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Efeitos das Variações de Velocidade e
Inclinação na Variabilidade do Sistema
Locomotor Durante a Corrida

Effects of Variations of Speed and Slope on
Locomotor System Variability During Running

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“Locomotion is a particularly richly studied but frustrating aspect of biology”

Loeb, G.E. (1989)

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Once, when I was upset about things that weren't going well on my project and asked my friend for advice, he told me that getting a masters degree is more about learning how to be a scientist than about doing great research. So I guess now is time for me to thank the people who have helped me "become a scientist". Although many people have assisted me in the last three years without whom I couldn't have finished this dissertation, I'd like to namely thank eight people: the first two are my parents for supporting me and mostly for not rushing me to finish. The third is my sister Vi for helping in literally every stage, from summarizing Randall's book to translating "constraints". The fourth is my professor ZéGui for being a great advisor and not kicking me out (I know I deserved sometimes). And lastly, my friends Zé, Thiago, Paulo, and Fefex who, for better or worse, share the responsibility with ZéGui for the scientist I am now. And Dus because I promised to mention you here.

LIST OF ABBREVIATIONS

a	Intercept of speed-and-slope models
b	Speed coefficient in speed-and-slope models
c	Interaction coefficient in speed-and-slope models
cv	Coefficient of variation of intervals between contractions
\hat{e}_1	x-axis of the gradient field
\hat{e}_2	y-axis of the gradient field
ECG	Electrocardiogram
EMG	Electromyogram
f_z	Function under the analysis of the gradient
GL	<i>Gastrocnemius lateralis</i>
GRF	Ground reaction force
HR	Heart rate
HRmax	Maximum heart rate
m	Muscle
MVC	Maximum voluntary contraction
rmssd	Quadratic mean of successive differences of intervals between contractions
st	Average interval between successive contractions
se	Standard error
sd	Standard deviation of intervals between contractions
u	Speed
U	Speed at which the subject's heart rate reached 86% HRmax
VL	<i>Vastus lateralis</i>
VM	<i>Vastus medialis</i>
y	Dependent variable
α	Intercept of HR models
β	HR coefficient in HR models
θ	Slope
σ	Standard deviation

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1 INTRODUCTION

1.1 BIOMECHANICS AND KINEMATICS OF RUNNING

1.1.1 Gait cycle

Running is the faster gait adopted by humans during terrestrial locomotion. Like walking and hopping, running is executed by repetition of cycles. In each of these cycles, a number of events take place from the moment one's foot touches the ground to the moment the same foot touches the ground again, that ultimately allows the body to move forward. This moment is referred to as initial contact, or heel strike, and is frequently depicted as the beginning of the gait cycle.

A gait cycle can be divided into two phases, according to the position of the foot in relation to the surface. Stance phase lasts from initial contact until toe-off. Throughout the stance phase, there is always contact between the foot and the ground. The moment when the foot leaves the ground marks the beginning of the swing phase, which ends when the same foot hits the ground again, initiating a new cycle.

The amount of time spent on each phase depends on the speed of locomotion. For amateur runners, stance phase lasts for about 36 to 39% of the gait cycle (NOVACHECK, 1997). As speed increases, less time is spent in the stance phase (MERO et al., 1992; NOVACHECK, 1997; HUNTER et al., 2014) so that the swing phase can account for almost 80% of the cycle in well-trained runners (NOVACHECK, 1997; SANTOS-CONCEJERO, 2014). During running, regardless of speed, the stance phase always accounts for less than half of the cycle. As a consequence, before initial contact and for some time after toe-off neither feet are in contact with the ground. This period of double float is often used to define a running gait and to differentiate it from walking (ALEXANDER, 1984; NOVACHECK, 1997; NICOLA & JEWISON, 2012).

1.1.2 Mechanical energy fluctuations

While some authors use the periods of double float to mark the distinction between running and walking gaits, others base such distinction on the differences in patterns of mechanical energy fluctuations (BIEWENER, 2006). During walking, oscillations in gravitational potential energy and kinetic energy of the body's center of mass are approximately 180 degrees out of phase, thus allowing considerable exchange between these forms of energy (FARLEY & FERRIS, 1998). For that reason, an inverted pendulum model has been used to describe this gait (FARLEY & FERRIS, 1998).

At higher speed gaits, however, oscillation in potential and kinetic energy are in phase and, therefore, a pendulum-like model does not properly describe variations of mechanical energy. In fact, the movements of the center of mass during running resemble those of a bouncing ball (NOVACHEK, 1997; FARLEY & FERRIS, 1998). Hence, it has been referred to as a bouncing gait.

Fluctuations in the mechanical energy of the center of mass during bouncing gaits can be quite precisely described by a single spring-mass model, where the leg acts like a spring (FARLEY & FERRIS, 1998).

From midswing to midstance, the center of mass is decelerating: the spring compresses and the resulting horizontal force is directed backward. During this phase, there is the net absorption of energy by the spring (NOVACHEK, 1997; DUGAN & BHAT, 2005; NICOLA & JEWISON, 2012). This period is referred to as absorption phase. During the second part of stance and the first half of swing, there is a net output of energy as the spring lengthens and the center of mass is accelerated upward and forward (NOVACHEK, 1997; DUGAN & BHAT, 2005; NICOLA & JEWISON, 2012). This period is referred to as generation phase. The point when absorption phase changes to generation is named stance phase reversal and the point when generation phase changes to absorption is swing phase reversal.

1.1.3 Muscle power

HAMNER et al. (2010) developed a model to address the issue of how muscle activity contributes to power generation that allows for the bouncing movement of the center of mass during running. They have shown that propulsion (horizontal acceleration of the center of mass) and support (vertical acceleration of the center of mass) during stance phase are primarily generated by leg muscles, more precisely, by the muscles acting on the knee and ankle joints.

During absorption phase, eccentric contraction of the quadriceps muscles (*rectus femoris*, *vastus lateralis* [VL], *vastus medialis* [VM] and *vastus intermedius*) is the main source of power for backward acceleration of the center of mass (braking) and vertical support (HAMNER et al., 2010; NICOLA & JEWISON; 2012). Quadriceps muscles start contracting at the late swing in preparation for ground contact. Activation of these muscles generates an extensor moment at the knee that, together with plantar flexors and hip extensors (*gluteus maximus* and hamstring muscles), keeps the leg joints from flexing excessively after heel strike, when the ground reaction force is about 2.2 times body weight (DUGAN & BHAT, 2005).

After stance phase reversal, the leg begins to extend. This marks the beginning of the generation phase. Concentric contraction of leg extensors pushes the body into the swing phase. Ankle plantar flexors (*gastrocnemius lateralis* [GL], *gastrocnemius medialis* and *soleus*) are the primary sources of power to accelerate the center of mass forward and upward. At toe-off, the ground reaction force is about 2.8 times body weight (DUGAN & BHAT, 2005) and, not surprisingly, the velocity of running is directly proportional to power output at this stage (NOVACHEK, 1997).

1.2 EFFECTS OF SPEED

There are primary two strategies that can be adopted in order to run faster. The first one is to change how often steps are taken (i.e. increase step frequency).

Alternatively (or in association) one can vary the distance traveled at each step (i. e., increase step length).

The first possibility requires more stride cycles to be completed within a given time when compared to slower speeds. Consequently, the average duration of stride cycles must be reduced. Several studies (WEYAND et al., 2000; NUMMELA et al., 2007; SCHACHE et al., 2011) have shown this strategy accounts for most of the variation at faster running speeds, above 5 - 7 m/s.

For most recreational runners, however, such speeds cannot be maintained for more than a few minutes and, thus, training, as well as race paces, are usually a lot slower. From speeds as low as 1.5 m/s up to 5 - 7 m/s (the precise number varies between studies), changes in velocity are primarily achieved by increasing stride length (KYROLAINEN et al., 1999; WEYAND et al., 2000; SCHACHE et al., 2011; TSUJI et al., 2015).

In order to increase the average distance covered at each stride cycle, one has to increase the acceleration of the center of mass. Accelerations of the center of mass are reflected as a force exerted against the ground. This force can be experimentally measured using a force platform placed over the running surface.

By conducting experiments in such way, several researchers have found a positive correlation between vertical and horizontal components of ground reaction force (GRF) and speed (HAMILL et al., 1983; MUNRO et al., 1987; KELLER et al., 1996; KYROLAINEN et al., 1999; WEYAND et al., 2000; KYROLAINEN et al., 2005; NUMMELA et al., 2007). In fact, GRF seems to be the primary factor associated with running speed. As an illustration, WEYAND et al. (2000) estimate that an increase of only 10% of body weight in vertical GRF is enough to alter maximum sprint speed by 1 m/s. Fig. 1 (published by KYROLAINEN et al., 2005) presents values of vertical and horizontal GRF over five sub-maximum speeds (4, 5, 5.5, 6 and 7 m/s) and maximum speed (8.5 ± 0.57 m/s).

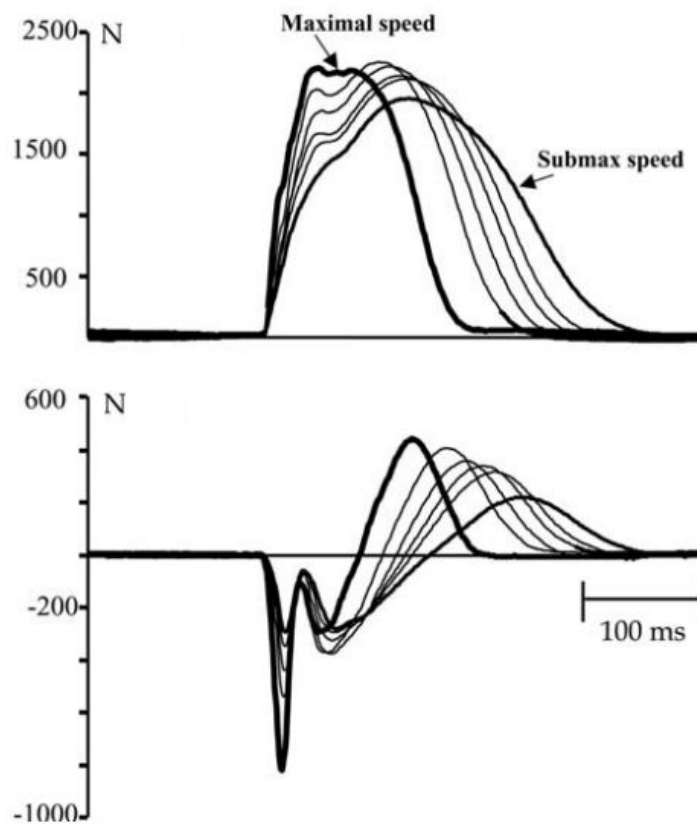


Figure 1 - Vertical (top) and horizontal (bottom) ground reaction forces at five sub-maximum running speeds (4, 5, 5.5, 6 and 7 m/s) and maximum speed (8.5 ± 0.57 m/s). (KYROLAINEN et al., 2005)

One aspect worth noticing on Fig. 1 is that, despite the differences in magnitude, the pattern of variation in GRF during a gait cycle remains roughly the same over the range of speeds. As discussed previously, the primary source of power for acceleration of the center of mass is force produced by muscles which, in turn, act on joints to adjust leg length and stiffness. Therefore, the similarity in the patterns of GRF across speeds suggests that changes in speed do not affect the overall mechanics of running.

SCHACHE et al. (2011) analyzed several variables of lower limb kinematics over speeds ranging from 3.5 m/s to maximum sprint and concluded that the profile of sagittal-plane torques for hip, knee and ankle joint was similar across all speeds. The magnitudes of torques and work produced and absorbed at these joints, on the other hand, were highly affected by speed.

From the work done and absorbed throughout a complete cycle, SCHACHE et al. (2011) concluded that the knee joint was predominantly an energy absorber. Although in this experiment total work done at the knee did not correlate with speed, other studies have found an increase in activity of knee extensors with increasing speeds: KYROLAINEN et al. (1999, 2005) observed an increase in the activity of *rectus femoris* and *vastus lateralis* with increasing speed in all phases of running. When speed increased from 3.25 - 4 m/s to maximum sprint, average values of electromyographic activity (EMG) increased about threefold during pre-activity (100 ms prior ground contact) and twofold during the braking phase for both muscles. At speeds above 6 m/s, the activity of VL exceeded maximum voluntary contraction (MVC) during both the pre-activity and the braking phase (Fig. 2). There was an increase in the activity of both muscles during push-off, the magnitude of forces (in % MVC) was a lot smaller than during the previous phases. TSUJI et al. (2015) also reported a significant increase in the activity of VM from 35.8 to 38.8% MVC (8.3% increase) when subjects went from jogging at 1.8 m/s to running at 2.8 m/s.

While knee was primarily responsible for energy absorption, analysis of work done and absorbed at these joints throughout a cycle led SCHACHE et al. (2011) to conclude that hip and ankle joints were predominantly energy generators. The studies mentioned above (KYROLAINEN et al., 1999; KYROLAINEN et al., 2005) reported an increase in activity of ankle plantar flexors and hip extensors when speeds progressed from 3.2 - 4 m/s to maximum sprint:

Biceps femoris activity increased with speed during all phases of running. At 4 m/s, EMG activity was about 20 - 25% of the values recorded during MVC. At maximum speed, EMG was either close to or slightly above MVC values. *Gluteus maximus* electromyographic activity increased with speed during pre-contact and braking phases, however, the changes were more discrete than those reported for *biceps femoris*.

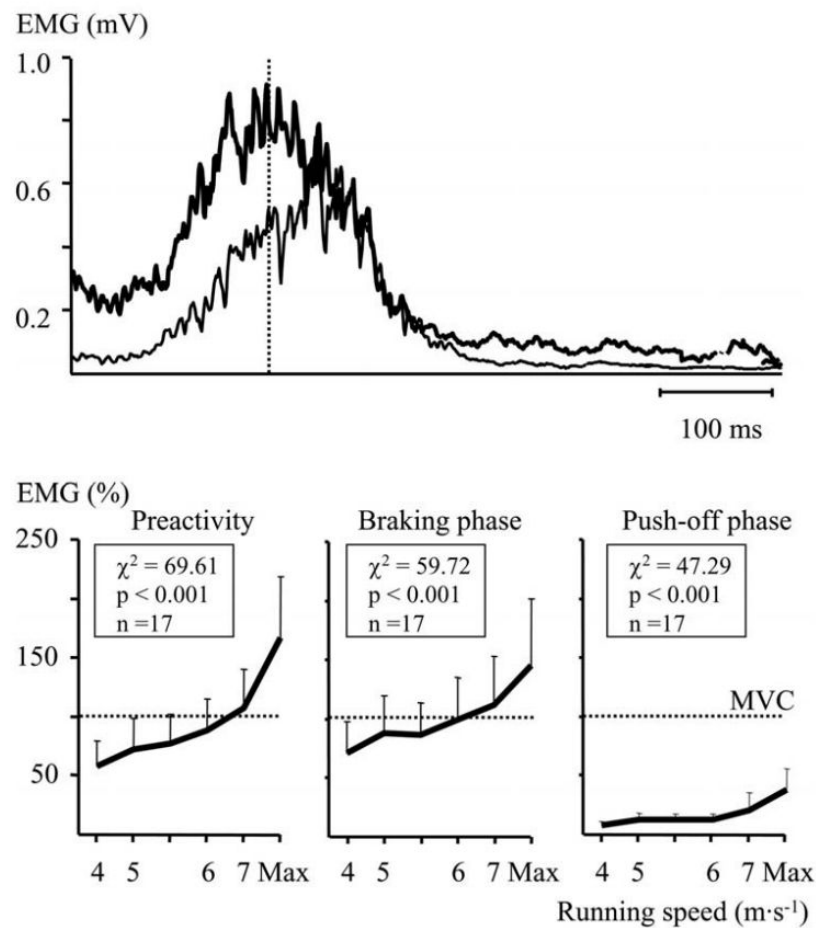


Figure 2 - Top: Electromyographic activity (EMG) pattern of *vastus lateralis* at 4 m/s (thin line) and at maximum speed (bold line), dashed vertical line marks the beginning of ground contact phase. Bottom: mean \pm standard deviation of relative EMG activity for three gait phases. The dashed horizontal line is 100% of the maximum voluntary contraction (MVC) (KYROLAINEN et al., 2005).

At the ankle, *gastrocnemius* EMG activity increased with speeds, mainly during pre-contact and braking phases. In all phases, values exceeded the ones recorded during MVC - in fact, during braking, they were already above MVC even at the speed of 4 m/s (KYROLAINEN et al., 1999; KYROLAINEN et al., 2005) (Fig. 3). TSUJI et al. (2015) also reported an increase in activity of medial head of *gastrocnemius* and *soleus* as speed progressed from 1.8 to 2.8 m/s.

