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Association between Ventilatory Thresholds Related to Aerobic Fitness and MCT1 A1470T Polymorphism

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Abstract

The purpose of this study was to verify the association between MCT1 polymorphism with physiological parameters related to aerobic fitness. A hundred fifty healthy male volunteers performed a maximal incremental running test to determine the speeds corresponding to Ventilatory Threshold (VT) and Respiratory Compensation Point (RCP). Participants were genotyped and divided in terciles based on the analyzed variables. Genotype frequencies were compared through chi-square test between lower (LT) and higher terciles (HT), with the lowest or highest values of each analyzed variable. MCT1 TT genotype was overrepresented in HT only for VT and showed a significantly higher odds ratio of belong to HT for VT compared only to AA (5.1). These results suggest that TT individuals could attain the VT and RCP at higher speeds, being able to sustain higher running speeds in lower exercise intensity domains. In other words, it is possible that individuals carrying the MCT1 TT genotype might run at higher speeds with lower fatigue signals, mimicking an inner aerobic fitness adaptation.

Keywords: Physical fitness; Aerobic evaluation; Genetics; MCT1

Abbreviations:

VT: Ventilatory Threshold; RCP: Respiratory Compensation Point; LT: Lower Tercile; HT: Higher Tercile; IPAQ: International Physical Activity Questionnaire

Introduction

Aerobic fitness has been traditionally evaluated during incremental exercise tests, through the determination of some physiological parameters, such as the maximal oxygen consumption (), the Ventilatory Threshold (VT), and the Respiratory Compensation Point (RCP) [1-3]. As exercise intensity increases, oxidative metabolism alone cannot maintain the rate of ATP resynthesis, enhancing the anaerobic metabolism contribution at high [4]. Consequently, lactate and H+ ions accumulate in blood during the early stages of a maximal incremental test [5], altering the ventilation response during exercise [6]. It has been demonstrated that the VT corresponds to the ventilatory compensation for the first increase in blood lactate concentration and carbon dioxide pressure [6], while the RCP corresponds to the onset of hyperventilation due to the excess production of carbon dioxide to buffer H+ ions via bicarbonate [7].

It is well known that physical status is dependent on a multifactorial phenotype, resulting from a complex interaction between environmental and genetic factors [8,9]. Among several genetic characteristics associated with physiological responses during exercise, the MCT1 gene polymorphism is a candidate that might have a significant influence on the gas exchange response to incremental exercise. The MCT1 gene encodes the monocarboxylate transporter 1, which is involved in the removal of lactate and H+ ions from active muscle cells and their uptake by inactive muscle cells, where lactate is oxidized [10,11].

A polymorphism in the MCT1 gene (A1470T), with a glutamic-to-aspartic acid at codon 490, was reported to result in a 40% enhancement of lactate removal after a forearm exercise in T allele carriers compared with that in A allele carriers [12]. In addition, a more recent study demonstrated that the MCT1 T allele is associated with less lactate accumulation after strength exercises [13]. Collectively, these results suggest that the MCT1 gene polymorphism could influence the lactate kinetics through both lactate influx to non-active or efflux out of the active [13], and gas exchange during exercise, known as important parameters to fitness status evaluation [1,14]. In regard of sports performance, the MCT1 A1470T results are quite inconclusive and still scarce, with only three studies analyzing the MCT1 genotypes frequencies among athletes. Ben-Zaken et al. [15] observed a higher frequency of T allele among swimmers (42%) compared to runners (27%, $p < 0.001$) of both long and short distances. Further, Fedotovskaya et al. [16] observed a higher frequency of the AA genotype (59.8%) among endurance-oriented athletes compared to control group (39%, $p < 0.001$). Lastly, Sawczuk et al. [17] showed that sprint/power athletes were more likely to possess the TT genotype (27%) compared to endurance athletes (14%, $p = 0.029$). Nevertheless, despite the interesting physiological link between MCT1 polymorphism and lactate kinetics, to date, no study has investigated its influence on ventilatory thresholds.

Therefore, regarding the important links with physiological processes, the aim of the present study was to analyze the influence of the MCT1 genotypes on the ventilatory thresholds that are traditionally associated with aerobic fitness. These results might help in

the future to identify a possible optimal endurance, enhancing the athletes' selection process and identification of inner fitness status, improving athletic and health benefits.

