DOCTORAL PROGRAMME IN BIOMEDICINE

IMPACT OF WHOLE-BODY ELECTROMYOSTIMULATION ON ENERGY METABOLISM IN ADULTS

Unai Perez de Arrilucea Le Floc'h

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UNAI ADRIAN PEREZ DE ARRILUCEA LE FLOC'H

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Doctoral thesis

Impact of whole-body electromyostimulation on energy metabolism in adults

Impacto de la electroestimulación global de cuerpo completo sobre el metabolismo energético en adultos

PROGRAMA DE DOCTORADO EN BIOMEDICINA

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Unai Adrian Perez de Arrilucea Le Floc'h

Directores: Jonatan Ruiz Ruiz y Francisco J. Amaro Gahete

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El doctorando **D. Unai Adrian Perez de Arrilucea Le Floc'h** ha realizado la presente Tesis Doctoral siendo beneficiario de un contrato laboral de la empresa WIEMSPRO S.L. (Identificador: E1820200306288-55). La presente tesis se enmarca dentro del proyecto Wiemslab: "Estudio sobre los efectos de la electroestimulación de cuerpo completo sobre la salud y el rendimiento físico" (REF: 30C0544900). No obstante, se declara que no existe ningún conflicto de interés en la realización de la presente Tesis Doctoral a pesar de utilizar el material y estar en nómina por dicha empresa.

“Si alguien puede refutarme, mostrarme que estoy cometiendo un error o que estoy mirando las cosas desde una perspectiva incorrecta, con mucho gusto cambiaré. Es la verdad lo que busco, y la verdad nunca perjudicó a nadie.”

- Marco Aurelio -

A mis padres, por hacerme a su imagen y semejanza,
por ser luz, fuerza, coraje y perseverancia,
por luchar contra viento y marea.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AUC: Area under the curve

BMI: Body mass index

Bpm: Beats per minute

ChoOx: Carbohydrate oxidation

CV: Coefficient of variance

EE: Energy expenditure

EMS: Electromyostimulation

FatMax: Intensity that elicit maximal fat oxidation

FatOx: Fat oxidation

FMI: Fat mass index

HR: Heart rate

Hz: Hertz

Kcal: Kilocalories

LMI: Lean mass index

mA: milliamps

Min: Minutes

MU: Motor unit

NutOx: Nutrients oxidation.

PA: Physical activity

RER: Respiratory exchange ratio

RPE: Rating of perceived exertion

SD: Standard deviation

SEM: Standard error of the mean

TENS: Transcutaneous electrical nerve stimulation

VAS: Visual analogue scale

VCO_2: Carbon dioxide production

VO_2: Oxygen consumption

VO_2max: Maximal oxygen uptake

VO_2peak: Peak oxygen uptake

WB-EMS: Whole-body electromyostimulation

WHO: World Health Organization

µsec: microseconds

ABSTRACT

Lack of time and the low motivation to exercise are the two main barriers for not being physically active. In recent decades, new training methodologies have emerged in order to, precisely, counteract these two problems. These new methodologies, which are thought to increase the intensity of exercise while reducing the volume of training, seem to offer the same or even better results compared to the more traditional programs. One tool that allows this increment of intensity is whole-body electromyostimulation (WB-EMS). The administration of electrical impulses through an artificial device increases the amount of skeletal muscle recruited, thus, elevating total energy expenditure during a training session. However, the electrical parameters used to optimize energy metabolism during a training session are currently unknown.

The overall aim of the present Doctoral Thesis is to investigate the effects of WB-EMS applied in different situations (i.e., at rest and during exercise) on energy metabolism and nutrients oxidation in young adults.

The results of the present Doctoral Thesis showed that the manipulation of electrical parameters plays a key role in energy metabolism kinetics. Our findings demonstrated that the application of different electrical frequencies and intensities can optimize the effects of WB-EMS on energy expenditure both at rest and during exercise. We found that 4 Hz at rest and 6 Hz during exercise are the optimal frequencies to increase the benefits of WB-EMS. Our data showed an increment of ≈ 604% in energy expenditure at rest and ≈ 44% during uphill walking (study I). Likewise, it was shown that the addition of WB-EMS to a basic physical activity recommendation (i.e., the recommended daily 10,000 steps per day) produces a significant increase (≈ 21%) in energy expenditure (study II).

In conclusion, this Doctoral Thesis shows that WB-EMS is an effective tool to increase energy expenditure at rest and during exercise. Moreover, WB-EMS is able to modify substrate oxidation in both conditions. Therefore, WB-EMS should be considered a promising therapy to help energy balance' management.

RESUMEN

La falta de tiempo y la escasa motivación hacia el ejercicio son las dos principales barreras que impiden que la población realice más ejercicio. En las últimas décadas han surgido nuevas metodologías de entrenamiento para, precisamente, contrarrestar estos dos problemas. Estas nuevas metodologías, que aumentan la intensidad del ejercicio al tiempo que reducen el volumen de entrenamiento, parecen ofrecer los mismos o incluso mejores resultados comparados con programas más tradicionales. Y una herramienta que permite aumentar la intensidad del ejercicio es la electroestimulación global de cuerpo completo (WB-EMS). La administración de corriente eléctrica a través de un dispositivo artificial aumenta el número de músculos contraídos provocando una elevación del gasto energético total durante la sesión de entrenamiento. Sin embargo, actualmente se desconocen los parámetros eléctricos que optimizan el uso de esta herramienta y su efecto en el metabolismo energético.

El objetivo general de la presente Tesis Doctoral es investigar los efectos de la WB-EMS en diferentes situaciones (i.e., en reposo y durante el ejercicio) sobre el metabolismo energético y la oxidación de nutrientes en adultos jóvenes.

Los resultados de la presente Tesis Doctoral mostraron que la manipulación de los parámetros eléctricos juega un papel clave en el metabolismo energético. Nuestros hallazgos demostraron que la aplicación de diferentes frecuencias e intensidades eléctricas puede optimizar los efectos de la WB-EMS sobre el gasto energético tanto en reposo como durante el ejercicio. Encontramos que 4 Hz en reposo y 6 Hz durante el ejercicio son las frecuencias óptimas para incrementar los beneficios de la WB-EMS. Nuestros datos mostraron un incremento de ≈ 604% en reposo y de ≈ 44% durante la marcha cuesta arriba (estudio I). Asimismo, se demostró que la adición de WB-EMS a una recomendación de actividad física básica (es decir, los 10.000 pasos diarios recomendados) produce un aumento significativo del ≈ 21% en el gasto energético (estudio II).

En conclusión, esta Tesis Doctoral demuestra que la WB-EMS es una herramienta eficaz para aumentar el gasto energético en reposo y durante el ejercicio. Además, la WB-EMS es capaz de modificar la oxidación de sustratos en ambas condiciones. Por lo tanto, WB-EMS debería considerarse una terapia prometedora para ayudar a los problemas relacionados con el desequilibrio energético.

GENERAL INTRODUCTION

1. ELECTROMYOSTIMULATION AS A NEW TRAINING METHOD

1.1. History of electromyostimulation

The first humans that discovered the application of an electrical stimulus were the ancient Egyptians. They used an electric catfish from the Nile to cure gout and migraine (1) and did not hesitate to capture that functions in art (Figure 1). However, they observed that the shock of a catfish is powerful enough to knock over a grown man, although it has never been known to be fatal (2). On the other hand, Aristotle also found that the torpedo fish could bring electrical impulses since it numbed other fish (3). Their thoughts were confirmed when Anthero, a freed slave who suffered from gout, stepped accidentally on an electrical fish (4) and felt the curative benefits from electrical stimulation. From then, every ancient writing registered that stepping on that fish could serve as a cure of gout (4).

Figure 1: The Palette of King Namer showed the importance of the catfish in its top

A long time after this discovery, Luigi Galvani (1737-1798) was the first scientist to prove that electrical artificial impulses could produce physical muscle contractions in animal models (5). His experiment was based on connecting an electrical lancet to the vertebral canal of a lower body half frog observing that, when electricity was applied, the muscles of the frog contracted vigorously (6). Together with his colleague Alessandro Volta, they discussed about the origin of those muscle contractions finally discovering what is called "electrophysiology" (7).

A few years after, Dr. Wall in 1967 and Dr. Kotz in 1971 applied, for the first time, an electrical impulse through a transcutaneous electrical nerve stimulation (TENS) as a treatment for muscle pain and to increase muscle strength, respectively (8, 9). Wall used electrical impulses with eight patients that suffered intense chronic pain observing a significant reduction after its application (10). By the way, Kotz applied an electrical impulse in athletes obtaining an improvement of 40% in muscle strength (9, 11). It was the first time that the electrical impulse applied though an artificial device was physiologically similar as the electrical impulse from the central nervous system in terms of muscular contraction (12). These researchers therefore proposed two different ways to use electrical stimulus: the electrotherapy and the electromyostimulation (EMS). Both scientists shared the idea that applying electrical stimulus using an artificial device could provide positive effects in humans. Nevertheless, the electrical parameters used and the application' aim were different.

Regarding the EMS as a part of the rehabilitation program in muscle injuries, Melzack and Wall developed specific methods to improve pain perception and pain treatment through electrical stimuli (8, 13-15). This research line has been subsequently developed in recent years proposing EMS as an effective way to improve chronic back pain, spasticity and multiple sclerosis, among others (16-18).

Regarding EMS in sport performance, after its first application in 1971 by Kots, other researchers investigated the potential benefits of training with local EMS to enhance muscle strength and performance (19-21). For example, Babault et al. showed an improvement in leg muscle strength and power after 12 weeks of EMS in knee extensor, plantar flexors and gluteus (22). Brocherie et al. also analyzed the effects of local EMS in ice hockey players showing that EMS training improved isokinetic strength of quadriceps femoris (23). Moreover, Maffiuletti et al. confirmed that performing a combined plyometric training with local EMS increase the height of vertical jump (24). Amaro-Gahete et al. had also reported that the application of whole-body electromyostimulation (WB-EMS) improves performance-related parameters in runners (25) .

Although local EMS has been used to improve muscular strength, the new tendency in WB-EMS application is focused on improving body composition (i.e., reducing fat mass and increasing lean mass) which can be explained by an increase in the total energy expenditure (EE) of the session. For example, Amaro-Gahete et al. examined the effects of WB-EMS combined with a high intensity interval training in physical fitness

(26). Lastly, Filipovic et al. summarized the effects of local EMS and WB-EMS of a big amount of studies performed in the first decade of XXI century in a systematic review and shown the benefits of training with this technology (27).

Lastly, in recent years, training with EMS for health purposes has gained great relevance among researchers (26, 28-31). Kemmler et al. have reported the effects of both local EMS and WB-EMS on exercise intensity and energy metabolism (32-38). They concluded that the EMS has an important impact on the EE of the session and its related consequences. There is also a growing trend towards WB-EMS training and health related outcomes (28, 39-41). Many studies, that will be discussed in part 2 of the introduction, are focusing on analyzing the effects of involuntary contraction motivated by EMS on health parameters such as glycemia, hypertension or dyslipidemia.

1.2. Physiology of muscle contraction: role of electromyostimulation

The musculoskeletal system works through electrical impulses originated in the central nervous system that travel though the spinal cord and the axon until myocytes conforming the motor unit (MU) of the skeletal muscle (Figure 2) (42). MU can be additionally excited by an exogenous electrical signal provided by EMS (43). Indeed, it has been suggested that maximal force exerted by the skeletal muscle in response to both voluntary and involuntary contractions is the result of the concurrent development of two different mechanisms: the recruitment of MUs and the discharged action potential (44).

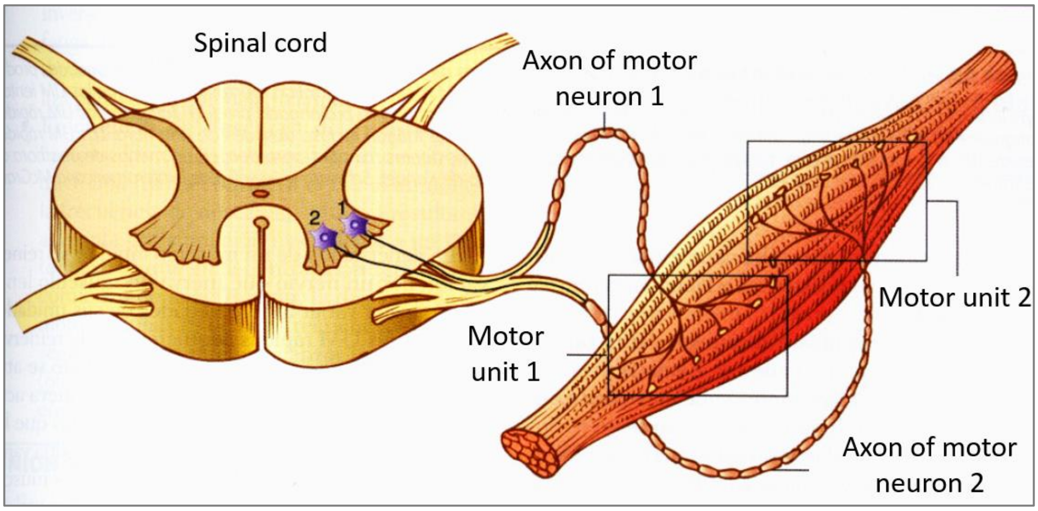

Figure 2: Anatomy of the neuromuscular system (45).

1.2.1. Recruitment of MUs

The recruitment of MUs is critical for force production. Henneman et al. reported in 1965, for the first time, the pattern of MUs activation in animal models (46). His theory, which was called as "*size principle*", basically expressed that the MUs size and their associated skeletal muscle fibers is determinant for muscle activation (47). According to this principle, the size of motor neurons determines their threshold to be excited, with small motor neurons being the first to be recruited (Figure 3) (48, 49).

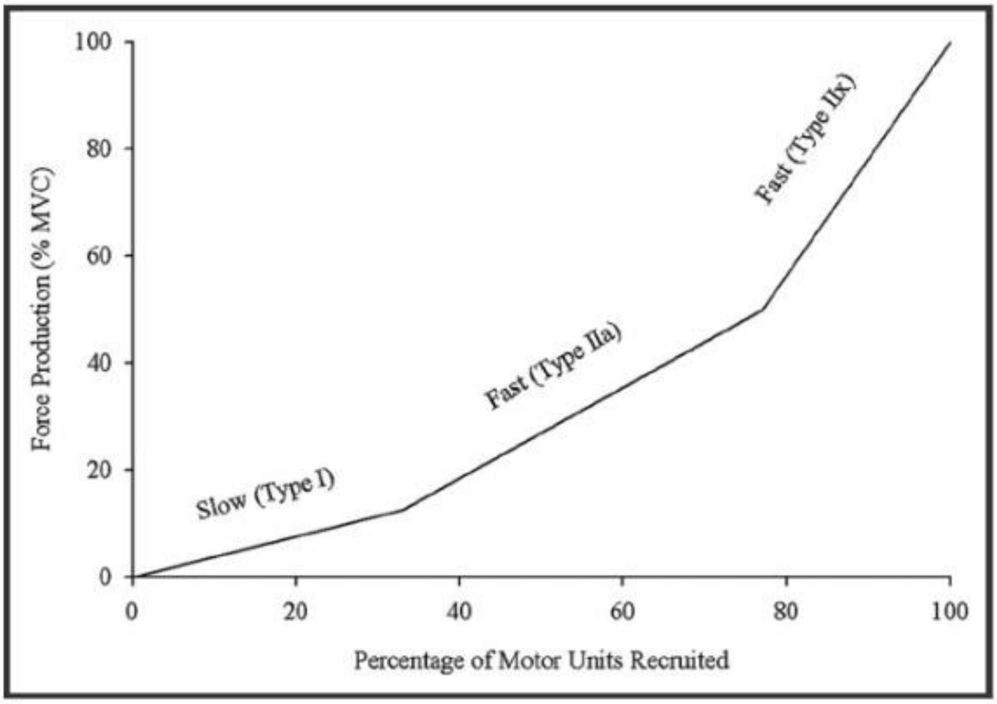

Figure 3: Graphic representation of the orderly recruitment of motor units during voluntary activation of skeletal muscle as described by Henneman et al. (46).

Considering this principle, it could be assumed that type I muscle fibers are usually innervated by type I motor neurons (they are also smaller in size), while type IIa and IIx muscle fibers are frequently innervated by motor neurons of larger size (50). That's the reason why there is a selective fiber utilization depending on the intensity of the exercise. For instance, exercises that did not need high levels of strength only recruited skeletal muscle fibers type I. However, when the intensity increases the skeletal muscle fibers recruited are, by order, type I and after type IIa and IIx (50).

1.2.2. Discharged action potential

The action potential is the first step in the chain of events leading to skeletal muscle contraction (51). It can be colloquially defined as the interchange of electricity between neurons. This electrical signal originated in the central nervous system is spread through the whole organism resulting in the activation of the motoneuron (52). Once the motoneuron is activated, muscle fibers are subsequently recruited and the skeletal muscle contraction produced. The discharge of action potential triggers a series of mechanisms allowing skeletal muscle contractions (53).

The discharged action potential between motoneuron and skeletal muscle fiber causes a depolarization of the cellular membrane with the subsequent release of acetylcholine, a molecule that binds to its cellular receptors and increases sodium and potassium permeability (Figure 4). This physiological mechanism is the main responsible of skeletal muscle contraction (54).

Considering that skeletal muscle contraction occurs due to the transmission of electric current between neurons, motor neurons, and skeletal muscle fibers, it results evident that EMS can provide the same electric stimuli through an artificial device. In the EMS, the transmission of the electric current is produced by an electrode placed on the top of the skeletal muscle and transmitted to the motor neuron (55). The physiological response to the EMS is similar than the response produced by the central nervous system (56). Motor neuron activation occurs in the same way that voluntary contraction and the acetylcholine response is also the same (Figure 4).

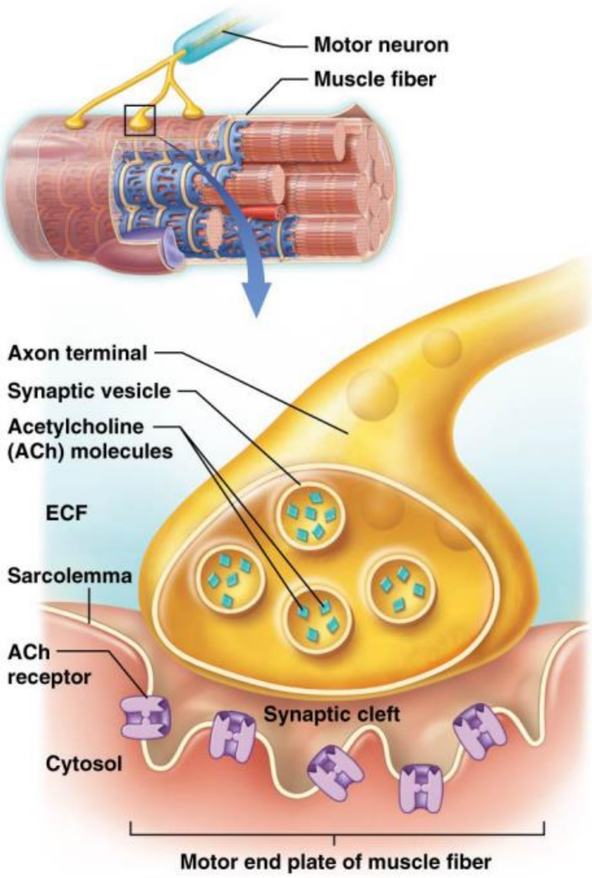

Figure 4: Anatomy and physiology of the release of acetylcholine in the motor unit (57)

The main difference between voluntary and involuntary contractions achieved through EMS is that the central nervous system is able to regulate the amount of electrical current needed whereas the EMS does not produce such an adjustment. The central nervous systems adjust following the next rule; the lower the charge, the lower the action potential and the lower the recruitment of fibers. However, EMS induces an action potential without considering any external load, being the skeletal muscle fibers recruitment not discriminated as it happens with voluntary contractions (58).

Although previous studies have suggested that there is no selection of the skeletal muscle fiber type when the contraction is induced by EMS, it is thought that certain electrical parameters could increase the proportion of fiber type I recruited, while others rise the proportion of fibers type IIa and IIx (59-62).

1.3. Electrical parameters

1.3.1. Electrical waveform

The application of electrical impulses through artificial devices is thought to achieve higher levels of strength and skeletal muscle mass development (29). EMS activates MUs to produce skeletal muscle contraction in healthy individuals and patients (63). However, the application of EMS must be carefully controlled since it is important to manage the location of the electrodes and the exercise programmed, as well as the regulation of electrical parameters selected.

