

Integrative Biomedical Research School XXX Cycle

DI MILANO

PhD Thesis

Current topics in locomotion physiology: a) muscle efficiency in heavily loaded gradient walking and b) heart rate off-kinetics as a predictor of VO_{2max}

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Preface

The human locomotion can be adapted to different conditions according to the environment. Walking on gradients and/or carrying (heavy) loads are some challenges that different population faced since long time ago and in the last decades they were studied by many researchers around the world (Heglund et al., 1995; Bastien et al., 2005; Minetti et al., 2006).

Since the pioneering paper of Margaria (1938) on gradient walking it is well known that the metabolic cost (the energy necessary to move one kilogram of body mass along one meter) keeps the same parabolic behaviour vs. the progression speed when walking on level and/or on gradient (Margaria, 1938). It is also known that minimum metabolic cost of walking occurs at a gradient of about -10% whereas it is higher steeper the (positive or negative) gradient (Minetti et al., 1993). The explanation of this finding was found on the partitioning of the positive and negative work that muscles have to perform when facing gradients (Minetti et al., 1993). All those results refer to unloaded walking. When subjects are loaded the metabolic cost increases proportionally to the extra load in all population, even if Nepalese porters are able to carry loads with a lower extra cost due to their long specialisation (Bastien et al., 2005). From a mechanical point of view data are scanty: it is known that the pendulumlike mechanical advantage of walking is impaired on gradient, whereas it could be enhanced when load are carried on the head (Heglund et al., 1995). An exhaustive description of mechanical changes due to both gradient and extra load and its implication on metabolic cost and efficiency deserve a new and accurate approach.

Furthermore, in the last years the interest in new technologies to assist the human health has grown also with the exercise monitoring. New useful and practical devices support this constant search, but a more precise control of physiological variables is still needed. In fact, along with this increasing number of devices, some issues about their functionality have been raised. This thesis will propose and discuss a new test to evaluate physical fitness by using the potential output of a smart watch in order to test both pros and cons of this new technology when applied in the field setting. Thus my main project was entitled

"A 'wearable' test for maximum aerobic power: real-time analysis of a 60-m sprint performance and heart rate off-kinetics" and was published in the journal *Frontiers in Physiology*. The proposed evaluation relies on a maximal sprint test (60-m) followed by a short recovery (5 min). Our aim was to define a multiple regression including sprint performance and physiological variables (as the heart rate off-kinetics) that could predict the aerobic fitness assessed in a standard laboratory test. According with these two focus, the thesis was subdivided in two chapters.

Chapter one presents the first topic of the thesis: muscle efficiency in heavily loaded gradient walking. The effects of loads (10, 20 and 40% of body weight), speeds (0.27 to 1.67 m/s) and gradients (15-25% uphill and downhill) on walking cost, mechanical work and efficiency were analysed.

Chapter two reports the findings of the second topic: heart rate off-kinetics as a predictor of $\dot{V}O_{2max}$. A new test for maximal aerobic power was developed based on the heart rate kinetics. This test consisted of only one maximal 60-m sprint where heart rate was recorded before, during and after the exercise.

CHAPTER 1

Muscle efficiency in heavily loaded gradient walking

Introduction

The human locomotion has been considerably analysed from both bioenergetics and biomechanical point of views (Saibene & Minetti, 2003; Cavagna, 2010). Since earliest times, hunting for food and escaping from predators already has proven how important is to comprehend this complex engineering that is our locomotor machine.

Different mechanical paradigms were defined and developed for each kind of locomotion. These models are based on the interrelationship between the mechanical energies of the body centre of mass (BCoM) in order to describe locomotion from a simple physics point of view. Walking and running were modelled as an 'inverted pendulum' and 'spring mass-model' (Saibene & Minetti, 2003; Blickhan, 1989).

The inverted pendulum is a theoretical model describing the mechanical paradigm of walking: potential (PE) and kinetic (KE) energies time course is out phase allowing an exchange, the sum of these two energies gives the total mechanical energy (TE = PE + KE). In a perfect pendulum without friction the energies will exchange forever so that no additional energy is needed to keep the pendulum oscillating and TE is constant. This peculiar exchange is addressed in the energy recovery proposed by Cavagna et al. (1976), where the percentage of energy saved by the pendulum is presented. Walking is not a 'perfect' pendulum, but the energy recovery is moderately high (up to 60%), and it is influenced among others by stride length (Minetti et al., 1995) and walking speed (Cavagna et al., 1976).

When analysing locomotion, the total mechanical work done is calculated as the sum of external and internal work, according to the Konig's theorem (Cavagna et al., 1963). The external work, W_{ext} , is the work done to rise and accelerate the BCoM with respect to the environment, and it is obtained from the increment of the total energy time course. The internal work, W_{int} , is the work

done to accelerate the body segments with respect to the BCoM (Cavagna et al., 1963). The total mechanical work (W_{tot}) done by muscle during locomotion is the sum of W_{ext} and W_{int} .

From the bioenergetics point of view, the net metabolic power of the investigated gait is divided by the progression speed in order to obtain the metabolic cost (C, J.kg⁻¹.m⁻¹) (Margaria, 1938; Schmidt-Nielsen, 1972; Di Prampero, 1986): the energy needed to move one kilogram of body mass along one meter. In walking, an increase in speed does not cause a proportional increasing of energy expenditure, then the cost vs. speed relationship shows a U-shape behaviour with a minimum (about 2 J.kg⁻¹.m⁻¹) at a speed of 1.1 – 1,4 m.s⁻¹, that can be considered an optimal speed since it minimizes the energy needed for travelling one meter (figure 1) (Margaria, 1938).

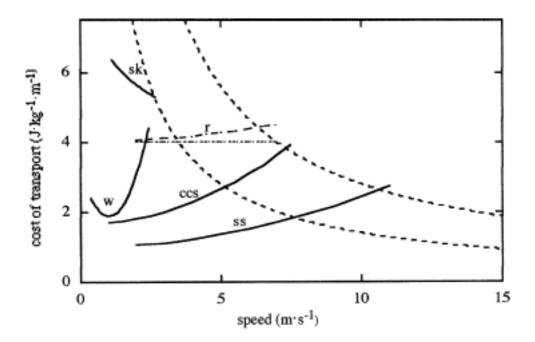


Figure 1. Cost of transport as a function of the speed for different types of human locomotion. w Walking, r running, ccs cross-country skiing, ss ice skating, sk skipping. The dashed curves represent the iso-metabolic power limit for a healthy normal subject (14 W.kg⁻¹, lower curve) and an athlete (28 W.kg⁻¹, upper curve). From Saibene & Minetti, 2003.

This optimal speed is also very close to the self-selected speed, highlighting that men usually move minimizing the energy expenditure (Cavagna & Kaneko, 1977). The concept of energy saving in human locomotion has been

widely reported both from a mechanical and bioenergetics perspective and it is a feature of human evolution (Alexander, 1991; Saibene & Minetti, 2003).

The parameter that relates mechanics and bioenergetics is the efficiency. Efficiency is the ratio between a mechanical output and a chemical input. The maximal muscular efficiency of the transformation of chemical energy into positive mechanical work by the muscles is about 25% for animals and humans (Heglund & Cavagna, 1987). The locomotion efficiency in level locomotion is usually expressed as the ratio between the positive work done by the muscles and metabolic cost (Cavagna & Kaneko, 1977). Efficiency can give an indication of the relative importance of the contractile and the elastic behaviour of human machine. In fact, a value greater than 25% (the muscular efficiency) shows that part of the work is performed by elastic elements (mainly tendons) without metabolic cost (Cavagna & Kaneko, 1977; Heglund & Cavagna, 1987).

When moving from level to gradient walking, C shows the same U-shape behaviour as function of speed uphill (Margaria, 1938), with the minimum (and maximal speed) that shifts leftwards to slower speed values. When walking downhill the U-shape was flatter, and a real minimum is hard to be detected (figure 2) (Margaria, 1938; Ardigò et al., 2003). Minetti et al. (1993) showed that when the minimum cost at each gradient is plotted against the gradient, another parabolic profile is present with a minimum at a gradient of -10%.

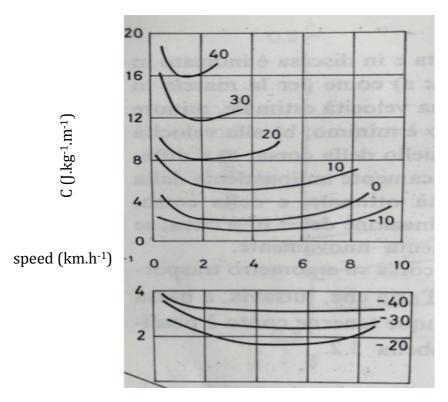


Figure 2. Metabolic cost (C, J kg⁻¹ m⁻¹) as a function of speed (km h⁻¹) at different gradients. From Margaria (1938).

This minimum was explained by the partitioning of positive and negative W_{ext} (figure 3). The positive external mechanical work, as stated before, is the work done to rise and accelerate BCoM, the negative is the work done to decelerate and lower BCoM (Minetti et al., 1993).

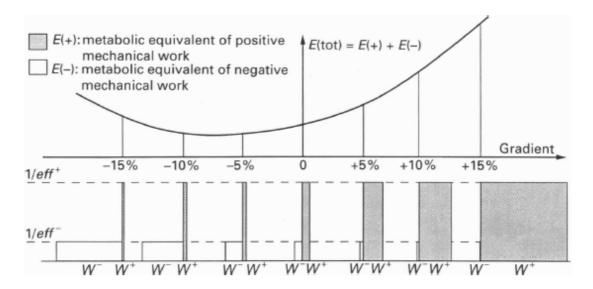


Figure 3. The positive and negative component (W^+ and W^- , widths of the bottom rectangles) constitutes one-half of the total mechanical work at level walking (zero gradient). They assume that to maintain the same speed uphill and downhill at different gradients both the total work increases and the contribution of one of its components becomes prominent (positive-uphill, negative-downhill). By multiplying the rectangle bases by the proper heights (which corresponds to dividing by the efficiency of positive and negative work), they obtain areas proportional to the metabolic equivalent of each component of the mechanical work (stippled and white patterns). The cumulative area for each gradient, given as the ordinate in the upper graph, has an asymmetric profile with a minimum corresponding to a certain gradient in the downhill zone. Adapted from Minetti et al. 1993.

The efficiency of negative work is 4-5 times higher than positive (Cavagna & Kaneko, 1977; Heglund & Cavagna, 1987) so that its metabolic contribution on level can be disregarded, however when moving downhill where the negative work becomes predominant (i>-15%), the amount of metabolic cost needed to perform that work is large and plays an important role (Minetti et al., 1993; 1994). When walking on level the partitioning between positive and negative work is the same (figure 4), whereas beyond 15% most of the mechanical external work is positive in uphill and negative in downhill walking (Minetti et al., 1993). This different partitioning affects also walking mechanics on gradient.

The displacement of BCoM becomes monotonic (only increasing, or decreasing, at positive or negative inclines, respectively). The pendulum-like mechanism is impaired: when walking uphill a lower value of energy recovery was reported because of a lower exchange between potential and kinetics energies (Minetti et al., 1993; Gomeñuka et al., 2014).