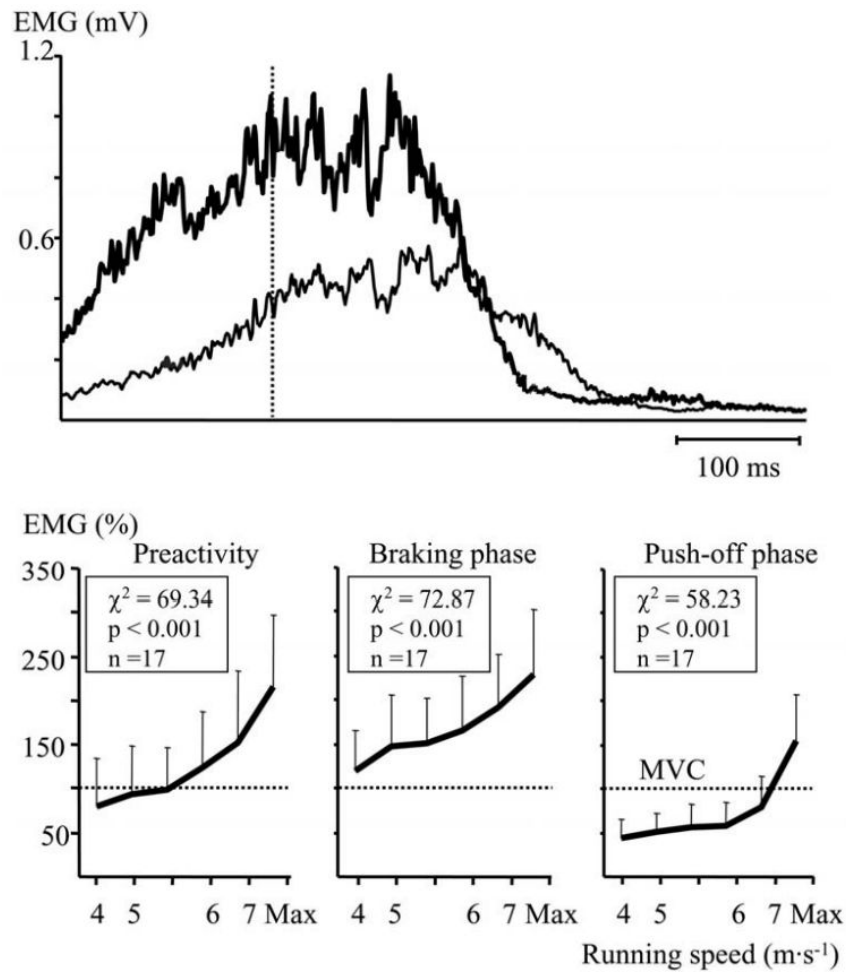


Figure 3 - Top: Electromyographic activity (EMG) pattern of *gastrocnemius* at 4 m/s (thin line) and at maximum speed (bold line), dashed vertical line marks the beginning of ground contact phase. Bottom: mean \pm standard deviation of relative EMG activity for three gait phases. The dashed horizontal line is 100% of maximum voluntary contraction (MVC) (KYROLAINEN et al., 2005).

In short, faster running is achieved by increasing the forces applied against the surface rather than by the frequency with which one does so. An increase in ground reaction force, in turn, requires the increased activity of muscles involved in braking (primarily muscles of the quadriceps group) and propulsion (hip extensors and ankle plantar flexors). Despite the changes in magnitude, the patterns of muscle activity and work performed at lower limb joints are not altered by the speed of running. In addition, KLUITENBERG et al. (2012) have shown that the profiles of GRF are similar between overground and treadmill running.

1.3 EFFECTS OF SLOPE

Comrades Marathon in South Africa is one of the most famous and challenging ultra races in the world. On Up years, runners start at Durban and finish in Pietermaritzburg, covering 87 km with an elevation gain of 1951 m. On Down years, the route is reversed - starting at Pietermaritzburg and finishing in Durban, after 90.1 km and 1095 m of elevation gain. The difference in elevation between up and down years is 856 m, which, on average, corresponds to about 1% incline throughout the race. This may not seem much, but when we look at race times, we find that Up year races are consistently slower than Down year races, despite the fact that it is about 3 km shorter. The male record time for the Up course is 5 hours, 24 minutes and 39 seconds, 6 minutes and 20 seconds more than the record for the Down course. For female athletes, the difference in record times is 14 minutes and 41 seconds. Evidently, other aspects besides incline may account for differences in race times, however, this example illustrates how even very small variations in surface slope may affect running performance.

Several studies have shown that self-selected pace is inversely proportional to gradient during hill running (STAAB et al., 1992; DAVEY et al., 1995; MASTROIANNI et al., 2000; TOWNSHEND et al., 2010; KAY, 2012; VERNILLO et al., 2017) - this relationship is not always true for downhill slopes: due to biomechanical and/or psychological constraints, runners cannot increase their speed at steeper negative slopes enough to achieve the same heart rate (HR) or rate oxygen consumption that they did at level ground (STAAB et al., 1992; TOWNSHEND et al., 2010). Therefore, it is of no surprise that uphill races (or parts of races) are typically run at slower paces than the ones with less elevation gain (KAY, 2012 [Fig. 4]).

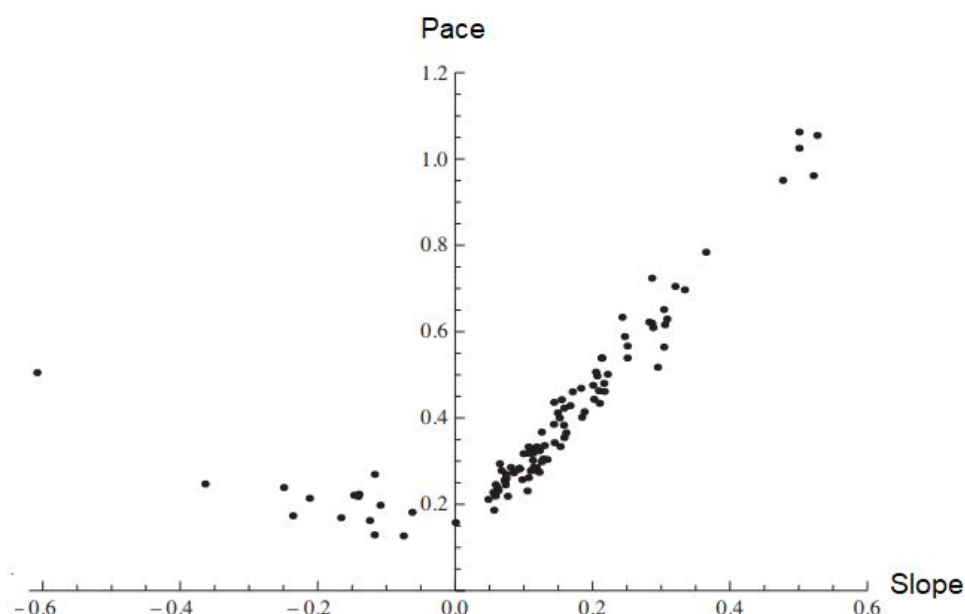


Figure 4 - Record pace (s/m) versus slope for ninety-one uphill and downhill races or race stages and the world 10 km track record (KAY, 2012)

Although extreme slopes are only present in a few races, it is unlikely that an athlete will not run on slightly inclined terrain (2%) during races or training, unless he or she does all workouts on a treadmill or track.

On level ground running, the energy cost required to cover a unit distance is constant - about $3.40 \text{ Jkg}^{-1}\text{m}^{-1}$ (MINETTI et al., 2002). During uphill running, both internal work (required to move limbs in relation to the body's center of mass) and external work (work to elevate the center of mass) increase linearly with the incline.

The increase in internal work arises mostly from the increase in stride frequency observed during uphill running, in comparison to level running at the same speed (MINETTI et al., 2002; VERNILLO et al., 2017). This is quite evident at steeper slopes: for instance, the twelve male runners studied by SWANSON & CALDWELL (2000) showed an average increase in stride frequency of 28% (from 1.39 to 1.78 Hz) when slope increased from 0% to 30%, at a speed of 4.5 m/s.

At smaller slopes, however, the increase in stride frequency is much more modest. SNYDER & FARLEY (2011) observed that, although their subjects seemed to choose higher stride frequencies when running at 5.24% slope than on 0% slope (2.8 m/s), the results were not significant. They concluded that the stride frequency

adopted was the same as the optimal stride frequency calculated for level ground. Moreover, the relationship between stride frequency and the metabolic cost was the same for uphill and level running. For most recreational long-distance runners, therefore, the increase in metabolic cost from the increase in stride frequency is of little significance, as they rarely train at slopes above the one studied by SNYDER & FARLEY (2011).

During uphill running, there is net positive external work done on the vertical axis. This means that, for the same speed, it requires an increase in power output compared to level running. Such an increase is proportional to the angle of incline - more precisely, the sine of the angle. Note that, during downhill running, net work in the vertical axis is negative and there is a decrease in total power output in comparison to level running.

Part of the energy used to elevate the center of mass during the propulsive phase is elastic energy stored in muscles and tendons during the absorption phase. At level running, this accounts for approximately 43% of the total positive mechanical energy at each cycle. At positive slopes, however, the maximum amount of energy that can be stored is reduced (VERNILLO et al., 2017). SNYDER & FARLEY (2011) estimated that the maximum possible elastic energy storage was reduced by 34% at the slope of 5.24%. The actual contribution of elastic energy also decreased. During level running, 54% of the positive power was from elastic energy while this value fell to 42% in uphill displacements.

In order to offset the reduction in the exploitation of stored elastic energy and the need to elevate the center of mass, greater power has to be produced during generation phase. GOTTSCHALL & KRAM (2005) found that when women run at 3 m/s, the parallel propulsive force peak, which reflects the active force produced during push off, increased from 169 N (about 0.3 body weight) at level ground to 216 N (28% increase), 253 N (50% increase) and 296 N (76% increase) at 3°, 6° and 9°, respectively. Normal propulsive force peak did not change significantly with the incline.

While running uphill requires more power to be generated during the propulsion phase, the amount of power absorbed during the first part of ground contact phase is reduced: in the same study, GOTTSCHALL & KRAM (2005) observed that normal impact peak decreased from 988 N at level ground to 840 N at 3° (15% decrease) and 767 N at 6° (22% decrease), although the results were only significant for the steeper slope. Parallel braking peak displayed a similar pattern, changing from -195 N to -157 N (19% decrease in magnitude), -122 N (38% decrease) and -89 N (54% decrease) at 3°, 6°, and 9°, respectively.

In line with GRF data, experiments conducted by ROBERTS & BELLIVEAU (2005) and TELHAN et al. (2010), under similar conditions as GOTTSCHALL & KRAM (2005) (3 - 3.5 m/s and 0 - 12° incline), have found a small but consistent increase of ankle power generation during uphill running and a decrease in knee power absorption, although the results were not significant for TELHAN et al. (2010). The most expressive change takes place at the hip joint: in ROBERTS & BELLIVEAU's experiment, work production at the hip during 0° incline was not different from zero and increased to about 0.25 J/kg at 6° and to 1 J/kg at 12°.

In summary, the studies mentioned above, as well as others reviewed by VERNILLO et al. (2017), indicate that graded running requires an increase in power production during late stance in order to overcome the reduction in elastic energy storage and the increased energy demand to elevate the center of mass. Although, power absorption during early stance decreases, it is not enough to match the increased energy demand during the push-off phase. Therefore, the total amount of energy for each stride cycle is higher than during level running at the same speed.

Similarly to what was reported for on the level ground, on hills most of the positive work is produced at the hip (55%) and ankle (32%) and most of the negative work is produced at the knee (58%), and, to a lesser extent, at the ankle (30%) (VERNILLO et al., 2017).

Regarding muscle activity, globally, uphill running requires higher activation than level running; more specifically of the *iliopsoas*, *gluteus maximus*, hip adductors, *vasti*, *gastrocnemius*, and *soleus* (GOSTILL et al., 1974; SLONIGER et al., 1997b;

VERNILLO et al., 2017), although the results are not always significant for smaller slopes. During the braking phase, higher activity has been measured in *gluteus maximus*, *vasti*, *gastrocnemius*, and *soleus*. These muscles also showed increased activity during the propulsion phase (VERNILLO et al., 2017).

For instance, using magnetic resonance imaging, SLONIGER et al. (1997b) found that lower limb muscle activity was 6% higher at 10% sloped running than level running at the same intensity (115% maximum oxygen uptake). The most marked changes happened for the *vasti* and *soleus*: these muscles showed an increase in both percentage of muscle volume activated (43% and 35%, respectively) and intensity of activity (23% and 14%). *Gastrocnemius* also showed increased intensity of activation (11% higher than level running), however, this was not statistically significant.

These findings are somewhat unexpected in face of the effects of sloped running on GRF. While the increased activity of hip extensors and ankle plantar flexors are easily explained by the necessity increasing power generation during push-off, one would expect a decrease in activity of the *vasti*, as power absorption by the knee has been shown to decrease with the slope. One hypothesis proposed by ROBERTS & BELLIVEAU (2005) suggests that some of the work performed by the hip during inclined running is generated by knee extensors and transferred to the hips by the hamstrings.

Most of the studies mentioned compared muscle activity in the level ground versus uphill running at the same speed. In reality, however, runners tend to reduce speed on hills which limits the applicability of the results reported. For this reason, VERNILLO et al. (2017) emphasized that future studies compare muscle activation during uphill and level running in similar conditions in terms of metabolic rate.

1.4 LOCOMOTION VARIABILITY

It is now widely accepted that motor behaviors display a certain degree of variability and this variability is not simply “noise” (HEIDERSCHEIT, 2000; JORDAN & NEWELL, 2008; MAIWALD et al., 2015).

During legged locomotion, the output of the system is given by temporal and spatial characteristics of strides. Studies done on walking patterns of elderly and clinical populations have shown that increased stride variability is an indicator of unsteady gait and is associated to increased risk of falling, aging and neurodegenerative diseases (HEIDERSCHEIT, 2000; JORDAN & NEWELL, 2008).

HEIDERSCHEIT (2000) emphasized the importance of not limiting the studies of locomotion to the variations in the outcome of the task - often called end-point variability. While higher variability of stride characteristics indicates an inability of the system to cope with ongoing constraints, measurements of the coupling between angles of lower limb joints (joint coordination variability) point to an opposing direction, where lower variability is associated with less healthy and pathological conditions (HAMILL et al., 1999; HEIDERSCHEIT, 2000; HAMILL et al., 2012).

These authors suggest that variability in the subtasks that contribute to the execution of the movement is an adaptive mechanism that enables the locomotor system to deal with internal and environmental constraints and to employ alternative ways to perform a given task. In other words, by allowing more flexibility to the degrees of freedom conveyed by joints and muscles, one could preserve the stability of gait.

Although the majority of the studies were done on walking, the same pattern seems to apply to running. MANN et al. (2015) investigated the correlation between previous running-related injuries and the variability of foot strike pattern during five 2-min runs at 80% to 120% of preferred running speed. The difference between experimental and control groups was not significant for the coefficient of variation of the foot strike pattern but detrended fluctuation analysis indicated that the runners from the control group had higher variability than those who reported a

running-related injury in the last twelve months. Moreover, they reported a linear decrease in the coefficient of variation of foot strike pattern as speed increased.

NAKAYAMA et al. (2010) reported a lower coefficient of variation of stride time in trained versus non-runners at speeds of 80, 100 and 120% of preferred speed. Detrended fluctuation analysis, on the other hand, indicated that trained runners had higher variability of stride time than non-runners at all speeds. For both estimators, the difference between groups remained even when subjects ran at the same absolute speed and neither correlated to speed.

Nonetheless, there is a dispute in the literature regarding this matter, as other reports present data that contradict this hypothesis. For instance, DAVIS (2017) observed increased joint coordination variability in individuals who underwent surgery for reconstruction of the anterior cruciate ligament in comparison to those of the control group. Likewise, HAUDUM et al. (2012) reported an increase in muscle variability of *tibialis anterior*, *gastrocnemius lateralis* and the *rectus femoris* when the movement was restrained by tubes attached to the subject's leg and hip during treadmill running.

An alternative hypothesis is that, while too little variability may result in an inability to find a solution to deal with imposed constraints, too much variability leads to an inability to adequately perform the task. This interpretation has led some authors to suggest that there is an optimum range of variability (STERGIOU & DECKER, 2011; HAUDUM et al., 2012).

1.4.1 A note on speed

Of all the variables that could potentially affect locomotion variability, speed is among the ones that have received the most attention. Although it could be interpreted in the context of varying constraints to the execution of the task, given the objectives of the current research, we thought it would be wise to describe its effects a little more thoroughly.

For both walking and running, stride variability appears to be minimum at preferred speed and increases for slower or faster paces, resulting in a U-shaped curve (HEIDERSCHEIT, 2000; JORDAN et al., 2007; JORDAN & NEWELL, 2008). This pattern is observed mostly when variability is measured using nonlinear methods and is in agreement with the hypothesis that increased end-point variability is an indicator of increased constraints.

On the other hand, when variability is measured by statistical estimators, such as coefficient of variation and standard deviation, it has been shown to be inversely proportional to speed (JORDAN et al., 2007; MANN et al., 2015).

It is important to highlight that interpretations regarding gait stability cannot be readily made from changes in variability unless the strategies adopted by the system to deal with the limitations and requirements of the environment are fully understood (BRUIJN et al., 2013). The authors illustrate this with the example of two subjects of similar built and patterns of motion who present the same stride variability during walking, but different stride widths. Despite the same variability, the one who adopts a narrow stride width is more likely to fall, thus, in this case, differences in instability are not reflected in gait variability.

Nevertheless, there is still no consensus among researchers given that studies have also described patterns dissimilar to the ones described above (JORDAN & NEWELL, 2008; NAKAYAMA et al., 2010; LINDSAY et al., 2014).

1.4.2 And a note on methods

A wide variety of methods have been employed to quantify movement variability. The choice of the estimator has critical implications for the interpretation of data and design of the experimental protocol. Moreover, several of the commonly used estimators lack validity (BRUIJN et al., 2013).

Among the methods used to access the capacity of the system to deal with small perturbations, Maximum Lyapunov Exponent and variability measures are the

ones that performed better across empirical and theoretical validations (BRUIJN et al., 2013).

Maximum Lyapunov Exponent calculates the average rate of divergence of trajectories after an infinitesimal perturbation and can be used in any type of kinematic data with more than 150 data points (BRUIJN et al., 2013). Although it is employed as an indicator of the capacity of the system to adapt to disturbances and exploit its degrees of freedom, the interpretation of the results is not straightforward.

Statistical measures, often standard deviation and coefficient of variation, can also be used for several kinematic variables in vectors of a considerable number of points, usually more than 200 observations (BRUIJN et al., 2013).

