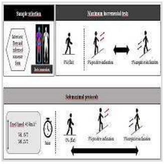

Mar 22, 2021

Obese locomotion on a positive and negative slope at iso-intensity: outcomes related to energetics, mechanics, perceived exertion, and hemodynamics. A study protocol.

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ABSTRACT

The main objective of this study is to propose a form of walking/running protocol that is capable of evaluating energy expenditure at different types of slopes, considering the same relative intensities (iso-intensity conditions), as well as to assess biomechanical and hemodynamic factors that may be associated. 11 obese adult men (BMI between 30 kg.m^{-2} and 39.9 kg.m^{-2}) aged between 18 and 35 years, physically inactive. Assessments will be carried out on six different days. On the first day, body composition, familiarization with the equipment, and a maximum incremental test without slope. In the next two days, the maximums tests in positive and negative slope will be performed. In the other three days, biomechanical, energetics, and cardiac variables will be collected in the following conditions: fixed speed (4.5 km.h^{-1}), speed relative to the first, and the second ventilatory threshold, being one day for tests on the positive slope, one on the negative slope and one without slope. From the second day, the tests will be distributed in random order and with an interval of, at least, 72 hours between tests. Metabolic data will be obtained using a gas analyzer, biomechanical data will be obtained through a kinematics system, with six infrared cameras, data Ground Reaction Forces (GRF) are obtained through force sensors installed on the treadmill, and hemodynamic parameters using a Signal-Morphology impedance device. The primary outcome is energy expenditure concerning slopes and speeds and the secondary outcomes are the mechanical work, mean GRF, and hemodynamic aspects. The data will be described by the mean and standard error values. The interactions between slopes and speeds will be tested with Generalized Estimating Equations (GEE) and Bonferroni post-hoc, adopting a significance level (α) of 0.05. Also, effect sizes (ES) will be calculated. The energy expenditure should be greater on the positive slope at all speeds. Higher production of positive mechanical work is expected on the positive slope and the impact peaks should be less in this condition. The negative slope should enable higher speeds relative to the thresholds, as well as lower cardiovascular load.

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Introduction

1 Obesity is considered one of the most important public health problems, making both treatment and prevention a priority (Di Cesare et al., 2016; Menéndez et al., 2019). Over 1.9 billion adults are overweight, of these, 650 million are characterized as obese. A concerning factor regarding these numbers is that overweight, obesity, and associated risk factors present a strong association with the incidence of morbidity and mortality. The main strategies to reduce the incidence of obesity and overweight include adequate dietary intake and engagement in regular physical activity, at least 150 minutes spread over most days of the week, seeking negative energy balance (WHO, 2017).
Systematic reviews with meta-analyses have demonstrated the benefits of walking, as predominantly aerobic activity, considering the sum of daily steps or especially in the form of periodized exercise. Walking/running benefits occur at the level of physical fitness with the prevention of cardiovascular disease risk (Hamer and Chida, 2008), weight control and loss (Richardson et al., 2008), as well as decrease of resting blood pressure and body fat (Murphy et al., 2007). Daily locomotion in an uncontrolled environment presents several challenges regarding the slope of the terrain, as well as the possibilities of increasing the slope of the surface in a controlled environment, such as on the treadmill (Lay et al., 2006).
Walking/running on a sloped surface requires adjustments in muscular recruitment and activation when compared to situations on level ground surfaces, which can result in differences in metabolic cost and biomechanical parameters (Kimel-Naor et al., 2017). In positive slopes, there is an increase in the magnitude and duration of the activity, which suggests that the muscular activation pattern may help to predict the increased metabolic cost, considering that in this condition there is a predominance of concentric contractions of propulsive muscles (Silder et al., 2012). On moderate positive slopes (3 – 9°) and at relatively slow walking speeds (0.5 – 1.0 $m.s^{-1}$) obese individuals can meet the recommended requirements for physical activity levels, which, in metabolic terms, is equivalent to walking on level terrain at higher speeds. For example, obese adults walking at 0.75 $m.s^{-1}$ and 6° slope are at an intensity of approximately 52% of VO_{2peak}, whereas walking on level terrain at 1.50 $m.s^{-1}$, the intensity is approximately 50% of VO_{2peak} (Ehlen et al., 2011).
Walking on a moderate positive slope can significantly reduce the risk of musculoskeletal and joint diseases in obese adults, due to the decrease of loads and joint movements when compared to walking on level terrain. Therefore, walking on moderate positive slopes and at relatively slow speeds can be a suitable form of physical activity in mechanical and energetic terms for obese adults to reach the recommended level of energy expenditure (Ehlen et al.,

