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Physiological Responses to Sprint Interval Exercise in a Fasted State in Active Men

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ABSTRACT

Introduction: Fasting is a method used to enhance metabolic flexibility toward lipid utilization. However, there are concerns about hypoglycemia or protein breakdown in exercise. The present study investigated the physiological responses to sprint interval exercise with different rest durations in a fasting state.

Methods: For this randomized crossover study, 10 healthy active men (aged 22.70 ± 1.15) were selected from eligible volunteers. In 2 separate sessions, following 14 hours of fasting, participants randomly performed 5×15s all-out sprints on an E894 MONARK ergometer with 30 or 60s rest intervals.

Results: Diastolic blood pressure and heart rate were lower with longer rest intervals. Blood glucose, insulin, and urea remained unchanged after both protocols compared with baseline levels. There was a marked increase in lactate levels after both protocols, independent of the rest duration. The immune response was significantly higher with longer rest intervals than with short rest intervals or baseline levels.

Conclusion: Sprint interval exercise performed in a fasted state did not induce hypoglycemia or evidence of protein degradation, suggesting that such training may be safe for active men. Longer rest intervals were associated with lower post-exercise heart rate and diastolic blood pressure, as well as an enhanced immune response. These findings suggest that coaches and practitioners may consider manipulating rest intervals to achieve targeted physiological adaptations.

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Introduction

Intermittent fasting (IF) is a widely adopted dietary pattern that helps conserve carbohydrates and promotes greater reliance on fat as a primary energy source [1]. Exercising during fasting increases adipose tissue lipolysis and peripheral fat oxidation via higher plasma catecholamines and cortisol concentrations and lower circulating insulin levels. A modest reduction in serum glucose levels can occur within a few hours of fasting, likely due to attenuated hepatic glycogen synthesis and glycolysis. Such modifications occur due to decreased insulin concentrations, increased glucagon levels, and enhanced sympathetic activity [2, 3]. These findings have encouraged athletes to incorporate fasting into their training protocols to enhance fat oxidation and spare glycogen stores.

High-intensity interval Exercise (HIIE) is a method commonly viewed in the context of health and athletic performance in all types of sports [4, 5]. Cycling-based HIIE as a sprint interval exercise (SIE) has been identified as a viable method for enhancing performance, with the nature and extent of adaptations contingent upon the work-to-rest ratio [6]. The absence of weight-bearing and minimal eccentric muscle contractions during stationary cycling presents runners and athletes with a low-impact alternative that may mitigate the risk of overuse injuries, thereby offering a pragmatic solution for optimizing training regimens [7].

Time-to-rest ratios have been studied, with some research showing that shorter-time SIEs may offer the same adaptations as longer work-to-rest ratios [8]. The effectiveness of different SIE protocols in eliciting specific adaptations related to physiological responses remains an area of

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interest. Research suggests that SIE protocol involving shorter work periods at supramaximal intensity may produce these adaptations more effectively than a protocol with longer work intervals at maximal intensity.

While no definitive conclusion has been reached, studies have indicated that a 2:1 work-to-rest ratio results in a higher accumulated oxygen deficit compared to other ratios [9]. Regarding metabolic responses, while the 2:1 SIE led to maximal values of VO_2, lactate, and ventilatory parameters within a few minutes, the 1:1 SIE allowed maintenance of moderately high values for a considerably longer period, especially for lactate and ventilatory parameters [10]. Also, the elimination of ammonia is significantly enhanced during high-intensity muscle activity, and the formation of urea increases. This is confirmed by the fact that the increased concentration of blood urea is directly proportional to the duration of high-intensity work [11]. Studies have also shown that HIIE provokes a greater immune response compared with moderate continuous exercise [12]. Lymphocyte-to-white blood cell ratio (LWR) and platelet-to-lymphocyte ratio (PLR) have been used as cost- and time-efficient markers of cellular immune response [13]. However, the immune response to high-intensity exercise while fasting is not clear.

