Modifications of viscoelastic properties and physiological parameters after performing uphill and downhill running trials

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ABSTRACT

BACKGROUND: Trail running performance depends on many factors, including energy cost of running, biomechanical parameters, and stiffness. The aim of this study was to examine the influence of different positive and negative slopes on metabolic cost, tight hemoglobin saturation, viscoelastic properties, and vertical peak impacts in physically active young runners.

METHODS: Nine healthy male volunteers (26±5 years) performed two separate uphill and downhill sessions on an instrumented treadmill; both sessions were completed in a random order at a constant running speed with variable slopes from 0% to ±20%. Oxygen uptake (VO_2), carbon dioxide production (VCO_2), pulmonary ventilation (VE), respiratory exchange ratio, heart rate (HR), muscle oxygen saturation, vertical impacts, and muscle tone and stiffness were assessed.

RESULTS: During downhill running, VO_{2peak} and VCO_2 significantly decreased, and impacts higher than 6G significantly increased with a negative slope. During uphill running, VO_{2peak}, VCO_2, VE, and maximum HR significantly increased. Minimum values of oxygen saturation and the vastus medialis tone significantly decreased and impacts of 4–5 G significantly increased with a positive slope.

CONCLUSIONS: Metabolic demand increased proportionally with the uphill slope and showed a linear negative relationship with a light and moderate downhill slope. Vertical impacts of high G-forces increased during downhill running, data that indicate the importance of our ability to attenuate impacts. Finally, muscle tone and stiffness remained stable at all times, results that demonstrated their acute adaptation to running in the absence of extreme fatigue.

Key words: trail running, muscle oxygen saturation, muscle tone, stiffness, VO_2,

Introduction

There has been an exponential increase in the worldwide popularity of trail running over recent years, with a growing number of participants (1). This ~~novel~~ modality is defined by the International Trail Running Association (ITRA) as a pedestrian foot race in natural environment with less than 20% of the race on asphalt roads and is classified in seven different categories depending on the elevation and distance of the race (2). Trail running performance depends on many factors that change according to the duration and inclination of the race analyzed; there are still ~~controversial~~ different opinions about these factors.

~~**On the one hand some authors consider maximal aerobic speed (MAS), fraction of maximal aerobic speed sustained (ratio between mean speed and MAS) and knee extensors force as performance determinants in the modality of mountain ultramarathon (3).**~~

~~**Likewise maximal sustainable power, energy cost of running and walking, psychological and motivational factors, and the ability to minimize lower limb tissue damage and muscle fatigue are considered as relevant factors to take into account for ultra-trail races (4).**~~

~~**On the other hand others suggest that lactate thresholds correlate with race performance times in short trail running races (21 and 31 km), finding that the anaerobic lactate threshold at a speed of 4 mmol/l and the individual lactate threshold correlated better ($r = 0.87$ and $r = 0.84$, respectively) than aerobic lactate threshold ($r = 0.65$) (5) .**~~

~~**Trail running races demand a great amount of energy; they require a good development of aerobic metabolism to cover this energetic expenditure (6). The oxygen uptake (VO_2) shows a general image of the body response to exercise, reflecting the adaptation of the cardiorespiratory system (VO_2 depends on cardiac output and the arterio-venous difference of oxygen) (7). The VO_2max (which establishes the upper limit of performance in endurance races and indicates the maximum aerobic capacity), the ability to sustain a high percentage of VO_2max and the energy required to cover a certain distance (also called running economy [RE]), are key determinant to take into account for running performance (8). Runners who consume the same or less energy (adenosine triphosphate production) at a higher running speed are considered more economical (29). Indeed, an increase of 5% in RE causes a rise of 3% in several factors**~~

~~(metabolic, cardiopulmonary, neuromuscular and biomechanical) that determine this value (8,11–16).~~

~~The energy cost of running increases linearly with running speed in level running (17), nevertheless the presence of slopes change this relationship as we can observe in some studies who have demonstrated that the energy cost of running is independent of speed and related only to the incline: it increases linearly with positive slope and decreases linearly with decline slope until reaching extreme negative slopes (from -20%) where the energy cost of running increases again (18–20).~~

Trail running races demand a great amount of energy what require a good development of aerobic metabolism to cover this energetic expenditure (6). It has been suggested that those runners who consume the same or less energy (adenosine triphosphate production) at a higher running speed are considered more economical (29), this aspect has been observed by the ability to sustain a high percentage of VO_2max and the energy required to cover a certain distance (also called running economy [RE]), (8). Changes in the running economy can cause alterations at the metabolic, cardiopulmonary, neuromuscular and biomechanical level during submaximal running (8,11–16). In addition, Balducci et al (2017) have established maximal aerobic speed (MAS), fraction of maximal aerobic speed sustained (ratio between mean speed and MAS) and knee extensors force as performance determinants in the modality of mountain ultramarathon (3). Complementary to these parameters the maximal sustainable power, psychological and motivational factors, and the ability to minimize lower limb tissue damage and muscle fatigue are considered as relevant factors to take into account for ultra-trail races (4).

The first aim of our study consists on analyze the relatioship between slope and energy cost of running. At level running there is a linear relationship between running speed and energy cost of running (17), but both trail running and many level running races own slopes that could change this relationship, as we can observe in some studies who have demonstrated that the energy cost of running is independent of speed and related only to the incline: it increases linearly with positive slope and decreases linearly with decline slope until reaching extreme negative slopes (from -20%) where the energy cost of running increases again (18–20).

