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# Influence of the *MCT1*-T1470A polymorphism (rs1049434) on repeated sprint ability and blood lactate accumulation in elite football players: A pilot study

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**Abstract.**

*Purpose.* The aim of this study is to investigate the influence of the *MCT1* T1470A polymorphism (rs1049434) on repeated sprint ability (RSA) performance and lactate accumulation after RSA testing. *Methods.* Twenty-six elite Italian male football players (age: 17.7±0.78 years; height 179.2±7.40 cm; weight:72.1±5.38kg) performed RSA testing (6x30-m sprints with an active recovery between sprints), and lactate measurements were obtained at 1,3,5,7, and 10 min post-exercise. Genotyping for the MCT1 T1470A polymorphism was performed using PCR. *Results.* Genotype distributions were in Hardy–Weinberg equilibrium, being 42% wildtype (A/A), 46% heterozygotes (T/A), and 12% mutated homozygotes (T/T). Significant differences between genotypic groups were found in the two final sprint times of the RSA test. Under a dominant model, carriers of the major A-allele (Glu-490) in the dominant model showed a significantly lower sprint time compared to footballers with the T/T (Asp/Asp) genotype (5th Sprint time: AA + TA = 4.60 sec vs TT = 4.97 sec, 95% CI= 0.07-0.67, P = 0.022; 6th Sprint: AA + TA = 4.56sec vs TT = 4.87sec, 95% CI= 0.05-0.57, P = 0.033).

*Conclusions.* The T1470A (Glu490Asp) polymorphism of *MCT1* was associated with RSA performance. Our findings suggest that the presence of the major A-allele (Glu-490) is favourable for RSA performance in football players.

Keywords: Monocarboxylate transporters; Lactate accumulation; Muscle fatigue; RSA, Soccer.

**Author contributions**

MM and CMC conceived and designed research. MM, LF, PC, MS, FP, FT and CMC conducted experiments. MM analysed data and wrote the manuscript. NK, PC, FT, and CMC revised the manuscript. All authors read and approved the final manuscript.

## Compliance with ethical standards.

**Conflict of interest.**

The authors have no conflict of interest to declare.

**Ethical approval**

Participants were fully informed of any risks or discomforts associated with the procedures before giving their written informed consent to participate. The procedures were approved by the Ethics Committees of the Azienda Ospedaliera Universitaria (AOU) of Cagliari University (Cagliari, Italy), and performed according to the Declaration of Helsinki.

**Informed consent.**

Participants gave written informed consent to participate in the present study and to its publication.

**Abbreviations**

AIC Akaike Information Criterion

ATP Adenosine triphosphate

BIC Bayesian information criterion

BLc Blood Lactate concentration

BLc-max Maximal blood lactate concentration

DNA Deoxyribonucleic acid

H+hydrogen ion

MCT Monocarboxylate transporter

*MCT1* Monocarboxylate Transporter-1 T1470A polymorphism

PCr Phosphocreatine

PCR Polymerase chain reaction

RSA Repeated Sprint Ability

Sdec sprint decrement

SNP Single-nucleotide polymorphism

S Sprint

$VO_2$max maximal oxygen uptake

**Introduction.**

Football is a multi-skilled complex sport requiring repetition of many different activities, such as jogging, sprinting, change of direction, tackling, and jumping (Bangsbo 2014). The ability to repeat high-intensity short duration efforts following short recovery periods is identified by repeated sprint ability (RSA) (Fitzsimons et al. 1993), which is considered a key factor in elite modern football (Rampini et al., 2009; Stølen et al. 2005).

Players are often required to repeatedly produce maximal or near-maximal sprints of short duration (1–7 s) with brief recovery periods, performing between 17 to 30 sprints during an official match according to their playing position (Di Salvo et al. 2010).

RSA is affected by both aerobic and anaerobic fitness determinants. Maximal oxygen uptake ($VO_2max$) influences recovery between repeated sprints by allowing efficient restoration of phosphocreatine (PCr) and adenosine triphosphate (ATP) stores from inorganic phosphates post-exercise (Bogdanis et al. 1996).

Consequently, the metabolic factors causally related with the energetic contribution in this kind of effort play an important role. Because of glycolytic energetic contribution, blood lactate (BL) concentration has been associated with reductions in strength and power output as well as RSA performance in football players (Rampini et al. 2009).

It has been argued that the considerable accumulation of hydrogen ions ($H^+$) in muscles and in the blood following sprinting may impair repeated-sprint performance (Bishop et al. 2003;2006; Sporis et al. 2009), which suggests that RSA may be improved by interventions that can increase $H^+$ removal from active muscles (Bishop et al. 2004a, 2004b).

Most membrane lactate transport (in symport with a proton) occurs via a monocarboxylate transporters (MCTs) that are stereospecific for L-lactate. Each molecule is shuttled through the membrane with a hydrogen ion ($H^+$), a process that confers MCTs an important role in pH regulation during exercise (Halestrap and Meredith, 2004; Juel and Halestrap, 1999).

The MCT protein family currently comprises 14 members (Halestrap and Meredith, 2004, Merezhinskaya and Fishbein, 2009; Morris and Felmlee, 2008) that are differentially distributed among different cell type and cellular components (Bonen et al., 2000a; McClelland et al. 2003). In skeletal muscles, the most important and well-described isoforms are MCT1 and MCT4 (Bonen, 2001).

In humans, MCT1 is predominantly found in oxidative muscles albeit also in small amounts in glycolytic muscles (Pilegaard et al. 1999). Its high affinity for lactate and greater expression in more oxidative fibres strongly suggest that a key physiological role of MCT1 is to take up lactate from circulation (Bonen et al. 2000b).