Firstly, it should be mentioned the existence of different types of electrical current. The type of waveforms determines the physiological response (64, 65). Depending on the shape, it has been described triangular, rectangular, or sinusoidal waveforms. Considering their symmetry, we can find symmetric and asymmetric waveforms. Lastly, according the phase (number of times the electrical current crosses the body) we can distinguish monophasic or biphasic waveforms (66).

Regarding the benefits in muscular strength and sport performance, four electrical waveforms have proved to be efficient: monophasic rectangular current, symmetrical biphasic rectangular current, asymmetrical biphasic rectangular current, and symmetrical biphasic sinusoidal current (61). Specifically, Laufer et al. discovered that monophasic and biphasic waveforms allowed greater muscular torques with lower fatigue than the triphasic ones (67). Moreover Stefanovska and Lodovnik found that performing 10 min of EMS with sinusoidal currents at high frequencies diminished the strengthening effects (68). In summary, the scientific evidence proposed that rectangular, symmetric and biphasic waveform as the best choice to increase strength levels (69-71).

Taking into account that skeletal muscle fibers have different orders of activation with EMS and without EMS, it is crucial to understand the potential combination of the available electrical parameters. Depending on the specific aim, different electrical parameters should be used to maximize the benefits of physical exercise (72).

1.3.2. Electrical frequency

The electrical frequency - measured in hertz (Hz) - is the number of pulses per second (Figure 5). The brain sends electrical impulses with different frequencies that determine the magnitude of skeletal muscle contraction (44). For instance, to induce slow

MUs activation and slow fibers contractions, the predominant electrical discharge from the nervous system ranged from 5 to 30 Hz (73, 74). However, to produce fast MUs activation and fast fibers contractions, the predominant electrical discharge should be between 30 and 60 Hz (73-76).

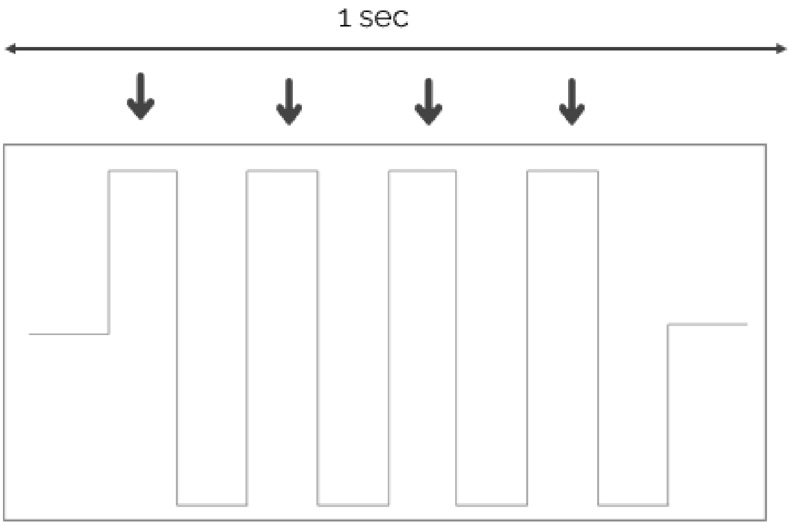

Figure 5: Number of electrical impulses during one second. It represents the electrical frequency of 4 impulses per second (4 Hertz)

Of note, artificial electrical frequency has similar nature than the electrical frequency created by the central nervous system. The electrical frequency is usually divided into low, medium and high. However, there is no scientific consensus regarding the range of this classification. For therapists, less than 100 Hz is considered low frequency while for sport scientists the range selected for high frequency should cover 66 to 100 Hz. Deley et al. proposed that less than 15 Hz was low frequency, while more than 40 Hz should be understood as high frequencies (77). However, Martínez et al. suggested that less than 40 Hz was usually considered as low frequency while more than 40 Hz would be high frequency (78). Traditionally, the difference between low frequency and medium/high frequency has been stipulated depending on the skeletal muscle tetanization (77). Below skeletal muscle tetanization the frequency was classified as low frequency, while above skeletal muscle tetanization it was classified as high frequency. In the absence of a scientific consensus on the distribution of the electrical frequencies, we proposed that the low frequency ranges from 1 Hz to 33 Hz, the medium frequency from 33 Hz to 66 Hz and the high frequency from 66 Hz to 100 Hz based on the current literature and the effects of WB-EMS in skeletal muscle mass observed in our laboratory.

This classification is important since different authors suggested that the application of different electrical frequencies would have different results in term of sport performance (79, 80). Binder-Macleod et al. showed that the higher the electrical frequency, the higher the force production, and the higher the fatigue and the muscular damage (81, 82), while others reported that low frequencies induces an increased angiogenic and blood flow effects (83, 84).

Finally, Filipovic et al. performed a systematic review in 2012 analyzing the differences between EMS methods (i.e., local EMS and WB-EMS; during isometric, dynamic, and isokinetic exercise; and with different impulse frequencies) (27). Although they found significant improvements with the application of EMS there is still no consensus regarding the electrical frequencies that optimize the benefits of EMS on different health and performance parameters.

1.3.3. Electrical intensity

The electrical intensity - measured in milliamps (mA) - is the amount of electrical current transferred from the artificial device to the human body (Figure 6) (61). The effectiveness of EMS depends on the sensory responses and the motor control during the neuromuscular recruitment (27). De Jesus Guirro et al. reported that these parameters are dependent on sex and age (85).

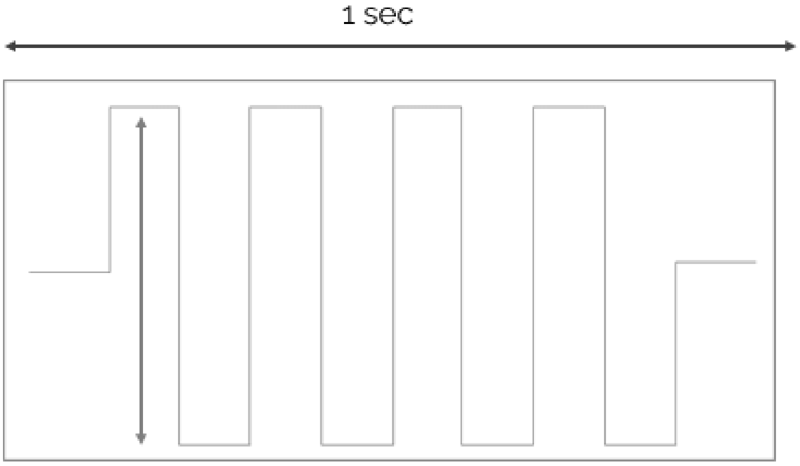

Figure 6: Quantity of electrical impulses during each impulse. It represents the amount of electrical intensity per impulse (e.g., 20 mA)

Regarding sensory and motor responses, a total of 4 levels have been described in the literature (86): (i) The sensory threshold is understood as the first lowest sensation such as itching, heat or tingling in response to EMS application. (ii) The motor threshold is reached when the skeletal muscle undergoes a contraction, although it is not strong enough to produce movements. (iii) The supramotor threshold occurs beyond motor threshold and is achieved when the EMS produces in an involuntary manner a 10% of maximal voluntary contraction (86). (iv) The pain threshold is defined as the threshold in which electric intensity supposes the initial perception of pain (86, 87). If the intensity is further increased until a threshold in which the participants could not assume any more stimulation, this point is called the maximal pain tolerance. Authors such as Delitto et al. suggested that if EMS does not reach pain threshold or maximal pain tolerance, the current intensity would not be enough to cause significant improvements in the skeletal muscles performance in quadriceps femoris (88). However, the new trends in WB-EMS application are investigating the effects of lower intensities on strength and energy metabolism (26, 89).

Current intensity is measured in mA, but just a few studies have reported such values in the scientific researches. Commonly, electric intensity is expressed as a % of maximal voluntary contraction or even with the rate of perceived exertion (RPE). Of note, the application of electrical intensity appears to be higher in trained than in sedentary people as well as in female than in males (20, 87).

1.3.4. Pulse width

The pulse width - measured in microseconds (μsec) - is defined as the duration of electric stimuli per pulse (Figure 7). Its management depends on chronaxie and rheobase of each specific skeletal muscle, terms that were defined by Louis Lapicque in 1926 (90). The rheobase and the chronaxie are the minimum intensity and time of stimulation required to generate an action potential, respectively (91, 92). In order to achieve skeletal muscle contractions, muscular chronaxie must be achieved through EMS.

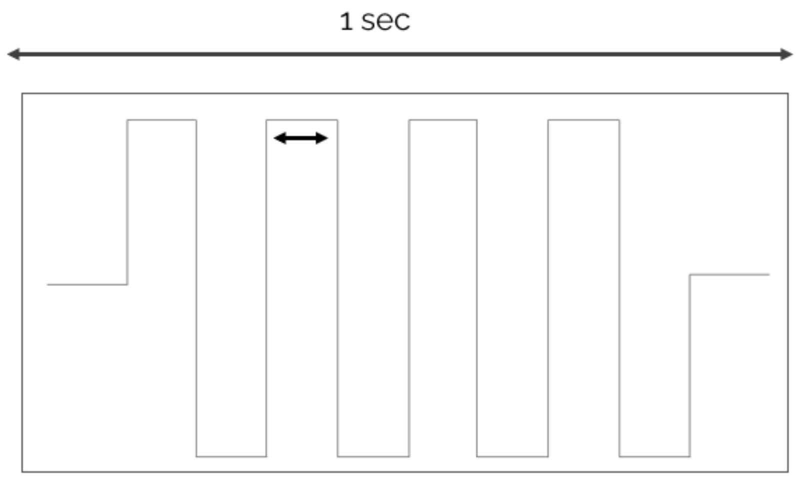

Figure 7: Time of one electrical impulse. It represents the amount of time per impulse (e.g., 200 microseconds (µsec))

The width pulse has motor and pain related consequences. For instance, regarding pain consequences Houson et al. concluded that width pulses lower than 60 µsec decrease the probability to activate pain receptors while pulses higher than 60 µsec active pain receptors. Regarding motor consequences, Bowman & Baker observed that 300 µsec produced more powerful contractions in quadriceps femoris than 50 µsec width pulses (93, 94). Moreover, previous studies have shown that the chronaxie is increased during exercise between 37.5% and 62.5%. This observation means that in order to reach any force production, the impulse width should be always higher than nervous chronaxie (95). Although it is known that lower impulse widths are related to better tolerance and less pain, some authors demonstrated that < 100 µsec could be the reason why some protocols with EMS did not achieve any significant improvement in muscle performance (60). The efficacy of conventional EMS involves pulse widths close to the nervous chronaxie between 100 and 400 µsec (96).

1.3.5. Duty Cycle

The duty cycle - measured in seconds - also called "working/resting time" is the ratio of active vs. inactive EMS (97). This variable can be expressed by the seconds when EMS is active and inactive (i.e., 30 sec active and 15 sec inactive) or by the ratio between both of them (i.e., 2:1). EMS is an artificial tool that produces involuntary contractions of the skeletal muscle mass trying to simulate voluntary contractions. Voluntary contractions are known to have a contraction and a relaxation time; the duty cycle is the electrical parameter focused on produce this similar effect.

EMS training should be organized and planned in a similar way that the used for strength training. If the aim of a specific mesocycle is to increase power force, the application of EMS must be in accordance with this objective. For example, if resting time ranged between 90 and 120 seconds, the EMS should be applied during the working time and remain inactive during resting periods.

Previous studies have suggested that the application of different duty cycle (e.g., 4:1 or 30:30) results in different physiological adaptations (61). Hultman et al. and Spriet et al. showed that an exercise protocol consisting of 45 min of EMS with a duty cycle of 1.6 sec active and 1.6 sec inactive decreased from 100% to 43% the force production in five min (98). Moreover, Selkowitz et al. found that longer periods of EMS (i.e., >10 sec) with no rest are not useful since the force production is significantly reduced after 8.2 sec (99). These authors subsequently tested the potential differences in force production between the application of an exercise protocol with 1.6 sec working time and 1.6 sec rest time vs. a continuously electrical current during 2 min and testing the skeletal muscle contraction. (100). They described that EMS protocols that applied electrical current in a continuous manner maintain higher levels of force production than short time intermittent current (100). Interestingly, Binder et al. showed that duty cycles with higher resting time proportion (specifically, double resting time than active) maintain better force production in contrast with the duty cycle characterized by lower resting time (82).

Depending on the aim of the EMS training program, the duty cycle will be different. For instance, previous studies trying to obtain higher maximal power outputs usually apply duty cycles with higher resting periods (23, 60, 101-103). But, Toca-Herrera et al. applied an exercise protocol with 10 sec active and 10 sec inactive getting an improvement in isometric force (104). The force production has many manifestations and it is of both scientific and practically interesting. Nowadays, it is common to see different applications of duty cycles in EMS training since there is no consensus about which duty cycle is better for both performance and health purposes.

1.3.6. Training time

The training time is the period in which athletes received superimposed EMS during the workout. It is registered in min, and usually covers between 10 and 20 min of the session (29). Previous evidence suggests that in order to increase the power production, it should be applied shorter training time (i.e., 8-12 min) (23, 60, 101-103). However, other works have used long-term training times; concretely, Valli et al. (105) applied 30 min of training time to increase quadriceps muscle voluntary maximal strength, while Grosset al. (106) programmed 1 hour session of electrical muscle stimulation in order to analyze its effects on EE and nutrients oxidation (NutOx).

There is a tendency to use the EMS in shorter training times arguing that longer applications could result in muscle damage and its potential health related consequences. Moreover, the idea of achieving the same results but in less time is highly attractive. However, if the aim of training is to achieve better results, the application of EMS during the same traditional time could increase the benefits of training.

2. ELECTROMYOSTIMULATION AND ENERGY METABOLISM

The management of energy metabolism and the control of energy balance are mandatory for the weight management and obesity treatment (107). It is well-known that patients with obesity should decrease energy intake while increasing EE and it is, therefore, believed that increasing EE daily could counteract the obesity incidence worldwide (108). In this sense, the World Health Organization (WHO) recommends the amount of physical activity (PA) necessary to maintain a healthy status achieving adequate daily levels of EE. Specifically, the PA guidelines recommends the combination of endurance (i.e., 150–300 min or 75-150 min of PA at moderate or vigorous intensity, respectively) and resistance exercise (i.e., ≥2 sessions/week) (109). Unfortunately, a relatively high number of adults (27.5%) and adolescents (81%) do not meet the PA recommendations (110, 111), commonly eluding to time constraints, the risk of injuries, and a lack of enthusiasm as the main barriers for that purpose (112). A recent systematic review and meta-analysis of 3.3 million participants across 31 countries showed that only 20% of adolescents and adults follow the WHO guidelines for both aerobic and muscle-strengthening activities and muscle-strengthening activities (113).

Due to the fact that people do not reach the levels of PA recommended by the WHO, it seems necessary to find alternative methodologies that motivate general population to do it and to achieve the EE necessary to maintain energy balance. One potential strategy to increase EE is to increase the intensity of training sessions and to introduce motivational and less time-consuming factors that help the compliance with PA recommendations. In this sense, EMS and WB-EMS may be a partial solution to this problem. However, to the best of our knowledge, there is no enough scientific evidence to suggest EMS or WB-EMS was an effective tool to significantly increase EE, thus combating the dramatical rates of obesity worldwide.

2.1. What is known about the effect of electromyostimulation on energy metabolism

The effects of local EMS and WB-EMS on energy metabolism are usually confused in both resting and during exercise conditions. Local EMS is characterized by applying current intensity in a selected skeletal muscle of the body and most of the studies performed with local EMS placed the electrodes on the legs (more specifically on quadriceps muscles). However, WB-EMS produces involuntary contractions in 8–12 different muscle groups. This fitness tendency is becoming increasingly popular worldwide as a potential and attractive alternative to traditional training methods to increase EE (35, 38) and simultaneously improving body composition and cardiometabolic health parameters (31, 33, 114). WB-EMS has attracted the researchers' attention for its recently published important implications on energy metabolism (38, 63). Compared to voluntary contractions - which represents a progressive skeletal muscle fiber recruitment (46) - WB-EMS presents a non-selective recruitment pattern of primarily fast-twitch MUs at relatively low force levels (115). This factor could enhance EE through the synchronous recruitment of skeletal muscle fibers (116).

Only a few studies have investigated the effects of EMS on EE at rest. Grosset et al. reported that 1 h of lower limb EMS applying a frequency of 5 Hz in resting conditions increased EE ($\Delta \approx 428\%$) (106). Minogue et al. also showed that 4 min of local 12 Hz EMS in the quadriceps augmented EE ($\Delta \approx 596\%$) (117). However, the effect of WB-EMS on EE at rest has not been analyzed yet. Kemmler et al. (38) showed that the application of high frequency (i.e., 80–85 Hz) WB-EMS at the subject's maximum tolerance in a 16-min training session consisting of slight weight-bearing movements increased EE ($\Delta \approx 17\%$) as compared to the same exercise protocol without WB-EMS. Similarly, Verch et al. (118) designed an exercise protocol based on WB-EMS, with 85

Hz of frequency during 10 min of walking and Nordic walking obtaining a ≈10% of increase in EE. Finally, Teschler et al. (119) observed that, after the application of WB-EMS in 16 healthy adults between 20 and 50 years old during dynamic squatting, the total EE increases ≈ 25% after training session.

The largest compendium of scientific articles investigating the effects of EMS and WB-EMS on energy metabolism has focused on its potential consequences on EE but not in NutOx. The respiratory exchange ratio (RER) is an indirect parameter that indicates the energy substrate that is predominantly oxidized during a given task. Only Grosset et al. have reported that 1 h of lower limb EMS applying 5 Hz at rest increased the RER (Δ ≈ 16%) (106) but, unfortunately, there is no further data regarding NutOx in the rest of the above-mentioned scientific works.

2.2. What is not known about the effect of electromyostimulation on energy metabolism

The EMS and WB-EMS training programs had almost always applied only one frequency (i.e., 85 Hz). To date, there small evidence that compared the effects of different electrical low frequencies at rest and during walking exercise with different intensities. This is because there is no consensus about what frequency elicits the highest increment in EE, none the type of exercise. Taking into account that the different electrical parameters have different physiological responses there is a need to understand better the effects of electrical current on human body. It remains unknown the effects of different electrical frequencies (between 1 Hz and 100 Hz) on EE and NutOx.

Moreover, previous studies used local EMS instead of WB-EMS and we cannot assume that these results will be the same with WB-EMS. In fact, the increase in the muscle mass involved by WB-EMS, might positively improve the effects of this technology. On the other hand, according to a recent systematic review that aimed to determine the effects of WB-EMS in energy metabolism, there is not enough scientific evidence to draw solid conclusions on the effects of WB-EMS on energy metabolism during exercise. That is the reason why examining the effects of WB-EMS on EE during an easy aerobic walking exercise (i.e., 10,000 steps) in healthy young people could be interesting in order to improve adherence and increase EE during exercise.

AIMS & HYPOTHESIS

OVERALL HYPOTHESIS

The overall hypothesis of the present Doctoral Thesis is that the manipulation of electricals parameters of WB-EMS could optimize its effects on energy metabolism and NutOx at rest and during exercise in young adults. Specifically, we hypothesize (i) that low frequencies would produce higher EE rates at rest and during exercise while increasing the RER, and (ii) that the application of WB-EMS during the recommended 10,000 steps would significantly rise EE while increasing fat oxidation (FatOx) values.

OVERALL AIM

The overall aim of the present Doctoral Thesis is to investigate the effects of WB-EMS on EE and NutOx at rest and during exercise in young adults.

SPECIFIC AIMS

Study I

The study I aimed to determine the effects of different WB-EMS electrical impulse frequencies (i.e., 1 Hz, 2 Hz, 4 Hz, 6 Hz, 8 Hz and 10 Hz) on EE and on RER at supine resting and during uphill walking in healthy young men.

Study II

The study II aimed to determine the effects of WB-EMS on EE and NutOx during walking (i.e., 10,000 steps) in healthy young adults.

MATERIALS AND METHODS

Table 1: Methodological overview of the studies included in the Doctoral thesis.

