UNIVERSIDAD ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
FACULDADE DE CIÊNCIAS – BAURU

POST GRADUATE COURSE IN MOVEMENT SCIENCES

DISSERTATION

EFFECTS OF MECHANICAL ENERGY ON ANAEROBIC CAPACITY DURING A SUPRAMAXIMAL TREADMILL RUNNING: IS THERE INFLUENCE BETWEEN RUNNERS AND ACTIVE INDIVIDUAL

Joel Abraham Martínez González

BAURU
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Joel Abraham Martínez González

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Dissertation presented to School of Science, Campus BAURU, São Paulo State University as part of the requirements to obtain the master degree title in Movement Sciences

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Faculdade de Ciências

ATA DA DEFESA PÚBLICA DA DISSERTAÇÃO DE MESTRADO DE JOEL ABRAHAM MARTINEZ GONZALEZ, DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA MOTRICIDADE, DA FACULDADE DE CIÊNCIAS - CÂMPUS DE BAURU.

Aos 29 dias do mês de março do ano de 2018, às 09:00 horas, no(a) Anfiteatro do Departamento de Educação Física, Faculdade de Ciências, Unesp Bauru, reuniu-se a Comissão Examinadora da Defesa Pública, composta pelos seguintes membros: Prof. Dr. ALESSANDRO MOURA ZAGATTO - Orientador(a) do(a) Departamento de Educação Física / UNESP - Faculdade de Ciências de Bauru - SP, Prof. Dr. FABIO AUGUSTO BARBIERI do(a) Departamento de Educação Física / Faculdade de Ciências de Bauru - SP, Prof. Dr. LEONARDO ALEXANDRE PEYRÉ TARTARUGA do(a) Escola Superior de Ed. Física, Laboratório de Pesquisa do Exercício / Universidade Federal do Rio Grande do Sul, sob a presidência do primeiro, a fim de proceder a arguição pública da DISSERTAÇÃO DE MESTRADO de JOEL ABRAHAM MARTINEZ GONZALEZ, intitulada **Capacidade Anaeróbia e a contribuição da energia mecânica: uma análise multifatorial**. Após a exposição, o discente foi arguido oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:____ APROVADO___. Nada mais havendo, foi lavrada a presente ata, que após lida e aprovada, foi assinada pelos membros da Comissão Examinadora.

Prof. Dr. ALESSANDRO MOURA ZAGATTO

Prof. Dr. FABIO AUGUSTO BARBIERI

Prof. Dr. LEONARDO ALEXANDRE PEYRÉ TARTARUGA

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ATESTADO DE APROVAÇÃO - DEFESA

Atestamos que **JOEL ABRAHAM MARTINEZ GONZALEZ**, RA nº: CMH160245, Passaporte nº G13712210, defendeu, no dia 29/03/2018, a dissertação intitulada **Capacidade Anaeróbia e a contribuição da energia mecânica: uma análise multifatorial**, junto ao Programa de Pós Graduação em CIÊNCIAS DA MOTRICIDADE, Curso de Mestrado Acadêmico, tendo sido 'APROVADO'.

Atestamos ainda que a obtenção do título dependerá de homologação pelo Órgão Colegiado competente.

Bauru, 29 de março de 2018

Caroline E. Bolla Rogeri
Supervisor Técnico de Seção
Seção Técnica de Pós-graduação

THIS PROJECT WAS POSSIBLE THANKS TO THE ORGANIZATION OF AMERICAN STATES AND COIMBRA GROUP

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Resumo

O objetivo do presente estudo foi determinar a influência de variáveis biomecânicas na capacidade anaeróbia e no desempenho avaliado com um único esforço supramáximo, e se essa relação é dependente da experiência de treinamento refletida na técnica de corrida. Participaram neste estudo descritivo 22 homens saudáveis (29,18 ± 7,13 anos, 175,27 ± 5,8 estatura e 71,54 ± 8,52 de massa), divididos em dois grupos, fisicamente ativo (n = 11) e corredores amadores (n = 11) com $\dot{V}O_{2máx}$ de 43,17 ± 4,46 mL·kg^{-1}·min^{-1} para atividade física e 55,45 ± 5,58 mL·kg^{-1}·min^{-1} para corredores amadores, respectivamente. Os voluntários realizaram três esforços supramáximos em dias diferentes a 115% da intensidade associada a $\dot{V}O_{2max}$ (i$\dot{V}O_{2max115\%}$) para estimar a capacidade anaeróbia (CA), calcular o trabalho mecânico total ($W_{[tot]}$) e testar a confiabilidade dos dados biomecânicos variáveis (isto é, trabalho mecânico e cinemática de corrida). Foram calculadas as correlações de Pairwise Person entre todas as variáveis biomecânicas e AC, bem como o desempenho de esforço supramáximo para verificar as correlações e uma análise de regressão múltipla foi calculada para obter o melhor preditor de AC e desempenho do esforço supramáximo. Uma ANOVA two way foi utilizada para verificar a confiabilidade dos cálculos do trabalho mecânico e verificar as diferenças nas variáveis biomecânicas ao longo do esforço supramáximo (dividido em quatro períodos de tempo). Os principais achados do estudo foram: 1) O tempo de balanço (SWT) e a frequência de passos (SF) foram encontrados associados a variáveis metabólicas nos corredores e somente o trabalho interno ($W_{[int]}$) em ativos. A análise de regressão pareada revelou que essas variáveis explicaram 52,6% e 42,4% da variabilidade na CA. 2) Além disso, em relação ao desempenho supramáximo, a energia potencial (E_{PE}) explica 47,2% da variabilidade em corredores e o SwT explica 40,8% da variabilidade em ativos. 3) o re-teste teve um efeito de aprendizagem entre os corredores, porque eles usaram 27,42% menos trabalho mecânico no teste válido. É possível concluir que SF é o melhor preditor de AC em corredores e o $W_{[int]}$ é o melhor preditor de AC em ativos. A familiarização afeta o trabalho mecânico de acordo com a experiência de corrida. No entanto, o trabalho mecânico pode precisar de mais de dois testes para se estabilizar.

Abstract

The purpose of the present study was to determine the influence of biomechanical variables on anaerobic capacity and performance assessed with a supramaximal effort and if this relationship is depending on training experience reflected in the running technique. 22 healthy men participated in this descriptive study (29.18 ± 7.13 years, 175.27 ± 5.8 height and 71.54 ± 8.52 of mass), divided in two groups, physical active (n=11) and amateur runners (n=11) with a $\dot{V}O_{2max}$ of 43.17±4.46 $mL \cdot kg^{-1} \cdot min^{-1}$ for physical active and 55.45±5.58 $mL \cdot kg^{-1} \cdot min^{-1}$ for amateur runners respectively. The volunteers performed three supramaximal efforts in different days at 115% of the intensity associated to $\dot{V}O_{2max}$ (i$\dot{V}O_{2max115\%}$) in order to estimate the anaerobic capacity (AC), calculate the total mechanical work ($W_{[tot]}$) and to test reliability of biomechanical variables (i.e. mechanical work and running kinematics). Pairwise Person correlations were calculated between all the biomechanical variables and AC as well as supramaximal effort performance to verify correlations and a multiple stepwise regression analysis were calculated in order to obtain the best predictor of AC and supramaximal effort performance. An ANOVA two way was used to verify the reliability of mechanical work calculations and to verify differences in biomechanical variables along the supramaximal effort (divided in four-time periods). The major findings of the study were:1) The swing time (SwT) and the step frequency (SF) were found to be associated with metabolic variables in the runners and only the internal work($W_{[int]}$) in physical active. Pairwise regression analysis revealed that these variables explained 52.6% and 42.4% of the variability in AC. 2) In addition, regarding the supramaximal performance, the potential energy (E_{PE}) explains 47.2% of the variability in runners and SwT explains 40.8% of the variability in physical active. 3) the retest had a learning effect among runners because they used 27.42% less mechanical work in the valid test. It is possible to conclude that SF is the best predictor of AC in runners and the $W_{[int]}$ is the best predictor of AC in physical actives. The familiarization affects the mechanical work according to running experience. However, mechanical work expenditure may need more than two trials to stabilized.

FIGURE LIST

TABLE LIST

LIST OF ABBREVIATIONS AND ACRONYMS

ATP	Adenosine triphosphate
EPOC	Excess post exercise oxygen consumption
$[La^-]$	Blood lactate concentrations
AC	Anaerobic capacity
CoM	Body center of mass
CT	Contact time
E_{KE}	Kinetic energy
E_{PE}	Potential energy
$EPOC_{fast}$	Excess post exercise oxygen consumption – fast component
FT	Flight time
iVO_{2max}	Intensity associated to $\dot{V}O_{2max}$
$iVO_{2max115\%}$	115% of the intensity associated to $\dot{V}O_{2max}$
MAOD	Maximum deficit accumulated of oxygen
$MAOD_{ALT}$	Alternative maximum deficit accumulated of oxygen
ME	Mechanical efficiency
MTC	Monocarboxylate-specific
NAD	Nicotinamide adenine dinucleotide
O_{2deb}	Oxygen debt
O_{2def}	Oxygen deficit
PCr	Phosphocreatine
SF	Steep frequency
SL	Steep length
TD	Touchdown
tlim	Time-to-exhaustion/supramaximal effort performance
TO	Toe-off
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2baseline}$	Oxygen uptake baseline or at rest
$\dot{V}O_{2max}$	Maximal oxygen uptake
$\dot{V}O_{2max\ EX}$	Maximal oxygen uptake reached at exhaustion point
$\dot{V}O_{2st}$	Oxygen volume stores
$E_{[La]}$	Glycolytic metabolic system
$E_{[pcr]}$	Phosphagen metabolic system
$W_{[ext]}$	External work
$W_{[int]}$	Internal work
$W_{[tot]}$	Total work

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1. Introduction

During a supramaximal exercise until exhaustion, the non-mitochondrial adenosine triphosphate (ATP) synthesis plays an important role to produce the energy required by the body. This metabolism is composed by two pathways, the phosphagen metabolic system ($E_{[pcr]}$) and the glycolytic metabolic system ($E_{[La]}$) (GASTIN, 2001; NOORDHOF, KONNING and FOSTER, 2010). The former refers to the degradations of phosphocreatine (PCr) stores and the latest refers to the partial degradation of glycogen with the subsequent blood lactate accumulation (FERRETTI, 2015). Theoretically, the anaerobic capacity (AC) is assumed as the sum of the contribution of both the $E_{[pcr]}$ and the $E_{[La]}$ and is the total energy that can be re-synthesized through non-mitochondrial pathways, for a specific effort and during a short duration high-intensity exercise (GREEN, 1994; MEDBØ, 1996).

The maximum accumulated oxygen deficit (MAOD) has been the most accepted method to estimate AC, however, it implies several visits to the laboratory. Currently the alternative maximal oxygen accumulated deficit ($MAOD_{ALT}$) to estimate AC was proposed by Bertuzzi et. al. (2010) which is capable of estimate the AC with the same accuracy as conventional MAOD plus the advantage of time reduction and the possibility to obtain values of both systems (i.e. $E_{[pcr]}$ and $E_{[La]}$). Recent studies have shown that AC seems to be influenced by some acute ingestion of ergogenics such as sodium bicarbonate and caffeine (BRISOLA, MIYAGI, *et al.*, 2015; DE POLI, MIYAGI, *et al.*, 2016) and have observed associations with mechanical properties such as MP and PP production assessed in cycle ergometers and treadmill (BERTUZZI, KISS, *et al.*, 2015; ZAGATTO, MIYAGI, *et al.*, 2017b).

There is extensive research into running biomechanics for a long period of time (CAVANAGH, 1990) whether running kinetics or kinematics influence metabolic variables, these research is focused on factors related with mitochondrial energy production and its impacts on endurance capabilities (i.e. running economy) (SAUNDERS, PYNE, *et al.*, 2004; TARTARUGA, BRISSWALTER, *et al.*, 2012; LACOUR and BOURDIN, 2015). In general, this kind of studies, agree that a good technique enables a runner to reduce the vertical displacement of the body center of mass (CoM) which is directly related to E_{PE} and has physiological implications, because its association with $\dot{V}O_2$, which is a characteristic of good running performance and a better running economy (WILLIAMS and CAVANAGH, 1987;

LUHTANEN, RAHKILA, *et al.*, 1990; MARTIN, HEISE and MORGAN, 1993; KYRÖLÄINEN, KOMI and BELLI, 1995).