MCT1 appears to contribute to increased metabolic use of lactate after muscle activity by facilitating lactate influx into myocytes and cardiocytes (Dubouchaud et al., 2000), a conclusion supported by studies reporting correlations between maximal muscle oxidative capacity and MCT1 content in both rats (McCullagh et al. 1996) and humans (Dubouchaud et al. 2000).

By contrast, MCT4 has a lower affinity but higher capacity for lactate transport (Dimmer et al. 2000), and it is expressed in fast twitch muscles where it mediates lactate efflux. The direction of the flux is primarily controlled by the gradient of lactate and protons (Juel, 1997).

The MCT1 transporter is encoded by *SLC16A1* (also known as *MCT1*) in humans. An A > T single nucleotide polymorphism (SNP) located in the protein-coding region (coding base 1470) of *MCT1* substitutes glutamic acid (Glu) for aspartic acid (Asp) at position 490. Merezhinskaya et al. (2000) found no clinical effects associated with this SNP (rs1049434) despite measuring a reduction of 35%–40% in the erythrocyte lactate transport rate of two patients carrying the minor T- allele (490-Asp). Ten years later, Cupeiro et al. (2010) showed increased lactate accumulation in blood during high-intensity circuit weight training in carriers of the *MCT1* T allele compared to those with an A/A (Glu/Glu) genotype. Previous studies have suggested that the rs1049434 polymorphism is also associated with physiological phenotypes showing that the mutated variant (containing Glu in the protein sequence and T in the DNA sequence) exhibits increased lactate transport via SLA16A1, as compared to the wild type Asp/A (Sasaki et al. 2015).

Moreover, a study of elite male rowers showed a higher mean BL concentration after an incremental test to exhaustion in *MCT1* T-allele carriers compared to those with an A/A genotype (Fedotovskaya et al. 2014).

Furthermore, in a study of elite male field hockey players, individuals with a homozygous T/T genotype showed a lower BL clearance rate (i.e. higher lactate accumulation) compared to individuals with a homozygous A/A genotype during active recovery following a maximal run test (Cupeiro et al. 2016).

In addition with sprint/power athletic status (Sawczuk et al. 2015), muscle injury (Massidda et al. 2015) and body composition (Massidda et al. 2016), the T allele and T/T genotype of the *MCT1* rs1049434 polymorphism were over-represented in Polish climbers (Saito et al. 2021) and have been associated with increased BL concentration after a 30-s Wingate anaerobic test and during intermittent sprint testing in Japanese wrestlers (Kikuchi et al. 2017).

In a study on 694 top-level football players from five different European countries (Massidda et al. 2018), we recently found an association between the A/A (Glu/Glu) genotype and status of football players playing the forward position, suggesting that the A/A genotype may confer an advantage for RSA, a key factor of football performance for forwards (Aziz et al. 2008).

More recently, a study of Brazilian and European endurance athletes showed that carriers of the major A allele (Glu-490) accumulated less lactate in blood in response to intense effort and exhibited higher $VO_2$ max compared with T/T genotype (Asp/Asp) carriers (Guilherme et al. 2021).

On the basis of the known relationship between BL accumulation and minor T allele (490-Asp) of rs1049434, and the assumption that football players who have greater lactate transport rates have a higher capacity to maintain their performance at intense levels of effort levels (e.g. the ability to perform repeated sprints at the highest possible speed), we hypothesised that the *MCT1* T1470A polymorphism affects RSA performance in football players. Thus, we performed a pilot study to investigate whether the rs1049434

polymorphism influenced RSA and BL accumulation after exercise in a cohort of elite football players from Italy.

## Methods

*Participants*.

Twenty-six male football players from Italy (age: 17.7±0.78 years; height 179.2±7.40 cm; body mass:72.1±5.38kg) participated in the study. Biological samples were collected in May 2019 at the end of the football season. All athletes played for the same team at an official Italian Football Championship (Primavera). All football players self-reported as Caucasian for ≥3 generations.

*Study design.*

In the current study, athletes underwent RSA testing to assess performance of repeated efforts, performance decrement, sprint time and circulating lactate levels after exercise. The study was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all participants, and the study was approved by the Ethics Committees of the Azienda Ospedaliera Universitaria (AOU) of Cagliari University (Cagliari, Italy).

*DNA analyses*

Genomic DNA was extracted from buccal swab using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Concentration and purity of extracted DNA was determined by spettrophotometer (Thermo Scientific NanoDrop). The average DNA concentration was 20 µg/mL, which is sufficient for polymerase chain reaction (PCR). Genomic DNA from subjects underwent PCR based on a previously described protocol (Cupeiro et al. 2010). The primers used for amplification were as follows: sense primer 5′-ACACATACTGGGCATGTGGC-3′ (1455–1474); antisense primer 5′-AAA TCCCATCAA TGA ACAACTGGTATGATTTCCAC-3′ (1807– 1841). Using primers and information on rs1049434 described by Cupeiro et al. (2010), we obtained the genomic sequence from GenBank. Using Nebcutter (http://tools. neb.com/NEBcutter2/index.php), we determined that the restriction enzyme BccI enables discrimination of T1470A alleles by restriction fragment length polymorphism analysis. The recognition site of BccI is 3′-GGTAG-5′ and results in the generation of three DNA fragments with the T allele (14, 171 and 202 bp), whereas only two fragments are generated with the A allele (14 and 373 bp). Heterozygotes (T/A) are identified by four fragments: 14, 171, 202 and 373 bp.

