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Evaluating bioenergetic pathway contributions from single to multiple sprints

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This study aims to investigate the changes in bioenergetic pathway contributions during repeated sprint exercises with an increasing number of repetitions. Twelve male amateur soccer players executed a single 20 m sprint and three repeated-sprint protocols (5 × 20 m, 10 × 20 m, 15 × 20 m with 15-second rest intervals), analyzing oxidative, glycolytic, and ATP-PCr energy pathways using the PCr-LA-O2 method. Findings revealed a significant decline in energy expenditure and performance outputs as the number of sprint repetitions increased. While the oxidative and ATP-PCr pathways' energy contributions significantly rose with more sprints, the glycolytic pathway's contribution notably increased only up to the 10 × 20 m protocol, then stabilized. Although the ATP-PCr pathway's energy contribution decreased slightly from sprints 1–5 to 11–15, it remained significantly higher than the oxidative and glycolytic pathways throughout. Initially, glycolytic contribution surpassed oxidative in sprints 1–5, equaled it in sprints 6–10, and fell below in sprints 11–15. Glycolytic activity, a major energy source initially (about 36%), diminished substantially with more sprints (below 7% in the 15th sprint). This indicates that the decrease in non-mitochondrial pathway energy, particularly glycolytic, outstrips the aerobic system's increased tolerance. These findings offer physiological insights into the relationship between performance decrement and bioenergetic metabolism in repeated sprints.

Keywords Bioenergetic, ATP, Oxidative, Glycolytic, Lactic acid

Repeated Sprint Ability (RSA) is a key fitness component for success in team sports and is characterized by short, maximal-intensity sprints followed by brief recovery periods[1–3]. Repeated sprint exercises are commonly used both as a training method and as a testing tool, as they closely mimic the dynamics of field-based sports[4,5]. RSA protocols offer a valuable model for studying the mechanisms behind performance decline during maximal-intensity efforts with short recovery intervals[6–8]. Recent studies have focused on how training strategies, recovery intervals, repetition numbers, and supplementation influence energy metabolism during RSA[9–12].

Understanding the contributions of different energy systems during exercise has long been of interest to scientists and coaches, as these findings help inform training prescriptions[3,4,7,12,13]. However, research into energy metabolism during RSA has only gained attention in the past decade, despite earlier muscle biopsy studies conducted in the 1990s[13–15]. More recent findings suggest that RSA-based exercises are highly efficient for many sports, as they engage all three energy pathways even in low-volume training sessions[1,3,11,16]. RSA protocols maximize anaerobic power by breaking down high-energy phosphates and enhancing glycolytic enzyme activity while also activating the oxidative system during recovery periods[7,12,13].

As the number of sprints increases without sufficient recovery, performance decline becomes inevitable, particularly in athletes with high initial power levels[16,17]. Indirect evidence points to the role of anaerobic energy pathways in maintaining repeated sprint performance, but this comes at the cost of metabolite accumulation and energy store depletion[13,16,18]. While research has explored the effects of factors like repetition number, sprint distance, training history, and supplementation on energy metabolism[6,8,11,17], the exact point at which fatigue mechanisms significantly affect muscle performance remains unclear.

There is a well-established but complex relationship between the benefits of increased ATP production from the glycolytic pathway during RSA and the potential negative effects of metabolite accumulation, particularly hydrogen ions (H^+) and inorganic phosphate (Pi). Previous studies indicate that although lactate

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and H^+accumulation suggest greater ATP production from glucose or glycogen, this does not always improve sprint performance[19,20]. In fact, while lactate can act as a buffer, delaying acidosis, Pi accumulation and a reduced capacity for ATP resynthesis are believed to play a more significant role in RSA-induced fatigue. For instance, glycolytic contributions have been shown to drop sharply during repeated sprints, decreasing from approximately 40% in the first sprint to as little as 9% in the final sprint of a 10×6-second protocol with 30-second rest intervals[1]. This sharp decline, often linked to metabolite buildup and impaired ATP production, raises questions about when glycolytic contributions become insufficient to sustain performance. The interplay between neuromuscular fatigue, muscle glycogen depletion, and Pi accumulation further complicates this scenario[19,21]. Therefore, this study aims to investigate how energy system contributions shift as sprint repetitions increase, offering new insights into the complex interactions between metabolic pathways and their impact on overall performance.

Methods

Participants

Twelve amateur male soccer players (age = 20.3 ± 1.5 years, weight = 69.4 ± 4.3 kg, height = 174 ± 5.4 cm) volunteered to participate in this study. Participants trained regularly (6–9 h competitive soccer training per week) for the last three years. Participants were informed about the possible risks and benefits and provided their informed written consent. Tests were conducted according to the principles of the Declaration of Helsinki and the ethics committee of the XXX Faculty, XXX University (ID: E-70400699-000-2200131871; Date: 28.04.2022) approved the entire study design. Participants were instructed to maintain their regular diet and not to perform strenuous exercise during the study period. Exclusion criteria were the following: contraindications to all-out effort testing, having any orthopedic injury, taking supplementation, and undergoing medical treatment.

Repeated sprint protocols

All testing sessions were conducted at the same time of day, between 16:00 and 18:00, to minimize the effects of diurnal performance variations. Prior to testing, participants completed a standardized warm-up consisting of 10 min of jogging and stretching, followed by three 5 m acceleration efforts. Long-duration high-intensity actions were minimized to avoid early fatigue or blood lactate accumulation. The tests began three minutes after completing the warm-up.

A single sprint of 20 m and three repeated-sprint protocols (5×20 m, 10×20 m, and 15×20 m, with 15-second rest intervals) were used in this study. On the first test day, participants' maximal sprint times over 20 m were measured twice, with a 3-minute rest interval between trials (Single 20 m sprint test). The better (shorter) sprint time was used to determine each participant's criterion score. Participants were required to achieve at least 95% of this criterion time in their first sprint. If they failed to meet this requirement, the test was terminated, and after a 5-minute rest, the test was restarted[22].

