¹	1.61 \pm 0.46	1.87 \pm 0.53	1.84 \pm 0.83	1.69 \pm 0.53	1.45 \pm 0.32	1.4-9.8
TSH	mUI ⁻¹	1.64 \pm 0.57	1.67 \pm 0.5	1.34 \pm 0.69	2.12 \pm 0.89 *	1.65 \pm 0.46	0.12-3.4
FT4	ng·l ⁻¹	0.98 \pm 0.13	1.05 \pm 0.16 *	1.06 \pm 0.17	1.00 \pm 0.16	0.97 \pm 0.13	7.0-18.5
Cortisol	mg·dl ⁻¹	13.4 \pm 2.2	9.0 \pm 1.9 *	9.3 \pm 2.3 *	10.3 \pm 2.1 *	10.6 \pm 2.6 *	5-30
Vitamins							
Vitamin A	mg·l ⁻¹	0.44 \pm 0.07	0.46 \pm 0.09	0.49 \pm 0.09	0.62 \pm 0.18 †	0.76 \pm 0.27 †	0.35-0.70
Vitamin E	mg·l ⁻¹	10.1 \pm 1.6	11.0 \pm 2.9	9.1 \pm 1.3	7.6 \pm 2.0 †	9.8 \pm 2.2	5-15

Significantly different from the pre-Ramadan phase (p<0.05)*, (p<0.01)†