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Ramadan Fasting: Physical and Performance Maintained, Health Challenged in Elite Adolescent Football

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A B S T R A C T

Objective: Adolescent Muslim athletes participating in elite sports during Ramadan Fasting (RF) face unique physiological and developmental challenges. This prospective cohort study aimed to observe the changes in body composition, hydration status, physiological performance, and hematological profiles in 20 elite Malaysian adolescent footballers (17.8 ± 0.8 years).

Methods: Measurements were taken at four phases (two weeks before Ramadan, BRF-2; mid, 2nd weeks of Ramadan, RF-2; late, 4th weeks of Ramadan, RF-4; and two weeks after Ramadan, ARF-6) using dual-time-point (morning/evening) blood and urine sampling, bioelectrical impedance, skinfolds, and the Yo-Yo Intermittent Run (YYIR) test.

Results: Results showed that energy balance, body composition, and aerobic performance (YYIR distance and HRmax) were successfully maintained throughout RF ($p>0.05$). However, significant dynamic fluid shifts were observed: morning measurements showed hemodilution, while late-afternoon Urine Specific Gravity was significantly higher in RF-4, indicating daily hemoconcentration and dehydration stress. Furthermore, while red blood cells (RBC) and haemoglobin (HB) showed transient morning reductions during RF, the most critical finding was the delayed post-fasting reduction in Mean Cell Volume (MCV) and persistent low Hematocrit (HCT) at ARF-6.

Conclusions: These findings suggest that elite performance is preserved through strong physiological adaptation and effective energy intake, but the RF period induced a subclinical iron deficiency stress that manifested as microcytosis post-Ramadan. Coaches and medical staff must implement rigorous post-Ramadan nutritional and detailed hematological screening to safeguard the long-term health of adolescent footballers.

Keywords: *Ramadan Fasting, Young Athletes, Elite Adolescents, Performance*

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1. Introduction

Adolescent Muslim athletes participating in sport during the holy month of Ramadan are of particular concern because of the potential impacts on their health and development. During Ramadan, Muslims are prohibited from eating, drinking from dawn to sunset, smoking, and engaging in sexual relations (1-3). Adolescent athletes who observe Ramadan may face challenges related to changes in circadian rhythms, hormonal fluctuations, dehydration, and nutritional intake (1, 4, 5). It has been reported that adolescents follow eating patterns that do not meet daily dietary recommendations and may be a trigger for eating disorders during Ramadan (1, 6).

In recent years, there has also been increased interest in studying Ramadan fasting (RF) and its effects on various aspects of health, including nutritional, biochemical, haematological, and performance parameters as a functional index during Ramadan (7-12). Several studies in athletes suggest that submaximal aerobic exercise, sprinting, agility, aerobic power, and muscle strength remain unchanged during Ramadan (2, 8, 13-18). These findings indicate that there may not be a decline in performance despite fasting. However, some studies suggest that aerobic capacity, anaerobic performance, mean power, peak power, speed, and agility of professional football players decrease during Ramadan in certain tests (14, 19-22). It remains unclear whether intermittent fasting has any significant detrimental effect on athletic performance in adolescents' populations.

Overall, RF affects dietary practices and daily habits, leading to inconsistent findings in studies regarding the impact of Ramadan on various aspects such as body weight, body fat mass, and physiological response (1, 12, 23-26). In addition, festive meals consumed during Ramadan have been associated with increased energy intake in certain regions (3, 4, 27). While young people tend to lose weight during Ramadan, they often regain it within a month after the period ends (1, 4, 12, 28). Adults may also experience similar changes, which may vary depending on individual metabolic rates and lifestyle factors (3, 11, 24, 29). Fasting causes changes in hormonal circadian rhythms, which may have different effects on adults and adolescents depending on their stage of hormonal development (4, 28-30).

Our main goal was to see how RF affects the body, hydration, and performance of young elite footballers who have to fast while training for national duty and competition. The results may also show how athletes adapt to fasting and provide insights into the impact of RF on various physiological factors from a young age and the perspective of the elite sports.

Crucially, most current literature relies on singular, daytime measurements, which fail to capture the dynamic fluid and metabolic shifts that occur in a 24-hour cycle of fasting and rehydration. Furthermore, the true physiological stress, particularly related to haematological recovery, may only become evident several weeks after the fasting period concludes. For this reason, it is particularly important to understand the physical and physiological changes in elite adolescent athletes who participate in sports during Ramadan, considering the temporal specificity of the physiological stress and the high demands placed on the erythropoietic system (2, 16, 31-33). This approach is vital so that appropriate evidence-based management and monitoring decisions can be formulated to ensure the well-being and competitive performance of these young athletes during and immediately following this period.

2. Methods and Materials

2.1 Participants

A total of 29 healthy male adolescent soccer players (mean age of 17.8 ± 0.6 years old) were recruited for this study. At the end of the study, only 20 players completed the study; nine players could not join the second phase of the study due to a scheduled game on the test day. The study protocol was approved by the Institutional Research Committee of the National Sports Institute of Malaysia and the Research Ethics Committee (Human) of Universiti Sains Malaysia under the protocol code ISNRP-016-2010. All procedures were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. After receiving a full description of the protocol, each player provided written informed consent. The players were in good mental and physical condition and were not taking any medication. Ramadan began on the 1st of August till the 30th of August 2011. The fasting period during the study was approximately 14 hours (between 05:30 h and 19:30 h). Participants were given strong verbal encouragement throughout the test.