Lighted photocell gates were positioned at the start and finish of the sprints. Athletes were instructed to monitor a 3-second countdown via the light system and begin sprinting once the final (green) light appeared. After crossing the finish gate, participants returned to the starting position (50 cm behind the start gate) to prepare for the next sprint. Repeated-sprint sessions were performed in a randomized, counterbalanced order, with 3–4 days separating each session.

Performance metrics, such as total time and the percentage of speed decrement, were calculated using the formulas provided by Glaister et al. (2008):

Percentage of speed decrement = (100 × (total sprint time ÷ ideal sprint time)) – 100.

Total sprint time = sum of sprint times from all sprints.

Ideal sprint time = the number of sprints × fastest sprint time[23].

Participants performed a general standardized warm-up protocol of 10 min composed of jogging and stretching followed by three 5 m acceleration efforts. Long-duration high-intensity actions were minimized to avoid blood lactate accumulation *[BLa]* and any fatigue effects. The tests started three min following the warm-up.

Anthropometric measurements were conducted using standardized methods on the first test day. The height was measured with a portable stadiometer (Holtain Ltd., Crosswell, Crymych, Dyfed, United Kingdom). Body mass and body fat were assessed using air displacement plethysmography (Bod Pod, Cosmed; Chicago, IL, USA) calibrated according to the manufacturer's guidelines. A breath-by-breath oxygen consumption (VO_2) measurement was carried out using a portable metabolic gas analyzer (K5b^2, Cosmed, Rome, Italy) 10 min pre-exercise, during the test, and 15 min post-exercise. Pre-exercise and in the 1st, 3rd, 5th, 7th, and 10th min post-exercise, blood lactate concentration was measured from capillary blood samples drawn from the fingertip of the left hand using a portable hand analyzer (Lactate Pro, Arkray, Japan). The laboratory temperature and humidity were kept constant during all test days at ~22 °C and ~45%.

Calculation of the energy pathway contributions

The contribution of oxidative, glycolytic, and ATP-PCr energy pathways was calculated by the PCr-LA-O_2method[24,25]. Oxygen uptake ($VO_{2)}$ was measured during the rest (10 min), exercise, and recovery (15 min) phases using a portable gas-exchange system in breath-by-breath mode. Before each test, the portable metabolic gas analyzer was calibrated according to the manufacturer's guidelines. Blood lactate concentrations were measured from capillary blood samples drawn from the fingertip of the left hand using a portable hand analyzer. Blood samples (20 µL) were collected at rest and following the exercises (1st, 3rd, 5th, 7th, and 10th min of recovery) to determine peak [BLa]. The delta lactate concentration (Δ[BLa]) was determined by the

difference between peak and baseline values. To avoid any possible effects of perspiration on the measurements, the fingertip was cleaned with alcohol and dried immediately before each measurement.

The oxidative pathway contribution was calculated from VO_2 above resting levels during the tests. The resting VO_2 levels were determined before all tests, and the average of the last five minutes of the 10 min measurement was accepted. Accordingly, VO_2 indicating the oxidative pathway contribution during the tests was calculated as the area under the curve of actual VO_2 minus the resting VO_2.

$$\text{Oxidative contribution}(kj) = \frac{[\text{actual } VO_2(ml) - \text{resting } VO_2(ml)]}{1000} \times 20.9 \quad (1)$$

The glycolytic system contribution was calculated from the Δ[BLa] (peak BLa minus resting BLa) with the O_2-lactate equivalent of 3.0 ml/mM/kg (assuming that the accumulation of 1 mM in lactate was equivalent to 3 ml O_2per kilogram of body mass)[26]. For multiple sprint sets, the glycolytic contribution was calculated cumulatively. For example, if the glycolytic contribution was 50 kJ for the 5-sprint protocol and 60 kJ for the 10-sprint protocol, the contribution for sprints 6–10 was determined to be 10 kJ. The same approach was applied to the 11–15 sprint set.)

$$\Delta[\text{BLa}]\ (\text{mM}) = \text{Peak BLa}\ (\text{mM}) - \text{Resting BLa}\ (\text{mM}) \quad (2)$$

$$\text{Glycolytic contribution}(kj) = \frac{[\Delta[BLa](mM) \times \text{body mass}(kg) \times 3(ml)]}{1000} \times 20.9 \quad (3)$$

The ATP-PCr pathway contribution was calculated by bi-exponential curves established to observe the fast and slow components of excess post-exercise oxygen consumption (EPOC) kinetic using OriginPro software (version 2019b; OriginLab, Northampton, MA, USA). The fast phase of EPOC (actual VO_2 – slow phase VO_2) was utilized as the representative of the ATP-PCr pathway contribution since ATP-PCr resynthesis occurs in this phase. Additionally, the energy contribution during the breaks was attributed to the ATP-PCr pathway since considering that rest intervals are predominantly devoted to the repayment of PCr stores. However, we assumed that the post-exercise replenishment of PCr stores was attributable to the oxidative system, neglecting a possible minor contribution of the glycolytic system to PCr resynthesis and VO_2 during the fast phase of EPOC totally serves the replenishment of PCr stores, neglecting the VO_2 consumed to rebind to myoglobin.

$$VO_2(t) = VO_2baseline + A1\left[e^{\frac{-(t-td)}{\tau 1}}\right] + A2\left[e^{\frac{-(t-td)}{\tau 2}}\right] \quad (4)$$

$$EPOC fast\, VO_2 = A1 \times \tau 1 \quad (5)$$

$$ATP - PCr\, contribution(kj) = EPOC fast\, VO_2 + (number\, of\, breaks \times integral\, of\, VO_2(EPOC_15_sec)) \times 20.9 \quad (6)$$

$EPOC_{fast}$ is ATP-PCr pathway contribution calculated from the oxygen kinetic during the fast component of EPOC, $VO_{2(t)}$ is the oxygen uptake at time t, $VO_{2baseline}$ is the asymptotic y-value of the curve, A is the amplitude, td is the time delay, τ is the time constant, $VO_{2(EPOC_15_sec)}$is the integral value of the curve for the first 15 s (recovery period between the sprints), and 1 and 2 denote the fast and slow components, respectively[12,27–29].