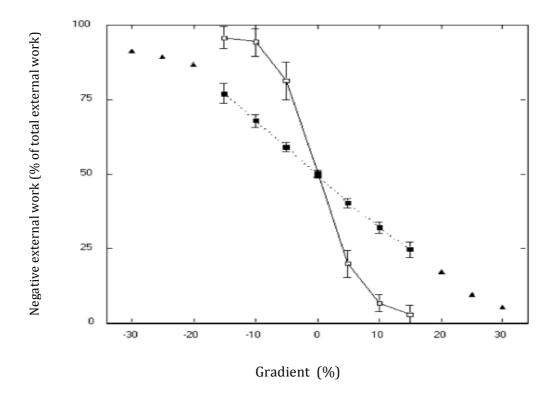


Figure 4. Negative external work (as a percentage of positive and negative external work) as a function of gradient; running data (filled squares and triangles) and walking values (open squares; Minetti et al., 1993) are both shown. Each value represents the fraction of the external mechanical work devoted to lower and decelerate the body centre of mass, while its complement to 100% is the fraction necessary to raise and accelerate it (from Minetti et al. 1994). In that paper the authors argued that the running curve superimposes on the walking curve after the effects of elastic structures on positive and negative work have been removed. Adapted from Saibene & Minetti (2003).

When walking carrying loads on level, the metabolic cost is higher (Huang & Kuo, 2014) but the U-shape remains the same (Bastien et al., 2005) and the few studies that investigated the mechanical work found no substantial differences on the level (Bastien et al., 2016) and on positive gradient (Gomeñuka et al., 2014). In the literature the ratio between negative and positive work efficiency during unloaded locomotion was found to be 5:1, whereas nothing is known about the influence of load on locomotion efficiency.

The analysis of muscular activity in locomotion may help to comprehend muscles activity during locomotion and their relation with BCoM displacement. Some investigations have reported the role of the main muscles when moving uphill or downhill (Lay et al., 2007; Pickle et al., 2016). They found that in uphill locomotion soleus and gastrocnemius had a significant role, whereas moving downhill a major activity (mean values and duration) of the knee extensors was reported (Lay et al., 2007; Pickle et al., 2016). However, they did not relate this activation to any mechanical work and neither they compare the activation during positive and negative locomotion.

Muscles when shortening are not able to exert the same force at different velocity, the force-velocity profile is hyperbolic (Woledge et al., 1985), with the maximal force that is exerted at zero velocity (isometric contraction) and zero force at maximal contraction velocity (figure 5). When lengthening the profile is different. During lengthening muscles are able to perform more force than the isometric contraction and this force production increases very sharply at the first negative velocity and then reaches a plateau (figure 5). It has to be said that the shortening (positive) side of the force-velocity, power and efficiency is well known, whereas less is known about the lengthening (negative) side.

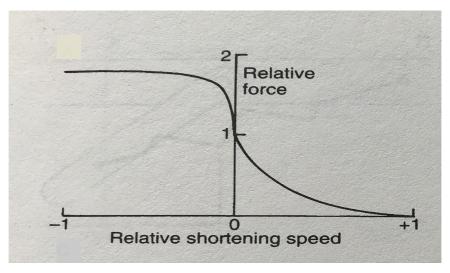


Figure 5. The force-velocity diagram of the muscle. Negative shortening speed refer to muscle lengthening, positive to muscle shortening. Adapted from Alexander (1999).

As already described, muscle shortening produced positive external work and lengthening negative external work. These two different modalities have a

different cost, in fact according to the force-velocity diagram when muscles are operating at the same velocity they can produce almost five time more force during lengthening. If the muscle is required to perform the same work by shortening or lengthening it will use 1/5 of its maximal force during lengthening, with a lower number of fibres activated that need less energy, so that the negative work is produced with less cost (see figure 3). The ratio between negative and positive efficiency (with efficiency obtained as the ratio between Wext + or Wext – and C) in human walking and running was found to be close to 5:1 (Minetti et al., 1993; 1994) that is in line with experiment at fibre level (Woledge et al., 1985). However, if the two contractions (positive and negative) are forced to occur at low contraction velocity (in the F-v diagram in figure 5 we are moving close to 0 v and isometric force), the difference in the activated muscle fibres should be no more 1/5. Thus, the cost of negative work should be higher, the negative efficiency lower and the ratio between negative and positive efficiency should move from 5:1 to smaller ratios.

The aim of this study was to analyse metabolic and mechanical aspects of loaded gradient walking in order to i) impair the positive and negative work efficiency and ii) give an exhaustive description of the load-gradient interaction on walking (mechanics and bioenergetics).

Material and Methods

Subjects

Three male subjects (29.0 ± 3.1 years, 1.74 ± 0.03 m height, 66.7 ± 7.0 kg mass; mean \pm SD) took part in the study. They were fully familiarised with the protocol and different weight-gradient-speed conditions during some familiarisation sessions.

Experimental Protocol

Subjects walked on a treadmill (Ergo LG, Woodway) at different speeds (from 0.28 to 1.94 m·s⁻¹), gradients (0, 15 and 25%, uphill and downhill), while carrying different loads with a weight vest on the trunk (0, 10, 20 and 40% of body weight). The protocol resulted in a combination of 20 conditions and 5-7 speed for each condition. During one experimental session subjects walked at one gradient, with one extra load, at all possible speeds that allowed to rely on the aerobic glycolysis (RER < 1) for four minutes. The gradient-load combination was randomly assigned to the subjects, whereas speeds were in crescent order from 0.28 to 1.67 m·s⁻¹ when walking uphill, from 0.56 to 1.94 m·s⁻¹ when moving downhill and from 0.28 to 1.94 m·s⁻¹ on level. At least 48 h intercurred between two consecutive sessions.

Data collection and Processing

Metabolic measurements

The experimental session started with 8 min of baseline $\dot{V}O_2$ (ml O_2 .kg 1 .min $^{-1}$) assessment (4 min in a seated and 4 min in a standing position) after that subjects were asked to walk on the treadmill for 4 min in each condition; in this way a steady state values for $\dot{V}O_2$ was reached and values of the last minute analysed. Respiratory gas was analysed breath by breath with three portable metabographs (one K4b² and two K5b², Cosmed, Rome, Italy. We were obliged to use three different apparatus for technical problems), and the metabolic cost (C, J kg $^{-1}$ m $^{-1}$, i.e., the metabolic energy needed to move 1 kg of body mass for a distance of 1 m) was calculated dividing the net $\dot{V}O_2$ (steady state $\dot{V}O_2$ – standing baseline $\dot{V}O_2$) by the progression speed (v, m s $^{-1}$) (Di Prampero, 1986). In order

to convert $\dot{V}O_2$ (ml O_2 .kg⁻¹.min⁻¹) to metabolic J the RER caloric equivalent (J/ml O_2) was taken into account.

Kinematics

Three-dimensional body motion was sampled by an eight-camera system (Vicon MX, Oxford Metrics), measuring at a sampling rate of 100 Hz the spatial coordinates of 18 reflective markers located bilaterally on the main joint centres (fifth metatarsal, calcaneus, lateral malleolus, femoral epicondyle, greater trochanter, glenohumeral axis, elbow axis, midpoint of the ulnar radius, and ear canal). in order to compute the BCoM position from an 11 rigid segments model (head-trunk, upper arms, lower arms, thighs, lower legs, and feet) (Minetti et al., 1993; Pavei et al., 2017) based on Dempster inertial parameters of body segments (Winter, 2005). When the vest was loaded the centre of mass location and radius of gyration of each segment remained the same, whereas the segment mass was scaled according to the added load and the percentage weight of each segment. Each acquisition lasted 1 min, during the last minute of the metabolic recording. From the BCoM 3D trajectory the time course of potential (PE) and kinetics (KE) energies were computed to obtain the total mechanical energy (TE = PE + KE).

The summation of all increases in TE time course constitutes the positive external work (W_{ext}^+ , J.kg⁻¹.m⁻¹) the work to accelerate and raise BCoM (Cavagna et al. 1963; Cavagna & Kaneko, 1977). On the other side the work done to decelerate and lower BCoM, which is calculated as the summation of all decreases in TE time course represents the negative external work (W_{ext}^- , J.kg⁻¹.m⁻¹, Minetti et al., 1993; 1994). The work necessary to rotate and accelerate limbs with respect to BCoM (internal work, W_{int} , Cavagna & Kaneko, 1977; Minetti, 1998) was calculated from the kinetic and rotational energy of the segment with respect to BCoM and summed to W_{ext} to obtain the total mechanical work (W_{tot} , J·kg⁻¹·m⁻¹). Energy Recovery, the ability of the system to save energy by acting like a pendulum-like system, was calculated on level according to Cavagna & Kaneko (1977). All kinematic data were processed with custom written LabView programs (release 2013, National Instruments).

Muscular activity

A wireless electromyographic (EMG) system (Trigno, Delsys Inc., Boston, MA, USA) recorded the surface EMG activity from eight lower limbs muscles of the right leg: gluteus medius [GM], vastus lateralis [VL], vastus medialis [VM], rectus femoris [RF], biceps femoris [BF], gastrocnemius medial head [Ga], soleus [Sol], and tibialis anterior [TA]. Parallel-bar EMG electrodes (DE-27x37x15-mm single differential surface EMG sensor with four 1-mm Ag contacts 5 mm apart) were placed longitudinally on each muscle according to standard recommendations (Stegeman & Hermens, 1999). The skin was shaved, slightly abraded, degreased, and disinfected with alcohol before attaching the electrodes to minimize impedance.

The EMG signal was sampled at 1000 Hz with Vicon software in order to have EMG and Kinematics signals synchronized. The root mean square amplitudes (RMS) of the EMG signal were calculated (with a time window of 50 ms) for each muscle and for each complete gait cycle in the 60 s of the acquisition. Gait cycle was defined as the interval between two consecutive heel strikes of the foot on the right side.

Results

Data are presented for one subject, the only that finished the whole experimental setup (20 conditions, 5-7 speeds for each condition), the other two subjects completed the 50% of the acquisitions and are not included yet. This delay was mainly caused by technical problems with the metabograph.

Metabolic Cost

Metabolic cost of walking unloaded across different gradients was comparable with Margaria's data (1938). Values are represented in figure 6 as a 3D surface with reference data (Margaria, 1938) in the mathematical description of Ardigò et al. (2003). The cost showed a parabolic profile versus speed at all gradient; when minimum cost was compared with gradient it showed a minimum around - 10% and increased at steeper (positive and negative) gradients.

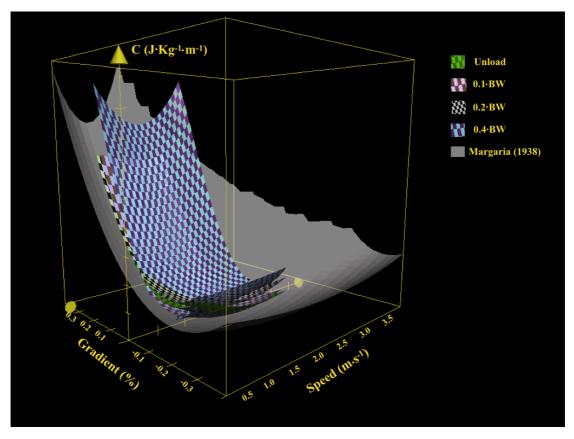


Figure 6. The 3D surface of metabolic cost (C, J kg $^{-1}$ m $^{-1}$) as function of gradient and speed (m s $^{-1}$) is represented. The uniform grey surface refers to Margaria's data (1938), the four checkerboard surfaces represent data from this study at the four different load conditions.