2 OBJECTIVES

In light of the effects of variations of speed and slope on muscle activity discussed so far, our primary objectives were to investigate (1) whether there is a correlation between speed and/or slope and the variability of the interval between contractions of muscles from the quadriceps and the plantar flexors groups and (2) if such correlations could be explained only by the changes in power output resulting from variations of these two parameters.

In order to design an experiment that adequately addressed the main objectives, it was necessary to impose restrictions regarding the characteristics of experimental subjects. However, we felt that this group did not accurately represent the widely heterogeneous population of recreational runners. Thus, our last objective was to (3) verify if the results observed for a restricted set of volunteers could be seen in a more diverse population of runners.

3 HYPOTHESIS

Based on the hypothesis that decreased variability of subtasks that contribute to the execution of the outcome task (in this case, intervals between contractions) is an indicator of increased constraints to the system and on the fact that speed and slope are positively correlated to activity of quadriceps and plantar flexors, we hypothesized that variability estimators of both muscles groups would be inversely related to speed and slope. Given that variations in muscle activity are the primary cause of changes in energy expenditure during running, we also expected variability estimators to be inversely proportional to power output.

Lastly, although we anticipated that age and fitness level could affect the values of variability between subjects, we expected to find similar patterns of response to changes in speed, slope and exercise intensity in the more restricted and in the wider group of runners.

4 MATERIALS AND METHODS

In all experimental procedures and data analyses, heart rate was used as a proxy to the power demand of the subject.

4.1 SUBJECTS

Seventeen healthy male trained runners volunteered for the study (eleven for Experiment 1 and six for Experiment 2). All subjects were non-smokers and had no chronic conditions or history of cardiovascular and muscular diseases that they were aware of and had been free from running-related injuries for at least six months. A 12-lead electrocardiogram (ECG) was performed before the experiments to confirm subjects had no cardiac problems. All volunteers gave written informed consent and procedures were approved by Comissão de Ética no Uso de Animais (CEUA IB-USP / CAAE 65361317.2.0000.5464).

We considered a candidate to be a trained runner and therefore fit for the study if he ran at least twice a week for the past one year and logged an average of 20 km or more per week. For Experiment 1, any person above 18 years of age and who fitted these criteria was accepted as a test subject. Since Experiment 2 aimed to collect data for a more homogeneous group, we imposed two further restrictions regarding age (20 - 45 years old) and fitness level (accessed through speed, as described below).

4.2 SPEED DETERMINATION

For each subject, a given running speed U was determined and used as a reference throughout the experiments. U was defined as the speed at which, at 0% incline, the subject's heart rate (HR) reached $86 \pm 9\%$ of his estimated maximum HR (HRmax), which corresponds to vigorous intensity exercise (ACSM, 2011). HRmax was estimated according to Equation 1, as suggested by TANAKA et al. (2001).

$$\text{HR}_{\text{max}} = 208 - 0.7 \times \text{age in years} \quad (1)$$

For the subjects who volunteered for Experiment 1, U was chosen during an interview and later verified in ECG records. Data from subjects whose chosen speeds were not within the desired HR zone were excluded from the analysis.

Since Experiment 2 required subjects to have a similar fitness, U was determined experimentally before the beginning of the first exercise section and only those runners whose U was between 11 km/h and 13 km/h were accepted as test subjects. After warming up for three minutes at 5 km/h, subjects run for four minutes at 11 km/h. From then on, speed was increased by 0.5 km/h every three minutes, until the subject reached the desired HR. The speed reached at this stage was adopted as U. HR was measured during the last minute of each speed stage using a Garmin Forerunner 620 HR monitor.

4.3 EXPERIMENTAL PROTOCOL

Experiments were conducted in the Laboratory of Energetics and Theoretical Physiology, at the Department of Physiology, Bioscience Institute, University of São Paulo between 7 and 10 a.m. Subjects ran on a motorized treadmill at room temperature ($21.3^{\circ}\text{C} \pm 2.3^{\circ}\text{C}$) and humidity ($62\% \pm 8.7\%$).

Subjects were instructed to wear appropriate running clothes and shoes and to abstain from caffeine and alcohol for at least twenty-four hours prior to each section. Although they were allowed to maintain their normal training schedule, we asked them to avoid doing unusually long or hard runs one day before the experiments and to plan their schedules so that they would not run on the same day before coming to experiment sections. Subject completed the test protocol within four weeks.

4.3.1 Experiment 1

Volunteers performed a total of nine 6-min target runs, resulting from the combination of three slopes ($\theta = 0\%$, 2.5% and 4%) and three speeds ($u = 0.8 U$, $0.9 U$ and $1 U$). These runs were divided into three exercise sections, which took place in different days.

Each exercise section consisted of a three-minute rest period in standing position, followed by a six-minute warm-up, three six-minutes target runs and, if the subject wished so, a recovery run/walk period. During each section, only one slope ($\theta = 0\%$, 2.5% or 4%) was used for the target runs and the order of the sections was randomly chosen. The three runs of each section ($u = 0.8 U$, $0.9 U$, and $1 U$) were done in an increasing-speed mode. This increasing speed protocol was adopted in order to allow for an adequate follow up of heart rate and variability (NATALI, 2015).

4.3.2 Experiment 2

The restriction adopted in Experiment 2 regarding U speed allowed us to expand the range of power developed by subjects during target runs by adding a fourth speed and a fourth incline. Therefore, Experiment 2 had a total of sixteen three-minute target runs, resulting for the combination of four slopes ($\theta = 0\%$, 2% , 4% and 6%) and four speeds ($u = 0.7 U$, $0.8 U$, $0.9 U$ and $1U$). These runs were divided among four exercise sections, one for each slope, which took place in separate days. Each section began with a three-minute rest period standing up on the treadmill and a three-minute jog at $0.8 U$ for warming up. Like in Experiment 1, target runs were done in an increasing-speed order.

In infrequent occasions, sweating caused the electrodes to detach from the subject's skin, compromising the quality of the signal. In those situations, experiments were interrupted between runs in order to replace them.

4.4 DATA ACQUISITION

Heart rate and intervals between successive muscle contractions were obtained from ECG and EMG records, respectively. Data were collected with Biopac Student Lab PRO MP36 (Biopac Systems, Inc.), at a sampling rate of 1000 Hz, on three simultaneous channels.

For ECG, three surface electrodes were attached to the subject's chest in a modified CM5 configuration. Equipment was set at AHA set up, with a low pass filter of 0.05 Hz and a high pass of 100 Hz.

For EMG, two surface electrodes were placed above the muscle belly of muscles belonging to the quadriceps and the plantar flexors muscle groups of the subject's left leg, according to the recommendations provided by the SENIAM PROJECT.

We collected signals from VL and GL in Experiment 1 and VM and GL in Experiment 2. The change from VL to VM was an attempt to overcome problems in signals due to noise sometimes observed during Experiment 1. Equipment was set at EMG configuration with low pass filter of 30 Hz and high pass at 500 Hz.

4.5 DATA PROCESSING

ECG and EMG signals were initially cut into vectors corresponding to each of the nine (Experiment 1) or sixteen (Experiment 2) target runs. From each vector, the first and last fifteen seconds were discarded in order to avoid noise associated with the transition between speed conditions.

4.5.1 ECG

R waves were detected using MatLab suite software (MathWorks Inc. R215b) scripts. Average HR was calculated from the last 256 interbeat intervals (approximately last 90 seconds of each vector).

4.5.2 EMG

Intervals between bursts of muscle activity were detected using R 3.5.1 (The R Project) scripts. Abnormally detected intervals were manually excluded from vectors and segments of 175 consecutive electrical steps were used for analysis.

4.6 ANALYSIS

Stride frequency and variability estimators were calculated from vectors of intervals between contractions as detailed below. When necessary, variables are indexed according to the muscles from which they were calculated.

1. st: interval between contractions (i.e., stride time).
2. sd: standard deviation
3. cv: coefficient of variation
4. rmssd: quadratic mean of successive differences

The analysis was divided into three parts, according to the questions addressed. For the first two questions, only data from Experiment 2 were used. In all stages of analysis, the significance level adopted was 0.05. Unless specified, analyses were done using relativized values in relation to those obtained in the [$\theta = 0^\circ$, $u = 1$ U] run. Models and gradients were based on populational mean values.

4.6.1 Question 1

In this question, we addressed the first aim of the study, which was to verify whether there is a correlation between estimators and speed and/or slope. It was answered in two stages: first, multiple regressions of the dependent variables (y) as a function of speed and slope were calculated for each estimator ("speed-and-slope models", Eq. 2). Data from VM and GL were fitted separately so that we obtained

estimates of three parameters (a, b and c) for each muscle. When necessary, parameters are indexed according to muscle group:

$$y = a + b \times u + c \times u\theta \quad (2)$$

Where y is the dependent variable (st and estimators), a , b and c are the parameters adjusted and $u\theta$ is the interaction between speed u and slope θ . Since the effects of slope can only be measured during motion, the model does not include a term for the effects of slope in isolation.

In order to compare the parameters of VM and VL, data from both muscles were combined and fitted to a model similar to the speed-and-slope regressions plus a dummy variable m for the effect of muscle group (Eq. 3).

$$y = a + am + b \times u + bm \times u + c \times u\theta + cm \times u\theta \quad (3)$$

Where y is the dependent variable (st and estimators), a , b and c are the parameters adjusted and $u\theta$ is the interaction between speed u and slope θ and m is the dummy variable for the muscle group.

On the second stage of analysis, the gradient $\nabla(\cdot)$ of the scalar field of the muscle contraction variability estimators (described above) and st as a function of speed u and slope θ was obtained (Eq. 4):

$$\nabla f_z = (\partial f_z / \partial u) \cdot \hat{e}_1 + (\partial f_z / \partial \theta) \cdot \hat{e}_2 \quad (4)$$

Where \hat{e}_1 and \hat{e}_2 form the orthogonal axes of the field - in this case, is simply speed as the x-axis and slope as the y-axis; and f_z is the function under analysis (st or one of the estimators). The gradient gives the direction and magnitude of the rate of variation of the function f_z . Therefore, for each pair $[\theta, u]$, it indicates to which independent variable the function is most sensitive and how much does it change. To obtain the gradients, intermediate values of estimators were interpolated so that surfaces are formed by a grid of $\theta = 0, 0.5, 1, 1.5 \dots 6\%$ and $u = 0.7, 0.75, 0.8 \dots 1$ U. The number of divisions was arbitrary.

4.6.2 Question 2

On this question, we sought to verify if there is a correlation between estimators (and st) with power output. The procedure adopted was similar to the first stage of analysis used on Question 1: a linear regression was fitted for each variable as a function of HR ("HR models") using data from VM and GL separately (Eq. 5):

$$y = \alpha + \beta \times \text{HR} \quad (5)$$

Where y is the dependent variable (st or an estimator), HR is the heart rate in % of estimated HRmax and α and β are the parameters adjusted.

Another model added a dummy variable for the muscle group to Eq. 5 in order to allow for comparisons between parameters α and β fitted for VM with those fitted for GL (Eq. 6):

$$y = \alpha + \alpha_m + \beta \times \text{HR} + \beta_m \times \text{HR} \quad (6)$$

Where y is the dependent variable, HR is the heart rate in % of estimated HRmax, α and β are the parameters adjusted and m is the dummy variable for the muscle group.

4.6.3 Question 3

Lastly, to answer the question from our third objective (do the patterns observed throughout Experiment 2 persist in a more diverse group, in terms of age and fitness, of runners?) we used the significant models created on Questions 1 and 2, which correlated variability estimators to either speed and slope (Eq. 2) or HR (Eq. 5), to estimate values for the combinations of u and θ used on runs on Experiment 1. These predicted values were then compared to the ones observed on Experiment 1 using a two-tailed paired t-test.

5 RESULTS AND DISCUSSION

The characteristics of the subjects who took part in Experiments 1 and 2 are presented in Tables 1 and 2.

Table 1 - Characteristics of subjects who volunteered for Experiment 1 (n = 11).

Subject	U (km/h)	HR _[0,U] (bpm)	HRmax (bpm)	%HRmax _[0,U]	Age (years)	Height (cm)	Weight (kg)
1	14.3	146	183	80	36	161	55
2	10.7	157	169	93	56	178	79
3	12	166	191	87	25	168	60
4	16	154	181	85	39	176	65
5	14.4	158	188	84	29	165	54
6	10	141	172	82	52	171	75
7	14	140	175	80	47	176	61
8	10	154	176	88	46	175	73
9	10.7	150	188	80	28	175	80
10	10.9	153	179	85	41	170	80
11	10.2	148	169	87	56	177	70
Mean	12.11	151.56	179.05	84.71	41.36	172	68.36
σ	2.16	7.72	7.76	4.08	11.08	5.50	9.86

U = speed at which, at level running, volunteer's heart rate (HR) attained 86% of their maximum heart rate (HRmax); HR_[0,U] = HR at run [θ = 0%, u = 1 U]; %HRmax_[0,U] = % of HRmax at run [θ = 0%, u = 1 U]; σ = standard deviation.

Table 2 - Characteristics of subjects who volunteered for Experiment 2 (n = 6).

Subject	U (km/h)	HR _[0,U] (bpm)	HRmax (bpm)	%HRmax _[0,U]	Age (years)	Height (cm)	Weight (kg)
1	13.0	147	179	82	42	177	83.7
2	12.0	170	191	89	24	168	78.5
3	12.0	155	178	87	43	163	71.0
4	11.0	165	193	86	22	166	53.0
5	12.5	153	185	83	33	172	84.7
6	11.0	164	188	87	29	174	84.0
Mean	11.92	159.00	85.69	185.48	32.17	170	75.82
σ	0.80	8.69	2.66	6.22	8.89	5.25	12.32

U = speed at which, at level running, volunteer's heart rate (HR) attained 86% of their maximum heart rate (HRmax); HR_[0,U] = HR at run [$\theta = 0\%$, $u = 1$ U]; %HRmax_[0,U] = % of HRmax at run [$\theta = 0\%$, $u = 1$ U]; σ = standard deviation.

The results of Experiments 1 and 2 are presented respectively on Tables 3 and 4 in absolute units and on Tables 5 and 6 in values relative to run [$\theta = 0\%$, $u = 1$ U]. Relative values are also shown in Figs. 5 (VL, Experiment 1), 6 (GL, Experiment 1), 7 (VM, Experiment 2) and 8 (GL, Experiment 2). These data were used to address Questions 1 - 3, which will be discussed next.

Table 3 - Results of Experiment 1 in absolute values (n = 11).

			HR (bpm)		st (ms)		sd (ms)		cv (%)		rmssd (ms)	
m	θ (%)	u (U)	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ
VL	0	0.8	130.1	8.6	700.0	56.7	17.3	4.1	2.49	0.60	27.5	7.0
		0.9	141.4	7.6	691.4	56.8	15.3	2.4	2.23	0.41	24.3	4.6
		1	152.2	8.0	682.0	60.3	15.4	3.5	2.27	0.53	24.7	6.3
	2.5	0.8	136.6	9.7	694.2	55.7	15.1	3.6	2.20	0.59	24.3	6.2
		0.9	151.2	9.6	684.4	58.7	16.8	4.2	2.51	0.83	26.5	7.6
		1	162.5	7.8	674.5	62.4	16.5	3.2	2.47	0.48	26.7	5.9
	4	0.8	147.7	11.5	687.8	60.7	17.5	3.9	2.58	0.67	27.7	6.6
		0.9	160.8	9.5	683.9	61.6	15.6	2.8	2.29	0.38	24.1	5.5
		1	171.7	7.5	684.7	45.2	17.7	3.7	2.60	0.58	28.4	7.7
GL	0	0.8	130.1	8.6	699.9	56.8	19.2	6.3	2.76	0.90	30.4	11.4
		0.9	141.4	7.6	691.5	56.8	15.5	3.9	2.24	0.49	24.7	6.7
		1	152.2	8.0	682.1	60.4	15.5	3.4	2.27	0.47	24.8	6.3
	2.5	0.8	136.6	9.7	699.5	55.9	16.5	6.2	2.35	0.84	28.0	11.1
		0.9	151.2	9.6	684.2	59.4	14.9	3.8	2.18	0.56	23.5	6.2
		1	162.5	7.8	674.4	62.7	13.9	3.0	2.07	0.48	22.1	5.3
	4	0.8	147.7	11.5	687.6	61.1	17.1	5.9	2.51	0.87	27.6	9.9
		0.9	160.8	9.5	678.1	65.6	14.2	3.8	2.10	0.52	22.0	6.6
		1	171.7	7.5	686.8	47.6	14.3	3.1	2.09	0.50	21.8	6.1

σ = standard deviation; m = muscle group; VL = *vastus lateralis*; GL = *gastrocnemius lateralis*; θ = slope; u = speed; U = speed at which, at level running, volunteer's heart rate (HR) attained 86% of their maximum heart rate; σ = standard deviation; st = stride time; sd = standard deviation of intervals between successive contractions; cv = coefficient of variation of successive contractions; rmssd = quadratic mean of intervals between successive contractions.