	STUDY I	STUDY II
Design	Within-subject repeated measures design	Crossover design
Participants' characteristics	Recreationally active males 10 (18-25 years old)	Healthy participants 13 (4 women, 24 ± 3 years old)
Independent variable	WB-EMS frequencies	WB-EMS application
Dependent variable	EE and RER	EE, RER and NutOx
Conditions	At rest and during uphill walking	Performing the 10,000 steps recommendation
Statistical analysis	Repeated-measures analysis of variance (ANOVA)	Paired sample T-test and area under the curve

STUDY I

Ten healthy and recreationally active males (18-25 years old) participated in the present study. A within-subject repeated measures design was used to compare the effects of different WB-EMS frequencies on EE and RER at rest and during uphill walking. The participants came to the laboratory in 3 sessions. On session 1 participants went through anthropometric and body composition measurements followed by a graded exercise test. On session 2, EE and RER were assessed at resting and unstimulated conditions during 30 min followed by the application of 6 different impulse frequencies, in random order, during 6 min each one and with a passive recovery of 10 min between them. On day 3, EE and RER were assessed during 30 min of uphill walking on a treadmill at a speed and grade eliciting an intensity of 60% of the maximal oxygen uptake (VO_2max) or peak oxygen uptake (VO_2peak) with the same order of 6 different impulse frequencies of the day 2. Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were recorded using a Vyntus CPX Metabolic Cart (Vyaire Medical Inc, Chicago, USA) and WB-EMS protocol was performed using an EMS device (Wiemspro®, Malaga, Spain) which simultaneously stimulates 8 muscle groups.

STUDY II

A total of 13 (n=4 women, aged: 24 ± 3 years) healthy participants with no previous experience in WB-EMS training were enrolled in the study. A crossover design was used to investigate the effects of WB-EMS during 10,000-steps walking test on energy metabolism and NutOx. The participants came to the laboratory in 2 sessions at least by 72 hours. On day 1, body composition and anthropometric values were measured. After that, participants performed a speed test to determine the speed at which they should perform the 10,000-steps walking test. The WB-EMS suit was put on and, depending on the experimental condition (i.e., with and without WB-EMS administration), the WB-EMS was connected or not. Once these procedures were finished, the 10,000-steps walking test began. We applied the Weir's stoichiometric equation to determine EE [EE (kcal/min) = $3.941*VO_2 + 1.106*VCO_2$] (120). The RER is determined as VCO_2/VO_2 and the substrate oxidation was estimated using the equations reported by Frayn (121):

- Carbohydrate oxidation (ChoOx) (g/min) = ($4,55*VCO_2 – 3,21*VO_2$).
- FatOx (g/min) = ($1,67*VO_2 – 1,67*VCO_2$).

RESULTS AND DISCUSSION

STUDY I

Acute effects of whole-body electromyostimulation on energy expenditure at resting and during uphill walking in healthy young men

Unai A. Perez-De-Arrilucea Le Floc'h, *Manuel Dote-Montero, Abraham Carlé-Calo, Guillermo Sánchez-Delgado, Jonatan R. Ruiz and Francisco J. Amaro-Gahete*

INTRODUCTION

Obesity is a public health problem worldwide since it is a risk factor for cardiovascular diseases and mortality (122-125). Excess body weight is mostly explained by an imbalance between energy intake and EE (126). Body weight regulation is highly influenced by genetics, physiology, and socioeconomic factors (127, 128). For instance, low EE (adjusted by body composition) is a known risk factor for increasing body weight and developing obesity (129-131). Similarly, adaptive thermogenesis, which is a reduction in EE beyond what can be predicted by changes in body weight and composition, is a barrier for further weight loss and might contribute to weight regain (132). In contrast, it seems that high EE achieved through PA can help to sustain weight loss (41). Therefore, interventions capable of increasing EE are of interest for managing body weight and preventing/treating obesity (133, 134).

It is well known that physical exercise is an effective strategy to (i) increase EE, (ii) improve body composition (135-139) and physical fitness (140-142), and (iii) reduce cardiometabolic risk factors (143-145). Indeed, the WHO has recently changed its PA guidelines recommending the combination of endurance (i.e., 150-300 minutes (min) of moderate and vigorous-intensity) and resistance exercise (i.e., >2 sessions/week) (109). Unfortunately, most people remain inactive (111), commonly eluding time constraints, the risk of injuries, and the lack of enthusiasm as barriers to sustain exercise (146-148). Exercise modalities that consume less time could be potential solutions to increase adherence to PA. In this context, WB-EMS training - which produces involuntary contractions in up to 14–18 regions or 8–12 different muscle groups - is becoming increasingly popular worldwide as a potential and attractive alternative to traditional training methods to increase EE (35, 38) and therefore improving body composition and cardiometabolic health (31, 114, 149).

Only a few studies have investigated the effects of EMS on EE and RER at rest. For instance, Grosset et al. (106) reported that 1 hour of lower limb EMS applying 5 Hz at rest increased EE ($\Delta \approx 428\%$) and RER ($\Delta \approx 16\%$). Minogue et al. (117) also showed that 4 min of local 12 Hz EMS in the quadriceps increases EE ($\Delta \approx 596\%$). However, both studies used only one frequency and local EMS. It remains unknown whether different electrical frequencies applied with WB-EMS at rest produce different EE and RER responses. Regarding the effect of EMS during exercise, Kemmler et al. (38) showed that the application of high frequency (i.e., 80-85 Hz) WB-EMS in a 16-min training session consisting on slight weight-bearing movements increased EE (Δ ≈ 17%) as compared to the same exercise without WB-EMS. Interestingly, Kemmler et al. (38) applied high frequencies (i.e., 80-85 Hz) at the subject's maximum tolerance levels. Similarly, Verch et al. (118) applied 85 Hz WB-EMS during 10 min of walking and Nordic walking finding an ≈10% increase in EE.

Electrical frequency appears to be inversely proportional to electrical intensity which is commonly applied at participant's maximal tolerance and highly influences the effects of WB-EMS (150). Hence, we hypothesized that applying lower frequencies will allow higher intensities and higher EE. The optimal electrical frequency to elicit the highest EE at supine resting and during uphill walking is currently unknown. Therefore, the present study aimed to determine the effects of different WB-EMS electrical frequencies in EE and RER at supine resting and during uphill walking.

METHODS

Participants

Ten healthy and recreationally active males (18-25 years old) participated in the present study. The inclusion criteria were: (i) no previous experience with WB-EMS training, (ii) having a stable body weight (variation of <5 kg in body weight over the previous 3 months), (iii) to show a normal weight status [body mass index (BMI) between 18.5-25.0 kg/m^2], (iv) not taking medications, (v) any chronic metabolic disease or cancer, and (vi) not suffering from any health problem that might be aggravated by exercise or WB-EMS, such as total endoprosthesis, epilepsy and abdomen/groin hernia. The participants signed a written informed consent before participation and were fully aware of the nature of the study. The study was approved by the Human Research Ethics Committee of the University of Granada (Nº 1092/CEIH/2020), registered as a clinical trial (NCT05218512) and was conducted following the latest revision of the Declaration of Helsinki (i.e., 2013).

Design

A within-subject repeated measures design was used to compare the effects of different WB-EMS frequencies on EE and RER at rest and during uphill walking (Figure 8). A wide range of impulse frequencies (i.e., 1 Hz, 2 Hz, 4 Hz, 6 Hz, 8 Hz, and 10 Hz) was applied in a randomized order, interposed by subsequent 10-minute recovery periods. After an initial screening session, participants completed two testing sessions, seven days apart. The study was conducted between October and November 2018 in the Sport and Health University Research Institute (iMUDS), Granada, Spain. On day 1 (screening), participants went through anthropometric and body composition measurements followed by a graded exercise test to determine the VO_2max or VO_2peak.

After seven days, on day 2 (first experimental session), EE and RER were assessed at resting and unstimulated conditions during 30 min followed by the application of 6 different impulse frequencies, in random order, during 6 min each one and with a passive recovery of 10 min between them. During the first 2 min of the 6 min application period, we adjusted the intensity (mA) at the participant's maximum tolerance, using the last 4 min of the bout to measure the effects of WB-EMS. On day 3 (second experimental session), EE and RER were assessed during 30 min of uphill walking on a treadmill at a speed and grade eliciting an intensity of 60% of the VO_2max or VO_2peak (without WB-EMS). The same 6 impulse frequencies were tested subsequently while the participants walked on the treadmill, with 10-minute recovery periods in between. Of note, the passive recovery was different in both experimental sessions. During the first experimental session, they waited 10 min lying on the stretcher without moving, while during the second experimental session, they waited 10 min seated steadily in a chair.

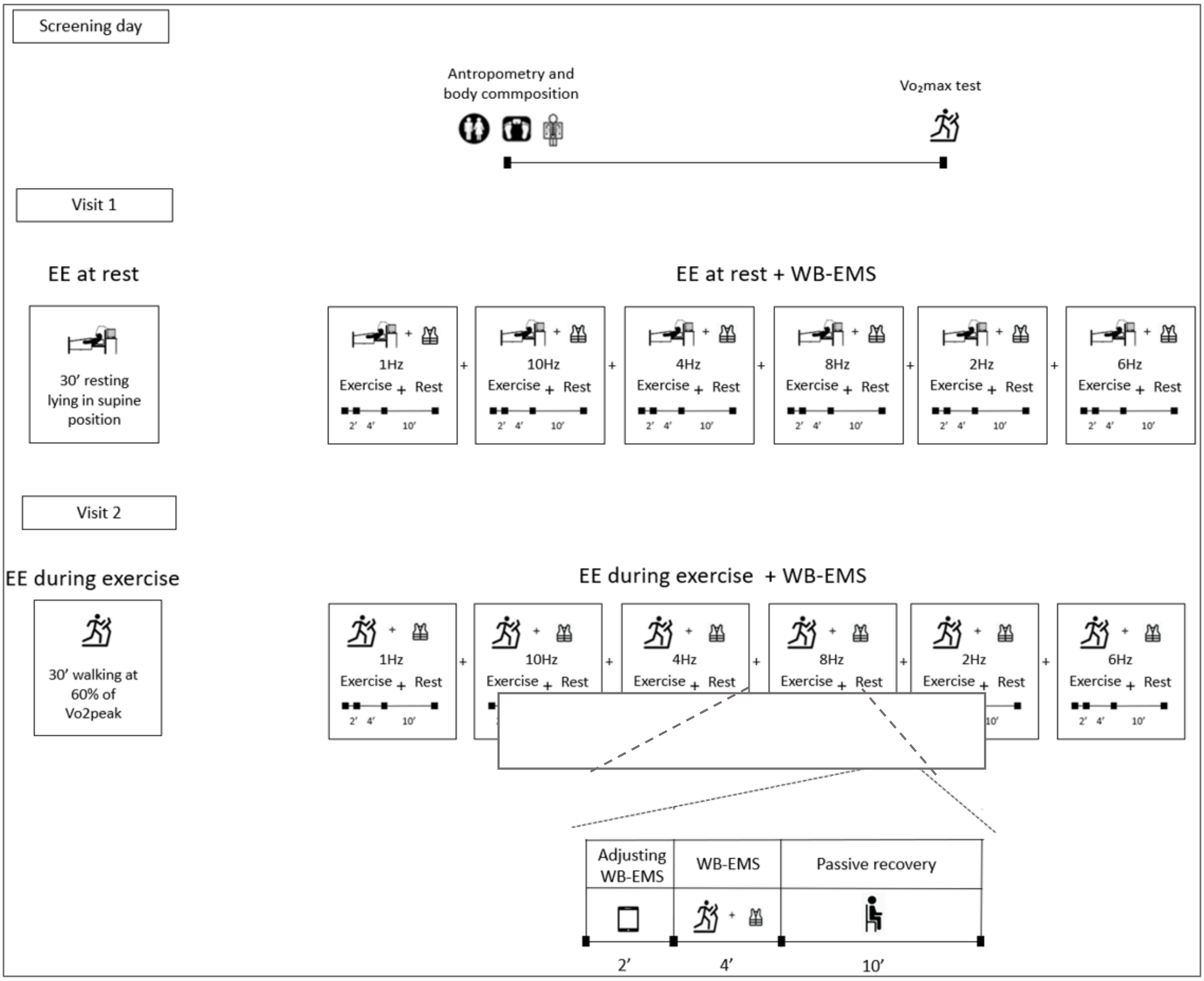

Figure 8. Study design. The different impulse frequencies were applied in a random order. Abbreviations: VO_2max, maximal oxygen uptake, EE, energy expenditure, WB-EMS, whole-body electromyostimulation.

The order of the impulse frequencies was randomly selected and counterbalanced. Participants followed the same order application on both days. Participants were instructed to replicate the same diet the day before each visit, to refrain from moderate (previous 24 hours) and vigorous PA (previous 48 hours), and to abstain from alcohol and caffeine consumption (previous 12 hours). All measurements were conducted in a temperature-controlled room (22-24ºC) and performed by the same researchers.

Procedures

Anthropometry and body composition

Body weight and height were measured using a Seca scale and stadiometer (Seca 799, GmbH & Co. KG, Hamburg, Germany) with participants being barefoot and wearing light clothing. Body composition was assessed by dual-energy X-ray absorptiometry using a Discovery Wi scanner (Hologic, Inc., Bedford, MA, USA), obtaining fat and lean mass. The fat mass index (FMI) and lean mass index (LMI) were calculated as fat body mass (kg)/height (m^2) and lean body mass (kg)/height (m^2), respectively.

VO_2max test

VO_2max was assessed during a maximum treadmill (H/P/Cosmos Pulsar treadmill, H/P/Cosmos Sport and Medical GMBH, Germany) exercise test with a progressive incremental protocol that has been extensively used and validated (151-154). The participants performed the modified Balke protocol (155) with 3 min stages. First, we estimated the maximum speed that allows comfortable walking (i.e., 4.5 km/h, 5.5 km/h, 6.5 km/h, or 7.5 km/h). Thereafter, they warmed up starting at 3.5 km/h and increasing 1 km/h every 3 min until the aforementioned speed was reached. Then, the grade was increased 2% every 3 min until volition exhaustion. VO_2 and VCO_2 were recorded using a Vyntus CPX Metabolic Cart (Vyaire Medical Inc, Chicago, USA). Participants were encouraged to invest maximum effort and provided measures of their rating of perceived exercise during the last 15 seconds (sec) of each stage, using the 6–20 Borg scale (156). The achievement of VO_2max was established as (157) : [i] showing a VO_2 change < 100 ml/min in the last 30 sec of the final stage, [ii] attaining a RER ≥ 1.1, and [iii] reaching a heart rate between ±10 beats/min of the theoretical maximal heart rate. The VO_2peak was considered when these criteria were not met (158) and all participant achieved the previously mentioned criteria.

Energy expenditure and RER at rest

EE and RER were measured while resting, laying in a supine position, between 8 AM and 11 AM after a 12-hour overnight fasting. The participants arrived at the research center by public transportation or by any motor vehicle avoiding any PA since waking up. We measured EE and RER in control conditions during a 30-min period with the above-mentioned metabolic cart. The participants were instructed not to move throughout the entire test. A silicone face-mask with a twin-tube sample line and a digital volume transducer was used for gas data collection. The measurements were subsequently recorded at 10 sec intervals for VO_2 and VCO_2. The gas analyzer was calibrated before every test using the manufacturer's automated flow and digital volume transducer calibration (i.e., 15.92% O_2 and 5.03% CO_2). The indirect calorimetry's measurement was performed in agreement with the recommended guidelines (159). Briefly, the participants were assessed in the same room, with controlled ambient temperature (22-24ºC), and by the same trained researchers. They laid on a reclined stretcher in a supine position for a minimum of 15 min before the EE and RER at rest measurement. Furthermore, the participants were instructed to breathe normally, and not to fidget, talk, or sleep while measurements were being taken.

For control (i.e., unstimulated) measurements, the first 5 min data were discarded, and records with a RER < 0.7 or > 1.0 were excluded. The coefficients of variance for VO_2, VCO_2 ventilation and RER were calculated and. the periods that met the steady-state criteria (i.e., coefficient of variance (CV) <10% for VO_2, VCO_2, and ventilation and CV < 5% for RER) were then selected. Finally, the period with the lowest CV was chosen for further analysis. EE was calculated using the stoichiometry equations of Weir (120) and expressed in kcal/min. Despite participants wore the face mask during the whole WB-EMS stimulated resting time, only the last 4 minutes of each stimulation bouts were considered in further analyses.

Energy expenditure and RER during uphill walking

EE and RER were measured while uphill walking on a treadmill. The grade of the treadmill was personally adjusted to the one that elicited 60% of the VO_2max/peak during the maximum effort test. The participants arrived in the same conditions as the first experimental session and had their unstimulated walking EE measured during 30 min followed by the application of the same order of impulse frequencies used in the first experimental session. Data analysis was performed following the same steps as during resting conditions.

Whole-Body Electromyostimulation protocol

The WB-EMS protocol was performed using an EMS device (Wiemspro®, Malaga, Spain) which simultaneously stimulates 8 muscle groups (i.e., upper legs, upper arms, upper back, gluteal, abdomen, chest, lower back, and shoulders; total size of electrodes: 2,800 cm2; Figure 9).

Figure 9. (a) Whole-body electromyostimulation suit; (b) Whole-body electromyostimulation devices that transmit electrical stimuli; (c) Whole-body electromyostimulation app to configure electrical stimuli.

Since no previous studies have investigated whether the application of different WB-EMS impulse frequencies modifies EE and RER at rest and during uphill walking, we conducted a pilot study selecting a large variety of impulse frequencies, ranging from 1 to 100 Hz. This pilot testing showed that lower frequencies (≤ 10 Hz) induced higher EE than those observed with high frequencies (> 66 Hz). Therefore, we restricted the frequency range applied in the present study from 1 to 10 Hz.

Several electrical parameters were set: a) the impulse frequency (i.e., the number of electrical pulses per time unit, measured by Hz); b) impulse intensity (i.e., the quantity of electricity, measured in mA); c) Impulse width (i.e., the time of each impulse, measured in microseconds – μsec); d) duty cycle [i.e., the ratio between time receiving electrical stimuli and the total cycle time (% duty cycle = 100/ [total time/on time])]. Impulse intensity (mA) was adjusted at the participant's maximum tolerance during the first 2 min of the 6 min application period (32, 38). Participants were asked to report the intensity of the electric impulse and the perceived intensity by using the reported perceived exertion (RPE) scale (156). Impulse width was kept fixed following general recommendations from each muscle group (160) (i.e., arms = 200 μsec, cervical = 200 μsec, chest = 200 μsec, dorsal = 250 μsec, abdominal = 300 μsec, glutes = 350 μsec and thighs = 400μsec). Finally, the duty cycle was fixed at 99% to find the physiological response to a continued electrical impulse. RPE and Visual Analogue Scale (VAS) were used to register pain perception just after the application of each frequency.

Statistical analyses

Descriptive variables are reported as mean ± standard deviation. The normality of the distribution of all variables was assessed by the Shapiro–Wilk statistic, visual check of histograms, and Q-Q plots. The data followed a normal distribution and, as a result, a repeated-measures analysis of variance (ANOVA) was used to compare the EE and RER elicited by different impulse frequencies (i.e., 1 Hz, 2 Hz, 4 Hz, 6 Hz, 8 Hz, and 10 Hz) at rest and during uphill walking. The Mauchly test indicated that the sphericity assumption (homogeneity) was met for the effects of impulse frequencies on the EE and RER at rest and during uphill walking ($p > 0.05$).

The effect size was measured by partial eta squared (η^2), and classified as small, medium, or large (<0.06, 0.06–0.14, and >0.14 respectively), following established guidelines (161). Post hoc Bonferroni tests with adjustment were performed to examine the difference between impulse frequencies. Significance was set at $p \leq 0.05$. All analyses were performed using the Statistical Package for the Social Sciences (SPSS, v. 25.0, IBM SPSS Statistics, IBM Corporation). Graphical presentations were prepared using GraphPad Prism 8 software (GraphPad Software, San Diego, CA, USA).

RESULTS

Descriptive data of the participants are shown in table 2. There were no WB-EMS-related adverse effects during the study course. Two participants did not perform the exercise part due to medical reasons (they got sick for personal reasons).

Table 2. Descriptive characteristics of the study participants.

	Mean	**SD**
Age (years)	21.6	(3.3)
Anthropometry and body composition		
Weight (kg)	77.0	(13.6)
Height (cm)	178.3	(8.2)
Body mass index (kg/m^2)	24.2	(3.8)
Lean mass index (kg/m^2)	17.4	(1.7)
Fat mass index (kg/m^2)	5.5	(2.4)
Fat mass (%)	22.3	(6.7)
Cardiorespiratory fitness		
VO_2max (ml/min)	3570.0	(555.9)
VO_2max (ml/kg/min)	46.8	(5.2)
Energy metabolism at rest		
EE at rest (kcal/min)	1.5	(0.2)
EE at rest (kcal/day)	2101.5	344.7
RER	0.794	(0.042)
Energy metabolism during uphill walking		
EE (kcal/min)	11.2	(3.2)
RER	0.898	(0.044)

Data are shown as means (standard deviation). EE, energy expenditure; RER, respiratory exchange ratio; VO_2max, maximum oxygen consumption.