Using near-infrared spectroscopy (NIRS) during exercise allows researchers to assess the skeletal muscle fractional oxygen extraction, which is a result of the dynamic balance between delivery and use of oxygen. This method discriminates between exercising muscles and the rest of the body, which is not possible using only global gas exchange (21). The skeletal muscle fractional oxygen extraction can only increase 3–4 times above resting values during exercise, whereas VO_2 can increase up to 100 times (22). Trail running performance depends on the capacity to satisfy the metabolic requirements of the exercising muscles. For this reason, fractional oxygen extraction, peripheral oxygen diffusion and oxidative metabolism increase, and PO_2 microvascular decreases. At rest, fractional oxygen extraction is approximately 25%, but during maximal exercise, it can increase to 75% (22). With incremental exercise, the arterial-venous O_2 difference increases, a phenomenon that leads to a decrease in venous O_2 saturation. Muscle tissue oxygen extraction quickly increases during the onset of exercise and thereafter, a more progressive increase until the steady state (23). Specifically these changes were analyzed after a trail running race, observing a impairment of oxidative function of skeletal muscle after the race, manifested by a reduced peak VO_2 and peak fractional O_2 extraction of vastus lateralis (24). During exercise, NIRS could be a very useful tool in order to monitor the exercise intensity, analyzing the tissue saturation index (TSI) (calculated by the oxygenated hemoglobin expressed as percentage to the total hemoglobin) (25), since heart rate does not reflect the metabolic and cardiorespiratory effort done in a reliable way, because it could overestimates the VO_2 and energy expenditure in exercise with a great contribution of anaerobic metabolism, dehydration, high temperatures and humidity (26). Based on these results, our aim is to isolate the effect of the slope on these variables and observe the specific influence of each slope in the pheriferal oxidative function in real time.

From a biomechanical point of view ~~trail runners also adopt different strike patterns depending on the slope, using mid-foot and fore-foot with uphill running and mid-foot or rear-foot with downhill running (20). This technique represents a protective strategy: running with a mid-foot or fore-foot strike diminishes the vertical impact peak during ground contact~~ (27)~~.~~ downhill running modifies the magnitude of an impact and thus produces greater peak forces than level running and uphill running. Consequently, the perpendicular peak impact accelerations decreases with the slope (20). These increases in vertical impacts might affect kinematic and metabolic responses during trail running.

Recently, many physiologists have begun to study the properties of relaxing muscle given their relationship with pathological processes. The muscle mechanical properties are muscle tone and stiffness. Muscle tone can be defined as the generated tension in response to changes in muscle length (28). Stiffness is the resistance to any deformation evoked by any pressure or the ratio of tension developed in muscle when it is stretched and contributes to muscle tone, and it is determined by elastic and absorbing properties of contractile apparatus and elasticity of tendons and connective tissue (28,29). ~~Muscle tone is key for controlling balance, stability, and posture through modifications in the passive muscle tension through changes in the viscoelastic intrinsic properties of the muscle without contractile activity, thus maintaining the posture with minimally increased energy costs for prolonged duration without fatigue(30).~~

Recent findings from several studies support the importance of stiffness in overall athletic performance, specifically from actions of quickly stretch-shortening cycle (31). The authors indicated that retain a higher stride frequency through owning higher vertical leg stiffness is key for improving performance time during maximal sprint running (32,33). Also the importance of the spring mass model characteristics as stiffness and the properties of the Achilles tendon to determine the energy cost of running in half marathon runners have also been demonstrated (34). However, the analysis of the isolated adaptation of the viscoelastic parameters of the main skeletal muscles involved in running during the performance of different slopes, both uphill and downhill is still unclear.

The aim of the present study was to analyze the influence of different degrees of positive and negative slopes on the metabolic cost, tight hemoglobin saturation, viscoelastic properties, and vertical peak impacts in physically active ~~young~~ runners. We hypothesized that uphill running races will increase muscle tone and stiffness of the triceps surae muscle and metabolic cost and will decrease muscle hemoglobin saturation, and vertical impact peaks. Furthermore, downhill running will increase muscle tone and stiffness of vastus medialis muscle, hemoglobin saturation, and vertical impact peaks and will decrease the metabolic cost.

Materials and methods

Participants

Nine healthy men (mean± standard deviation [SD]: age 26 ± 5 years; body mass 71.7 ± 8.1 kg; height: 173 ± 0 cm) volunteered for this study. The participants were students of "Sport Sciences" at Universidad San Jorge (Spain). All of them accomplished the minimum requirement of a 10 km running race in less than 50 min, none of them had health problems that prevent the practice of physical activity, and none had suffered skeletal muscle injuries during the last 3 months. Each participant signed a written informed consent form. This study was approved by the ethics committee of the institution (Ref 004-18/19). All participants were informed about the experimental procedures and were free to abandon the study.

Experimental design

The study comprised two sessions. During the first one, each athlete followed a standardized warm-up (run 5 min at 8 km/h, perform 5 single leg squat jumps, 5 lunges with each leg, and 5 bilateral maximal counter-movement jumps) to increase the neuromuscular activation.
After warming up, the participants ran for 5 min on an inclinable treadmill (HP Cosmos Pulsar 4.0, h/p/cosmos sports & medical gmbh, Germany) at five uphill angles (0, 5, 10, 15, and 20%), with a 10 min rest between trials. During the second session, the participants followed the same protocol (same warm-up) but changed to negative slopes, performing 5 min on the treadmill at ~~five~~ four downhill angles (~~0,~~ -5, -10, -15, and -20%), again with a10 min rest between trials. The orders of the slopes for each subject were randomized for each session. The speed was set to 10 km/h for all the slopes except for 15% and 20% in uphill, where the speeds were 8 and 6 km/h, respectively (due to the inability of the participants to run at those speeds with such slopes). The participants were tested on two different days with a week rest between the sessions.

Ventilatory parameters

A breath-by-breath gas exchange protocol was used to obtain ventilatory information (K5, Cosmedsrl, Italy) (35). Subjects wore a facial mask that covers the nose and mouth to analyze the expired air and assess the rates of oxygen uptake (VO_2), carbon dioxide production (VCO_2), pulmonary ventilation (VE), and respiratory exchange ratio (RER). Moreover, the analyzer was connected to a chest band that allowed us to assess the heart rate

(HR). We analyzed the peak and mean value of each ventilatory parameter during the 5 minutes of each trial.

Hemoglobin saturation data acquisition

The Humon hex near-infrared spectroscopy (NIRS) device (HUMON, USA) was used in this study. It measures localized muscle oxygen saturation (SmO_2). This device uses the different absorption light spectra of oxyhemoglobin (HbO_2) and deoxyhemoglobin (Hb). This value can be utilized to determine the muscle oxygen saturation (Eq. 1)

$$SmO_2 = \frac{HbO_2}{HbO_2 + Hb} * 100\% \qquad \text{Eq.1}$$

The Humon hex device uses two light sources and three photodetectors to measure the intensity of the propagated light in the tissue. The device is 6.0 x 5.7 x 1.4 cm; it has a plastic case for contact with the skin. The wearable device is attached to the leg (quadriceps muscle) with a strap, and the smartphone app allows for the collection of *in vivo* data during exercise. The Humon hex is clinically validated as real-time oxygen wearable for endurance sports by Harvard Medical School and the Biomechanical Center of Massachusetts General Hospital **(36)**.