Both athletes and the general population widely apply fasting strategies. However, there are concerns regarding the impact of fasting on high-intensity exercise outcomes, particularly concerning protein degradation and immune fluctuations. Therefore, examining the acute physiological response to SIE with varying rest intervals while in a fasted state could offer valuable insights for developing future training strategies for athletes.

Materials and Methods

Study Design

This randomized crossover study investigated the physiological response to SIE following a 14-hour overnight fast with different rest intervals. This research followed the Declaration of Helsinki (1964) and has been approved by the Ethics Committee in Biomedical Research, University of Guilan (IR.GUILAN.REC.1403.050).

Participants

Upon an announcement, fourteen 18-25-year-old males volunteered for this study. After a clear explanation of the research protocol and examining the eligibility criteria, ten healthy men (aged: 22.70 ± 1.15, weight: 79.90 ± 7.56 kg, height: 180.6 ± 5.89 cm, and body mass index (BMI): 22.94 ± 1.75 kg / m^{-2}) with normal BMI, at least one year of regular exercise, and no history of cardiometabolic diseases or musculoskeletal injuries were selected (estimated sample size: 9, $\alpha = 0.05$, effect size = 0.5, and actual statistical power = 0.85, G Power software). One person refused to participate in the SIE protocol, and three volunteers did not meet the research criteria. Selected participants were fully informed about the research protocol and completed and signed a written informed consent form.

Procedure

In the first session, after 14 hours of overnight fasting, pre-test blood samples were collected from the antecubital vein. The participants were asked to continue their daily activities during the research and maintain their usual diet, but refrain from consuming caffeine and vigorous activity 48 hours before the trials. The last meal before 8 pm was consumed similarly (~500 kcal, containing 70% carbohydrate, 20% protein, and 10% fat), and the participants were only allowed to drink water and zero-calorie beverages for 14 hours. The subjects randomly performed a 1:2 or 1:4 SIE protocol in two sessions separated by at least 72 hours of rest. The randomization sequence was generated using the Random Allocation Software before the study's commencement to ensure an unbiased distribution of conditions among participants. Participants warmed up for 15 minutes, including static/dynamic stretches and light cycling on a stationary bicycle. SIE protocol consisted of 5 sprints with maximal speed on the Monark E894 ergometer, with adjusted handlebar and seat height, and a 5% of the body weight basket dropped from the beginning of cycling for each participant. They were also asked to perform their maximum effort in work intervals and passive sitting on the bike in rest intervals. Work-to-rest ratio for 1:2 and 1:4 HIIE was considered 15s: 30s and 15s: 60s, respectively. Both trials were held between 08:00 to 12:00, in similar times and conditions for each participant's testing.

Blood pressure was measured using a digital device (Omron M6) before (after 15 minutes of sitting rest) and immediately after exercise. The heart rate was monitored before and during

protocol using an H10 Polar belt and the Polar Beat application on an Android mobile phone. After 5 minutes of cooling down, blood samples were collected in both EDTA and coagulating tubes to obtain serum and plasma samples and transferred to the laboratory. Serum glucose concentrations were determined using an enzymatic colorimetric method (Glucose Oxidase-Peroxidase; Pars Azmun, Tehran, Iran). Serum insulin levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Monobind Inc., Lake Forest, CA, USA). Plasma lactate concentrations were assessed using a colorimetric assay kit (ZellBio GmbH, Ulm, Germany). Serum urea levels were determined by an enzymatic colorimetric method using a commercial kit (Pars Azmun, Tehran, Iran. A complete blood count (CBC), including white blood cell (WBC) and platelet counts, was performed using an automated hematology analyzer (Sysmex KX-21N, Sysmex Corporation, Kobe, Japan) following the manufacturer's protocol.