2.2 Experimental Design

All the measurements were made on four different occasions: two weeks before Ramadan (BRF-2), the second

(RF-2) and fourth (RF-4) week of Ramadan, and two weeks after the end of Ramadan (ARF-6). All morning measurements (Food intake, anthropometric measurements, body composition, and venous blood) were investigated approximately 2 hours after awaking (in non-RF month) or 2 hours after Sahour (in RF month). Players reported to the nutrition and exercise physiology laboratories for the morning session. The evening measurement session started with the venous blood draw, followed by hydration, body composition, and physical performance tests. The physical performance tests - Yo-Yo Intermittent Recovery Test Level 1 (YYIR) were performed between 17:00 h and 18:00 h in the Biomechanics Hall at the National Sports Institute. All assessments were performed under identical laboratory conditions and by the same examiners on each occasion. During RF month, the players' calorie intake during Sahour was individualized by dividing their calorie intake from the 24-hour diet recall in the BRF-2 into three (representing three main meals: Sahour, Breaking-fast, Moreh). The menu was adjusted based on the calculated caloric needs.

2.3 Body Composition

Body weight was measured to the nearest 0.1 kg using a digital scale, and stature was measured to the nearest 0.1 cm using a stadiometer (Seca 767, Hamburg, Germany) with subjects in light clothing and without shoes. Body Mass Index (BMI) was calculated by dividing body weight in kilograms by the square of stature in meters. Body fat percentage (BF), fat-free mass (FFM), and total body water (TBW) content were assessed using a hand-to-foot bioelectrical impedance analyzer (Inbody 230, Tokyo, Japan). Bioelectrical impedance was performed following the manufacturer's instructions, and was used during both morning and evening sessions. Body fatness was also estimated from skinfold thickness. The thickness of seven skinfolds (biceps, subscapular, triceps, supraspinal, abdominal, front thigh, and medial calf) was measured with a Harpenden Skinfold Calliper (Holtain Ltd, UK) to the nearest 0.2 mm on the right side of the body. Skinfold thickness was measured in duplicate or triplicate, and the mean of the two closest values was used for analysis. Girths of five sites (arm relaxed, arm flexed, waist, hip, and calf), and two breadths (humerus and femur) were measured using Executive Thinline Lufkin Tape and small sliding calliper (Rosscaft, Canada). The anthropometric measurements were performed by ISAK-trained anthropometrics, and this method was used only on the morning of the test day. In non-RF month, athletes were asked to fast at night starting at midnight until the morning assessment.

2.4 Hydration status

The hydration status was assessed via urine specific gravity (USG) measurements using a refractometer (Atago, USA), which was collected twice: in the morning (at waking) and evening (before training) of the testing day.

2.5 Dietary Intake and Energy Expenditure

Each player was requested to keep three-day records of food intake and activity patterns, including a weekend day. The data were analyzed by Food Processor II software (Nutrition System, ESHA Research). Daily calorie intake was extracted. Energy Expenditure (EE) was calculated as the total 24-hour EE by summing the EE of individual activities over the 24 hours, estimated using the metabolic equivalent (MET) values derived from the Compendium of Physical Activities formula: EE (kcal/min) = METs x 3.5 x Body Weight (kg) / 200.

2.6 Hematological Measure

Blood sample (morning) was taken at 07:00 h. A blood sample (evening) was taken at 16:00 h (1 hour before the YYIR test). Players provided venous blood samples (~5 ml) from an antecubital vein into a plain vacutainer tube in a seated position. Samples were poured into tubes with EDTA (for hematology) and into tubes with lithium heparin (for biochemistry/plasma analysis). Heparinized blood samples were centrifuged within the first 5 min after blood withdrawal, and plasma was harvested. Hematological levels, including Red Blood Cells (RBC), Hemoglobin (HB), Hematocrit (HCT), Mean Red Cell Volume (MCV), White Blood Cells (WBC), Platelets (PLT), and Glucose (GLU) were analyzed.

2.7 Physical Performance Assessment

Physical performance was assessed by the (YYIR). Before starting the tests, the participants warmed up for 10 minutes. This test was performed according to the guidelines established by Bangsbo (1996). The test consists of repeated 2 x 20-meter runs at progressively faster speeds dictated by audio cues on a compact disc. Each run is separated by a 10-second active rest period (jogging to the 5m marker and back). The YYIR is terminated when the player fails to reach the front line in time twice or is unable to cover another shuttle at the dictated speed.

2.8 Physiological Measure

Heart rate (HR) was recorded at 5-second intervals using HR monitors (Polar Team2, Finland) during the YYIR test. Peak HR was taken as the highest HR achieved immediately after the players stopped. Blood lactate by finger prick was taken before the test, immediately after the test, and 2 minutes after the test.