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2011; Browning et al., 2013). Previous studies demonstrated that a positive slope favors the decrease of joint impact in eutrophic and obese individuals (Browning, 2012; Strutzenberger et al., 2017). However, no studies were found evaluating the joint impact of walking on a negative slope in obese people, but only speculations that this type of exercise may promote greater joint impact than walking on level terrain (Browning et al., 2013). There is a relationship between negative inclination, rating of perceived exertion (RPE) and walking speed, in which at the same absolute speed compared to walking on level ground, the rating of RPE is lower, mainly due to the lower relative load (%VO_{2max}) (Agarwal et al., 2017b), due to a higher proportion of negative $W_{mec}$and its high efficiency (~100%) (Minetti et al., 1993). However, under the same relative load, which represents greater absolute speeds in the negative slope, no studies were found confirming or rejecting the hypothesis of lower RPE in this condition. Unlike the positive slope, in the negative slope, there is a predominance of eccentric contractions (Minetti et al., 1993), which are used as a strategy in clinical practice for eccentric activities (Geremia et al., 2019).

Another system presenting specific responses to the type of muscular contraction during exercise is the cardiovascular. Through a non-invasive Signal-Morphology impedance device, hemodynamic parameters such as heart rate (HR), stroke volume (SV), and cardiac output (CO) can be accurately measured. It is speculated that in predominantly eccentric activities, such as walking on a negative slope, there is less overload on this system. This response can be attributed to the smaller amount of muscle mass used, under the same relative load, when compared to a predominantly concentric muscular activity, which represents lower recruitment and firing rates of motor units (Agarwal et al., 2017a).

To our knowledge, the study of Browning et al. (2013) was the pioneer in the research on walking on negative slope of obese adults, in which the authors verified that, at a 3° negative slope and at a speed of 1.25 $m.s^{-1}$, obese individuals can reach an intensity level close to 40% of VO_{2peak}, suggesting that this slope condition can be a good alternative for moderate intensities of physical exercise. The authors suggest that future studies with greater samples, control group, standardized measurements, and walking protocols are needed to improve the results found. Besides, speed is one of the critical points of the comparison analysis between different slopes, because if the absolute speed is the same at different inclinations, the relative intensity will be different. No studies were found developing a robust methodology to compare mechanical and energetic outcomes in different slopes, but in the same relative intensity, that is, iso-intensity (for example, speeds referring to the first ventilatory threshold (VT1), and second ventilatory threshold (VT2)).

To answer some of these questions, it is necessary to conduct an integrative analysis between biomechanics and physiology of locomotion, named Locomotion Physiomechanics (Peyré-Tartaruga and Coertjens, 2018). According to a classic study, a better understanding of the metabolic outcomes' behavior must be accompanied by biomechanical analysis. Muscle mechanical work (W_{mec}) can be estimated through kinematic analysis and is basically divided into two parts: 1) external Wmec (W_{ext}) which considers the work performed to elevate and accelerate the center of mass (CM) concerning the environment and 2) internal Wmec (W_{int}), which considers the work performed to accelerate the body segments in relation to the CM. These variables, when analyzed together, can provide important information on how the locomotor machinery and the pendulum system are working to generate the energy needed to perform the task, including at different types of slope, which helps us to understand the main mechanisms associated with energy expenditure. To our knowledge, no studies have yet been conducted seeking a comparison of iso-intensity at different slopes measuring the locomotion physiomechanics, especially energy expenditure, to verify if there are differences caused by the type of slope.

The objective of the present study is to propose a form of walking/running protocol that is capable of evaluating energy expenditure at different types of slopes (-5, 0, +5%), considering the same relative intensities (iso-intensity conditions, at VT1 and VT2 relative speeds), as well as to assess biomechanical and hemodynamic factors that may be associated.

2

VICON Motion Capture System
Motion Capture System

Vicon **003125**

by six infrared cameras, accompanied by Nexus software (v. 1.8.2).

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K5 Portable Metabolic System
Portable Metabolic System

Cosmed **US Patent 9581539**

The K5 uses the latest technology, including COSMED's 4th generation opto-electronic reader and proven and reliable highly linear and rapid response O2 (GFC) and CO2 (NDIR) sensors.

