



Article Acute Effects of Padel Match Play on Circulating Substrates, Metabolites, Energy Balance Enzymes, and Muscle Damage Biomarkers: Sex Differences

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Abstract: This study aimed to analyze the effects of padel match play on circulating substrates, metabolites, energy balance enzymes, and muscle damage biomarkers and evaluate possible sexrelated differences. Twenty-two trained padel players (13 female and 9 male young-adult players) were recruited for this study in which simulated padel matches were analyzed. Circulating levels of substrates (glucose -BG- and triglycerides -TGs-), metabolites (creatinine -Cr- and urea), energy balance enzymes (lipoprotein lipase -LPL-), and muscle damage biomarkers (creatine kinase -CK-, lactate dehydrogenase -LDH-, and fatty acid-binding protein 3 -FABP-3-) were assessed both pre- and post-padel competition. Time analysis of padel matches reported a real time-total time ratio of 0.4. Moreover, players' mean heart rate during padel matches represented around 75% of their individual maximum value. Unaltered BG levels and a slight decrease in TGs were observed post-exercise. Cr, urea, LPL, CK, LDH, and FABP-3 levels increased after padel matches when total group was considered. Moreover, sex-related differences in Cr, CK, and LDH blood concentrations were found in both pre- and post-padel competition. According to our results, the padel competition could be defined as a low- or moderate-impact sport in which aerobic energy system contribution is prevalent although anaerobic metabolism also plays a key role in performing padel shots and other explosive actions. Considering that male and female players exercised at the same relative intensity during padel matches, sex differences found in muscle damage biomarkers could be due to the greater muscle mass in males.

Keywords: padel; physiological demands; energy supply; circulating markers; muscular disorders

1. Introduction

Padel could be defined as a new doubles racket sport since the first record of this sport practice date back to the mid-20th century. Despite this, padel has enjoyed a growing popularity during the last decades. According to the International Padel Federation (IPF), over 25 million people play padel in over 90 countries worldwide [1]. Similarly, padel has attracted considerable scientific interest in the last few years. To our best knowledge, since 2011, when the first article was published [2], more than 150 padel studies have been performed by different sports scientists who have focused on topics related to padel performance. A recent scoping review [3] showed that the most interesting research topics in padel are "match analysis", "training", "epidemiology of injuries", "biomechanical analysis", and "physiology and physical performance". In fact, several studies have been conducted under a physiological perspective to (a) describe and compare anthropometric and physical fitness attributes of padel players according to their competitive level, and



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). also to establish a functional anthropometric and physical profile [4]; (b) examine the fitness characteristics and to identify the influence of gender and practice experience between young amateur padel players [5,6]; (c) monitor heart rate variability (HRV) responses during a padel match and characterize them using linear and non-linear metrics; and (d) evaluate the responses of several myokines in trained players and to determine whether these responses were sex-dependent [7].

Although the physiological demands of padel are already well-known (match play oxygen consumption below 50% of VO_{2max} and mean HR between 80 and 85% of maximum have been previously reported) [2], there is a lack of information on the metabolic effects of a padel match play. Only few studies have reported the impact of padel on energy regulation or metabolic systems. Bartolomé et al. [8] evaluated the urinary excretion of seven essential trace minerals after a padel match. Although creatinine, arsenic, and selenium concentrations did not change, nickel, zinc, and copper urinary levels showed significant increases, whereas a decrease in lithium concentrations was also reported. As the authors stated, these exercise-induced responses failed to explain to what extent padel has an impact on the energy balance system. In another study, researchers assessed different biochemical and hematological markers after a padel match in professional players. Sexrelated differences in blood red cells count, hemoglobin, glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), lactate dehydrogenase (LDH), albumin, urea, and uric acid were found in resting conditions. These differences remained after padel match where significant increases in urea blood levels were also observed in female players [9]. Similar results were obtained by Pradas et al. [10], who determined the impact of a simulated padel match competition on hematological and urinary parameters. Moreover, no red blood cells were found in urine after padel match but a significant increase in micro-albuminuria was observed independently of sex.