*Measurement of Repeated Sprint Ability.*

Following an active running warm-up (i.e., jogging to striding intensity) for approximately 10 min at 70% of the HRmax, football players completed three to five sprints of short duration (i.e., 20-30 m) at 85% of the HRmax. Athletes were then given a 5-min recovery before commencing the repeated-sprint test. To measure the Repeated Sprint Ability, we followed the protocol previously published by Spencer et al. (2005)

modified regarding the change of direction within each sprint. In detail, all athletes performed 6x30 meter sprint test with an active recovery (approximately 3.1-3.3 m $s^{-1}$) between sprints. On completion of each 30-m sprint, subjects were required to decelerate to a walk by the 10-m cone, turn sharply and commence the jog recovery (Figure 1). A distance of 40 m of jogging was completed within approximately 12-13 s, in order to allow 4-5s of passive recovery before the commencement of the next sprint repetition. During the jogging component, constant verbal feedback was provided to ensure that the pacing of the jog recovery was completed within approximately 12-13 s. The timing for each subsequent sprint was standardized. A 5-s warning was given, the subject then assumed the ready position and waited for the countdown. On the 'go' command, the subject broke the light beam of the electronic timing gates (Polifemo Radio Light; Microgate, Bolzano, Italy) and respective 10-m split times were recorded (i.e., 0-10 m, 10-20 m and 20-30 m) throughout each sprint (S). A standardised starting position, with the front foot placed 10 cm behind the start line, which was instrumented with timing gates, was used to minimize any variation in sprint start technique.

Participants were required to give an all-out maximal effort in each sprint and constant verbal feedback was provided to fully motivate the football player to give the maximum performance.

The 30-meter times in each sprint (S1, S2, S3, S4, S5, S6) expressed in seconds (s) were considered. The percent sprint decrement ($S_{dec}$) was calculated as follows: $S_{dec}$ (%)=[(S1+S2+S3+S4+S5+S6)/(Sbest*6)-1)*100] (Girad et al. 2011). The ideal time (Sbest) was the best time (usually the first sprint) while 6 was the total number of sprints performed by athletes. The mean sprint time (RSA-mean) was also calculated.

*Blood lactate Measurement.*

Capillary blood samples were obtained from the fingertip at baseline (pre-exercise) and at 1, 3, 5, 7 and 10 min after completion of RSA testing. During the first minute after test completion, football players performed active recovery (1 min jogging) prior to sitting in a chair for passive recovery. Samples were analysed using a Lactate Pro LT-1710 portable lactate analyser (Arkray, Kyoto, Japan) to obtain BL concentrations at baseline and immediately after 1 (Lc1), 3 (Lc3), 5 (Lc5), 7 (Lc7) and 10 (Lc10) min post-RSA testing. Maximal lactate concentration throughout the whole study (BLc-max) was determined for each football player. Prior to lactate measurements, equipment calibration was performed according to the manufacturer's recommendations. The suitability and reproducibility of the analyser were previously established throughout the physiological range of 1.0–18.0 mmol/L (Pyne et al 2000).

*Statistical analyses*

Chi-squared test was used to test for Hardy–Weinberg equilibrium. A linear regression models were used to assess the proportion of variation in the RSA parameters and BL concentration after exercise explained by the *MCT-1*. In each case, analyses were performed by comparing A/A (reference group) versus T/A, A/A versus T/T (co-dominant effect), A/A versus T/T + T/A (recessive effect), and A/A + T/A (reference group) versus 1470T/T (dominant effect). To confirm the results obtained using the linear regressions models we analyzed the data also by mean of the One-way analyses of variance (ANOVA). The analyses were performed

using SNPStats (https://www.snpstats.net/start.htm). Furthermore, to interpret differences between means, Cohen's d effect sizes were calculated and interpreted using the criteria of Hopkins and colleagues, where ≤0.2 is trivial, >0.2–0.6 is small, >0.6–1.2 is moderate, and >1.2 is large (Hopkins et al. 2009). Alpha was set at 0.05.

## Results

Genotype distributions were in Hardy-Weinberg equilibrium, comprising 42% homozygous A/A (Glu/Glu), 46% heterozygous T/A (Glu/Asp) and 12% homozygous T/T (Asp/Asp) and in line with European controls in 1000 Genomes Selection Browser 1.0 (Pybus et al. 2014). We found no significant differences between genotypic groups and age, height or body mass (all $p > 0.05$).

### *RSA performance*

No significant differences were observed between *MCT1* genotypes and mean RSA (Figure 2). However, the mean time difference between A/A genotype and T/T genotype showed a moderate to large effect size (RSA mean time: AA=4.41sec vs TT=4.61sec; d=1.10; r=0.48). Moreover, we found evidence of an association between the *MCT1* polymorphism and time of the two final sprints (Tables 1, 2). Under a dominant model, carriers of the A allele (Glu-490) showed a significantly lower sprint time in S5 (Table 1) and S6 (Table 2) compared to footballers with the T/T genotype (S5: AA + TA = 4.60 sec vs TT = 4.97 sec, 95% CI= 0.07-0.67, p = 0.022; S6: AA + TA = 4.56sec vs TT = 4.87sec, 95% CI= 0.05-0.57, p = 0.033). The mean time difference between carriers of the A-allele and T/T genotype showed a large effect size in both 5$^{th}$ and 6$^{th}$ sprint (5S: d=1.44, r=0.58; S&: d=1.51, r=0.60).

The results were confirmed also by the analyses of variance (S5:F=6.01, p=0.021; S6:F=5.29, p=0.030) Despite no significant differences between genotypes and $S_{dec}$ (%), it was interesting to note a small effect size for a lower $S_{dec}$ (%) in carriers of the A allele compared to those with a homozygous T/T genotype ($S_{dec}$ (%): AA + TA = 4.84% vs TT = 5.80%, 95% CI=-1.51-3.43, d=0.38; r=0.18; p = 0.45).