All the subjects were familiar with the testing methods used in the study because they were routinely performed as standard scientific follow-up testing during the season. To avoid the influence of changes in circadian rhythms, all performance tests were conducted at the same time of day (between 17:00 h and 18:00 h) and in the same order under standard environmental conditions.

2.9 Statistical Analyses

Continuous data were summarized by descriptive statistics (mean and standard deviation). Changes over time were analyzed by repeated-measures analysis of variance (ANOVA) with week as the repeated factor using statistical package software Minitab 21. p-values less than 0.05 were considered significant. Tukey's post-hoc method was used conjunction with repeated-measures ANOVA to identify means that are significantly different from each other.

Gemini's large language model (LLM) tool was used to enhance the grammar, clarity and structure of the written content of the results and discussion. This was done to improve the understanding of the readers. Gemini was also used to generate figures and charts.

3. Results

The study investigated the effect of RF on body composition, hydration status, physiological and haematological responses in elite adolescent footballers, comparing measurements taken two weeks before Ramadan (BRF-2), during the 2nd (RF-2) and 4th (RF-4) weeks of Ramadan, and two weeks after Ramadan (ARF-6).

3.1 Changes in Body Composition

Body composition showed no statistically significant changes throughout the study period, regardless of the measurement method used. Skinfold Method were no significant differences ($p>0.05$) observed in body weight, BMI, Sum of 7 Skinfolds Thickness (Table 1 & 2), or any individual skinfold site/girth measurement (e.g., Triceps, Biceps, Abdominal, Thigh, Calf, Waist, Hip) across the four time points (BRF-2, RF-2, RF-4, ARF-6).

Table 1. Changes in energy intake, energy expenditure and body composition (skinfold method) of elite adolescent footballers during the month of Ramadan

Parameters	Mean ± SD				F	p-value
	BRF-2	RF-2	RF-4	ARF-6		
Body Weight (kg)	67.09 ± 6.96	67.27 ± 6.60	66.27 ± 6.56	67.00 ± 6.71	0.086	0.967
BMI (kg/m2)	22.74 ± 1.90	22.69 ± 1.67	22.41 ± 1.72	22.55 ± 1.66	0.150	0.929
Sum 7 Skinfolds Thickness (mm)	50.14 ± 13.99	50.18 ± 13.91	47.69 ± 14.37	51.53 ± 12.99	0.267	0.849
Energy Intake (kcal)	2841.80 ± 812.51	2321.85 ± 547.40	2628.90 ± 822.32	2550.40 ± 499.09	1.957	0.128
Energy Expenditure (kcal)	2841.90 ± 477.40	2607.15 ± 509.84	2531.20 ± 467.52	2844.05 ± 406.88	2.381	0.076

Note: Data presented as Mean ± SD. P-value > 0.05 indicates no significant difference across time points. BRF-2 = two weeks before Ramadan; RF-2 = 2nd week of Ramadan; RF-4 = 4th week of Ramadan; ARF-6 = two weeks after Ramadan

Table 2. 7-site of skinfolds and girth measurements of elite adolescent footballers during the month of Ramadan

Parameters	Mean ± SD				F	p-value
	BRF-2	RF-2	RF-4	ARF-6		
Skinfolds site						
Triceps	7.28 ± 2.19	7.05 ± 2.08	6.81 ± 2.02	7.50 ± 1.95	0.418	0.741
Subscapular	8.43 ± 1.46	8.49 ± 1.53	8.30 ± 1.56	8.70 ± 1.29	0.253	0.859
Biceps	4.17 ± 1.39	4.54 ± 1.29	4.03 ± 1.04	4.49 ± 1.39	0.728	0.538

Supraspinale	6.67 ± 2.76	6.47 ± 2.90	6.24 ± 2.94	6.71 ± 2.82	0.115	0.951
Abdominal	9.94 ± 3.88	9.66 ± 3.84	9.12 ± 3.94	10.64 ± 3.90	0.528	0.664
Mid-thigh	7.90 ± 2.29	8.02 ± 2.36	7.64 ± 2.46	8.03 ± 2.25	0.122	0.947
Medial calf	5.75 ± 1.85	5.97 ± 1.98	5.57 ± 1.75	5.49 ± 1.36	0.301	0.825
Girth						
Arm relax	28.15 ± 1.78	27.90 ± 1.80	27.61 ± 1.79	27.98 ± 1.79	0.316	0.814
Arm flex	30.11 ± 1.87	29.84 ± 1.66	29.71 ± 1.71	29.90 ± 1.72	0.183	0.908
Waist	73.14 ± 3.28	74.44 ± 3.23	73.75 ± 3.32	73.74 ± 3.42	0.514	0.674
Hip	93.57 ± 3.57	93.71 ± 3.23	92.66 ± 3.22	93.35 ± 3.67	0.371	0.774
Calf	37.66 ± 2.55	37.81 ± 2.52	37.50 ± 2.54	37.58 ± 2.47	0.053	0.984
Breadth						
Humerus	6.76 ± 0.37	6.81 ± 0.31	6.74 ± 0.31	6.90 ± 0.26	0.986	0.404
Femur	9.61 ± 0.50	9.68 ± 0.45	9.63 ± 0.49	9.70 ± 0.43	0.156	0.925

Note: Data presented as Mean ± SD. P-value > 0.05 indicates no significant difference across time points. BRF-2 = two weeks before Ramadan; RF-2 = 2nd week of Ramadan; RF-4 = 4th week of Ramadan; ARF-6 = two weeks after Ramadan

Bioelectrical Impedance Analysis (BIA) (Table 3) also found no significant changes (p>0.05) in Percentage of Body Fat (PBF), Total Body Water (TBW), Fat Mass, or Muscle Mass when comparing morning (am) and afternoon (pm) measurements across the four time points.