Viscoelastic parameters

In order to assess viscoelastic parameters, we used a Myoton-Pro (Myoton AS, Tallinn, Estonia) to record damped natural oscillation of soft tissues as the acceleration signal and thus analyze viscoelastic parameters as tone and stiffness. A low force mechanical impulse was applied to cause a damped oscillation, and information was recorded with a three-axis accelerometer. The medial and lateral gastrocnemius, biceps femoris, and vastus medialis of both legs were assessed. To perform standardized measurements, we first made marks on the skin in each muscle measured in order to always asses the same anatomical point with the device. Marks were made based on the instructions from Surface Electromyography for the Non-Invasive Assessment of Muscles (SENIAM; 16) for the next skeletalmuscles: gastrocnemius medialis, gastrocnemius lateralis, biceps femoris and vastus medialis of each leg.

- ~~Gastrocnemius medialis: on the most prominent bulge of the muscle;~~
- ~~Gastrocnemius lateralis: at one third of the line between the head of the fibula and the heel;~~

- ~~Biceps femoris: at 50% on the line between the ischial tuberosity and the lateral epicondyle of the tibia;~~
- ~~Vastus medialis: at 80% on the line between the anterior spinailiaca superior and the joint space in front of the anterior border of the medial ligament.~~

Impact data acquisition

A STATSports Apex (STATSports Group, Newry, UK) device can calculate over 50 metrics in real time. It integrates 100 Hz three-axis accelerometer technology and the collected information can identify maximum accelerometer impacts of 2 g in a 0.1 s period. For this study, accelerometers were placed in the upper back and we used the percentage of impacts in each positive and negative slope and sorted the impacts in six zones: zone 1, impacts between 3 and 4 G-force; zone 2, impacts between 4 and 5 G-force; zone 3, impacts between 5 and 6 G-force; zone 4, impacts between 6 and 7 G-force; zone 5, impacts between 7 and 8 G-force; zone 6, impacts between 8 and 9 G-force. We decided to dismiss those impacts below 3 G-force and above 9 G-force. All data were collected and organized with Excel® (2016, Microsoft, Inc., Redmond WA) for further statistical analysis.

Statistical analysis

We analyzed the data using SPSS (IBM SPSS version 22.0, Chicago, IL, USA). Descriptive statistics are presented as the mean ± SD. The Shapiro-Wilk normality test was used to determine the distribution (uphill stiffness of all muscles, uphill VO_2, VE and HR; and downhill VO_2, VCO_2, RER, and SbO_2 were normally distributed. The rest of analyzed variables were not normally distributed). The effect of slope on dependent variables was assessed using Kruskal–Wallis analysis of variance (ANOVA; non-normal data) orrepeated measures ANOVA (normal data). We followed up with Kruskal–Wallis or Bonferroni post hoc tests when significant differences were detected. The comparison between the same inclination with opposite slope was analyzed using related samples analysis (T-test/Wilcoxon). A pvalue<0.05 was considered statistically significant. Additionally, we analyzed the clinical changes using Cohen's d effect size (ES). The scale used to interpret Cohen's d was: d=0.2–0.5, a "small" ES d=0.5–0.8, a "medium" sizes, and d≥0.8, a "large" ES **(38)**.

Results

Table I summarizes the values of all variables in relation to the slopes in uphill or downhill running. Table II summarizes the comparison of the uphill and downhill slope with the same inclination.

Ventilatory parameters

Ventilatory parameters presented several significant changes. VO_2 peak (mL/min/kg) significantly increased when the slope changed from 0% to 10% (difference of means mean standard unit difference= 23.2, p<0.001, ES=3.50), to 15% (difference of means mean standard unit difference =24.8, p<0.001, ES=2.45), and to 20% (difference of means mean standard unit difference=28, p<0.001, ES=3.72). There was also a significant change from 5% to 20% (difference of means mean standard unit difference =14.9, p= 0.0042, ES=1.82). We also observed significant changes in downhill running, where the VO_2 peak decreased from a slope of 0% to 15% (difference of means mean standard unit difference= -12.2, p=0.016, ES=1.91) and to 20% (difference of means mean standard unit difference= -11.4, p=0.03, ES=2.20; Figure 1A). (Table I)

Comparing the positive slope with their negative counterpart, we also observed significant changes in all slopes: between 5% vs -5% (difference of means = 15.9, p= 0.003, ES=1.95), 10% vs -10% (difference of means =30.5, p<0.001, ES=3.91, 15% vs -15% difference of means =37, p<0.001, ES=3.44) and 20% vs-20% (difference of means =39.2, p<0.001, ES=5.52). (Table II)

The pulmonary ventilation peak (VE; L/min) significantly increased when the slope changed from 0% to 10% (difference of means mean standard unit difference=65.7, p=0.01, ES=3.14), to 15% (difference of means mean standard unit difference=64.5, p=0.01, ES=2.52), and to 20% (difference of means mean standard unit difference =83.5, p<0.001, ES=3.12).There was also a change from 5 to 20% (difference of means mean standard unit difference=55.9, p<0.001, ES=1.93) in uphill running. However, we cannot affirm the same with downhill running because there were no significant changes (Figure 1B). (Table I)

Comparing the positive slope with their negative counterpart we observed significant changes in all slopes: between 5% vs -5% (difference of means =31.4 , p= 0.004, ES=1.85), 10% vs -10% (difference of means =70.5, p<0.001, ES=4.09, 15% vs -15% difference of means =74.2, $p<0.001$, ES=3.14) and 20% vs-20% (difference of means =90.1, $p<0.001$, ES=3.66). (Table II)