Effects of WB-EMS on energy expenditure at rest

There were significant differences in EE at rest across impulse frequencies [$F_{(6,54)} = 43.23$, $p < 0.001$, $\eta^2 = 0.828$]. Post hoc analyses indicated differences in EE between unstimulated and all the stimulated impulse frequencies (all $p \leq 0.001$; Figure

10A). We noted an increase of EE from 1.47 kcal/min at rest conditions to 6.23 kcal/min (Δ = 322.8%) at 1 Hz, 8.11 kcal/min (Δ = 450.8%) at 2 Hz, 10.36 kcal/min (Δ = 603.6%) at 4 Hz, 9.52 kcal/min (Δ = 546.6%) at 6 Hz, 8.51 kcal/min (Δ = 477.9%) at 8 Hz and 7.37 kcal/min (Δ = 361.3%) at 10 Hz. There were also significant differences in RER at rest across impulse frequencies [F (6,54) = 13.20, p < 0.001, $\eta^2 = 0.595$]. Post hoc analyses indicated differences between the unstimulated period and 4 Hz (Δ = + 0.21; Δ = 25.9%; p =0.008), 6 Hz (Δ = + 0.22; Δ = 28.1%; p = 0.004), 8 Hz (Δ = + 0.26; Δ = 32.3%; p < 0.001) and 10 Hz (Δ = + 0.24; Δ = 30.6%; p = 0.016) (Figure 10B). We conducted an individual analysis of the EE and RER at rest to observe the differences between participants (Figure 11A; Figure 11B).

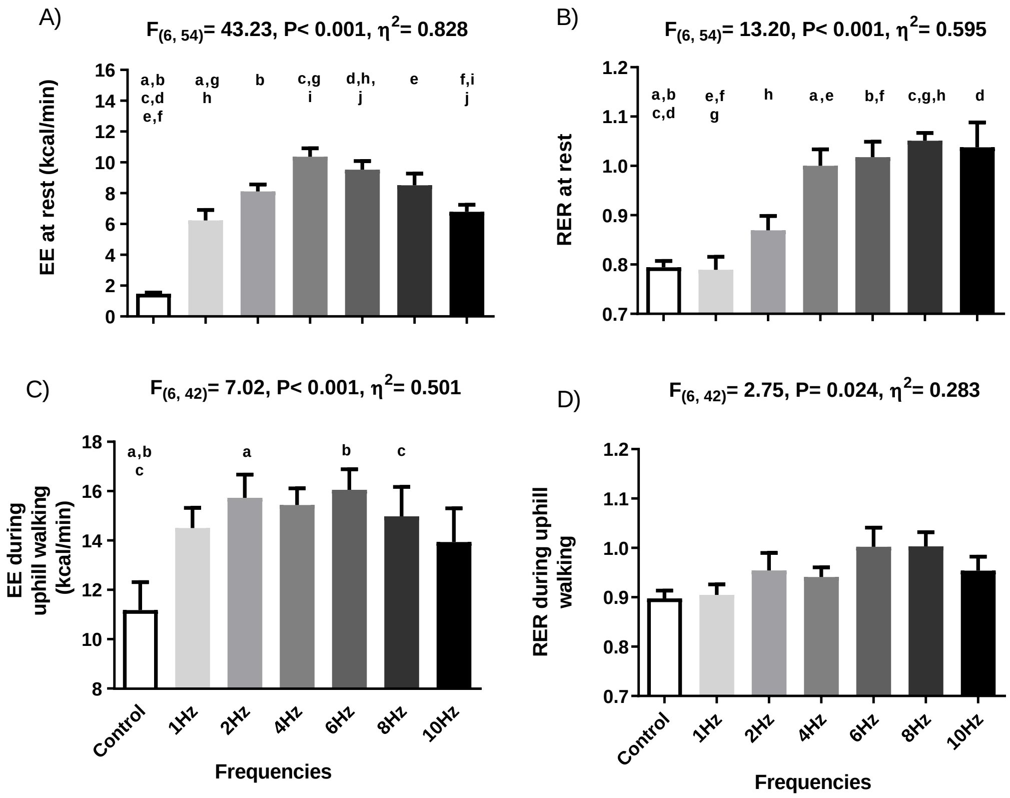

Figure 10. (**A**) Energy expenditure (EE) at rest (n=10) when applying different frequencies of whole-body electromyostimulation; (**B**) respiratory exchange ratio (RER) at rest (n=10) when applying different frequencies of whole-body electromyostimulation; (**C**) EE during uphill walking (n=8) when applying different frequencies of whole-body electromyostimulation; (**D**) RER during uphill walking when applying different frequencies of whole-body electromyostimulation. EE when applying different frequencies of whole-body electromyostimulation. *P*-values from repeated measures analysis of variance (ANOVA). Similar

letters represent differences between experimental conditions as determined by post-hoc Bonferroni analysis.

There were significant differences between frequencies in participants' pain perception [F (5,29) = 9.48 $p < 0.001$, η^2=0.613] at rest. Pos-hoc analysis showed that there were notorious disparities in perceived pain between control conditions and 6 Hz (p = 0.001) and 10 Hz (p = 0.013) in the majority of the body muscles (Figure 12). There were no significant differences between the intensities applied on each frequency (all $p > 0.071$) except for 2 Hz and 8 Hz in whole body intensity (p = 0.011), upper back (p = 0.026), lower back (p = 0.009), gluteal (p = 0.044) and hamstrings (p = 0.044) at rest (Figure 13).

Effects of WB-EMS on energy expenditure during uphill walking

There were significant differences across impulse frequencies in EE during uphill walking [F (6,42) = 7,02 $p < 0.001$, η^2=0.501]. Post hoc analyses indicated differences in EE between the unstimulated condition and 2 Hz, 6 Hz, and 8 Hz (all $p \leq 0.027$) but not for 1 Hz, 4 Hz, and 10 Hz (all $p \geq 0.063$) (Figure 10C). We observed an increase of EE from 11.18 kcal/min during unstimulated uphill walking to 15.73 kcal/min (Δ = 38.1%) at 2 Hz, 16.05 kcal/min (Δ = 43.56%) at 6 Hz, and 14.97 kcal/min (Δ = 33.94%) at 8 Hz. There were also significant differences across impulse frequencies in RER during uphill walking [F (6,42) = 2,75 p = 0.024, η^2=0.283]. However, post hoc analyses indicated no significant differences in RER between control conditions and any of the frequencies applied (all $p \geq 0.247$; Figure 10D). Specific EE and RER data of each participant during uphill walking is shown in Figure 11C and Figure 11D.

There were significant differences across impulse frequencies in pain perception during uphill walking [F (6,36) = 10.28 $p < 0.001$, η^2=0.632]. Pos-hoc analysis only showed higher pain perception at 4 Hz in low back (p = 0.041), hamstrings (p = 0.007) and gluteal (p = 0.046) compared to control conditions (Figure 14). There were no significant differences between the intensities applied in the majority of the body muscles during uphill exercise except for gluteal at 1 Hz (p = 0.046), 6 Hz (p = 0.046), 8 Hz (p = 0.007), and 10 Hz (p = 0.008) (Figure 15) but not in other muscle groups. We also measured the specific substrate oxidation during the tests but in a reduced time (4 minutes) which is represented in Figure 16.

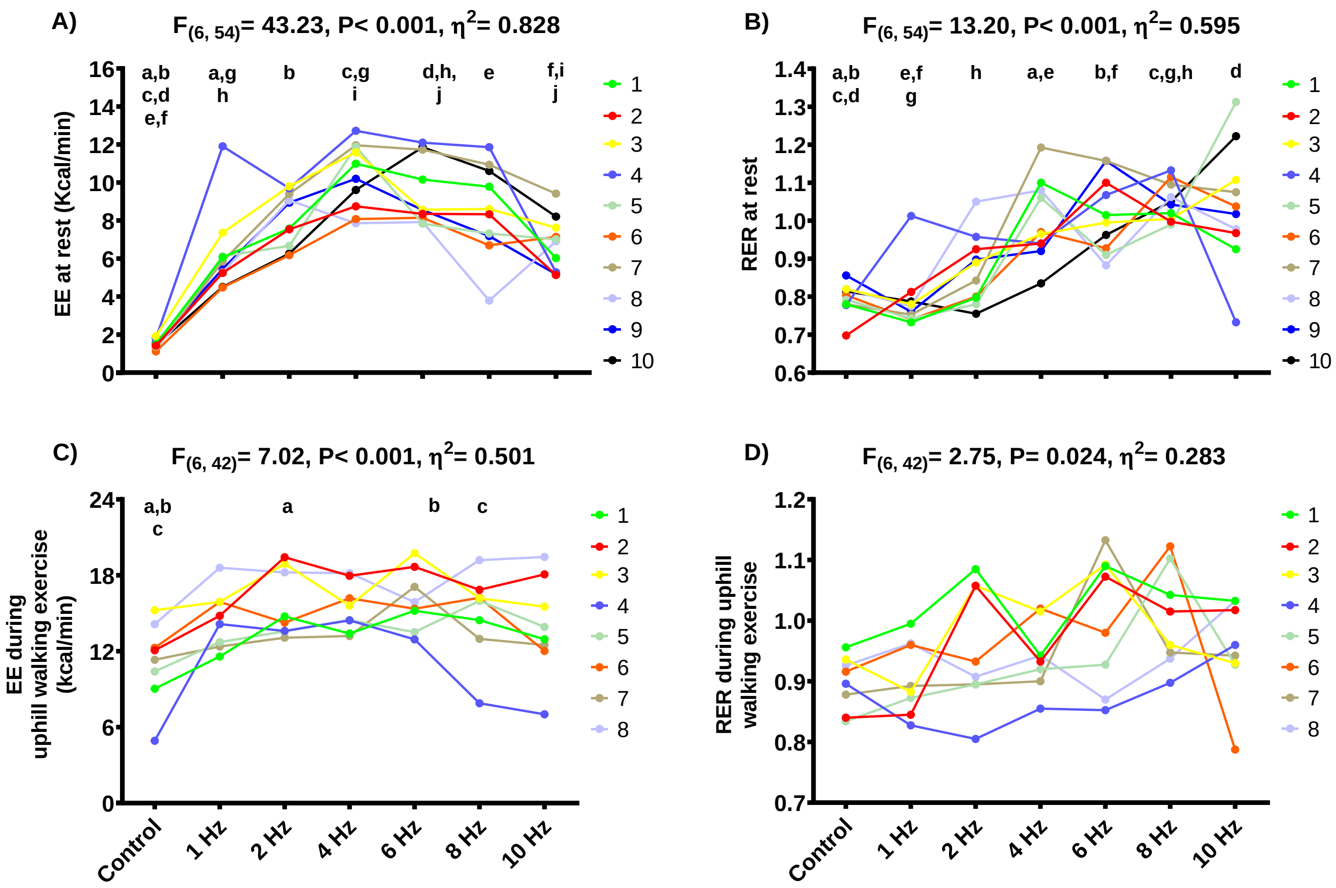

Figure 11. Individual values of energy expenditure (EE) and respiratory exchange ratio (RER) at rest (n=10) and during uphill walking (n=8) when applying different frequencies of whole-body electromyostimulation. *p*-values from repeated measures analysis of variance (ANOVA). Similar letters represent differences between experimental conditions as determined by post-hoc Bonferroni analysis. Crow data are presented as mean of 4 minutes in each frequency.

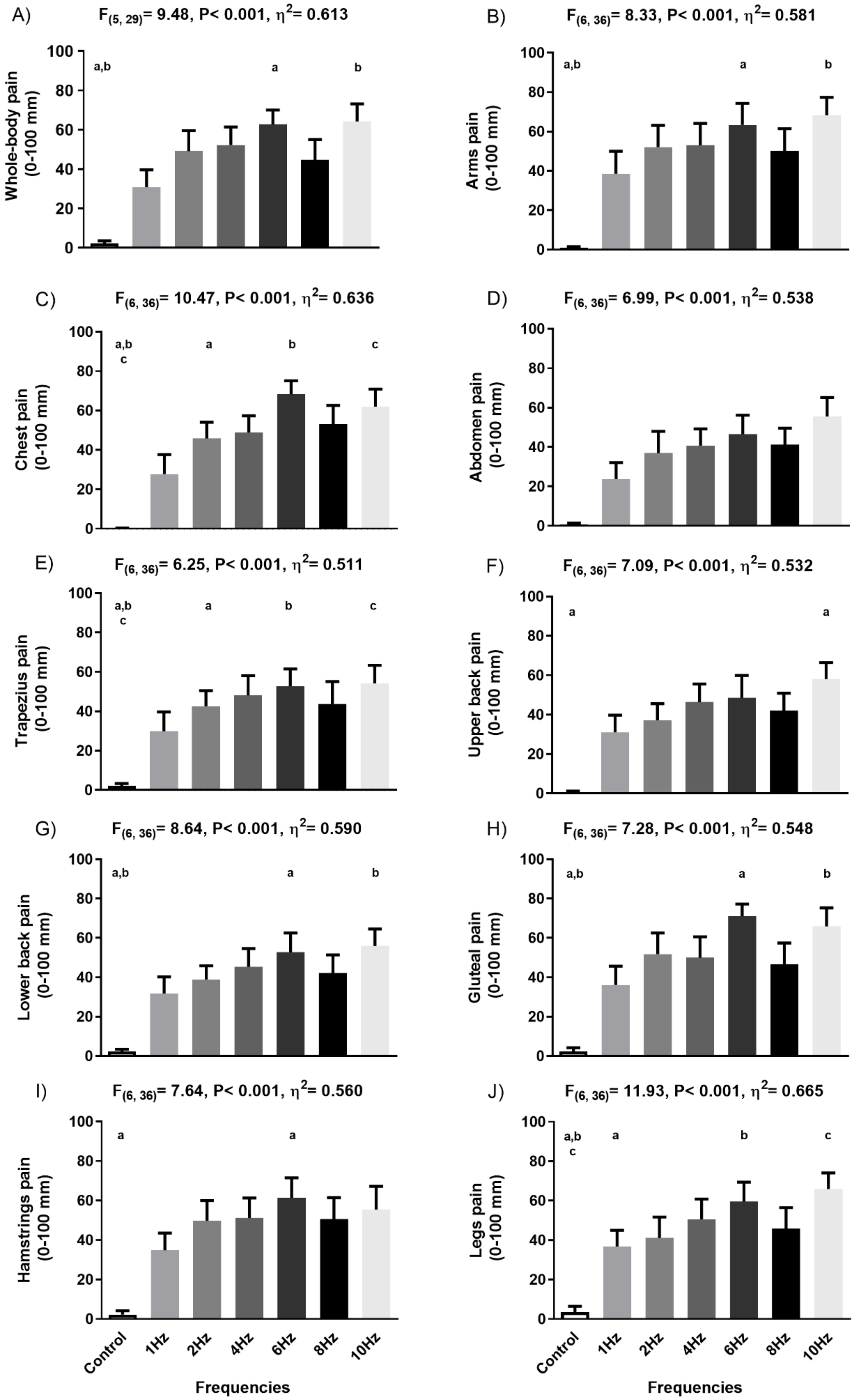

Figure 12. Pain perception in different anatomic locations after applying whole-body electromyostimulation at different frequencies at rest (n=7). Visual analogue scales (VAS) ranges from 0 to 100, being 0 "no pain", and 100 "the maximum tolerable pain". *p*-values from repeated measures analysis of variance (ANOVA). Similar letters represent differences between experimental conditions as determined by post-hoc Bonferroni analysis. Data are presented as mean and standard error of the mean (SEM).

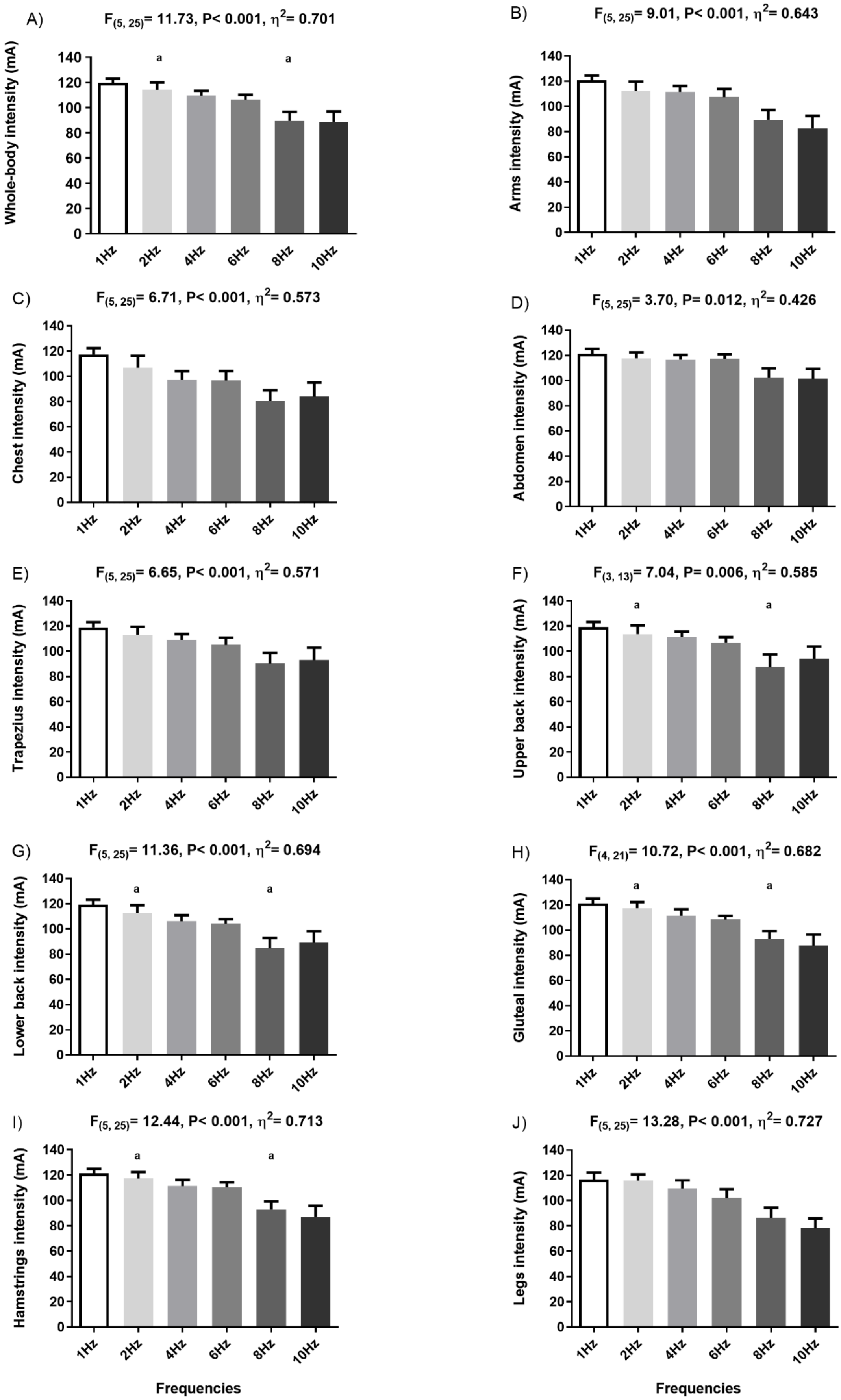

Figure 13. Impulse intensity at rest (n=6) applying whole-body electromyostimulation with different frequencies. Impulse intensity ranges from 0 mA to 125 mA, being 0 mA "no intensity", and 125 mA "all intensity possible". *p*-values from repeated measures analysis of variance (ANOVA). Similar letters represent differences between experimental conditions as determined by post-hoc Bonferroni analysis. Data are presented as mean and standard error of the mean (SEM).