Table 3. Changes in body composition of elite adolescent footballers during the month of Ramadan by using bio-electrical impedance analysis method (In-body 230)

Parameters		Mean ± SD				F	p-value
		BRF-2	RF-2	RF-4	ARF-6		
PBF (%)	am	14.20 ± 2.91	13.16 ± 2.63	12.63 ± 2.61	13.94 ± 2.92	1.349	0.265
	pm	14.46 ± 2.82	13.67 ± 3.14	13.15 ± 2.87	13.97 ± 2.63	0.730	0.537
TBW (kg)	am	41.81 ± 3.93	42.72 ± 4.19	42.32 ± 4.21	42.18 ± 4.21	0.167	0.918
	pm	42.38 ± 4.01	41.83 ± 4.07	41.66 ± 4.06	42.69 ± 4.08	0.265	0.850
Fat mass (kg)	am	9.55 ± 2.50	8.88 ± 2.11	8.40 ± 2.00	9.43 ± 2.28	1.119	0.347
	pm	9.86 ± 2.41	9.11 ± 2.50	8.69 ± 2.27	9.47 ± 2.17	0.913	0.439
Muscle mass (kg)	am	32.53 ± 3.22	32.18 ± 3.48	32.89 ± 3.41	32.81 ± 3.47	0.126	0.945
	pm	32.83 ± 3.30	32.45 ± 3.33	32.41 ± 3.33	33.16 ± 3.36	0.225	0.879

Note: Data presented as Mean ± SD. P-value > 0.05 indicates no significant difference across time points. PBF = Percentage of body fat; TBW = Total body water; BRF-2 = two weeks before Ramadan; RF-2 = 2nd week of Ramadan; RF-4 = 4th week of Ramadan; ARF-6 = two weeks after Ramadan

3.2 Energy Balance: Energy Intake (EI) and Energy Expenditure (EE)

The study found no significant difference in Energy Intake (p=0.128) or Energy Expenditure (p=0.076) throughout the study (Table 1). While Energy Intake appeared lowest during RF-2 (2332.85 ± 547.40 kcal) and Energy Expenditure appeared lowest during RF-4 (2531.20 ± 467.52 kcal), these changes were not statistically significant.

3.3 Hydration Status (Urine Specific Gravity - USG)

Overall, hydration status (measured by Urine Specific Gravity - USG) showed no significant overall difference across the four-time points (Figure 1).

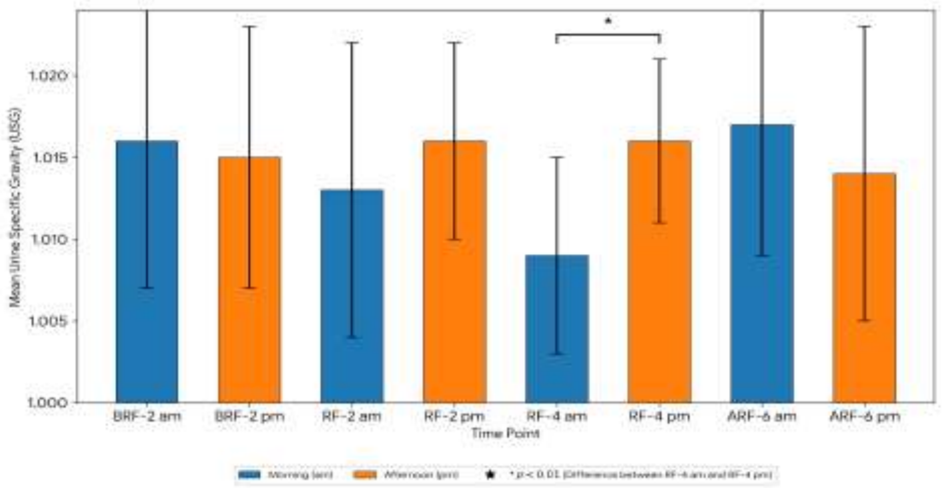

Figure 1. Comparison of AM and PM USG of Elite Adolescent Footballers during Ramadan Fasting Month. This figure illustrates the mean morning and afternoon Urine Specific Gravity (USG) across four time points. The symbol* indicates a significant difference between AM and PM measurement at $p < 0.01$ during the fourth week of Ramadan (RF-4).

Abbreviations: AM: Morning; PM: Afternoon; USG: Urine Specific Gravity; BRF-2: 2 weeks before Ramadan; RF-2: 2nd week of Ramadan; RF-4: 4th week of Ramadan; ARF-6: 2 weeks after Ramadan.