When loads were applied to the subject, the +10% condition did not show any difference, +20% had small increases, whereas +40% caused an increase in cost that was speed and gradient dependent as shown in figure 7. The average C increase at each gradient was greatest at +25% (4 J.kg⁻¹.m⁻¹) and lowest at -11% (0.1 J.kg⁻¹.m⁻¹), when reported as percentage of unload condition the peak of increase was at +15% with an extra cost of 42%, the lowest extra cost was at 11% with 0.05% (figure 8, Extra cost surface).

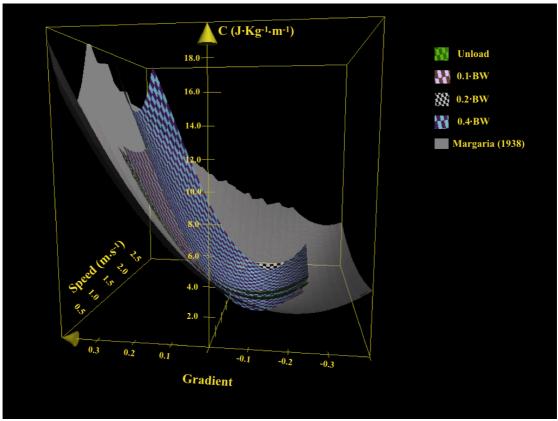


Figure 7. The 3D surface of metabolic cost (C, J kg^{-1} m^{-1}) as function of gradient and speed (m s^{-1}) is presented. From this perspective the difference in cost across different loaded conditions can be better appreciated, in particular 0.4BW showed the highest cost whereas no appreciable differences can be seen between unload and 0.1BW.

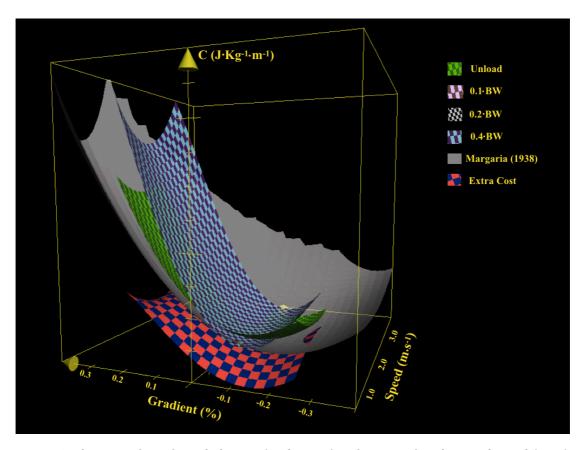


Figure 8. The 3D surface of metabolic cost (C, J kg^{-1} m^{-1}) as function of gradient and speed (m s^{-1}) is presented. With respect to the previous two graphs here the checkerboard pink and blue surface represent the Extra Cost of 0.4BW compared with unload. It can be seen that this extra cost is not constant, it can be described as a function of both speed and gradient.

Mechanical Work

Internal work (Wint) increased with speed, did not change with gradient and decreased with load (figure 9).

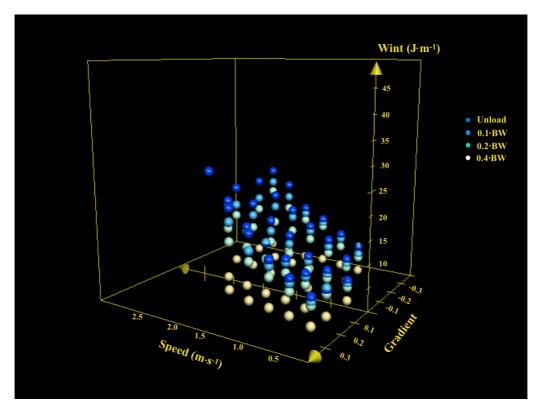


Figure 9. The internal work values (Wint, J m^{-1}) are presented as function of gradient and speed (m s⁻¹). Different colours represent the different loaded conditions.

External work (Wext) positive for uphill and negative for downhill gradients increased with gradient and load but was mainly speed independent at gradient (figure 10). The partitioning of positive and negative external work was, as supposed, very close to 100% in the two gradient conditions (figure 11).

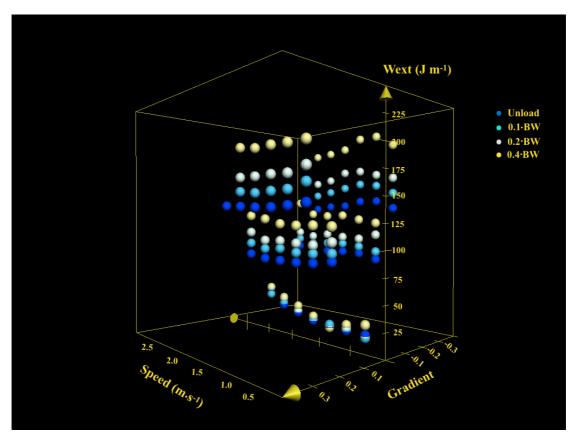


Figure 10. The external work values (Wext, J m^{-1}) are presented as function of gradient and speed (m s^{-1}). Different colours represent the different loaded conditions.

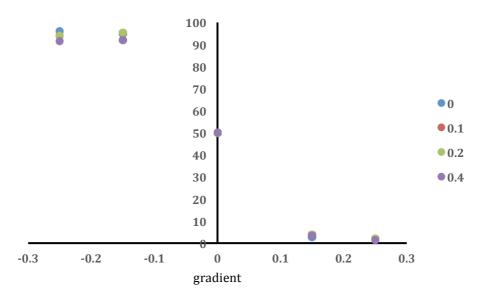


Figure 11. Negative external work (as a percentage of external work) as a function of gradient. Different colours represent the different loaded conditions. It can be seen that no differences in partitioning are present among load conditions and in the analysed gradients the external work was almost totally negative downhill and positive uphill.

Total work (Wtot) as sum of positive or negative external work and internal work increased with gradient and loads. On level and at -15% it increased also with speed (figure 12).

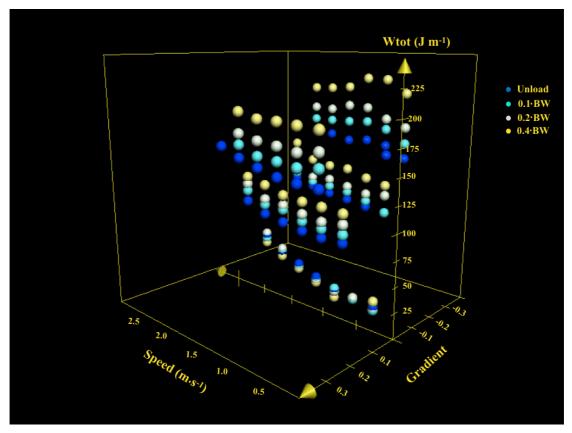


Figure 12. The total mechanical work values (Wtot, J m⁻¹) are presented as function of gradient and speed (m s⁻¹). Different colours represent the different loaded conditions.

Efficiency

Efficiency was calculated as the ratio between Wtot and C, with Wtot that was composed of negative or positive external work when walking downhill or uphill and internal work. When moving uphill efficiency was mostly speed independent, the load effect was not well defined with small differences among conditions (±3%) (figure 13). In downhill part the efficiency vs. speed curve followed a parabolic profile with a peak at the intermediate speed. The load effect was not so clear. (figure 13). As for the ratio between negative and positive work efficiency (figure 14) there was not a visible trend with added masses: the ratio was about 4:1 with fluctuations among speeds and loads.

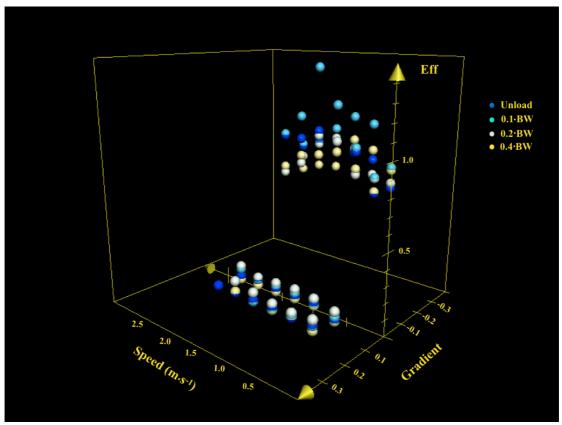


Figure 13. The mechanical efficiency (Eff) is presented as function of gradient and speed (m s⁻¹) Different colours represent the different loaded conditions.

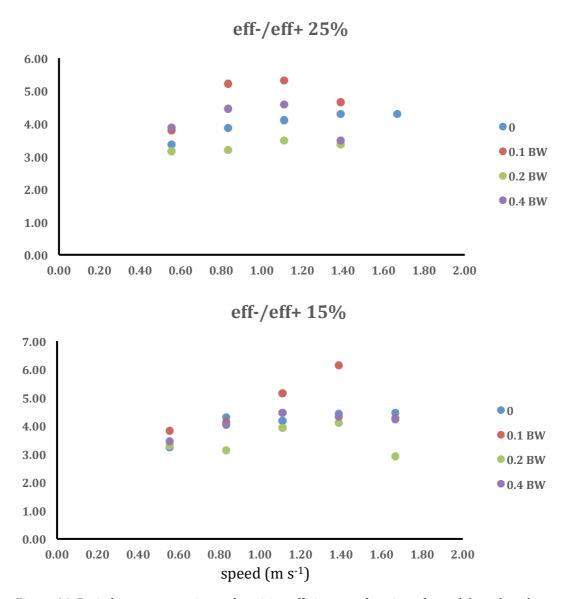


Figure 14. Ratio between negative and positive efficiency as function of speed (m s^{-1}) at the two gradients analysed (15 – 25%). Different colours represent the different loaded conditions.

Discussion

The metabolic cost of loaded walking on gradient showed some interesting points of discussion. First of all, unloaded data were superimposed to the reference data of Margaria (1938) and the surface representation (as for Ardigò et al., 2003) showed no differences between the two series/surfaces. This underlines that at all gradients the cost versus speed behaviour is parabolic with a minimum that slightly change to slower speed. Moreover, the minimum at intermediate negative gradient was again confirmed and was similar to previous papers (Margaria, 1938; Minetti et al., 1993).

When loaded, the metabolic cost of the subject increased markedly only at the highest load (+40%BW) whereas was unchanged with 10%BW of extra load and only slightly at +20%BW. This is quite different from previous studies where the increase in metabolic cost should be directly proportional to the extra load carried (Bastien et al., 2005b). This difference could be partially attributed to the experimental setup: extra load was carried with a weighted vest where loads were symmetrically placed in the front and rear side, whereas all the other studies used a normal backpack that could have enhanced rotational torques and muscular work to counterbalance them. Since also mechanical work was calculated, and then BCoM position was computed, it was very important to keep the center of mass of the trunk in the same relative position and the vest well addressed this need. Another interesting finding of the extra cost was the inconstancy across speeds and gradients. Bastien et al. (2005b) were among the first to report that extra cost related to the extra load increased as a function of walking speed on level. Looking at figure 8, present data with 40%BW load confirm those results on level and negative gradient, whereas for the walking uphill part it seems that a constant increase is shown across speeds; it cannot be excluded that the lower speed range would help this constancy. What is new with present data is the difference in extra cost, calculated as average difference across speeds, at the different gradients. As shown in figure 8 the minimum extra cost (analysed as percentage in order to avoid discrepancies among gradients) occurs at -11% and it increases with steeper (positive and negative) gradients. The "expected-target" value of +40% in extra cost was confirmed only at steepest

negative slope and above +15%, so that the increase in cost due to the added mass seems not so strictly defined and speed and gradient dependent. The cost vs. speed relation on gradient with loads keeps the parabolic profile of the unloaded condition without appreciable differences in shape and minimum, these results confirm and extend Gomeñuka et al. (2016) data with lower extra load.