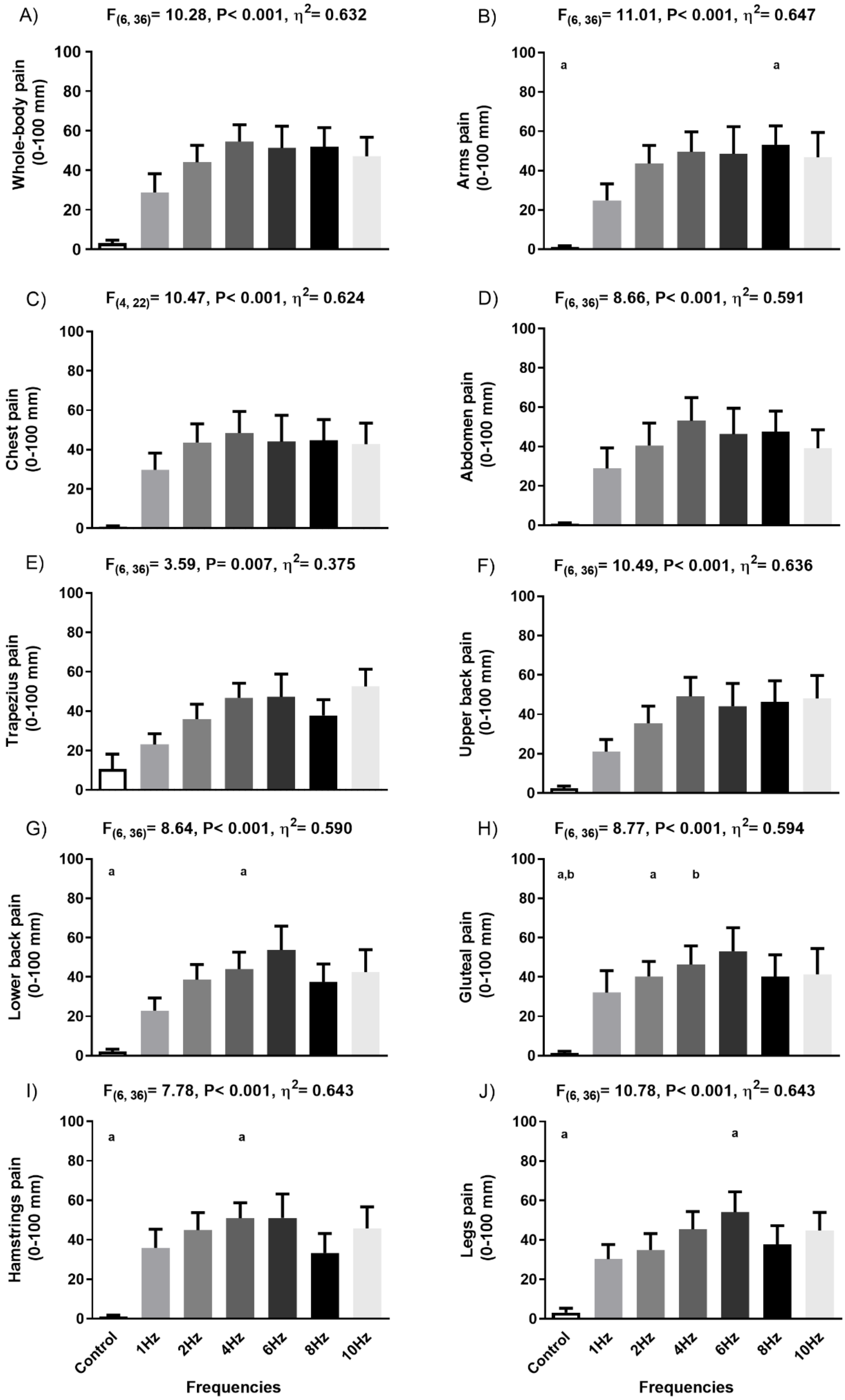

Figure 14. Pain perception in different anatomic locations after applying whole-body electromyostimulation at different frequencies during uphill walking (n=7). Visual analogue scales (VAS) ranges from 0 to 100, being 0 "no pain", and 100 "the maximum tolerable pain". *p*-values from repeated measures analysis of variance (ANOVA). Similar letters represent differences between experimental conditions as determined by post-hoc Bonferroni analysis. Data are presented as mean and standard error of the mean (SEM).

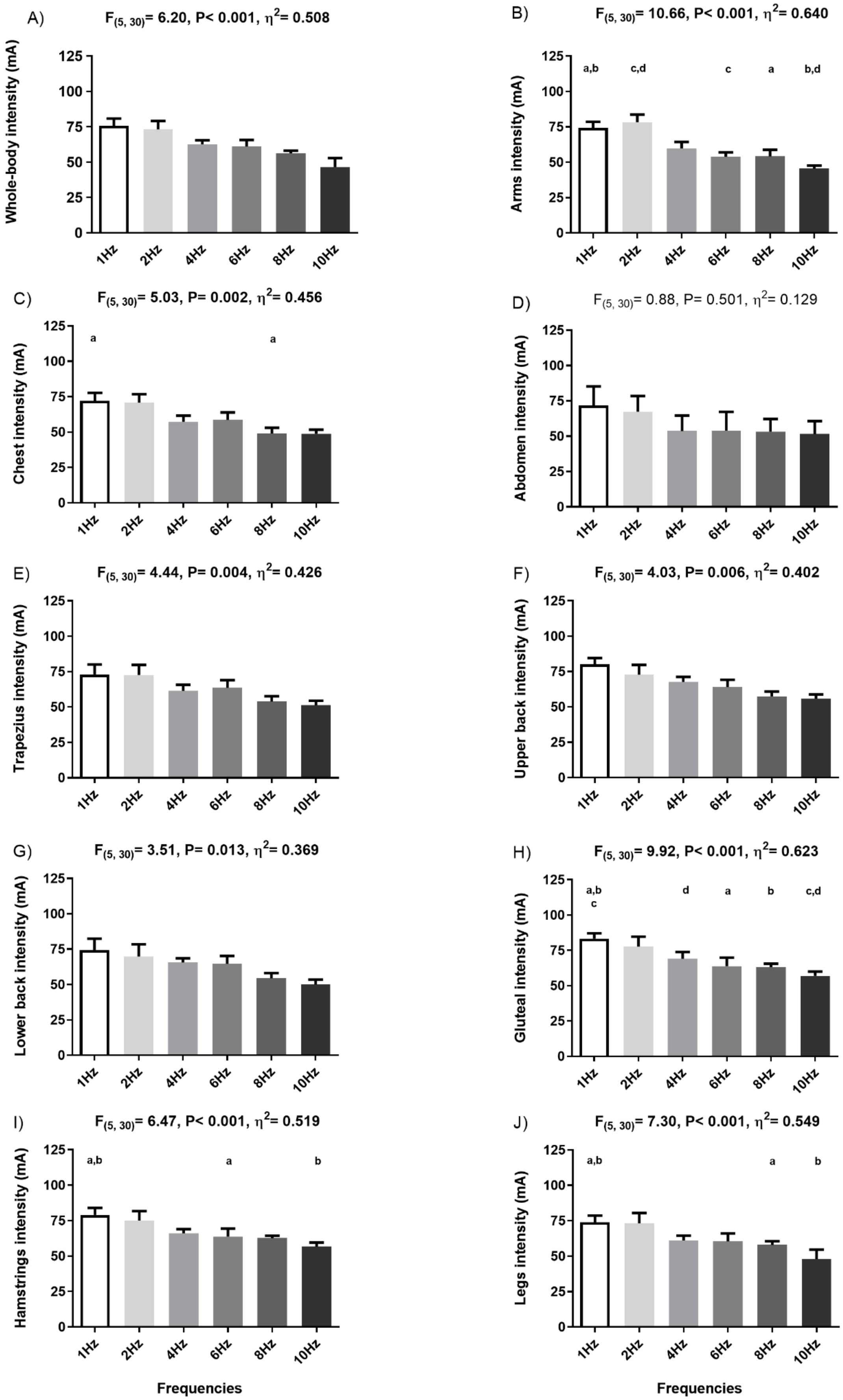

Figure 15. Impulse intensity during uphill walking (n=7) applying whole-body electromyostimulation with different frequencies. Impulse intensity ranges from 0 mA to 125 mA, being 0 mA "no intensity", and 125 mA "all intensity possible”. *p*-values from repeated measures analysis of variance (ANOVA). Similar letters represent differences between experimental conditions as determined by post-hoc Bonferroni analysis. Data are presented as mean and standard error of the mean (SEM).

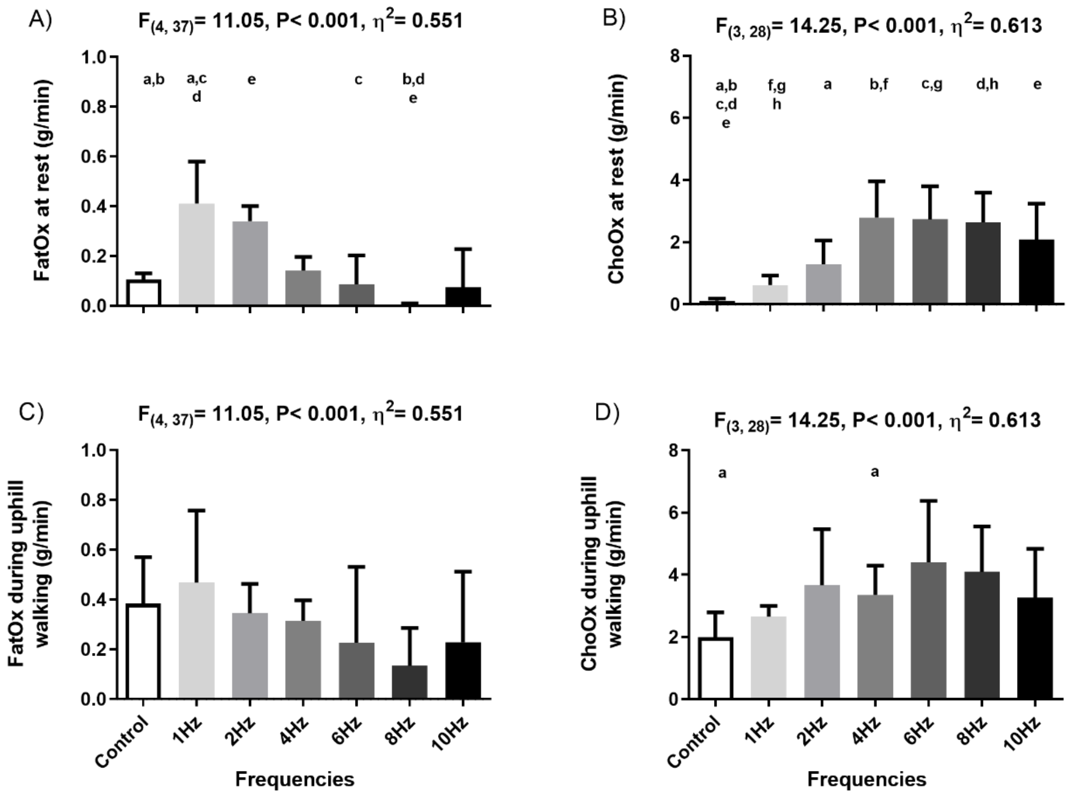

Figure 16. Fat oxidation (FatOx) and carbohydrate oxidation (ChoOx) at rest (n=10) and during uphill walking (n=8) when applying different frequencies of whole-body electromyostimulation. *P*-values from repeated measures analysis of variance (ANOVA). Similar letters represent differences between experimental conditions as determined by post-hoc Bonferroni analysis. Data are presented as mean and standard error of the mean (SEM).

DISCUSSION

To the best of our knowledge, this is the first study aiming to elucidate which is the WB-EMS impulse frequency that elicits the highest increase of EE at rest and during uphill walking. We observed that 4 Hz induces the highest EE at rest (Δ = 8.89 ± 1.49 kcal/min; Δ = 603.60%), whereas 6 Hz seems to produce the most extensive EE during uphill walking (Δ = 4.87 ± 0.84 kcal/min; Δ = 43.56%). There were also other frequencies that elicit significant increases in EE at rest (e.g., 6 Hz, Δ = 8.05 ± 1.52 kcal/min, Δ = 603.60% and 8 Hz, Δ = 7.03 ± 2.16 kcal/min, Δ = 477.87%) compared to unstimulated conditions and during uphill walking (e.g., 2 Hz, Δ = 4.55 ± 0.54 kcal/min, Δ = 40.70% and 8 Hz, Δ = 3.79 ± 0.18 kcal/min, Δ = 33.94%). A significant increase in RER at rest was induced by 4 Hz, 6 Hz, 8 Hz and 10 Hz, but not by low frequencies such as 1 Hz and

2 Hz. Lastly, there were no significant effects of WB-EMS on RER during exercise. These results suggest that a single bout of WB-EMS at 4 Hz and 6 Hz (adjusting impulse intensity to the participant's maximum tolerance) induces the highest increase in EE at rest and during uphill walking, respectively. Although 4 Hz seems to be the frequency that elicit the highest increase in EE there were no significant differences between 4 Hz, 6 Hz and 8 Hz at rest. Similarly, during uphill walking we did not find significant differences between 6 Hz, 2 Hz y 8 Hz. Intriguingly, although 1 Hz and 2 Hz do not change RER values, they increase EE at rest suggesting a potential impact on substrate utilization.

There are previous studies investigating the impact of local EMS on EE at rest. Grosset et al. (106) compared the EE induced by involuntary skeletal muscle contractions and reported that 1 hour of lower limb EMS in adults with obesity (i.e., isometric knee extension contraction at 5 Hz in a lying position without a determined duty cycle and at the maximum tolerable intensity). Local EMS increased EE rates (Δ = + 240.8 kcal/h; ≈ 428%) as compared to resting conditions. We observed a similar effect in EE although we found a higher increment (Δ = 603.60%), which is expectable since we used WB-EMS instead of local EMS. Minogue et al. (117) tested the effect of local EMS (i.e., symmetric biphasic pulse, intensity of 200 mA, phase duration of 600 µsec and interphase interval of 100 µsec) in the quadriceps muscles on EE applying different impulse frequencies during 4 min (i.e., 1 Hz, 2 Hz, 4 Hz, 5 Hz, 6 Hz, 8 Hz, 10 Hz, and 12 Hz) while seating at rest. They observed the highest EE at 12 Hz (≈ 2.8 kcal/min; Δ ≈ 596%), while the EE was increased (≈ 1.6 kcal/min; Δ ≈ 294%) when selecting 4 Hz. This increase is lower than the one observed in our study for 4 Hz (Δ = 8.89 ± 1.49 kcal/min; Δ = 603.60%). This discrepancy is likely explained because Minogue et al. (117) used local EMS in quadriceps muscles with participants seated, while we applied a WB-EMS protocol while participants were lying. Another reason could be that they used different electrical parameters such as duty cycle (99% vs 50%) among others.

Regarding WB-EMS stimulation during exercise, Kemmler et al. (38) showed an increase of Δ = + 1 kcal/min (≈ 17%) on EE, while we reported Δ = 4.87 ± 0.84 kcal/min (43.56%). These differences could be due the exercise performed and WB-EMS settings used. In the study by Kemmler et al. (38), participants performed light weight-bearing movements with high fixed frequencies (i.e., 80-85 Hz), while we performed uphill walking with low frequencies (i.e., 1Hz-10Hz). We cannot compare the electrical intensity since they did not report the one, they applied. On the other hand, Verch et al. (118) aimed to determine the differences in VO_2 comparing Nordic walking and walking with and without WB-EMS. They continuously applied WB-EMS during 10 min with the following pattern: 9 sec at 85 Hz and 1 sec at 7 Hz, with a pulse width 350 μsec in every muscle stimulated at the individual tolerated maximum intensity (118). Interestingly, they found a ≈ 10% increase in VO_2 elicited by WB-EMS (118). The comparison between Verch et al. (118) findings and our study's results is hard since electrical parameters and time of application are not equivalent. However, we observed a much bigger increase in EE than Verch et al. (118).

The RER indicates the prevalence of one substrate utilization (i.e., fat vs. carbohydrate utilization), provided that some assumptions are met (162). We observed lower RER when applying 1 Hz and 2 Hz at rest suggesting higher fat utilization compared to the rest of the frequencies. Importantly, these frequencies increased EE without modifying RER, which suggest that low frequency WB-EMS preferentially induce FatOx, a fact that could be extremely interesting since there is enough evidence to think that low-fat utilization is a risk factor to develop obesity and weight gain (163). Higher frequencies are usually associated with a higher activation of fast-twitch type IIX and IIA muscle fibers, which consume glucose almost exclusively and in turn would explain the higher RER (164). In this sense, Hamada et al. (165) compared RER after the application of EMS in different conditions: (i) involuntary lower limb muscle contractions at 20 Hz with duty cycle of 1-sec stimulation / 1-sec pause for 20 min vs. (ii) voluntary skeletal muscle contractions consisting on cycling at the same intensity obtained with EMS during 20 min (165). They found a higher increase of RER during the application of local EMS at 20 Hz than during the voluntary skeletal muscle contraction (165). Although the increase in RER observed by Hamada et al. (165) was similar to the one observed in our study, this comparison should be performed cautiously since electrical parameters and time of application were quite different. On the other hand, Minogue et al. (117) found the highest increase in RER at 12 Hz ($\Delta \approx + 0.16$; $\Delta \approx 18\%$) while we obtained the highest increase in RER at 8 Hz ($\Delta = + 0.26$; $\Delta = 32.3\%$). Grosset et al. (106) observed an increment in RER ($\Delta = 16\% \pm 4\%$) in response to a 1-hour bout of maximally tolerated low-frequency electrical muscle stimulation at 5 Hz (lying position) in people with obesity. We observed similar RER values using 4 Hz, 6 Hz, 8 Hz y 10 Hz ($\Delta \approx 29\% \pm 3\%$), but not with 1 Hz and 2 Hz ($\Delta \approx 4\% \pm 7\%$), during just 4 min.

Electrical frequencies and intensities are inversely proportional and the relationship between both parameters could explain the differences observed in EE. Intensity has been positioned as a crucial parameter to modulate EE. For instance, Hsu et al. (166) demonstrated that higher impulse intensities induced greater EE rates during the application of local EMS. The effects of WB-EMS are dependent of impulse intensities and since the above-mentioned studies did not report the intensities applied, we cannot compare them. This electrical parameter could be the reason why we observed different responses in EE and RER compared to other studies. Based on our results there is a tendency to reduce the intensity while the frequency increases, however, we did not find significant differences in intensity between frequencies in both conditions (i.e., resting and during uphill walking). Moreover, we only found differences in pain perception between 6 Hz and 10 Hz at rest, whereas no differences were noted during uphill walking. Thus, we can assume that the results are neither influenced by intensity or pain perception. However, when participants remained at rest, the whole-body intensity was higher than when they were walking (i.e., 104 ± 19 mA vs 62 ± 16 mA; $p < 0.001$) due to the incompatibility to walk with high intensities. This fact would explain why RER values rise to higher levels at rest than during uphill walking. Concretely, we found that RER values at 10 Hz at rest were 1.04 ± 0.16 while during uphill walking were 0.95 ± 0.08 at the same frequency.

We showed that the application of low-frequencies of WB-EMS at rest produces induce an EE level similar to the one observed during uphill walking (i.e., at 60% of the VO_2max). This could be of interest for individuals with severe obesity problems since long aerobic training sessions at moderate intensity could lead to joint and biomechanical imbalance increasing injury risk (167), as well as for people with reduced mobility. It is believed that high EE achieved through PA and/or exercise can help to sustain weight loss (41). Therefore, interventions capable of increasing EE might result in a better management of body weight and obesity (134). Moreover, since it has been suggested that most exercise interventions are not usually attractive for these patients, in part attributed to the uncomfortable feelings experienced during the exercise session (168, 169), WB-EMS training sessions could be an attractive alternative to enhance the motivation of this cohort. However, we cannot underestimate that although our participants did not report extreme pain, they anecdotally reported discomfort while walking with the WB-EMS at maximum intensity which could also result in poor adherence.

STRENGTH AND LIMITATIONS

This study had several strengths and limitations. One of the strengths is that the technology applied allowed us to record the impulse intensity applied. Moreover, it allowed us to increase and decrease the intensity of each body muscle independently. However, we found also several limitations. Although we found significant differences between frequencies, the limited sample size should be considered. Moreover, we only measured the effects of each frequency during 4 min, not enough time to talk about substrate oxidation despite being measured. This is an acute study and, for this reason, the data cannot be extrapolated to possible chronic adaptations such as a decrease in fat mass. Furthermore, WB-EMS equipment still has a high price, which means that this technology is not accessible to everyone. Our study only included young male adults with no previous experience with WB-EMS and our results cannot be extended to other populations.