**Table 1. *MCT1* association with the 5$^{th}$ sprint time (seconds) of the RSA test.**

| Model | Genotype | n. | Mean Time (SE) | Difference (95% CI) | P-value | AIC | BIC |
|---|---|---|---|---|---|---|---|
| **Co-dominant** | A/A | 11 | 4.54 (0.07) | 0.00 | | | |
| | T/A | 12 | 4.65 (0.07) | 0.11 (-0.09-0.32) | 0.045* | 6.2 | 11.3 |
| | T/T | 3 | 4.97 (0.17) | 0.43 (0.11-0.75) | | | |
| **Dominant (A)** | A/A-T/A | 23 | 4.60 (0.05) | 0.00 | 0.023* | 5.6 | 9.3 |
| | T/T | 3 | 4.97 (0.17) | 0.37 (0.07-0.67) | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | A/A | 11 | 4.54 (0.07) | 0.00 | | | |
| **Recessive (A)** | T/A-TT | 15 | 4.71 (0.07) | 0.18 (-0.03-0.38) | 0.10 | 8.3 | 12.1 |

*Statistically significant. AIC= Akaike Information Criterion; BIC= Bayesian information criterion RSA= Repeated sprint ability

**Table 2.** ***MCT1*** **association with the 6th sprint time (seconds) of the RSA test.**

| **Model** | **Genotype** | **n.** | **Mean Time (SE)** | **Difference (95% CI)** | **P-value** | **AIC** | **BIC** |
|---|---|---|---|---|---|---|---|
| **Co-dominant** | A/A | 11 | 4.52 (0.06) | 0.00 | | | |
| | T/A | 12 | 4.60 (0.07) | 0.08 (-0.10-0.26) | 0.067 | -0.9 | 4.2 |
| | T/T | 3 | 4.87 (0.12) | 0.43 (0.11-0.75) | | | |
| | A/A-T/A | 23 | 4.56 (0.06) | 0.00 | | | |
| **Dominant (A)** | T/T | 3 | 4.87 (0.17) | 0.31 (0.05-0.57) | 0.030* | -1.9 | 1.8 |
| | A/A | 11 | 4.52 (0.07) | 0.00 | | | |
| **Recessive (A)** | T/A-TT | 15 | 4.65 (0.07) | 0.14 (-0.04-0.31) | 0.15 | 1 | 4.7 |

*Statistically significant. AIC= Akaike Information Criterion; BIC= Bayesian information criterion RSA= Repeated sprint ability

*Blood lactate concentration*

We found no evidence of an association between genotype and mean lactate levels (Lc1, Lc3, Lc5, Lc7 and Lc10) and L-max after RSA (all $p > 0.05$) (Table 3), but we found a moderate effect size for increased mean Lc1 and Lc3 in carriers of the minor T allele compared with individuals homozygous for the A/A genotype (Lc1-mean: AA + TA = 11.4 mmol / L vs TT = 14.4 mmol / L, 95% CI=-1.10-7.00, d=0.95; r=0.42; p=0.17; Lc3-mean: AA+AT = 11.5 mmol/ L vs TT = 14.1 mmol / L, 95% CI=-0.745-1.98, d=0.76; r=0.37) (Figure 4 and 5).

**Table 3. *MCT1* association with the mean lactate levels (Lc1, Lc3, Lc5, Lc7 and Lc10) and L-max after RSA test .**

| Genotype | n. | Lc1 mml/L (SD) | Lc3 mml/L (SD) | Lc5 mml/L (SD) | Lc7 mml/L (SD) | Lc10 mml/L (SD) | Lmax mm/L (SD) |
|---|---|---|---|---|---|---|---|
| A/A | 11 | 11.47±4.0 | 11.46±3.5 | 11.15±3.7 | 10.04±4.0 | 9.40±4.4 | 13.26±3.0 |
| T/A | 12 | 11.37±2.8 | 11.63±3.5 | 10.8±3.9 | 10.31±3.5 | 9.40±3.3 | 12.75±3.5 |
| T/T | 3 | 14.37±4.0 | 14.17±2.8 | 11.5±1.6 | 10.08±1.3 | 9.80±0.6 | 14.46±2.8 |

## Discussion

To our best knowledge, the current study is the first investigation to determine whether the *MCT-1* T1470A polymorphism influences RSA performance and BL concentration after exercise in elite football players. Compared to individuals with the T/T genotype, we discovered that carriers of the A allele (Glu-490) exhibited significantly better times in the two final sprints of the RSA test, with trends towards for lower performance decrement and decreased BL concentration during the first minute of active recovery time.

It is important to note that a good RSA is best determined by a high average sprint performance, with or without a low fatigue index or low decrements of performance. For instance, a marathon runner with a low average sprint performance and very low fatigue index or very low performance decrement would not be classified as having good RSA (Bishop et al. 2011; Girard et al. 2011).

In our study, the differences in the sprint performance (RSA mean) between A/A and T/T genotypes was about 0.20 seconds. A change of 0.20 s represents an improvement of 0.03 s per sprint, over six sprints. An improvement of 0.03 s per sprint is approximately 20-22 cm over a 30-m sprint (assuming a sprint time of between 4.3 and 4.6 s). This distance could be enough to have an advantage over an opposition player (i.e., intercept a pass, take possession of the ball, jumping during a header etc.).

Moreover, in our study, we found evidence of an association between the *MCT1* polymorphism and last two sprints of the RSA test, which are most affected by fatigue. In humans, expression of MCT1 is correlated with lactate removal (Green et al. 2002; Thomas et al. 2005) and the amount of lactate oxidised into muscle cells (Bonen et al. 2000a). A study conducted on 15 humans with different training status showed that the blood lactate removal ability after a 1-min all-out test was significantly related to MCT1 content (Thomas et al. 2005). On the basis of our findings, we speculate that the greater decline in sprint speed (i.e. longer time to cover a fixed distance) in players with the T/T genotype compared to carriers of the A allele may be because of the impaired functionality of MCT1 under conditions of prolonged activity and insufficient recovery, resulting in the observed decrease in BL clearance rate and higher BL accumulation in T/T individuals. Our mean Lc1 was 3 mmol/L higher in T/T individuals compared to A-allele carriers, which is similar to that found in previous research (Cupeiro et al. 2012; 2016; Guilherme et al. 2021).