Table 4 - Results of Experiment 2 in absolute values (n = 6).

m	θ (%)	u (U)	HR (bpm)		st (ms)		sd (ms)		cv (%)		rmssd (ms)	
			Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ
VM	0	0.7	126.7	17.7	724.5	14.7	39.2	40.5	5.38	5.50	59.3	57.7
		0.8	138.6	15.9	707.7	16.0	19.2	5.2	2.72	0.77	29.7	7.8
		0.9	147.9	15.1	697.8	20.9	19.7	9.2	2.83	1.34	32.2	16.1
		1.0	157.5	12.4	688.5	19.4	25.1	8.3	3.66	1.23	36.0	11.4
	2	0.7	133.1	15.1	711.8	13.1	25.5	5.7	3.58	0.80	40.4	8.0
		0.8	148.9	16.7	699.4	15.2	23.0	11.5	3.31	1.68	38.5	19.8
		0.9	158.0	15.2	687.8	22.1	22.1	8.1	3.22	1.19	32.4	9.4
		1.0	168.1	15.1	677.3	33.8	20.3	6.3	3.00	0.92	29.5	9.5
	4	0.7	139.1	14.2	712.7	15.0	17.9	5.3	2.51	0.75	26.6	6.6
		0.8	155.5	14.3	699.1	25.0	20.4	11.5	2.91	1.64	30.6	16.0
		0.9	167.9	11.4	687.2	26.0	16.0	5.1	2.32	0.73	23.9	8.4
		1.0	180.2	10.7	682.2	28.7	16.1	5.6	2.36	0.83	21.7	3.6
	6	0.7	145.0	11.8	709.0	25.2	24.8	12.3	3.54	1.89	36.9	16.5
		0.8	163.2	10.6	698.8	20.8	16.3	2.1	2.33	0.26	25.3	2.6
		0.9	177.2	8.0	690.9	22.7	15.9	4.0	2.30	0.61	23.8	5.1
		1.0	186.3	6.7	665.0	10.9	14.7	2.3	2.21	0.39	21.7	4.0
GL	0	0.7	126.7	17.7	721.1	14.7	22.7	6.7	3.14	0.89	35.3	10.5
		0.8	138.6	15.9	707.9	16.0	18.2	6.8	2.56	0.95	28.0	12.4
		0.9	147.9	15.1	697.9	21.0	17.2	5.9	2.46	0.85	27.5	10.6
		1.0	157.5	12.4	687.7	19.3	21.0	5.6	3.05	0.76	28.1	8.6
	2	0.7	133.1	15.1	711.9	12.9	21.2	4.6	2.98	0.67	33.1	7.5
		0.8	148.9	16.7	699.1	15.0	18.1	2.7	2.58	0.36	27.9	5.1

			HR (bpm)		st (ms)		sd (ms)		cv (%)		rmssd (ms)	
m	θ (%)	u (U)	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ
GL	2	0.9	158.0	15.2	688.3	21.8	18.4	2.7	2.67	0.37	25.1	3.4
		1.0	168.1	15.1	677.5	33.7	17.3	3.1	2.56	0.46	25.6	1.1
	4	0.7	139.1	14.2	712.8	14.6	17.9	5.0	2.51	0.68	26.6	4.9
		0.8	155.5	14.3	700.0	25.0	14.3	4.0	2.04	0.54	22.0	6.4
		0.9	167.9	11.4	687.3	25.9	14.9	1.9	2.17	0.23	21.9	3.1
		1.0	180.2	10.7	681.7	29.4	17.1	4.8	2.53	0.79	22.0	3.3
	6	0.7	145.0	11.8	707.9	26.5	23.0	9.3	3.23	1.26	36.9	16.2
		0.8	163.2	10.6	697.3	23.0	19.9	8.5	2.86	1.21	31.3	14.1
		0.9	177.2	8.0	690.9	22.9	18.9	5.9	2.72	0.82	28.5	9.7
		1.0	186.3	6.7	664.7	10.6	15.5	5.0	2.34	0.74	20.5	4.7

σ = standard deviation; m = muscle group; VM = *vastus medialis*; GL = *gastrocnemius lateralis*; θ = slope; u = speed; U = speed at which, at level running, volunteer's heart rate (HR) attained 86% of their maximum heart rate; st = stride time; sd = standard deviation of intervals between successive contractions; cv = coefficient of variation of successive contractions; rmssd = quadratic mean of intervals between successive contractions.

Table 5 - Results of Experiment 1 in values relative to run [$u = 1U$, $\theta = 0\%$] ($n = 11$).

			HR		st		sd		cv		rmssd	
m	θ (%)	u (U)	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ
	0	0.8	0.854	0.023	1.027	0.020	1.151	0.308	1.120	0.299	1.145	0.322
		0.9	0.929	0.020	1.014	0.010	1.014	0.143	0.999	0.142	1.006	0.158
		1	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
VL	2.5	0.8	0.897	0.035	1.019	0.023	1.000	0.226	0.980	0.210	1.013	0.240
		0.9	0.990	0.030	1.004	0.014	1.110	0.246	1.106	0.245	1.095	0.268
		1	1.068	0.026	0.989	0.012	1.094	0.199	1.106	0.197	1.111	0.252
	4	0.8	0.970	0.050	1.009	0.020	1.173	0.327	1.164	0.326	1.162	0.320
		0.9	1.057	0.048	0.992	0.018	1.042	0.268	1.051	0.273	1.019	0.296
		1	1.125	0.031	0.986	0.014	1.190	0.303	1.207	0.309	1.191	0.348
	0	0.8	0.854	0.023	1.027	0.020	1.238	0.272	1.208	0.275	1.219	0.292
		0.9	0.929	0.020	1.014	0.010	1.015	0.175	1.001	0.172	1.009	0.166
		1	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
GL	2.5	0.8	0.897	0.035	1.015	0.022	1.087	0.301	1.069	0.292	1.111	0.292
		0.9	0.990	0.030	1.003	0.015	0.963	0.136	0.960	0.135	0.949	0.125
		1	1.068	0.026	0.988	0.012	0.911	0.158	0.922	0.162	0.910	0.185
	4	0.8	0.970	0.050	1.008	0.020	1.115	0.322	1.108	0.329	1.118	0.315
		0.9	1.057	0.048	0.995	0.018	0.926	0.201	0.932	0.206	0.901	0.241
		1	1.125	0.031	0.988	0.012	0.917	0.236	0.926	0.233	0.870	0.270

σ = standard deviation; m = muscle group; VL = *vastus lateralis*; GL = *gastrocnemius lateralis*; θ = slope; u = speed; U = speed at which, at level running, volunteer's heart rate (HR) attained 86% of their maximum heart rate; st = stride time; sd = standard deviation of intervals between successive contractions; cv = coefficient of variation of successive contractions; rmssd = quadratic mean of intervals between successive contractions.

Table 6 - Results of Experiment 2 in values relative to run [$u = 1U$, $\theta = 0^\circ$] ($n = 6$).

m	θ (%)	u (U)	HR		st		sd		cv		rmssd	
			Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ
VM	0	0.7	0.832	0.102	1.053	0.033	1.536	1.426	1.453	1.329	1.647	1.562
		0.8	0.895	0.062	1.028	0.018	0.823	0.271	0.804	0.273	0.871	0.255
		0.9	0.946	0.038	1.013	0.009	0.803	0.246	0.794	0.246	0.881	0.211
		1.0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
	2	0.7	0.862	0.090	1.034	0.034	1.171	0.563	1.142	0.572	1.239	0.493
		0.8	0.940	0.059	1.015	0.022	0.963	0.413	0.954	0.430	1.070	0.428
		0.9	0.986	0.040	0.998	0.014	0.961	0.367	0.966	0.376	0.946	0.273
		1.0	1.042	0.052	0.982	0.021	0.865	0.243	0.883	0.255	0.850	0.219
	4	0.7	0.895	0.066	1.035	0.014	0.840	0.310	0.812	0.302	0.861	0.282
		0.8	1.000	0.048	1.015	0.014	0.955	0.514	0.942	0.514	0.968	0.429
		0.9	1.082	0.063	0.997	0.008	0.725	0.213	0.726	0.210	0.748	0.204
		1.0	1.148	0.076	0.985	0.010	0.797	0.226	0.810	0.234	0.733	0.143
	6	0.7	0.951	0.044	1.030	0.041	1.037	0.437	1.016	0.452	1.105	0.522
		0.8	1.040	0.051	1.015	0.030	0.717	0.280	0.703	0.260	0.773	0.305
		0.9	1.116	0.091	1.000	0.018	0.711	0.239	0.709	0.230	0.730	0.284
		1.0	1.164	0.126	0.973	0.005	0.643	0.327	0.661	0.335	0.666	0.378
GL	0	0.7	0.832	0.102	1.049	0.033	1.096	0.230	1.044	0.218	1.270	0.151
		0.8	0.895	0.062	1.030	0.019	0.863	0.198	0.839	0.193	0.982	0.197
		0.9	0.946	0.038	1.015	0.011	0.816	0.174	0.805	0.177	0.970	0.183
		1.0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000
	2	0.7	0.862	0.090	1.036	0.035	1.079	0.392	1.039	0.368	1.225	0.447
		0.8	0.940	0.059	1.017	0.022	0.895	0.168	0.881	0.170	1.004	0.188
		0.9	0.986	0.040	1.000	0.013	0.908	0.154	0.908	0.153	0.913	0.177
		1.0	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000

			HR		st		sd		cv		rmssd	
m	θ (%)	u (U)	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ
GL	2	0.9	0.986	0.040	1.000	0.013	0.908	0.154	0.908	0.153	0.913	0.177
		1.0	1.042	0.052	0.984	0.021	0.877	0.265	0.895	0.283	0.949	0.256
	4	0.7	0.895	0.066	1.037	0.013	0.947	0.281	0.914	0.276	1.065	0.087
		0.8	1.000	0.048	1.018	0.011	0.742	0.179	0.729	0.178	0.875	0.172
		0.9	1.082	0.063	0.999	0.007	0.786	0.107	0.787	0.111	0.883	0.093
		1.0	1.148	0.076	0.985	0.012	0.902	0.436	0.919	0.453	0.861	0.238
	6	0.7	0.951	0.044	1.030	0.041	1.122	0.424	1.092	0.426	1.326	0.483
		0.8	1.040	0.051	1.014	0.031	0.966	0.376	0.956	0.387	1.120	0.401
		0.9	1.116	0.091	1.000	0.019	0.854	0.165	0.854	0.168	0.953	0.135
		1.0	1.164	0.126	0.972	0.006	0.775	0.313	0.798	0.327	0.749	0.226

σ = standard deviation; m = muscle group; VM = *vastus medialis*; GL = *gastrocnemius lateralis*; θ = slope; u = speed; U = speed at which, at level running, volunteer's heart rate (HR) attained 86% of their maximum heart rate; st = stride time; sd = standard deviation of intervals between successive contractions; cv = coefficient of variation of successive contractions; rmssd = quadratic mean of intervals between successive contractions.

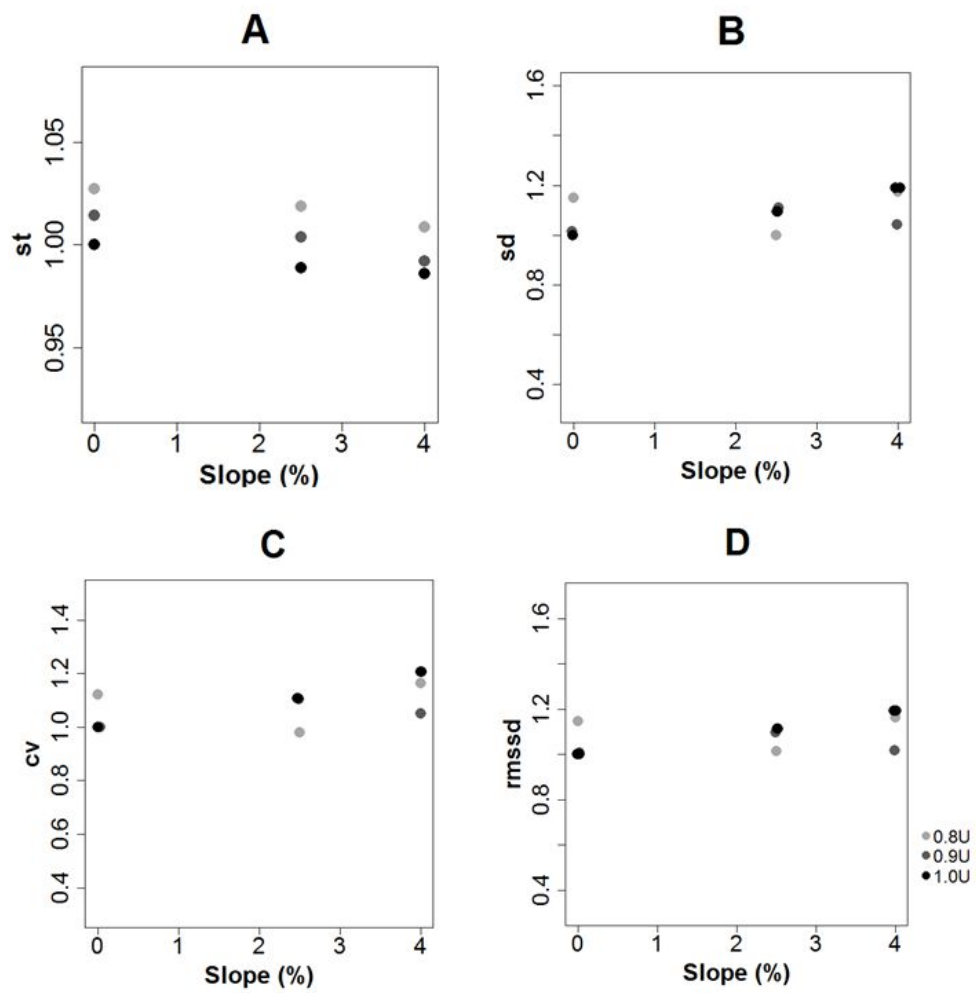


Figure 5 - Mean values of st and variability estimators of *vastus lateralis* for target runs of Experiment 1 (n = 11). Values on y-axis are relative to run [$\theta = 0\%$, $u = 1$ U]. A = stride time (st); B = standard deviation of intervals between successive contractions (sd); C = coefficient of variation of successive contractions (cv); D = quadratic mean of intervals between successive contractions (rmssd).

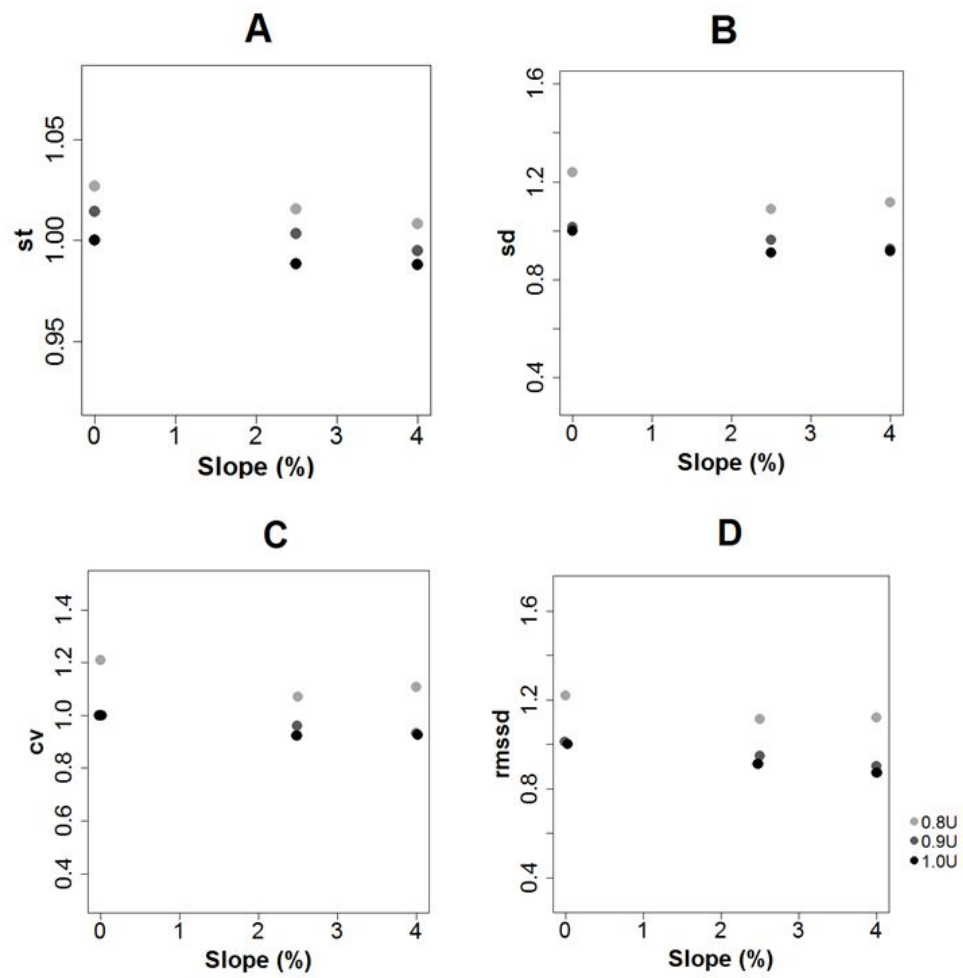


Figure 6 - Mean values of st and variability estimators of *gastrocnemius lateralis* for target runs of Experiment 1 (n = 11). Values on y-axis are relative to run [θ = 0%, u = 1 U]. A = stride time (st); B = standard deviation of intervals between successive contractions (sd); C = coefficient of variation of successive contractions (cv); D = quadratic mean of intervals between successive contractions (rmsd).