However, scarce literature has addressed the influence of mechanical work in supramaximal efforts and as a consequence with AC and supramaximal performance. Keir et. al. (2012) is one of the few who investigated the non-mitochondrial energy production and the mechanical variables. AC was estimating by using accumulated oxygen deficit (AOD) method, in order to verify differences between each speed bound and mechanical efficiency. Nevertheless, would be expected a negative relationship between $W_{[int]}$ and AC in runners due to a better leg recovery which implies less inertial resistance and, therefore, less work done by the leg transportation. The contrary would be expected in physical active people. Thus, due to the fact that a better technique tends to be more economical reflected in lower CoM's vertical oscillations while running would be factors that could potentiate AC and supramaximal performance.

2. Literature review

2.1. ATP turnover and anaerobic capacity

The energy required by a contracting muscle is supplied by the degradation of the molecular bounds of ATP, and its production relies on three processes: non-mitochondrial and mitochondrial pathways which in conjunction compose the three systems of energy production, $E_{[pcr]}$, $E_{[La]}$ and oxidative system (BAKER, MCCORMICK and ROBERGS, 2010). The activation of those systems implies that the muscle must breakdown the ATP molecule through the ATPase enzyme and transferred to the myosin molecule on order to activate the cross-bridge cycle formed between the myosin and actin proteins. This reaction implies the formation of adenosine di-phosphate (ADP) and phosphate (Pi) (ATP $\longrightarrow$ ADP + Pi) in a process called ATP hydrolysis. This biochemical reaction is reversible through phosphocreatine hydrolysis that can regenerates the ATP for short period of time without oxygen and does not implies several metabolic reactions, although, it does require additional energy to happens (ATP $\longleftarrow$ ADP + Pi). The cycle formed by ATP hydrolysis and phosphocreatine hydrolysis to re-synthesize ATP, whether by non-mitochondrial or mitochondrial pathways is entitled as ATP turnover (SCOTT, 2005).

At the onset of exercise and mainly during an intense effort the ATP turnover from $E_{[pcr]}$ is managed by two isoforms of creatine kinase enzyme (e-CK). The e-CK that catalyze the degradation of PCr to form ATP in the cytoplasm, and the mi-CK that reacts due to an increase of creatine (Cr) which moves freely through the mitochondrial outer membrane. As a consequence, increasing the ADP concentrations which in turn allows the proteins (e.g. translocators) to transport the ADP into the mitochondrial inner membrane resulting in the activation of ATP production by oxidative phosphorylation (WALSH, TONKONOGI, *et al.*, 2001; GRASSI, 2005). Additionally, it has been demonstrated that the PCr is a powerful regulator of mitochondrial respiration activating more rapid the oxidative phosphorylation than free Cr (GREENHAFF, 2001; WALSH, TONKONOGI, *et al.*, 2001), and acts as a temporal buffer that prevents the increase of ADP product of an abrupt increase on the demands of ATP during a muscle contraction (GRASSI, 2005).

If the exercise is maintained longer than few seconds and once the AMP has activated the mitochondrial respiration the glucose / glycogen begins to play a more active role on the ATP turnover now within the glycolysis. The glycolysis, starts when the glycogenolysis increases the quantity of glucose-6-phospate that subsequently activate a sequence of 8 reactions. Biochemically the process is divided in two phases, which in short, ends up generating two molecules of lactate plus two usable molecules of ATP from the exergonic reactions between the glyceraldehyde-3-phosphate and the inorganic phosphate (BAKER, MCCORMICK and ROBERGS, 2010).

It has proven by direct measurements that during an intense exercise the ATP re-synthesized by non-mitochondrial pathways, accounts ~20%, whereas the catabolism of carbohydrates by the glycogenosis / glycolysis, contributes ~53% (BANGSBO, GOLLINCK and GRAHAM, 1990), obviously, the remaining energy is provided by mitochondrial respiration. Thus, together $E_{[pcr]}$ and $E_{[La]}$, constitute the anaerobic capacity which is the total energy that can be re-synthesized through non-mitochondrial pathways, mainly in a short duration-high intense exercise (GREEN, 1994; MEDBØ, 1996; NOORDHOF, KONNING and FOSTER, 2010).

Thus, AC represents a physiological marker which provide the necessary information to determine the bioenergetic profile during training and competitions but due to the complex biochemical processes because the three systems are interlinked and work simultaneously, getting access to both $E_{[pcr]}$ and $E_{[La]}$ (and thus AC) it is somehow possible by assuming the non-mitochondrial processes are activated in the whole body (at least in the largest body mass) during a constant load exercise at high intensity.

2.2. The oxygen deficit concept

Estimating anaerobic capacity has been of great interest to scientists for more than half a century due to alterations in human physiology when the body is performing exercise and the possible implications to physical health and sports training. One of the greatest achievements on this field is the term maximal accumulated oxygen deficit (MAOD), introduced by Medbø for the first time in 1988 that was possible thanks to the studies made by two great scientists, Schack August Steenberg Krogh (1874-1949) and Johannes Lindhard of the University of Copenhagen, Denmark. The Krogh and Lindhard studies were crucial not only to MAOD but to exercise physiology theory because they introduced the physiological principle of O_{2def} from observations on $\dot{V}O_2$ kinetics in humans. As a result, they concluded that $\dot{V}O_2$ does not increase immediately after the beginning of physical exercise activity, but rather after several minutes later until the plateau is reached (BERTUZZI, LIMA SILVA, *et al.*, 2008; NOORDHOF, KONNING and FOSTER, 2010).

The energy used during the deficit phase corresponds to non-mitochondrial energy sources and is derived mainly from PCr stores and subsequently blood lactate production / concentration ($[La^-]$) (GASTIN, 1994). Years later, Hermansen and his co-workers (1969) retook the principle in a series of experiments and showed how calculate the accumulated oxygen deficit by means of the integral between the curve of oxygen demanded and O_2 consumed and thus influencing studies for at least 15 years. It was until late 1980 decade when Medbø et. at. (1988) redefined the concept and developed the most accepted non-invasive method to estimate AC based on Hermansen's proposal, the maximal accumulated oxygen deficit (MAOD). MAOD assumes the anaerobic capacity could be estimated through the difference between the demand of O_2 linearly estimated and the accumulated oxygen consumption ($\dot{V}O_2$) during a supramaximal exercise. Therefore, the concept of O_{2def} has been used as a way to estimate the metabolic production of ATP from non-mitochondrial pathways. Hence, the O_{2def} is also of great interest to estimate the phosphagen metabolism (i.e. E_{PCr}) of the ATP resynthesize under high-intensity exercise conditions (GRAHAM, 1996; MEDBØ, 1996).

2.3. The oxygen debt concept

Hill hypothesized that the oxygen debt (O_{2deb}) is the amount of oxygen consumed after exercise to restore the deficit occurred at the onset of the exercise and the entire phenomenon was due to the oxidation of lactate. They also noticed that the O_{2deb} has two phases the fast and the slow component. Initially, it was believed the fast and slow component had a role in removal of lactate that could have escaped by diffusion from the muscle to the blood stream (HARRIS, 1969; GAESSER and BROOKS, 1984).

Later Margaria et. al. (1933) based on Lundsgaard's discovery of the phosphagens, took back the Hill's idea of fast component and slow component of O_{2deb} but with a different meaning because he proved that during the fast component of O_{2deb} there is no removal of lactate, instead, the volume of O_2 stores ($\dot{V}O_{2st}$) and PCr are recovered in this phase and consequently the slow component of O_{2deb} is in part used in glycogenesis processes. Moreover, they were the first to provide a calculation of

O_{2deb} in order to estimate the $E_{[pcr]}$ contributions (GAESSER and BROOKS, 1984; DI PRAMPERO and FERRETTI, 1999).

Green and Dawson (1993) highlighted some studies regarding the calculations of oxygen consumed during O_{2deb}, for instance the study of Katch (1973) that reinforces the validity of the biexponential equation $Y=c+a_1e^{-k_1t}+a_2e^{-k_2t}$ which fits well the curve of O_2, and also the work of Roberts and Morton (1978) who confirmed that it is possible to obtain reliable measurements of O_{2deb} as they reported a good test-retest correlation ($r = 0.89$) using the biexponential equation. Besides they improved the process to perform the calculations that takes into account the oxygen offset (i.e. the delay in the fall of oxygen) and the approximate a suitable value of O_2 baseline.

Currently, the excessive post-exercise oxygen consumption (EPOC) is a more accepted concept to refer to O_{2deb} because it implies that the energy used during O_{2deb} serves to restore homeostasis and involves other physiological adjustments (i.e. respiratory, circulatory, hormonal, ionic and thermal) besides the PCr restoration.

2.4. Blood lactate concentrations its significance in supramaximal efforts

With the same importance of the O_{2def} and EPOC concepts to the knowledge of sports / exercise science bioenergetics, is the lactate metabolite which showed up thanks to the contribution of Fletcher and Hopkins (1907) who described the acid lactic production on an amphibian muscle, marking the beginning of the acid lactic era in the study of muscular physiology (BROOKS and GLADDEN, 2003).

Lactate is considered a sub-product of glycolytic system that is catalyzed through the enzyme lactate dehydrogenase (LDH) in the cells' cytoplasm and is formed from a glucose / glycogen molecule. Lactate plays an important role in the nicotinamide adenine dinucleotide (NAD) redox reaction (NAD^+/NADH), within the ATP production (ROBERGS, GHIASVAND and PARKER, 2004), helps to remove the excess pyruvate and also acts as a proton buffering that retards the muscular acidosis, hence, sustaining high production rates of $E_{[La]}$ for longer periods of time even after few seconds (BAKER, MCCORMICK and ROBERGS, 2010).

From the bioenergetic point of view Margaria et. al. (1963) observed the rate at which lactic acid is produced and accumulated in blood is constant and depend on the intensity (i.e. velocity or power). A linear relationship was possible where the higher slope is the higher is the intensity applied. Later di Prampero (1981) calculated the rate of diffusion of lactate through different compartments of blood to whole-blood lactate concentrations at equilibrium from which one can obtain the oxygen equivalents of 1 mmol of lactate concentration with the same linear relationship. The energy equivalent of blood lactate accumulation is a key concept of such an importance in the analysis of the whole-body energy expenditure during the supramaximal exercise besides represents one of the cornerstones of the alternative maximal accumulated oxygen deficit that we will discuss later.

2.5. Anaerobic capacity and maximal accumulated oxygen deficit

2.5.1. The maximal accumulated oxygen deficit method

As previously mentioned the MAOD method considers the principle of O_{2def} and assumes that AC could have been accessed by the concomitant difference between an O_2 demand and the accumulated $\dot{V}O_2$ during supramaximal exercise. The O_2 demand is obtained by linear regression between the O_2 uptake and the intensity at submaximal efforts (between 30% to 90% of $i\dot{V}O_{2max}$). Then, the accumulated $\dot{V}O_2$ is calculated by mathematical integration (MEDBØ, 1996; NOORDHOF, KONNING and FOSTER, 2010).

Regarding to the validity of MAOD, Bangsbo et. al. (1990) is capable of providing satisfactory estimates of muscle lactate production, degradation of ATP and PCr in exercises that involved small muscle groups. Bangsbo and coworkers observed in an experiment that extracted muscular biopsy samples and arterial-venous's blood from the muscle rectus femoral, that the total ATP production by direct methods amounted to 83.1 mmol ATP (kg wet wt^{-1}), which was extremely well related with 94.7 mmol ATP (kg wet wt^{-1}) estimated with indirect method (i.e. MAOD) (BANGSBO, GOLLINCK and GRAHAM, 1990; BERTUZZI, LIMA SILVA, *et al.*, 2008). Although, MAOD is the most accepted method to estimate anaerobic capacity its determination involves the measurements of several submaximal tests to establish the O_2 linear estimations fact that makes it unfeasible in the field (NOORDHOF, KONNING and FOSTER, 2010).