Further, we posit that it is during these last two final sprints that BL concentrations reached a minimal threshold (approximately 10 mM/L), which taxes the MCT1 polymorphic (Asp/Asp) isoform, as previously hypothesizsed (Cupeiro et al. 2010, 2012). Moreover, the biological effect of the minor allele becomes evident at higher lactate levels (Fishbein, 1986) when a saturation phenomenon for lactate transport can result in lactate accumulation (Thomas et al. 2012).

Additional support comes from a study on 16 young male elite field hockey players in which individuals with the T/T genotype have lower BL decrease than those with the A/A genotype during a 10-20-min period of active recovery after 400-m maximal run test. This period was the time when BL concentration reached minimal threshold (approximately 10 mM/L), thereby affecting the *MCT1* Asp/Asp isoform (Cupeiro et al. 2016).

It is important to highlight that, although the difference observed in performance between genotypes could be due to the different lactate levels indicate by the effect size, we believe that larger sample sizes are needed to assure this hypothesis.

Probably due to the small sample size, our hypothesis was a possibility that has not been supported by significant differences in lactate levels between genotypes, and therefore we cannot exclude the hypothesis that other factors, different to the lactate levels, could have affected our results. Among these factors there is the unequal resynthesis of phosphocreatine (PCr) during recovery times due to the different VO2max of the athletes (Tomlin et al. 2001). RSA is closely related to oxidative capacity for PCr recovery (Aziz and Chia, 2010) and the aerobic energy system plays a key role in the return to homeostasis during the rest intervals of repeated sprints (Gaitanos et al. 1993). Therefore, it has been suggested that a high level of aerobic capacity enhances the ability to recover from repeated high-intensity exercise (Rankovic et al. 2010).

Oxygen consumption increases linearly with exercise intensity, and individuals with higher $VO_2$ max can reach and sustain higher exercise intensities prior to excessive lactate accumulation and fatigue. Further, some studies found a significant relationship between $VO_2$ max and RSA (Aziz et al. 2007; da Silva et al. 2010).

Fedotovskaya et al. (2014) found that the frequencies of the A allele and A/A genotype were significantly higher in Russian endurance-oriented athletes (n = 142) compared with controls (n = 467), indicating that the A allele (Glu-490), which is the lower lactate accumulation variant, is favourable for endurance performance. A similar finding was recently reported in a study (n = 1,280) and European (n = 867) cohorts in which the A allele (Glu-490) was overrepresented in endurance athletes (Guilherme et al. 2021). In an assessment of Caucasian endurance athletes, they also found evidence of an association between the *MCT1* genotype and $VO_2$ max, with individuals homozygous for the A/A genotype exhibiting the highest values of $VO_2$ max (Guilherme et al. 2021). In the present study, individuals with the T/T genotype may have increased muscle fatigue during the last two sprints of the RSA test, resulting in a significantly longer time to complete these final sprints compared to A-allele carriers. Since we did not collect the VO2max in our athletes, we cannot know if there were significant differences in VO2 max among *MCT1* genotypes.

From a physiological point of view, there are correlations between sprint decrement and both muscle buffer capacity and changes in blood pH (Bishop et al. 2003, 2004a, 2004b, 2006). Proposed factors responsible for

these performance decrements have been previously reviewed (Glaister, 2005) and include limitations to energy supply and accumulation of metabolites, such as inorganic phosphate and $H^+$.

Previous research has shown that the extent of PCr degradation and $H^+$ accumulation, which increases with repeated bouts of maximal exercise (Balsom et al. 1992), is associated with muscle fatigue in football players. In particular, $H^+$ dissociated from lactate in exercising muscle and blood can lead to a drop in pH (i.e. lactic acidosis), which affects body functions (Sahlin 1983) in a number of ways, including inhibition of glycolysis and lipolysis (Fredholm and Hjemdahl, 1978), increased water content of muscles (Sahlin et al. 1978), decreased lactate efflux from muscles (Hirche et al. 1975), increased requirements for calcium ions ($Ca^{2+}$) (Donaldson et al. 1978), decreased myosin ATPase activity (Shalder 1967), increased protein binding of $Ca^{2+}$ in sarcoplasmic reticulum (Nakamura and Schwarts 1972) and increased potassium ions ($K^+$) in the extracellular space (Saltin et al. 1981). As body functions are sensitive to changes in pH, the main outcome of these negative mechanisms is inhibition of exercise performance capacity by accelerating local muscle fatigue.

In a pilot study of 10 men who performed three sessions of controlled circuit weight training (CWT), it was found that carriers of the T allele showed higher lactate accumulation and higher lactate peak after exercise (Cupeiro et al. 2010). The researchers hypothesised that these observed increases in lactate accumulation and peak in T-allele carriers at the end of a CWT session may be because of the impaired functionality of the MCT1 isoform, which would lead to impaired lactate transport into less active muscle cells for oxidation and thus increased BL concentration (Juel et al. 1999; McCullagh et al. 1996).

To our knowledge, this is the first study to investigate the potential influence of the *MCT1* T1470A polymorphism on RSA. However, the main limitation of our pilot study is the small sample size. Nevertheless, we believe that this limitation was mitigated by the strong homogeneity of the study cohort. It was composed of elite athletes of the same team that shared the same training requirements and diet regime for over a year, which is important for substrate utilisation and, in turn, lactate production (Chen et al. 2008; Wu et al. 2003).