Although these previous studies indicate that both inter-individual differences and exercise intensity variability are two of the main factors that could explain the lack of clear responses of these biomarkers, there is no doubt about the need for new studies to clarify these biochemical responses and where they are coherent to the physiological cost of padel competition. Thus, the main aim of this study was to analyze the effects of padel match play on circulating parameters including substrates, metabolites, energy balance enzymes, and muscle damage biomarkers. In addition, possible sex-related differences were evaluated.

2. Materials and Methods

2.1. Participants

A total of 22 trained padel players (13 female in early-mid follicular phase after menstruation, and 9 male young-adult players) with more than five years of experience in the professional circuit World Padel Tour participated voluntarily in this study. Subjects' characteristics are shown in Table 1. All participants gave their informed consent for inclusion prior to participation. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Department of Health and Consumption of the Government of Aragon, Spain (code: 21/2012; date: 19 December 2012).

Table 1. Padel players' characteristics.

| | Females | Males | Total | Sig. (CI = 95%) | Effect Size (d) |
|-------------------------|-----------------|----------------|----------------|-----------------|-----------------|
| Age (years) | 29.0 ± 3.8 | 25.2 ± 7.9 | 27.3 ± 6.2 | p = 0.169 | 0.61 |
| Height (cm) | 167.1 ± 5.6 | 176.6 ± 2.4 | 171.4 ± 6.5 | p < 0.001 | 2.20 |
| Weight (kg) | 60.7 ± 4.5 | 77.0 ± 6.5 | 68.0 ± 9.9 | p < 0.001 | 2.91 |
| $BMI(kg/m^2)$ | 21.7 ± 1.0 | 24.6 ± 1.8 | 23.0 ± 2.0 | p < 0.001 | 1.99 |
| Body fat (%) | 20.2 ± 2.1 | 13.3 ± 5.4 | 17.1 ± 5.2 | p = 0.001 | 1.68 |
| Muscle mass (%) | 37.1 ± 2.9 | 43.8 ± 1.8 | 40.1 ± 4.2 | p < 0.001 | 2.77 |
| VO_{2max} (L/min) | 2.89 ± 0.39 | 4.41 ± 0.53 | 3.57 ± 0.89 | p < 0.001 | 3.27 |
| HR _{max} (bpm) | 186.3 ± 7.8 | 188.3 ± 11.3 | 187.2 ± 9.3 | p = 0.636 | 0.21 |

BMI—body mass index; VO_{2max} —maximum oxygen consumption; HR_{max} —maximum heart rate measured in the graded exercise test; d—Cohen's d.

The calculations for sample size and power were based on urea responses to a padel match competition reported by a previous study [9]. Considering means and standard deviations found by these authors in both pre- and post-match), the a priori sample size calculation (G*Power v.3.1) with ES = 0.59 established that a sample of 19 would be sufficient to obtain a statistical power of 0.8 (p < 0.05). Therefore, our sample size of 22 allowed us to overcome a power of 84%.

2.2. Experimental Approach

Participants were evaluated in two separate testing sessions between 7 and 9 days apart. In the first session, subjects' body composition analysis was assessed (bioelectrical impedance, TANITA BC–418MA, Amsterdam, The Netherlands). Moreover, participants' maximum oxygen consumption (VO_{2max}) and maximum heart rate (HR_{max}) were assessed using an incremental running test on a treadmill (Pulsar HP Cosmos, Nussdorf, Germany) equipped with a gas analyzer (Oxycon Pro. Jaegger, Germany) and heart rate monitor (Cosmos, Nussdorf, Germany). After a warm-up period of 5 min of brisk walking (6 km/h), the initial speed was set at 8 km/h, increasing by 1 km/h every minute until volitional exhaustion. The treadmill slope was kept at 1%. VO_{2max} was defined following the ACSM criteria [11], whereas HR_{max} was determined as the highest HR value reached during the running test.