2.6. Alternative maximal accumulated oxygen deficit method

The need to increase the effectiveness of protocols that help to determine the energy non-mitochondrial energy production, has led researchers to propound evaluation forms increasingly close to real training or competition situations as well as to improve the applicability of such assessments in experimental conditions (BENEKE, BEYER, *et al.*, 2004; BERTUZZI, FRANCHINI, *et al.*, 2007; ZAGATTO and GOBATTO, 2012).

In this regard, Beneke et. al. (2002) based on Margarias et. al. (1933) and di Prampero et. al. (1999) studies, estimated, the extent at which the 30 seconds Wingate test requests the non-mitochondrial energy sources. On this study they estimate $E_{[pcr]}$ by the adjustment of a biexponential equation on Excess post exercise oxygen consumption – fast component ($EPOC_{fast}$) and $E_{[La]}$ expressed in equivalents of oxygen. Bertuzzi et. al. (2010) summed both estimations (i.e. $E_{[pcr]}$ and $E_{[La]}$) in order to obtain an AC estimative, and then, compared with MAOD. The methods were not different ($p = 0.60$) and were highly correlated ($r = 0.78$; $p = 0.014$) and such sum was named as alternative maximal accumulated oxygen deficit ($MAOD_{ALT}$).

According to Zagatto et. al. (2016) and Miyagi et. al. (2017) $MAOD_{ALT}$, is a valid method where the intensity of 115% of $i\dot{V}O_{2max}$ presented the highest concordance when compared with MAOD in treadmill and cycle ergometer. Besides, the test-retest was verified and the results leads to consider $MAOD_{ALT}$ as highly repeatable method also at 115% of $i\dot{V}O_{2max}$ since a small effect size value (≤ 0.45), an intra-class correlation (≥ 0.77) and limits of agreement of 95%, were found. Those studies reinforce the idea that states, to deplete the non-mitochondrial energy stores it is necessary to choose an effort at a certain intensity that allows the total usage of non-mitochondrial energy sources prior the accumulation of H^+ that deactivates glycolytic enzymes preventing access to these energy sources.

2.6.1. $MAOD_{ALT}$ physiological assumptions

The $MAOD_{ALT}$ method is based on three physiological assumptions, i) the oxygen consumed during $EPOC_{fast}$ it is assumed equivalent to the oxygen consumed during the deficit, ii) during the $EPOC_{fast}$ the PCr should be restored and iii) blood lactate reflect the contribution of $E_{[La]}$ in O_2 equivalents.

There is a considerable amount of studies that are consistent with the theories about O_{2def} and O_{2deb} are symmetric and quantitatively equal between exercise and recovery kinetics of $\dot{V}O_2$ reinforcing the first $MAOD_{ALT}$ assumptions (PATERSON and WHIPP, 1991; ENGELEN, PORSZASZ, *et al.*, 1996; ÖZYENER, ROSSITER, *et al.*, 2001; CLEUZIOU, PERREY, *et al.*, 2003).

Although, the recovery of PCr after a high intense effort is determined by several factors the (SAHLIN, HARRIS and HULTMAN, 1975; SAHLIN, HARRIS and HULTMAN, 1979; ARNOLD, MATTHEWS and RADDA, 1984; MCMAHON and JENKINS, 2002), Piiper and Spiller (1970) apud Bertuzzi and Souza (2009), observed that the O_2 availability is most influential factor of PCr recovery is after an effort since they observed that the ATP-PCr responses and the $\dot{V}O_2$ (EPOC) had a similar tendency during the initial minutes of recovery. They compared a muscle sample with measurements of $\dot{V}O_2$ by arterial-venous difference. Concluding that probably the recovery $\dot{V}O_2$ guarantees the recovery of PCr in that phase. In that sense, Harris et. al. (1976) extracted muscular tissue after a bout of isometric knee extension while a pneumatic cuff (inflated to a pressure of 240mm Hg) was placed around the volunteers' thigh causing blood occlusion. The degraded PCr was measured at different moments during the 6 minutes period recovery. The recovery of PCr was completely inhibited when the circulation of the thigh was occluded. Furthermore, Haseler et. al. (1999) measured the fraction of inspired O_2 (F_{IO2}) as a tool to assess the O_2 availability during the recovery through nuclear magnetic resonance spectroscopy (^{31}P-NMR). The subjects were lying down supine in a whole-body magnetic resonance imaging system while they performed a submaximal plantar flexion at an intensity of ~60%. Also, ^{31}P-MRS samples were collected during the exercise when the subjects breathed different concentrations of oxygen (10%, 20% and 100% of O_2 concentrations). They reported that the PCr recovery is sensitive to oxidative capacity where the kinetics of PCr were statistically slower in hypoxic conditions ($\tau = 33.5 \pm 4.1$ s) than hyperoxia conditions ($\tau = 20.0 \pm 1.8$ s) and both different from normal condition of O_2 concentrations ($\tau = 25.0 \pm 2.7$ s) ($p < 0.05$).

On the other hand, the lactate metabolite has been quantitatively related to all the energy output from $E_{[La]}$ which made it a marker of the same energetic system. As briefly mentioned the school of Milan headed by Margaria (1963) took advantage of this physiological discovery. They were able to demonstrate by means of a series of supramaximal exercises at several intensities and inclines that the rate of lactate production/accumulation in blood (and consequently in muscle), increases linearly as the metabolic power increases (e.g. $\dot{V}O_2$). Then, it was possible to calculate the energetic equivalent (in units of O_2) of 1 mmol of lactate per body mass just considering the slope of the regression line as a measure of the amount of energy released by the lactate production, (which is also a measure of the rate of lactic acid increases in blood)

(DI PRAMPERO, 1981). To do so, two conditions were assumed, that the peak blood lactate attained between the 5th and the 8th minutes of EPOC i) is the result of an equilibrium of the actual lactate concentrations in both extra and intra cellular fluids, and ii) the time constants of lactate disappearance in the different body fluid compartments are the same (DI PRAMPERO and FERRETTI, 1999).

2.6.2. Anaerobic capacity estimated by $MAOD_{ALT}$ state of the art

Probably the current research of AC has been oriented in three main topics; i) bioenergetics' estimations of competition simulations and/or training actions in a specific sport modality, ii) the use of ergogenic substances to enhance the AC, and, iii) $MAOD_{ALT}$ extra features that seek relationships with other methods and/or protocols.

Beneke et. al. (2004) was probably one of the first to capitalize the estimation of $E_{[pcr]}$ through $EPOC_{fast}$ and the $E_{[La]}$ by means of lactate oxygen equivalent. They evaluated ten well trained karatekas that were about to compete in the European Championship with a simulated competition test consisted on two to four fights organized and rated as the last year qualifying rounds in order to estimate the karate-fighting bioenergetics in competition. They also simulated the rest time intervals between separate fights, using as a parameter previous competition, hence, 17 minutes between fight 1 and 2, 15 minutes between fight 2 and 3 and 9 minutes between fight 3 and 4 were considered. The breath by breath gas exchange was measure during and after each fight, as well as blood lactate samples, collected right after a fight and along the whole recovery time. They concluded that in spite of the fact that the karate kumite fighting is composed by high intensity activities that accounted around 7.4% and 17.9% of non-mitochondrial metabolism (i.e. $E_{[pcr]}$ and $E_{[La]}$ respectively) its overall metabolism is mainly supplied by mitochondrial respiration that accounted 74.7% of energy supplies.

It is known that diverse ergogenic substances could allow to get access to non-mitochondrial stores through an effect within the extracellular buffering system which would allow to stay for longer time in an intense effort, therefore, deplete in a greater extent those stores meaning a better estimation of AC. That is the case of sodium bicarbonate ($NaHCO_3$) which has been proven is responsible for an increase of blood lactate concentrations post exercise, plus to be the responsible to slow the constant time during the $EPOC_{fast}$. Brisola et. al. (2016) verified the effects of acute $NaHCO_3$ supplementation on AC in a double-blind, crossover, placebo-controlled study, estimated by $MAOD_{ALT}$ and the likely correlations with the 200 and 400-meter dash in a track. It was reported a statistical significant alternatively with a likely positive effect on $MAOD_{ALT}$ after acute supplementation with $NaHCO_3$. Likewise, other ergogenics have been investigated, such as taurine (MILIONI, DE SOUZA MALTA , *et al.*, 2016) and caffeine (DE POLI, MIYAGI, *et al.*, 2016), but $MAOD_{ALT}$ does not seem to be influenced after an acute supplementation with these ergogenic substances, although, it is important to point out that the caffeine had a negative effect on $E_{[pcr]}$ reducing its time constant in addition to having a possibly positive effect on $E_{[La]}$.

Separately, Zagatto et. al. (2017) investigated the sensitivity of $MAOD_{ALT}$ to detect differences on AC among individuals with different levels of physical fitness. Less trained individuals (LT), moderately trained individuals (MT), runners (ET) and rugby players (RG), performed a supramaximal effort at 115% to estimate AC through $MAOD_{ALT}$. The authors reported meaningful differences between all the possible combinations within the groups, which allows us to infer that $MAOD_{ALT}$ is able to detect differences between groups with different levels of AC as it was the case of this investigation.

Also, recently Bertuzzi et. al. (2015) analyzed non-mitochondrial parameters ($E_{[pcr]}$ and $E_{[La]}$) of $MAOD_{ALT}$ and the mechanical variables such as MP and PP of 30 seconds Wingate test (WAnt-30) in cycle ergometer. As expected there were positively correlations between $E_{[pcr]}$ and PP ($r= 0.71$; $p<0.05$) and between MP and $E_{[La]}$ ($r= 0.72$; $p<0.05$). Therefore, authors suggested that individuals with higher PP could also have higher $E_{[pcr]}$ contributions and, also that the ability to exert high values of mechanical power during the WAnt-30 is an expression of $E_{[La]}$ contributions. These results are similar with those reported by Zagatto et. al. (2017) in a 30 seconds all-out tethered running effort. Where the $E_{[La]}$ was positively associated with MP ($r = 0.58$; $p < 0.03$) as well as the $MAOD_{ALT}$'s absolute value was correlated with MP ($r = 0.58$; $p =0.03$), total work ($r = 0.57$; $p = 0.03$) and mean force ($r = 0.79$; $p = 0.001$). The significant correlations observed in both studies highlight the importance of energy production by non-mitochondrial pathways to maintain short intense efforts and into the production of mechanical work. As a result of all the evidence reported so far, seems that $MAOD_{ALT}$ is capable of estimate the AC with the same accuracy as conventional MAOD plus the advantage of time reduction and the possibility to obtain values of both systems, the $E_{[pcr]}$ and the $E_{[La]}$.

2.7. Biomechanical variables as a determinant of anaerobic capacity and supramaximal effort performance

The total mechanical work ($W_{[tot]}$) represents the sum of W_{int} and the W_{ext}. The $W_{[ext]}$ is related to the CoM‘s interaction with the environment and, it is also, the energy required to overcome the gravitational force as the CoM‘s moves vertically in a sagittal plane under normal circumstances, whether during daily live locomotion tasks or locomotion exercises (e.g. running, walking, cycling) (SAIBENE and MINETTI, 2003). Contrary, the $W_{[int]}$ is the work responsible to accelerate the segments within its center of mass and both have been considered two separate identities (FENN, 1930; CAVAGNA and KANEKO, 1977; NARDELLO, ARDIGÒ and MINETTI, 2011; TARTARUGA, BRISSWALTER, *et al.*, 2012).

The W_{int} and W_{ext} are calculated from the movements of the CoM, the segment's center of mass as well as anthropometric parameters considering the work-energy theorem. These variations of energy are forms of energy production within the moving segments and are expressed as the potential energy (E_{PE}) and kinetic translational energy (E_{KE}). Where the E_{PE} is the energy related to the force of gravity, the E_{KE} is the energy due to the motion (therefore linked to the CoM's velocity and W_{ext}) and E_{KE} is also related with the acceleration of the segments with respect to the CoM in the planes of action.