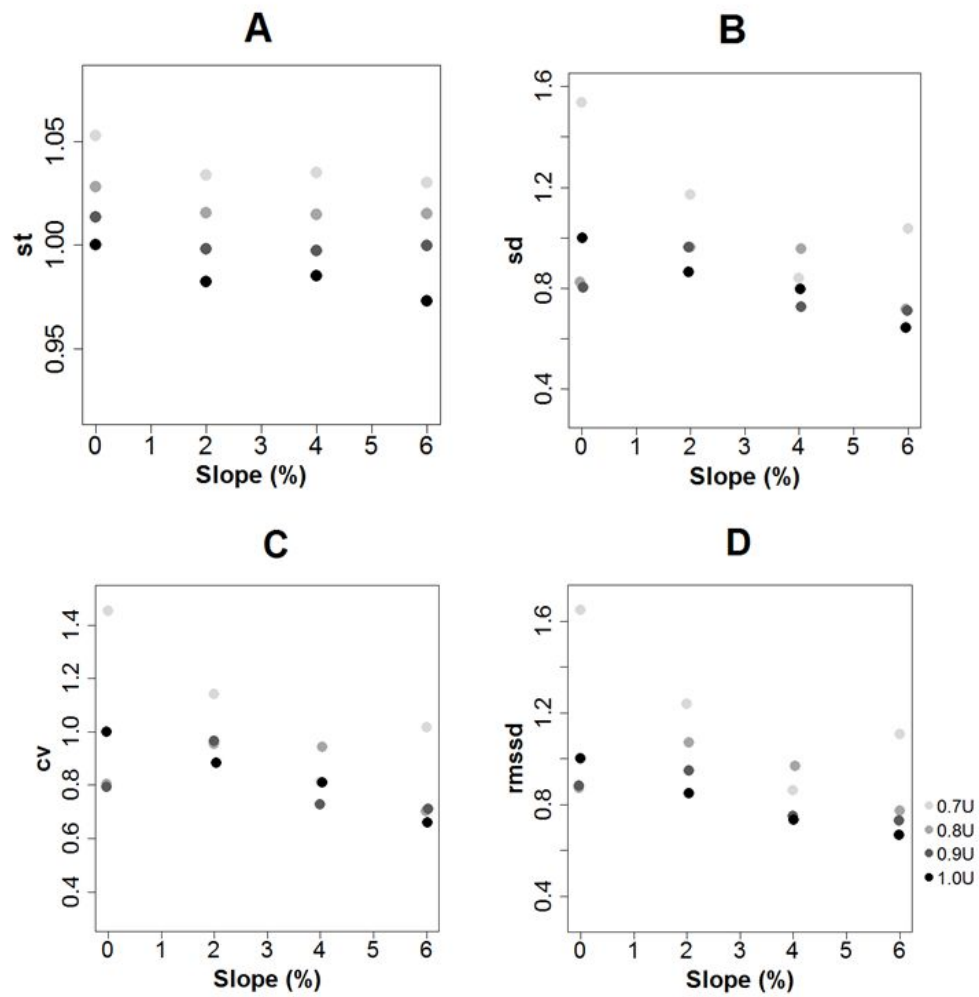


Figure 7 - Mean values of st and variability estimators of *vastus medialis* for target runs of Experiment 2 ($n = 6$). Values on y-axis are relative to run [$\theta = 0\%$, $u = 1$ U]. A = stride time (st); B = standard deviation of intervals between successive contractions (sd); C = coefficient of variation of successive contractions (cv); D = quadratic mean of intervals between successive contractions (rmsd).

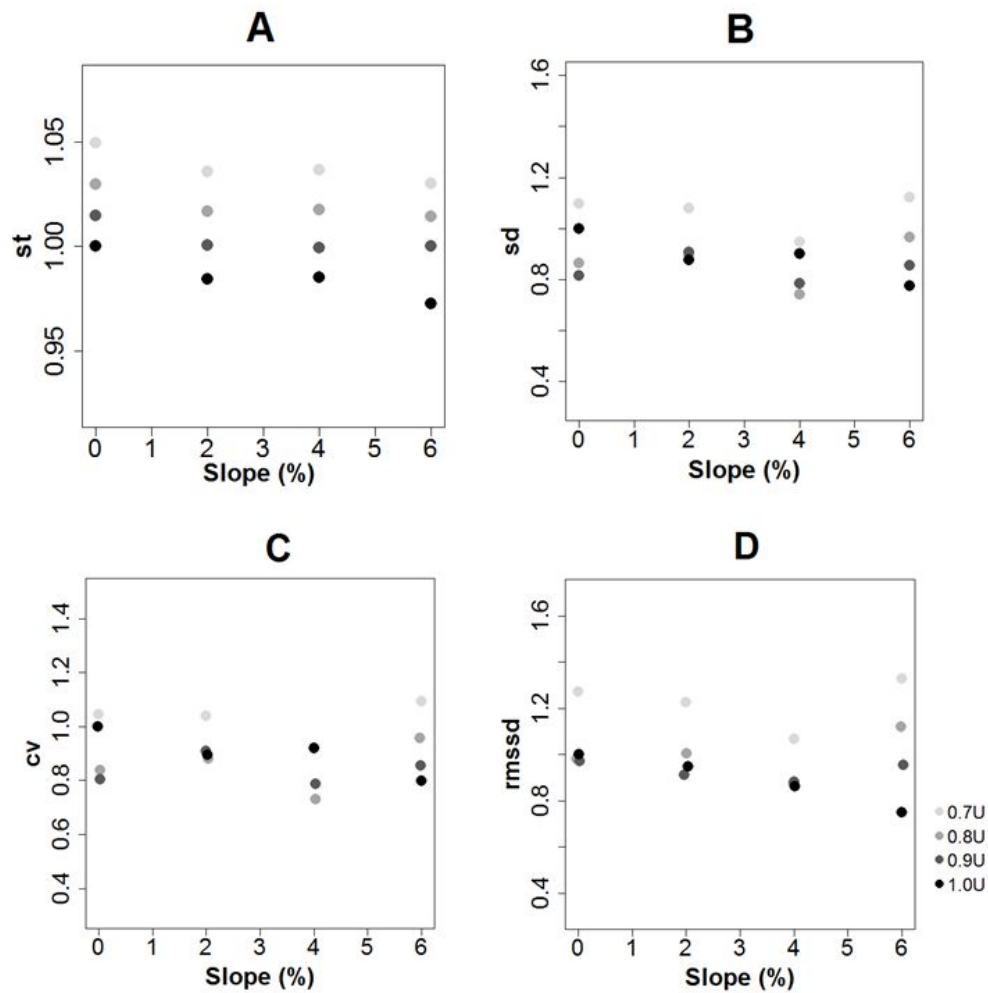


Figure 8 - Mean values of st and variability estimators of *gastrocnemius lateralis* for target runs of Experiment 2 (n = 6). Values on y-axis are relative to run [$\theta = 0\%$, $u = 1$ U]. A = stride time (st); B = standard deviation of intervals between successive contractions (sd); C = coefficient of variation of successive contractions (cv); D = quadratic mean of intervals between successive contractions (rmssd).

5.1 QUESTION 1

5.1.1 Linear regressions (speed-and-slope models)

Stride time and all estimators of the variability of VM contractions showed a tendency to decrease with speed and with the interaction speed-slope (Table 7), although the correlation between cv and the interaction speed-slope did not reach significance ($p = 0.06$). This pattern was only observed for st_{GL} , which was expected,

given that both muscles have one burst of activity at each stride cycle. While sd_{GL} and $rmssd_{GL}$ decreased with speed, neither correlated to the interaction speed-slope and cv_{GL} did not correlate to speed nor the interaction (Table 7).

As discussed in the introduction, it has been suggested that variability of subtasks (in this case, intervals between contractions) is higher in situations of increased demand (i.e., force production) or constraints to the locomotor system (HAMILL et al, 1999; HEIDERSCHEIT, 2000; HAMILL et al.,2012). Additionally, it is well known that increases in slope and speed of running require an increase in muscle activity of quadriceps and ankle extensors (SLONIGER et al., 1997b; KYROLAINEN et al.,1999; KYROLAINEN et al., 2005; ABE et al., 2011; TSUJI et al., 2015; VERNILLO et al., 2017). Thus, our results were expected based on data from the literature and are in line with our hypothesis.

At first glance, GL had lower absolute values of a, b and c for almost all variability estimators. In other words, variability of GL appears to start from lower levels and to be less responsive than variability of VM to changes in slope and speed. However, the differences between parameters estimated for the two muscles were not significant (st: $p_a = 0.98$, $p_b = 0.92$, $p_c = 0.92$; sd: $p_a = 0.28$, $p_b = 0.43$, $p_c = 0.09$; cv: $p_a = 0.26$, $p_b = 0.40$, $p_c = 0.08$; rmssd: $p_a = 0.72$, $p_b = 0.83$, $p_c = 0.08$).

Previous experiments indicate that, at a given speed, *gastrocnemius* is more active in comparison to *vastii*: EMG data collected of elite middle-distance runners show that activation of VL goes from ~70% MVC at 4 m/s to ~150% MVC at maximum sprint during braking phase and from less than 10% MVC to ~40% MVC during push-off phase. For the same range of speeds, EMG activity of *gastrocnemius* increases from ~120% MVC to ~225% MVC during braking phase and from ~50% MVC to ~150% MVC during push-off phase (KYROLAINEN et al., 2005). Moreover, SLONIGER et al. (1997b) reported that, on level-ground running at 115% of maximum oxygen consumption, the percentage of muscle volume activated was 53% for *vastii* and 68% for *gastrocnemius*. When slope was increased to 10% and speed reduced so that exercise intensity was kept constant, the volume activated increased to 76% for both muscles. There was also a small (roughly 5%) yet significant

increase in the intensity of muscle activation (i.e., force produced per unit volume) for the *vastus* during inclined running.

Table 7 - Results of the multiple regressions fitted for data of Experiment 2 as a function of speed u and slope θ (speed-and-slope models).

m	y	a (se)	b (se)	c (se)	p_a	p_b	p_c	F(2,13)	R^2	p
VM	st	1.159 (0.010)	-0.164 (0.012)	-0.003 (0.001)	<0.01	<0.01	<0.01	122.65	0.94	<0.01
	sd	1.778 (0.308)	-0.862 (0.364)	-0.053 (0.021)	<0.01	0.03	0.02	7.31	0.46	<0.01
	cv	1.624 (0.293)	-0.704 (0.347)	-0.050 (0.020)	<0.01	0.06	0.03	6.27	0.41	0.01
	rmssd	2.045 (0.315)	-1.121 (0.373)	-0.058 (0.021)	<0.01	0.01	0.02	9.89	0.54	<0.01
GL	st	1.158 (0.008)	-0.163 (0.009)	-0.003 (0.001)	<0.01	<0.01	<0.01	191.25	0.96	<0.01
	sd	1.376 (0.194)	-0.514 (0.230)	-0.010 (0.013)	<0.01	0.04	0.47	3.15	0.22	0.08
	cv	1.219 (0.190)	-0.351 (0.225)	-0.007 (0.013)	<0.01	0.14	0.62	1.52	0.07	0.25
	rmssd	1.911 (0.199)	-1.026 (0.235)	-0.012 (0.014)	<0.01	<0.01	0.41	10.84	0.57	<0.01

m = muscle; VM = *vastus medialis*; GL = *gastrocnemius lateralis*; y = dependent variable; st = stride time; sd = standard deviation of intervals between successive contractions; cv = coefficient of variation of successive contractions; rmssd = quadratic mean of intervals between successive contractions; a, b and c = parameters estimated; se = standard error; p_a , p_b and p_c = p-values for each parameter; F(degrees of freedom) = F-statistic for indicated degrees of freedom; R^2 = adjusted R^2 ; p = p-value for the model.

5.1.2 Gradients and interpolated values

While multiple regressions provided an overview of the behavior of a given estimator throughout the sixteen target runs, the surfaces created with interpolated values (Figs. 9 [VM] and 10 [GL]) and the vectorial fields results from the gradients (Figs. 11 [VM] and 12 [GL]) allow higher detailing of the behavior of the estimators within the range of the speeds and slopes covered.

5.1.2.1 st

The surface plots of interpolated values and gradients show that the behavior of st as a function of speed was not entirely linear. Little change is observed at speeds above 0.85 U and the magnitude of variations is much greater at lower speeds. Nonetheless, the absolute variations of this variable are quite small: for instance, the decrease in st_{VM} when speed increased from 0.7 U to 0.8 U was of 16.8 ms (a reduction of 0.0327 Hz in stride frequency) while the decrease from 0.9 U to 1 U was of 9.3 ms (a reduction of 0.0193 Hz in stride frequency). Thus, it is unlikely that the changes in the magnitude of the effects of speed have any physiological significance, at least for the range of speeds that would be naturally adopted by runners. In addition, R^2 values of speed-and-slope models ($R^2 = 0.94$ for VM and $R^2 = 0.96$ for GL), indicate that linear regressions explain most of the variations in this variable across the range of speeds and slopes used in this study.

5.1.2.2 Variability estimators

The surfaces of sd, cv, and rmssd are quite similar within each muscle and between muscles. In general terms, results of surfaces are in accordance with the hypothesis that variability is inversely proportional to muscle strength in relation to its maximum capacity: Variability of both muscles is higher at [low θ , low u] combinations and decreases at intermediate speeds and slopes, attaining their minimum values at [high θ , high u] combinations.

In spite of the similarities in the surfaces of VM and GL, the maximum values of variability estimators are higher for VM (about 1.5 normalized units) than it is for GL (about 1.1 normalized units), which is also in agreement with the results of KYROLAINEN et al. (2005) and SLONIGER et al. (1997b) regarding muscle activity.

Based on data from KYROLAINEN and colleagues (2005) we expected the difference in variability observed at [low θ , low u] to remain at higher speeds (and perhaps higher powers?), however, this was not the case in the current study. Instead, our results agree with those reported by SLONIGER et al. (1997b), in which, as power increases, the difference in activation between muscles diminishes and both lose variability. At [high θ , high u] estimators of both muscles decrease to about 60% of the values measured at [$\theta = 0\%$, $u = 1$ U].

It is interesting to note that although several of the linear models were significant, surfaces and gradients suggest that the behavior of estimators is not linear across the range speeds and slopes used in this experiment. At [low θ , high u] and at [high θ , low u], there is a slight increase in variability in comparison to the intermediate slopes and speeds. Furthermore, the responsiveness to changes in θ and u vary along the combinations: At low to intermediate slopes (roughly $\theta < 1\%$ for VM and $\theta < 2.5\%$ for GL) both muscles are most responsive to changes in speed, displaying a somewhat U-shaped pattern with minimum variability at $u = 0.9$ U. At slopes steeper than 3%, further changes in speed and slope result in a minimum variation of estimators.

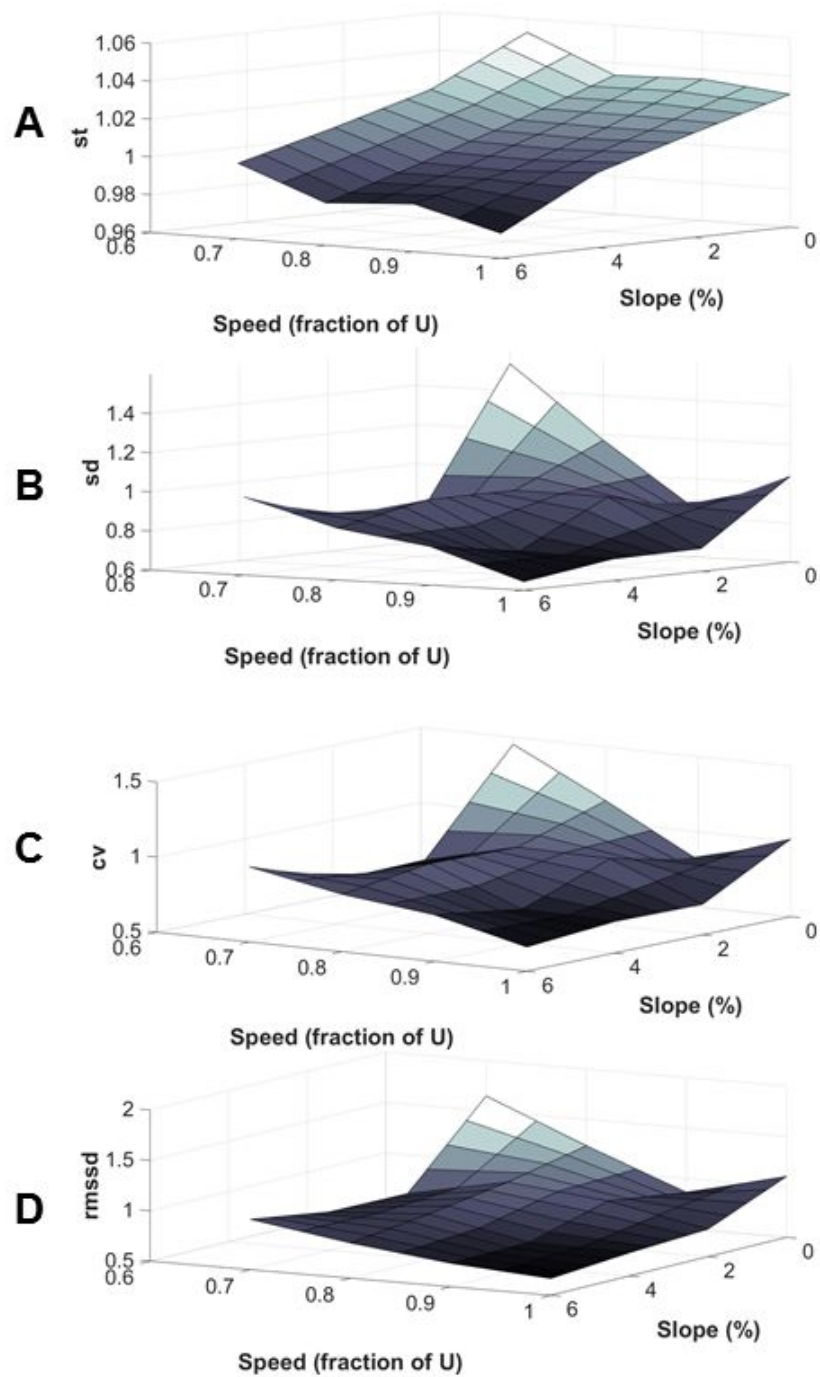


Figure 9 - Surfaces of st and variability estimators of *vastus medialis* for target runs of Experiment 2 ($n = 6$) relative to run [$\theta = 0\%$, $U = 1$ u]. Data were interpolated in order to increase resolution. U = speed at which, at level running, volunteer's heart rate attained 86% of their maximum heart rate. A = stride time (st); B = standard deviation of intervals between successive contractions (sd); C = coefficient of variation of successive contractions (cv); D = quadratic mean of intervals between successive contractions ($rmssd$). Darker shades of blue indicate lower values of the estimator.