Statistical Analysis

All Data are presented as mean ± standard deviation. The Shapiro-Wilk test was used to test the normality of the data distribution. In case of abnormality, data were normalized using the Inverse distribution function method with fractional ranks of data and original mean and SD of the data distribution [14]. Repeated measurements analysis of variance and Bonferroni tests were used to compare means between trials. In the case of the sphericity assumption violated by the Mauchly test, Greenhouse-Geisser was used to determine the significance of the F value. Partial eta squared (η_p^2) and observed power (OP) are reported in case of a significant F value. Data analysis was done using IBM SPSS software, version 27 ($P < 0.05$). GraphPad Prism 10.2.2 was used for graph illustration.

Results

Blood Pressure and Heart Rate

With systolic blood pressure (SBP), the main effect of time was significant ($F = 10.70$, $P = 0.010$, $\eta_p^2 = 0.543$, $OP = 0.829$, 95% CI = (-32.14, -5.86)), but the main effect of the trial ($P = 0.079$) and interaction effect of time*trial ($P = 0.178$) were not statistically significant. It shows that the effect of both protocols on SBP was similar (Figure 1, A). The main effect of the trial for diastolic blood pressure (DBP) was significant ($F = 6.321$, $P = 0.033$, $\eta_p^2 = 0.413$, $OP = 0.611$, 95% CI = (0.63, 11.97)). However, the main effect of time ($P = 0.730$) and the interaction effect of time*trial ($P = 0.120$) were not significant, and the mean DBP was ~13% lower after 1:4 compared with 1:2 SIE (Figure 1, B).

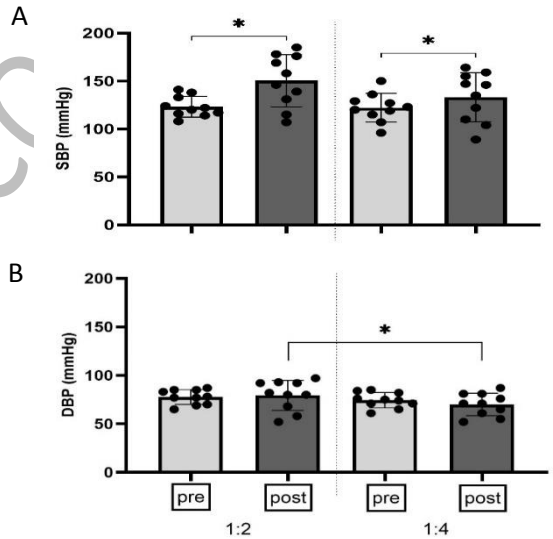

Figure 1. Mean ± SD of (A) systolic blood pressure (SBP) and (B) diastolic blood pressure (DBP). SBP increased with both protocols and there was no significant difference between protocols. Post-exercise DBP was significantly lower after 1:4 HIIE. *: significant difference at $P < 0.05$.

The main effect of time (F = 372.39, P < 0.001, η_p^2 = 0.976, OP = 1.000, 95% CI for Pre-test vs post1 (-88.59, -49.41); Post2 (-99.80, -70.80); Post3 (-106.25, -79.86); Post4 (-110.27, -83.23); Post5: -112.91, -83.79)) and trial (F = 5.270, P = 0.047, η_p^2 = 0.369, OP = 0.535, 95% CI = (0.08, 10.29)) for heart rate (HR) was significant but the time*trial interaction effect was not statistically significant (P = 0.227). HR increased after each work interval of both protocols. After the first and second intervals, HR had no difference in trials, but in three subsequent intervals, HR was higher in 1:2 SIE (P < 0.05) (Figure 2).