These seems to be the first data on walking loaded mechanics on gradient above +15% and downhill, since Gomeñuka et al. (2014) analysed mechanical work at 0, +7 and +15%. The choice of slopes greater than ±15% was driven by the idea that above that gradient the partitioning between positive and negative work was predominant by negative work downhill and positive uphill (Minetti et al., 1993). Looking at figure 11 the assumption was right since in all gradient/load condition the value was above 93%. Thanks to these results the subsequent analysis on efficiency can be safely done and will regard only positive and negative work parts. A proof of the monotonically ascent or descent of BCoM at these slopes was confirmed in figure 15, where Wext is shown as a function of speed and gradient (as in figure 10) together with two planes that represent the vertical mechanical work. Vertical mechanical work is the work that has to be done to lift (positive gradient) or absorbed in lowering (negative gradient) the BCoM, and it relies only on the difference in potential energy according to the equation

$$W_{vert} = m g \sin(\arctan|i|)$$

where m is the mass (kg), g is gravity acceleration (9.81 m s⁻²) and i is the gradient (Minetti et al., 2002). The planes pass through both unloaded and +40%BW data (the other two added loads have been omitted for clarity but showed the same interpolation) highlighting that most of the external (positive or negative) work is done to lift or absorbed in lowering BCoM. The increase in external work is to be ascribed only to the increase in total mass and the absence of speed effect on gradient could be attributed to the greater amount of potential energy compared with level walking, where, instead, the great kinetic energy fluctuations at increasing speed also contribute to the increase in external work.

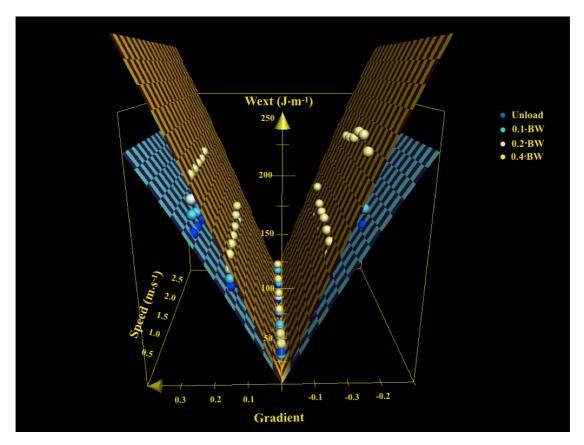


Figure 15. The total mechanical work values (Wtot, J m⁻¹) are presented as function of gradient and speed (m s⁻¹) with different colours that represent different loaded conditions. The blue checkerboard plane represents the vertical work of the unloaded condition, while the yellow one refers to 0.4BW extra load. In both cases the planes interpolate completely (positive and negative gradient parts) the experimental points highlighting the predominance of vertical work in the mechanical external work.

Mechanical internal work increased with speed regardless the gradient, this is in agreement with previous papers (Nardello et al., 2011; Gomeñuka et al., 2014; Minetti, 1998) and it is probably due to the changes in its determinants: an increase in stride frequency and a decrease in duty factor driven by the speed change (Minetti, 1998). On the other hand, Wint decreased with load, this is a consequence of the percentage distribution of the segment mass due to the extra load on the trunk. The internal work is calculated based on rotational and linear kinetic energies of limbs with respect to BCoM and both include the percentage mass of the limb. From the anthropometric table it is known that the trunk has more weight in the weighted mean for BCoM compared with limbs, moreover the extra load is placed on the trunk augmenting its percentage weight. In unloaded walking limbs periodic movements determine the greatest part of Wint, whereas trunk movements are small and give a marginal contribution to Wint. In the loaded condition even if limbs movements are the same their percentage mass is

lower and consequently their work. On the other hand, the trunk has a greater weight but its movements with respect to BCoM are smaller than limbs, they require less work and then Wint in heaviest loaded condition (+40%BW) becomes smaller than unloaded condition. As the sum of the two mechanical work described before and with predominance of Wext, Wtot can only increase as a function of the added mass, by following the same trend of Wext.

In unload condition, efficiency of positive work was lower than negative work at the two investigated gradients, as reported in previous studies (Minetti et al., 1993, Minetti et al., 2002). When loads were applied there was not an appreciable change in efficiency versus gradient and/or speed, results were overlapped and it was difficult to find a significant trend (figure 13). In this situation, also the ratio of negative and positive efficiency remained almost constant among gradients and speeds (figure 14). We thought that adding masses the gap between positive and negative efficiency could be reduced due to a higher muscular activation that would have moved closer positive and negative cost with the same (obviously) total mechanical work.

The mechanical work was the same, the cost was still different even with an extra load that challenge the aerobic capacity of our subject, so that a higher load will be not carried for four minutes. Speeds were analysed paired so that muscles should have operated at the same velocity, probably it was not enough slow for diminishing the difference between the positive and negative part of the Force-Velocity diagram. In fact, very low contraction velocities (and movements) could let muscles operate very close to an isometric contraction. In this isometric condition there is no more difference in the muscle force, velocity, power and efficiency when contraction is performed to resist a load or to support it. At the same time i) if walking uphill slowly (let's say at 0.1 m s⁻¹) is easily achievable, it would be demanding from a neuromuscular point of view downhill and probably subjects could find such slow speeds to be awkward; ii) the $\dot{V}O_2$ difference between baseline and very slow walking speeds could be quite small (when moving downhill) to be accurately detected; and iii) when walking very slow the support phases are longer with an increase muscular demand for stabilization (at the back for example) and balance of the single support leg. These can lead to higher level of co-contractions of agonist and antagonist muscles of the lower

limbs (or the trunk) that would increase the metabolic consumption, but it is not considered in and neither produce any mechanical work. When efficiency is computed we could assist to a decreased efficiency at these very low speeds just because the denominator accounts for the co-contraction extra cost, but the numerator does not. So that also the ratio between negative and positive efficiency could change, but we are not confident that the whole change is due to a real change in work efficiency.

Nevertheless, we are working now with the new acquisitions in this direction with very slow speeds paired uphill and downhill with the heaviest (+40%BW) load in order to get close enough to the isometric condition and no differences between positive and negative part of the F-v diagram.

If nothing will change and these are the results, it means that in human walking the ratio between negative and positive efficiency is always the same whichever (aerobic) combination of load/gradient and speed is chosen. And the real upper limit for loaded gradient locomotion is the subject metabolic (aerobic) power.

CHAPTER 2

Heart rate off-kinetics as a predictor of \dot{V} O_{2max}

Introduction

Wearable sensors

During last years we faced a great increase of new technological devices in the health and sports market due to lower cost and size of many sensors that can monitor and measure physics, geographic, physiological etc. variables. Wearable devices are appealing because they are lightweight, can be worn close to and/or on the skin surface, and detect, analyse, and transmit information about various internal and external variables (Halson et al., 2016).

Mobile health devices (i.e. wearable and wireless sensors) are expanding into the network commerce accurate data for metabolic energy expenditure during different activities (Parak & Korhonen, 2014; Wallen et al., 2016; Chowdhury et al., 2017). In this perspective it is worth noting wearable sensors updating periodically its models to an effective and accurate biofeedback. Among these devices, the smartwatch developed by Apple Inc. (Cupertino, California, USA) has been featured its straightforwardness and reliability to obtain physiological data from physical exercise (Chowdhury et al., 2017).

Some authors have pointed out the main limitations of current wearable devices: a) the need to place devices at specific anatomical locations; b) movement artefact; c) the sampling frequency; d) the capability to monitor only a few of selected variables (as opposed to a suite of them); e) the lack of measurement of environmental factors (e.g. temperature, humidity, altitude, UV radiation); f) the uncertainty in accuracy of data interpretation (by athletes/algorithm vs. trained professional); and, f) the inability to transmit data indoors, underwater, and in built-up areas; h) and interference from other physiological responses (e.g. vasoconstriction, hypovolemia) (Duking et al., 2016). Based on this perspective few studies have emphasized the accuracy of these wrist-worn devices during rest, exercise and daily of life activities (Spierer et al., 2015; Wallen et al., 2016; Chowdhury et al., 2017).

One device that has attained up to now a quite wide acceptance is the Apple Watch (Cupertino, California, USA) due to the simplicity to obtain HR. HR is acquired by blood oxygenation pulsations (plethysmography) from its red and green lights sensors fixed behind the main wrist of the watch (infrared lightemitting diodes – LEDs) and photodetectors. The measurement of blood fluctuations (volume changing) results in HR data (figure 2). Nevertheless, the time interval between every beat (R-R) provided by plethysmography device (pulse oximeter) is still unknown and a 5 seconds mean is usually provided. Probably manufacturers still do not release the algorithms used into own devices to avoid major details to the concurrent commerce (Van Hees et al., 2016). On the other hand, traditional HR monitors (with the elastic thoracic-band) provides the R-R period and/or t HR beat-by-beat according to consumers' requirement.

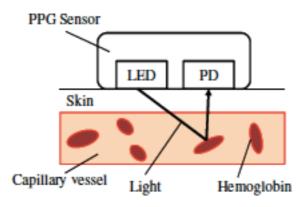


Figure 1. Structure and modus operandi of photoplestymography (PPG) by using reflected light. LED represents Light Emitting Diode technology and PD the Photo Detector. Adapted from Fukushima et al. (2012).

The major challenge nowadays for the companies is to produce a device, which is able to track the best HR signal and then to provide reliable physical activity outputs (such as energy expenditure) on the device itself. For instance, Chowdhury et al. (2017) reported that four different smartwatches (Apple Watch, Microsoft Band, Fitbit Charge HR and Jawbone UP24) gives weak or lower results when compared with controlled laboratory conditions. Moreover, any consumer monitors are able to perform as well as the research-grade devices although, in some (but not all) cases, estimates were close to criterion

measurements. Differently, El-Amrawy & Nounou (2015) investigated the accuracy of 17 wearable devices during walking with three different step counts (200, 500 and 1000, repeated 40 times) compared with heart rate. They found a reasonable result (not exceeding 20% deviation of HR) from the selected fitness trackers, then estimating properly energy expenditure.

Some researchers have proposed novel techniques in order to estimate accurately HR signals through smartwatches (Coolbaugh et al., 2014; Salehizadeh et al., 2015). This is because strenuous and high intensity exercise can result in severe motion-corrupted artifacts in plethysmography signals, making accurate HR estimation difficult (Salehizadeh et al., 2015; Zakynthinaki, 2015). Likewise, this bias also does not supply a beat-to-beat detection accuracy required by proper HR analysis, which makes a calculation over an average time (i.e. 3-5 s). Consequently, HR data from vigorous and quick exercises have not been mentioned as the general increasing into this subject (Ostojic et al., 2010; 2011).

In this context, data of HR from smartwatches seems more reliable during submaximal exercises avoiding sudden movements (Parak et al., 2015). However, a faster activity still requires more consideration by wearable devices. Even though, the manufacturers have released new devices with new algorithms and technologies trying to include this important factor (see Appendix section for the current gadgets).