Maximum HR (beats per mimute [bpm]) in uphill running was significantly increased when the slope changed from slope 0% to 5% (difference of means mean standard unit difference=24.7, p=0.002, ES=1.62), to 10% (difference of means mean standard unit difference=38.4, p=0.002, ES=2.68),to 15% (difference of means mean standard unit difference=38.4, p=0.002, ES=2.40), and to 20% (difference of means mean standard unit difference=38.7, p=0.002, ES=2.78; Figure 1C). However, we did not observe significant changes with downhill running.(Table I)

Comparing the positive slope with their negative counterpart we observed significant changes in all slopes. Between 5% vs -5% (difference of means = 35.6, p= 0.013, ES=2.25), 10% vs -10% (difference of means =54.1, p= 0.001, ES=4.09, 15% vs -15% difference of means =56.3, p=0.001, ES=3.64) and 20% vs-20% (difference of means =51.3, p=0.002, ES=4.87). (Table II)

In addition to these results, we found that VCO_2 (mL/min/kg) was significantly increased in uphill running when the slope changed from slope 0% to 5% (difference of means mean standard unit difference=8.1, p=0.001, ES=1.70), to 10% (difference of means mean standard unit difference=18.8, p=0.001, ES=2.35), to 15% (difference of means mean standard unit difference=18.7, p=0.001, ES=1.99), and to 20% (difference of means mean standard unit difference=19.8, p=0.001, ES=1.85). Similar to VO_2 values, there were significant decreases in downhill running, specifically from 0 to 10% (difference of means mean standard unit difference= -6.6, p=0.024, ES=1.68), to 15% (difference of means mean standard unit difference= -6.7, p=0.031, ES=1.40), and to 20% (difference of means mean standard unit difference= -6.6, p=0.045, ES=1.46; Figure 1D). Nevertheless, no significant changes were found in RER values. (Table I)

Comparing the positive slope with their negative counterpart we observed significant changes in all slopes: between 5% vs -5% (difference of means = 10.5, p= 0.006, ES=1.84), 10% vs -10% (difference of means =25.4, p= 0.001, ES=3.34, 15% vs -15% difference of means =25.4, p= 0.001, ES=2.69) and 20% vs-20% (difference of means =26.4, p= 0.002, ES=2.47). (Table II)

Saturation data

For NIRS values, we found significant decreases in the minimum, mean, and maximal values of oxygen saturation percentage only in uphill running.

There were changes in minimum values when the slope changed from 0% to 5% (difference of means ~~mean standard unit difference~~=4.6, p=0.003, ES= 0.77), to 10% (difference of means ~~mean standard unit difference~~= -17.6, p=0.003, ES= 1.72), to 15% (difference of means ~~mean standard unit difference~~= -11.4, p=0.003, ES= 1.28), and to 20% (difference of means ~~mean standard unit differences~~ = -16.3, p=0.003, ES= 2.20; Figure 2A).

With respect to mean values, there were significant changes when the slope increased from 0% to 5% (difference of means ~~mean standard unit difference~~ = -3.1, p =0.006, ES= 0.62), to 10% (difference of means ~~mean standard unit difference~~= -13.4, p=0.006, ES= 1.62), to 15% (difference of means ~~mean standard unit difference~~ = -8, p=0.006, ES= 1.42), and to 20% (difference of means ~~mean standard unit difference~~= -9.6, p=0.006, ES= 1.81; Figure 2B). (Table I)

In the minimum values of oxygen saturation percentage we also observed significant changes between 10% vs -10% (difference of means = -15.8, p= 0.008, ES=2.84) and 20% vs -20% (difference of means = -13.8, p= 0.042, ES=1.51). In the mean values of oxygen saturation percentage we observed significant changes between 10% vs -10% (difference of means = -13.4, p=0.003 , ES=1.74), 15% vs -15% % (difference of means = -8.7, p= 0.021, ES=1.80) and 20% vs -20% (difference of means = -9.4, p=0.015 , ES=1.86) (Table II)

Impact data

For impact variables, there were only significant changes in uphill running in the percentage of impacts in Zone 2 (4–5 G), with decreases when the slope changedfrom 0% to 10% (difference of means ~~mean standard unit difference~~= -20.5, p=0.049, ES=0.80), to 15% (difference of means ~~mean standard unit difference~~= -26.6, p=0.049, ES= 1.31), and to 20% (difference of means ~~mean standard unit difference~~= -30.3, p=0.049, ES=1.63; Figure 3A).

For downhill running, there were impact increases in Zone 4 (6–7 G) when the slope changed from 0% to 5% (difference of means ~~mean standard unit difference~~=6.8, p=0.015, ES= 0.89), to 10% (difference of means ~~mean standard unit difference~~=13.5, p=0.015, ES=1.26), 15% (difference of means ~~mean standard unit difference~~=23.9, p=0.015, ES=2.79), and to 20% (difference of means mean standard unit difference=15.2, p=0.015, ES=2.08; Figure 3B).

In Zone 5 (7–8 G), the differences were noted when the slope changed from 5% to 10% (difference of means mean standard unit difference=1.52, p=0.003, ES=0.69), to 15% (difference of means mean standard unit difference=10.8, p=0.003, ES=2.76), and to 20% (difference of means mean standard unit difference=9.98, p=0.003, ES=1.27), as well as from 10% to 15% (difference of means mean standard unit difference=9.26, p=0.003, ES=2.24) and to 20% (difference of means mean standard unit difference=8.46, p=0.003, ES=1.06; Figure 3C). Finally, in Zone 6 (8–9 G), the differences were when the slope changed from 10% to 15% (difference of means mean standard unit difference=2.18, p=0.023, ES=0.87) and to 20% (difference of means mean standard unit difference=4.22, p=0.023, ES=0.94; Figure 3D). (Table I)