The bi-exponential model presented the time constants of 30.7 ± 3.7, 38.4 ± 5.1, 40.4 ± 6.6, and 41.9 ± 5.1 and the amplitude values were 1706 ± 547, 2569 ± 661, 2957 ± 505, and 2962 ± 556 for single20m, 5 × 20 m, 10 × 20 m, and 15 × 20 m protocols, respectively. Mean R^2 values for all protocols were above 0.90. For each sprint protocol (5 × 20 m, 10 × 20 m, and 15 × 20 m), the energy contributions of the oxidative, glycolytic, and ATP-PCr pathways were calculated cumulatively. For example, if the glycolytic contribution was measured as 50 kJ for the 5-sprint protocol and 60 kJ for the 10-sprint protocol, the glycolytic contribution for sprints 6–10 was determined to be 10 kJ (i.e., the difference between the 10-sprint and 5-sprint protocols). Similarly, for the 15-sprint protocol, the glycolytic contribution for sprints 11–15 was determined by subtracting the total glycolytic contribution of the 10-sprint protocol from that of the 15-sprint protocol. This cumulative approach was applied consistently across all energy systems (oxidative, glycolytic, and ATP-PCr pathways), being allowed to assess the incremental energy demands for each set of sprints. Liter oxygen values obtained from each energy pathway were presented in kilojoules by multiplying by 20.9 and expressed as absolute terms and relative percentages of total metabolic work.

Statistical analyses

The data are reported as mean and standard deviation. Data normality was verified using the Kolmogorov–Smirnov test. The assumptions of sphericity were assessed by Mauchly's test. Whenever an assumption was violated, the Greenhouse–Geisser correction if the epsilon (ε) value was < 0.75 and the Huynh-Feldt correction if ε was > 0.75 were applied on the degree of freedom. A one-way analysis of variance for repeated measures was used to compare the variables related to different repeated sprint protocols. When a difference was found, a Bonferroni post hoc test was applied. The data were analyzed using the Statistical Package for the Social Sciences version 24.0 (IBM Corp., Armonk, NY, USA). The alpha level was set at 0.05 for all analyses.

Results

The sprint times recorded during 5 × 20 m, 10 × 20 m, and 15 × 20 m RSA protocols are displayed in Fig. 1. The highest performance outputs (shortest sprint times) were recorded during the first sprints and performance outputs were significantly greater than the values attained in the next sprints. The mean and slowest sprint times

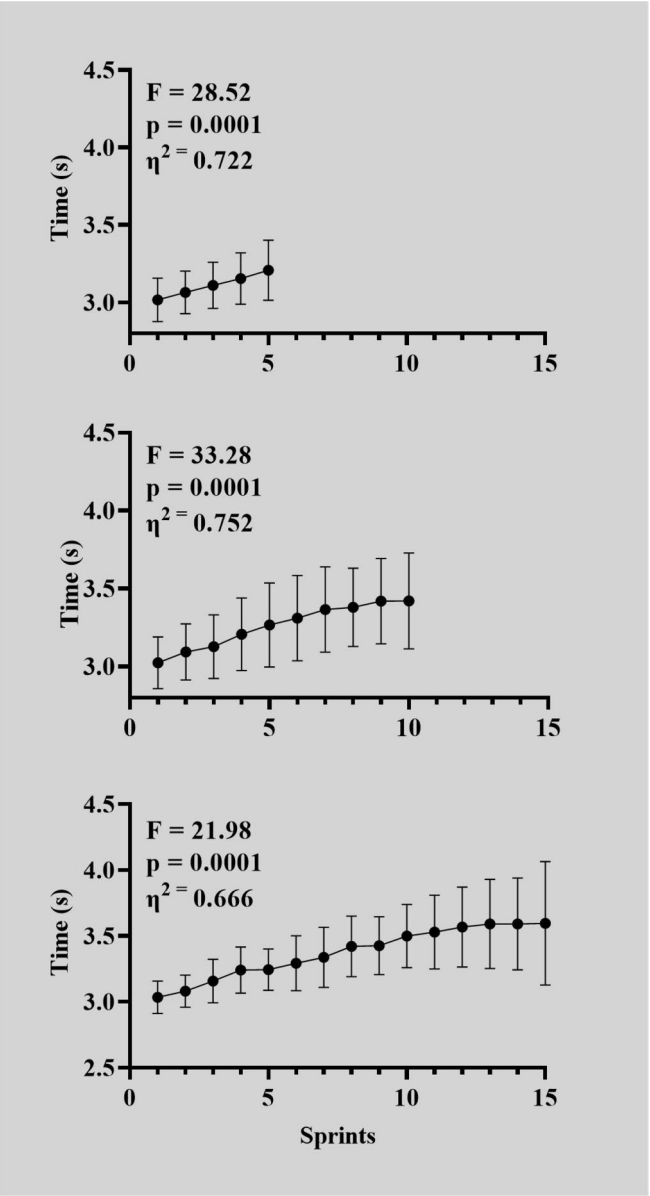

Fig. 1. Sprint performance during 5 × 20 m, 10 × 20 m, and 15 × 20 m RSA protocols.

were significantly different among the four protocols and sprint times significantly increased as the number of repetitions augmented (Table 1). TEE was significantly increased whereas EE per sprint was significantly decreased as the number of repetitions augmented. About EE per sprint, the P_{dec} (%) calculated from the 5 × 20 m, 10 × 20 m, and 15 × 20 m were significantly different among the three protocols and significantly increased as the number of repetitions augmented (Table 1).