Heart rate

One of the most used parameters for assessing health and physical fitness status is the heart rate (HR). The first written description of HR can be dated back to the 300 Before Christ, when a Greek scientist and physician was probably the first to describe the timing of the pulse, which can be considered the first HR description.

During these two millennia, technology and knowledge made progresses for analysing beat-to-beat pulse variability, the electrocardiogram and HR variability in time and frequency domain (Billman, 2011). In the early sixties exercise physiologists (among the others also Margaria et al. (1965)) assessed HR during different exercises and they showed the close relation between HR and oxygen consumption ($\dot{V}O_2$, an index of aerobic fitness). This relationship is linear at submaximal intensity with a variation at maximal intensity. Based on this evidence $\dot{V}O_{2peak}$ can be determined from HR values recorded at submaximal work rates (Daanen et al., 2012) in this way the maximal aerobic capacity can be assessed in different population, without causing subject's exhaustion. Thus, a reliable assessment of exercise intensity and control became a more convenient tools compared with the oxygen consumption that can be assessed in laboratory conditions).

The HR behaviour during exercise mirrors the $\dot{V}O_2$ patterns mostly because of the Fick law (Wieling et al., 2016). As for $\dot{V}O_2$ an on-kinetics, at the onset of exercise, a plateau, during exercise and an off-kinetics, after exercise and during the recovery, can be described also for HR. The steady state is usually considered for setting exercise intensity, whereas the two transitions (on- and off-) have been mainly addressed to heart capability of adjust the rhythm and metabolic demand. The HR off-kinetics depict the decrease in HR after exercise, from peak to rest condition and has been shown to be an indicator of mortality (Kriatselis et al., 2012; Coolbaugh et al., 2014) and fitness level (Koeneman et al., 2011; Wallen et al., 2016) Based on these features different studies have focused on the HR off-kinetics (8et al., 2007; Coote, 2009) and they define different indexes that better identify specific relationship with the investigated variables, so that it is quite difficult to make a comparison. The HR off-kinetics is modelled as a mono-

exponential decay (Figure 2) (Coote, 2009) this allows to define different parts on the curve.

Some studies focused their attention on the first phase of the decay where the slope is steeper and then the decay is faster, and analysed the difference between the peak of HR after exercise and its value after 30-60s of recovery (Lamberts et al., 2004; Ostojic et al., 2010). This is considered the simplest noninvasive analysis of HR recovery that can estimate cardiac autonomic recovery through both sympathetic and parasympathetic mechanisms (Duking et al., 2016). This balance between sympathetic and parasympathetic responses plays a major role during and after exercise (Figure 2) (Coote, 2009). At the beginning of exercise, a vagal (parasympathetic) inhibition occurs driven by central system and tetanoreceptor in the muscles, simultaneously with an increased sympathetic activity (more influenced by central command and muscle metaboreceptors) (Ostojic et al., 2011). At the end of the exercise the mirror tends to occur (increasing of vagal activity and withdrawal of sympathetic activation). These activities can be influenced by exercise related to factors such as intensity and volume (Goldberger et al., 2006; Lepretre et al., 2012). The analysis of the fast HR recovery is usually performed to assess the para/sympathetic interplay on HR. However, because of the workloaddependency (type of exercise, volume, intensity) of the kinetics, it becomes hard to compare different studies especially when just the fast off-phase is analysed (Ostojic et al., 2011; Haddad et al., 2012). When fitting the curve only in the first 30 s in order to explain only the fast time constant, some authors used a mathematics curve based on semi-logarithmic analysis (Buchheit et al., 2007; Peçanha et al., 2017): the HR decay is represented by logarithm analysis, and fitted as a first-degree polynomial equation. With this approach it is possible to obtain the time constant for the fast HR recovery as the negative reciprocal of the slope of the fitted line (-1/slope; see Figure 3).

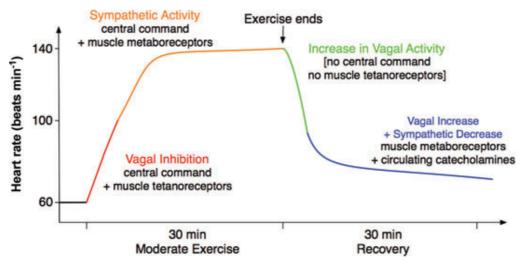


Figure 2. Changes in heart rate during and following exercise. Adapted from Coote (2009).

After 60 s the HR presents a more graduated decline (Peçanha et al., 2017). This second phase of the HR decay is primarily influenced by a slower sympathetic withdrawal, which was shown by using pharmacological blockades (Coote, 2009). In order to analyse the sympathetic behaviour and the investigation needs a much longer recovery that can arrive to 300 s (Peçanha et al., 2017). In this way it is possible to describe both parasympathetic reactivation and sympathetic withdrawal (8et al., 2007).

Even if some studies focus only on the first (fast) or second (slow) recovery phase, the analysis of the whole HR off-kinetics can give a whole picture of the all system (Adabag & Pierpont, 2013). With this approach the off-kinetics is modelled and fitted with a mono-exponential decay and the time constant of HR recovery, Tau (τ) , which denotes the time to reach 63% of the steady state response is calculated (Coote, 2009; Peçanha et al., 2017). The mono-exponential analysis presents an asymptotic value of HR (mean basal value), the maximal amplitude of HR and τ

$$HR(t) = a_0 + a_1 \cdot e^{\left(-t/\tau\right)}$$

Where a_0 is the asymptotic value of HR; a_1 is defined as maximum value of HR after exercise; t is time and τ is the time constant to reach 27% (since this is a off kinetics tau equals to 1-63%) of HR maximum excursion.

For Pierpont et al. (2000) τ is a useful tool for representing HR recovery after submaximal exercises. They found that τ is not influenced by the intensity of the exercise, but they highlighted that the recovery time should be long enough for reaching the baseline values again (Pierpont et al., 2000). Moreover, HR recovery could be analysed by HR variability after exercise. This assessment can be accomplished both in time and frequency domain by exploiting the interbeat (RR) interval between heartbeats (Peçanha et al., 2017). The HR variability is commonly studied under controlled environments, which can include long-term monitoring during recovering (around 5 min and 24 hours) (Dupuy et al., 2012). Heart rate variability is generated by the integrated action of parasympathetic and sympathetic branches of the autonomic nervous system on the sinus node. The difference for this evaluation consists to find how variable are the heartbeats (by the time or frequency) instead of single value after a determined time period.

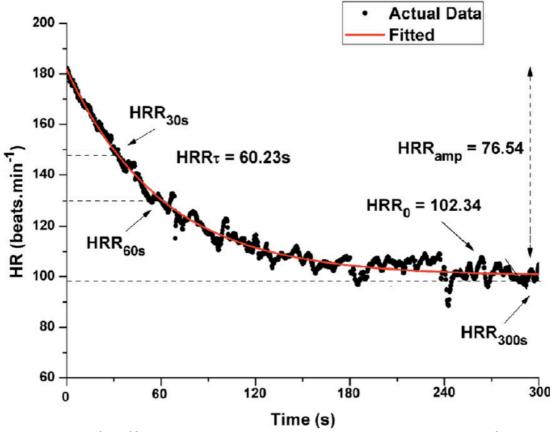


Figure 3. Examples of heart rate recovery assessment. HRR30s, HRR60s, HRR300s: heart rate after 30, 60 and 300 s of recovery. HRRt = time-constant of heart rate recovery after a first order exponential fitting in the entire recovery curve. HR_{amp} is the difference between peak HR and HR_0 , HR_0 = asymptotic value of HR. Adapted from Peçanha et al. 2017.

The aforementioned literature shows that researchers are looking for similar values that could be representative of HR recovery. Their findings try to combine simple and practical methodologies with more complex one. The use of short and reliable tests/equations could reach a larger number of practitioners via common used devices, such as heart rate monitor.

Fitness variable predictions

In the last decades researchers have tried to find a simple model to predict aerobic fitness (Weyand et al., 2001; Mier et al., 2004; Nemeth et al., 2009). When HR was analysed, these trials have focused on off-kinetics due to easiest data collection when compared to exercise being performed (Dupuy et al., 2012; Haddad et al., 2012). Also, we have to know that certain tests are considered neither quick nor useful, because they use, for example, a long time pre-exercise, or require several bouts to complete the test (Weyand et al., 2001; Magrani et al., 2010). At the same time, it is hard to find consistent HR data for $\dot{V}O_{2peak}$ prediction in fast tests (Boullosa et al., 2014).

Several studies have reported good relationship between HR off-kinetics and aerobic power (Watson et al., 2016), when performing long protocols (more than 30 min) even if without a methodological consensus (Otsuki et al., 2007; Ostojic et al., 2010; Mourot et al., 2015; Peinado et al., 2014). Even though, the phase-off (recovery) of HR has been shown to be advantageous also because utilizes a chest belt to detect HR signals. However, the difficulty to detect HR signals during a movement requiring intensive activity it is known mainly due to motion artefact-corrupted signals (El-Amrawy & Nounou, 2015; Parak et al., 2015). Unfortunately this bias is also significant for smartwatches due to responsive mechanism to collect/calculate data from pulse (Fukushima et al., 2012). Albeit facing difficulties with smart devices for indicating maximal aerobic capacity, HR monitor by chest belt is still considered gold standard for this purpose when exploiting HR as main input to predict $\dot{V}O_{2peak}$ (Lai & Kim, 2015). Besides that, when performing submaximal exercise, both materials are able to provide accurate estimates for aerobic power (independent of device) due to absence of the aforementioned problems (Fukushima et al., 2012; Lai & Kim, 2015). In this contest, the tests most used are based on cyclic movements, such as cycling, walking, running and stepping (Peçanha et al., 2017).

In general, walk tests are more accessible to anybody (from novel users to experienced subjects), since it is safely, and useful when running is not advisable, such as in obese or elderly people. Moreover, the studies based on running exercise require a higher intensity and longer time when compared with walking and stepping. A recent review of Sartor et al. (2013) analysed various studies

that estimate $\dot{V}O_{2peak}$ from submaximal tests: 28 studies involved walking and/or stepping, while 11 studies explored running (Swain et al., 2004; Davies et al., 2008; Mier et al., 2004; Nemeth et al., 2009). Running tests can estimate aerobic fitness with a small standard error of the estimate (SEE, range was ~ 2.87-5.98 ml.kg⁻¹.min⁻¹) as well as walking locomotion (Sartor et al., 2013). Although running can require some more attentions (as longer time for familiarization by untrained individuals), shows good correlation between HR and $\dot{V}O_2$ during entire data collect (resting, running and recovery) (Daanen et al., 2012).

The running protocol for $\dot{V}O_{2peak}$ estimate from HR off-kinetics data may used steady or unsteady speeds and various series of intervals (Ostojic et al., 2010; Buchheit et al., 2014), and these methods can be accomplished in laboratory or field conditions depending on the purpose of the study. Simpler the method, it is easier to be accomplished into the field conditions and then it is more accessible to the most of the population. In this line, as precursor through running activity, Cooper (1968) estimated maximal aerobic power by distance travelled during a maximal running test (12 min) at athletic track field (the only predictor variable was the covered distance). When HR is introduce as dependent factor to estimate $\dot{V}O_{2peak}$, the time required for data collection during exercise varied between 10 and 30 min (Kline et al., 1987; Weyand et al., 2001). The advantage to analyse off-kinetics of HR is a shorter time for the evaluation after exercise, which can vary between the 30 s and 5 min (Peçanha et al., 2017). Thus, the only concern could be linked to finding shorter and economic exercise protocols.