Materials and Methods

Subjects

A hundred fifty healthy, moderately active male individuals voluntarily participated in this study. All participants were nonsmokers, free of neuromuscular and cardiovascular dysfunctions and not taking any medication at the time of data collection. They received a verbal explanation about the possible benefits, risks and discomforts associated with the study, and they provided written informed consent before participation. All individual data were anonymized, and the individual results of the physical tests were sent to the participants if requested. All experimental procedures were conducted according to the principles of the Declaration of Helsinki and were also previously approved by the ethics committee of the School of Physical Education and Sport of the University of São Paulo.

Experimental design

All participants reported to the laboratory twice. During the first visit, DNA was collected using the mouthwash method. The MCT1 genotypes were determined after all other experimental procedures were completed, and the investigators who were involved with data collection or analysis were unaware of the genotypes during the course of the study. The participants were then required to fill out an International Physical Activity Questionnaire (IPAQ-short version) to determine their physical activity levels. A moderate activity level (from 600 to 2999 Met-min.week-1) was criterion for inclusion in the study. Finally, anthropometric measurements (height, body mass and body composition) were acquired. During the second visit, a maximal incremental treadmill test was performed to determine VO_2max, VT, and RCP. The tests were performed at the same time of day in a controlled-temperature room (20-24°C) 2-3 h after the last meal. All individuals were asked to refrain from any exhaustive or unaccustomed exercise for the 48 h preceding the test. They were also instructed to wear standard running shoes and to avoid taking nutritional supplements throughout the experimental period.

Anthropometric measurements

All anthropometric measurements were taken according to the procedures described by Lohman [18]. Skinfold thickness was measured at eight body sites to the nearest 0.2 mm using a Harpenden caliper (West Sussex, UK). The median of three values was used for data analysis. Measurements were performed by the same experienced investigator. Body density was predicted using the generalized equation of Jackson and Pollock [19] and body fat was estimated using the equation of Brozek et al. [20].

Physical activity level determination

The participants received a detailed explanation about IPAQ (short version) and were asked to fill it out. One investigator remained close to the participants during the IPAQ completion period to assuage any doubts. The major aim of IPAQ is to determine the total amount of walking, moderate and vigorous physical activity and to generate a total score for the weekly energy expenditure from physical activity, expressed in METs. Total physical activity was calculated assuming metabolic equivalents of 3.3, 4.0 and 8.0 METs for low, moderate and intense activities, respectively. Low activity represents <599 MET-min.week-1, moderate activity represents a minimum of 600 Met-min.week^{-1}, and high activity represents a minimum of 3000 Met-min.week^{-1} [21].

Maximal incremental running test

The subjects performed a maximal incremental test on a motor-driven treadmill (model TK35, CEFISE, Nova Odessa). After a 3-min warm-up at 8 km. h^{-1}, the treadmill speed was increased by 1 km.h^{-1} every minute until exhaustion with strong encouragement to continue the exercise as long as possible. Gas exchanges were measured breath-by-breath using a gas analyzer (Cortex Metalyzer 3B, Cortex Biophysik, Leipzig, Germany) and were subsequently averaged over 30 s intervals throughout the test. Before each test, the gas analyzer was calibrated according to the manufacturer's recommendations. Maximal heart rate was defined as the highest value measured during the test. VT was determined at the point of a nonlinear increase in therelationship. RCP was determined at the point of a concomitant nonlinear increase in, a constant increase in the relationship, and the first decrease in the expiratory fraction of CO_2 [14]. These thresholds were determined by two independent investigators who were blinded to the participants' identification and genotype data, and did not co-author the present study. When the investigators disagreed, a third independent investigator was consulted. A third investigator determined the VT and RCP in less than 10% of the tests.was determined when two or more of the following criteria were met: an increase in oxygen uptake of less than 2.1 ml·kg^{-1}·min^{-1} between two consecutive stages, a respiratory exchange ratio greater than 1.1 and the attainment of a heart rate ≥90% of the predicted maximal heart rate (i.e., 220-age) [22].

Genotyping

Mouthwash-derived cells were subjected to overnight digestion with proteinase K. Nucleotides were separated from cellular debris by density gradient centrifugation using chloroform. Genomic DNA was then precipitated by isopropyl alcohol, isolated by centrifugation and resuspended in TE buffer. DNA quantification was performed using a spectrophotometer (NanoDrop, ND 2000, USA), and the concentration was adjusted to 1 µg/µL for subsequent storage at -20°C.