Note: Vertical bars represent standard deviation (SD).

A notable finding was observed in the 4th week of Ramadan (RF-4), where the afternoon USG (1.016) was significantly higher than the morning USG (1.009, $p < 0.01$). This suggests that by the end of Ramadan, the footballers exhibited inferior hydration status in the afternoon compared to the morning.

3.4 Hematological and Biochemical Changes

Interesting, time-dependent changes were observed in the hematological profile and blood GLU (Table 4) during the morning. RBC, HB, and HCT all experienced a significant reduction by the 2nd week of Ramadan (RF-2) compared to baseline. MCV, which measures RBC size, showed no change during the fasting month itself, but experienced a significant reduction at ARF-6 (two weeks post-Ramadan). Morning GLU levels maintained stability during the initial fasting period but increased gradually during RF-4 and peaked significantly at ARF-6. WBCand PLT counts showed no significant changes throughout the entire study period.

In the afternoon, RBC, HB, and HCT were generally increased during the fasting weeks compared to morning values, consistent with hemoconcentration. WBC, MCV, and PLT showed no significant changes compared to baseline. Evening GLU was reduced during RF-2 compared to BRF-2 and peaked post-Ramadan at ARF-6.

3.5 Physiological Performance

The YYIR test results indicated no detrimental effect of RF on the footballers' physiological performance capacity (Figure 2).

Table 4. Hematological changes of elite adolescent footballers during the month of Ramadan

Ramadan Parameter		BRF-2	RF-2	RF-4	ARF-6	Trend During Ramadan (RF-2 to RF-4)	Post-Ramadan (ARF-6) Status
White Blood Cell (103/uL)	am	$^{a}8.0 \pm 1.6$	$^{a}8.6 \pm 2.4$	$^{a}8.3 \pm 2.4$	$^{a}7.9 \pm 2.5$	No significant changes	
	pm	$^{a}7.9 \pm 1.8$	$^{a}7.5 \pm 1.6$	$^{a}7.5 \pm 1.8$	$^{a}8.2 \pm 1.8$		
Red Blood Cell (106/uL)	am	$^{a}5.31 \pm 0.2$	$^{b}5.02 \pm 0.3$	$^{ab}5.18 \pm 0.3$	$^{a}5.31 \pm 0.3$	Significantly reduced (BRF-2 vs. RF-2)	Returned to baseline (BRF-2 level)
	pm	$^{ab}5.04 \pm 0.3$	$^{ab}5.23 \pm 0.3$	$^{ab}5.27 \pm 0.3$	$^{ab}5.15 \pm 0.4$	Varied but generally maintained	
Hemoglobin (g/dL)	am	$^{a}16.6 \pm 1.0$	$^{b}15.6 \pm 0.9$	$^{ab}16.2 \pm 0.9$	$^{ab}16.5 \pm 1.3$	Significantly reduced (BRF-2 vs. RF-2)	Approached but did not return to baseline (BRF-2 level)

	pm	^{ab}15.9 ± 1.0	^{ab}16.3 ± 0.9	^{ab}16.5 ± 0.8	^{ab}16.4 ± 1.1	Varied but generally maintained	
Hematocrit (%)	am	^{a}50.4 ± 2.7	^{ab}47.8 ± 2.3	^{ab}49.1 ± 2.3	^{ab}47.8 ± 3.3	Significantly reduced (BRF-2 vs. RF-2)	Did not return to baseline (remainted significantly lower than BRF-2 level)
	pm	^{b}45.5 ± 2.2	^{ab}49.5 ± 2.6	^{a}49.8 ± 2.2	^{ab}49.1 ± 2.9	Increased during Ramadan fasting (e.g., HCT RF-4 vs. BRF-2)	Did not return to baseline (BRF-2 level)
Mean Cell Volume (fL)	am	^{a}95.2 ± 6.1	^{a}95.2 ± 5.6	^{a}95.0 ± 5.4	^{b}86.0 ± 4.2	No significant change during fasting	Significantly reduced (microcytosis) after Ramadan (ARF-6 vs BRF-2)
	pm	^{a}95.2 ± 5.5	^{a}94.8 ± 5.7	^{a}94.8 ± 5.3	^{a}94.9 ± 5.8	No significant changes	
Platelet (103/uL)	am	^{a}290.0 ± 50.0	^{a}278.0 ± 57.0	^{a}289.0 ± 50.0	^{a}303.0 ± 55.0	No significant changes	
	pm	^{a}284.0 ± 50.0	^{a}284.0 ± 62.0	^{a}298.0 ± 55.0	^{a}275.0 ± 43.0	No significant changes	
Glucose (mmol/l)	am	^{c}5.07 ± 0.2	^{c}5.07 ± 0.8	^{bc}5.27 ± 0.8	^{ab}5.66 ± 0.5	Gradual increased at RF-4 vs. RF-2	Further increased at ARF-6 compared to RF-2
	pm	^{a}6.08 ± 0.7	^{bc}5.42 ± 0.7	^{ab}5.69 ± 0.5	^{a}6.14 ± 0.8	Reduced during RF-2 compared to BRF-2	Peaked after Ramadan at ARF-6