Actually, besides longer protocols, the major part of these studies has not been exploited off-kinetics of HR as estimation for fitness aerobic (Kline et al., 1987; Swank et al., 2001; Weyand et al., 2001). Instead of a predicting $\dot{V}O_{2peak}$, the most recent analyses choose to estimate energy expenditure during daily routine activities from algorithms embedded in the studied device. The system generally is composed of HR data originated from R-R interval, or by average time windows of that interval (Peçanha et al., 2017).

Usually, the studies that aimed to estimate $\dot{V}O_{2peak}$ during running used traditional HR monitor, which analyses HR from R-R interval (MacMillan et al., 2006; Lamberts et al., 2010; Ostojic et al., 2010, and 2011; Buchheit et al., 2014) and they showed that athletes report a faster HR recovery to performance. Ostojic et al. (2010 and 2011) reported significant faster HR recovery during the first 20-30 s for athletes with higher aerobic capacity following after a maximal exercise. This fast decreasing suggests a strong influence of autonomic system playing an important role in ultra short-term cardiovascular responses to exercise. Therefore, this higher $\dot{V}O_{2peak}$ can be associated with faster HR recovery by autonomic control due to the rapid analyses.

Although the shorter recovery phase, the studies usually have proposed maximal protocols requiring exercise test lasting at least fifteen minutes (Pierpont et al., 2000; Ostojic et al., 2011). In this line, the type of methodology utilized, such as number of beats recovered within a given time (e.g. 60 s, HRR₆₀ s), or fitting via exponential models might influence the fitness/performance control estimated (Seiler et al., 2007; Thomson et al., 2015; Peçanha et al., 2017). The recovery time would be inclined to the analysis accomplished: a long ('slower') evaluation would be associated to mono-exponential modeling. It is known that longer analysis can respond with a better capture of the overall HR responses compared with a short one since both the initial fast component and the delayed recovery phase are considered (Buchheit et al., 2014). Likewise, a longer recovery time has been required to better understand off-kinetics behavior (MacMillan et al., 2006).

It is a challenge for current researchers to estimate aerobic fitness while running, from off-kinetics of HR, by combining a protocol composed by shorter running tests and recovery. Up to now, authors reporting off-kinetics of HR of running do not used quick protocols, instead of cycling, walking and stepping (MacMillan et al., 2006; Ostojic et al., 2010 and 2011). They proposed a longer maximal ramp protocol of running (that can last from 10 to 15 minutes) combined with a certain time for rest and/or recovery (5-10 minutes) in order to control performance/fitness. This kind of protocols requires young and healthy people with no physical restrictions to support a maximal effort.

Besides maximal aerobic ramp tests, some authors have proposed sprinting tests to evaluate off-kinetics of HR (Ostojic et al., 2010; Al Haddad et al., 2012; Vernillo et al., 2015). These studies reported results from intermittent exercise where a number of series with resting intervals where performed (Ostojic et al., 2010; Vernillo et al., 2015) and then that cannot be applied to the examples cited above.

Therefore, the shorter off-kinetics of HR requires more attention by researchers during its analysis, which currently has not been well addressed. The sprint running has demonstrated reliable values for subsequent recovery analyses (Al Haddad et al., 2012; Vernillo et al., 2015). Even though, recent studies have preferred explore multiple bouts and its recovery after a total of series performed (Ostojic et al., 2010; Vernillo et al., 2015). Still, which could be the response of HR after a single sprint bout, and how it would estimate aerobic power since authors have shown good correlations between anaerobic efforts and $\dot{V}O_{2peak}$?

Article

My study on the feasibility of sprint performance and HR off-kinetics on the $\dot{V}O_{2peak}$ estimation has been recently published in a paper in *Frontiers in Physiology* entitled "A 'wearable' test for maximum aerobic power: real-time analysis of a 60-m sprint performance and heart rate off-kinetics" (Storniolo, JL., Pavei, G., and Minetti, AE., 2017). The following paragraphs will address this publication.

A 'wearable' test for maximum aerobic power: real-time analysis of a 60-m sprint performance and heart rate off-kinetics

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ABSTRACT

Maximum aerobic power ($\dot{V}O_{2peak}$) as an indicator of body fitness is today a very well known concept not just for athletes but also for the layman. Unfortunately, the accurate measurement of that variable has remained a complex and exhaustive laboratory procedure, which makes it inaccessible to many active people. In this paper we propose a quick estimate of it, mainly based on the heart rate off-kinetics immediately after an all-out 60-m sprint run. The design of this test took into account the recent availability of wrist wearable, heart band free, multi-sensor smart devices, which could also inertially detect the different phases of the sprint and check the distance run. 25 subjects undertook the 60-m test outdoor and a $\dot{V}O_{2peak}$ test on the laboratory treadmill. Running average speed, HR excursion during the sprint and the time constant (τ) of HR exponential decay in the off-kinetics were fed into a multiple regression, with measured $\dot{V}O_{2peak}$ as the dependent variable. Statistics revealed that within the investigated range (25-55 ml 02/(kg.min⁻¹)), despite a tendency to overestimate low values and underestimate high values, the three predictors confidently estimate individual $\dot{V}O_{2peak}$ (R²= 0.65, p<0.001). The same analysis has been performed on a 5-s averaged time course of the same measured HR off kinetics, as these are the most time resolved data for HR provided by many modern smart

watches. Results indicate that despite of the substantial reduction in sample size, predicted $\dot{V}O_{2peak}$ still explain 59% of the variability of the measured $\dot{V}O_{2peak}$.

INTRODUCTION

In the last few decades we assisted to a growing interest toward personally keeping one's health in a better shape, a condition that would enrich the entire life and likely prevents early deterioration of many body functions. This passes, among others, through the development and maintenance of a maximum oxygen consumption $(\dot{V}O_{2peak})$ higher than for a sedentary. However, portable professional metabographs are out of reach for most of the athletes, not to mention the laymen, who represents the vast majority of the potential audience in the need to periodically check the aerobic fitness level.

At the same time, the progress in terms of portable technology (tablets, 'smart' phones, bracelets/bands and watches) makes us move equipped with a redundancy of sensors. In addition to the ubiquitary camera, most of the devices bring GPSs, accelerometers, gyroscopes, magnetometers, proximity sensors and, most recently, infrared emitter/detector LED systems to measure heart rate (HR) in real-time. Although not all of them provide data accurately and precisely enough to compete with the analogous laboratory equipment (Chowdhury et al., 2017), their improvement is just a matter of time and scenarios for new and different biomedical tests could be certainly hypothesized to be implemented in the near future.

Submaximal metabolic effort such as walking, running, hiking, swimming at moderate speed has been classically included in the activity monitor function of 'smart' portable/wearable devices. The estimate of burned calories is obtained from short-term average HR, average speed and from accelerometry-based recognition of locomotion type (Chowdhury et al., 2017).

Differently, no estimate of $\dot{V}O_{2peak}$ from smart devices has been implemented so far, to the authors' knowledge. Potential reasons for this is, as mentioned, the infancy of wearable sensor technology that strives to compete with professional analogues. Our challenge in the present investigation was to design a simple test exploiting sensors already incorporated in smart watches/bracelets.

The idea originated from transport engineering: race car engines increase and decrease rpm (revolutions per minute, a 'sound' particularly apparent when gear is disengaged) much faster than in a normal car. In the biological realm we face a similar phenomenon: athletes display a faster $\dot{V}O_2$ increase (at the start of a heavy exercise) and decrease (during the recovery) than sedentary subjects (for a review see Jones and Poole 2005; Rossiter, 2011). HR is a fundamental determinant of $\dot{V}O_{2peak}$ on- and off-kinetics, since its time course closely mimics the changes in gas exchange (Hickson et al., 1978; Hagberg et al., 1980; Norris and Petersen, 1998). Thus, similarly to engine rpm, HR kinetics is expected to be faster the higher the metabolic power of human engine (Darr et al. 1988; Sugawara et al. 2001; Otsuki et al., 2007; Ostojic et al., 2010; Ostojic et al., 2011; Watson et al., 2017). This applies to other important kinetics, such as the enzymatic chain (Timmons et al., 1998), within the whole metabolic/mechanical 'turn on/off' process of muscular exercise.

A very short maximal sprint (60-m) was adopted in order to design a quick test that could be performed in a non-specialized environment: only rubber soles and a short straight path, in addition to the smart watch, would be necessary. We decided to use only the HR off-kinetics because even more professional HR sensors (i.e. the thoracic belt) have troubles to detect just the heart signal when many other muscles in the body are intensively activated, as during maximal propulsion.

Aim of the study was to propose a simple methodology and algorithm predicting individual aerobic fitness, and check its adherence to experimentally measured $\dot{V}O_{2peak}$ values. This test could be implemented in many smart wearable devices and would certainly benefit from the inevitable improvement in sensor technology.

MATHERIAL AND METHODS

Subjects

Twenty-five subjects (7 women and 18 men, 25.0 ± 5.0 yr, 1.77 ± 0.08 m height, 71.4 ± 8.6 kg body mass; mean \pm SD) took part in the study; they were physically active subjects involved either in recreational activity or in amateur sport activity with a maximum of four sessions per week. The study was

approved by the Ethics Committee of the University of Milan, and participants, after becoming aware of the potential risks involved in the experimental sessions, gave written informed consent.

Experimental protocol

Subjects performed two different tests in different days separated by a minimum of 48 h: a 60-m maximal sprint accomplished on an outdoor athletic track and an incremental exercise test for the determination of $\dot{V}O_{2peak}$ in the laboratory. Participants were instructed to arrive at the experimental session in a rested and fully hydrated state and to avoid strenuous exercise in the 24 h preceding each testing session. In addition, they were told to avoid alcohol (24 h) and caffeine (6 h) intake before the exercise test.

Data acquisition

Field test

The first session consisted of a 60-m maximal sprint trial preceded by a short warm-up (5 min with jogging and stretching) and 10 min of resting period (5 min in a seated and 5 min in a standing position). Heart rate (HR) was recorded beat-by-beat throughout all phases of the sprint test (rest, running and 5 min of recovery) by a heart rate monitor with transmitter belt (Polar S410, Kempele, Finland). All tests were performed at the same time of day (10-11 a.m.) to limit the influences of circadian rhythm on muscle performance and heart rate response/variability. 60-m sprint duration was recorded by using a manual stopwatch and the average running speed (v_{test}, m.s⁻¹) was obtained. Subjects were encouraged to accomplish their best performance.

Laboratory test

Peak aerobic power $(\dot{V}O_{2peak})$ was determined with an incremental running test performed on a treadmill (Ergo LG, Woodway). After 10 minutes of standing resting period, the protocol began with subjects running at 9 km.h⁻¹ for 4 minutes, then the belt speed was increased by 1 km.h⁻¹ every minute until volitional exhaustion. Pulmonary ventilation ($\dot{V}E$, BTPS), O₂ consumption ($\dot{V}O_2$), and CO₂ output ($\dot{V}CO_2$), both STPD, were determined breath by breath by a

portable metabograph (K4b2, Cosmed, Italy).