MCT1 genotyping was performed with predesigned 5' nuclease assays (TaqMan SNP Genotyping Assay, Applied Biosystems, Foster City, CA). The polymerase chain reaction (PCR) conditions were as follows: initialization hold at 95°C for 5 min and 45 cycles of denaturation at 95°C for 15 s and annealing and extension at 60°C for 60 s. Fluorescence was measured with the Rotor-Gene Q (Qiagen) sequence detection system using green (A allele) (VIC probe: GACTTTCCTCCTCCTTGGGCCCTCC) and yellow (T allele) (FAM probe: TCTGTGTCTTTCTGGTCCGGAGATT) channels to distinguish the genotypes. To ensure a proper internal control, for each batch of analysis we used positive and negative controls from different DNA aliquots that were previously genotyped by the same method, according to recent recommendations for replicating genotype-phenotype association studies [23]. The determination of MCT1 genotypes through the fluorescence behavior was performed by three

experienced and independent investigators who were blinded to the participant's data.

Statistical Analysis

Data normality was assessed through the Kolmogorov-Smirnov test. The Hardy-Weinberg Equilibrium was evaluated by the chi-squared test. The magnitude of difference in VT and RCP between MCT1 genotypes was compared through the one-way ANOVA. In order to obtain the highest and lowest physiological values, the sample was divided in terciles based on the VT and RCP values and the intermediate tercile was excluded. A Student's t test for independent samples was performed to confirm the differences of the VT and RCP between the lower (LT) (n = 50) and higher (HT) (n = 50) terciles. The MCT1 genotype frequencies between LT and HT were compared through the chi-squared test. To further verify the influence of the genotype on ventilatory thresholds, we compared the chances for individuals with different genotypes of the MCT1 gene to be in the LT or HT through an odds ratio analysis. Additionally, the same analysis was applied to verify the recessive effect (TT vs. AA + AT). All analyses were conducted using the SPSS software (version 17.0), and the significance level was set at $\alpha = 0.05$.

Results

Genotype frequencies and characteristics of the participants

Table 1 shows the age, physical activity level, and anthropometric and physiological characteristics of the participants. The genotyping was completely successful in all participants. Replication analysis of 50% of the samples using a different genotyping method (i.e., restriction fragment length polymorphism) was 100% successful. The genotype distributions were in agreement with the Hardy-Weinberg Equilibrium (Figure 1). The genotype distributions of the MCT1 gene polymorphism was comparable to the distributions reported in the public databases for other populations (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs = 1049434).

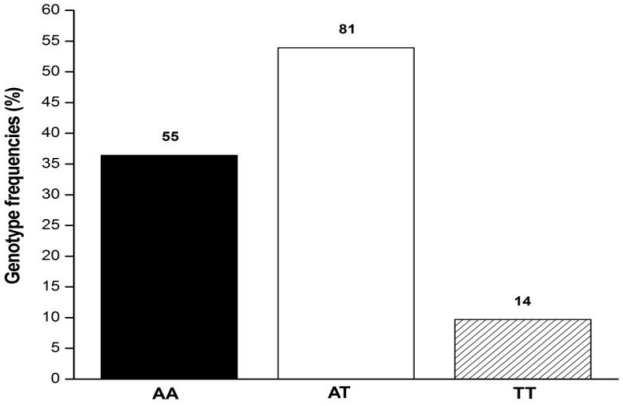

Figure 1: Genotype frequencies for MCT1 genotypes (AA, AT and TT) (n = 150). Data on top of bars are total number of subjects per genotype.

	Mean ± SD	95% CI
Age (years)	25.2 ± 4.0	24.7-26.2
IPAQ (MET-min.week^{-1})	1309 ± 291	1261-1358
Body mass (Kg)	77.8 ± 13.9	75.3-80.1
Height (cm)	173.9 ± 21.4	168.9-178.9
Body fat (%)	13.3 ± 4.2	12.4-14.2
(mL.kg^{-1}.min^{-1})	46.7 ± 5.6	45.7-47.7
HRMAX (bpm)	192 ± 8	190-194
VT (km.h^{-1})	9.9 ± 1.4	9.7-10.2
RCP (km.h^{-1})	13.2 ± 1.4	12.8-13.6

Data are means ± standard deviations. 95% CI: 95% confidence interval; IPAQ: International Physical Activity Questionnaire; : maximal oxygen consumption; HRMAX: maximal heart rate; VT: speed associated to ventilatory threshold; RCP: speed associated to respiratory compensation point.