Historically, a pioneer study regarding $W_{[tot]}$ was introduced by Fenn (1930), he studied the mechanical work done against gravity and the metabolic expenditure of some locomotion tasks such as walking and running. Later, Cavagna and Kaneko (1977), established a linear relationship between the mechanical work done (i.e. $W_{[ext]}$ and $W_{[int]}$) and the intensity (i.e. velocity) while walking and running, where it was observed a decrease in $W_{[ext]}$ while an increase in $W_{[int]}$ when the intensity was above 20 $km \cdot hr^{-1}$. They, also, proposed a linear predictor of $W_{[tot]}$ as a dependent of horizontal velocity and they suggested despite the same mechanical power by the endurance runner and the sprint runner the absolute velocity of the former could be due to a better skill ability to transform the mechanical energy into forward speed.

There has been a lot of research into running biomechanics for a long period of time (CAVANAGH, 1990) whether running kinetic influence metabolic variables these research is focused on factors related with mitochondrial energy production and its impacts on endurance capabilities (i.e. running economy) (SAUNDERS, PYNE, *et al.*, 2004; TARTARUGA, BRISSWALTER, *et al.*, 2012; LACOUR and BOURDIN, 2015).

As an example, Tartaruga et. al. (2012) observed significant correlations between several biomechanical variables (i.e. vertical oscillations of the CoM, SF, SL and SwT) running economy and concluding that a e better technique implies a better economy at least for the examined sample (recreational endurance runners). In contrast, Kyröläinen, Beli and Komi (2001) did not find exclusive parameters to explain running economy in elite runners. Additionally, in a study with competitive Kenyans distance runners Moose et.al. (2014) concluded that elite running performance is influenced by several factors besides running economy. Despite the controversy, in general, this kind of studies, agree that a good technique enables a runner to reduce the vertical displacement of CoM which is directly related to E_{PE} and has physiological implications because its association with $\dot{V}O_2$ is a characteristic of good running performance and a better running economy (WILLIAMS and CAVANAGH, 1987; LUHTANEN, RAHKILA, *et al.*, 1990; MARTIN, HEISE and MORGAN, 1993; KYRÖLÄINEN, KOMI and BELLI, 1995; ANDERSON, 2013).

As a matter of fact, a more efficient way of running could enhance AC leading to improvements on the supramaximal performance. In other words, less mechanical work expenditure would be negatively related with AC and supramaximal performance and at the same time the mechanical work variables would have different associations with those metabolic variables according to the technical abilities acquired with training. This idea is reinforced by the study of, Heise and Martin (2001) where they observed a greater expenditure of $W_{[ext]}$ (i.e. expressed as vertical impulse) in less economical endurance runners. Furthermore, Morin et. al. (2011) observed athletes (i.e. one long jumper) had a better use of energy in a horizontal way than a team sports player (i.e. one basketball player).

On the other hand, $W_{[int]}$ is linked to SF, speed and inertial values, thus, $W_{[int]}$ could influence negatively the AC in runners due to a less inertial resistance needed to run at supramaximal intensity. To support this idea Leskinen et. al. (2009) in a study with national and elite middle-distance runners observed a faster recovery of the leg during the SwT in elite runners due to higher flexion velocity of the knee joint, fact, that reduces the inertial resistance during running, hence, the $W_{[int]}$. On the contrary, would be expected in physical active a slower knee flexion velocity and a greater inertial resistance, therefore, more $W_{[int]}$ required with a positive relationship with AC.

As a consensus, longer SwT and contact time (CT) could be metabolically expensive and as a consequence of long-term training the chronic adaptations can influence the way to harness the mechanical work during a supramaximal effort since such kinematic variables CT, SwT and SL were statistically different among sprint runners, endurance distance runners and a control group (MOORE, 2016; BUSHNELL and HUNTER, 2007; SANTOS-CONCEJERO, GRANADOS, *et al.*, 2013).

Due to the mechanical work calculations proposed by Cavagna and Kaneko (1977) have been fully tested in running exercises at several speeds it is well known the trend and the factors that influence the mechanical work and energy changes yet there are not previous works that determined its associations with non-mitochondrial energy sources (WILLEMS, CAVANAGH and HEGLUND, 1995; SAIBENE and MINETTI, 2003). The only study linked with nonmitochondrial energy sources and mechanical work variables was proposed by Keir et. al. (2012) who investigated the addition of AC to the total metabolic energy expended during several submaximal and one supramaximal intensity in order to verified differences between each speed bound and mechanical efficiency (ME). They concluded that the non-mitochondrial sources have an influence on the rate at which the metabolic energy is used as mechanical energy during the movement. Currently, it is hard to say on what extent such kinematics kinetics are associated to AC and supramaximal effort performance and what would be its behavior on runners who have been trained in endurance running compared with non-trained people. Therefore, the first step is to determine the relationship between those physiological markers and the biomechanical variables.

3. Purposes

3.1. Main purpose

To determine the influence of mechanical variables on anaerobic capacity determined during a supramaximal running effort and if this possible influence is modified according running experience (amateur runners and physical active volunteers).

3.2. Secondary purposes

- To verify the reliability of the mechanical work variables between the test and re-test.

- To verify the correlation between kinetic and kinematical variables with anaerobic capacity in each group through a stepwise regression analysis.

- To verify the kinetic and kinematical variables importance in running performance.

4. Methodology

4.1. Volunteers

Prior any test the volunteers were informed about the procedures, the study's risks that are very little since this research will use non-invasive methods for measuring physiological variables and biomechanical variables during a test on a treadmill. However, it is important to stress that the potential risks are: musculoskeletal injuries to run over a treadmill, these injuries may happen due a lack of practice of running on a treadmill or a distraction while the volunteer is running. Another possible risk is the discomfort that the high intense exercise may cause and in some unlikely cases when the volunteer has a heart disease may cause cardiopulmonary complications mainly in the non-trained group. All the procedures in this study were approved by to the ethics research committee of São Paulo State University (Campus Bauru; CAAE: 62044416.0.0000.5398) and will attend the Helsinki Declaration.

Participated 22 healthy male volunteers with good health and no musculoskeletal injury background. Further, the volunteers were allocated in two groups, physical active (23.36 ± 3.61 years, 173.73 ± 4.67 cm height and 71.33 ± 10.74 kg of body mass), and amateur runners (35.00 ± 4.40 years, 176.82 ± 6.19 cm height and 71.75 ± 6.07 kg of body mass). The inclusion criterion for runners was ate least two year of training experience and to be training at the moment of the tests.

Additionally, all the volunteers were instructed to avoid any substance aid that affect the performance and physiological responses (i.e. alcohol, caffeine, sodium bicarbonate) and they were informed to avoid perform strenuous exercise 24h before each exercise session. Further, the volunteers reported had not taken ergogenic substances like chronic creatine ingestion in the previous three months.

4.2. Study protocol

The volunteers visited the laboratory four times with at least 48 hours between each visit (Fig. 1). On the first visit, a graded exercise test (GXT) was performed until voluntary exhaustion to measure the maximal oxygen consumption ($\dot{V}O_{2max}$) and to determine the $i\dot{V}O_{2max115\%}$. In the next three visits a supramaximal constant load effort at $i\dot{V}O_{2max\ 115\%}$ was performed in order to estimate $MAOD_{ALT}$ (the first 2 efforts were applied as familiarizations). Prior every effort a 5 minutes run at 8 km·h^{-1} was performed as a warm up.

All the efforts were performed on a motorized treadmill (ATL, Inbramed, Inbrasport, Porto Alegre, RS, Brazil) following the indications of Jones and Doust (1996) who recommends to incline 1% the treadmill to equalize outdoor energy cost conditions. During all efforts, participants were verbally encouraged to perform maximally.

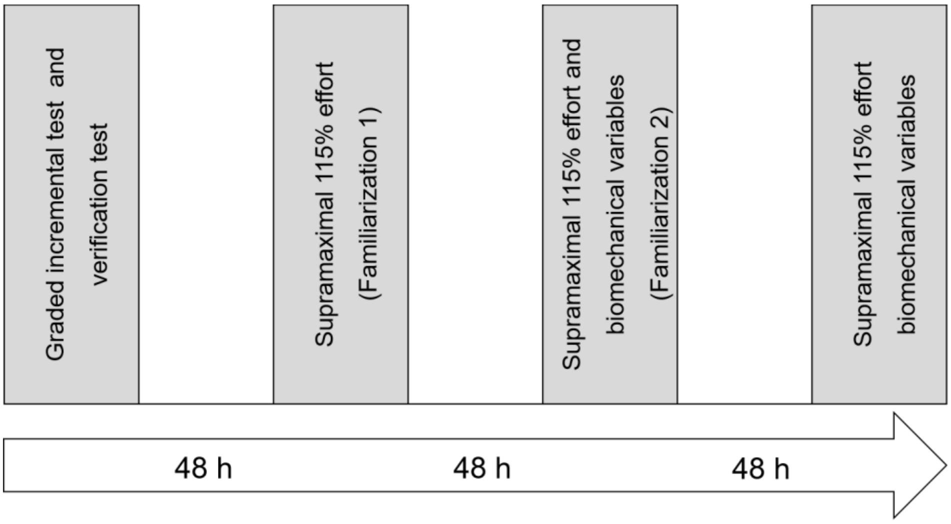

FIGURE 1: Study design

4.3. Physiological analysis

The metabolic parameters were measured breath by breath with a Cosmed Quark CPET stationary gas analyzer (Quark CPET, Cosmed, Rome, Italy), coupled with a heart rate transmitter (Wireless HR 138 Monitor, Cosmed, Rome, Italy). The gas analyzer was calibrated before each test using an ambient air sample and a high-precision gas mixture (3.98% CO_2, 16.02% O_2 and balanced N_2; White Martins Gases Industrials Ltda, Osasco, SP, Brazil), whereas the turbine was calibrated before each test and verified after each test using a 3-L calibration syringe (Hans-Rudolf, Kansas City, MO, USA) in accordance with the manufacturer's instructions. The raw data was smoothing with a moving average of 5 and then interpolated to each second.

Furthermore, to determine the peak blood lactate concentration ($[La^-]_{peak}$), blood samples from the earlobe (40µL) were taken at 3rd, 5th min after GXT test and at rest, 3rd, 5th, and 7th min after supramaximal test and transferred to Eppendorf tubes containing 80µL of sodium fluoride 1%. The samples were analyzed in duplicate using a biochemical analyzer YSI 2900 (Yellow Spring Instruments, Ohio, EUA) with a measurement error ranging ±2%. The Borg scale (6-20) was used to assess the rate of perceived exertion (RPE) on every effort.

4.4. Graded exercise test (GXT)

The GXT started with an initial 8 $km \cdot h^{-1}$ and was incremented in 1.5 $km \cdot h^{-1}$ every two minutes (ZAGATTO, NAKAMURA, *et al.*, 2017a). The oxygen consumption ($\dot{V}O_2$) was measured during the whole test and the highest average (i.e., values from the last 30-s of effort) was assumed as $\dot{V}O_{2max}$, considering the verification of plateau as a main criterion (variation in $\dot{V}O_2$ < 2.1 $mL \cdot kg^{-1} \cdot min^{-1}$ between the final and penultimate stage of exercise). As a secondary criterion to determine $\dot{V}O_{2max}$, we followed a traditional one i) maximal heart rate > age-predicted maximum (220-age), ii) maximal [La] > 8 $mmol \cdot L^{-1}$, and iii) RER > 1.10. (HOWLEY, BASSETT and WELCH, 1995; MIDGLEY and MCNAUGHTON, 2007). Additionally, a verification test on the same day was performed to corroborate the true $\dot{V}O_{2max}$ values where the volunteers ran after a 5-minutes of passive rest at 110% of $i\dot{V}O_{2max}$ until voluntary exhaustion. The highest value of the last 30 seconds was calculated and compared with the $\dot{V}O_{2max}$ of the incremental test (ROSSITER, KOWALCHUK and WHIPP, 2006; SCHARHAG-ROSENBERGER, CARLSOHN, *et al.*, 2011). In this study 12 volunteers were not able to finish the last stage but were able to reach at least 30 seconds, in those cases the $i\dot{V}O_{2max}$ was calculated according the recommendation of Kuipers et. al. (1985).