CONCLUSION

- Relatively low frequencies of WB-EMS are an effective tool to increase EE at rest and during uphill walking in young healthy adults. Specifically, although 4 Hz is the frequency that elicits the major increment in EE at rest, other frequencies such as 2 Hz, 6 Hz and 8 Hz also did this compared to unstimulated conditions.
- The frequency that produces the highest increment in EE during uphill walking is 6 Hz, having a similar effect with 2 Hz and 8 Hz, compared to unstimulated conditions.
- Low frequencies (1 Hz and 2 Hz) of WB-EMS do not increase the RER values despite an increase in EE at rest suggesting that it could have an impact on fuel utilization rates.

STUDY II

Whole-body electromyostimulation increases energy expenditure during walking in healthy young adults

Unai A. Perez-De-Arrilucea Le Floc'h,
Miguel A. de-la-Torre-Tallon, Jonatan R. Ruiz and Francisco J. Amaro-Gahete

INTRODUCTION

PA guidelines are created and promoted by governmental and non-governmental agencies to provide further directions for health maintenance in the general population (170). Indeed, it is one of the most powerful strategies (i) to lower the risk of all-cause mortality, (ii) to decrease the incidence of multiple medical co-morbidities, and (iii) to improve the overall quality of life (171-173). Physical inactivity is considered a major public health threat since it is strongly associated with the development of different cardiometabolic disorders that produce a notable economic burden (174).

The WHO recommends meeting a total of 150–300 minutes (min) of aerobic PA at moderate-intensity or 75–150 min of aerobic PA at vigorous-intensity (170). Previous studies have suggested that walking should be considered an appropriate way to reach the aforementioned recommendations (175), with a higher number of daily steps being strongly linked to decreased all-cause morbi-mortality rates (176). Normative data indicate that healthy individuals typically register between 4,000 and 18,000 steps per day, being 10,000 steps per day a reasonable number for being in a healthy status for this population (177).

However, physical inactivity rates are continuously increasing worldwide during the last decade (178). Concretely, almost 40% of the Spanish population have a sedentary lifestyle (179) referring to 'Not having enough time' as the major external barrier to do PA (180). A secondary barrier highlighted by these individuals is the increased use of technology for daily tasks, as well as the difficulty in feeling motivated to be more active (178). Altogether, are the most important reasons for the increased incidence of obesity and other cardiometabolic diseases (175). Obesity is mainly characterized by an energy imbalance (i.e., energy intake vs. EE) (181, 182). It is well-known that increasing EE is the most efficient strategy to reduce body weight and fat mass (183) and improving the individuals' motivation seems to be crucial to produce a life behavior change related to augment PA levels (184, 185).

In this context, WB-EMS is an increasingly popular training method that has become a focus of research in recent years (186). WB-EMS has attracted the researchers' attention for its important implications on energy metabolism (38, 63). Compared to voluntary exercise which represents a progressive muscle fiber recruitment (46), WB-EMS presents a non-selective recruitment pattern of primarily fast-twitch motor units at relatively low force levels (115), a factor that could enhance EE through the synchronous recruitment of muscle fibers (116).

Kemmler et al. (38) showed an increase of ≈ 17% in EE during low-intensity resistance exercises with WB-EMS compared to the same exercise workout without WB-EMS. Similarly, Verch et al. (118) also observed a significant increment in EE performing Nordic walking with WB-EMS vs. unstimulated conditions. However, both studies applied different electric parameters (i.e., frequency, intensity, wide pulse, duty cycle, and time of training) since there is no consensus regarding which ones are the most optimal to rise EE during exercise. According to a recent systematic review that aimed to determine the effects of WB-EMS in energy metabolism, there is not enough scientific evidence to draw solid conclusions on the effects of WB-EMS in energy metabolism (30). Nevertheless, a recently published study focused on comparisons of electrical frequencies have suggested that low frequencies (i.e., 4 Hz and 6 Hz) seem to boost the increase in EE over high frequencies as used by Kemmler et al. (38) and Verch et al. (118).

Gathering data about the increase in EE could be interesting for the inclusion of WB-EMS for people who need to increase their daily EE as part of their routines. If this hypothesis is confirmed, this strategy will undoubtedly have an impact on the control and management of obesity and its comorbidities.

Therefore, this study aimed to determine the effect of WB-EMS on EE and substrate oxidation during aerobic walking exercise (i.e., 10,000 steps) in healthy young adults.

METHODS

Participants

A total of 13 (n=4 women, aged: 24 ± 3 years) healthy participants with no previous experience in WB-EMS training were enrolled in the study. One woman left the study due to medical reasons. All of them provided oral and written informed consent to participate in the current project. The study complied with the revised ethical guidelines of the Declaration of Helsinki (revision of 2013) and approved by the Portal de Ética de la Investigación Biomédica de Andalucía (1172-N-22), and it was carried out at the Instituto Mixto Universitario Deporte y Salud (IMUDS, Granada, Spain) of the University of Granada.

The inclusion criteria were as follows: adults aged between 18 and 30 years old, body mass index between 18.5-25 kg/m^2, not to be physically active, not having made a weight loss program in the last 3 months, to have stable body weight during the last 3 months (changes <3 kg), and acceptance of informed consent. The exclusion criteria were the following: taking some type of drug, being pregnant, suffering from cardiovascular disease, cancer, diabetes, hypertension, hyperthyroidism or hypothyroidism, wearing an implantable cardiac pacemaker or prosthesis, and having any non-controlled medical condition that could alter the results of the study.

Design

A crossover design was used to investigate the effects of WB-EMS during 10,000-steps walking test on energy metabolism and substrate oxidation. The timeline of the present study can be seen in Figure 17. Each participant attended the laboratory for 2 days separated at least by 72 hours to complete the 10,000 steps walking test with and without WB-EMS in a randomized order. They were instructed (i) not to engage in any moderate intensity exercise (within the previous 24 hours) or vigorous intensity exercise (within the previous 48 hours), (ii) to fast 6-8 hours before the test (iii) to abstain from alcohol or stimulants (within the last 6 hours), and (iv) not to alter their sleeping habits.

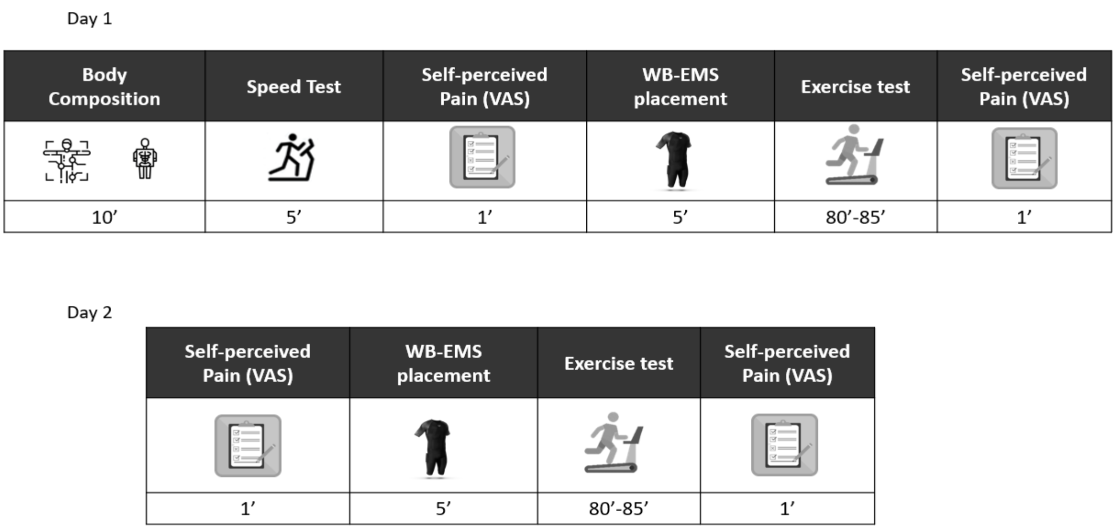

Figure 17. Study Design. Abbreviations: WB-EMS: Whole-body electromyostimulation; VAS: Visual analog scale.

On the first day, the participant came to the lab and signed the written informed consent. Subsequently, the anthropometric and body composition evaluations were completed. Weight and height were measured without shoes and wearing light clothes and a digital integrated scale and stadiometer was used (SECA, Hamburg, Germany). BMI was calculated as weight (kg)/height (m^2). Body composition (i.e., lean mass and fat mass) was determined by Dual Energy X-ray Absorptiometry (Discovery Wi scanner, Hologic, Inc., Bedford, MA, USA.). After that, participants performed a speed test to determine the speed at which they should perform the 10,000-steps walking test. This test consisted of starting by walking for one min at a speed of 3.5 km/h, increasing the speed by 1 km/h every min until achieving a comfortable walking rhythm (i.e., 10-12 on Borg Scale) without running (156).

Previously to 10,000-steps walking test, participants completed a pain test through the VAS to measure the perceived muscle pain in response to WB-EMS (i.e., quadriceps, biceps femoris, gluteus, abdomen, pectorals, lower back, upper back, biceps, and triceps). Afterward, the WB-EMS suit was put on. The WB-EMS suit was to be worn on both days in order to reduce any burden. However, the WB-EMS device that provides the electrical stimulation was only activated on the WB-EMS trial. Once these procedures were finished, the 10,000-steps walking test began.

The ergometer used was a treadmill (H/P/Cosmos Pulsar treadmill, H/P/Cosmos Sport & Medical GMBH, Germany) and heart rate was continuously evaluated using a heart rate monitor previously validated (187) that was placed on the forearm. The 10,000 steps were measured through the Samsung Health app (188) (Samsung Electronics Co., Ltd., Suwon, South Korea) using a smartphone that the participant carried in their pocket. This application was checked every 10 min to control the steps count. During the test, EE and substrate oxidation were estimated by indirect calorimetry and two lactate measurements (at 5,000 and 10,000 steps) were made through a prick in the index finger of the hand and evaluated with a lactate analyzer. Finally, the pain perception test was performed just after the end of the 10,000-steps walking test. On the second day, the same process was carried out without performing the anthropometric analysis and the speed test, and in the opposite condition of day one.

WB-EMS was implemented through a suit (Wiemspro®, Malaga, Spain) that covers the entire body and stimulates various muscle groups (i.e., quadriceps, biceps femoris, gluteus, abdomen, chest, lower back, upper back, biceps, and triceps) (Figure 18). The suit has several electrodes placed in the previously mentioned areas. To note, the effects of WB-EMS are dependent of the selected: (i) electrical frequency (i.e., number of pulses per second, measured in Hz), (ii) electrical intensity (i.e., the amount of electrical current applied, measured in mA), (iii) wide pulse (i.e., the time of application of each electrical impulse, measured in µsec) that ranges from 200-400 µsec depending on the muscle group, and (iv) duty cycle (i.e., the time that WB-EMS is active vs. inactive, measured in seconds).

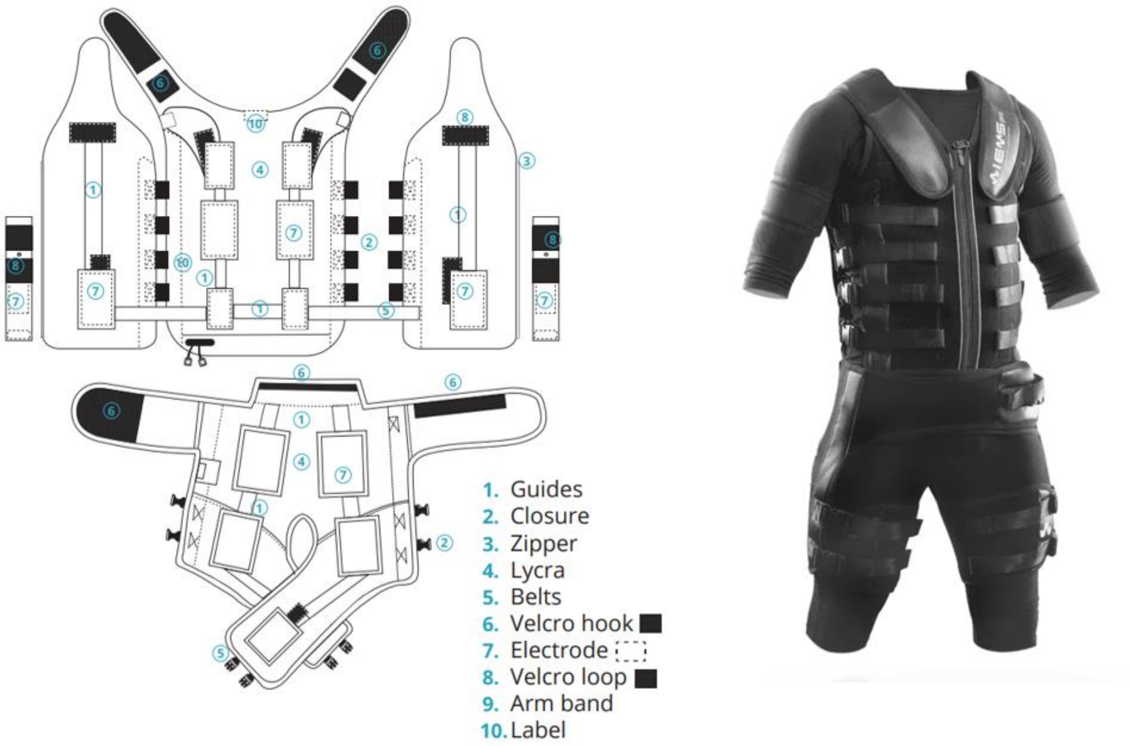

Figure 18. Whole body electromyostimulation EMS suit (Wiemspro®, Malaga, Spain).

The electric current is handled through a remote tablet and software (Wiemspro®, Malaga, Spain), and was applied using the following parameters based on a recently published manuscript conducted in our lab aiming to maximumly increase EE:

- Frequency: 21 Hz.
- Intensity: 6/10 on the subjective intensity perception scale.
- Pulse width: quadriceps = 400 µs, biceps femoris = 350 µs, gluteus = 350 µs, abdomen = 300 µs, lower back = 250 µs, upper back = 250 µs, chest = 200 µs, and biceps = 200 µs and triceps = 200 µs.
- Duty cycle: 40-20 (66%).

Procedures

Gases exchange parameters during the 10,000-steps walking test

Gas exchange data were obtained and exported from the metabolic cart (Medical Graphics Corp, St Paul, MN, USA.) to Excel for Windows (Microsoft Corporation, ABQ, New Mexico, United States). The EE was calculated from the analysis of VO_2 consumption and VCO_2. We applied the Weir's stoichiometric equation to determine EE [EE (kcal/min) = 3.941*VO_2 + 1.106*VCO_2] (120). The RER is determined as VCO_2/VO_2 and the substrate oxidation was estimated using the equations reported by Frayn (121):

- Carbohydrate oxidation (ChoOx) (g/min) = (4,55*VCO_2 – 3,21*VO_2)
- Fat oxidation (FatOx) (g/min) = (1,67*VO_2 – 1,67*VCO_2)

To calculate the EE through the test, the ≈80 min duration steady stage was split into 5 min stages, and the average EE of each stage was calculated. Therefore, a total of ≈16 mean EE values (one per each stage) were obtained. Subsequently, the area under the curve (AUC, trapezoidal rule) and the AUC expressing it as a percentage of its baseline data were calculated. The same procedure was conducted to calculate the RER, FatOx, and ChoOx during the test. The obtained parameters were used in the subsequent analyses.

Lactate, heart rate, pain perception, and Rate of Perceived Exertion (RPE)

The blood lactate concentration is sensitive to changes in exercise intensity and was measured at 5,000 and 10,000 steps using a Lactate Pro 2 (Lactate Pro, Fact Canada, Quesnel, BC, Canada). Heart rate was measured during the whole test using an infrared heart rate monitor (OH1 polar, Kempele, Finland). The perceived pain was evaluated through a VAS (189) where 0 was “No pain” and 10 was “Unbearable pain”. Using the Borg scale (156), perceived exertion was estimated through a scale from 6 to 20, with 6 being no exertion at all and 20 being maximal exertion.

Statistical analyses

At the end of the 10,000-steps walking test, data of energy metabolism and substrate oxidation were exported and explored. Initially, a descriptive analysis of the selected sample was carried out. Data normality was assessed using the Shapiro-Wilk test, histograms, and Q-Q plots. As the data followed a normal distribution, T-Student test and area under the curve (AUC) was performed for paired samples to compare the change in dependent variables between both conditions. Linear mixed model analyses were used to examine the kinetics of gas exchange parameters during exercise with and without WB-EMS. All tests were performed using the statistical package SPSS, v. 25.0, IBM SPSS Statistics, IBM Corporation, and graphs were presented using GraphPad Prism 8 software, San Diego, CA, USA.

RESULTS

Descriptive data of the participants and mean value and five minutes mean of each muscle impulse intensity are shown in Table 3, Table 4 and Figure 23. According to the protocol, all participants finished the test, and no adverse effects related to WB-EMS were noted.

Table 3. Descriptive characteristics of the study participants.

	N	Mean	SD
Age (years)	13	24.1	± 3.3
Sex (%)			
Men	8	61.5	
Women	5	38.5	
Anthropometry and body composition			
Body mass (kg)	13	69.3	± 11.2
Height (m)	13	1.7	± 0.1
Body mass index (kg/m^2)	13	23.6	± 2.7
Lean mass (kg)	13	48.1	± 9.6
Lean mass index (kg/m^2)	13	16.2	± 2.3
Fat mass (kg)	13	16.9	± 5.0
Fat mass index (kg/m^2)	13	5.8	± 1.7
Body fat percentage (%)	13	25.2	± 6.8
Visceral adipose tissue (g)	13	240.0	± 122.6

Data are shown as means (standard deviation).

Table 4. Whole-body electromyostimulation (WB-EMS) intensity measured in milliamps of the study participants.

	N	Mean		SD
Legs (mA)	13	29.8	±	5.9
Hamstrings (mA)	13	30.7	±	6.2
Gluteal (mA)	13	29.2	±	6.4
Low back (mA)	13	30.2	±	7.2
Upper back (mA)	13	33.8	±	7.1
Abdomen (mA)	13	29.4	±	7.3
Chest (mA)	13	19.6	±	8.0
Arms (mA)	13	21.7	±	5.7
Global RPE (0-10)	13	6.1	±	0.2

Data are presented as mean and standard deviation (SD). RPE, rating of perceived exertion; mA, milliamps.

We found a significant increase in EE (27.9 ± 5.4 kcal/min vs. 33.8 ± 7.0 kcal/min; Δ 5.9 kcal/min; 21%; $p < 0.001$), VO_2 (1.1 ± 0.2 L/min vs. 1.4 ± 0.3 L/min; Δ 0.3 L/min; 27%; $p < 0.001$), and VCO_2 (1.0 ± 0.2 L/min vs. 1.2 ± 0.3 L/min; Δ 0.2 L/min; 20%; $p = 0.001$) comparing unstimulated conditions vs. WB-EMS (**Figure 19, Panel A, E, and G**). We did not find significant differences in RER (0.89 ± 0.06 vs. 0.86 ± 0.07; Δ 0.03; 3%; $p = 0.149$) between conditions (**Figure 19, Panel C**).