Table 1: Characteristics of the participants (n = 150).

Magnitude of difference in ventilatory threshold and respiratory compensation point between MCT1 genotypes

It was observed no significant differences between MCT1 genotypes (AA, AT, and TT) for the VT and RCP (Table 2).

	AA	AT	TT	F-value	p-value
VT (km.h^{-1})	10.1 ± 1.5	10.2 ± 1.2	10.1 ± 1.1	0.117	0.838
RCP (km.h^{-1})	13.4 ± 1.4	13.7 ± 1.5	12.8 ± 1.9	1.289	0.231

Data are mean ± standard deviation. VT: speed associated with the ventilatory threshold; RCP: speed associated with the respiratory compensation point. F- and P- values are outcomes of the one-way ANOVA.

Table 2: Analysis of variance (ANOVA) results for the comparison of the ventilatory threshold and respiratory compensation point between MCT1 genotypes (n = 150).

Lower and higher terciles for the ventilatory threshold and for the respiratory compensation point

The sample (n = 150) was divided in terciles based on the VT and RCP values to separate the individuals who were able to attain these two parameters at the lowest and highest speeds for further comparisons of the genotype frequencies. When the sample was separated based on the VT values, the values for the LT (8.8 ± 0.9 km.h^{-1}; 95%CI: 8.5-9.1) and HT (11.4 ± 0.6 km.h^{-1}; 95%CI: 11.2-11.6) were significantly different ($p < 0.0005$). Similarly, the RCP values for the LT (12.0 ± 0.8 km.h^{-1}; 95% CI: 11.8-12.3) and HT (15.0 ± 1.1 km.h^{-1}; 95%CI: 14.6-15.3) also significantly differed ($p < 0.0005$).

MCT1 genotype distributions through the terciles

No differences were observed for age ($p = 0.346$), height ($p = 0.098$), body mass ($p = 0.979$) and body fat ($p = 0.507$) between the MCT1 genotypes. For the VT, the MCT1 TT genotype was overrepresented in the HT compared with the LT ($p = 0.013$). However, for the RCP

values, no differences between the LT and HT were observed for the MCT1 genotypes distribution (p = 0.103) (Table 3).

Ventilatory threshold						
Tercile	AA	AT	TT	Total	χ^2 (df = 2)	p
Lower	26(52)	21(41)	3(6)	50(100)	8.68	0.013*
Higher	12(24)	31(62)	7(14)	50(100)		
Respiratory compensation point						
Tercile	AA	AT	TT	Total	χ^2 (df = 2)	p
Lower	24(48)	21(42)	5(10)	50(100)	4.56	0.103
Higher	14(28)	31(61)	5(10)	50(100)		

Data are total numbers and percentages (%) per MCT1 genotype on terciles for the speed associated to ventilatory threshold and respiratory compensation point. *Genotype distributions significantly different between terciles.

Table 3: MCT1 genotype frequencies by tercile based on the speed associated to ventilatory threshold and respiratory compensation point (n = 100).

When the sample was divided based on the VT values, it was observed a significant odds ratio between the genotypes in the HT for the MCT1 gene (Figure 2). These results indicated an odds ratio of 5.1 (95% CI: 1.6-12.8; p = 0.036) for carriers of the TT genotype to be in HT for VT compared with the AA carriers. No statistically significant odds ratio was observed for the RCP in any of the comparisons.

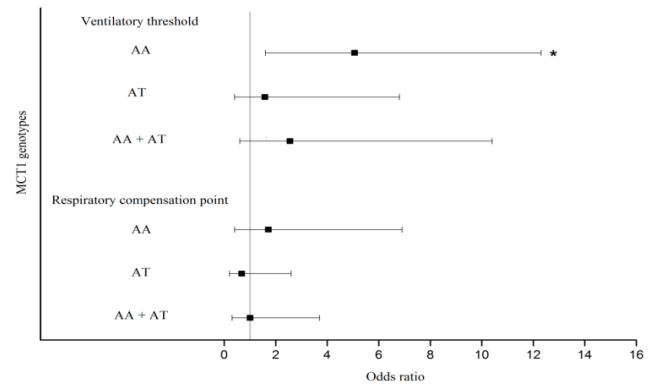

Figure 2: Data are odds ratio values (± 95% CI) of MCT1 TT genotype to be in higher tercile for VT and RCP compared to other MCT1 genotypes. OR: Odds Ratio; HT: Higher Tercile. *Statistically significant (p < 0.05).