The second session consisted of participating in a simulated padel competition following the International Padel Federation rules. The participants were paired based on sex and performance level. All matches were played to the best of three sets; in case of six equal games, a tie-break was played. Before each match, players performed a standardized 15 min warm-up. Total playing time (TPT, full time of the match from the beginning to the end, considering the periods of game and rest), total resting time (TRT, sum of periods in which the ball was not in play), and real playing time (RPT, total playing time minus total resting time) were measured for each match. Moreover, players' HR was continuously recorded during the competition as average values over 5 s (Polar Team System, Kempele, Finland). This competitive event was held on outdoor courts with a relative environmental humidity and temperature of $45.7 \pm 7.3\%$ and 24.1 ± 7.1 degrees Celsius, respectively.

Both sessions were conducted between 9:00 a.m. and 12:00 a.m., and participants were instructed to avoid strenuous physical activity during the previous 24 h and to abstain from food (overnight fasting), caffeine, and alcohol 12 h before testing. Nevertheless, players were allowed to hydrate ad libitum during the competition (bottled mineral water).

2.3. Blood Sampling

In the second session, two 5 mL blood samples (pre- and post-padel competition) were drawn from the antecubital vein of each participant. Blood samples were collected in Vacutainer tubes (BD Vacutainer, Plymouth, UK) containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. The first sample was collected 90 min before the competition (fasting conditions), and the second was collected within 10 min after the matches. Blood samples were immediately put on ice and transferred to a laboratory for processing.

2.4. Hematological and Biochemical Assessment

Hematological parameters (hematocrit and hemoglobin) were determined using the Coulter counter model ACT Diff (Beckman Coulter Inc., Brea, CA, USA). On the other hand, substrates (glucose -BG- and triglycerides -TGs-), metabolites (creatinine -Cr- and urea), energy balance enzymes (lipoprotein lipase -LPL-), and muscle damage biomarkers (creatine kinase -CK- and lactate dehydrogenase -LDH-) were determined in whole blood by using spectrophotometric IFCC techniques (BTS-350 Semiautomatic Analyzer, Biosystems Diagnostics, Mylapore, India) [12]. Among these last biomarkers it is worth highlighting the assessment of fatty acid-binding protein 3 (FABP-3) using Simplex ProcartaPlex[®] for Luminex 200 System (ThermoFisher Scientific, Austin, TX, USA). Complete biochemistry

was processed at the laboratory of San Jorge University Hospital, with an Advia 1650 Chemistry Analyzer (Siemens, Berlin, Germany).

Lastly, and although no significant variations in hematocrit and hemoglobin were observed after exercise, changes in plasma volume were calculated following the methods of Dill and Costill [13]. Thus, blood levels of substrates (glucose and creatinine), metabolites (urea and uric acid), energy balance enzymes (FABP-3 and LPL), and muscle damage biomarkers (CK and LDH) were individually corrected according to the formula described by Matomäki [14].

2.5. Statistical Analysis

The data are expressed as mean \pm standard deviation (sd). The Shapiro–Wilk test was applied to test for a normal distribution of variables. Parametric and non-parametric tests (two-way ANOVA-time × sex interaction-, Wilcoxon signed-rank, and Mann–Whitney tests) were used where appropriate to determine both intragroup and intergroup differences considering pre- and post-padel competition time points. Moreover, effect size (ES) was calculated using d-value proposed by Cohen [15]. Thus, the ES was interpreted as trivial when d < 0.19; small when d = 0.20; medium when d = 0.50; and large when d = 0.80. For all tests, a *p*-value of <0.05 was used to indicate statistical significance.

3. Results

3.1. Participant's Characteristics and Cardiorespiratory Fitness

As it is shown in Table 1, body composition variables and VO_{2max} showed significant sex-related differences.

3.2. Characteristics of Simulated Padel Competition

Figure 1 shows time analysis of padel matches. In general, TPT lasted just over an hour showing RPT:TPT ratio of 0.4. Although TPT and TRT showed sex-related differences (both variables were higher in males), no differences were found in RPT. On the other hand, HR_{mean} and HR_{max} during padel competition were similar in both females and males. Likewise, considering that HR_{max} measured during maximal exercise test did not report sex differences, the percentage of HR_{mean} on reference HR_{max} (graded exercise test) did not show any statistical significance (Figure 2).