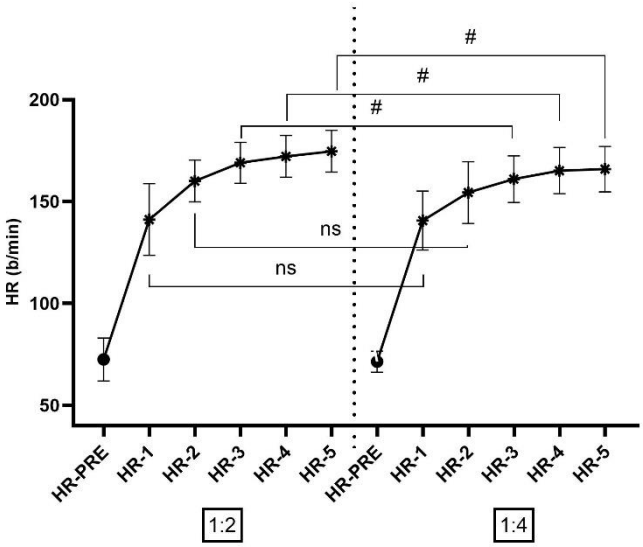

Figure 2. Mean ± SD of heart rate (HR) pre-exercise and after 5 work intervals. HR increased after each work interval in both protocols. The last three intervals induced lower HR in 1:4 HIIE compared with 1:2 HIIE. *: significant difference compared with pre-test; #: significant difference between trials (P < 0.05).

Blood Markers

Although RM ANOVA showed a significant F value for glucose levels (*F = 5.686, P = 0.012*, η_p^2 = 0.387, OP = 0.798), pairwise comparisons by the Bonferroni test showed no significant difference between trials (*P ≥ 0.05*) and mean glucose levels were not statistically different after 1:2 and 1:4 SIE compared with the control condition (Figure 3, A).

RM ANOVA showed a significant F value for insulin levels (F = 5.476, P = 0.014, η_p^2 = 0.378, OP = 0.782, 95% CI for control vs 1:2 HIIE (-13.48, -0.5)). Based on a pairwise comparison of Bonferroni, insulin levels only increased after 1:2 HIIE (*P = 0.035*) compared with the control trial (Figure 3, B).

There was a significant F value for lactate levels (F = 222.91, P < 0.001, η_p^2 = 0.961, OP = 1.000, 95% CI for control vs 1:2 HIIE (-18.80, -14.71) and 1:4 HIIE (-20.02, -13.14)) and based on the Bonferroni test, lactate levels increased significantly with both protocols compared with the control condition (P < 0.001). Still, it was not different between 1:2 and 1:4 SIE (P = 1.000) (Figure 3, C).

Urea levels had no significant difference between trials (P = 0.839) (Figure 3, D).

Lymphocyte count was different between trials (F = 21.27, P < 0.001, η_p^2 = 0.703, OP = 1.000, 95% CI for control vs 1:2 HIIE (-3.41, -1.09) and 1:4 HIIE (-3.79, -1.07)). WBC showed a significant difference between trials (F = 32.08, P < 0.001, ηp^2 = 0.781, OP = 1.000, 95% CI for baseline vs 1:2 HIIE (-6.08, -1.93)). Platelets were also different between trials (F = 15.69, P < 0.001, η_p^2 = 0.635, OP = 0.998, 95% CI for control vs 1:2 HIIE (-100.52, -23.48) and 1:4 HIIE (-111.25, -19.55)). LWR also showed a significant F value (F = 23.33, P < 0.001, η_p^2 = 0.722, OP = 0.994, 95% CI for control vs 1:2 HIIE (-0.15, -0.007) and 1:4 HIIE (-0.69, -0.18) and 1:2 vs 1:4 HIIE (-0.59, -0.14)). Further pairwise comparisons revealed that LWR significantly increased after 1:2 and 1:4 SIE and was ~80% higher after 1:4 compared with the 1:2 protocol (Figure 3, E). A significant F value was also observed for PLR (F = 6.395, P = 0.008, η_p^2 = 0.415, OP = 0.845, 95% CI for control vs 1:4 HIIE (6.38, 55.43)). PLR was significantly lower after 1:4 SIE compared with baseline (P = 0.015) (Figure 3, F).