Values are mean ± SD. Means of each parameter in the same row that do not share same alphabet letter are significant different
BRF-2 = two weeks before Ramadan; RF-2 = 2nd week of Ramadan; RF-4 = 4th week of Ramadan; ARF-6 = two weeks after Ramadan

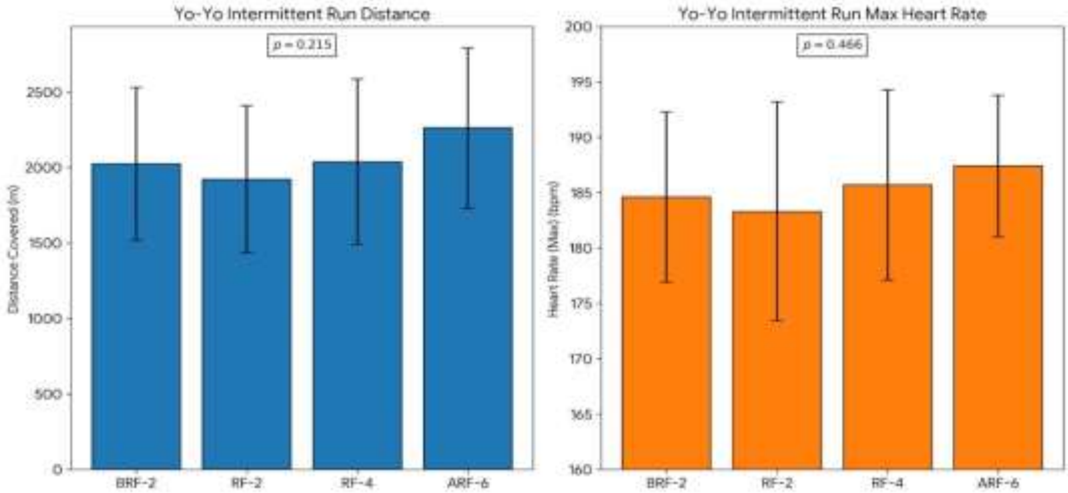

Figure 2. Yo-Yo Intermittent Run (YYIR) Test Result Before, During, and After Ramadan Fasting Month. This figure displays the mean distance covered and maximum heart rate during the YYIR test. Left panel shows distance covered in meters (m); right panel shows maximum heart rate in beats per minute (bpm). No significant differences ($p > 0.05$) were observed across time points.

Abbreviations: YYIR: Yo-Yo Intermittent Run; HR: Heart Rate; bpm: beats per minutes; m: meters; BRF-2: 2 weeks before Ramadan; RF-2: 2nd week of Ramadan; RF-4: 4th week of Ramadan; ARF-6: 2 weeks after Ramadan.

Note: Vertical bars represent standard deviation (SD).

Performance Markers: Distance Covered (p=0.215) and Maximum HR- (p=0.466) achieved during the YYIR test showed no significant difference across the before, during, and after Ramadan time points (Figure 2). The blood lactate response (Figure 3) measured before, during, and after the YYIR test was also not significantly different ($p > 0.05$), despite a slight, non-significant decrease observed during RF-2.

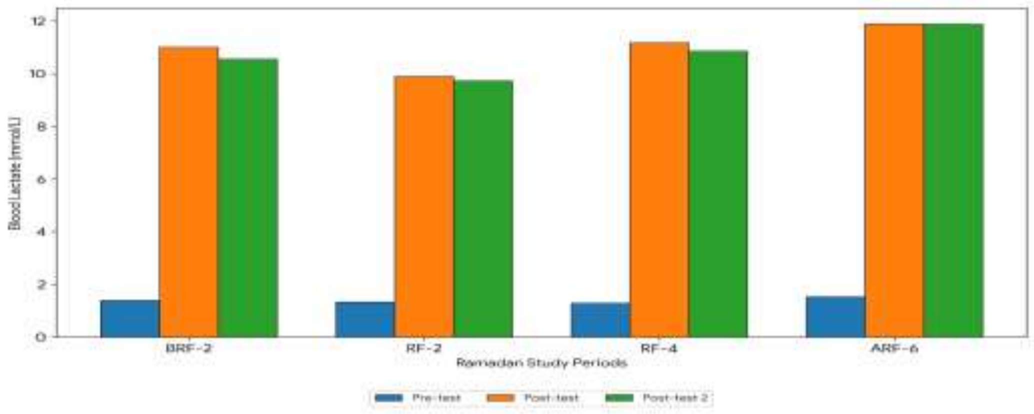

Figure 3. Blood Lactate Before, During, and After Yo-Yo Intermittent Run (YYIR) Test Before, During, and After Ramadan Fasting Month. Data points show lactate levels response at three stages (Pre-test, Post-test, and Post-test 2) of the YYIR test across four study periods.

Abbreviations: mmol/L: millimoles per liter; YYIR: Yo-Yo Intermittent Run; BRF-2: 2 weeks before Ramadan; RF-2: 2nd week of Ramadan; RF-4: 4th week of Ramadan; ARF-6: 2 weeks after Ramadan.