Figure 1. Characteristics of padel match competition (time analysis). TPT—total playing time; RPT—real playing time; TRT—total resting time; * p < 0.05; ** p < 0.01.

On the other hand, volumes of water intake during padel match ranged between 735 mL and 861 mL for female and male players, respectively. Nevertheless, and as mentioned above, plasma volume changes were not relevant since a slight but not statistically significant increase ($1.1 \pm 2.3\%$) for the total group was found.



Figure 2. Players' heart rates during padel match competition.

3.3. Biochemical Analysis

Table 2 shows biochemical parameters measured including substrates, metabolites, energy balance enzymes, and muscle damage biomarkers. Sex differences in Cr, CK, and LDH blood concentrations were found in both pre- and post-padel match. In addition, LDL responses after exercise were also higher in female than male players. On the other hand, and except for BG, TGs, and LDL, the intra-group analysis reported significant differences in all evaluated parameters when total group was considered, with higher values after padel matches. The same results were found in females but, in addition to BG and TGs, LDH showed no differences in males.

Table 2. Biochemical determinations in padel players before (pre) and after (post) padel match play.

| Biomarkers | Female Players | Females Intragroup Contrast | Male Players | Males Intragroup Contrast | Total Players | Total Intergroup Contrast | Sex Intergroup Contrast |
|-----------------------|--|---|--|---|--|------------------------------------|--|
| BG pre BG post | $\begin{array}{c} 89.15 \pm 11.93 \\ (81.94 \hbox{-} 96.36) \\ 98.23 \pm 16.99 \\ (87.96 \hbox{-} 108.49) \end{array}$ | a p = 0.118 d = 0.84 | $\begin{array}{c} 92.78 \pm 11.04 \\ (84.29 - 101.27) \\ 92.78 \pm 13.40 \\ (82.47 - 103.08) \end{array}$ | a p = 1 d = 0 | $\begin{array}{c} 90.64 \pm 11.45 \\ (85.56 - 95.71) \\ 96.00 \pm 15.52 \\ (89.12 - 102.88) \end{array}$ | a p = 0.161 d = 0.39 | ^a $p = 0.527$ d = 0.31 ^a $p = 0.431$ d = 0.36 |
| TGs pre TGs post | $\begin{array}{c} 96.07 \pm 61.93 \\ (58.65 - 133.50) \\ 82.23 \pm 62.70 \\ (44.34 - 120.12) \end{array}$ | ^b p = 0.116 d = 0.22 | $\begin{array}{c} 117.33 \pm 73.339 \\ (60.96 - 173.70) \\ 107.89 \pm 75.39 \\ (49.94 - 165.84) \end{array}$ | ^b p = 0.553 d = 0.13 | $\begin{array}{c} 104.77 \pm 65.99 \\ (75.51 {-} 134.03) \\ 92.73 \pm 67.66 \\ (62.72 {-} 122.73) \end{array}$ | c p = 0.94 d = 0.18 | p = 0.401 d = 0.31 p = 0.395 d = 0.37 |
| Cr pre Cr post | $\begin{array}{c} 0.78 \pm 0.08 \\ (0.73 0.83) \\ 0.88 \pm 0.12 \\ (0.81 0.95) \end{array}$ | ^a <i>p</i> < 0.001 d = 0.98 | $\begin{array}{c} 1.06 \pm 0.12 \\ (0.971.16) \\ 1.21 \pm 0.11 \\ (1.121.29) \end{array}$ | a p < 0.001 d = 1.30 | $\begin{array}{c} 0.90 \pm 0.17 \\ (0.82 0.97) \\ 1.01 \pm 0.20 \\ (0.92 1.10) \end{array}$ | a p < 0.001 d = 0.59 | $a^{a} p < 0.001$ d = 2.74 $a^{a} p < 0.001$ d = 2.87 |