4.5. Supramaximal test ($MAOD_{ALT115\%}$) and estimations

Prior the supramaximal effort the volunteers remained seated during 10 minutes in order to determine the oxygen uptake baseline ($\dot{V}O_{2baseline}$) (consider as the $\dot{V}O_2$ average of the last 2 minutes of rest) and of $[La^-]_{baseline}$. The volunteers ran at $i\dot{V}O_{2max115\%}$ until voluntary exhaustion while tlim was recorded as well as $\dot{V}O_2$ measurements during the test and for 10 minutes after the supramaximal effort test to measure EPOC (BERTUZZI, FRANCHINI, *et al.*, 2010; ZAGATTO, BERTUZZI, *et al.*, 2016) (Fig. 2).

FIGURE 2: Supramaximal effort in treadmill

$MAOD_{ALT}$ was estimated as the sum of oxygen equivalents of $E_{[pcr]}$ metabolic contributions form $EPOC_{FAST}$ and the $E_{[La]}$ metabolic contributions (ZAGATTO and GOBATTO, 2012; ZAGATTO, MIYAGI, *et al.*, 2017b). The $E_{[pcr]}$ was considered as the product between the first amplitude and the first time constant (Eq. 2) estimated by means of a nonlinear adjustment of a biexponential equation (Eq. 1) with a baseline, two-amplitudes and two-time constants (MARGARIA, EDWARDS and DILL, 1933; KATCH, 1973; ROBERTS and MORTON, 1978; BERTUZZI, FRANCHINI, *et al.*, 2010).

Eq. 1: $\dot{V}O_{2(t)} = \dot{V}O_{2baseline} + A_1[e^{-(t-\delta)/t1}] + A_2[e^{-(t-\delta)/t2}]$

Eq.2: $EPOC_{fast} = A_1 \times \tau_1$

Where $\dot{V}O_{2(t)}$ is the oxygen uptake at time t, $\dot{V}O_{2baseline}$ is the oxygen uptake at baseline. A is the amplitude, δ is the time delay and τ as time constant. The $_1$ and $_2$ subscripts represent the fast and slow components respectively.

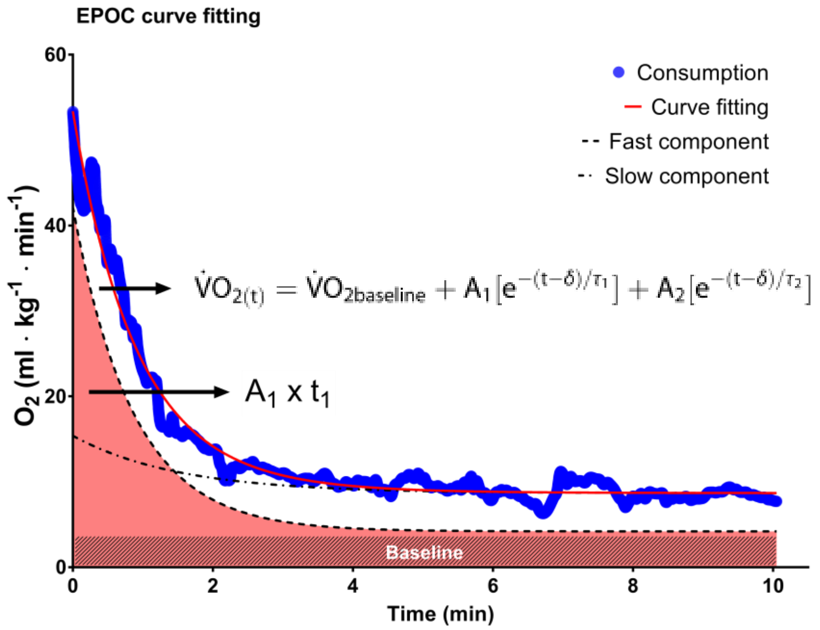

FIGURE 3: Curve fitting on EPOC$_{fast}$ and the area that represents the E$_{[pcr]}$ system contributions

Finally, to estimate the lactic $W_{[La]}$ it was subtracted the $[La^-]_{baseline}$ from the $[La^-]_{peak}$, where a value of 1 mmol · L^{-1} of lactate was considered as equivalent of 3.0 ml. O_2 · kg^{-1} (DI PRAMPERO, 1981; DI PRAMPERO and FERRETTI, 1999). All the computations were performed using OriginPro 2017 software (Origin Lab Corporation, Microcal, Massachusetts, USA). $E_{[pcr]}$, $E_{[La]}$ and AC were considered equal to 20.9 J·mLO$_2$, for runners and 21.3 J·mLO$_2$ for physical active.

4.6. Kinematic and kinetic data

4.6.1. Acquisition and processing of kinematic data

The kinematic data was measured using the Vicon System (Vicon®, MX System, EUA) and the motion capture was collected at a sampling rate of 250Hz by means of eight “bonita” B3 cameras (Vicon®, MX System, EUA). The cameras recorded the 3D position of 39 markers positioned on the body sides during the entire treadmill supramaximal effort and were calibrated before each test according manufacturer’s instructions.

The body was divided into 15 rigid body segments (head, thorax, pelvis, arms, forearms, hands, thighs, legs, and feet) modeled as described in Vicon Plug-in-Gait model (PlugInGait FullBody) (Nexus 1.81 version, Oxford Metrics UK). Each segment’s mass, center of mass position and radius of gyration were taken from Vicon

Documentation anthropometric tables (VICON MOTION SYSTEMS, 2018). The data (e.g. markers trajectories, segment velocities and angular velocities) was filtered with a non-adaptive filter of 5^{th} order Butterworth filter with an 8.5 Hz cut off frequency (NARDELLO, ARDIGÒ and MINETTI, 2011). All kinematic data was processed through a custom-written Matlab script (Mathworks, Natick, M.A.).

4.6.2. Kinematic and Kinetic calculations

To calculate the biomechanical variables first was necessary to distinguish the “toe-off” and “touchdown” kinematic events by using the algorithm from Handsaker et. al. (2016). Briefly, the algorithm considered the peak vertical acceleration of the heel marker as touchdown and the peak jerk of the toe marker as toe-off to identify such events.

Furthermore, the supramaximal effort was divided in four periods (time windows) approximately the same length of time (~5 seconds) and then we selected the last 20 steps of each period and we calculated the kinematics and kinetic variables. We followed the proposal of by Padulo et. al. (2014) to calculate the kinematics of running (Fig. 4).

- *Contact time (CT):* the time between the initial foot/shoe contact time (i.e. touch down) with the ground and the last foot/shoe contact (i.e. toe-off) before the takeoff.
- *Flight time (FT):* the time between the toe-off and the next touchdown of the contra-lateral foot.
- *Swing time (SwT):* the time from toe-off to the next touchdown of the same foot (i.e. left or right).
- *Step frequency (SF):* was determined from the inverse of the total step time of the same leg (expressed in CT and FT): $Equ. 3\; SF = 1/(CT + FT)$.
- *Step length (SL):* the proportion between the speed of the belt and the step frequency: $Equ. 4\; SL = Speed/SF$.

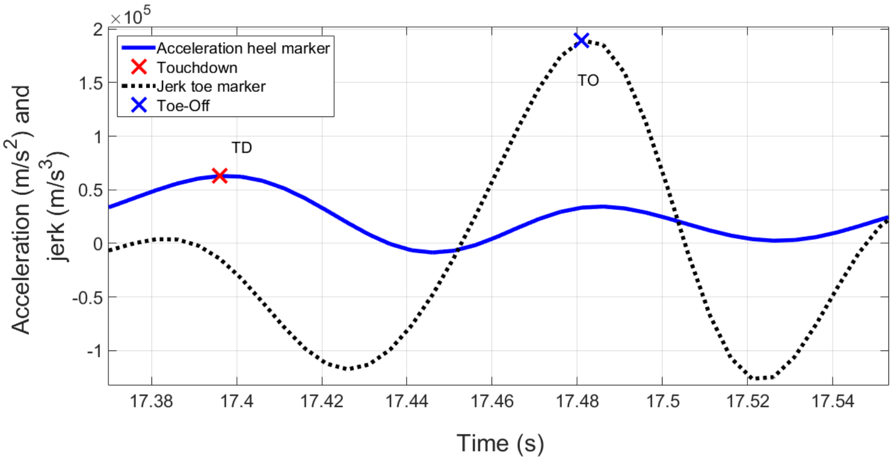

FIGURE 4: Touchdown and toe-off instances estimated using kinematic algorithm.

To calculate the $W_{[int]}$ of each body segment, initially, it was computed the absolute angular speed and the relative speed of its center of mass with respect to the CoM, in order to obtain the rotational and linear kinetic energies (Eq. 5). Then the rotational and linear kinetic energies were obtained and the internal total work was computed as the mean sum of E_{PE} and E_{KE} of each segment during the 20 steps.

Eq. 5: $$W_{[int]} = 1/2 \sum_{n=1}^{17} m_s V_s^2 + I_s \omega_s^2$$

Where s represents each segment, m_s is the mass of each segment (kg), v_s is the velocity ($m \cdot s^{-1}$) with respect to the CoM, ω_s is the angular velocity of the segment's center of mass (sCM) (rad·s) with respect to the CoM and I_s represents the inertial parameters of the segment ($kg \cdot m^2$).

The $W_{[ext]}$ (Eq. 8) was calculated by motion analysis using the method Cavagna and Kaneko (1977) that considered the changes of E_{PE} (Eq. 6) and the E_{KE} (Eq. 7) in vertical and horizontal planes to calculate the total energy changes of the system during the time period analyzed.

Eq. 6: $$E_{PE} = m_{wb} \bullet g \bullet \Delta h$$

Eq. 7: $$E_{KE} = 1/2\, m_{wb} \bullet (v_{max}^2 - v_{min}^2)$$

Eq. 8: $$W_{ext} = E_{PE} + horizontal\ E_{KE} + vertical\ E_{KE}$$

Where m is the body mass, g the gravitational force and Δh the change in vertical oscillations of CoM and v is the speed at two instants of time.

Both $W_{[ext]}$ and $W_{[int]}$ were taken as the absolute value in their variation during the period of time already mentioned (J), and then, $W_{[tot]}$ (J) was considered as the sum of the $W_{[ext]}$ and the $W_{[int]}$ (Eq. 9).

Eq. 9: $$W_{tot} = W_{int} + W_{ext}$$

4.7. Analysis and statistics

The data was first organized in Microsoft Excel® and then exported to Statistical Package for Social Science (SPSS™ version [19] for Windows™) to further analysis. The data normality of each variable was examined using the Shapiro-Wilk test to allow the use of parametric analysis.

In order to find the best predictors, we first calculated the variance explained by the combination of biomechanical variables and the dependent variable (i.e. AC and tlim) with a stepwise multiple linear regression. In this case the analysis was performed separately for each group and including all the biomechanical variables (i.e. E_{KE}, E_{PE}, $W_{[int]}$, $W_{[ext]}$, $W_{[tot]}$, CT, FT, SwT, SF, SL) as predictors of the dependent variable. Then we calculated a bivariate Pearson's correlations with data of each group (n = 11) separately. The correlation coefficient was classified as very weak to negligible (0 to 0.2), weak (0.2 to 0.4), moderate (0.4 to 0.7), strong (0.7 to 0.9) and very strong (0.9 to 1.0).

In order to test the reliability of the mechanical work estimations an ANOVA two-way repeated measures (groups / test-retest), the ICC, effect size (ES) and the 95% limits of agreement (LoA) were used. A Mauchly sphericity test was applied to the data, and the sphericity was assumed to be violated when the F test was significant. In case of sphericity violation, the Greenhouse-Geisser Epsilon correction was used. The ESs was obtained in each statistical analysis was shown and interpreted as proposed by Hopkins with ES<0.2 considered as trivial, 0.2-0.5 small, 0.6-1.1 moderate, 1.2-1.9 large, and >2 very large. Additionally, to test variability of each biomechanical variable in each time window and ANOVA two way was used (group / four-time windows). On this study, the significance level of 5% was assumed in all cases.

5. Results

The mean, standard deviation, interval of confidence and percentage of change (%C) of physiological responses during the GXT are shown in table 1, pointing out that results demonstrate all aerobic power parameters were significant greater in runner than physical active except for the [La$^-$], being equal in both groups. The $\dot{V}O_2$ attained after the verification test (supramaximal effort at 110% of i$\dot{V}O_{2max}$) did not differ ($p > 0.05$) within groups from the $\dot{V}O_2$ at exhaustion moment measured after the GXT.