Despite the strong points mentioned above, we believe that our study has some additional limitations represented by the lack of control over other variables of interest such as the blood lactate concentration during the RSA test, the value of VO2max of the athletes and their body composition, all factors that could have affected the results of the present study.

**Conclusions.**

In conclusion, we found evidence of an association between the *MCT1* T1470A polymorphism and RSA. Individuals with a T/T genotype exhibited decreased performance, taking more time to complete the final two sprints compared to A-allele carriers. Although our findings are tempered by the small sample size of our cohort, they nevertheless contribute to better understanding the role of MCT1 on RSA and lactate clearance in football. Further studies are needed to replicate our findings in larger cohorts of football players from different countries.

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**Figure Legends**

**Fig.1** Schematic of the 6x30 m over ground repeated sprint test with an active recovery.

**Fig.2** RSA mean time as function of MCT1 genotypes.

**Fig.3** Lactate concentration during the first minute after RSA test as function of MCT1 genotypes.

**Fig.4** Lactate concentration during the third minute after RSA test as function of MCT1 genotypes.

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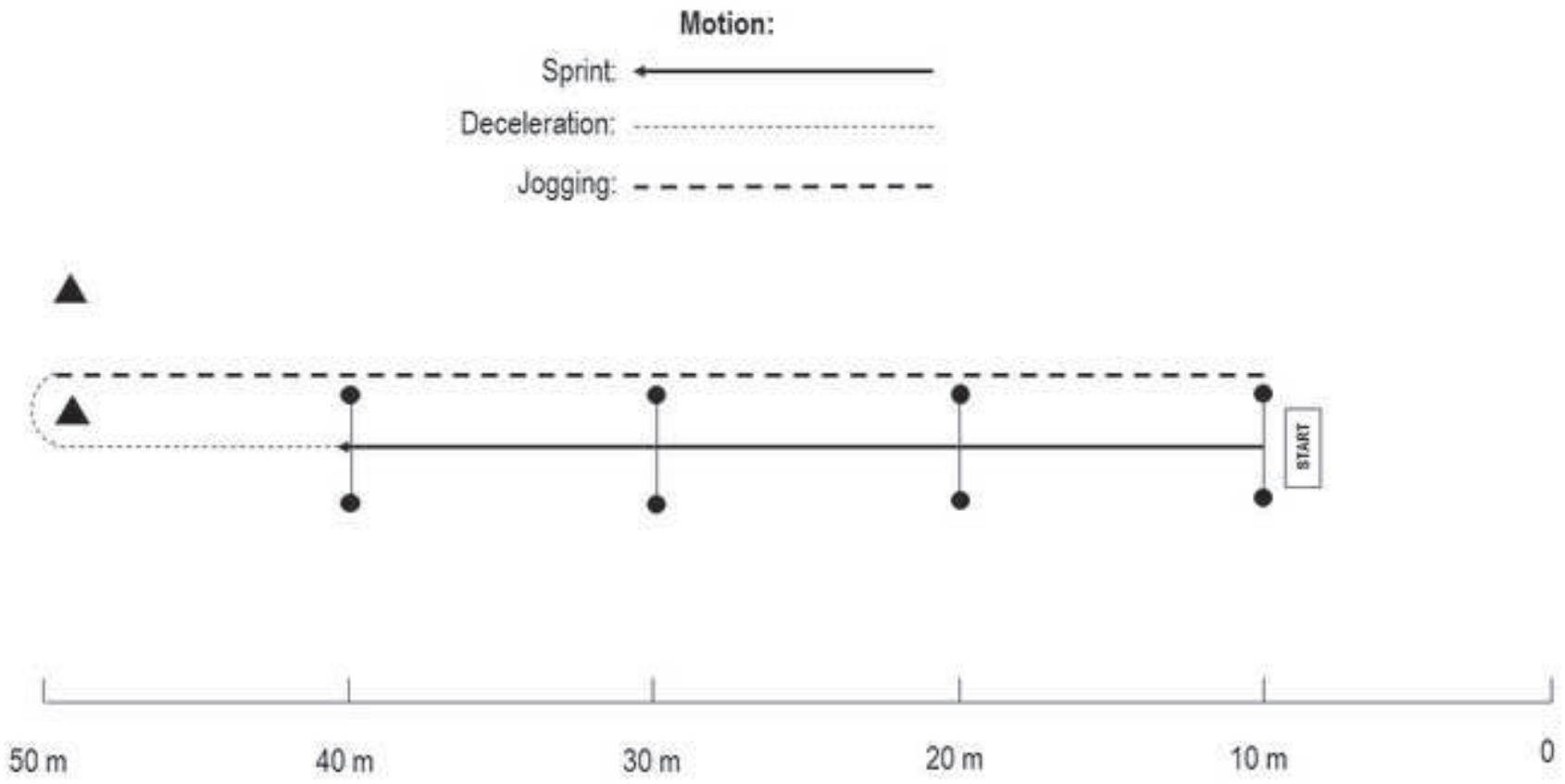