Data analysis

Anthropometric

Body mass and height of subjects were measured using a stadiometer (A. Vandoni, Italy). The subjects were asked to maintain a relax position for both measurements (height and body weight).

Maximal oxygen consumption $(\dot{V}O_{2peak})$

 $\dot{V}O_{2peak}$ values were taken as the highest 30s average $\dot{V}O_2$ value attained before the subject's volitional exhaustion. These data were collected and analysed as ml.kg⁻¹.min⁻¹. At rest and at various times (5, 7 and 9 min) during recovery, 0.6 µL of capillary blood was obtained from a preheated earlobe for the determination of blood lactate concentration ([La]_b) (Lactate Plus, Nova Biomedical). Thus, values higher than 8 mmol.L⁻¹ were accepted to ensure a $\dot{V}O_{2peak}$ state. Besides that, respiratory exchange ratio (RER) was calculated as the ratio of $\dot{V}CO_2$ to $\dot{V}O_2$, and values higher than 1.1 were used to confirm $\dot{V}O_{2peak}$ as well as a HR higher than predicted value (220 – age; Tanaka et al., 2001).

Heart rate kinetics

Heart rate off-kinetics (HR decrease after 60-m sprint) was modelled according to a mono-exponential function of time by using a Least Squares Method (minimizing the sum of squared vertical distances between experimental points and the exponential curve):

$$HR(t) = HR_{baseline} + Ampl \cdot e^{\left(-t/\tau_{off}\right)}$$
 (1)

where $HR_{baseline}$ is the average of HR (bpm) during the last 60 s of the recovery period; is the asymptotic amplitude for the exponential term (maximal HR values – $HR_{baseline}$, bpm); τ_{off} is the time constant (s) of the exponential, i.e. the time from the end of the sprint to reach 27% of HR maximum excursion (which corresponds to HR = $HR_{baseline}$ + Ampl (1 - 63%)). The velocity of HR decays after

the sprint (v_{off} , s^{-1}) was inferred as the reciprocal of τ_{off} . Also, the heart rate range from the sprint start to the beginning of the off-kinetics phase (Δ HR) was calculated (Fig. 1).

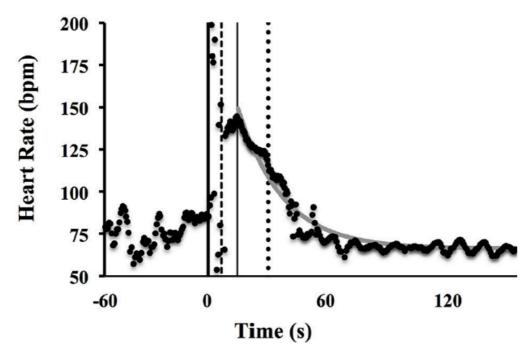


Figure 1. Example of a representative heart rate time course from rest to recovery of the maximal sprint test. The thick vertical line coincides with the start of the sprint, the dashed vertical line with the end of the sprint, the thin vertical line represents the start decay of HR, while the dotted line expresses the τ off and the grey continuous curve represents the mono-exponential best fit.

The input data available for the mathematical routine were R-R intervals (raw data, .hrm) from heart rate monitor. A time delay (t_{delay}) after final test was calculated due to a short constant time present for peak of HR in all subjects (2-5 s). Then, this variable was also a confounding factor that enabled avoid consequents error to the final analysis.

All data have been analyzed with purposely written LabView programs (release 13, National Instruments).

Statistics

Data are presented as mean \pm standard deviation (SD). Multiple linear regression analysis was adopted to explain the variance of the individual $\dot{V}O_{2peak}$, based on independent variables v_{test} , v_{off} and ΔHR . Linear regressions were used to analyse correlations between variables and residuals of predicted $(\hat{V}O_{2peak})$ vs. measured $\dot{V}O_{2peak}$.

A Bland-Altman test was carried out to assess the repeatability of $\hat{V}O_{2peak}$ from sprint field test by verifying agreement between both $\dot{V}O_{2peak}$ values (measured and estimated).

The Standard Error of the Estimate (SEE) was calculated to measure the accuracy of the prediction and to compare it to other published predictors of $\dot{V}O_{2peak}$.s

Statistical significance was granted at p \leq 0.05. Statistical analysis was performed by using SPSS v20 (IBM, USA).

RESULTS

 $\dot{V}O_{2peak}$ range was 29.1-56.6 ml.kg⁻¹.min⁻¹ (mean \pm SD, 42.5 \pm 8.7 ml.kg⁻¹.min⁻¹). Peak net blood lactate concentration after the incremental test was 8.5 \pm 1.3 mM. All groups attained maximal HR values corresponding to 95% of the age predicted maximum and RER values > 1.1. Thus, taking into account also [La]_b peak values, it can be assumed that subjects reached the maximum exercise capacity.

The v_{test} and v_{off} were significantly related to $\dot{V}O_{2peak}$ (r = 0.74, p < 0.001; r = 0.43, p = 0.03, respectively) (Fig. 2A, B), whereas Δ HR was not related to $\dot{V}O_{2peak}$ (r = -0.18, p = 0.39) (Fig. 2C). Multiple regression analysis ($\dot{V}O_{2peak}$ = 7.46· v_{test} + 261.4· v_{off} - 0.19· Δ HR) showed that a linear combination of v_{test} , v_{off} and Δ HR from the sprint test explained 65% of the $\dot{V}O_{2peak}$ variance (R² = 0.65, p < 0.001) (Fig. 3).

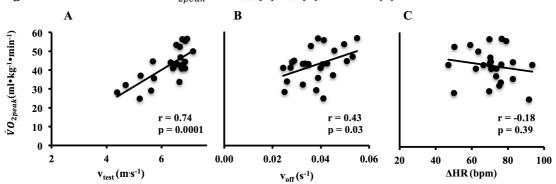


Figure 2. Relation between $\hat{V}O_{2peak}$ and v_{test} (A), v_{off} (B), and Δ HR (C) are illustrated.

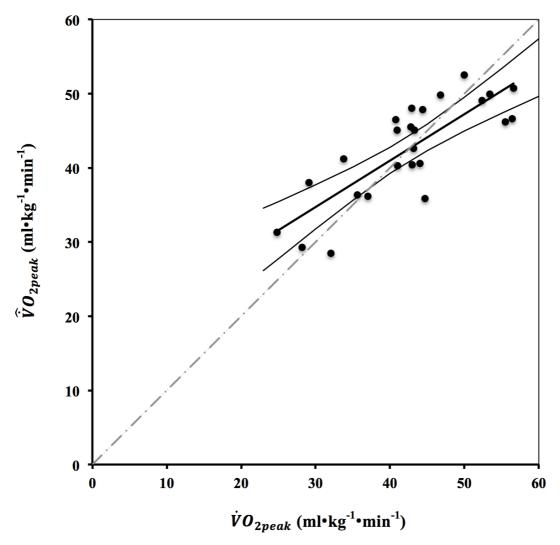


Figure 3. Relation between measured $\dot{V}O_{2peak}$ and predicted $\hat{V}O_{2peak}$ estimated from multiple linear regression $\dot{V}O_{2peak}$ = 7.46· v_{test} + 261.4· v_{off} - 0.19·ΔHR Trend line (thick black line) expresses the linear regression between the variables ($\hat{V}O_{2peak}$ = 0.62· $\dot{V}O_{2peak}$ + 15.9; r = 0.80). The thin black lines are the confidence interval (95%) of the trend line, while the dashed grey line is the identity line.

A paired t-test did not show significant difference (p > 0.05) between measured $\dot{V}O_{2peak}$ and the predicted value ($\dot{V}O_{2peak}$.) and the SEE was 5.28 ml.kg⁻¹.min⁻¹. Fig. 4 shows the Bland-Altman plot VO_{2diff} ($\dot{V}O_{2peak} - \dot{V}O_{2peak}$) vs. mean $\dot{V}O_{2peak}$, both for a beat-to-beat analysis and for a 5s average of HR off-kinetics (see below).

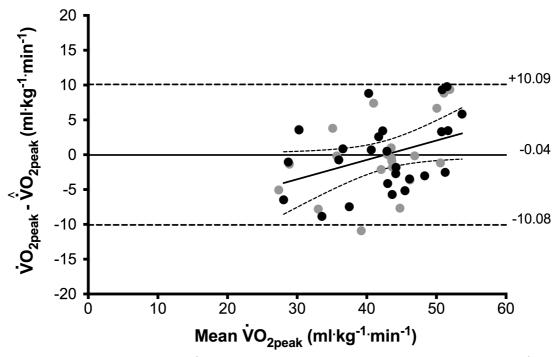


Figure 4. Bland-Altman plot of $\dot{V}O_{2peak}$ difference (measured - predicted values) vs. mean $\dot{V}O_{2peak}$ (black circles). Solid line (average bias = -0.04 ml.kg⁻¹.min⁻¹); dashed line indicates 95% limits of agreement. The trend line equation expresses y = 0.28x - 11.9, with r = 0.40 (p = 0.04) with confidence intervals (95%). Grey circles: predicted values based on 5s average of HR time course (see Discussion).

DISCUSSION

The idea behind this investigation has been to find a simple and short test that could reasonably predict individual $\dot{V}O_{2peak}$, based on signals from sensors that constitute the current 'equipment' inside mobile/smart devices (phones or watches).

The software algorithm has been designed as to use the most meaningful part of the post-sprint HR time course: it was noted that signal is often still increasing or almost constant before starting the decay towards the resting value (Fig. 1 and 5). Thus, in order to better quantify HR off-kinetics, a routine trimmed the data and fed the statistical procedure (exponential regression Least Squares Method) with just-decay values. We did not analyse the on-kinetics because, as expected, during the sprint, HR values are scattered (see the data between the first two vertical lines in Fig. 1 and 5) presumably due to both the interferences of the contracting thoracic muscles and of the belt vibrations on the ECG signal. However, as an indicator of the on-kinetics the overall HR variation from rest baseline to the beginning of the off-kinetics (Δ HR) was

included in the model.

The Multiple Regression has been designed to correlate a measure of metabolic power (= metabolic work / time, $\dot{V}O_{2peak}$) to three predictors: as two of them originally have units with time at the denominator (v_{test} and Δ HR), we decided to transform $\tau_{\rm off}$ into $v_{off} = \frac{1}{\tau_{off}}$, to increase the 'linearity' of their statistical effect.

Despite of the short duration and the simplicity of the test, its reliability in predicting the 'real' $\dot{V}O_{2peak}$ values has been checked in terms of a significant correlation both of the Multiple Regression and by an acceptable standard error of the estimate (also in relation to previous literature, see Fig. 6 and below). Among the predictors, when individually compared to $\dot{V}O_{2peak}$, only Δ HR does not significantly correlate (albeit negatively) with $\dot{V}O_{2peak}$ (see Fig. 2C). This can be ascribed to two contrasting effects: 1) Δ HR should be higher in highly fit subjects due to their faster on-kinetics, and 2) highly fit subjects (as shown by a significant correlation in Fig. 2A) run the same 60-m in a shorter time, thus not allowing HR to reach a high value. However, the regression made just by v_{test} and v_{off} as variates explains a smaller portion of the experimental $\dot{V}O_{2peak}$ variance (55%), witnessing the value of Δ HR in the multiple regression models.