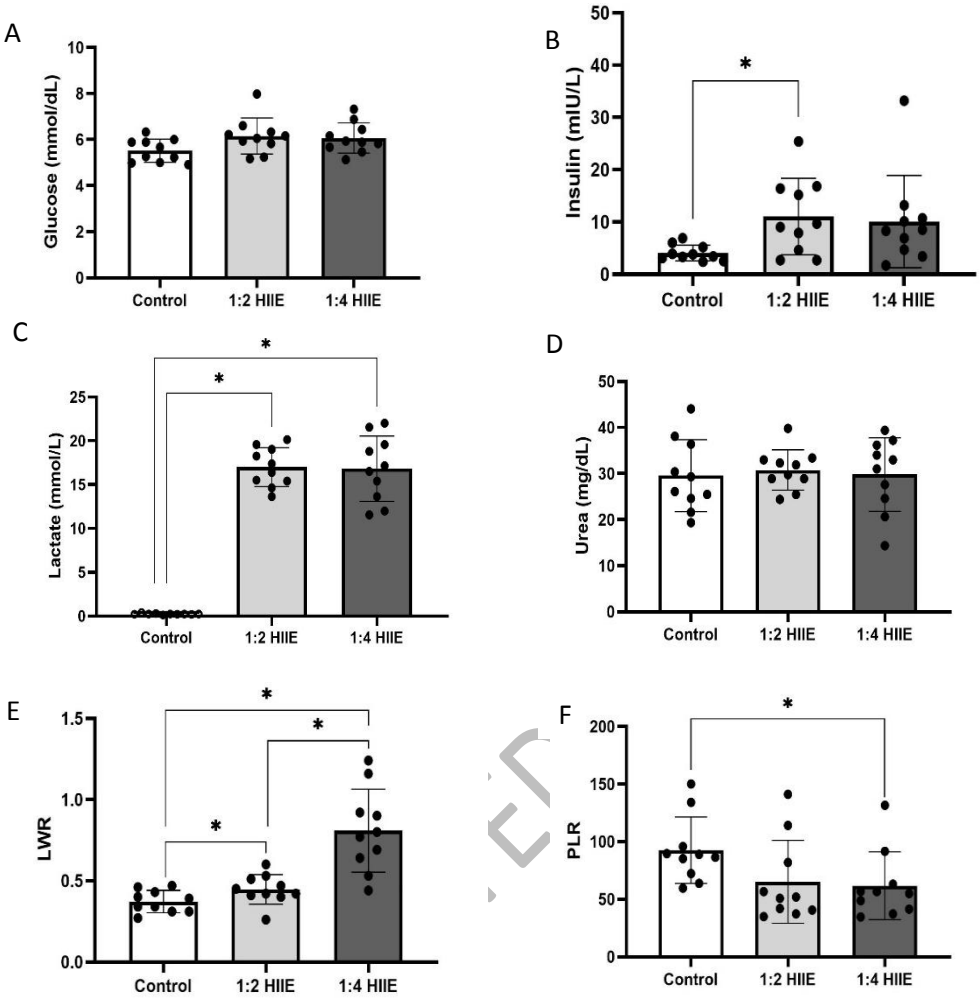

Figure 3. Mean ± SD of (A) Glucose and (B) Insulin (C) Lactate, (D) Urea levels, (E) lymphocyte to WBC ratio (LWR), and (F) platelet to lymphocyte ratio (PLR) in trials. Glucose and Urea levels were similar in both protocols compared with baseline levels. 1:2 HIIE increased insulin levels compared to baseline levels. Lactate levels increased similarly after both protocols. 1:4 HIIE increased LWR compared with baseline levels and 1:2 HIIE and lowered PLR compared with baseline levels. *: Significant difference at $P < 0.05$.

Discussion

This randomized controlled study was conducted in two counterbalanced trials in active males to investigate the effect of SIE with two work-to-rest ratios following a 14-hour overnight fast on selected physiological indices. To our knowledge, this is the first study that considers the work-to-rest ratio of all-out sprint cycling in a fasting state.