Comparing the positive slope with their negative counterpart we observed significant changes in several zones. In Zone 1 (3-4 G), between 5% vs -5% (difference of means = 35.6, p= 0.045, ES=1.41) and 15% vs -15% (difference of means =67.2, p=0.006 , ES=5.18). In Zone 2 (4-5 G) between 20% vs -20% % (difference of means = -17.7, p= 0.01, ES=1.77). In Zone 3 (5-6 G) between 5% vs -5% (difference of means = -12.2, p= 0.043, ES=0.93), 10% vs -10% (difference of means = -16.1, p= 0.015, ES=1.78), 15% vs -15% (difference of means = -19.5, p= 0.004, ES=3.07) and 20% vs-20% (difference of means = -16.3, p=0.003 , ES=2.57). In Zone 4 (6-7 G) between 10% vs -10% (difference of means = -13.4, p= 0.007, ES=1.26), 15% vs -15% (difference of means = -23.9, p= 0.003, ES=2.8) and 20% vs-20% (difference of means = -15.3, p=0.003 , ES=2.1). In Zone 5 (7-8 G) between 10% vs -10% (difference of means = -2.84, p=0.011 , ES=1.56), 15% vs -15% (difference of means = -12.1, p= 0.003, ES=3.27) and 20% vs -20% (difference of means = -11.3, p= 0.003, ES=1.46). Finally in Zone 6 (8-9 G) between 15% vs -15% (difference of means = -3.58, p= 0.003, ES=2.72) and 20% vs-20% (difference of means = -5.62, p= 0.003, ES=1.44). (Table II)

Viscoelastic parameters

As shown in Figure 4, only muscle tone significantly changed in uphill running. Specifically, the vastus medialis tone decreased from a slope of 10% to 20% (difference of means mean standard unit difference= -0.8, p= 0.004, ES=0.64) and significantly increased from 0% to 10% (difference of means mean standard unit difference=0.7, p= 0.004, ES=0.61) and 5% to

10% (difference of means mean standard unit difference=0.2, p=0.045, ES=0.12). There were no significant differences in positive slopes for the other muscles. Furthermore, muscle tone did not differ for any muscle during downhill running. With respect to muscle stiffness, there were no changes in any muscle for uphill or downhill running. (Table I)

Comparing the positive slope with their negative counterpart we only have observed significant changes in stiffness values of biceps femoris muscle between 10% vs -10% (difference of means = -34.9, p= 0.033, ES=0.79). (Table II)

Discussion

This study explored the possibility of finding viscoelastic, impacts, ventilatory, and muscle oxygenation changes at different uphill and downhill slopes. The use of an uphill and downhill running test on a treadmill at a given velocity allowed us to better differentiate the specific influence of the slope and reasonably replicate overground graded running effect **(39)**. We identified significant increases in ventilatory demand with downhill and uphill slope, as well as increases in vertical impact accelerations with downhill slope. However, there were no changes in muscle tone and stiffness.

Ventilatory parameters

Most studies related to trail running performance in this discipline suggested that the oxygen cost of running depends on incline rather than running speed as expressed in terms of oxygen uptake (18,19). In this sense we have observed that at all negative slopes and light and moderate positive slopes whose speeds were the same, the oxygen uptake increased linearly with slope, agreeing with the literature. With extreme positive slopes VO_2 continued to increase but to a lesser extent, possibly due to a progressive decrease in running speed. Regarding downhill running, VO_2 decreased with negative slope, founding the lowest values of VO_2 with extreme downhill slopes, agreeing with recent researches (19,40). according with recent researches VO_2 values decreased to -20% (reaching a plateau) (19,40). We found minimum values of VO_2 with extreme downhill slopes.

In this sense, our results agree with the literature from the point of view of the relationship with the slope, because at all negative slopes and light and moderate positive slopes whose speeds were the same, the oxygen uptake increased linearly with slope.

Indeed, with a higher incline, with a higher VO_2 and extreme positive slopes (10%, 15%, and 20%), VO_2 stabilized possibly due to a progressive decrease in running speed. In addition, VO_2 decreased with downhill slopes, according with recent researches VO_2 values decreased to −20% (reaching a plateau) (19,40). We found minimum values of VO_2 with extreme downhill slopes.

We also found important significant changes in VCO_2, where there were similar CO_2 values with negative slopes (lower values compared to level running), but in uphill running this production increased up to the 10% gradient. With regards to oxygen uptake, the production of CO_2 seems similar with 10%, 15%, and 20% slopes.From 10% gradient, the production of CO_2 stabilized. Related to our results, there are other studies that also observed an increase of lactate levels, which are related to VCO_2, CO_2 is generated when lactate is increased during exercise because its hydrogen concentrations are buffered by bicarbonate (HCO_3^-; 22, 28). The highest increase of lactate values and CO_2 production were found with moderate uphill slopes, while with extreme positive slopes no major increases were observed; just as it happens in our study (19). These results could be a consequence of the reduction of running speed with positive inclination increase, carried out both in Minetti's study and in ours.

The highest values of lactate were found with moderate uphill slopes. Slightly lower values were previously noted with respect to the maximum values observed with extreme uphill slopes (19). We made similar observations; the VCO_2 values for the more positive gradient slopes were similar and, as Minetti did, we also reduced running speed with as positive inclination increased. The elevated VCO_2 demonstrated the aerobic-anaerobic transition, which prevailed to a greater extent anaerobic energy system compared with level running. The main explanation for this transition might be the increase of positive mechanical work with the slope and the higher concentric activation requirement of skeletal muscle (20).

In relation with previous parameters, We also analysed pulmonary ventilation (VE) and maximum HR. These values were always similar downhill, but uphill, VE increased with slope. The 20% slope was an inflexion point because when the slope was 10 or 15% the VE values were similar. HR maximum values also increased with slope, and athletes reached the same maximum values at 10, 15, and 20% slopes.

Recent studies demonstrated that during uphill running, HR and VE increase, accompanied with the disturbance in the acid-base balance. (19). Therefore, an elevated effort during a prolonged slope might lead to fatigue due to exceeding the maximum steady state. Although running economy depends on more factors, if VE increases, the oxygen uptake and running economy will also increase (some studies have found a moderate correlation between changes in VE and RE) (12). Therefore, those participants with lower pulmonary ventilation at a given running velocity might have better running economy due to the decreased aerobic demand. Although more research is necessary, pulmonary ventilation could represent up to 6–7% of the total oxygen cost of exercise (19).

Contrasting same inclinations uphill versus downhill, we have observed that uphill running entails a higher cellular oxygen demand, which determines a quick physiological response of all factors involved in the transport of oxygen from atmosphere to the mithocondria with the aim of increasing the oxygen supply. At the pulmonary level, with an increase in ventilation and gas exchange; at the cardiocirculatory level, with an increase in cardiac output and therefore heart rate and at the skeletal muscle microcirculation level, as we will see below, with an increase in oxygen extraction in skeletal muscle.