The HR off-kinetics (voff, Fig. 2B) is positively related to VO2peak: fitter subjects showed a faster decay; this result is in line with previous literature (Darr et al., 1988; Sugawara et al., 2001; Yamamoto et al., 2001; Carnethon et al., 2005; Giallauria et al., 2005; Ostojic et al., 2011; de Mendonca et al., 2017). HR off-kinetics is characterised by a coordinated interaction of parasympathetic reactivation and sympathetic withdrawal (Perini et al., 1989; Imai et al., 1994 Dewland et al., 2007; Borresen and Lambert, 2008; Maeder et al., 2009; Daanen,

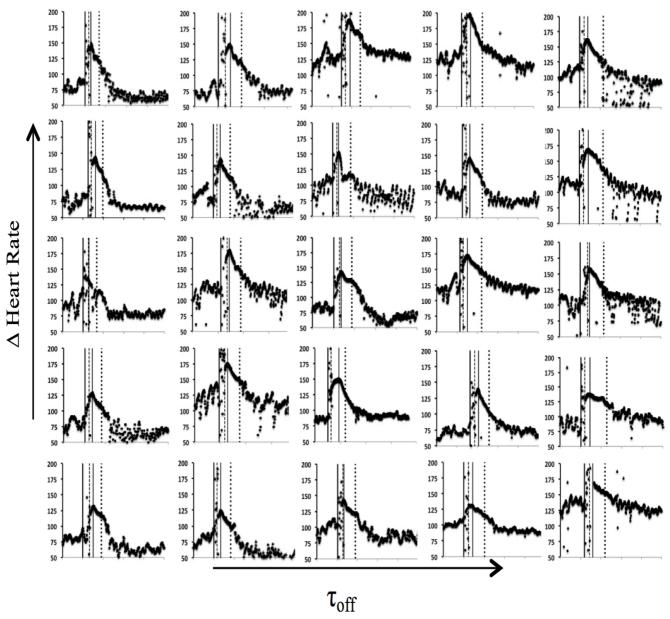


Figure 5. Heart rate recording of each participant (n = 25) during the sprint test and their respective markers: thick black vertical line as start of the sprint, dashed vertical line representing end of the sprint, thin vertical black line denotes the start decay of HR, and dotted line expresses the $\tau_{\rm off}$.

et al., 2012) and it seems that in trained subjects the adaptations in the efferent parasympathetic pathway could accelerate the vagus-mediated heart rate recovery (Imai et al., 1994; Dewland et al., 2007). On the other hand Hagberg et al. (1979) found that this faster recovery was not related to a more rapid recovery of the sympathetic response to exercise. Exercise intensity and type have been shown to influence the speed/tau of HR off-kinetics due to different energetic contribution and released metabolites during exercise and recovery

(Pierpont et al., 2000; Buchheit et al., 2007; Borresen and Lambert, 2008; Al Haddad et al., 2009; Nakamura et al., 2009; do Nascimento Salvador et al., 2016). Such heterogeneity in exercise related factors and different indexes used for defining the off-kinetics make a comparison of decays among different studies quite troublesome.

In the present study, subjects performed a shorter effort than those present in literature, reached a maximal HR of 150±20 bpm, which is the 79% of the maximal HR of the incremental test, in about 20 s. At the beginning of the exercise, the onset of HR is characterized by the fast vagal withdraw and the slower sympathetic activation. Even if our exercise duration was very short it seems that both mechanisms would have been activated in order to reach (and then recover from) the 79% of the maximal HR.

As shown in Fig. 3 and 4, both these regressions slightly overestimate and underestimate real values at low and high $\dot{V}O_{2peak}$, respectively. The predictive equation was verified with a random sampling approach. From the whole sample (n=25), 12 subjects were randomly extracted and used as a new control group for the predictions of the multiple regression that was performed on the other 13 subjects. This process was performed 35 times (taking care of avoiding duplicated group composition). We obtained 35 new predictive equations and average discrepancies between the measured and predicted $\dot{V}O_{2peak}$. The mean SEE was 6.31±0.82, similar to 5.28 obtained from processing the whole sample.

Although we used lab-quality sensor technology, the implementation of the proposed test on consumer wearables could manage the whole experimental protocol locally: continuous beat-by-beat HR would be used both to monitor/warn the subjects on the most appropriate time at which to start the 60-m sprint (i.e. when a rest steady state is reached) and to collect the recovery phase. GPS and 3-axis accelerometers could provide where and when, respectively, the 60-m sprint started and ended, from which the overall distance travelled can be checked and the average speed calculated.

The use of a 'traditional' thoracic belt sensor (Polar S410, Kempele, Finland) has been driven by the need of the most accurate, beat-by-beat HR sensor. There is no such a capability, so far, in most consumer wrist-wearable devices. Even Apple Watch (Apple Inc., California, USA), which has been

mentioned for a very high reliability in processing physiological data during physical exercise (Chowdhury et al., 2017; Wang et al., 2017), does not output beat-to-beat intervals. Blood oxygenation pulsations (photo-plethysmography) are detected by photodetectors measuring the bounced back infrared light emitted by LED diodes located between wrist and watch. The fluctuations in blood colour absorption due to the local volume changes are measured resulting in HR data. A few studies have emphasized the accuracy of these wrist-worn devices (Spierer et al., 2015; Wallen et al., 2016; Chowdhury et al., 2017; Wang et al., 2017) during rest and exercise. Nevertheless, no system at present seems to be confident enough to deliver single beat interval/frequency, probably due to motion induced artifacts, a problem that could be solved by sensor redundancy and/or signal processing enhancing signal-to-noise ratio.

Current technology confines the time resolution of most of those devices (Parak et al., 2015) to about 5 seconds, within which an average heart frequency is computed. Although our investigation is particularly meant for next, beat-by-beat sensors, we tested the predictive ability of the proposed algorithm when HR data was provided at 0.2 Hz (as in the actual versions). This was achieved by manipulating the recorded single-beat sequences as to obtain an average value every 5s. In Fig. 4 grey points reflect the approximation involved in using 5s average HR data, which resulted quite similar to single-beat regressions (R² = 59%).

The proposed, indirect $\dot{V}O_{2peak}$ test is certainly not meant to replace the usual direct metabolic measurements and protocols done in a research or clinical laboratory setting, where a much higher accuracy is required in the assessment of subject/athlete's aerobic fitness. By using a portable smart device with multiple sensors and the suggested algorithm, a handle $\dot{V}O_{2peak}$ estimation is at reach for individuals who could later decide whether or not to deepen the awareness of their health status. However when compared with many other $\dot{V}O_{2peak}$ predicting submaximal protocols done on similar subjects, based on different physical activities and with much longer exercise duration (thus more distressful conditions, quoted in Fig. 6), the SEE was quite similar: 5.28 for the 60-m sprint vs. 4.63 \pm 1.58 (average) of the literature.

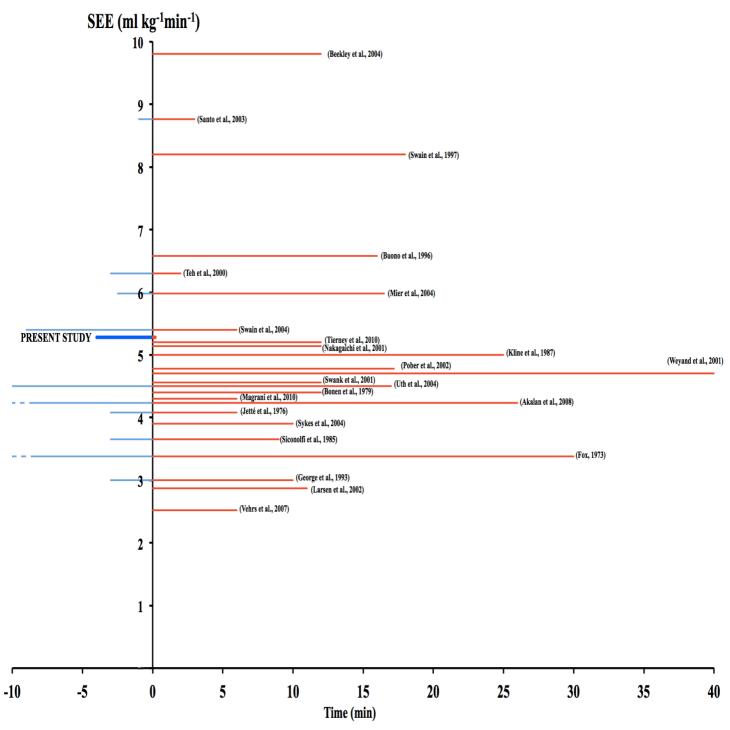


Figure 6. Accuracy of the $\dot{V}O_{2peak}$ prediction, as SEE (ml.kg⁻¹.min⁻¹), is presented in relation to average protocol duration (t, min) clustered in exercise (red positive bars) and rest (blue negative bars) time. Present data is shown as thick lines. SEE of other predictive equations on submaximal protocols are shown for comparison with their bibliographic reference. A more detailed discussion about the quoted investigations can be found in Sartor et al., 2013 review.

The proposed test leaves space for improvement: a) HR kinetics are known (Astrand et al., 1986) to be affected by a number of conditions (age, body and ambient temperature, over-training, altitude, fatigue, hydration, etc.) here not taken into account, b) off-kinetics only have been considered, but new processing techniques (e.g. Salehizadeh et al., 2016) could allow to include HR on-kinetics to better infer $\dot{V}O_{2peak}$, and c) new refined modeling approaches (e.g. Zakinthinaky, 2015) could help to incorporate in the algorithm and detect slightly differences in the delayed off-kinetics start that could better estimate the fitness level, d) a greater sample size with inter-subject repeatability could enhance the power of the predictive equation.

Results from the current investigation encourage to develop new simple methods to infer individual physiologic variables by exploiting the current and next portable technology. Watches and bracelets are the perfect candidates, with respect to smart phones, because of their small size and the increased computational power capable to sample and process the data on-board, with no immediate need of external connection.

CONCLUSION

Then, a simple and short test (60-m sprint run) could reasonably predict individual VO_{2peak} based on the heart rate off-kinetics immediately after the sprint. This test can be easily managed by all individuals with the new wrist wearable, heart band free, multi-sensor smart devices and the proposed algorithm.

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Appendix

Table 1. Technical features of current smartwatches on commerce.

Device Model	Operator System	Display	Processor	Onboard storage (GB)	Battery	Connectivity
Apple Watch 3	WatchOS 4	1.53" OLED	S2 - Dualcore	8 / 16	18 hours	Wi-Fi; Bluetooth; NFC;
LG Watch Style	Android 4.3+	1.2" 360 x 360 P- OLED	Snapdragon Wear 2100	4	Up to 24 hours	Wi-Fi; Bluetooth;
Samsung Gear 3	Tizen OS	1.3" 360 x 360 Super AMOLED	Dual-core 1.0GHz	4	3 days	Wi-Fi; Bluetooth; 4G;
LG Watch Sport	Android Wear 2.0	1.38" OLED	Snapdragon Wear 2100	4	16 hours	Wi-Fi; Bluetooth; NFC;
Fitbit Ionic	Fitbit OS	TBC, 1000 nits	Dual-core 1.0GHz	2.5	2-3 days	Wi-Fi; Bluetooth;
Moto 360	Android Wear 2.0	1.37" or 1.56" LCD	Quad-core 1.2GHz	4	1.5-2 days	Wi-Fi; Bluetooth;

AMOLED: Active-matrix organic light-emitting diode;

LCD: liquid crystal display;

NFC: Near Field Communication;

OLED: Organic light-emitting diode;