SIE is a short-term, intense exercise method that is believed to have beneficial effects on health and athletic performance [15]. SIE constitutes a particularly intense variant of HIIE that can be distinguished as repeated bouts performed with near-maximal to "all-out" effort. This characterization coincides with the highest intensity classification identified in training zone models or exercise prescription guidelines, including the extreme-intensity domain, anaerobic speed reserve, or near-maximal to maximal intensity classification [6]. We hypothesized that rest intervals of SIE in the fasting state have a different physiological effect.

Blood Pressure and Heart Rate

Based on data from pre- and post-exercise monitoring of BP and HR, both SIE protocols

elevated post-exercise SBP similarly, but post-exercise DBP was significantly lower following 1:4 SIE. Tan et al. reported that sprint interval exercise (4*30s sprint: 2min rest) while in a fasting state (> 10 hours of fasting) led to a notable reduction in blood pressure compared with the fed state [16]. However, the acute response of blood pressure to SIE in fasting is unclear. Kriel et al. reported that 1:4 SIE (30s: 2min) increased SBP but did not change DBP [17]. Some other studies have suggested that HIIT does not promote a significant difference in SBP but DBP [18]. The different observations may be related to the duration of work and rest intervals. Longer work intervals seem to elevate SBP, while longer rest intervals may lower DBP [19]. As exercise intensity increases, SBP typically exhibits a linear increase, reflecting increased metabolic demands, sympathetic activation, and thermoregulatory responses that collectively increase blood flow to the active muscles [20]. The decrease in DBP, is probably due to metabolic vasodilation in the exercising muscles, ensuring adequate oxygen delivery and waste removal [21]. It is highly evidenced that chronic intermittent fasting and calorie restriction can reduce BP by linking different pathways involving inflammation, reactive oxygen species (ROS), nitric oxide (NO), and others [22], but the acute effect of fasting on exercise BP needs to be further investigated.

It was anticipated that HR would increase during the SIE [19], and this was indeed observed. Notably, HR remained elevated when shorter rest intervals were employed. Conversely, when longer recovery periods were implemented during the 1:4 SIE protocol, HR was observed to be lower, similar to another research [23], possibly attributable to increased parasympathetic activity during recovery intervals [24].

Blood Markers

Post-exercise blood levels of glucose, insulin, urea, lactate, and the LWR and PLR were compared with a baseline level. The glucose levels did not show any significant difference relevant to the SIE protocol. The fasting glucose levels were near normal values (5.51 ± 0.49 mmol/dL). Although the glucose level was ~6 mmol/dL after 1:2 and 1:4 SIE, it was not statistically different from the baseline. Despite this, it is reported that a modest reduction in serum glucose levels can occur within a few hours of fasting (fasting glucose levels of 3.3 and 3.9 mmol/L), likely due to attenuated hepatic glycogen synthesis and glycolysis due to decreased insulin concentrations, and also increased glucagon levels and enhanced sympathetic activity [1]. Continuing fasting duration may attenuate insulin levels and activate gluconeogenesis through amino acids, glycerol, and ketone body availability [25]. Free fatty acids (FFAs) can be the main energy source of exercise during fasting because of enhanced adipose tissue lipolysis [1]. However, we observed increased levels of insulin after SIE, but it was only significant with the 1:2 protocol. Baseline fasted-insulin levels were 4.05 ± 0.48 mIU/L, increasing to 11.04 ± 2.31 and 10.07 ± 2.80 mIU/L after 1:2 and 1:4 SIE. During the absorptive state (after eating), insulin levels will increase, potentially reaching levels between 30-70 mIU/L or higher, depending on the meal and individual factors. But, usually, for a fasting glucose level of about 5 mmol/L, the insulin ranges between 5 and 10 mU/L (35–70 pmol/L), with some variation depending on the insulin assay usedIn contrast [26], and it is reported that longer fasting periods (36h vs 12h) are related to more reduced blood glucose and insulin levels [27]. Also, a meta-analysis revealed that fasting plus exercise has no or minimal effect on glucose and insulin levels, but the time of fasting or exercise mode was not precisely documented by the authors [28]. Adams (2013) reported that in intense exercise (>80% VO_2max), unlike at lesser intensities, glucose is the exclusive muscle fuel. Catecholamine levels rise markedly, causing glucose production to rise seven- to eightfold while glucose utilization is only increased three- to fourfold. In people without diabetes, there is a small blood glucose increase during intense exercise that increases further immediately at exhaustion and persists for up to 1 hour. Plasma insulin levels rise, correcting the glucose level and restoring muscle glycogen [29]. This may explain the higher glucose and insulin levels observed in our study. However, more studies are needed to measure insulin levels during fasted state exercise.