Lunar Prodigy
Dual-Energy X-Ray absorptiometry

General Electric **856-9**

Lunar Prodigy model, General Electric Company

Load Cells for Treadmill
Load Cells for Treadmill

Instor **784426-8**

three-dimensional load cells with low-pass and second-order filter with a cut-off frequency of 30Hz. Data were collected at 1000Hz per channel with Instor software (Porto Alegre, Brasil) and a custom LabVIEW system (National Instruments, Austin, USA), as previously described by Da Rosa et al. (2019).

Super-ATL
Treadmill

Inbramed **10318090009**

tilt capability of 26%, speed capability of 32 km/h

Protocol

3 Objectives and method validation

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The main objective of this method is to propose a way of comparing walking/running in isso isso-intensity condition at different slopes. Considering that physiological, biomechanical, and hemodynamic responses have to be specific according to the slope type (level, positive or negative), we realized that there was no validated/published protocol that could make it possible to compare this set of variables at speeds relative to the specific VT of each slope.

Step-by-step procedures

Study Population

The sample size was obtained through the calculation performed in the G*POWER 3.1.6 software, wherein a significance level of 0.05 and a power of 80% were adopted, based on effect size values for energy expenditure from the study of Browning et al. (2013), considering -3% and +3% slopes. Therefore, the calculation demonstrated a need for at least 11 participants.

Besides, men aged 18-35 years, grade 1 (Body mass index (BMI) between 30 and 34.9 $kg.m^{-2}$) and grade 2 obese (BMI between 35 and 39.9 $kg.m^{-2}$) will be included, who were not practicing regular physical exercise for at least three months, and non-smoking. Regular physical exercise practice was defined as the performance of any physical training modality for at least 20 minutes in three or more days of the week. The exclusion criteria of the study will include the presence of a history of musculoskeletal and joint injuries, chronic diseases associated with cardiovascular and respiratory problems, and any other muscular or joint impairment that prevents physical exercise performance safely.

3.1 *General aspects of the tests*

Collections will be performed at the Laboratory of Exercise Research, Biodynamics Sector, at the School of Physical Education, Physiotherapy and Dance of UFRGS. Each participant will have to attend the laboratory in six days to carry out the assessments. Individuals will be instructed to keep their eating and physical activity habits unchanged during the collection period. All walking/running tests will be performed on the treadmill.

On the first day, reading and explanation of the Free and Informed Consent Form (FICF) will be held, indicating the risks and benefits of participating in the research, and the entire testing protocol will be explained. After agreeing on the procedures and signing the FICF, the individuals will undergo a body composition assessment via dual-energy x-ray, having followed the preparation recommendations informed by the equipment manual and using the appropriate clothing. The evaluation is conducted with the individual in the supine position, with the hands along the body, without moving, using the full-body protocol

After the body composition assessment, the individuals will be familiarized with the treadmill and other equipment. Thereafter, the maximal incremental test on the treadmill will be performed on the level condition, using the portable gas analyzer along with the cardiac monitor. In the next two visits, maximal tests in positive and negative slope conditions will be carried out, in a randomized order. The data obtained in the three maximal tests will be analyzed by two experienced physiologists, in an independent and blinded manner, for the determination of VT1 and VT2 and thus, the ideal speeds for the submaximal protocols will be determined.

On the other three days of collection, submaximal protocols will be performed at the speeds referring to the thresholds determined in the maximal tests, wherein each day a slope condition (positive, negative, and level) will be carried out. First, the placement of the reflective points required for the three-dimensional reconstruction in the kinematics system will be performed. After that, the electrodes for hemodynamic monitoring, and the silicone mask of the respiratory gas analyzer will be placed. Thereafter, test protocols in the following conditions will be performed: speed referring to the VT1; speed referring to the VT2; and fixed speed (4.5 $km.h^{-1}$). The order will be randomized, and each condition will be executed for five minutes, followed by at least 15 minutes of rest or until the resting values (HR and VO_2) are re-established. VO_2data and kinematic and kinetic records will simultaneously occur after three minutes and 30 seconds of walking, until four minutes and thirty seconds, considering that in this period it is already possible to obtain steady-state values. Tests will be scheduled in the same time range on different days for everyone, to eliminate possible differences arising from the circadian rhythm. Individuals will be oriented to keep their eating and physical activity habits unchanged during the collection period.

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3.2 *Maximal tests protocols*

Before starting all protocols, the gas analyzer equipment will be calibrated for air volume (with a 3L calibration syringe), humidity (with a specific device), and gas concentrations (by using a cylinder with a standard concentration of mixed gas with 16% of O_2, 5% of CO_2 and nitrogen for balance).