Legend: Pre-test refers to baseline levels; Post-test refers to levels immediately following exercise; Post-test 2 refers to recovery levels

4. Discussion

The primary objective of this study was to determine the effect of RF on elite adolescent football players. The study found no significant changes in body weight, BMI, body fat, muscle mass, or body size throughout the RF month, as confirmed by both skinfold and bioelectrical impedance analysis BIA methods (Table 1, 2, and 3). This finding aligns with numerous studies conducted on young footballers and healthy populations, which reported similar stability in body composition during RF (5, 10, 11, 23, 28, 34). Conversely, some research on female athletes and physically active males has reported a reduction in total body weight and fat mass (11, 16, 24, 25).

The key factor explaining the maintenance of body composition in the present cohort is energy balance (13). This study found no significant difference in Energy Intake or Energy Expenditure across the phases (Table 1), suggesting that the calorie intake during the compressed feeding window (sunset to sunrise) was sufficient to match the energy demands, thereby preventing catabolic loss. The observed stability of body composition is consistent with other studies on athletes where energy intake was maintained during RF (5, 8, 10, 11, 25). However, overall changes in energy intake and body composition remain highly individualized, depending on factors like dietary habits, training intensity, and geographical region (3, 4, 9, 11).

Despite prolonged daytime fluid restriction, the overall hydration status, measured by Urine Specific Gravity (USG), was maintained throughout RF, consistent with findings in other young male athletes (5, 25, 31, 32). This may be attributed to a water retention mechanism enabled by renal function, allowing active individuals to better adapt to dehydration (5, 25, 31, 35). However, the significant finding during RF-4 where afternoon USG was significantly inferior compared to morning USG (Figure 1) suggests a dynamic fluid imbalance during the fasting day. This is supported by the corresponding hematological changes:

Morning Hemodilution (am): The observed reduction in RBC, HB, and HCT in the morning (RF-2) compared to baseline is likely due to hemodilution (7, 25, 34, 36). Following the large fluid intake at Sahour, the body increases plasma volume overnight, temporarily diluting the blood component concentrations.

Afternoon Hemoconcentration (pm): Conversely, the increase in HCT and HB in the afternoon during RF strongly indicates hypohydration and hemoconcentration (5, 25, 31, 34). The daytime fluid restriction, combined with training, reduces central circulating blood volume, concentrating the blood components. This hemoconcentration increases blood viscosity, imposing greater cardiovascular strain and raising the risk of heat illness during afternoon training (22, 25, 37). Therefore, coaches should shorten afternoon training duration and focus on lower-intensity drills to maximize safety and training efficacy, allowing players to rehydrate immediately after Iftar (5, 9, 22, 30, 32).

The hematological data highlights a physiological stress on the erythropoietic system, with effects persisting beyond the fasting month.

Transient RBC Stress: The morning reduction in RBC, HB, and HCT during RF-2 suggests a transient effect on oxygen-carrying capacity (8, 25, 31, 36). While this could theoretically impair aerobic capacity and increase fatigue, the maintenance of YYIR performance suggests that the athletes compensated effectively, or the reduction was sub-clinical in terms of performance impact (8, 13, 15, 23, 25). While RBC returned to baseline after Ramadan (ARF-6), HB only approached baseline, and HCT did not return to baseline, suggesting incomplete recovery.

Delayed MCV Reduction and Iron Status: The most concerning finding is the significant reduction in MCV at ARF-6, two weeks after RF was completed. This microcytosis is a strong clinical signal for impaired erythropoiesis, most commonly linked to a developing or subclinical iron deficiency (7, 34, 36, 38). The delay in the MCV drop is likely due to the lifespan of red blood cells; the deficiency became apparent when new, smaller cells were produced under conditions of iron shortage, exacerbated by the combined stress of fasting, inadequate post-fasting nutritional correction, and high training volumes (exercise-induced hemolysis). This finding is similar to that reported by Attarzadeh et al. (3, 9, 31, 36). However, the diagnosis of subclinical iron deficiency in this study is inferred from indirect evidence (microcytosis) rather than direct measurement, this remain inconclusive without confirmation from a full iron panel.

GLU Fluctuations: Blood GLU was maintained in the morning during RF, gradually increasing until peaking after Ramadan (ARF-6), likely due to the return of regular feeding patterns and counter-regulatory hormone release (4, 11, 12, 38). The reduced evening GLU at RF-2 suggests a need for adjustment, leading to the recommendation that coaches may need to reduce training intensity during the initial weeks of RF to allow time for metabolic adaptation.

WBC and PLT Stability: The absence of changes in WBC and PLT is a positive indicator, suggesting that RF did not compromise the footballers' immune function or coagulation ability, contradicting some previous findings that linked nutrient deficiency to decreased PLTs- (7, 12, 34, 36).