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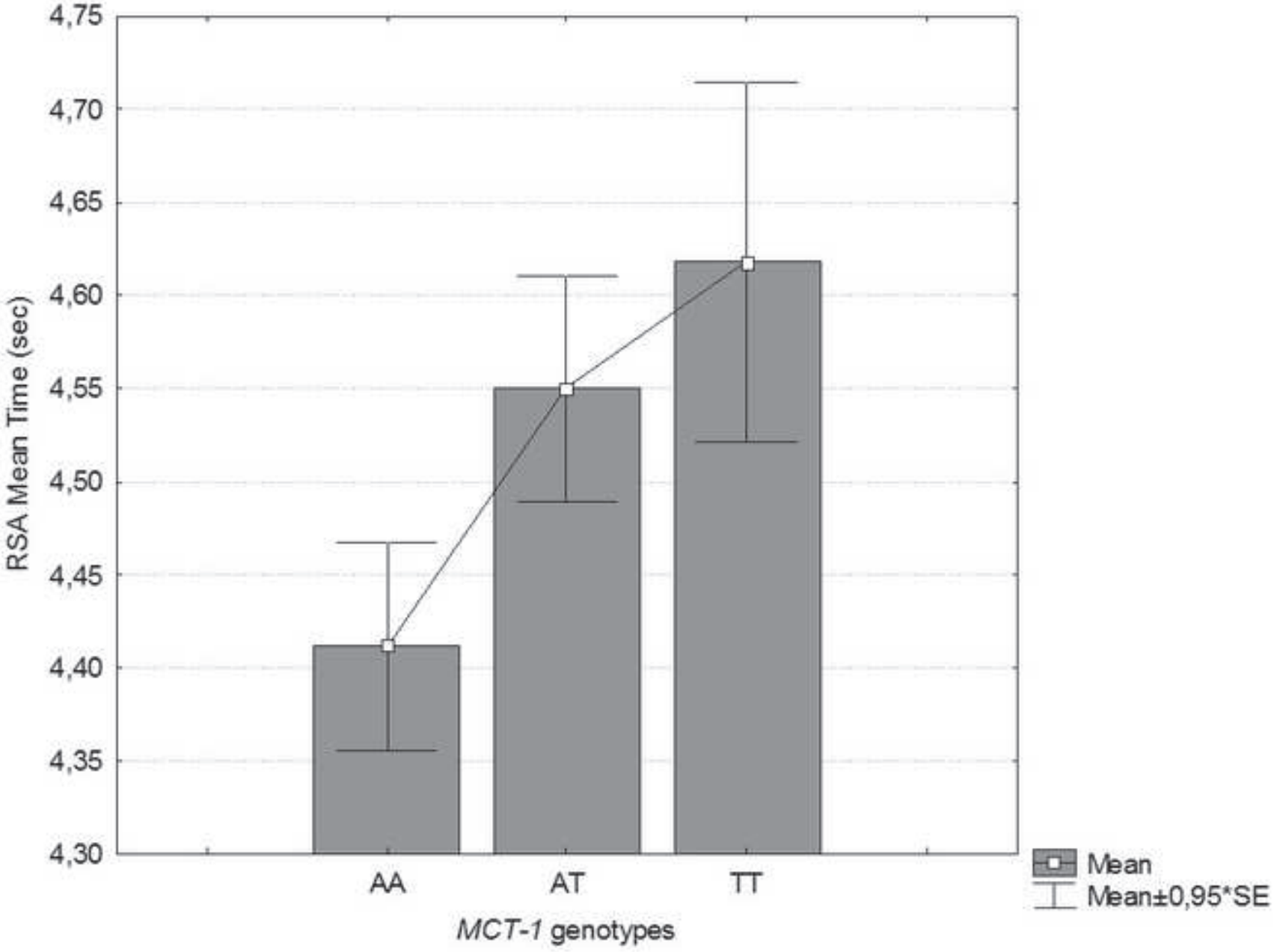

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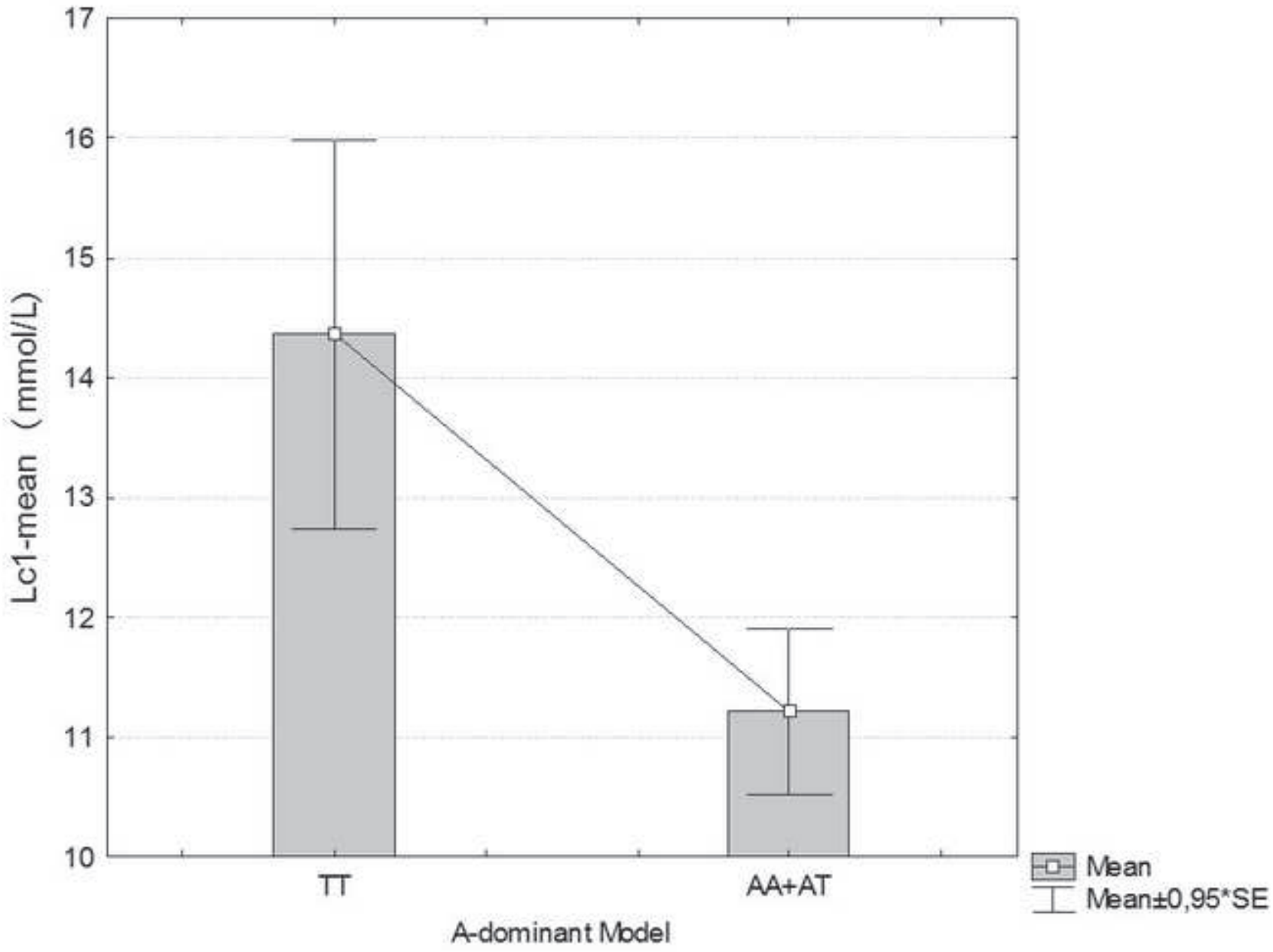