Regarding absolute energy system contribution during Single20m, 5 × 20 m, 10 × 20 m, and 15 × 20 m RSA protocols, oxidative and ATP-PCr pathway's contributions were significantly different among the four protocols and significantly increased as the number of repetitions augmented. However, glycolytic contribution significantly increased from the Single20m protocol to the 10 × 20 m protocol but remained unchanged between 10 × 20 m and 15 × 20 m protocols (Table 1). In regard to relative energy system contribution, oxidative contribution was significantly different among the four protocols and significantly increased as the number of repetitions augmented. However, glycolytic contribution was significantly greater in the 5 × 20 m protocol than the 10 × 20 m and 15 × 20 m protocols and glycolytic contribution was significantly greater in the Single20m protocol than the 15 × 20 m protocol, but there was no significant difference between other pairwise comparisons. ATP-PCr pathway contribution was significantly lowest in the 5 × 20 m protocol and significantly increased from the 5 × 20 m protocol to the 15 × 20 m protocol as the number of repetitions augmented (Table 1).

Figure 2 shows the absolute energy system contribution during sprints. Oxidative contribution during sprints 1–5 was lower than in sprints 6–10 and sprints 11–15, whereas glycolytic contribution during sprints 1–5 was higher than in sprints 6–10 and sprints 11–15, however, no difference in the ATP-PCr pathway contribution.

	Single20m	5 × 20 m	10 × 20 m	15 × 20 m	F	*p*
Oxidative (kj)	1.9 ± 0.6	14.4 ± 3.5[a]	33.8 ± 4.9[ab]	55.6 ± 6.1[abc]	497.3	< 0.001
Glycolytic (kj)	11.2 ± 4.9	48.8 ± 10.1[a]	60.1 ± 8.2[ab]	63.1 ± 10.5[ab]	144.6	< 0.001
ATP-PCr (kj)	21.7 ± 7.6	75.9 ± 23.7[a]	142.2 ± 29.6[ab]	199.5 ± 46.1[abc]	126.6	< 0.001
TEE (kj)	34.9 ± 8.4	139.1 ± 26.8[a]	236.1 ± 30.6[ab]	400.5 ± 45.6[abc]	280.4	< 0.001
EE per sprint	34.9 ± 8.4	27.8 ± 5.4[a]	23.6 ± 3.1 [ab]	21.2 ± 3.2 [ab]	26.82	< 0.001
Oxidative (%)	5.6 ± 1.2	10.3 ± 1.1[a]	14.3 ± 1.3[ab]	17.6 ± 1.4[abc]	216.1	< 0.001
Glycolytic (%)	32.7 ± 12.5	36.2 ± 8.9	26.1 ± 6.0[b]	20.3 ± 5.0[abc]	14.22	< 0.001
ATP-PCr (%)	61.7 ± 12.2	53.5 ± 8.4[a]	59.6 ± 6.3	62.1 ± 5.7[b]	4.57	0.026
Mean time (s)	3.00 ± 0.15	3.11 ± 0.15[a]	3.26 ± 0.23[a]	3.38 ± 0.22[abc]	31.27	< 0.001
Slowest time (s)	3.00 ± 0.15	3.21 ± 0.19 [a]	3.44 ± 0.29 [ab]	3.71 ± 0.45[abc]	21.77	< 0.001
P_{dec} (%)	-	3.10 ± 1.24	7.82 ± 3.83[b]	11.1 ± 4.57[bc]	30.65	< 0.001

Table 1. Metabolic outputs of Single20m, 5 × 20 m, 10 × 20 m, and 15 × 20 m RSA protocols. TEE: total energy expenditure; P_{dec}: Percentage of performance decrement; [a] significantly different from Single20m; [b] significantly different from 5 × 20 m; [c] significantly different from 10 × 20 m.

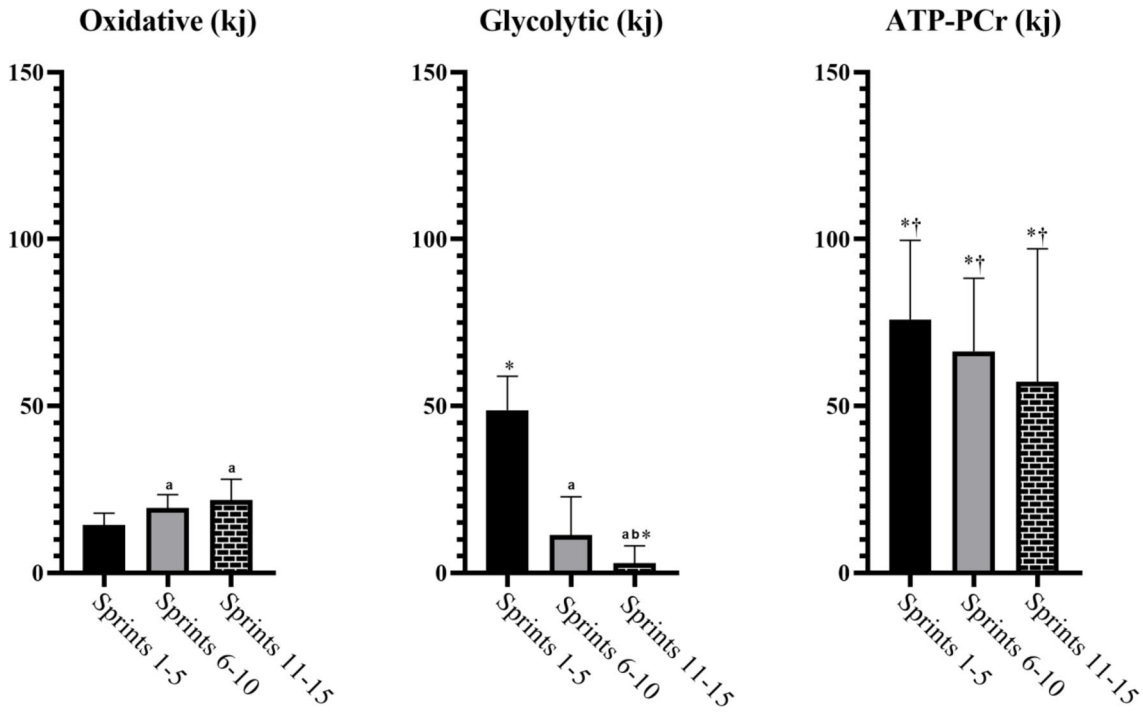

Fig. 2. Absolute energy contribution (kj) during sprints 1–5, sprints 6–10, and sprints 11–15. ***Note***: [a] significantly different from sprints 1–5 for the same energy pathway; [b] significantly different from sprints 6–10 for the same energy pathway; [*] significantly different from oxidative for the same sprints; [†] significantly different from glycolytic for the same sprints.