Hemoglobin saturation data

Whole-body VO_2 ~~Global oxygen uptake~~ depends on cardiac output and arteriovenous oxygen difference, mainly produced by the active skeletal muscle oxygen consumption (VO_{2m}) in exercise. We assessed the hemoglobin oxygen saturation (SbO_2) with NIRS in the quadriceps femoris muscle, a measure that might be influenced by VO_{2m}. We observed that SbO_2 decreased at 5 and 10% slopes when running uphill, perhaps because VO_{2m} increased. At 15% and 20% slopes, SbO_2 values were similar, as were the global VO_2 values (possibly due to reduced speed running). The VO_{2m} response and maximum HR might limit the VO_2 response because they stabilized at 15% and 20% uphill slopes. A decrease in muscle oxygenation would indicate a more pronounced increase in muscle oxygen utilization compared to muscle oxygen transport. Shibuya et al. found a significant linear relationship between percentage of SbO_2 at exhaustion and VO_2max; lower levels of SbO_2 correspond to lower levels of VO_2max (41). Moreover, low blood pH contributes to decrease muscle oxygenation. The elevated CO_2 production that is linked with H^+ accumulation—and joined with core temperature increase—helps to modify the oxygen-hemoglobin dissociation curve and allow the separation between oxygen and hemoglobin inside skeletal muscle (42).

A recent study, De Lorey et al. (43) analyzed changes in muscle deoxygenation with NIRS in vastus lateralis during constant-load and leg-cycling exercise. They observed a delay before an increase in muscle deoxygenation at the onset of exercise. Subsequently, a rapid muscle deoxygenation occurred that reflects the increase of oxygen extraction in the active muscle because of an increase of muscle oxygen consumption. Differences among subjects might be due to individual variability in local muscle blood flow regulation (43). NIRS studies also demonstrated that muscle reoxygenation after exercise might be important to establish muscle performance and training status (44).

Impact data

Vertical impact accelerations were affected by inclination, with a decreasing percentage of heavy vertical impacts with positive slope and increasing when downhill. The impact force at foot contact is determined by landing velocity, passive shoe stiffness, and lower limb mass. If running kinematics do not change drastically between slopes, then only landing velocity should influence impact force (45).

There are reportedly higher impact forces in downhill running (compared to level and uphill running) at a given running speed (46). The slope can affect impacts, and running kinematics can help to increase shock attenuation. Running with heel strike (rear-foot strike) lowers hip flexion, increases dorsiflexion, promotes greater knee flexion, and augment stride duration/length to reduce shock attenuation in downhill running (20).

These results may be mainly due to the fact that running kinematics can vary between slopes and even between subjects; being a factor that determines both the impacts and the metabolic parameters (20). Trail runners also adopt different strike patterns depending on the slope, using mid-foot and fore-foot with uphill running and mid-foot or rear-foot with downhill running (20). This technique represents a protective strategy: running with a mid-foot or fore-foot strike diminishes the vertical impact peak during ground contact (27). One of the limitations of our study may be the influence of running kinematic and strike pattern (not analayzed), factors that could perharps better explain the results found.

Viscoelastic parameters

Our results on muscle tone and stiffness are consistent with other research, where leg stiffness remained stable throughout the race protocol after the initial adjustment of the running kinematic (47). The only significant change was in muscle tone of the vastus medialis. This muscle is used for running uphill and downhill by acting as a knee stabilizer and extensor, and its tone changed symmetrically when running uphill or downhill. Therefore, the most important determinant of muscle tone might be the slope. Additionally, the other muscle tone and stiffness data were quite stable, without any change between positive or negative slopes.

Uphill running requires more pronounced activation patterns of lower limb muscles, but electromyography (EMG) activity is different among muscles. Compared with level running, uphill running requires more activation of the gluteus maximus, adductor muscles, iliopsoas, hamstrings, tibialis anterior, gastrocnemius, and quadriceps muscles (20). However, there were no changes in muscle tone in the gastrocnemius and biceps femoris, so muscle tone might not be related to EMG activity levels.

Regarding stiffness, a minimum level is required to achieve an adequate use of the stretch-shortening cycle. The stiffness required normally increases with the activity demands. Several researchers have found that leg stiffness improves jump and sprint performance, but there is scant research regarding running distance performance. It seems that a greater stiffness level could decrease VO_2 and the energy cost of running (48). However, the magnitude of stiffness required to optimize performance has not yet been theorized. A runner who utilizes optimal lower limb stiffness could perhaps better use the elastic energy and reduce positive mechanical work during uphill running. These adaptations might reduce fatigue and the risk of injury (49).

The lack of significant changes both in muscle tone and stiffness may possibly be due to the protocol performed, since the measurements were taken post-trial, instead of during it. Likewise the short duration of each trial may also have influenced these values.

Conclusions

The ventilatory parameters increased proportionally with the uphill slope (elevated VO_2, VCO_2, VE, and HR) and showed a linear negative relationship with downhill slope and the presence of a plateau with extreme downhill values. These results indicate that on the one hand performance and running speed in uphill running may be dependent on VO_2, VO_{2m}, and running economy. On the other hand, downhill running performance might be dependent of kinetic pattern and the capacity of the impacts' attenuation.

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NOTES

Conflicts of interest.— The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding.— This work was supported by the Operative Program ERDF Aragon 2014-2020, “Building Europe from Aragon”, Research Group ValorA, Grant No. S08_20R, and by Fondo Social Europeo “Construyendo Europa desde Aragón”. Pablo.J.BASCUAS is supported by Universidad San Jorge and Banco Sabadell predoctoral grant (“Beca formación investigadores fundación banco Sabadell”).

Authors' contributions.— All authors contributed equally.

Acknowledgements.—

Table I: Mean ± standard deviation and analysis of variance p values for metabolic, biomechanical and viscoelastic parameters depending on slopes. Statistical significance was calculated from Bonferroni or Kruskal–Wallis post hoc tests. *p<0.05; **p<0.01; ***p<0.001.Abbreivations—VO_2: oxygen uptake; VCO_2: carbon dioxide discharge; VE: ventilation; HR: heart rate; RQ: respiratory exchange ratio; SbO2: hemoglobin oxygen saturation.