Individuals will be initially positioned sitting in a chair on the treadmill, where the silicone mask of the gas analyzer will be placed. Gases collections will start with the individual at rest for 15 minutes and the median for every 10 points during the last 3 minutes will be considered as resting values. At the end of the 15 minutes of rest, resting blood pressure will be measured for control and release for testing. Considering the specific fixed slopes of each of the three tests (-5%, 0% e +5%) and to adjust the protocol of maximal voluntary exhaustion test (duration between 7 and 12 min) for the study sample, a series of pilot tests were carried out and the following protocols for maximal tests were developed:

•Level Ground (0%) and positive inclination (5%): initial speed of 3.0 $km.h^{-1}$, for 3 minutes, with an increase of 0.5 $km.h^{-1}$ every 1 min.

•Negative Slope (-5%): initial speed of 4.0 $km.h^{-1}$, for 3 minutes, with an increase of 1.0 $km.h^{-1}$every 1 min.

Heart rate will be continuously registered and the perceived effort (Borg Rating of Perceived Exertion 6-20) (Borg, 1982) will be registered at the end of each stage. The test will be interrupted when the participant signals through manual gestures, being instructed to signal only when they reach a state of exhaustion. The assessment will be considered valid when at least two of the following criteria is reached at the end of the test (Mclaughlin et al., 2010): 1) obtainment of the estimated HR_{max} (220 - age); 2) obtainment of a respiratory exchange ratio (RER) greater than 1.1; 3) perceived exertion greater than 17.

3.3 *Submaximal tests protocols*

From the results of the analyzed maximal tests, the three last collection days will be carried out, one day for each condition: level ground, positive and negative slopes. Initially, the period of 15 minutes of rest will be repeated, and at the end, resting blood pressure will be measured for control and release for testing.

In these protocols, after the initial resting period, the electrodes of the hemodynamic monitoring system will be placed. For that, trichotomy and asepsis of the skin will be carried out (with abrasive gel and alcohol) at the electrode placement sites. With the dry skin, 6 electrodes will be fixed for hemodynamic monitoring (FS- 50, Skintact®, Austria) in the following sites: left lateral region of the neck, supraclavicular fossa, the center of the sternum, V1 and V6 standardized positions for electrocardiogram and parallel to the spine at the xiphoid process level. Using the specific software of the device, paired via Bluetooth, the equipment calibration will be performed.

The next step will be the placement of reflective points of the kinematics system, adhered to the skin with double-sided silicone tape (3M®), following the model proposed by (Minetti et al., 1993) and recently described by Oliveira et al. (2020), on the following anatomical points: ear canal, acromion, lateral epicondyle of the humerus, midpoint of the distal radioulnar joint, greater trochanter, femoral epicondyle, lateral malleolus, calcaneus, and fifth metatarsal. The last preparation step will be the placement of the respiratory gas analysis mask.

3.4 **Data analysis**

Energetics

Maximal tests

The energetic variables of the maximum test will be collected throughout the test. The ventilatory method will be used to determine VT1 and VT2, from VO_2, Ventilation (VE), and carbon dioxide production (VCO_2) curves, independently by two experienced physiologists, according to the methodology previously described (Binder et al., 2008; Cassirame et al., 2015):

•VT1: from the break in linearity of the VE/VO_2 ratio;

•VT2: from the break in the linearity of the VE/VCO_2 ratio;

•VO_{2peak}: the second highest VO_2 value in the last stage of the test.

In a complementary way, the Ventilation/time curve will be analyzed for possible checking of the determination of thresholds. For submaximal tests, the relative speeds of each slope in which the VT1

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and VT2 were found.

3.5 Submaximal tests

The collection of energy variables occurs during the five-minute test. For the analysis, we will consider the values obtained in 1min, between 3min30s from the beginning to 4min30s, to evaluate the steady-state in the conditions of inclines and speeds (Ferretti et al., 2017).

•Caloric expenditure: in kcal.min-1, obtained by multiplying VO_2 (L/min) and the metabolic equivalent of RER (eqRER);

•Cost of Transport: in $J.kg^{-1}.m^{-1}$, obtained for VO_{2net} multiplied by 60 and eqRER and divided by speed;

•VO_2: in $ml.kg^{-1}.min^{-1}$, were considered as the average values during the walk/run during the steady-state period.