Additionally, the glycolytic contribution during sprints 1–5 was greater than the oxidative contribution, no difference during sprints 6–10, and the glycolytic contribution during sprints 11–15 was lower than the oxidative contribution. ATP-PCr pathway contribution was greater than oxidative and glycolytic contributions during all sprint parts.

Figure 3 shows the relative energy system contribution during sprints. Oxidative contribution was significantly different among sprints 1–5, sprints 6–10, and sprints 11–15 and significantly increased as the number of repetitions augmented. Glycolytic contribution significantly decreased from sprints 1–5 to sprints 6–10 but remained unchanged between sprints 6–10 and sprints 11–15. On the contrary, ATP-PCr pathway contribution significantly increased from sprints 1–5 to sprints 6–10 but remained unchanged between sprints 6–10 and sprints 11–15. Additionally, Fig. 4 shows that TEE significantly decreased from sprints 1–5 to sprints 6–10 but remained unchanged between sprints 6–10 and sprints 11–15.

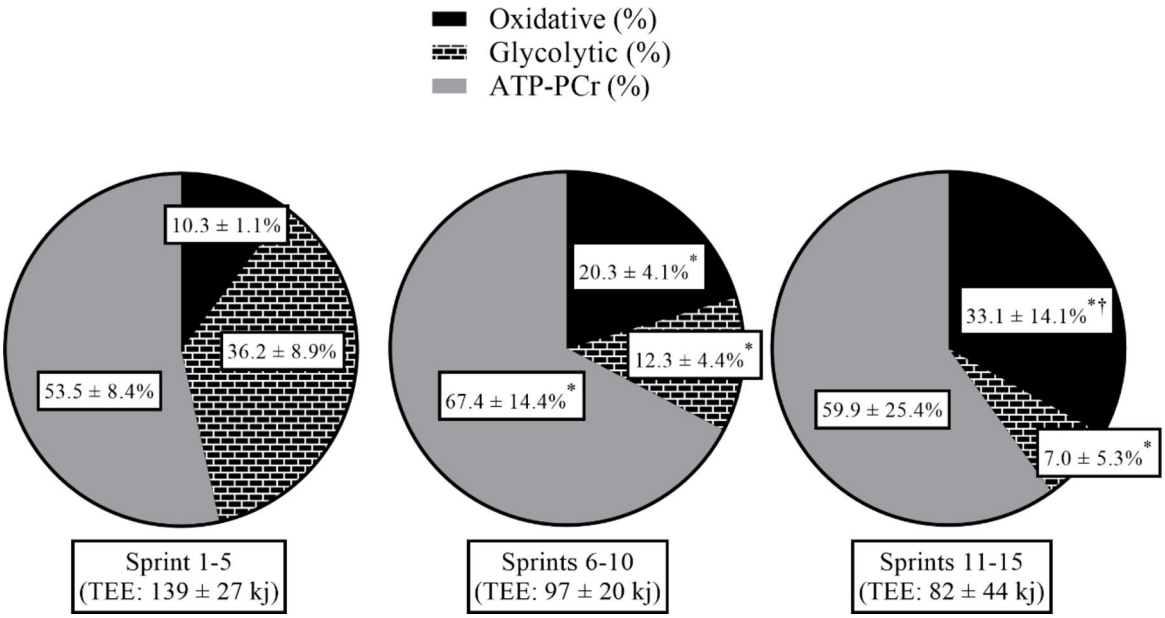

Fig. 3. Relative energy contribution (%) during sprints 1–5, sprints 6–10, and sprints 11–15. **Note:** TEE: total energy expenditure; * significantly different from sprints 1–5 for the same energy pathway; † significantly different from sprint 6–10 for the same energy pathway.

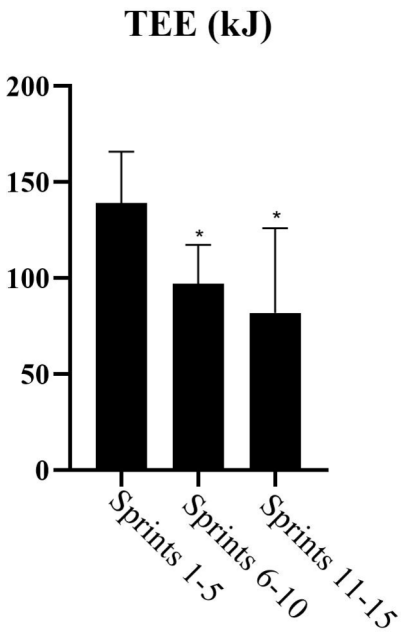

Fig. 4. Total energy expenditure during sprints 1–5, sprints 6–10, and sprints 11–15. ***Note***: * significantly different from sprints 1–5.

Discussion

This study is the first to compare changes in energy metabolism during sprint exercises as the number of repetitions increases. The main findings revealed that both energy expenditure per sprint and performance outputs significantly decreased with more repetitions. During RSA protocols, the absolute contributions of the oxidative and ATP-PCr pathways significantly increased with additional sprints, while the glycolytic contribution increased only up to the 10 × 20 m protocol and then plateaued between the 10 × 20 m and 15 × 20 m protocols. Additionally, the oxidative contribution during sprints 1–5 was lower than during sprints 6–10 and 11–15, while the glycolytic contribution showed the opposite pattern, being higher in sprints 1–5 and decreasing in later sprints. No significant changes were observed in the ATP-PCr pathway across these stages. The findings

confirmed that as the number of repetitions increases, the contribution of non-mitochondrial pathways, especially glycolysis, decreases, while the aerobic system becomes more dominant. This imbalance between the reduced energy supply from non-mitochondrial pathways and the aerobic system's capacity to compensate forms the physiological basis for the observed performance decline.