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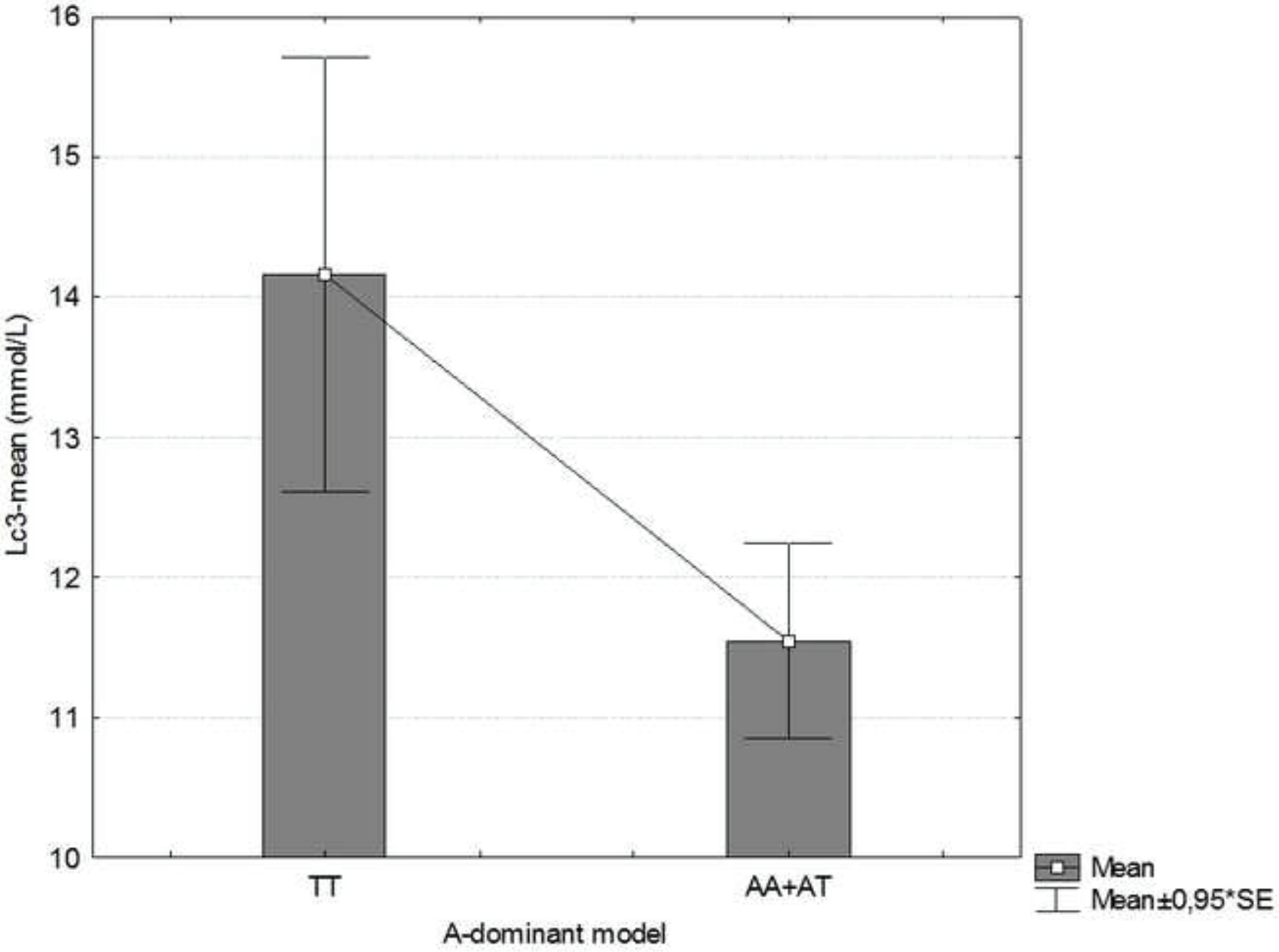

# Influence of the *MCT1*-A1470T polymorphism (rs1049434) on repeated sprint ability and blood lactate accumulation in elite football players: A pilot study

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**Author contributions**

MM and CMC conceived and designed research. MM, LF, PC, MS, FP, FT and CMC conducted experiments. MM analysed data and wrote the manuscript. NK, PC, FT, and CMC revised the manuscript. All authors read and approved the final manuscript.