| Urea pre Urea post | $\begin{array}{c} 34.70 \pm 8.04 \\ (29.83 - 39.55) \\ 39.00 \pm 9.42 \\ (33.31 - 44.69) \end{array}$ | ^a <i>p</i> < 0.001 d = 0.49 | $\begin{array}{c} 40.33 \pm 8.38 \\ (33.89 {-}46.77) \\ 45.89 \pm 8.58 \\ (39.29 {-}52.48) \end{array}$ | ^a <i>p</i> < 0.001 d = 0.65 | $\begin{array}{c} 37.00 \pm 8.47 \\ (33.24 40.76) \\ 41.82 \pm 9.52 \\ (37.59 46.04) \end{array}$ | a p < 0.001 d = 0.53 | p = 0.112 d = 0.68 p = 0.123 d = 0.76 |
| LDL pre LDL post | $\begin{array}{c} 29.54 \pm 16.79 \\ (19.39 - 39.68) \\ 30.54 \pm 17.29 \\ (20.09 - 40.99) \end{array}$ | ^b p = 0.033 d = 0.06 | $\begin{array}{c} 22.11 \pm 10.03 \\ (14.40\mathchar`-29.82) \\ 20.44 \pm 9.49 \\ (13.15\mathchar`-27.74) \end{array}$ | ^b p = 0.040 d = 0.17 | $\begin{array}{c} 26.50 \pm 14.61 \\ (20.0232.98) \\ 26.41 \pm 15.20 \\ (19.6733.15) \end{array}$ | p = 0.003 d = 0 | $p^{c} p = 0.269$ d = 0.54 $p^{c} p = 0.128$ d = 0.72 |
| CK pre CK post | $\begin{array}{c} 115.54 \pm 62.93 \\ (77.51 - 153.56) \\ 147.23 \pm 97.88 \\ (88.08 - 206.38) \end{array}$ | ^b p = 0.001 d = 0.38 | $\begin{array}{c} 262.89 \pm 189.02 \\ (117.59 - 408.18) \\ 308.22 \pm 187.12 \\ (164.39 - 452.06) \end{array}$ | ^b p = 0.008 d = 0.24 | $\begin{array}{c} 175.82 \pm 146.19 \\ (110.99240.64) \\ 213.09 \pm 159.30 \\ (142.46283.72) \end{array}$ | ^c p < 0.001 d = 0.24 | p = 0.012 d = 1.04 p = 0.016 d = 1.08 |
| LDH pre LDH post | $\begin{array}{c} 170.38 \pm 25.28 \\ (155.11 - 185.66) \\ 182.69 \pm 20.92 \\ (170.05 - 195.33) \end{array}$ | a p = 0.008 d = 0.53 | $\begin{array}{c} 231.22\pm 30.44\\ (207.83-254.62)\\ 249.67\pm 38.75\\ (219.87-279.46)\end{array}$ | a p = 0.066 d = 0.53 | $\begin{array}{c} 195.27 \pm 40.69 \\ (177.23 - 213.31) \\ 210.09 \pm 44.25 \\ (190.47 - 229.71) \end{array}$ | a p = 0.002 d = 0.35 | ^a $p < 0.001$ d = 2.17 ^a $p < 0.001$ d = 2.15 |

| Biomarkers | Female Players | Females Intragroup Contrast | Male Players | Males Intragroup Contrast | Total Players | Total Intergroup Contrast | Sex Intergroup Contrast |
|-------------|--|--------------------------------------|---|---------------------------------|--|------------------------------|------------------------------|
| FABP-3 pre | $\begin{array}{c} 1667.69 \pm 597.78 \\ (1306.45 2028.93) \end{array}$ | ^b $p = 0.004$ d = 1.09 | 1945.57 ± 590.78 (1491.45–2399.69) | b p < 0.001 d = 2.52 | $\begin{array}{c} 1781.37 \pm 597.26 \\ (1516.56 - 2046.18) \end{array}$ | c p < 0.001 d = 1.42 | $^{c} p = 0.389$ d = 0.47 |
| FABP-3 post | $\begin{array}{c} 3218.73 \pm 1921.99 \\ (2062.71 4374.74) \end{array}$ | | (3163.88 - 4532.36) | | $\begin{array}{c} 3476.21 \pm 1579.04 \\ (2776.10 4176.31) \end{array}$ | | p = 0.376 d = 0.42 |

Table 2. Cont.