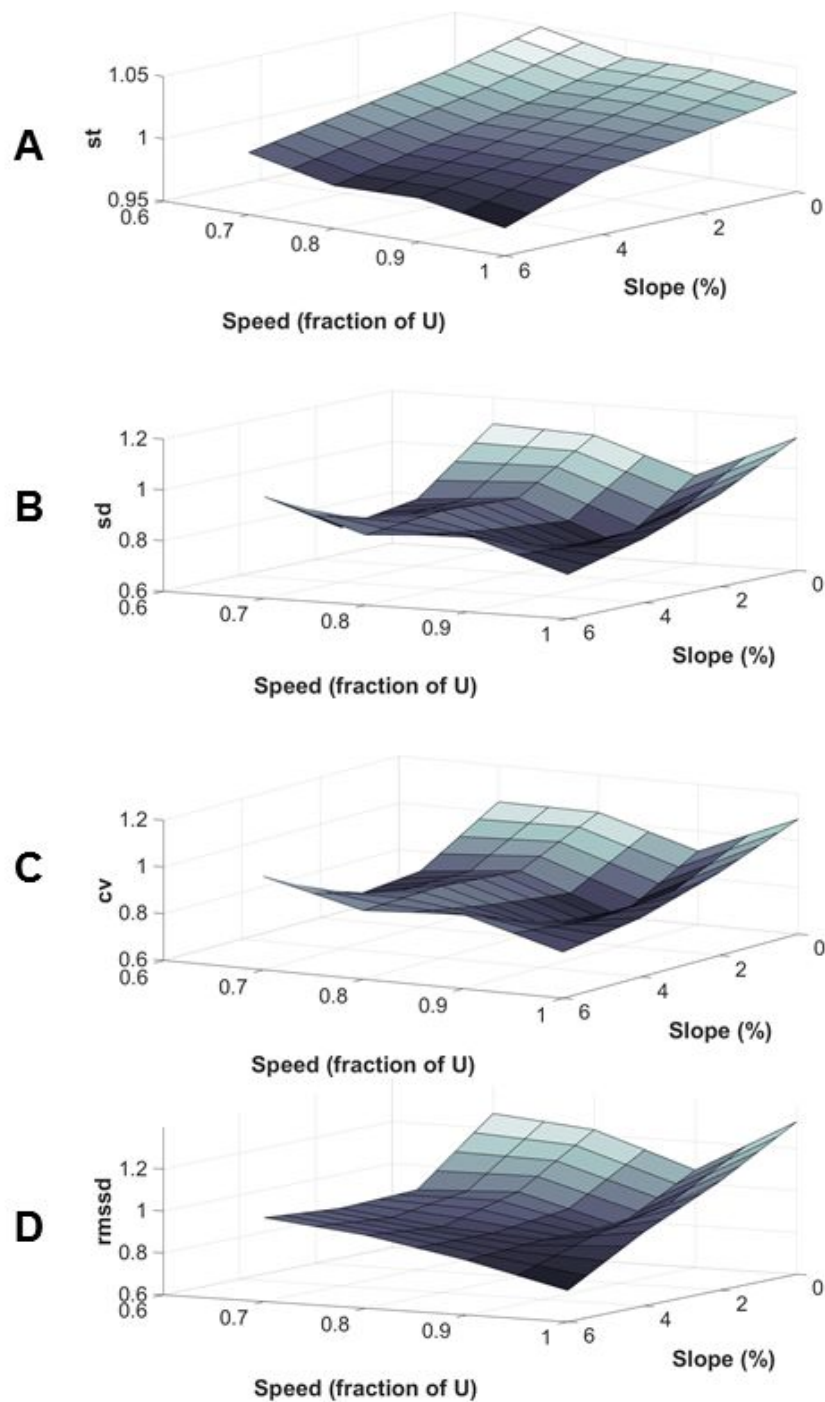


Figure 10 - Surfaces of st and variability estimators of *gastrocnemius lateralis* for target runs of Experiment 2 ($n = 6$) relative to run [$\theta = 0\%$, $U = 1$ u]. Data were interpolated in order to increase resolution. U = speed at which, at level running, volunteer's heart rate attained 86% of their maximum heart rate. A = stride time (st); B = standard deviation of intervals between successive contractions (sd); C = coefficient of variation of successive contractions (cv); D = quadratic mean of intervals between successive contractions (rmsd). Darker shades of blue indicate lower values of the estimator.

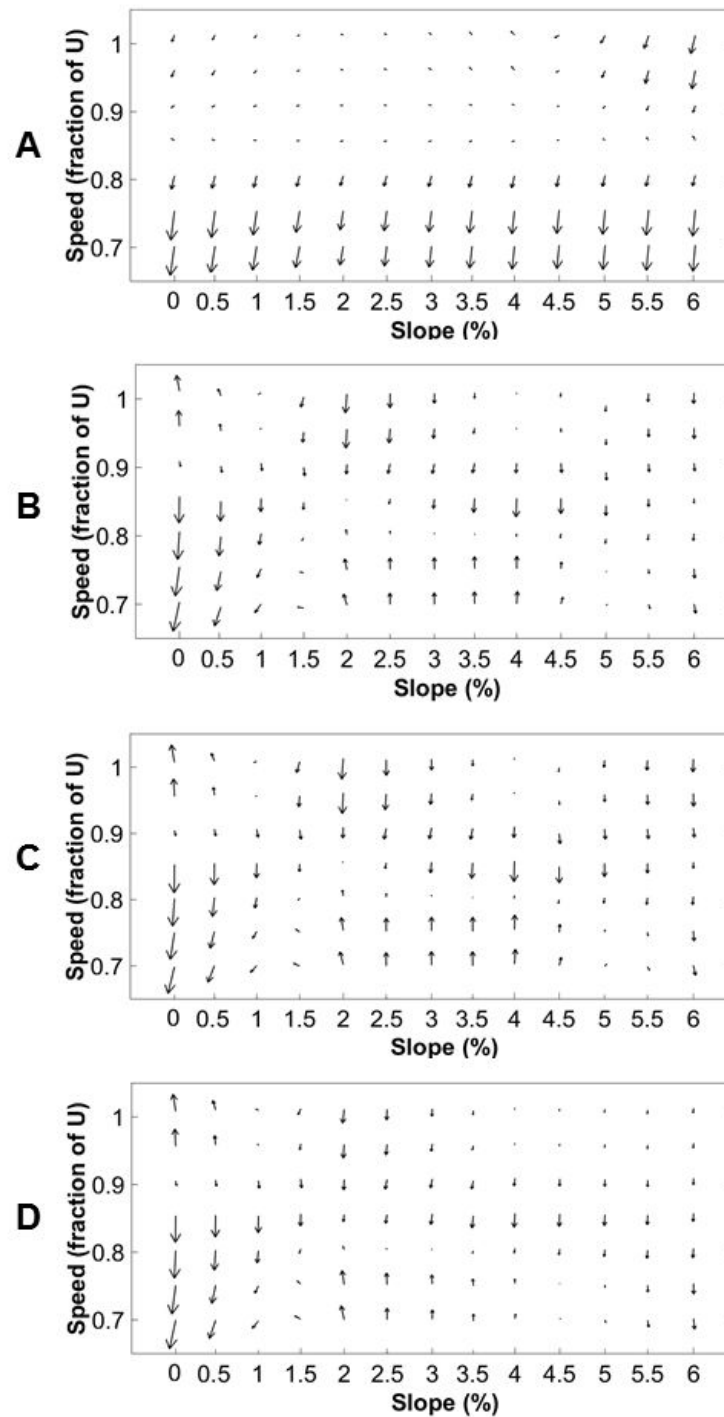


Figure 11 - Gradients of st and variability estimators of *vastus medialis* for target runs of Experiment 2 ($n = 6$) relative to run $[\theta = 0\%, U = 1 \text{ u}]$. U = speed at which, at level running, volunteer's heart rate attained 86% of their maximum heart rate. A = stride time (st); B = standard deviation of intervals between successive contractions (sd); C = coefficient of variation of successive contractions (cv); D = quadratic mean of intervals between successive contractions (rmssd).

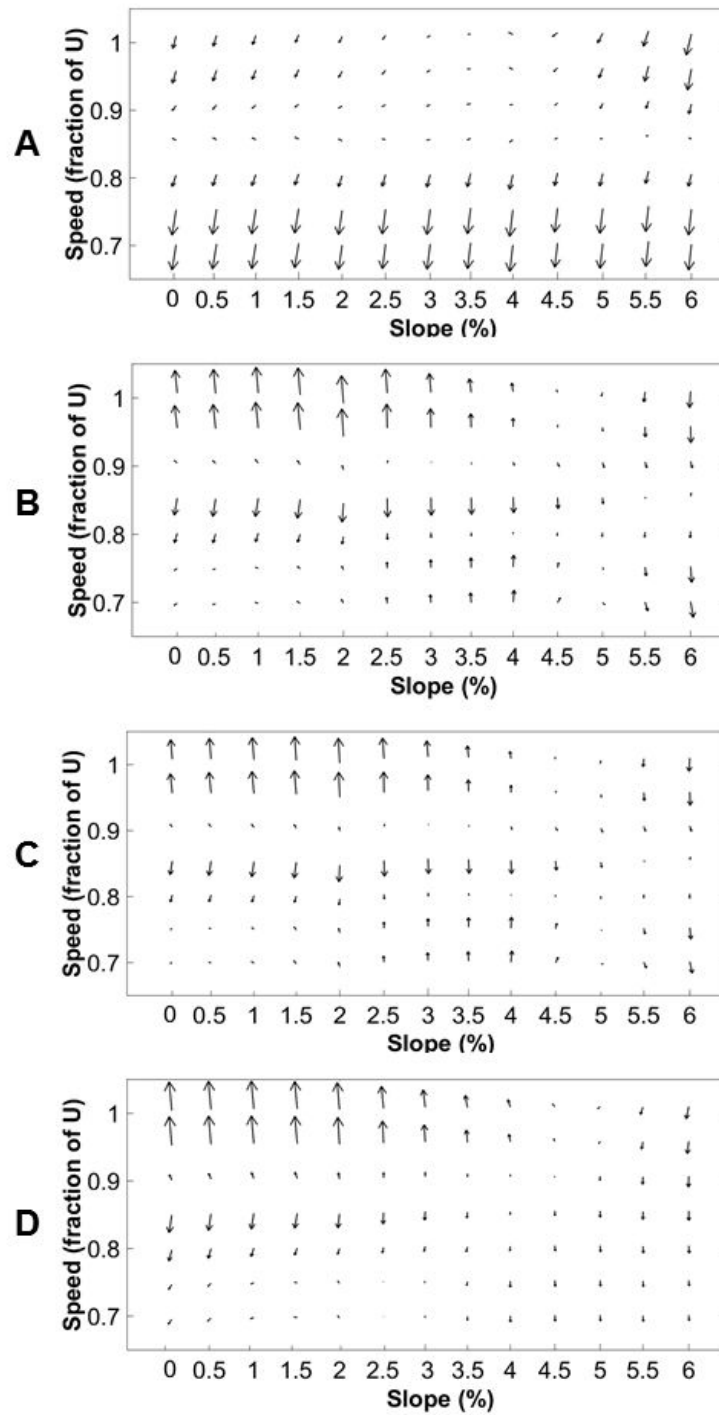


Figure 12 - Gradients of st and variability estimators of *gastrocnemius lateralis* for target runs of Experiment 2 ($n = 6$) relative to run $[\theta = 0\%, U = 1 \text{ u}]$. U = speed at which, at level running, volunteer's heart rate attained 86% of their maximum heart rate. A = stride time (st); B = standard deviation of intervals between successive contractions (sd); C = coefficient of variation of successive contractions (cv); D = quadratic mean of intervals between successive contractions (rmsd).

5.1.3 In summary

While previous studies have reported that slight slopes such as the ones used in this study were not sufficient to elicit significant changes in stride frequency (SNYDER & FARLEY, 2011) and muscle activity (YOKOZAWA et al., 2007), in our experiment, stride time as well as muscle contraction variability correlated negatively to both speed and interaction speed-slope: for VM, an effect was observed for speed as well as the interaction speed-slope while the variability of GL was sensitive only to changes in speed (the difference between the two muscles was not significant though).

Moreover, our data suggest that muscle variability is inversely proportional to strength in relation to its maximum capacity. This hypothesis is compatible with reports of muscle activation in the literature and with the hypothesis that loss of variability in subtasks is an indicator of increased constraints to the system: as muscles are required to produce more force, their flexibility to assume alternative states is diminished and, maybe because of that, so is their capacity to respond to further changes.

Lastly, although speed-and-slope models were often statistically significant, the behavior of the variability estimators is not linear across speeds and slopes. At low speed and slow slope combinations, estimators are most responsive to changes in speed while at higher powers further variations in θ and u have little impact on variability.

5.2 QUESTION 2

5.2.1 Linear regressions (HR models)

Similarly to the procedure used in the previous question, we used linear regressions to verify whether estimators and st correlated to HR. The results are presented in Table 8 and graphically in Figs. 13 (VM) and 14 (GL): st and all

variability estimators of VM and GL correlated negatively with HR, although such tendency did not reach statistical significance for cv_{GL} ($p = 0.09$).

Table 8 - Results of the multiple regressions fitted for data of Experiment 2 as a function of HR (HR models).

m	y	α (se)	β (se)	p_α	p_β	F(1,14)	R^2	p
VM	st	1.203 (0.026)	-0.194 (0.026)	<0.01	<0.01	55.19	0.78	<0.01
	sd	2.480 (0.400)	-1.580 (0.401)	<0.01	<0.01	15.56	0.49	<0.01
	cv	2.279 (0.381)	-1.390 (0.381)	<0.01	<0.01	13.27	0.45	<0.01
	rmssd	2.816 (0.410)	-1.885 (0.411)	<0.01	<0.01	21.08	0.57	<0.01
GL	st	1.204 (0.024)	-0.194 (0.024)	<0.01	<0.01	63.53	0.80	<0.01
	sd	1.555 (0.254)	-0.645 (0.254)	<0.01	0.02	6.44	0.27	0.02
	cv	1.353 (0.246)	-0.452 (0.246)	<0.01	0.09	3.37	0.14	0.09
	rmssd	2.121 (0.296)	-1.119 (0.297)	<0.01	<0.01	14.23	0.47	<0.01

m = muscle; VM = *vastus medialis*; GL = *gastrocnemius lateralis*; y = dependent variable; st = stride time; sd = standard deviation of intervals between successive contractions; cv = coefficient of variation of successive contractions; rmssd = quadratic mean of intervals between successive contractions; α and β = parameters estimated; se = standard error; p_α and p_β = p-values for each parameter; F(degrees of freedom) = F-statistic for indicated degrees of freedom; R^2 = adjusted R^2 ; p = p-value for the model.

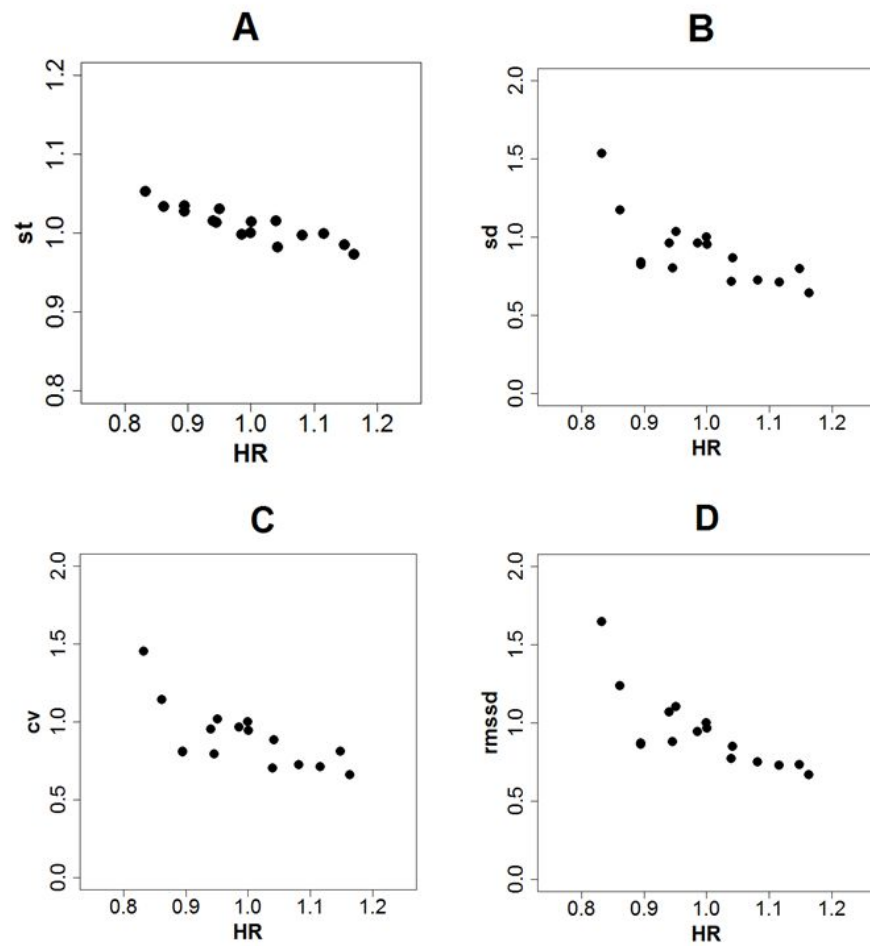


Figure 13 - Mean values of st and variability estimators of *vastus medialis* versus heart rate for target runs of Experiment 2 ($n = 6$) relative to run $[\theta = 0\%, u = 1 \text{ U}]$. HR = heart rate relative to $[\theta = 0\%, u = 1 \text{ U}]$. A = stride time (st); B = standard deviation of intervals between successive contractions (sd); C = coefficient of variation of successive contractions (cv); D = quadratic mean of intervals between successive contractions (rmssd). Each circle corresponds to the population average for one of the sixteen combinations $[\theta, u]$.