Crucially, this study indicated no adverse effects of RF on the primary physical performance measure. The YYIR distance covered (Figure 2), Maximum HR (Figure 2), and blood lactate (Figure 3) response were all maintained across all phases. These results are in agreement with other research on endurance capacity in athletes during RF (1, 8, 9, 15, 25). The maintenance of performance, despite the observed physiological stress markers HCT and MCV, underscores the high degree of adaptation in elite athletes, but it simultaneously highlights the need for rigorous post-RF nutritional and medical monitoring to ensure full hematological recovery before major competitions.

5. Conclusion

The study concluded that RF did not significantly impair the body composition, energy balance, or physiological performance capacity (as measured by the YYIR test) of elite adolescent footballers. The athletes effectively adapted to the feeding schedule by maintaining adequate energy and nutrient intake, thereby preserving measures such as body weight, muscle mass, and running endurance. However, the study identified significant time-dependent physiological stress markers that require careful management:

Dynamic Hydration Imbalance: The footballers experienced a daily cycle of hemodilution in the morning (following the Sahour meal) and hemoconcentration (inferior hydration status) by the late afternoon (RF-4). This mandates the scheduling of high-intensity training sessions to the post-Iftar window and limiting afternoon sessions to low-intensity technical work.

Delayed Hematological Stress: The most critical finding was the significant reduction in -HCT- persisting at two weeks post-Ramadan (ARF-6) and the delayed onset of microcytosis (MCV reduction) at ARF-6. These findings are strong clinical indicators of a subclinical iron deficiency that manifested after the fasting period, likely due to increased RBC turnover combined with compromised nutritional recovery.

Recommendations and Future Research: To safeguard the long-term health and performance potential of these elite young athletes, the following evidence-based strategies are commended:

- **Post-Fasting Nutritional Recovery:** Implement rigorous nutritional guidance (focusing on erythropoietic nutrient profile to support hematological recovery) in the immediate post-RF period.
- **Nutrients Bioavailability:** Future studies should investigate the specific intake of iron, folic acid, vitamin B12, and Vitamin C during the feeding window and also prioritize identifying iron inhibitor factors (such as phytates or tannins) consumed during Sahour and Iftar, as these can impact iron absorption.

- **Comprehensive Iron Profiling:** As this study inferred subclinical iron deficiency from delayed microcytosis, future research could prioritize serum ferritin and transferrin saturation to definitely confirm iron status.
- **Erythropoietic Tracking:** Longitudinal studies are required to track the specific timeline of erythropoietic recovery beyond ARF-6. -

Clinical Implications: The findings of this study provide specific practical applications for the multidisciplinary team supporting elite adolescent athletes:

- **For Coaches:** Periodized training, shift high-intensity sessions to the post-Iftar window to ensure athletes are hydrated and fueled. Limit late-afternoon sessions to low-intensity technical work or shorter durations to minimize cardiovascular strain caused by daily hemoconcentration.
- **For Team Physicians:** Implement detailed blood screenings during, and two to four weeks after Ramadan, to detect delayed physiological stresses for example microcytosis condition and persistent low HCT.
- **For Dietitians/Nutritionists:** Focus on the quality of micronutrient intake (iron and B vitamins) during the restricted feeding window to support red blood cell synthesis. Dietitians/Nutritionists should also develop individualized rehydration protocols for the period between Iftar and Sahour to counteract the dynamic fluid imbalances observed throughout the day.

Limitations: While this study provides valuable insights into the effects of RF on elite adolescent footballers, several limitations need to be acknowledged:

- **Sample Size and Generalizability:** The final sample size was limited to 20 male participants. This small cohort is insufficient to generalize the findings to other teams, different sport disciplines, female athletes, or non-elite populations.
- **Incomplete Iron Status Markers:** The diagnosis of subclinical iron deficiency was inferred from a delayed MCV drop rather than definitively confirmed via gold standard serum ferritin, transferrin saturation, or serum iron.
- **Dietary Quality:** While 3-day food records were utilized to maintain energy balance, the study did not specifically analyse the intake levels of key micronutrients such as iron, Vitamin C, B^{12}, and folate. Furthermore, the specific nutritional quality of Sahour versus Iftar meals were not accurately investigated.
- **Training Intensity Control:** The study utilized the athletes' existing national youth team training schedule. It did not involve an experimental design where training intensity was controlled or manipulated, it is difficult to draw definitive causal conclusions regarding whether performance was maintained through physiological adaptation to RF or adjustments in training intensity.

Authors' Contributions

S.M.I, R.S., A.M., A.B.S., were responsible for the conceptualization, design and direction of the project. S.M.I., G.K.W., R.S., A.M., A.B.S., performed the experiments, carried out data collection, and contributed to data analysis. G.K.W. took the lead in interpreting the results and writing the original draft of the manuscript, with all authors providing critical review and input to the final version.

Declaration

In order to improve the academic writing and language quality of this manuscript, the authors used the language model ChatGPT. The authors take full responsibility for the content of the manuscript.

Transparency Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Declaration of Interest

The authors report no conflict of interest.

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Ethical Considerations

Ethical approval for this study was obtained from the Institutional Research Committee of the National Sports Institute of Malaysia (ISN) and the Research Ethics Committee (Human) of Universiti Sains Malaysia (USM).

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