Lactate levels were highly increased after both SIE protocols (~17 mmol/L), and there was no difference between protocols. Animal studies have also shown that a single session of HIIE raises lactate levels to 375%, independent of work-to-rest ratios [30]. The normal resting

value of lactate is less than 1 mmol/L. In the current study, the fasted lactate level was approximately 0.23 mmol/L. Following HIIE, there is increased neuronal activation in the prefrontal cortex. Neurons can use muscle-derived lactate to meet these heightened energy demands [31]. Lactate may serve as an alternative fuel for the brain during fasting, potentially sparing glucose. Acute sprint exercise eliciting peak blood lactate accumulation may increase brain-derived neurotrophic factor (BDNF) expression in the brain [32] and may give rise to enduring cognitive and neural benefits [31]. However, the lactate response to high-intensity exercise in fasting needs to be investigated.

Urea formation during muscular activity is a violation of the balance of adenosine triphosphate (ATP) in working muscles, as well as increased protein catabolism [11]. We questioned whether high-intensity exercise in a fasting state might lead to protein degradation. However, our SIE protocols did not increase blood urea formation. Control of the dynamics of urea after a set of training loads indicates the balance of anabolic and catabolic processes in the athlete [11] and it seems SIE in a fasting state may not harm protein metabolism.

The lymphocyte-to-white blood cell ratio (LWR) [33] and platelets-to-lymphocyte ratio (PLR) [13], are blood markers of the systemic inflammatory response. As we observed, LWR increased significantly after 1:2 (~22%) and 1:4 (~119%) SIE protocols compared with the baseline, and the difference was also significant. PLR was lower after 1:4, but it was not different from the 1:2 protocol. It seems all-out sprint exercises provoke a greater immune response than moderate continuous exercise [12, 34]. It is also known that a single exercise session only induces a transient immune response [35]. Lymphocytes elevate during exercise and decrease shortly after exercise cessation. Within 24 hours, baseline levels are usually restored [13]. It is suggested that active rest intervals may attenuate the increased immune response to SIE [34]. Interestingly, we observed much higher LWR after longer rest intervals in the 1:4 SIE protocol. Perhaps the active rest intervals may help to reduce the immune response to 1:4 high-intensity protocols.

This study is, to our knowledge, the first to investigate the effects of the work-to-rest ratio of SIE protocols conducted in a fasted state on physiological responses. However, it had several limitations, including a small sample size and recruiting only men as participants. Additionally, we were unable to measure postprandial levels of parameters before and after exercise.

Conclusion

We found that the work-to-rest ratio in fasted-state SIE protocols may influence heart rate, diastolic blood pressure, and immune response. However, it does not appear to induce hypoglycemia, lactate production, or protein degradation. Athletes and coaches may benefit from fasting and manipulating the intensity of SIE protocols. Finally, we suggest conducting a similar protocol in females, with comparisons to postprandial physiological responses.

Declarations

Funding

This research did not receive specific funding.

Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

Code of Ethics

This study is approved by the Ethics Committee in Biomedical Research, University of Guilan (IR.GUILAN.REC.1403.050).

Author Contributions

E.MS: performing protocol, data collection, writing the draft; A. D: study design, supervision, revision of manuscript; M. E: study design, supervision, data analysis, writing and revising the draft.

Artificial Intelligence

We have not used any AI tools or technologies to prepare this manuscript.

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