As expected, the contribution of the ATP-PCr system decreased from the first sprints to the last in our protocol, although it remained the dominant system in each sprint. Our findings showed that the greatest reduction occurred in the glycolytic pathway. During the first 5 sprints, the glycolytic pathway contributed 48.8 kJ (36.2% of total energy), but this dropped to approximately 3 kJ (~7%) during the final 5 sprints, a reduction far beyond what the aerobic system could compensate for. On the other hand, while the oxidative system is typically considered negligible in short-duration tasks like sprints[4,30], we found a significant oxidative contribution during the final 5 sprints (approximately 22 kJ, or 33%), which exceeded the glycolytic contribution in the later stages of the protocol.

The capacity for PCr resynthesis, muscular buffering mechanisms, and aerobic fitness can help delay the negative effects of glycolytic by-products such as H^+accumulation and lactate[16,18,31]. The removal of intracellular $H^+$and lactate during repeated sprints is facilitated by intracellular buffering systems and membrane transporters, particularly monocarboxylate transporters[32]. Interestingly, increased $H^+$and lactate accumulation serves as an effective stimulus to upregulate the number of these transporters[19,32]. Therefore, the accumulation of these metabolites is necessary to enhance the body's capacity to clear them, which is activated through specific training. As highlighted by the complex metabolic interactions in repeated sprints, improving performance likely depends on dominant phosphagen system activity during early sprints, optimal replenishment of high-energy phosphates during short recovery periods, and effective glycolytic contribution during later sprints. Thus, the negative effects of intramuscular acidosis caused by H^+ and lactate accumulation can be mitigated.

Research has shown that muscle buffering capacity and changes in muscle and blood pH significantly influence performance[6,8,13]. In our study, the first 5 sprints exhibited the highest glycolytic activity (48.8 kJ and 36.2%), leading to a smaller performance decrement (3.1%). As predicted[1], this early anaerobic glycolytic activity contributed to a smaller performance drop compared to later sprints, where the glycolytic contribution fell to 11.3 kJ (12.3%), and the performance decrement increased to 4.7% during sprints 6–10. This suggests that minimal glycolytic activity and maximal phosphagen system contribution during early sprints, combined with efficient replenishment of high-energy phosphates during recovery, can prevent a significant rise in muscle and blood H^+ levels. Moreover, contrary to the common belief that RSA and $VO_{2max}$are strongly correlated[33,34], recent literature indicates only moderate-to-low relationships between these concepts[35]. While improved aerobic capacity undoubtedly enhances the replenishment of high-energy phosphates and myoglobin stores during short breaks, the metabolic demand is driven primarily by the amount of high-energy phosphates consumed during each sprint. Thus, optimizing ATP-CP consumption during sprints may allow for better utilization of oxidative capacity during recovery periods.

Sprint exercises rapidly deplete intramuscular phosphocreatine (PCr) and increase inorganic phosphate levels, which then activate anaerobic glycolysis[31]. In studies involving 10 × 6-second maximal cycling efforts, anaerobic glycolysis was responsible for about 40% of the ATP during the first sprint, but this contribution dropped below 10% by the final sprint[1]. In our 15 × 20 m repeated sprint protocol, we observed an even more dramatic reduction, with glycolytic contribution dropping from 36.2% during the first 5 sprints to about 7% during the last 5 sprints. This rapid decline in glycolytic activity during later sprints was accompanied by a significant performance drop. Despite this, glycolytic ATP production is known to enhance peak sprint performance during early sprints, which highlights the paradox of whether maximal glycolytic activity improves overall RSA performance[20,31,36]. There appears to be a need for further exploration of the balance between energy gained from early maximal glycolytic activity and the performance decrement caused by acidosis and other factors in later sprints.

Muscle fatigue during repeated sprint activities (RSA) is a multifactorial process involving complex interactions between metabolic, neuromuscular, and biochemical mechanisms[16,17]. While cellular acidosis has traditionally been seen as a major contributor to fatigue, more recent evidence highlights other significant factors, such as phosphate accumulation, muscle glycogen availability, and neuromuscular impairments, all of which contribute to performance decline[19,21,37].

Phosphate accumulation, particularly the rise in inorganic phosphate, is known to interfere with excitation-contraction coupling by disrupting calcium release from the sarcoplasmic reticulum and reducing muscle fiber calcium sensitivity[19,36,38]. This impairs force production, particularly during high-intensity efforts involving fast-twitch fibers, which are heavily recruited in RSA. In our study, as the number of sprints increased from 5 × 20 m to 15 × 20 m, we observed a significant decline in performance outputs. This supports the idea that Pi accumulation contributes to reduced ATP resynthesis and subsequent impairments in muscle function, especially in later sprints.

Muscle glycogen availability and its utilization also play a critical role in RSA-induced fatigue. Glycogen serves as an essential energy source for both anaerobic and aerobic pathways, and its depletion—or the reduced ability to utilize available glycogen—limits ATP resynthesis, leading to decreased endurance and performance[17,20,37]. In repeated sprints, glycogen stores may not be fully depleted, but the efficiency of glycogen use may be impaired, particularly in fast-twitch fibers that rely heavily on glycolysis. Our findings reflect this, as we observed a significant decrease in glycolytic contribution from sprints 1–5 to sprints 6–10, which then plateaued from sprints 6–10 to sprints 11–15. This suggests that both glycogen availability and its utilization may play a role in the reduction of glycolytic activity in later sprints.