Additionally, we found significant differences across conditions in the total AUC of EE, RER, and VCO_2 (all $p < 0.032$; **Figure 19, Panel B, D, and H**). There were no significant differences in total AUC of VO_2 between conditions ($p = 0.061$; **Figure 19, Panel F**)

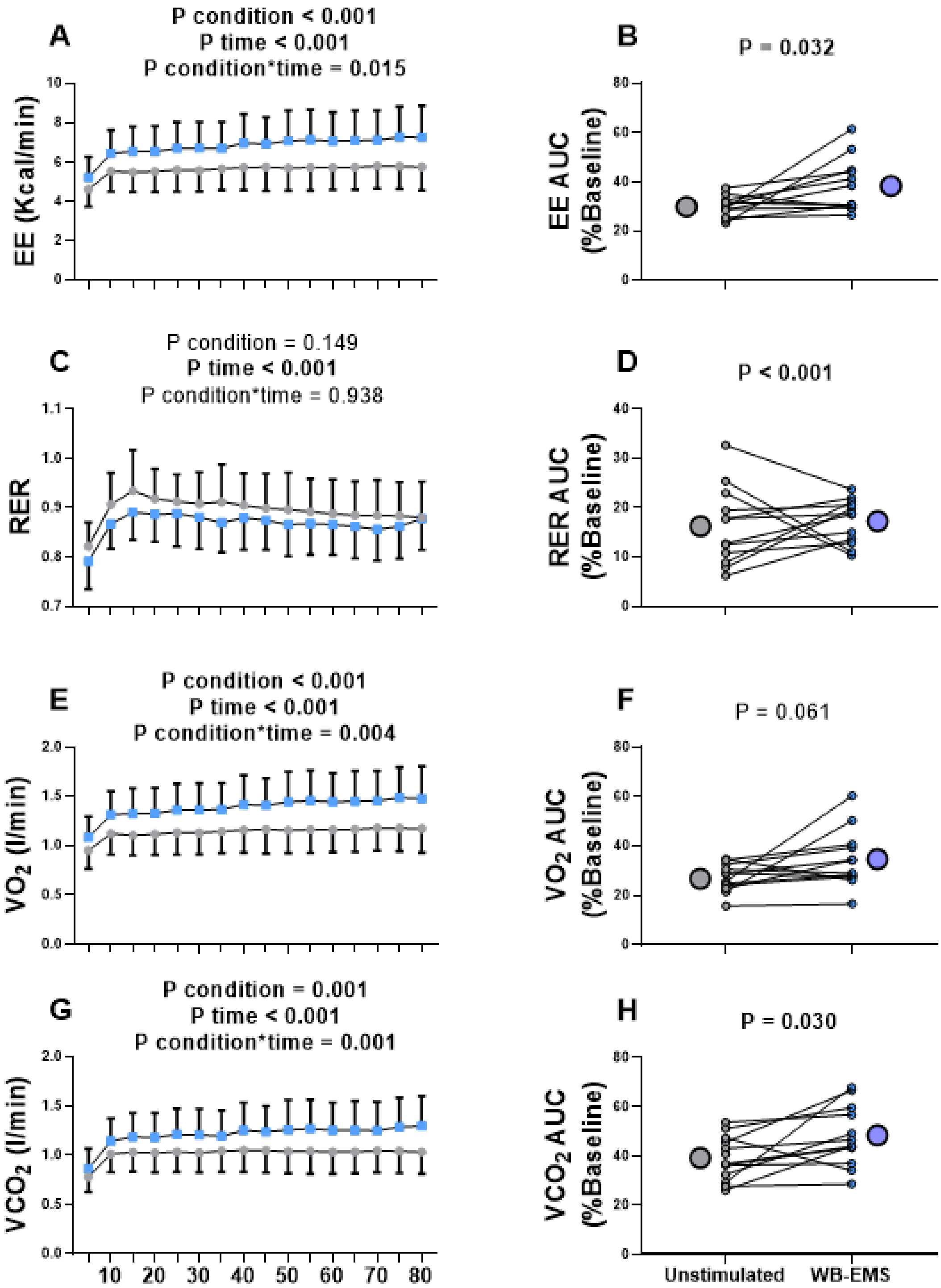

Figure 19. Effects of WB-EMS on EE, RER, VO_2, and VCO_2 during walking exercise (n=12). **Panels A, C, E,** and **G** show the kinetics of EE, RER, VO_2 and VCO_2 across these conditions. **Panels B, D, F,** and **H** show P values from paired t-test comparing AUC expressed as a percentage of its baseline. Data are presented as mean and standard deviation (SD). In panels B, D, F, and H, each single point (grey) or square (blue) represents the mean value of each 5 min period in unstimulated and WB-EMS conditions respectively. AUC: area under the curve, EE: energy expenditure, RER: respiratory exchange ratio, VO_2: oxygen volume, VCO_2: carbon dioxide volume.

Regarding substrate oxidation, no differences across unstimulated and WB-EMS conditions were found in the total FatOx (0.20 ± 0.04 gr/min vs. 0.30 ± 0.4 gr/min; Δ 0.10 gr/min; 50%; p = 0.067) and ChoOx **(**0.98 ± 0.14 gr/min vs. 1.05 ± 0.17 gr/min; Δ 0.07 gr/min; 7%; p > 0.688) (**Figure 20; Panels A and C**). The kinetics of FatOx values showed a significant decrement in the first part of the test, while they were increased after 30 min (effect of time, p < 0.001; **Figure 20; Panel A**). On the contrary, the kinetics of ChoOx showed a significant increase in the first 15 min of the walking test, whereas they remained stable until the end of the test (effect of time, p < 0.001; **Figure 20; Panel C**). The interaction condition*time had not a significant effect neither in FatOx or in ChoOx (all p > 0.178; **Figure 20; Panels A and C)**.

Regarding AUC there were significant differences across conditions in FatOx and ChoOx (all p < 0.032)

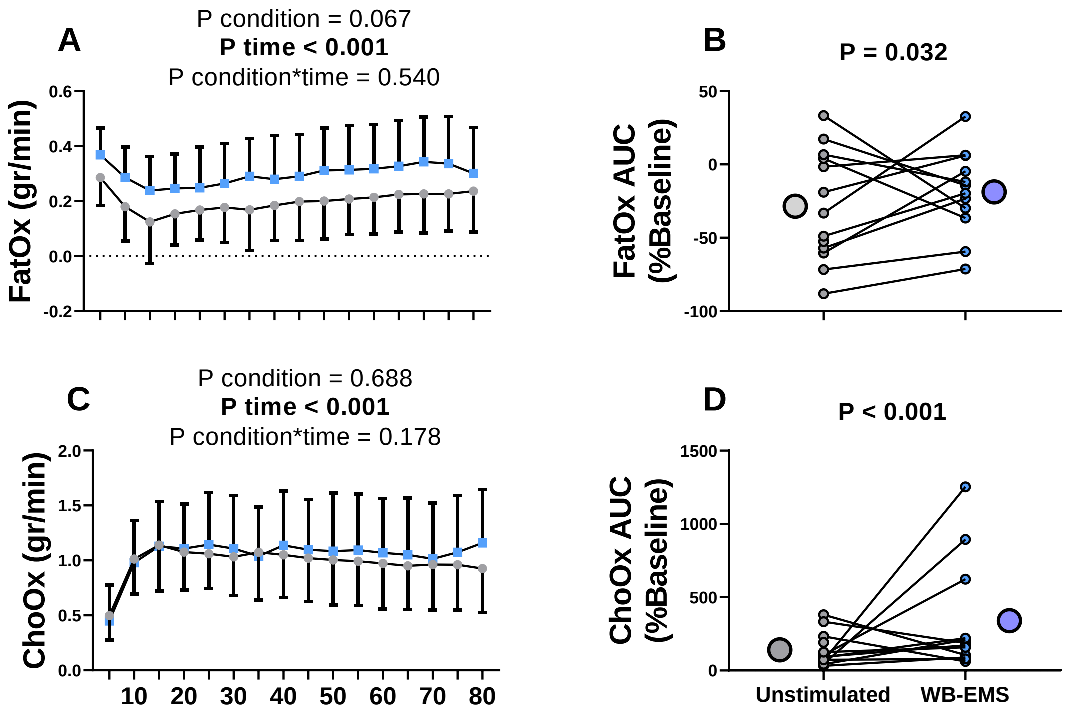

Figure 20. Effects of WB-EMS on FatOx and ChoOx (n=12). **Panels A** and **C** show the kinetics FatOx and ChoOx across these conditions. **Panels B** and **D** show P values from paired t-test comparing AUC expressed as a percentage of its baseline. Data are presented as mean and standard deviation (SD). In panels B and D, each single point (grey) or square (blue) represents the mean value of each 5 min period in unstimulated and WB-EMS conditions respectively. AUC: area under the curve, FatOx: fat oxidation and ChoOx: carbohydrate oxidation. Individual values of EE, RER, FatOx, and ChoOx are presented in figure 21.

Figure 21. Individual values of EE, RER, FatOx, and ChoOx in unstimulated and WB-EMS conditions (n=12). Row data are presented as a mean of 5 minutes in each condition. EE: energy expenditure, RER: respiratory exchange ratio, VO_2: oxygen volume, VCO_2: carbon dioxide volume, FatOx: fat oxidation and ChoOx: carbohydrate oxidation.

No significant differences were observed across conditions in blood lactate concentration at 5,000 steps (1.8 ± 1.1 mmol/L vs. 2.1 ± 0.9 mmol/L; Δ 0.3 mmol/L; 17%; p = 0.052) and at 10,000 (1.6 ± 0.9 mmol/L vs. 2.0 ± 0.8 mmol/L; Δ 0.4 mmol/L; 25%; p = 0.194) (**Figure 22; Panel A**). Similarly, we did not find significant differences between time extraction (i.e., 5,000 and 10,000) in unstimulated condition (1.8 ± 1.1 mmol/L vs. 1.6 ± 0.9 mmol/L; Δ 0.2 mmol/L; 11%; p = 0.580) and WB-EMS condition (2.1 ± 0.9 mmol/L vs. 2.0 ± 0.8 mmol/L; Δ 0.1 mmol/L; 5%; p = 0.952). Regarding pain perception, there were also no significant differences between conditions before and after the 10,000-steps walking test (2.0 ± 12.7 vs. -6.5 ± 11.2; Δ 8.5; 428%; p = 0.122) (**Figure 22; Panel D**). The analysis of heart rate showed a significant increase in WB-EMS conditions compared to unstimulated (117± 14 beats per minute (bpm) vs. 107 ± 12 bpm; Δ 10 bpm; 9%; p < 0.001) **(Figure 22; Panel B**). Lastly, there were significant differences in RPE before and after the 10,000-steps walking test in unstimulated conditions (6.1 ± 0.3 vs. 8.0 ± 2.5; Δ 1.9; 31%; p = 0.015) and WB-EMS conditions, (6.0 ± 0 vs. 11.0 ± 3.4; Δ 5.0 mmol/L; 83%; p < 0.001) (**Figure 22; Panel C**). Although we did not find significant differences in RPE across conditions before the test (6.1 ± 0.3 vs. 6.0 ± 0; Δ 0.1; 2%; p = 0.337) we did observe differences after the test (8.0 ± 2.5 vs. 11.0 ± 3.4; Δ 3; 38%; p = 0.005).

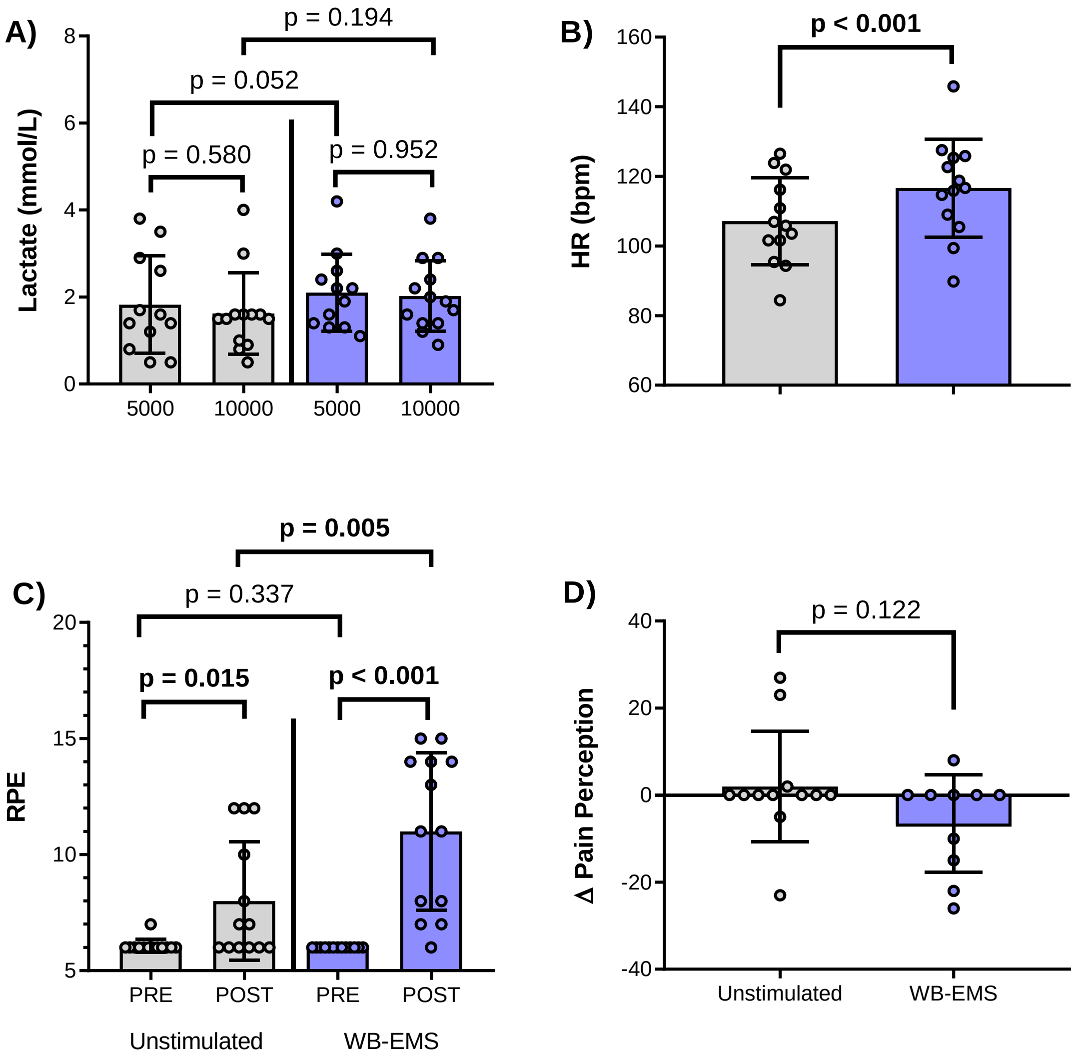

Figure 22. Lactate concentration, heart rate (HR), rate of perceived exertion (RPE), and Δ pain perception during treadmill walking in unstimulated (grey) and whole-body electromyostimulation (blue) conditions (n=13). P-values from paired samples t-test. Data are presented as mean and standard deviation (SD).

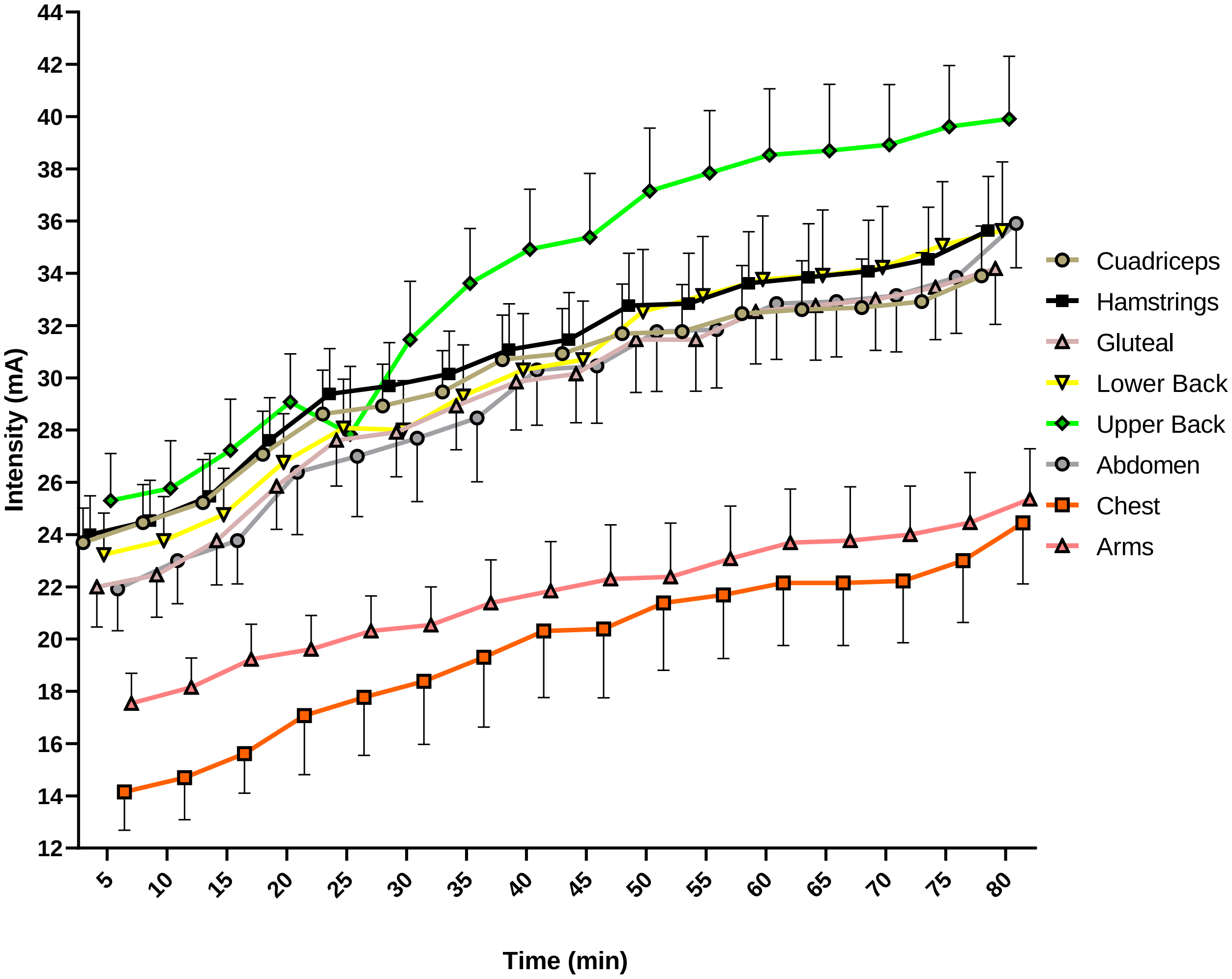

Figure 23. Descriptive data of the impulse intensities in each body muscle along the whole test. Data are presented as means of all participants (n=13). Each single shape represents the mean value of each 5 min period in different body muscle. mA, milliamps.

DISCUSSION

This is the first study that analyses the impact of wearing WB-EMS on energy metabolism and NutOx during a 10,000-steps walking exercise. The major finding of this study is that superimposed WB-EMS increases EE during walking by ≈ 21% in young adults. Additionally, we observed a higher FatOx during WB-EMS (≈ 50%) compared to unstimulated conditions, whereas no effect was observed on ChoOx (≈ 7%). The observed increment in EE could be an essential tool for the control and management of energy balance in people with obesity and its comorbidities. Moreover, giving the fact that 'Not having enough time' is the main reason not to perform PA, we confirm that WB-EMS could reduce a total of 25% the time of performing the recommended 10,000-steps walking with the same EE.

Several studies have already reported that EMS can be used to potentially increase the metabolic demands during exercise, but it is unknown whether this effect is limited to a single muscle or affects the entire body (30, 38, 116, 118). Our results concur with those reported by Kemmler et al. (38) that found an increase in EE of ≈ 17% during an acute bout of resistance exercise with WB-EMS compared to unstimulated conditions. This study also showed a significant increase in RPE (≈ 23%) during the application of WB-EMS although it was lower than the ≈ 38% that we obtained in our study. These differences might be partly explained by the application of different electrical parameters (i.e., frequency [21 vs. 85 Hz], intensity [6/10 vs. maximum tolerated], or duty cycle [40/20 vs. 4/4 seconds]) in the WB-EMS protocol between both studies as well as the differences in the type of exercise (i.e., walking at low intensity on a treadmill vs. resistance exercise).

Verch et al. (118) determined the differences in VO_2 between conventional walking and Nordic walking with and without superimposed WB-EMS. They found a significant increase in VO_2 (≈ 25%) when using WB-EMS which was slightly lower than those achieved in our study (≈ 27%). Interestingly, our participants showed higher values in post-exercise RPE during the application of WB-EMS (≈ 38%) compared with the data reported by Verch et al. (≈11%). These results could be explained by the differences in the familiarization protocols with WB-EMS and in the electrical parameters (i.e., frequency [21 vs. 85 Hz], intensity [6/10 vs. maximum tolerated], or working/resting time [40/20 vs. 9/1 seconds]) or the time of exercise session. Concretely, we designed an 80 min test while they conducted just 10 minutes. However, we found a Δ 3.0 points in RPE scale between before and after 10,000-steps walking test while Verch et al. found a difference of Δ1.2 points.

Regarding blood lactate concentration, Verch et al. (118) found statistically significant differences using WB-EMS compared to unstimulated conditions (31%) while no significant differences were observed in our study (25%). This difference can be possibly caused by the fact that time application was different between studies, as well as the duty cycle.

After comparing our results with those obtained by Kemmler et al. (38) and Verch et al. (118), we conclude that the WB-EMS protocol is the main factor of variability across studies. In our study, the frequency was 21 Hz while both studies applied 85 Hz (38, 118). In addition, we selected an intensity of 6/10 (self-perceived perception) which is the equivalent of 28 ± 5 mA while any of the studies reported these data. Similarly, the duty cycle in our research (i.e., 40 seconds WB-EMS – 20 seconds rest) was considerably longer than in both studies (i.e., 4 seconds WB-EMS – 4 seconds rest and 9 seconds WB-EMS – 1 second rest, respectively), a fact that could produce a greater sense of exertion in the participants, as well as, a higher effect of WB-EMS.