Inclination	**-20%**	**-15%**	**-10%**	**-5%**	**0%**	**5%**	**10%**	**15%**	**20%**	**p value uphill**	**p value downhill**
Metabolic parameters											
VO_2 peak (mL/min/kg)	38.2 ± 4.08	37.4 ± 7.3	42.3 ± 7.86	46.8 ± 9.19	49.6 ± 5.27	62.7 ± 6.93	72.8 ± 7.73	74.4 ± 13.3	77.6 ± 9.22	**<0.001*** **	**0.009*** **
VO_2 (mL/min/kg)	28.2 ± 3.25	29 ± 4.49	30.1 ± 3.65	34.7 ± 3.8	40.2 ± 4.46	52.7 ± 7.12	62.8 ± 8.03	60.7 ± 8.25	61.8 ±9.88	**<0.001*** **	**<0.001*** **
VCO_2 (mL/min/kg)	23.9 ± 4.4	23.8 ± 4.93	23.9 ± 3.08	28.5 ± 5.99	30.5 ± 4.6	38.6 ± 4.91	49.3 ± 10.3	49.2 ± 12.4	50.3 ± 14.4	**0.001*** *	**0.007*** *
VE peak (L/min)	71.3 ± 7.73	68.2 ± 9.08	73.1 ± 9.09	74.1 ± 7.78	77.9 ± 16.7	105.5 ± 22.7	143.6 ± 24.4	142.4 ± 32.1	161.4 ± 33.9	**<0.001*** **	0.608
HR mean (bpm)	122 ± 11.7	117.9 ± 17	119.1 ± 18.1	124.3 ± 16.5	134.3 ± 16.2	156.6 ± 12.2	171.3 ± 10	171 ± 12.2	172.7 ± 11.7	**<0.001*** **	0.071
HR peak (bpm)	129.4 ± 12.8	124.1 ± 17.2	126.3 ± 19	131.1 ± 19.1	142 ± 18.1	166.7 ± 11.6	180.4 ± 9.1	180.4 ± 13.5	180.7 ± 7.58	**0.002*** *	0.053
RQ peak	0.99 ± 0.09	0.93 ± 0.15	0.94 ± 0.15	0.93± 0.11	0.91 ± 0.11	0.81 ± 0.04	0.86 ± 0.06	0.87 ± 0.09	0.92 ± 0.12	0.355	0.598
Saturation data											
Min SbO_2 (%)	66.6 ± 9.83	67.3 ± 5.75	67.3 ± 4.71	67.8 ± 5.3	69.1± 6.28	64.5 ± 5.65	51.5 ± 13	57.7 ± 9.79	52.8 ± 8.39	**0.003*** *	0.998
Mean SbO_2 (%)	71.6 ± 4.93	72.5 ± 3.56	71.8 ± 3.06	71.3 ± 5.04	71.8 ± 5.39	68.7 ± 4.53	58.4 ± 10.4	63.8 ± 5.84	62.2 ± 5.18	**0.006*** *	0.938
Impact data											
Impacts Zone 1 (%)	31.6 ± 14.9	27 ± 13.7	38.2 ± 15.1	43.7 ± 14.9	60.6 ± 33.9	79.3 ± 32.4	84.7 ± 33.3	94.2 ± 12.2	81.3 ± 39.9	0.155	0.355
Impacts Zone 2 (%)	19.8 ± 13.7	13.6 ± 4.92	24.4 ± 13.8	28.4 ± 11.8	32.3 ± 25.9	18.2 ± 20.1	11.8 ± 25	5.71 ± 12.1	2.03 ± 3.76	**0.049***	0.357
Impacts Zone 3 (%)	16.3 ± 8.95	19.6 ± 9.64	19.4 ± 10	17.6 ± 13.8	6.8 ± 9.93	5.33 ± 12.5	3.26 ± 7.95	0.05 ± 0.12	0	0.114	0.232
Impacts Zone 4 (%)	15.3 ± 10.3	24 ± 12.1	13.6 ± 15.1	6.92 ± 10.8	0.2 ± 0.31	0.5 ± 1.26	0.13 ± 0.32	0.01 ± 0.04	0	0.47	**0.015***
Impacts Zone 5 (%)	11.3 ± 10.9	12.1 ± 5.23	2.84 ± 2.57	1.32 ± 1.77	0	0.01 ± 0.04	0	0	0	0.406	**0.003*** *
Impacts Zone 6 (%)	5.62 ± 5.52	3.58 ± 1.86	1.4 ± 3.01	1.96 ± 3.6	0	0	0	0	0	1	**0.023***
Viscoelastic parameters											
Muscle tone gastrocnemius medialis (Hz)	15.4 ± 0.83	15.6 ± 1.25	15.6 ± 1.04	15.7 ± 1.07	15.9 ± 1.25	15.8 ± 1.2	15.7 ± 1.1	16.1 ± 1.46	15.9 ± 1.51	0.875	0.289
Muscle tone gastrocnemius lateralis (Hz)	16.8 ± 1.78	17 ± 1.63	16.5 ± 1.64	17 ± 2.19	16.7 ± 1.7	17 ± 1.68	16.8 ± 1.53	16.9 ± 1.74	16.8 ± 1.7	0.22	0.256
Muscle tone biceps femoris (Hz)	16.2 ± 1.53	16.2 ± 1.48	16.4 ± 1.55	16.3 ± 1.52	16.1 ± 1.44	16 ± 1.72	15.8 ± 1.56	16.1 ± 1.77	16 ± 1.65	0.79	0.351
Muscle tone vastus medialis (Hz)	13.9 ± 1.58	14.3 ± 1.47	14.1 ± 1.52	14.2 ± 1.63	13.7 ± 0.81	14.2 ± 1.87	14.4 ± 1.4	14 ± 1.05	13.6 ± 1.04	**0.034***	0.273
Stiffness medialis gastrocnemius (N/m)	266.8 ± 16.2	273.5 ± 19.9	272.6 ± 17.6	270.7 ± 21	276.1 ± 24.3	273.1 ± 24.8	272.4 ± 24.5	277.8 ± 31.5	276.2 ± 30	0.447	0.678
Stiffness lateralis gastrocnemius (N/m)	299.8 ± 32.8	301.2 ± 30.7	297.1 ± 30.9	306.2 ± 47.2	296.7 ± 38.5	303.1 ± 42.3	293.2 ± 24.5	300 ± 39.3	301.8 ± 40.4	0.225	0.228
Stiffness biceps femoris (N/m)	304.1 ± 33.8	314.5 ± 47.6	320.2 ± 49.3	308.2 ± 39.2	293.6 ± 28.2	289.7 ± 40.5	285.3 ± 37.6	305.9 ± 40.8	300 ± 46.4	0.306	0.61
Stiffness vastus medialis (N/m)	256 ± 37	262.8 ± 42.4	253.8 ± 35.3	258.3 ± 36	243.8 ± 29.3	257.4 ± 46	263.3 ± 36.1	254.3 ± 29.9	245.5 ± 28.4	0.136	0.09