TABLE 1: Mean, standard deviation and IC 95% of physiological responses of GXT.

Variable	Active	Runner
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1})	43.17±4.46 (40.17-46.17)	55.45±5.58 (51.70-59.20)*
vt$\dot{V}O_{2peak}$ (mL·kg^{-1}·min^{-1})	42.51±3.97 (39.84-45.17)	54.73±6.50 (50.36-59.10)*
$\dot{V}O_{2max}$ (L·min^{-1})	3.07±0.41 (2.71-3.33)	3.95±0.25 (3.79-4.12)*
i$\dot{V}O_{2max}$ (km·h^{-1})	14.28±1.15 (13.51-15.05)	17.60±1.33 (16.70-18.50)*
[La$^-$] peak (mmol·L^{-1})	10.90±1.59 (9.83-11.97)	10.70±1.73 (9.52-11.85)

The intensity of the third supramaximal constant velocity effort was different ($p < 0.05$) between groups being greater for the group of runners than the physical active group (14.28±1.15 and 17.60±1.33 km·h^{-1}) due to its physical fitness level. The performance was greater in physical active that in runners expressed in seconds ($p < 0.05$). Differences were found between both $EPOC_{fast}$ parameters; amplitude 1 and first-time constant being 17.04% and 22.93% greater in runners (table 2).

TABLE 2: Mean, standard deviation and IC 95% of physiological responses of supramaximal effort

Variable	Active	Runner
tLim (s)	133.09 ± 25.31 (116.09 – 150.10)	108.64 ± 23.94 (92.55 – 124.72)*
vt$\dot{V}O_{2peak}$ (mL·kg^{-1}·min^{-1})	42.51±3.97 (39.84 – 45.17)	54.73±6.50 (50.36 – 59.10)*
$\dot{V}O_{2max\ EX}$ (mL·kg^{-1}·min^{-1})	41.41 ± 3.88 (38.80 – 44.01)	49.11 ± 5.66 (45.31 – 52.92)
i$\dot{V}O_{2max}$ (km·h^{-1})	14.28±1.15 (13.51 – 15.05)	17.60±1.33 (16.70 – 18.50)*
Δ [La$^-$] (mmol·L^{-1})	11.15 ± 1.89 (9.88 – 12.42)	11.87 ± 1.55 (10.83 – 12.92)

The figure 5 shows the comparison between groups of metabolic variables, where, differences were found in anaerobic capacity and $E_{[pcr]}$ expressed in both, absolute and relative values ($p < 0.05$). When expressed in relative values the AC (69.89±8.56 mL·kg^{-1} for runners and 56.22±8.34 mL·kg^{-1} for physical active) was 16.51% greater in runners, mainly, due to the $E_{[pcr]}$ (34.51±6.80 mL·kg^{-1} for runners and 23.47±5.03 mL·kg^{-1} for physical active) contributions which was 32.22% also greater in runners.

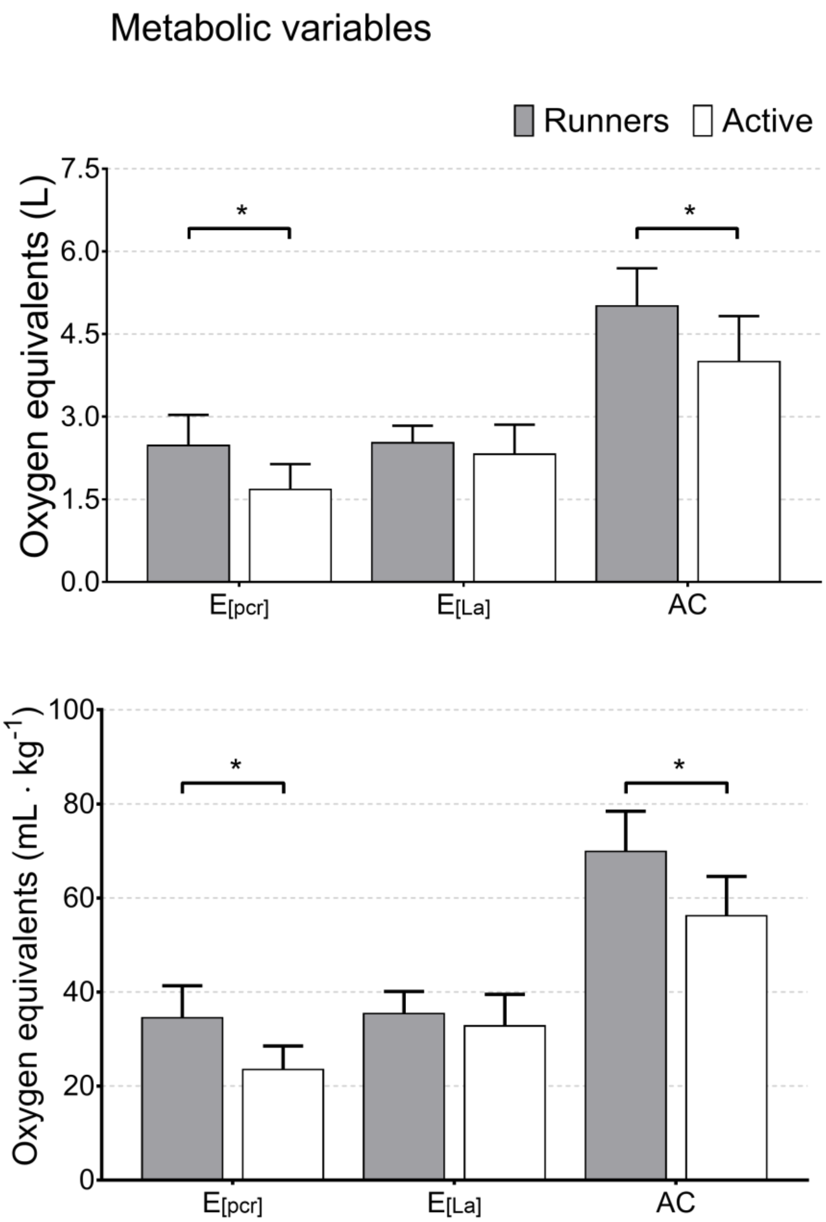

*FIGURE 5: Metabolic variables achieved during the supramaximal effort. Oxygen equivalents in absolute and relative values of, phosphagen system, $E_{[pcr]}$; glycolytic system, $E_{[La]}$; and anaerobic capacity, AC, * $p < 0.05$.*

The kinetic variables compared between groups, the $W_{[int]}$ was different between groups in both absolute and relative values whereas differences were found in E_{PE} and $W_{[ext]}$ only in relative values ($p < 0.05$). The E_{PE} (0.41±0.06 J·kg^{-1}·m^{-1} for runners and 0.54±0.08 J·kg^{-1}·m^{-1} for physical active) and $W_{[ext]}$ (1.96±0.22 J·kg^{-1}·m^{-1} for runners and 2.30±0.36 J·kg^{-1}·m^{-1} for physical active) were 28.24% and 15.77% greater in the physical active group respectively. The $W_{[int]}$ (0.64±0.10 $J \cdot kg^{-1} \cdot m^{-1}$ for runners and 0.54±0.09 $J \cdot kg^{-1} \cdot m^{-1}$ for physical active) was 8.82% greater in the runners group (Fig. 6).

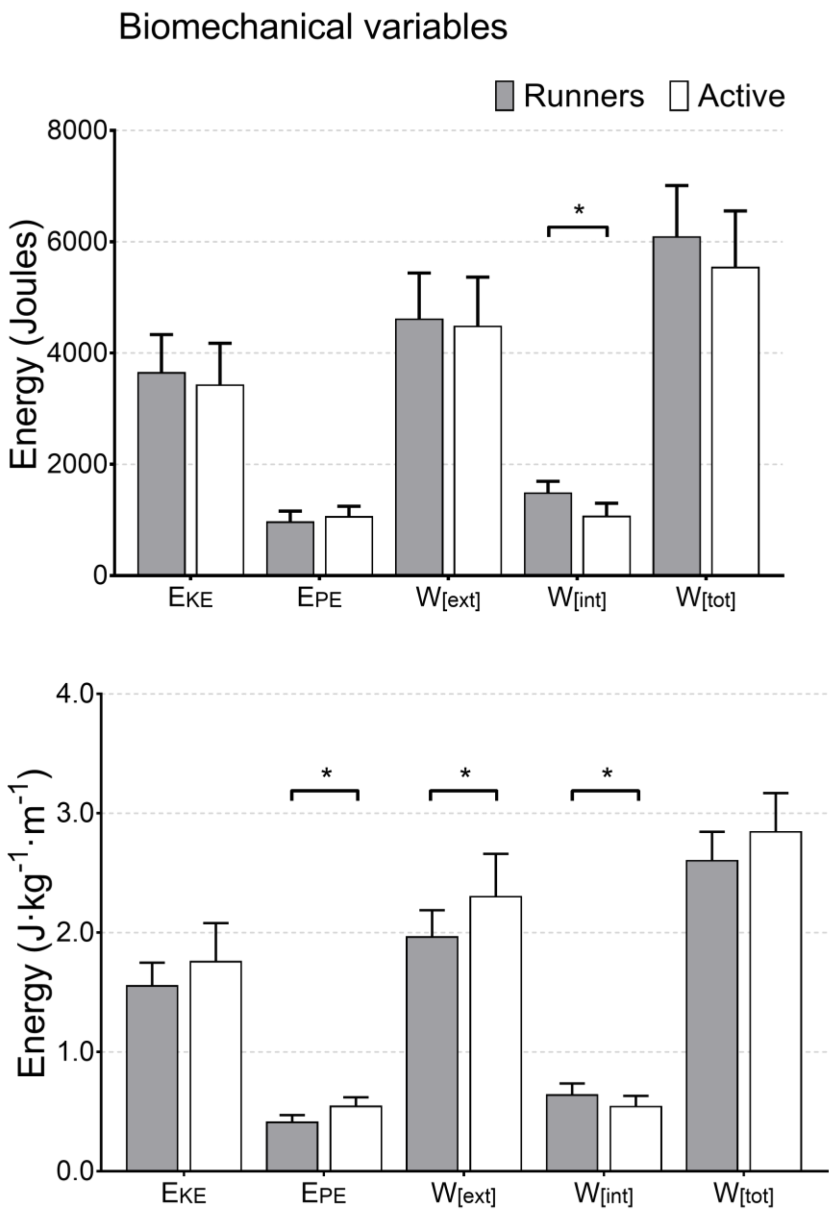

*FIGURE 6: Biomechanical variables achieved during the supramaximal effort. Energies in absolute and relative values of, kinetic energy, E_{KE}; potential energy, E_{PE}; external energy, $W_{[ext]}$; internal energy $W_{[int]}$; and total energy, $W_{[tot]}$; * $p < 0.05$.*

Table 3 demonstrates that most of the kinematic variables remained statistically equal but there were only significant differences between groups in SF (p = 0.049) and SL (p = 0.000). There were no differences between test-retest.

TABLE 3: Mean, standard deviation and IC 95% of kinematic variables estimated during the supramaximal effort.

Variables	Active	Runner
Contac time(s)	0.192 ± 0.018 (0.18 – 0.205)	0.188 ± 0.014 (0.179 – 0.198)
Flight time (s)	0.373 ± 0.034 (0.270 – 0.645)	0.378 ± 0.060 (0.338 – 0.405)
Swing time (s)	0.526 ± 0.054 (0.490 – 0.562)	0.508 ± 0.043 (0.479 – 0.537)
Step frequency (step·s)	3.147 ± 0.234 (2.990 – 3.303)	3.367 ± 0.260 (3.193 – 3.542)*
Step length (m)	1.440 ± 0.119 (1.361 – 1.520)	1.677 ± 0.126 (1.592 – 1.761)*

The table 4 shows no significant differences between familiarization and the valid test in non-mitochondrial energy contributions (i.e. phosphagen and glycolytic systems) as well as anaerobic capacity, all of them with a trivial effect size and a significant ICC's ($p < 0.05$). The kinetic variables were all significant different in runners group showing a decrease between familiarization and the valid test, in the physical active group tend to increase from test to test and only the kinetic energy, internal and total work were not different but with a small, trivial and small effect size respectively.