3.6 *Mechanics*

Mechanical Work

The kinematic model will be performed as described by (Minetti et al., 1993), with bilateral placement of nine anatomical markers (14 mm in diameter). The motion capture system is composed of six infrared cameras with a sampling frequency of 100Hz. The calibration procedure will be carried out according to the supplier's manual. After placing the markers, the static calibration will be conducted with the participant in an upright standing position, with the shoulders abducted and flexed elbow, both 90°, for 5s, to construct the model, and the data from 15 consecutive strides will be considered to analysis. Kinematic data will be filtered through a fourth-order Butterworth low-pass digital filter using a cut-off frequency defined by residual analysis technique (limited at 6–12 Hz). The mechanical work will be calculated according to previously described methodologies (Cavagna and Kaneko, 1977; Willems et al., 1995). From the three-dimensional positions of the 18 anatomical markers, will build a spatial model of 11 rigid segments: head–neck–trunk, upper arms, lower arms, thighs, lower legs, and feet (Minetti et al., 1993; Nardello et al., 2011). The characteristics of each body segment (CM and radius of gyration) will be determined according to results of Dexa exam, as suggested by (Browning, 2012) and detailed described by (Matrangola et al., 2008). Based on these characteristics, the three-dimensional trajectory of the CM will be calculated. The kinematic collection will be performed only in submaximal tests.

Finally, based on the CM data, the time course of potential (Ep) and kinetic (Ek) energies (using three-dimensional coordinates: z, vertical; x, anteroposterior; and y, mediolateral) will be computed to determine W_{tot}, W_{ext}, and W_{int}, as recently described by Oliveira et al. (2020), and briefly described below:

•W_{tot}: summation of the $W_{ext}$and W_{int} modules

•W_{ext}: The summation of all increases over the E_{ext} time course resulted in the positive external mechanical work, W_{ext}. The Wext corresponds to the work necessary to lift and accelerate the Body CM within the environment.

•W_{int}: The work necessary to rotate and accelerate the limbs concerning the CM. It was calculated through the summation of the positive increments of rotational energy from the body segments and the translational energy from the body segments relative to the CM.

3.7 Kinetic: impact force

We will also calculate the impact peak magnitude. Values will be expressed as total load (N), and normalized to body weight (%) (Wallace et al., 2018). The average of the same 15 strides of kinematic analysis will be considered for each inclination and speed condition.

3.8 Cardiac Hemodynamics

We will analyze the following parameters regarding hemodynamics. Data gathering will be carried out throughout the submaximal test. We will use for this analysis only the data from the 1min steady-state period for each speed.

•Heart Rate (HR), in bpm, will be considered as the average HR during this 1min period;

•Stroke Volume (SV), in ml, will be considered as the average of the estimated amount of blood ejected from the left ventricle by beating during this 1min period;

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•Cardiac Output (CO), in L.min^{-1}, will be considered as the product average between HR and VS during this 1min period.

4 *Statistical analysis*
Statistical Package for the Social Sciences version 17.0 (SPSS Inc., Chicago, DE, USA) will be used. Descriptive analyses will be presented as the mean and standard error. Comparisons between the variables of submaximal protocols will be analyzed with Generalized Estimating Equations (GEE) with two factors (slope and speed) and Bonferroni post hoc test. Also, effect sizes (ES) will be calculated through Cohen's d value with the following classifications (Hopkins, 2002): trivial ($d \leq 0.2$), small ($d < 0.2$), moderate ($d > 0.6$),large ($d > 1.2$), or very large ($d \geq 2.0$), to compare the main differences between the slopes. A significance level of $\alpha = 0.05$ will be adopted.

5

A potential strategy for obese adults to achieve higher levels of energy expenditure can be walking/running on inclined surfaces. In positive slope conditions, lower limb muscles perform a greater proportion of positive mechanical work for increasing gravitational potential energy in each step and most of this work is performed by hips and knees muscles and joints when compared to level walking (Sawicki and Ferris, 2009). The combination of lower speeds at positive slope versus walking on level ground at higher speeds will result in similar metabolic cost between the conditions (Ehlen et al., 2011; Dewolf et al., 2017), which can mean the advantage of locomotion on an inclined level. On the other hand, the negative slope may favor the development of higher speeds with less perceived effort, and generally require less load on the cardiovascular system, a favorable condition for individuals with low exercise tolerance.

Advantages
The protocol aims to define iso-intensity conditions for comparing the mechanical and energetic factors of locomotion. aims to enable a better understanding of the relationship of these variables and the possible advantages found depending on the type of inclination, thus increasing the possibilities of exercise prescription safely and efficiently.

Limitations
Despite the possible benefits of this method, we observe some limitations: i) only one level of inclination for each condition (positive and negative); ii) the speeds determined by the iso-intensities of VT1 and VT2 can happen while some individuals are running and others are walking; iii) there is no comparator group.

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