Discussion

The present study aimed to investigate the association between MCT1 gene polymorphism and selected physiological parameters traditionally associated with aerobic fitness. It has been suggested that the MCT1 gene is able to influence lactate kinetics during exercise [12,13]. However, to the best of our knowledge, no study has investigated the impact of MCT1 gene polymorphism on the ventilatory thresholds traditionally associated with aerobic fitness. Our main findings showed a higher proportion of the MCT1 TT genotype among individuals with higher VT speeds and that the participants with this genotype are more likely to present the highest values of this physiological variable. However, no association between MCT1 genotypes and RCP was observed.

It is interesting to highlight that the two statistical analyses performed in the present study resulted in quite conflicting outcomes. A one-way ANOVA showed no significant differences between MCT1 genotypes in the magnitude of difference in the VT and RCP. Otherwise, the chi-squared and odds ratio tests, also performed in previous studies [24-26], showed significant results associating MCT1 TT genotype with VT. To date, there was no gold standard for statistical analysis in sports and physical activity genetics. While the ANOVA could be considered a more traditional statistical test to compare the magnitude of difference of the dependent variables, it is well accepted that the magnitude of influence of a single polymorphism is small and a little part of a complex genotype profile [27,28], explaining the non-significant ANOVA results in the present study. Thus, the analysis of the frequency of individuals with higher fitness level carrying different MCT1 genotypes could be an interesting method to detect smaller, but recurrent, differences between genotypes. In this view, we decided to focus the discussion on the chi squared and odds ratio results. However, it is important that future studies could determine a more standardized statistical method to analyze genetics influence on physical performance, in order to facilitate comparisons among different studies.

Our results showed that an individual carrying the MCT1 TT genotype was more likely to be performing at highest speeds when reaching VT (p < 0.05). VT is related to the first increase in blood H+ ions and lactate levels during incremental exercise [6], which are mainly transported from muscle by the MCT family of transporters [10]. Utilizing a circuit-training mode, Cupeiro et al. [13] observed less lactate accumulation in individuals with the TT genotype. Thus, it is possible that the TT genotype might delay the blood lactate accumulation. However, due the relatively small number of subjects carrying the TT genotype, these results should be replicated in a bigger sample to reinforce our findings.

It has been postulated that VT corresponds to the ventilatory response to the first increase in blood lactate levels and H+ ions during incremental exercise [6]. During dynamic exercises, approximately 80% of muscle lactate is transported across the sarcolemma by the MCT family of transporters [10]. More recently, a polymorphism in the MCT1 gene has been suggested to play an important role in muscle lactate transport [12] and accumulation [13], as described above, suggesting that MCT1 TT genotype increases blood lactate clearance via the transport of lactate from exercised to non-exercised muscle cells, in which lactate can be oxidized. Thus, our findings suggest that the TT genotype could increase the lactate influx to non-exercised muscles, delaying ventilatory compensation for the first increase in blood lactate concentration and carbon dioxide pressure.

The present study has some limitations. First, the subjects in the present investigation were characterized as physically active, as evidenced by the IPAQ outcomes. Thus, caution should be exercised in extrapolating these findings to highly trained endurance runners. Second, the present study was conducted using a relatively small sample size. Therefore, our findings must be confirmed in a larger cohort of subjects. Lastly, the MCT1 polymorphism data should be analyzed carefully due the small number of TT subjects, and should be replicated in other studies, in order to verify the recurrence of our results. On the other hand, it is important to notice that our cohort was

composed exclusively of men. In addition, no differences in physical activity level, age, and anthropometric measurements were observed among the individuals with different MCT1 genotypes. This finding appears to be particularly important because training status [29], age and gender [30] can influence VT and RCP. Therefore, it is reasonable to assume that some of the potential confounding variables were controlled in the current study.

Conclusion

In conclusion, the present study provides novel findings associating the MCT1 gene polymorphism with physiological variables that are connected to endurance performance. The MCT1 TT genotype was associated with the occurrence of VT at higher speeds. The MCT1 TT genotype could possibly lead to less blood lactate accumulation, what might be associated with a better aerobic profile during exercise, which is beneficial for endurance performance.

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