TABLE 4: Test-retest reliability for biomechanical variables.

Variables		Test	re-Test	E.S.	95% LoA	Magnitude Inference	ICC	%C
$E_{[Pcr]}$ ($mL \cdot kg^{-1}$)	Active	24.56 ± 5.71	23.47 ± 5.03	-0.18	-0.82 – 0.46	Trivial effect	0.73 (-0.01 to 0.93)*	-4.57
	Runner	32.59 ± 9.32	34.51 ± 6.80	0.19	-0.41 – 0.79	Trivial effect	0.87 (0.51 to 0.96)*	5.70
$E_{[La]}$ ($mL \cdot kg^{-1}$)	Active	32.14 ± 6.48	32.75 ± 6.73	0.09	-0.61 – 0.78	Trivial effect	0.69 (-0.14-0.92)*	1.91
	Runner	35.12 ± 4.98	35.39 ± 4.70	0.05	-0.61 – 0.71	Trivial effect	0.91 (0.68 to 0.98)*	0.76
AC ($mL \cdot kg^{-1}$)	Active	56.70 ± 9.85	56.22 ± 8.34	-0.04	-0.68 – 0.59	Trivial effect	0.71 (-0.09 to 0.92)*	0.85
	Runner	67.71 ± 12.27	69.89 ± 8.56	0.16	-0.43 – 0.75	Trivial effect	0.90 (0.62 to 0.97)*	3.17
E_{KE} ($J \cdot kg^{-1} \cdot m^{-1}$)	Active	1.45 ± 0.47	1.75 ± 0.32	0.59	0.01 – 1.18	Small effect	0.66 (-0.25 to 0.91)*	18.96
	Runner	2.00 ± 0.58	1.55 ± 0.20*	-0.72	-1.24 – 0.19	Moderate effect	0.07 (-2.44 to 0.75)	-25.23
E_{PE} ($J \cdot kg^{-1} \cdot m^{-1}$)	Active	0.45 ± 0.08	0.54 ± 0.08*	1.01	0.35 – 1.66	Moderate effect	0.27 (-1.72 to 0.80)	18.20
	Runner	0.56 ± 0.13	0.41 ± 0.06*	-1.03	-1.47 – -0.48	Moderate effect	0.07 (-2.47 to 0.75)	-30.41
$W_{[ext]}$ ($J \cdot kg^{-1} \cdot m^{-1}$)	Active	1.90 ± 0.53	2.30 ± 0.36*	0.69	0.10 – 1.28	Moderate effect	0.59 (-0.52 to 0.89)	18.82
	Runner	2.56 ± 0.70	1.96 ± 0.22*	-0.79	-1.31 – -0.27	Moderate effect	-0.01 (-2.75 to 0.73)	-26.37
$W_{[int]}$ ($J \cdot kg^{-1} \cdot m^{-1}$)	Active	0.54 ± 0.09	0.58 ± 0.19	-0.19	-0.74 – 0.35	Trivial effect	0.51 (-0.81 to 0.87)	-6.97
	Runner	0.87 ± 0.26	0.64 ± 0.10*	-0.81	-1.34 – -0.28	Moderate effect	0.46 (-1.06 to 0.85)	-30.59
$W_{[tot]}$ ($J \cdot kg^{-1} \cdot m^{-1}$)	Active	2.49 ± 0.33	2.84 ± 0.57	0.57	0.01 – 1.13	Small effect	0.43 (-1.31 to 0.85)	13.37
	Runner	3.43 ± 0.91	2.60 ± 0.24*	-0.84	-1.36 – -0.32	Moderate effect	-0.09 (-3.05 to 0.71)	-27.42

The figure 7 shows results from the stepwise analysis. The main biomechanical predictor of anaerobic capacity in the runner group was step frequency ($y = -1.53 + 1.18 \cdot x$) with 52.60%, contrary, in the physical active group the main predictor was internal work ($y = 1.01 + 1.89 \cdot x$) with 42.30% of the variance explained. Additionally, the performance was better explained by potential energy ($y = -2.23 + 271.25 \cdot x$) with 49.90% in the runners group, and swig time ($y = -24.28 + 299.19 \cdot x$) 40.80% in the physical active group.

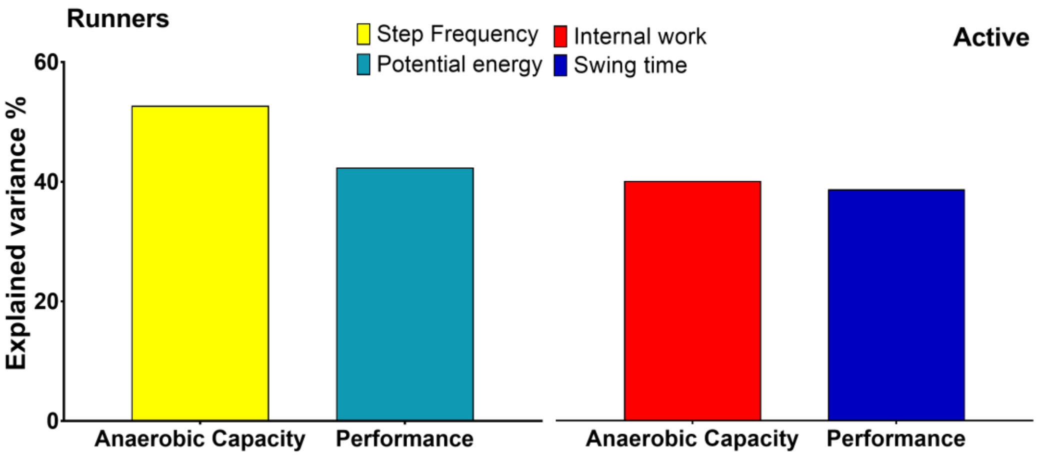

FIGURE 7: kinetic and kinematic determinants for anaerobic capacity and supramaximal effort performance (i.e. tlim) from the stepwise multiple linear regression analysis.

Table 5 shows all the Pearson's correlations, where it is worth noting that, even though, in the runners group of the ten biomechanical variables only two were strongly and significant correlated with AC: SF (p = 0.011) and SwT (p = 0.046), three were moderate and five were weak correlated but not significant. When the biomechanical variables were analyzed with performance just one was strongly and significant correlated; potential energy (p = 0.015) and regarding the remaining nine variables, two were moderate and seven were weak also without significance. Contrarily, in the physical active group, internal work was correlated with anaerobic capacity (p = 0.030), and only one was correlated with performance as well as swing time (p = 0.035). The remaining variables fluctuated between moderate and weak correlated but without statistical significance.

TABLE 5: Correlation coefficient and IC 95% between the biomechanical (i.e. kinetic and kinematic) variables as predictors of anaerobic capacity and performance (i.e. tlim).

Variables		Dependent variable: Anaerobic Capacity	Dependent variable: Performance
Kinetic			
E_{KE}	*Active*	-0.137 (-0.45 to 0.21)	0.419 (0.10 to 0.66)
	Runner	-0.314 (-0.59 to 0.02)	0.197 (-0.15 to 0.50)
E_{PE}	*Active*	-0.194 (-0.50 to 0.15)	0.395 (0.07 to 0.64)
	Runner	-0.566 (-0.76 to -0.29)	0.707 (0.49 to 0.84)*
$W_{[ext]}$	*Active*	-0.164 (-0.47 to 0.18)	0.460 (0.15 to 0.69)
	Runner	-0.429 (-0.11 to -0.67)	0.367 (0.04 to 0.62)
$W_{[int]}$	*Active*	0.650 (0.40 to 0.81)*	-0.419 (-0.10 to -0.66)
	Runner	0.430 (0.11 to 0.67)	-0.216 (-0.51 to 0.13)
$W_{[tot]}$	*Active*	-0.002 (-0.34 to 0.33)	0.394 (0.07 to 0.64)
	Runner	-0.225 (-0.52 to 0.12)	0.254 (-0.09 to 0.54)
Kinematic			
CT	*Active*	-0.162 (-0.47 to 0.18)	0.031 (-0.31 to 0.36)
	Runner	-0.400 (-0.08 to -0.65)	0.077 (-0.26 to 0.40)
FT	*Active*	-0.521 (-0.23 to -0.73)	0.261 (-0.08 to 0.55)
	Runner	-0.285 (-0.56 to 0.05)	-0.163 (-0.47 to 0.18)
SwT	*Active*	-0.525 (-0.23 to -0.73)	0.638 (0.39 to 0.80)*
	Runner	-0.611 (-0.35 to -0.78)*	0.531 (0.24 to 0.73)
SF	*Active*	0.513 (0.22 to 0.72)	-0.450 (-0.14 to -0.68)
	Runner	0.726 (0.52 to 0.85)*	-0.518 (-0.22 to -0.73)
SL	*Active*	-0.432 (-0.12 to -0.67)	0.065 (-0.27 to 0.39)
	Runner	0.184 (-0.16 to 0.49)	-0.254 (-0.54 to 0.09)

*Bold letter and * significant correlation p < 0.05*

In table 5 is evident that when the correlations were expressed in joule per kilogram per second (tlim) in the runner group there are significant correlations between all the kinetic variables and the performance. Where the external work variables are moderately associated with performance (i.e. E_{KE} -0.792, E_{PE} -0.681 and $W_{[ext]}$ -0.793, all $p < 0.05$), while internal (-0.838; $p < 0.05$) and total mechanical work (-0.859; $p < 0.05$) were strongly associated. In the physical active group, the associations are less clear, showing no significant correlations with performance.

Finally, figure 10 and 11 demonstrates the main difference in mechanical external energy analyzed in each time window (i.e. 20 steps each) between the groups and the physical active shows a variability in the two energies and also in the total external energy. While the external mechanical energy expenditure in runners was more stable and even did not show any differences in potential energy along the four time-windows. Contrarily to $W_{[ext]}$, in $W_{[int]}$ and $W_{[tot]}$ both groups had similar trends and expressing a fluctuation of the work in the four times analyzed also being greater in the last part of the test in both groups.

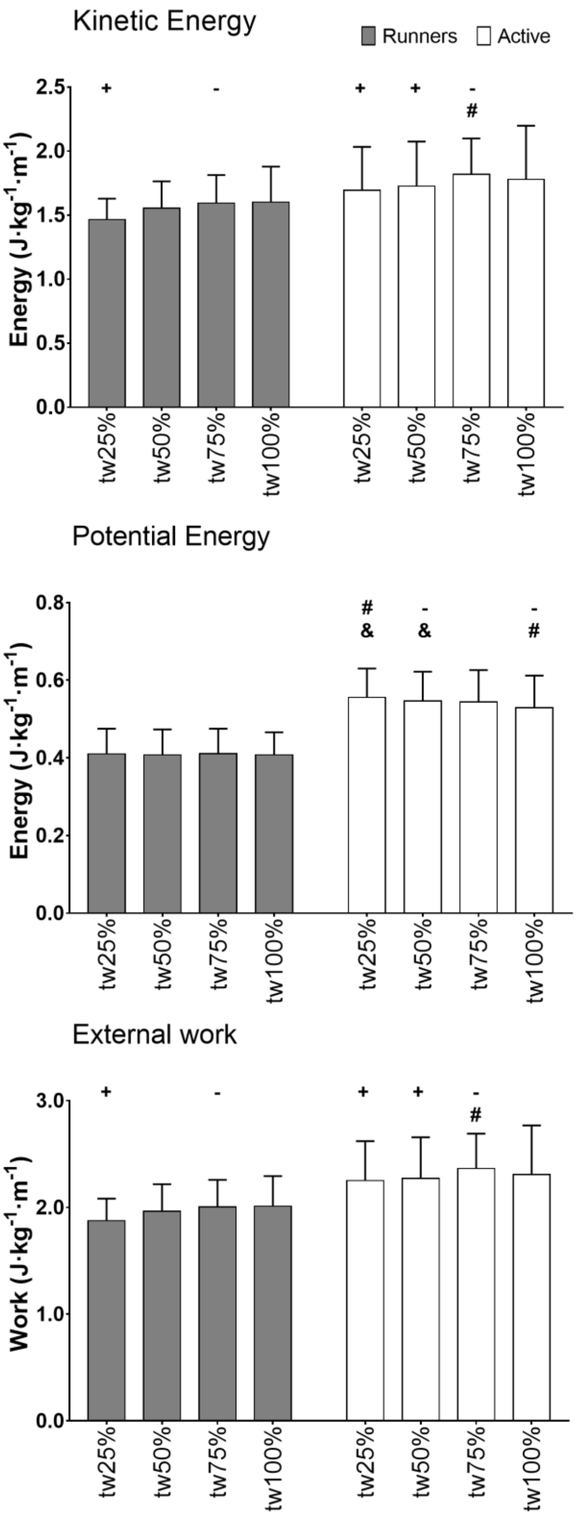

FIGURE 8: time window analysis of the external work; - differences with the first time window, # differences with the second time window; + differences with the third time window; & differences with the fourth time window; time window of the first quarter of the test (tw 25%), time window of the second quarter of the test (tw 50%), time window of the third quarter of the test (tw 75%) and time window of the fourth quarter of the test (tw 100%).