BG—blood glucose (mg/dL); TGs—triglycerides (mg/dL); Cr—creatinine (mg/dL); LDL—lipoprotein lipase (IU/L); CK—creatine kinase (U/L); LDH—lactate dehydrogenase (U/L); FABP-3—fatty acid binding protein-3 (ng/mL). Data are expressed as mean \pm sd; range of values in brackets represent 95% confidence intervals (CI) of the mean; *p*: *p*-value (95% CI); ^a ANOVA contrast test for normally distributed data (BG, Cr, urea, and LDH); ^b Wilcoxon signed-rank test (for not normally distributed data: TGs, LDL, CK, and FABP-3); ^c Mann–Whitney test (for not normally distributed data: TGs, LDL, CK, and FABP-3); d: effect size Cohen's d; italics are used to highlight statistical significance.

4. Discussion

The aims of this study were to analyze the effects of padel match play on circulating substrates, metabolites, energy balance enzymes, and muscle damage biomarkers and to evaluate possible sex-related differences. Accordingly, our results suggest that aerobic metabolism is the main source of energy during padel match play since decreases in TGs and increases in LDL concentrations were assessed. However, anaerobic metabolism contribution (involved in padel shots and repeated sprint actions) was also confirmed by the increases in some metabolites such as Cr and urea, and muscle-damage biomarkers (LDH and CK). Moreover, sex-related differences were found in aerobic fitness and body composition, but also in muscle-specific enzyme and metabolites, since the response of Cr, CK, and LDH were higher in male players.

Although previous studies have been focused on hematological changes, urinary excretion of trace minerals and muscle damage biomarkers [8,10], there are still gaps of knowledge about the biochemical responses during padel competitive practice. Only the study conducted by Pradas et al. [9] tried to explore and identify these physiological changes by assessing several hematological and biochemical markers. As a novelty, this is the first study measuring exercise-related substrates, metabolites, and energy balance enzymes in order to explain the metabolic requirements of padel. Additionally, part of this novelty lies in its experimental control since blood markers were individually corrected using plasma volume changes to avoid the impact of different hydration states due to ad libitum water intake during padel matches.

Apart from the above, padel players' characteristics (e.g., age, body composition, VO_{2max}, etc.) in our study does not differ significantly from those reported by other authors who examined fitness level, specific performance outcomes, and physiological responses in trained padel players [6,8,9]. On the other hand, padel match play characteristics observed in our study were also similar to those reported previously for both simulated and real padel competition [2,7,9,16–18]. However, it is important to point out that padel matches, which last just over 80 min on average, show a RT:TT ratio of 0.4 (this means that 60% of match duration is down time). Not forgetting that padel is played in doubles with intra-pair alternating actions, previous studies that examined the dynamics of padel match play reported an intermittent activity, which combines short but high-intensity actions (0.7–1.5 per second) and low-intensity efforts (9–15 s) during rallies, interspersed by 20 s of rest in between, leading to longer breaks of 90 s [2,18–20]. Moreover, and considering the HR_{mean} assessed during padel matches ($75.2 \pm 7.9\%$ regarding HR_{max} for total group), padel could be defined as a low- or moderate-impact sport. Nevertheless, and as it was mentioned before, the main strength of this study is to determine the impact of padel competition by measuring the responses of different biomarkers checking their physiological coherence.