Neuromuscular fatigue, defined as a reduced ability of the central nervous system to activate muscles efficiently, is another factor contributing to RSA fatigue. High-intensity repeated efforts reduce motor unit recruitment and firing frequency, which in turn limits force production[16,17]. Neuromuscular impairments can also amplify metabolic stress during repeated sprints by increasing the reliance on glycolysis and accelerating

the accumulation of metabolic byproducts like Pi and H^+. Additionally, extracellular potassium levels, which rise during intense exercise, can further disrupt muscle excitation and contraction processes, contributing to muscle fatigue[17,39]. Although our study primarily focused on metabolic contributions, the observed performance decline after the initial sprints suggests that neuromuscular fatigue, alongside other mechanisms such as ion imbalance and metabolite accumulation, likely plays a significant role in later stages of RSA.

Limitations

This study contains some limitations in calculating the contributions of the glycolytic and ATP-PCr energy pathways. Lactate concentrations were determined at resting and following the protocols, therefore, it did not allow for observation of data on lactate kinetics during each sprint. A possible methodological limitation of this study is that the oxygen uptake during the breaks was attributed to ATP and PCr replenishment. However, it is well known that there are several factors, especially depleted myoglobin stores, related to the demand for oxygen uptake, thus, this conjecture possibly led to an overestimation of the contribution of the ATP-PCr pathway.

Additionally, as discussed earlier in this manuscript, using the La-PCr-O_2 method has some inherent limitations when compared to methods like muscle biopsy. While muscle biopsies can provide direct insight into PCr depletion and lactate accumulation, they pose their own challenges. For instance, the time delay in obtaining muscle samples (typically 8–10 s post-exercise) can result in missing the early recovery phases, where significant PCr resynthesis might already have occurred. Moreover, muscle biopsies are usually limited to a single muscle group, which may not fully capture the metabolic contributions of other muscles involved in sprint activity, such as respiratory and upper limb muscles, which can account for a significant portion of total energy expenditure.

On the other hand, the La-PCr-O_2 method allows continuous tracking of energy system contributions throughout the entire exercise and recovery phases. Although we cannot precisely track the specific cellular use of oxygen (e.g., PCr resynthesis or binding to myoglobin), this method offers valuable insight into overall metabolic responses. In addition, irregular breathing patterns during recovery periods and the delayed metabolic response complicate the separation of certain metabolic events, and this limitation applies to both biopsy and gas-exchange methods. Despite these limitations, the present approach allowed us to observe the changes in energy metabolism during consecutive sprints due to the accumulation of repetitions, providing a comprehensive understanding of the dynamics of energy system contributions.

Conclusion

This study provides a comprehensive analysis of how bioenergetic pathway contributions change during repeated sprint activities as the number of repetitions increases. We observed a progressive decline in energy expenditure per sprint and performance outputs, with oxidative and ATP-PCr pathways becoming increasingly dominant as repetitions augmented. While glycolytic activity initially played a major role, contributing approximately 36% during the first five sprints, its contribution fell below 7% by the 15th sprint, highlighting its limited capacity to sustain performance over time. Despite a slight decrease in ATP-PCr pathway contribution from sprints 1–5 to sprints 11–15, it remained consistently higher than the oxidative and glycolytic contributions across all stages. The inability of the aerobic system to fully compensate for the reduced glycolytic energy supply underscores the physiological challenges of maintaining high-intensity performance in repeated sprints. These findings contribute to a deeper understanding of energy system dynamics in RSA and suggest that future research should focus on optimizing the balance between these pathways to delay fatigue and improve performance sustainability.

Data availability

The data supporting this study's findings are available upon reasonable request with corresponding authors.

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References

1. Girard, O., Mendez-Villanueva, A. & Bishop, D. Repeated-sprint ability—part I. *Sports Med.* **41** (8), 673–694 (2011).
2. Gharbi, Z. et al. Aerobic and anaerobic determinants of repeated sprint ability in team sports athletes. *Biology Sport*. **32** (3), 207–212 (2015).
3. Turner, A. N. & Stewart, P. F. Repeat sprint ability. *Strength. Conditioning J.* **35** (1), 37–41 (2013).
4. Bishop, D. et al. The validity of a repeated sprint ability test. *J. Sci. Med. Sport*. **4** (1), 19–29 (2001).
5. Spencer, M. et al. Physiological and metabolic responses of repeated-sprint activities. *Sports Med.* **35** (12), 1025–1044 (2005).
6. Ulupınar, S., Hazır, T., Kin, A. & İşler *The Contribution of Energy Systems in Repeated-Sprint Protocols: The Effect of Distance, Rest, and Repetition*p. 1–7 (Research Quarterly for Exercise and Sport, 2021).
7. Milioni, F. et al. Energy systems contribution in the running-based anaerobic sprint test. *Int. J. Sports Med.* **38** (03), 226–232 (2017).
8. Mendez-Villanueva, A. et al. The recovery of repeated-sprint exercise is associated with PCr resynthesis, while muscle pH and EMG amplitude remain depressed. *PloS One*. **7** (12), e51977 (2012).
9. Spencer, M. et al. Performance and metabolism in repeated sprint exercise: effect of recovery intensity. *Eur. J. Appl. Physiol.* **103** (5), 545–552 (2008).
10. Gharbi, Z. et al. Effect of the number of sprint repetitions on the variation of blood lactate concentration in repeated sprint sessions. *Biology Sport*. **31** (2), 151–156 (2014).
11. McGinley, C. & Bishop, D. J. Influence of training intensity on adaptations in acid/base transport proteins, muscle buffer capacity, and repeated-sprint ability in active men. *J. Appl. Physiol.* **121** (6), 1290–1305 (2016).
12. Ulupınar, S. et al. Effects of sprint distance and repetition number on energy system contributions in soccer players. *J. Exerc. Sci. Fit.* **19** (3), 182–188 (2021).
13. Bogdanis, G. C. et al. Contribution of phosphocreatine and aerobic metabolism to energy supply during repeated sprint exercise. *J. Appl. Physiol.* **80** (3), 876–884 (1996).