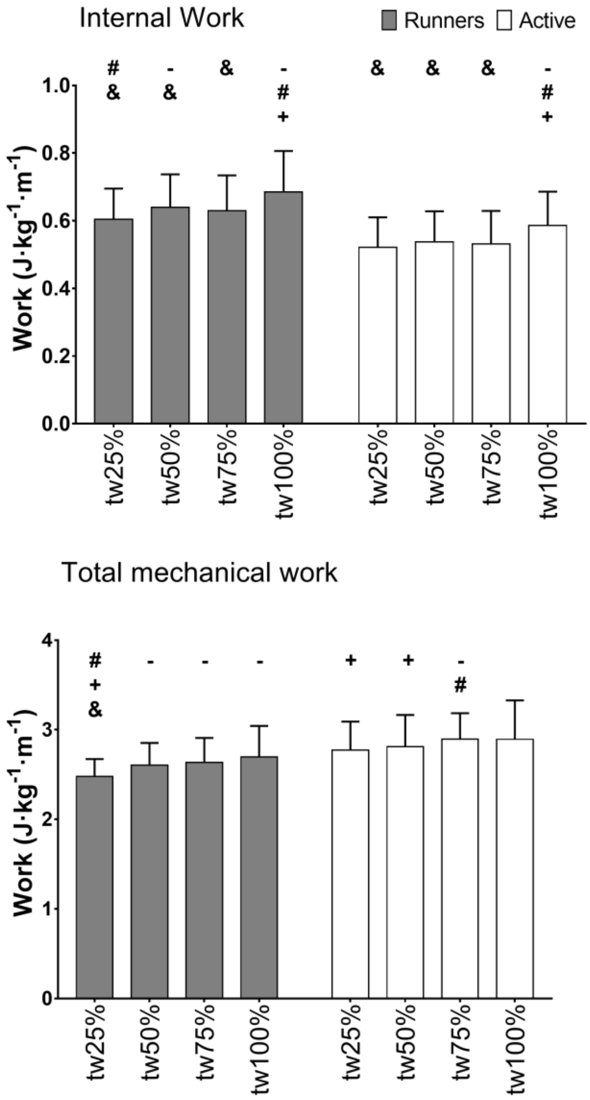

FIGURE 9: time window analysis of the internal and total work; - differences with the first time window, # differences with the second time window; + differences with the third time window; & differences with the fourth time window; time window of the first quarter of the test (tw 25%), time window of the second quarter of the test (tw 50%), time window of the third quarter of the test (tw 75%) and time window of the fourth quarter of the test (tw 100%).

6. Discussion

In order to investigate the relationships between the mechanical work and anaerobic capacity the present work assessed 22 volunteers divided in two groups (amateur runners and physical active), using contemporary estimations of the AC, kinematics and a total mechanical work during a supramaximal effort. It was a pioneering study that covers issues with few references within the specialized literature such as mechanical contributions during supramaximal intensities (and non-mitochondrial as a consequence).

The $EPOC_{fast}$ parameters and the metabolic variables observed in the present work are in agreement with those reported in the literature (KATCH, 1973; ROBERTS and MORTON, 1978; BENEKE, POLLMANN, *et al.*, 2002). Additionally, the $W_{[ext]}$ (798.75 $J{\cdot}s^{-1}$ for runners and 729.42 $J{\cdot}s^{-1}$ for physical active) and its components expressed in relative values, the E_{KE} (633.22 $J{\cdot}s^{-1}$ for runners and 557.86 $J{\cdot}s^{-1}$ for physical active) and the E_{PE} (165.53 $J{\cdot}s^{-1}$ for runners and 171.56 $J{\cdot}s^{-1}$ for physical active) are in line with those estimated by Keir et. al. (2012). However, overestimated when compared with Williams et. al. (1995) probably due to the noise in the velocity CoM's velocity and the errors of anthropometric tables. On the other hand, $W_{[int]}$ (261.92 $J{\cdot}s^{-1}$ for runners and 197.95 $J{\cdot}s^{-1}$ for physical active) was slightly lower compared with the former study, probably due to lower intensity of the supramaximal effort in this work.

The major findings of the present study are: 1) two biomechanical variables were found to be associated with metabolic variables in the runners group (i.e. SwT and SF) and only one in the physical active group (i.e. $W_{[int]}$). Pairwise regression analysis revealed that these variables explained 52.6% and 42.4% of the variability in AC, 2) the E_{PE} explains 47.2% for runners while SwT explains 40.8% the variability of performance, 3) runners had an $W_{[ext]}$ and $W_{[tot]}$ lower than the physically active group that is mainly due to a smaller vertical distance oscillation, horizontal as well as vertical speed variations of the CoM, 4) familiarization test had a learning effect among runners because they used 27.42% less mechanical work in the valid test, 5) the supramaximal effort performance was better explained by the kinetic variables when they were expressed as a rate (joules per kilogram per second).

The biomechanical variables seem to contribute in two ways with the non-mitochondrial energy sources. In the first place, $W_{[ext]}$ plays a role similar to the observed in submaximal efforts where the smaller the $W_{[ext]}$ the better the economy of running (MOORE, 2016). The $W_{[ext]}$ is linked to the vertical oscillations of CoM because one of the sources of energy that integrates it, is gravity, which in turn is supported by the smaller changes in E_{PE} in runners than in physical active observed in this study. Although we did not observed relationship between $W_{[ext]}$ and AC in any group, we indeed, observed a strong relationship between E_{PE} and supramaximal performance in runners. Therefore, people with a better technique tend to spend less energy during running which in turn decreases the $W_{[ext]}$ what favors a better economy of movement but also a better ability to potentiate the AC and supramaximal performance. Furthermore, the $W_{[ext]}$ follows the same trend in the two groups analyzed, as it was observed in the time window analysis where the runners tended to be more economical also with a relatively low variability in the energies (i.e. E_{KE} and E_{PE}) along the supramaximal effort. By contrast, the $W_{[ext]}$ in physical active group was more variable likely to several technical adjustments during the effort. In the second place, Cavagna, Heglund and Willems (2005) observed higher $W_{[int]}$ values to be liked to lower CoM vertical oscillations. This finding was also observed only in runners group. Interestingly, and despite the $W_{[int]}$ was greater in runners, it was not a factor that explain the AC, which indeed, was in physical active group. This fact might be explained by the quantity of muscle mass involved during the supramaximal effort (i.e. muscles that are not yet in a state of peripheral fatigue), by the SF, but also, by a worst technique by the physical active group which might increases the angular inertial resistance increasing the energy required to the movement.

Likewise, it is possible to extract from kinematic variables a few additional explanations of the associations with AC. Ruiter et. al. (2013) and Cavagna and Williams (1982) have observed that an optimum and self-selected SF is better to improve the economy, this finding are in agreement whit our results study where a moderate correlation between SF and AC in the runner was found. A higher SF value is linked to an increased in stance leg stiffness, energy absorptions at the joint involved in the motion and reduced CoM vertical oscillations (SCHUBERT, KEMPF and HEIDERSCHEIT, 2014). Additionally, the runners tend to stabilize this kinematic variable along the whole supramaximal effort, contrarily, there was not an overstriding pattern in physical active group that could have explained the low economy of these volunteers, however, the variability analysis did not show differences in any time window.

It was observed a positive influence of familiarization in runners group since they reduced 27.47% the $W_{[tot]}$, however, there was not observed an influence in AC and/or supramaximal performance. The greater reduction was in the $W_{[int]}$ with 30.59% followed by E_{PE} with 30.41%. Contrarily, in physical active group a generalized increase of all the mechanical variables was observed. The biggest increase was observed in E_{KE} with 18.96% followed by the $W_{[ext]}$ with 18.82% and finally, $W_{[tot]}$ in 13.37%. To the best of our knowledge this is a first study that assess the reliability of mechanical work estimations. The reduction in mechanical work demand by runners might be due to an improvement in intra-limb coordination and a reduction of mechanical efficiency, whereas, the reason why metabolic variables remained equal is due to the fact that some metabolic energy is not exhibit as mechanical work such as isometric contractions and co-contractions (WILLIAMS K., 1985, apud SPARROW W. and IRIZARRY-LOPEZ V., 1987).

On the other hand, the biomechanical variables interaction with performance are more complex to understand because it was not observed an improvement in performance, however, correlations between the rate of mechanical work expenditure and kinetic variables along the supramaximal effort explain better the runners' performance. When the mechanical work was expressed in $J \cdot kg^{-1} \cdot s^{-1}$ all the kinetic variables turned negatively correlated with performances of which the following can be discussed: the more economical way of mechanical expenditure is likely to improve the performance, even though the runners showed a better economy did not improved the performance. It seems that their enzymatic potential can deplete faster the nonmitochondrial energy reserves and the whole biochemical processes independent of the way as the mechanical work is expressed. Furthermore, probably their type of training does not allow them to take advantage of their mechanical economy. Our volunteers were amateur endurance runners which train mostly below the intensity associated to $\dot{V}O_{2max}$ (iVO_{2max}) their bodies are adapted to run at those submaximal intensities, therefore, it is likely that they do not activate all the biochemical processes such as buffering capacity or glycolytic enzymes (pyruvate dehydrogenase) in the same extent as would do a middle-distance runner (i.e. 800 meters to 1500).

Finally, the interactions between AC and biomechanical variables seems to be very complex to understand and due to the fact that the kinetic analysis observed in both groups presented the same relationship tendencies (i.e. positive and negative slopes), individual functional and structural characteristics that interact positively in a subject could be a negative factor in another runner (WILLIAMS and CAVANAGH, 1987; KYRÖLÄINEN, BELLI and KOMI, 2001). Future studies would be important to understand individual structural and functional characteristics that influence uneconomical aspects in running mechanics in conjunction with a protocol that allow constant feedback about the elapsed time which in turn could limit the metabolic demand centrally regulated by the brain (ANSLEY, ROBSON, *et al.*, 2004). Likewise, as proposed by Billat and Hamard et. al. (2009) perception of the time limit during the effort is still an unexplored issue that could potentiate the AC in conjunction with the mechanical variables.

7. Conclusion

Based on the results presented above, it is possible to conclude that step frequency is the best predictor of anaerobic capacity in runners and the internal work is the best predictor of AC in physical actives, and that these factors are altered according to running experience. Additionally, the rate of mechanical work expenditure is negative correlated between with supramaximal performance. Finally, the familiarization affects the mechanical work according to running experience. In runners the mechanical variables trend to decrease whereas in physical active trend to increase. Therefore, mechanical work expenditure was not reliable in this study and may need more than two trials to stabilized.

8. Limitations

A small cohort could be a limitation in the present work during the Pearson correlations and to establish a stepwise linear model as well as the heterogeneity of in the physical fitness and the kinematics of the runner group. Additionally, AC is an important tool of the training assessments in middle distance runners (i.e. 800 and 1500), thus, the runners in this work were amateur endurance runners with few physiological adaptations in supramaximal intensities (i.e. > 100% iVO2max) that could be the reason of lower time limit in this group compared with physical active group. Finally, the main source of $W_{[ext]}$ is the ground reaction forces so the use of other biomechanical models could reveal other aspects that the present model does not take into account.

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