Regarding energy-related substrates, BG and TGs were assessed. Contrary to previous studies [9], and although a slight increase was observed in female players, BG concen-

trations remained unchanged during padel competition at about 90–100 mg/dL. This lack of BG response could be explained by the low-impact activity performed; in fact, during moderate-intensity exercise in healthy subjects (i.e., without diabetes), increased glucose uptake by muscles is balanced by an equal rise in hepatic glucose production, and blood glucose levels remain unchanged [21,22]. Nonetheless, increased BG levels could be observed after longer padel matches since catecholamines stimulate glucose production only during moderate-intensity exercise greater than 2 h of duration [23]. On the other hand, TGs levels measured after padel matches showed a non-significant decrease in both female and male players but also in the total group. These results are similar to those previously described by Pradas et al. [9] and also seem to be consistent with the energy requirements of 80 min of moderate intensity exercise roughly half of the energy is derived from fatty acids. However, to ensure the supply of fatty acids it is necessary that LPL hydrolyze TGs molecules. In our study, a significant increase in blood LPL concentration was found after padel matches, which could explain the TGs-lowering effect.

On the other hand, it is generally accepted that widespread alterations in metabolite levels are known to occur when a biological system is in a dysregulated or exacerbated state (e.g., during exercise). Accordingly, changes in different metabolites induced by moderate or intense exercise regimes have been noted for many decades [25]. In our study, Cr and urea were assessed as energy-related metabolites. As expected, circulating Cr and urea levels significantly increased after padel matches in both male and female players (and also in the total group), although sex-related differences in both pre- and post-exercise were found. Previous studies have concluded that light to moderate exercise results in an increase in net protein catabolism and an increase in Cr [26]. Moreover, and considering that the origin of Cr from creatine, which is stored mainly in muscle tissue, it seems reasonable that male Cr concentrations were higher than those measured in female players [27]. Nevertheless, the increases in Cr observed after exercise were moderate, not exceeding the reference range established for healthy adults [28].

Blood urea concentrations measured in both male and female players showed significant increases after padel competition. These increases were also found in the total group which could be explained by an enhanced protein catabolism due to the energy requirements of padel match play. Very similar results were reported by Pradas et al. [9], who also observed sex-related differences in urea responses after padel competition. Although our results did not show such differences, pre- and post-exercise urea concentrations were slightly higher in males than in females. Nevertheless, it is important to note that pre-exercise blood urea levels found in our study were close to the upper limit of the reference values which is consistent with previous studies that showed high resting urea concentration because of the continual stress of training and sports competition [28].

Exercise-induced muscle damage is a well-documented phenomenon particularly resulting from vigorous exercise which results in muscle micro-injuries and, consequently, in a loss of muscle function due to overexertion. CK, an intracellular enzyme present in greatest amounts in skeletal muscle, is typically used as a marker of muscle damage [29]. In our study, blood CK levels showed a huge increase from pre- to post-exercise evaluation in male and female groups (also in the total group). However, sex-related differences were also found since circulating CK concentrations were higher in male than female players in both assessment time points. These results are in line with those reported from studies in which serum CK levels increased significantly after simulated padel competition and sex differences were also found [9]. As occurred with Cr, CK is a cytoplasmic enzyme abundant in skeletal muscle, so it was to be hoped that resting levels and post-exercise circulating CK concentrations were higher in male players.

Although, in general, padel could be classified as low-moderate impact sport, successive shots and explosive actions during rallies seem to induce disruptions in muscle fibers and the release of CK to the bloodstream. In fact, exercise-induced muscle damage

and, consequently, increases in blood CK levels are related to highly repeated muscle contractions [30].

Another typically muscle damage biomarker used in sports science research is LDH. As CK and other intracellular molecules and enzymes, the appearance of increased levels of this biomarker in the blood indicates to changes in muscle fiber membrane permeability as well as cellular disruption, damage, and death, which has been observed in response to exercise [31]. Supporting the results reported by Pradas et al. [9], our findings showed increases in blood LDH concentrations when total group was considering. Accordingly with these previous data, sex-related differences were also found in both pre- and post-padel matches assessments since LDH levels were higher in male than in female players. However, pre- to post-exercise analysis performed in our study revealed significant increases in females but not in males which was not observed in previously published data [9].