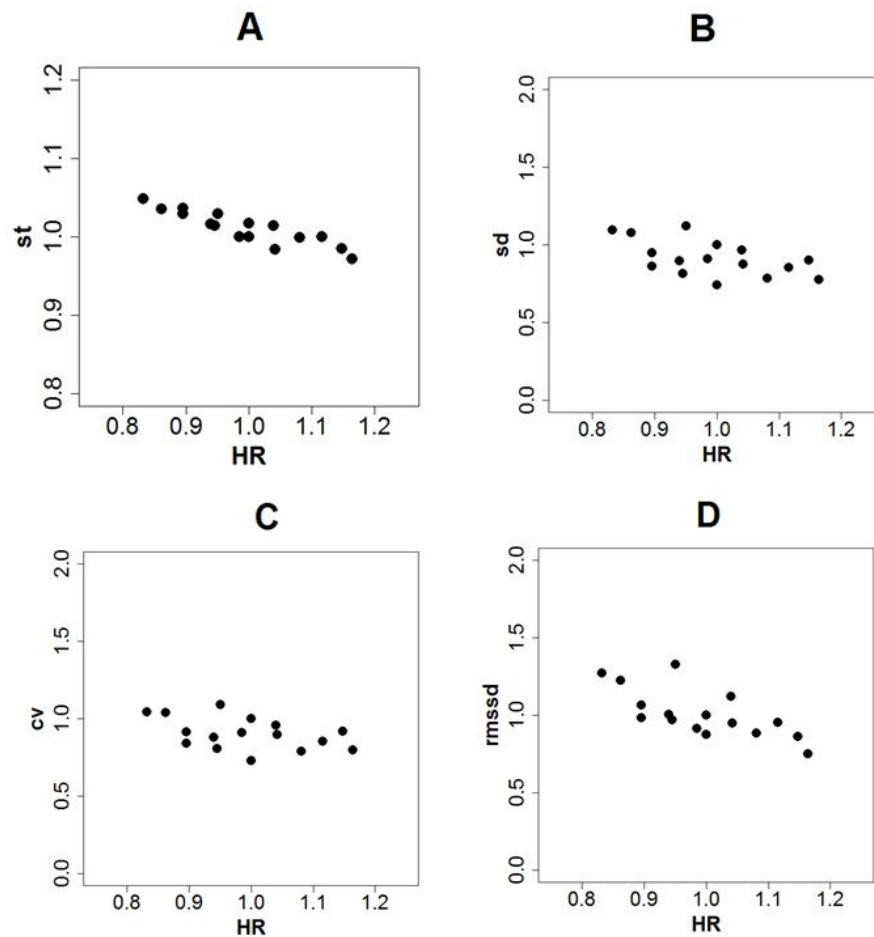


Figure 14 - Mean values of st and variability estimators of *gastrocnemius lateralis* versus heart rate for target runs of Experiment 2 ($n = 6$) relative to run [$\theta = 0\%$, $u = 1$ U]. HR = heart rate relative to [$\theta = 0\%$, $u = 1$ U]. A = stride time (st); B = standard deviation of intervals between successive contractions (sd); C = coefficient of variation of successive contractions (cv); D = quadratic mean of intervals between successive contractions (rmssd). Each circle corresponds to the population average for one of the sixteen combinations [θ, u].

As expected given the results of the speed-and-slope models of Question 1, regressions initially suggest that GL starts from lower levels of variability and is less responsive to changes in power than VL. However, once again, differences between muscles were not significant except for cv (st: $p_\alpha = 0.98$, $p_\beta = 1$; sd: $p_\alpha = 0.06$, $p_\beta = 0.06$; cv: $p_\alpha = 0.05$, $p_\beta = 0.05$; rmssd: $p_\alpha = 0.18$, $p_\beta = 0.14$). Since there is a strong correlation between speed and slope with power output, it is not surprising that the tendencies observed in Question 1 when comparing muscles would also be present

in correlations with HR. As previously suggested, these differences could arise from differences in muscle activation and force produced in relation to MVC.

5.2.2 Speed and slope or metabolic rate?

With the exception of stride time, whose dependency on slope and speed has been widely described in the literature (see Introduction) and cv_{GL} , the remaining variability estimators could be equally well predicted by speed-and-slope models and HR models for both GL ($p[st] < 0.01$, $p[sd] = 0.65$, $p[rmssd] = 0.06$) and VM ($p[st] < 0.01$, $p[sd] = 0.78$, $p[cv] = 0.75$, $p[rmssd] = 0.77$). Since cv_{GL} did not correlate to speed and slope nor to HR, there was no reason to compare models for this variable as the neutral model is statistically an equally good option.

For most estimators, this implicates that the same value may be attained through different combinations of speed and slope. Or, in an inversion of the reasoning, we can anticipate that energy expenditure would be a function of speed and slope with level lines representing similar demands due to different combinations of u and θ . This is a result confirmed elsewhere (ELDASH, 2019).

It is important to mention that the volume of muscle activated is higher during hill running than during level running at the same oxygen consumption (SLONIGER et al., 1997a,b). Furthermore, GOSTILL et al. (1974) have shown there are also differences in the rate of glycogen utilization by muscles: glycogen depletion of *vastus lateralis*, *gastrocnemius*, and *soleus* muscles were 3.2, 1.39 and 1.36 higher (respectively) after a 2 h run at 6% than after a 2 h level run at the intensity (75% of maximum oxygen consumption). These results suggest that at inclined running the contribution of anaerobic system to energy production is increased. As a consequence HR may be an underestimation of the actual metabolic demand. Nonetheless, as far as our results allow for deduction, for the range of slopes used in this study, HR is still a good approximation to energy consumption.

In summary, our results suggest that variability is inversely proportional to the force produced by muscles, in relation to their maximum capacity, in a way that the

higher the %MVC, the lower the variability between contractions. Hence, different combinations of speed and slope can lead to the same values of variability as long as power output remains constant.

5.3 QUESTION 3

By looking at the average values of age (Experiment 1 = 41.3 ± 11.1 years old and Experiment 2 = 32.2 ± 8.9 years old) and speed U (Experiment 1 = 12.1 ± 2.2 km/h and Experiment 2 = 11.9 ± 0.8 km/h) of all subjects that engaged in the present study, one would think that these populations were not different. However, unlike Experiment 2, Experiment 1 imposed no restrictions regarding age and fitness so that the range of these variables was much wider (age = 25 - 56 years old, U = 10 - 16 km/h) in comparison to those of Experiment 2 (age = 22 - 42 years old, U = 11 - 13 km/h). Thus, our aim in this question was to verify if, in spite of the effects that age (CHRISTOU, 2011; SILVERNAIL et al., 2015; BOYER et al., 2016; HERSSENS et al., 2018) and fitness level (FUJII et al., 2009; NAKAYAMA et al., 2010; MO & CHOW, 2018) may have on gait variability, the patterns reported in Questions 1 and 2 could also be observed in a more heterogeneous group of runners.

Table 9 shows the results of the t-test comparing observed values with those estimated by the models of Questions 1 and 2. The same data is plotted in Figs. 15 (VL) and 16 (GL) for speed-and-slope models and Figs. 17 (VL) and 18 (GL) for HR models as the ratio between estimated and observed values. Only models that succeeded in explaining variation in estimators of Experiment 2 were used to estimate variables for Experiment 1. To further comprehend the behavior of the variables in Experiment 1, we used the same procedure of Questions 1 and 2 to create regressions of estimators as a function of speed and the interaction speed-slope and estimators as a function of HR to data of Experiment 1. The parameters of these models are shown in Tables 10 and 11 respectively.

Table 9 - t-tests results for comparisons between predicted and observed values of st and estimators for Experiment 1.

m	Model	y	t	p
VL	Speed-and-slope	st	0.185	0.858
		sd	-3.563	0.007
		cv	-3.546	0.008
		rmssd	-2.721	0.026
	HR	st	6.754	0.000
		sd	-2.928	0.019
		cv	-3.086	0.015
		rmssd	-1.970	0.084
GL	Speed-and-slope	st	0.696	0.506
		rmssd	-2.437	0.041
	HR	st	5.325	0.001
		sd	-4.560	0.002
		rmssd	0.351	0.735

Predicted values were estimated using significant speed-and-slope models and HR models. m = muscle; VL = *vastus lateralis*; GL = *gastrocnemius lateralis*; y = dependent variable; st = stride time; sd = standard deviation of intervals between successive contractions; cv = coefficient of variation of successive contractions; rmssd = quadratic mean of intervals between successive contraction; t = t-statistic (8 degrees of freedom).

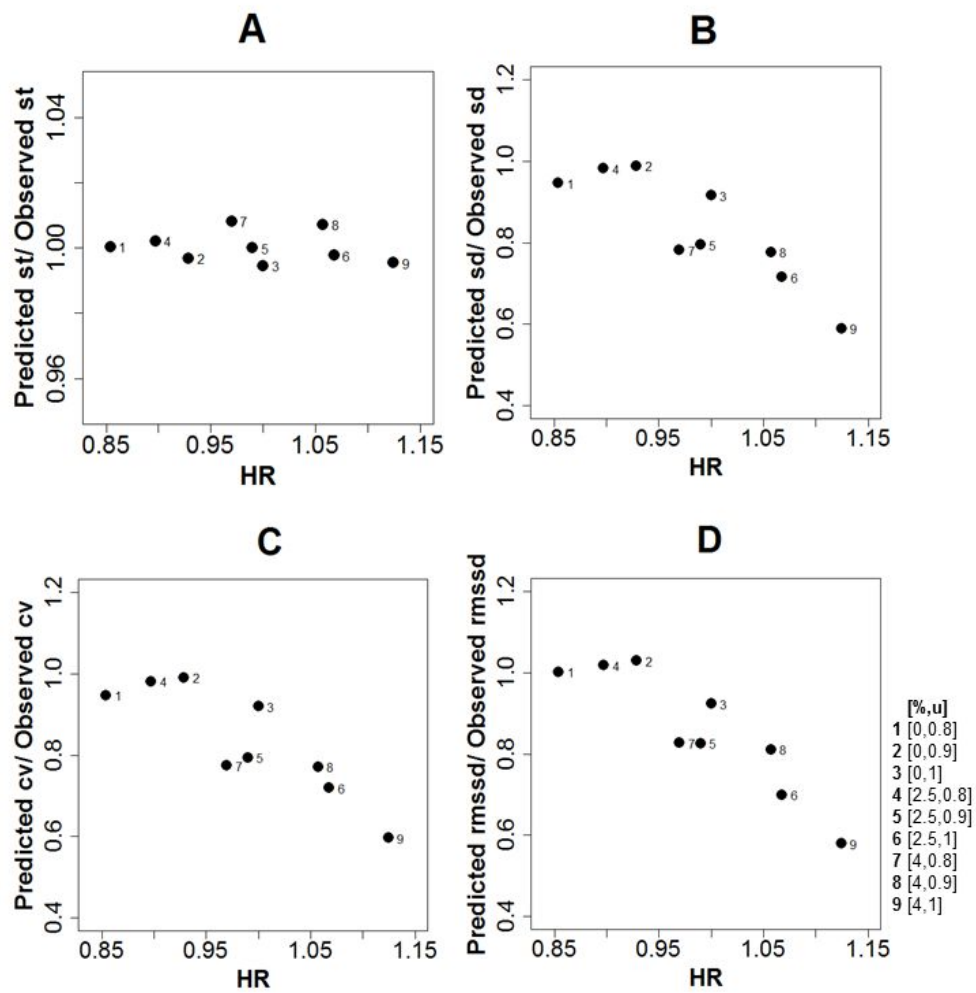


Figure 15 - Ratio between values estimated using the speed-and-slope models and values observed of st and variability estimators of *vastus lateralis* as a function of heart rate for target runs of Experiment 1 ($n = 11$). HR = heart rate relative to run [$\theta = 0\%$, $u = 1U$]. A = stride time (st); B = standard deviation of intervals between successive contractions (sd); C = coefficient of variation of successive contractions (cv); D = quadratic mean of intervals between successive contractions (rmssd).

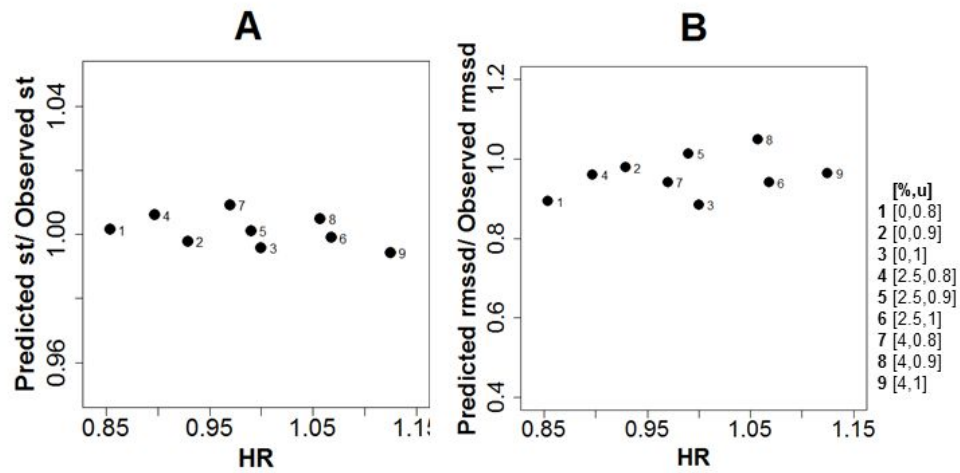


Figure 16 - Ratio between values estimated using the speed-and-slope models and values observed of st and variability estimators of *gastrocnemius lateralis* as a function of heart rate for target runs of Experiment 1 ($n = 11$). Predicted values were only estimated for the variables whose models were significant ($p < 0.05$). HR = heart rate relative to run [$\theta = 0\%$, $u = 1U$]. A = stride time (st), B = quadratic mean of intervals between successive contractions (rmssd).

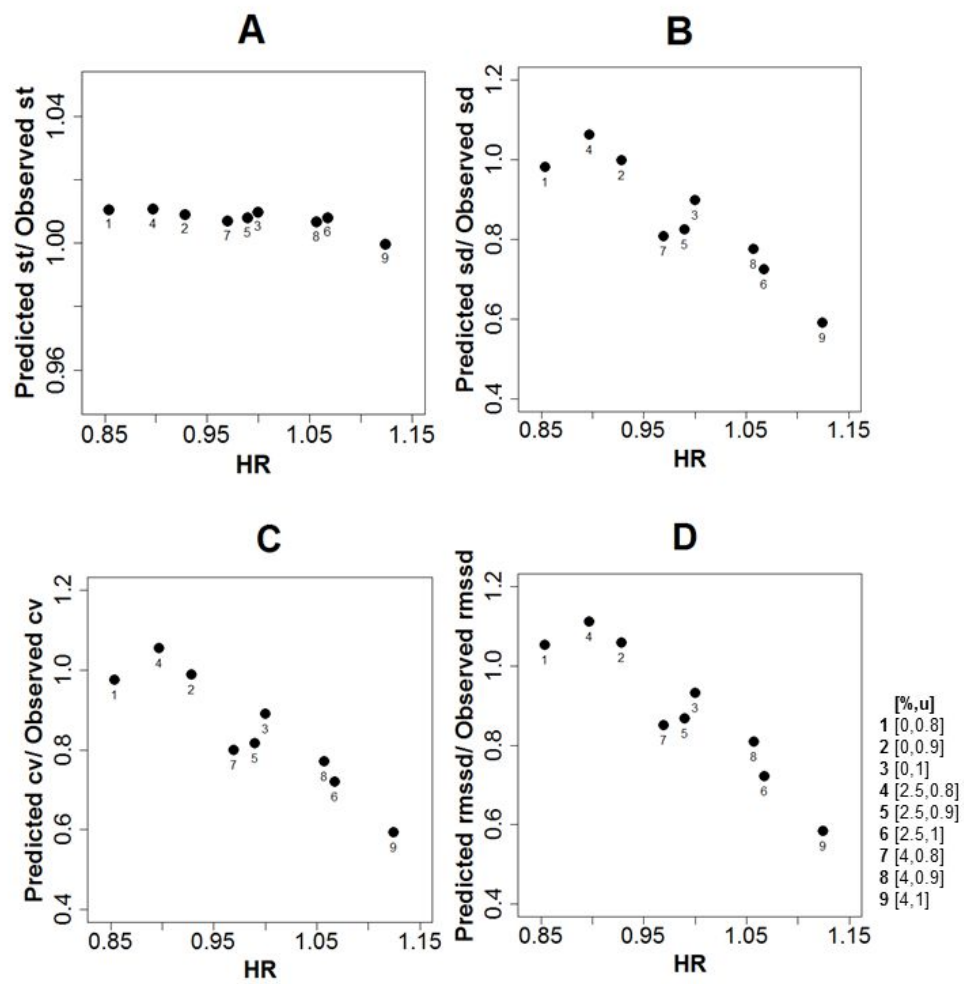


Figure 17 - Ratio between values estimated using the HR models and values observed of st and variability estimators of *vastus lateralis* as a function of heart rate for target runs of Experiment 1 ($n = 11$). HR = heart rate relative to $[\theta = 0\%, u = 1U]$. A = stride time (st); B = standard deviation of intervals between successive contractions (sd); C = coefficient of variation of successive contractions (cv); D = quadratic mean of intervals between successive contractions (rmssd).