14. Wadley, G. & Rossignol, P. L. The relationship between repeated sprint ability and the aerobic and anaerobic energy systems. *J. Sci. Med. Sport*. **1** (2), 100–110 (1998).
15. Dawson, B. et al. Changes in performance, muscle metabolites, enzymes and fibre types after short sprint training. *Eur. J. Appl. Physiol. Occup. Physiol.* **78** (2), 163–169 (1998).
16. Bishop, D., Girard, O. & Mendez-Villanueva, A. Repeated-sprint ability—part II. *Sports Med.* **41** (9), 741–756 (2011).
17. Mendez-Villanueva, A., Hamer, P. & Bishop, D. Fatigue in repeated-sprint exercise is related to muscle power factors and reduced neuromuscular activity. *Eur. J. Appl. Physiol.* **103** (4), 411–419 (2008).
18. Bishop, D. et al. Induced metabolic alkalosis affects muscle metabolism and repeated-sprint ability. *Med. Sci. Sports. Exerc.* **36** (5), 807–813 (2004).
19. Fitts, R. H. Muscle fatigue: the cellular aspects. *Am. J. Sports Med.* **24** (6_suppl), S9–S13 (1996).
20. Robergs, R. A., Ghiasvand, F. & Parker, D. *Biochemistry of exercise-induced Metabolic Acidosis* (American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2004).
21. Milioni, F. et al. *Effect of β-alanine Supplementation during high-intensity Interval Training on Repeated Sprint Ability Performance and Neuromuscular Fatigue* (Journal of applied physiology, 2019).
22. Spencer, M. et al. Physiological and metabolic responses of repeated-sprint activities: specific to field-based team sports. *Sports Med.* **35**, 1025–1044 (2005).
23. Glaister, M. et al. The reliability and validity of fatigue measures during multiple-sprint work: an issue revisited. *J. Strength. Conditioning Res.* **22** (5), 1597–1601 (2008).
24. Beneke, R. et al. How anaerobic is the Wingate Anaerobic Test for humans? *Eur. J. Appl. Physiol.* **87** (4), 388–392 (2002).
25. Artioli, G. G. et al. *Determining the contribution of the energy systems during exercise*. *JoVE (Journal Visualized Experiments)*, (61): p. e3413. (2012).
26. di Prampero, P. E. & Ferretti, G. The energetics of anaerobic muscle metabolism: a reappraisal of older and recent concepts. *Respir. Physiol.* **118** (2–3), 103–115 (1999).
27. Tortu, E., Hazir, T. & Kin-Isler, A. Energy System contributions in repeated Sprint tests: protocol and sex comparison. *J. Hum. Kinetics*. **92**, 87 (2024).
28. Latzel, R. et al. Energetic profile of the basketball exercise simulation test in junior elite players. *Int. J. Sports Physiol. Perform.* **13** (6), 810–815 (2018).
29. Panissa, V. L. et al. Is oxygen uptake measurement enough to estimate energy expenditure during high-intensity intermittent exercise? Quantification of anaerobic contribution by different methods. *Front. Physiol.* **9**, 868 (2018).
30. Perrey, S. et al. Neural and muscular adjustments following repeated running sprints. *Eur. J. Appl. Physiol.* **109** (6), 1027–1036 (2010).
31. Crowther, G. J. et al. Control of glycolysis in contracting skeletal muscle. I. turning it on. *Am. J. Physiology-Endocrinology Metabolism*. **282** (1), E67–E73 (2002).
32. Juel, C. Muscle pH regulation: role of training. *Acta Physiol. Scand.* **162** (3), 359–366 (1998).
33. Tomlin, D. L. & Wenger, H. A. The relationship between aerobic fitness and recovery from high intensity intermittent exercise. *Sports Med.* **31**, 1–11 (2001).
34. Brown, P., Hughes, M. & Tong, R. Relationship between VO^ sub 2max^ and repeated sprint ability using non-motorised treadmill ergometry. *J. Sports Med. Phys. Fitness*. **47** (2), 186 (2007).
35. Ulupınar, S. et al. *Low-to-moderate correlations between repeated Sprint ability and aerobic capacity: a systematic review and Meta-analysis*. *Strength. Conditioning J.*, : p. 101519. (2022).
36. Milioni, F. et al. Time Course of Recovery after Cycling repeated sprints. *Med. Sci. Sports. Exerc.* **53** (2), 413–420 (2021).
37. Billaut, F. & Bishop, D. Muscle fatigue in males and females during multiple-sprint exercise. *Sports Med.* **39**, 257–278 (2009).
38. Place, N. & Westerblad, H. *Metabolic Factors in Skeletal Muscle Fatigue, in Exercise Metabolism*p. 377–399 (Springer, 2022).
39. Lindinger, M. I. & Cairns, S. P. Regulation of muscle potassium: exercise performance, fatigue and health implications. *Eur. J. Appl. Physiol.* **121** (3), 721–748 (2021).

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Author contributions
Authors contributed to the concept and design (S. U., S. Ö., C. G., I. O., F. Ö., N. F. K., F. K., H. H. Y. and L. P. A.), acquisition of the data (S. U., H. H. Y., C. G., F. Ö. and S. Ö.), analysis (S. U. and C. G.) and interpretation (S. U., S. Ö., C. G., I. O., F. Ö., N. F. K., F. K., H. H. Y. and L. P. A.), drafting and revision (S. U., S. Ö., C. G., I. O., F. Ö., N. F. K., F. K., H. H. Y. and L. P. A.), final approval (S. U., S. Ö., C. G., I. O., F. Ö., N. F. K., F. K., H. H. Y. and L. P. A.) and agreement to be accountable (S. U., S. Ö., C. G., I. O., F. Ö., N. F. K., F. K., H. H. Y. and L. P. A.). The guarantor (S. U.) accepts full responsibility for the work and the conduct of the study, has access to the data and controls the decision to publish. The corresponding authors attest that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Competing interests
The authors declare no competing interests.

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