A novelty of this study was using FABP-3 as muscle damage biomarker. Although FABP-3 was initially investigated as a marker of cardiac injury in experimental animal models, this fatty-acid binding protein has been proposed as a muscle damage biomarker due to its presence in skeletal muscle. In fact, it has been reported that circulating FABP-3 levels increase and decrease more rapidly than CK, indicating that FABP-3 is more useful than CK for the early detection of skeletal muscle injuries and the monitoring of injury during repeated exercise bouts [32]. Considering the total group, two-fold significant increases in blood FABP-3 levels were observed. Moreover, similar responses were also reported in both male and female groups after padel matches, although no statistical sexrelated differences were found. While previous studies reported higher serum FABP-3 levels in males than in females, and the gender difference was attributed to larger muscle mass in males [33], our results seem to be in accordance with those reported by Ishimura et al. [34], who did not find sex differences in FABP-3 resting levels.

Our results have practical implications for both improving padel performance (they could help to coaches to gain a better understanding of metabolic demands of padel match play and to design better training programs) and avoiding adverse effects of training overloads which could lead to impaired recovery of damaged muscles, increasing the susceptibility to injury. This last point is important given the popularity and social characteristics of padel, with many inexperienced or beginners players joining padel clubs and participating in a number of amateur leagues and tournaments. In fact, the increases in circulating muscle damage biomarkers observed (LDH, CK, FABP-3) inform about the impact of padel match play in trained players; however, how would it affect players whose padel movements and strokes are not well-skilled? Future studies should be focused on the use of these biomarkers for monitoring the impact of padel on the musculoskeletal system of this population.

Finally, as with most one group pre-post study designs in sports science research, the present findings should be interpreted with caution due to several limitations. Firstly, we use a convenience sampling of trained padel players who regularly participate in padel competition events. Although the sample size was calculated to achieve adequate study power, morphological and performance characteristics of players could compromise external validity of the results. Additionally, female players who participated in this study were in early-mid follicular phase. Although this menstrual phase does not appear to affect glucose, lipids, and protein metabolism during exercise (and does not seem to alter muscledamage biomarkers either) [35–37], the effects of menstruation, ovulation, or luteal phase on these circulating substrates and metabolites should be taken into account. Secondly, some methodological aspects regarding biochemical measures must be considered. All substrates, metabolites, energy balance enzymes, and muscle damage biomarkers were determined in whole blood using spectrophotometry techniques which could limit comparisons between studies. Moreover, determining total CK (including muscular -MM-, heart -MB-, and brain -BB- isoforms) and not counting on CK values obtained 24-48 h after a padel match competition could affect the understanding of our results. Lastly, although the effect of plasma volume changes on the studied biomarkers was controlled, sweat loss volume and

sweat metabolites were not assessed, which could underestimate Cr and urea responses. In any case, due to the exploratory nature of our analysis, these results could serve as preliminary findings implicated for further studies.

5. Conclusions

In this study, we have analyzed the acute effects of padel match play on different circulating substrates, metabolites, energy balance enzymes, and muscle damage biomarkers in both male and female players, evaluating possible sex-related differences. The results reported here confirm that padel can be defined as a low- or moderate-impact sport. Unaltered blood glucose levels, decreases in TGs, and increases in LDL concentrations indicate the prevalence of aerobic energy system contribution. Nevertheless, padel match play is characterized also by successive shots and explosive actions in which powerful muscle contractions and anaerobic energy system play a key role. The results of the present study seem to support this anaerobic energy contribution since increased levels of Cr, urea, and muscle damage biomarkers such as CK and LDH were found.

Apart for the above conclusions, this work has presented a novel approach to evaluating the impact of padel on muscular system. The use of FABP-3 as muscular damage biomarker could allow earlier assessment of padel-induced skeletal muscle alterations than does CK, being also particularly suited to the assessment of recurrent padel-induced injuries. Nevertheless, further research is needed to clarify the usefulness of FABP-3 and other alternative biomarkers in monitoring both acute and long-term impacts of padel on players' fitness and health status.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to patient privacy concerns.

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