Table 2: Analysis of related samples (T-test/Wilcoxon) and Cohen's d effect size (ES) for metabolic, biomechanical and viscoelastic parameters. *p<0.05; **p<0.01; ***p<0.001.Abbreivations—VO_2: oxygen uptake; VCO_2: carbon dioxide discharge; VE: ventilation; HR: heart rate; RQ: respiratory exchange ratio; SbO2: hemoglobin oxygen saturation.

Inclination	**-5% vs 5%**		**-10% vs 10%**		**-15% vs 15%**		**20% vs 20%**	
Metabolic parameters	**p-value**	**ES**	**p-value**	**ES**	**p-value**	**ES**	**p-value**	**ES**
VO_2 peak (mL/min/kg)	**0.003****	1.95	**<0.001*****	3.91	**<0.001*****	3.44	**<0.001*****	5.52
VO_2 (mL/min/kg)	**<0.001*****	3.15	**<0.001*****	5.24	**<0.001*****	4.77	**<0.001*****	4.56
VCO_2 (mL/min/kg)	**0.006****	1.84	**0.001****	3.34	**0.001****	2.69	**0.002****	2.47
VE peak (L/min)	**0.004****	1.85	**<0.001*****	4.09	**<0.001*****	3.14	**<0.001*****	3.66
HR mean (bpm)	**0.001****	2.22	**<0.001*****	3.82	**<0.001*****	3.59	**<0.001*****	4.33
HR peak (bpm)	**0.013***	2.25	**0.001****	4.09	**0.001****	3.64	**0.002****	4.87
RQ peak	**0.010***	1.45	0.352	0.70	0.271	0.48	0.200	0.66
Saturation data								
Min SbO_2 (%)	0.510	0.60	**0.008****	2.84	0.059	1.19	**0.042***	1.51
Mean SbO_2 (%)	0.720	0.54	**0.003****	1.74	**0.021***	1.80	**0.015***	1.86
Impact data								
Impacts Zone 1 (%)	**0.045***	1.41	0.054	1.79	**0.006****	5.18	0.067	1.65
Impacts Zone 2 (%)	0.465	0.62	0.067	0.62	0.065	0.84	**0.01***	1.77
Impacts Zone 3 (%)	**0.043***	0.93	**0.015***	1.78	**0.004****	3.07	**0.003****	2.57
Impacts Zone 4 (%)	0.059	0.83	**0.007****	1.26	**0.003****	2.8	**0.003****	2.1
Impacts Zone 5 (%)	0.112	1.04	**0.011***	1.56	**0.003****	3.27	**0.003****	1.46
Impacts Zone 6 (%)	0.104	0.76	0.104	0.66	**0.003****	2.72	**0.003****	1.44
Viscoelastic parameters								
Muscle tone gastrocnemius medialis(Hz)	0.801	0.08	0.742	0.09	0.269	0.37	0.129	0.41
Muscle tone gastrocnemius lateralis (Hz)	0.963	0	0.434	0.19	0.740	0.05	0.885	0
Muscle tone biceps femoris (Hz)	0.623	0.18	0.305	0.45	0.759	0.06	0.665	0.12
Muscle tone vastus medialis (Hz)	0.976	0	0.224	0.20	0.282	0.23	0.356	0.22
Stiffness medialis gastrocnemius (N/m)	0.756	0.10	0.975	0.01	0.597	0.16	0.196	0.39
Stiffness lateralis gastrocnemius (N/m)	0.818	0.07	0.603	0.12	0.968	0.03	0.823	0.05
Stiffness biceps femoris (N/m)	0.150	0.46	**0.033***	0.79	0.583	0.19	0.738	0.10
Stiffness vastus medialis (N/m)	0.930	0.02	0.295	0.27	0.425	0.38	0.297	0.31

TITLES OF FIGURES

Figure 1.— Differences in A) peak oxygen uptake (VO_2 peak, mL/min/kg), B) peak ventilation (VEpeak, L/min), C) peak heart rate (HRpeak, beats per minute[bpm]), and D) mean carbon dioxide discharge (VCO2 mean, mL/min/kg) between slopes. Statistical significance was calculated from Bonferroni or Kruskal–Wallis post hoc tests: *p<0.05; **p<0.01; ***p<0.001.

Figure 2.— Figure 2: Differences in A) mean and B) minimum values of hemoglobin oxygen saturation (SbO_2,%) between slopes. Statistical significance was calculated from Bonferroni or Kruskal–Wallis post hoc tests: *p<0.05; **p<0.01; ***p<0.001.

Figure 3.— Figure 3: Differences in values forA) zone 2 impacts (4–5 G), B) zone 4 impacts (6–7 G), C) zone 5 impacts (7–8 G), and D) zone 6 impacts (8-9 G)between slopes. Statistical significance was calculated from Bonferroni or Kruskal–Wallis post hoc tests: *p <0.05; **p<0.01; ***p<0.001.

Figure 4.— Figure 4: Differences inmuscle tone of the vastus medialis (Hz) between slopes. Statistical significance was calculated from Bonferroni or Kruskal–Wallis post hoc tests: *p <0.05; **p <0.01, ***p <0.001