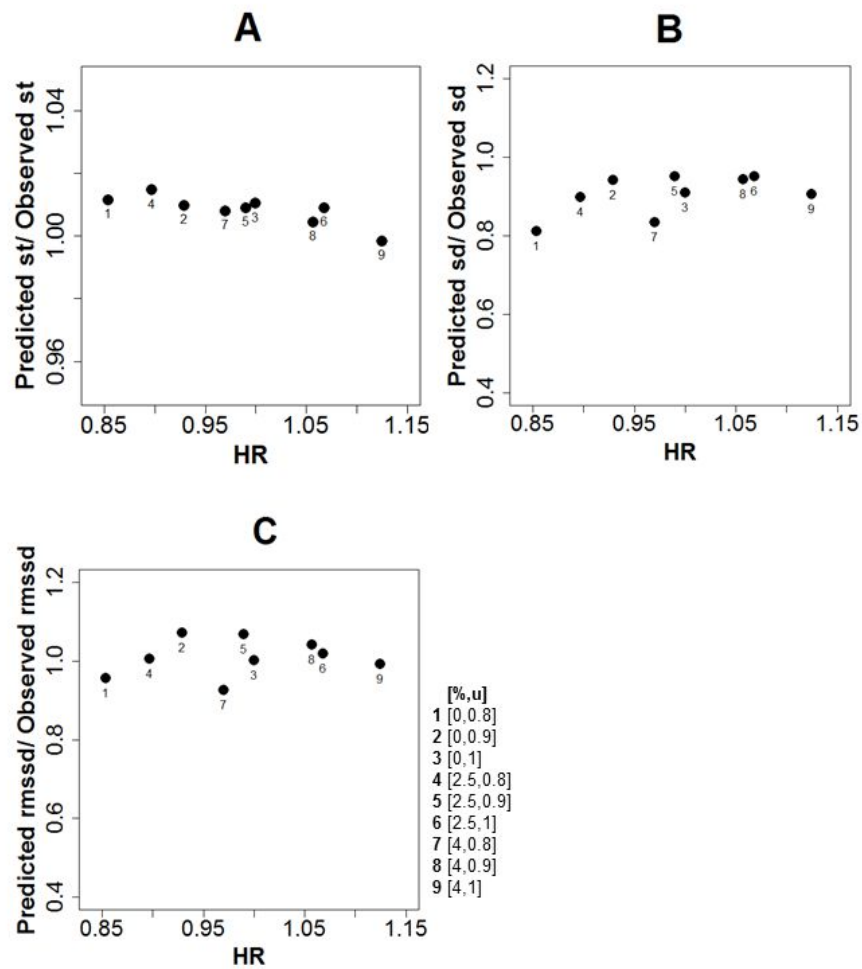


Figure 18 - Ratio between values estimated using the HR models and values observed of st and variability estimators of *gastrocnemius lateralis* as a function of heart rate (HR) for target runs of Experiment 1 ($n = 11$). Predicted values were only estimated for the variables whose models were significant ($p < 0.05$). HR = heart rate relative to run [$\theta = 0\%$, $u = 1U$]. A = stride time (st); B = standard deviation of intervals between successive contractions (sd); C = quadratic mean of intervals between successive contractions (rmssd).

Table 10 - Results of the multiple regressions fitted for data of Experiment 1 as a function of speed u and slope θ .

m	y	a (se)	b (se)	c (se)	p_a	p_b	p_c	F(2,6)	R^2	p
VL	st	1.124 (0.010)	-0.122 (0.011)	-0.005 (0.001)	<0.01	<0.01	<0.01	100.87	0.96	<0.01
	sd	1.146 (0.281)	-0.115 (0.313)	0.022 (0.017)	0.01	0.73	0.24	0.89	-0.03	0.46
	cv	1.008 (0.277)	0.021 (0.309)	0.028 (0.017)	0.01	0.95	0.15	1.42	0.10	0.31
	rmssd	1.109 (0.285)	-0.075 (0.317)	0.021 (0.017)	0.01	0.82	0.26	0.76	-0.06	0.51
GL	st	1.116 (0.009)	-0.114 (0.010)	-0.005 (0.001)	<0.01	<0.01	<0.01	103.47	0.96	<0.01
	sd	1.938 (0.200)	-0.960 (0.223)	-0.028 (0.012)	<0.01	0.01	0.06	13.36	0.76	0.01
	cv	1.818 (0.194)	-0.843 (0.216)	-0.024 (0.012)	<0.01	0.01	0.09	10.67	0.71	0.01
	rmssd	2.013 (0.190)	-1.045 (0.012)	-0.032 (0.012)	<0.01	<0.01	0.03	17.89	0.81	<0.01

m = muscle; VL = *vastus lateralis*; GL = *gastrocnemius lateralis*; y = dependent variable; st = stride time; sd = standard deviation of intervals between successive contractions; cv = coefficient of variation of successive contractions; rmssd = quadratic mean of intervals between successive contractions; a, b and c are the parameters estimated; se = standard error; p_a , p_b and p_c = p-values for each parameter; F(degrees of freedom) = F-statistic for indicated degrees of freedom; R^2 = adjusted R^2 ; p = p-value for the model.

Table 11 - Results of the multiple regressions fitted for data of Experiment 1 as a function of HR.

m	y	α (se)	β (se)	p_α	p_β	F(1,7)	R ²	p
VM	st	1.164 (0.009)	-0.161 (0.009)	<0.01	<0.01	353.62	0.98	<0.01
	sd	0.877 (0.315)	0.211 (0.318)	0.03	0.53	0.44	-0.08	0.53
	cv	0.694 (0.309)	0.392 (0.311)	0.06	0.25	1.58	0.07	0.25
	rmssd	0.872 (0.313)	0.213 (0.316)	0.03	0.52	0.45	-0.07	0.52
GL	st	1.151 (0.011)	-0.148 (0.011)	<0.01	<0.01	168.81	0.95	<0.01
	sd	2.113 (0.230)	-1.107 (0.232)	<0.01	<0.01	22.77	0.73	<0.01
	cv	1.955 (0.222)	-0.953 (0.224)	<0.01	<0.01	18.08	0.68	<0.01
	rmssd	2.228 (0.214)	-1.233 (0.216)	<0.01	<0.01	32.47	0.80	<0.01

m = muscle; VL = *vastus lateralis*; GL = *gastrocnemius lateralis*; y = dependent variable; st = stride time; sd = standard deviation of intervals between successive contractions; cv = coefficient of variation of successive contractions; rmssd = quadratic mean of intervals between successive contractions; α and β are the parameters estimated; se = standard error; p_α and p_β = p-values for each parameter; F(degrees of freedom) = F-statistic for indicated degrees of freedom; R² = adjusted R²; p = p-value for the model.

5.3.1 st

Despite the fact that both speed-and-slope and HR models were significant for data of Experiment 2, st is better described as a function of speed and the interaction

speed-slope $p < 0.01$). This model was also more adequate to predict data for subjects of Experiment 1 (Table 9, $p = 0.858$ for VM and $p = 0.506$ for GL).

In this experiment, the correlation between absolute values of stride time (at $[\theta = 0\%, u = 1 \text{ U}]$) and age was not significant (st_{VL} : $R^2 = 0.12$, $p = 0.158$; st_{GL} : $R^2 = 0.12$, $p = 0.156$), but st correlated positively with speed U (st_{VL} : $R^2 = 0.30$, $p = 0.046$; st_{GL} : $R^2 = 0.30$, $p = 0.045$). Our results suggest that regardless of the effects of speed U , runners of different ages and fitness levels show a similar response to changes in speed and slope and in HR.

5.3.2 sd and cv

Contrary to our hypothesis, this was not the case for sd and cv . Neither speed-and-slope nor HR models were able to adequately predict values of sd and cv for VL and GL.

At first, one would be tempted to think that the models of GL failed to predict sd and cv of Experiment 1 due to increased noise in data caused by variations of age and fitness level among subjects. However, when we applied the same procedure done in Questions 1 and 2 to estimate the parameters of speed-and-slope and HR models from data of Experiment 1, we observed (to our surprise), that all models were significant (Tables 10 and 11).

Even though the differences did not reach statistical significance, the results of Experiment 2 indicate that the magnitude of the response of sd and cv to changes in speed and intensity of exercise is somewhat low in GL, at least when compared to VM. Also, t -tests confirmed that there were no significant differences (all $p = 1$) in residuals between VM and GL for any of the models. As a consequence, the effects of manipulations in u , θ and energy expenditure would be more difficult to identify in GL than in VM. Thus, it is possible that the six runners sampled in Experiment 2 were not sufficient to adequately estimate the parameters of the models that would describe the entire population. This hypothesis offers an explanation to why the

models based on data from Experiment 2 failed to explain data of Experiment 1 and why models created from Experiment 1 ($n = 11$) are overall more significant.

On the other hand, for VM this does not seem to be the case. The magnitudes of changes in sd_{VM} and cv_{VM} were greater for VM than for GL in Experiment 2 and the p-values of models and of parameters were considerably lower. Moreover, none of the models fitted for data of sd and cv of Experiment 1 (Tables 10 and 11) were significant, in spite of the fact that the sample size in Experiment 1 is almost twice that of Experiment 2.

As evidenced by the values of ratio < 1 in Figs. 15 and 17, models have a tendency to overestimate variability, especially at higher exercise intensities. One possible explanation for this is the fact that data of Experiment 1 is from VL contractions but, due to technical difficulties, we changed to VM in Experiment 2: Even though both muscles belong to the same group and have similar functions during running, there is evidence suggesting that the ratio between activity of VM and VL may change in response to variations of speed and/or slope (SANTOS et al., 2007; ZUNIGA & MALEK, 2013; JENSEN et al., 2015), although for most of these studies, differences between muscles did not reach significance.

The exception is the study of ZUNIGA & MALEK (2013). While the authors did not detect a significant difference between *vasti* activity in speeds ranging from 4.8 to 12.9 km/h, the ratio VM/VL was higher than 1 for all six speeds. Moreover, there is a small but highly significant ($p < 0.01$, $R^2 = 0.919$) positive correlation between the ratio VM/VL and speed. Yet, in a very similar experiment (CAMIC et al., 2015) no correlation between VM/VL and exercise intensity was detected ($p = 0.912$).

5.3.3 rmssd

At first glance, t-tests indicate that HR models of Experiment 2 succeeded in estimating the values of rmssd of Experiment 1. However, a closer look suggests that this is an artifact: the model fitted for data of Experiment 1 was not significant ($p = 0.523$) from which we conclude that there is no correlation between these variables.

Moreover, similarly to sd and cv , Figs. 15 and 17 show that the HR model overestimates values of $rmssd$ for data of Experiment 1 as power increases (ratio estimated/observed $\propto -1.86 \times HR$, $p < 0.01$), reaching a ratio as low as 0.58 in the run [$\theta = 4\%$, $u = 1 U$]. Models of speed-and-slope also failed to predict data.

The results for $rmssd_{GL}$ were more coherent: t-test indicates that HR model adequately predicted values for Experiment 1 and, unlike $rmssd_{VL}$, the parameters of the model based on Experiment 2 ($\alpha = 2.1205$, $\beta = -1.1185$) and those of the model fitted for the observed values ($\alpha = 2.2279$, $\beta = -1.2333$) are quite similar. The fact that the HR but not the sleep-and-slope model was adequate for data of Experiment 1 further supports our hypothesis that contraction variability is a function of power output (i.e., force production) regardless of the combination of speed and slope.

Lastly, even though the subjects who took part in Experiment 1 constituted a more diverse group than those who volunteered for Experiment 2, speed-and-slope and HR models of $rmssd_{GL}$ fitted for data of Experiment 1 had higher power of explanation ($R^2 = 0.809$ and $R^2 = 0.797$, respectively) than the ones fitted for Experiment 2 ($R^2 = 0.567$ and $R^2 = 0.469$). As discussed for sd_{GL} and cv_{GL} , we attribute this to the small sample of Experiment 2.

In summary, due to methodological issues, we were not able to provide a conclusive answer to Question 3. Nonetheless, the combined results of Experiments 1 and 2 indicate that patterns of variation in estimators of variability are similar among a broad group of subjects.

5.3.3.1 $rmssd$ as an estimator

Most studies that use statistical methods to estimate gait variability focus on sd and cv . In this study, we opted to include $rmssd$. All estimators of variability of VM and of GL showed a similar pattern of variation across speeds and slopes and HR in all analyses done, however $rmssd$ had higher correlations to both HR and speed and slope, as evidenced by the adjusted R^2 values in significant models. Moreover, it was the only estimator to demonstrate an effect of slope for GL (Experiment 1), which

would be expected according to our hypothesis that variability is proportional to muscle force. Thus, we suggest that further studies should include this variable among variability estimators.

6 CONCLUSION

1. Variability of contractions of muscles from the quadriceps and plantar flexors groups are inversely proportional to the force produced in relation to their maximum capacity. As a consequence, different combinations of speed and slope could result in the same levels of variability, despite the potential differences in muscle metabolism.
2. The relationship between muscle variability estimators and heart rate is not linear. Estimators are more responsive to changes in speed and slope at smaller inclines. At higher powers, the magnitude of variation of estimators diminishes to the point where further increments in speed or in slope lead to almost no changes in variability.
3. Our results support the hypothesis that while low end-point variability is an indicator of a well-adjusted system, a decrease in variability of the subtasks involved in the execution of the output movement indicates an increase in constraints to the motor system.
4. Unlike variability estimators, intervals between contractions are better explained by the effects of speed and slope rather than exercise intensity. Both variables lead to a small increase in stride frequency.

7 RESUMO

Durante a corrida, uma série de movimentos é realizada de forma cíclica que, em última análise, fazem com que o corpo se desloque. Cada ciclo é dividido em uma fase em que ocorre absorção de trabalho conforme o centro de massa é rebaixado e desacelerado e uma fase em que ocorre produção de trabalho, conforme o centro de massa é impulsionado para cima e para frente. Os músculos do quadríceps e flexores plantares são os principais responsáveis pela absorção e geração de trabalho, respectivamente. Variações na velocidade da corrida e na inclinação do terreno requerem ajustes da força muscular e, como consequência, da frequência cardíaca. Neste contexto, análises de variabilidade oferecem uma maneira de medir a adequação do sistema muscular às variações na demanda. Assim, buscamos investigar a relação entre a variabilidade das contrações de músculos do quadríceps e flexores plantares com alterações na velocidade, inclinação e potência; bem como verificar se os padrões observados em um grupo restrito de indivíduos se aplicavam a uma amostra mais variada. Para isso, coletamos dados de intervalos entre contrações do vasto e gastrocnêmio de dezessete voluntários durante corridas realizadas em diferentes combinações de velocidade e inclinação da esteira. Os sujeitos foram divididos em dois grupos experimentais: no Experimento 1, não houve restrições quanto à idade e condicionamento, enquanto que no Experimento 2 essas variáveis foram controladas. Regressões lineares e gradientes mostraram que, embora os estimadores de variabilidade correlacionem negativamente com velocidade e inclinação, eles são melhor explicados por mudanças no consumo energético (i.e., frequência cardíaca). Assim, concluímos que a variabilidade é inversamente proporcional à força muscular, em relação à sua capacidade máxima. Como consequência, diferentes combinações de velocidade e inclinação podem resultar na mesma variabilidade, apesar das diferenças no perfil metabólico. Devido a complicações metodológicas, não pudemos chegar a uma conclusão definitiva em relação a comparação entre voluntários dos Experimentos 1 e 2, embora os resultados sugiram que corredores respondam a mudanças de velocidade e inclinação de maneira semelhante, independentemente de diferenças de idade e nível de condicionamento.

8 ABSTRACT

Running is achieved by cyclic execution of movements that ultimately allows a person to move. Each cycle is divided into two phases. The first phase is where power is absorbed as the body's center of mass is lowered and decelerated. The second being where power is generated and the center of mass is propelled upwards and forwards. Power absorption is primarily done by quadriceps muscles while plantar flexors generate most of the power during push-off phase. Variations in speed and slope are met by adjustments in the same direction of force produced by lower limb muscles and, due to changes in energy expenditure, of heart rate. In this context, analysis of variability offer a non-invasive way to measure how well-adjusted muscles are to cope with variations in demand. Thus, our aim was to investigate the relationship between the variability of contractions of muscles from the quadriceps and plantar flexors with changes in speed and slope and in heart rate. Secondly, we sought to verify if the patterns observed in a restricted group of subjects could also be noted in a wider population. To answer these questions, we used electromyography to collect data of intervals between successive contractions of *vasti* and *gastrocnemius* muscles of seventeen runners across several target runs. These runs were chosen with several different speeds and slopes. Runners were divided into two experimental groups: Experiment 1 contained no restrictions regarding age and fitness level while Experiment 2 did control for these variables. Linear regressions and gradients showed that even though variability estimators correlated negatively to speed and/or slope, they could be better explained by changes in heart rate. Thus our main conclusion was that variability between contractions is inversely proportional to muscle activation, in relation to its maximum capacity. As a consequence, different combinations of speed and slope could result in the same variability despite differences in metabolic profile, as long as the power output remains constant. Due to methodological issues, we could not reach a decisive conclusion regarding the comparison between the more restricted and more heterogeneous groups of volunteers, although results suggest that the runners respond to changes of speed and slope in a similar way regardless of differences in age and fitness level.

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