STRENGTH AND LIMITATIONS

The study II has an important strength that should be highlighted: (i) the technology employed allows to increase or decrease independently any muscle group improving EMS administration. Nevertheless, certain limitations should be mentioned: (i) our study only included 4 young women. Due to the fact that there is no consensus regarding the optimal WB-EMS parameters to increase EE, we selected 21 Hz and a comfortable RPE to achieve the 10,000-steps test. We do not know if selecting other parameters could improve the results. (ii) participants had no prior experience with WB-EMS training and, therefore, it is unknown if these findings apply to athletes who have previously trained with WB-EMS training. (iii) such conclusions do not apply to individuals with a different age range or different biological characteristics (i.e., people with obesity),

CONCLUSION

- The inclusion of WB-EMS during aerobic walking exercise is an effective tool to increase EE in healthy young people.
- Despite no significant differences were observed in substrate oxidation rates, there was an increment of 50% in FatOx.

GENERAL DISCUSSION

The present Doctoral Thesis aimed to investigate whether the manipulation of electrical parameters can optimize the effects of WB-EMS on energy metabolism and NutOx at rest and during exercise in young adults. Consequently, we analyze the effects of WB-EMS application with different electrical frequencies in both conditions (**Study I)**. In addition, we also applied WB-EMS during a common PA recommendation (i.e., 10,000 steps) to test its potential effects on energy metabolism and NutOx (**Study II)**.

This Doctoral Thesis emerged from the idea that the use of WB-EMS with health-related purposes could be improved taking into consideration previously published studies. The common and traditional application of EMS and WB-EMS has followed fix electrical parameters (i.e., 85 Hz, duty cycle of 4-4 at maximum intensity and during isometric exercises). However, these parameters have been employed aiming to improve skeletal muscle power and strength. On the other hand, these electrical parameters were also applied during plyometric training interventions demonstrating significant improvements of muscular performance. However, little was known about their effects in energy metabolism and substrate oxidation. Finally, there is not enough evidence that support why these electrical parameters are selected and whether the application of a wide range of electrical frequencies can induce different responses in terms of energy metabolism.

IMPACT OF LOW ELECTRICAL FREQUENCIES ON ENERGY METABOLISM

Understanding the effects of different low electrical frequencies on energy metabolism could help to optimize its application with different aims and with different populations that have special biological characteristics. In order to understand how the WB-EMS can help to balance energy metabolism and aiming to clarify its use in healthy individuals, we designed a study that compare the effects of low frequencies on EE and RER. We found, in **study I,** that 4 Hz increased the EE about 604% at rest while 6 Hz did it about 44% during uphill walking. We also observed that the application of other frequencies also increases the EE in both conditions but with lower magnitude. On the other hand, the lowest frequencies (i.e., 1 Hz a 2 Hz) seem to maintain the RER while the other frequencies applied (i.e., 4 Hz, 6 Hz, 8 Hz and 10 Hz) increased the RER at rest. Lastly, there were no significant effects of WB-EMS on RER during exercise.

The findings obtained in **study I** partially concur with other studies such as the Grosset et al. that found an increment of ≈ 428% using local EMS (106), or Minogue et al. that found an increment of ≈ 596% with the application of 12 Hz (117). Regarding the use of WB-EMS during exercise Kemmler et al. reported an increment of ≈ 17% (38) while we obtained an increase of ≈ 44%. On the other hand, we observed similar results regarding RER than those found by the above-mentioned works both at rest and during exercise (106, 165). The main differences between studies could be explained by the substantial differences in exercise interventions and the different electrical stimuli applied.

The most interesting finding obtained in the **study I** was that the application of WB-EMS at low-frequencies at rest produces an EE similar to the one observed during uphill walking (i.e., at 60% of the VO_2max or VO_2peak) without WB-EMS. This could be of interest for individuals with severe obesity problems since long aerobic training sessions at moderate intensity could lead to joint and biomechanical imbalances increasing injury risk (167). It has been demonstrated that high EE induced by PA and/or exercise can help to sustain weight loss (41). Therefore, interventions capable of increasing EE might result in a better balance of energy metabolism and its related diseases (134). Moreover, given that it has been suggested that most exercise interventions are not usually attractive – partially attributed to the uncomfortable feelings experienced during the exercise session (168, 169) the WB-EMS application during training could be a potential alternative to enhance the motivation of the individuals. However, we cannot underestimate that, although our participants did not report extreme pain, they anecdotally registered discomfort while walking with the WB-EMS at maximum intensity, a factor that could result in low adherence rates.

The previous results motivated us to perform the **study II** in which the electrical frequency was increased to minimize the discomfort during walking while electrical intensity was reduced. No evidence is available regarding if the application of WB-EMS could reduce the time of training. We therefore proceeded with these calculations in **study II.** In summary, the **study II** highlighted the differences in terms of EE between performing 10,000 steps with and without WB-EMS concluding that WB-EMS at low intensity (i.e., 6/10) increased EE by ≈ 21% during planned PA. Our results concur with those reported by Kemmler et al. (38) that found an increase in EE of ≈ 17% during an acute bout of resistance exercise with WB-EMS compared to unstimulated conditions.

The mayor differences between both studies were (i) that we applied moderate electrical intensities while Kemmler et al. selected the maximum tolerable intensity (38), and (ii) that we performed a common recommendation of walking exercise while they conducted a light-bearing training circuit. An additional remarkable conclusion derived from the **study II** was that ChoOx remain similar between conditions, whereas FatOx increased under the application of WB-EMS (≈ 50%). Surprisingly, while increasing the intensity of exercise in response to WB-EMS has been related to higher ChoOx, our study showed for the first time the opposite effect obtaining that WB-EMS may help to increase the intensity of exercise to a point in which the maximal FatOx occurs (FatMax).

Previous studies have highlighted that 'Not having enough time' seems to be the main reason to not performing enough PA. To counteract this health-related problem, in **study II**, we calculated how much time of exercise and how many steps should be completed adding WB-EMS to obtain a similar EE than the produced by 10,000 steps without WB-EMS. These new findings indicate that performing ≈ 8,500 steps with WB-EMS with an intensity of 6/10 was the equivalent stimuli to generate the EE related to completing 10,000 steps (with a 20 minutes reduction of PA volume).

In summary, the results derived from **study I** and **study II** demonstrated that the manipulation of WB-EMS parameters produces different metabolic responses on EE and substrate oxidation, and that the application of WB-EMS can reduce the training volume getting similar effects on energy metabolism.

GENERAL LIMITATIONS

The results presented in this Doctoral Thesis need to be considered with some cautions since there are some limitations:

- This Doctoral Thesis is composed by two studies performed in healthy adults and the results obtained do not apply to other populations.
- We included men in **study I** and four women in **study II.** Thus, similar studies including women are desirable.
- The data of EE were taken with two different metabolic carts and the reliability of both devices should be taken into account.
- The **study II** was performed in standardized laboratory conditions, a different situation than performing the 10,000 steps outdoors.
- There was not any specific familiarization session which could affects the results obtained.

CONCLUSION & FUTURE PERSPECTIVES

GENERAL CONCLUSION

In conclusion, this Doctoral Thesis shows that WB-EMS is an effective tool to increase EE at rest and during exercise in young adults. Moreover, WB-EMS is able to modify substrate oxidation in both conditions while reducing training volume. Therefore, WB-EMS should be considered a promising therapy to help energy balance' management.

SPECIFIC CONCLUSIONS

Study I

- Relatively low frequencies of WB-EMS are an effective stimulus to increase EE at rest and during uphill walking in healthy young men. Specifically, although 4 Hz was the frequency that elicits the major increment in EE at rest, other frequencies such as 2 Hz, 6 Hz and 8 Hz also produced this effect compared to unstimulated conditions.
- The frequency that induced the highest increment of EE during uphill walking was 6 Hz – with similar effects than those produced with 2 Hz and 8 Hz - compared to unstimulated conditions.
- Low frequencies (i.e., 1 Hz and 2 Hz) of WB-EMS did not increase the RER values despite an increase in EE at rest suggesting that it could have an impact on fuel utilization rates.

Study II

- The inclusion of WB-EMS during aerobic walking exercise is an effective tool to increase EE in healthy young adults.
- Despite no significant differences were observed in substrate oxidation rates, there was an increment of 50% in FatOx.

FUTURE PERSPECTIVES

Future perspectives studies with different electrical parameters and different exercises should be performed in order to clarify the effects of WB-EMS on EE and fuel utilization. First of all, the type of exercise might be critical in theses sense. The effects of WB-EMS might be different during aerobic and resistance training, and at moderate and vigorous intensity. Moreover, the stimulation time might be similar to those performed in traditional training and not only 4 min.

The ergometer' type used during the WB-EMS training sessions seems to have a critical impact on energy metabolism' adaptations. This point could be explained because the muscle groups recruited are highly dependent on the ergometer selected (e.g.., elliptic, rowing machine, an air bike or a skierg). Therefore, future studies are needed to elucidate whether the effects of performing aerobic exercise while applying WB-EMS are similar or not on energy metabolism when different ergometers are compared.

Regarding electrical parameters, we showed that the selection of impulse frequencies is crucial in the effects on energy metabolism. Thus, future trials should compare the effects of low, medium and high electrical frequencies on energy metabolism. Determining the electrical frequency that elicit the highest increment in EE could be crucial for people who are attempting to improve the energy balance.

On the other hand, it has been shown that impulse intensity and impulse frequency are inversely proportional. However, nowadays, we are not able to clarify if the effects of low frequencies and high intensities on EE are better than high frequencies with low intensities. Future perspectives should determinate the relationship between impulse frequency and impulse intensity and we believe that creating a variable that give a general quantification of WB-EMS is needed to improve its effects.

Finally, we only included healthy young adults in both studies. It might be beneficial to investigate these effects in other population such as older individuals and patients with obesity. Furthermore, future studies should emphasize in WB-EMS applications that, while reducing heavily the time of training, achieved the same results than traditional long trainings.

ANNEXES

MANUSCRIPTS DERIVED FROM THE DOCTORAL THESIS

- **Unai A. Perez-De-Arrilucea Le Floc'h**, Manuel Dote-Montero, Abraham Carlé-Calo Montero, Guillermo Sánchez-Delgado, Jonatan R. Ruiz and Francisco J. Amaro-Gahete. Acute Effects of Whole-Body Electromyostimulation on Energy Expenditure at Resting and during Uphill Walking in Healthy Young Men. Metabolites. 2022;12(9):781.

- **Unai A. Perez-De-Arrilucea Le Floc'h,** Miguel A. de-la-Torre-Tallon, Jonatan R. Ruiz and Francisco J. Amaro-Gahete. *Submitted.*

SHORT CURRICULUM VITAE

Unai Adrian Perez de Arrilucea Le Floc'h
Date of birth: 17/02/1994, Vitoria-Gasteiz, España
Email: unaiperez@correo.ugr.es
ORCID profile: 0000-0002-6856-4529

Education

09/2013 – 07/2017: Bachelor's degree in Sports Sciences (Grade: 8.2/10), University of the Basque Country, Spain.

09/2016 – 03/2017: ERASMUS student of Bachelor's degree in Sports Sciences (9.6/10), University of Urbino Carlo Bo, Le Marche, Italy

09/2017 – 05/2018: Certificate IV in Massage Therapy, Australian College of Sport and Fitness, Melbourne, Australia

09/2018 – 09/2019: Master's degree in Researching in Physical Activity and Sports (Grade: 9.4/10), University of Granada, Spain

09/2020 – 07/2021: Master's degree in Teacher Training in Obligatory Secondary and Upper Secondary School Education, Vocational Training and Languages (Grade: 9.0/10), University of Granada, Spain.

03/2020 – 03/2023: PhD Student in Biomedicine, University of Granada, Spain.

International internship

01/2018 – 05/2018 **Location:** Institute of sport, exercise & active living (ISEAL), College of Sport and Exercise Science, Victoria University, Melbourne, Australia
Project: The effects of exercise markers on mitochondrial dynamicsand mitochondrial remodeling.
Supervisors: Javier Botella & David Bishop

Previous and current positions

2018 - 2019	Research Initiation Fellow. Department of Physical Education and Sport, School of Sport Sciences, University of Granada.

Supervision

2020 – 2021	Co-supervisor for 1 master thesis; Master's degree in food and sport for health (Food & Fit), University of Granada.
2021 – 2022	Co-supervisor for 1 master thesis; Master's Degree in Human Nutrition, University of Granada

Research experience

2020 – 2023: **Wiems Laboratory Project**: Effects of whole-body electromyostimulation on body composition and muscular strength in humans.
Funded by WiemsPro S.L.
Principal investigator: Francisco Jose Amaro Gahete
Role: Project manager data collection and participation on manuscripts.

2019-2020: **ACTIFOX project**: ACTivating Fat OXidation Through Capsinoids (ACTIFOX). effects of the acute ingestion of dihydrocapsiate during aerobic exercise on energy metabolism in adults with overweight/obesity.
Funded by Consejería de Conocimiento, Investigación y Universidades, Proyectos I+D+i del Programa Operativo del Fondo Europeo de Desarrollo Regional (FEDER 2018, ref. B.CTS. 377.UGR18).
Principal investigator: Jonatan R Ruiz
Role: Project Assistant, data collection and participation on data base.

2018 – 2019 **Project:** Validity of four commercially available metabolic carts for assessing resting metabolic rate and respiratory exchange ratio in non-ventilated humans
Funded by Spanish Ministry of Economy and Competitiveness via Retos de la Sociedad grant DEP2016-79512-R, and European Regional Development Fund.
Principal investigator: Jonatan Ruiz Ruiz
Role: Data collection.

Publications

- **Perez De Arrilucea Le Floc'h UA**, Dote Montero M, Carlé Calo A, Sánchez Delgado G, Ruiz JR, Amaro Gahete FJ. Acute Effects of Whole-Body Electromyostimulation on Energy Expenditure at Resting and during Uphill Walking in Healthy Young Men. Metabolites. 2022;12(9):781.
- Osuna-Prieto FJ, Acosta FM, **Perez de Arrilucea Le Floc'h UA,** Riquelme-Gallego B, Merchan- Ramirez E, Xu H, de la Cruz-Marquez JC, Amaro-Gahete FM, Llamas-Elvira JM, Trivino Ibanez EM, Segura-Carretero A, Ruiz JR. Dihydrocapsiate does not increase energy expenditure nor fat oxidation during aerobic exercise in men with overweight/obesity: a randomized, triple-blinded, placebo-controlled, crossover trial. Journal of the International Society of Sports Nutrition. 2022;19(1):417-36.

Other merits

- **7 communications** or **posters** both in **national and international conferences.**

2022 **I Congreso Internacional sobre Optimización del Entrenamiento de Fuerza y Rendimiento Neuromuscular**

Autores: Unai Pérez de Arrilucea Le Floc'h, Francisco J. Amaro Gahete. **Título aportación:** La electroestimulación global de cuerpo complete aumenta la carga de entrenamiento y precisa de mayor tiempo de recuperación. **Entidad Organizadora:** Red de Entrenamiento de Fuerza, Universidad de Granada **Título del Congreso:** I Congreso Internacional sobre Optimización del Entrenamiento de Fuerza y Rendimiento Neuromuscular. **Ámbito del Congreso:** Internacional. **Tipo de participación:** Comunicación oral. **Lugar de celebración:** Granada. **Fecha de celebración:** 7/10/2022 – 8/10/2022.

2022 **III Congreso Nacional - V Jornadas de Investigadores/as en Formación: Fomentando la interdisciplinariedad (JIFFI)**

Autores: Unai Pérez de Arrilucea Le Floc'h, Miguel De La Torre; Abraham Carle-Calo; Francisco J. Amaro-Gahete y Jonatan Ruiz Ruiz. **Título aportación:** Efecto de la electroestimulación global de cuerpo completo sobre el metabolismo energético y la oxidación de sustratos energéticos durante la práctica recomendada de actividad física (i.e., 10.000 pasos). **Entidad Organizadora:** Universidad de Granada **Título del Congreso:** III Congreso Nacional - V Jornadas de Investigadores/as en Formación: Fomentando la interdisciplinariedad (JIFFI). **Ámbito del Congreso: N**acional. **Tipo de participación:** Comunicación oral. **Lugar de celebración:** Granada. **Fecha de celebración:** 22/06/2022 – 24/06/2022.

2022 **XXII Congreso Sociedad Andaluza de Cancerología**

Autores: Unai Pérez de Arrilucea Le Floc'h, Ana Serradilla, Mª Ángeles Samaniego, Ángel Acosta-Rojas, Alicia Villegas-Sánchez, Eugenio Fernández-Miranda, Antonio Sáez-Cantos, Francisco J. Montilla-Oballe, Aida Tórtola-Navarro. **Título aportación:** Impact of covid-19 confinement on physical activity levels in Spanish cancer survivors. **Entidad Organizadora:** Sociedad Andaluza de Cancerología. **Título del Congreso:** XXII Congreso Sociedad Andaluza de Cancerología. **Ámbito del Congreso:** Nacional. **Tipo de participación:** Comunicación oral. **Lugar de celebración:** Granada. **Fecha de celebración:** 17/02/2022 – 19/02/2022.

2022 **II Congreso Investigación PTS Granada**

Autores: Unai A. Perez de Arrilucea Le Floc'h, Manuel Dote-Montero; Abraham Carle-Calo; Guillermo Sanchez-Delgado; Jonatan R. Ruiz; Francisco J. Amaro-Gahete. **Título aportación:** Effect of an aerobic exercise program with whole-body electromyostimulation on physical condition and body composition in healthy adults. **Entidad Organizadora:** Fundación PTS Granada. **Título del Congreso:** II Congreso Investigación PTS Granada **Ámbito del Congreso:** Nacional. **Tipo de participación:** Comunicación oral. **Lugar de celebración:** Granada. **Fecha de celebración:** 09/02/2022 – 11/02/2022.

2021 **Fundación Estadio Fundación – Aula Estadio Aretoa**

Tipo de participación: Ponente. **Tipo de actividad:** Seminario orientado a la la importancia del ejercicio físico y del cáncer. **Nombre de la actividad:** Da el paso, el ejercicio físico mejora tu vida. **Objetivos del curso:** Dar a conocer la importancia del ejercicio físico en la sociedad vitoriana. **Perfil de los destinatarios:** Adultos abonados al Estadio. **Entidad organizadora:** Fundación Estadio. **Horas:** 2. **Lugar de celebración:** Vitoria-Gasteiz. **Fecha:** 05/05/2021.

2020 **VII Simposio EXERNET: "Prescripción del ejercicio físico basado en la evidencia"**

Autores: Francisco J. Osuna-Prieto*, Francisco M. Acosta*, **Unai A. Perez de Arrilucea Le Floc'h**, Blanca Riquelme-Gallego, Elisa Merchan-Ramirez, Huiwen Xu, Juan Carlos de la Cruz-Márquez, Francisco M. Amaro-Gahete, Jose A. Llamas-Elvira, Eva M. Triviño, Antonio Segura-Carretero, Jonatan R. Ruiz. **Título aportación:** The acute ingestion of dihydrocapsiate does not increase energy expenditure nor fat oxidation during aerobic exercise in overweight/obese men: a randomized, triple-blinded, placebo-controlled, crossover trial. **Entidad Organizadora:** Red Española de Investigación en Ejercicio Físico y Salud. **Título del Congreso:** VII Simposio EXERNET: "Prescripción del ejercicio físico basado en la evidencia". **Ámbito del Congreso:** Internacional. **Tipo de participación:** Póster. **Lugar de celebración:** Cuenca. **Fecha de celebración:** 22/09/2020.

2020 **VII Simposio EXERNET: "Prescripción del ejercicio físico basado en la evidencia"**

Autores: Unai A. Perez de Arrilucea Le Floc'h, Manuel Dote-Montero; Abraham Carle-Calo; Guillermo Sanchez-Delgado; Jonatan R. Ruiz; Francisco J. Amaro-Gahete. **Título aportación:** Acute effects of whole-body electromyostimulation training on energy metabolism at resting and during aerobic exercise in healthy young men. **Entidad Organizadora:** Red Española de Investigación en Ejercicio Físico y Salud. **Título del Congreso:** VII Simposio EXERNET: "Prescripción del ejercicio físico basado en la evidencia". **Ámbito del Congreso:** Internacional. **Tipo de participación:** Póster. **Lugar de celebración:** Cuenca. **Fecha de celebración:** 22/09/2020.

ACKNOWLEDGEMENTS

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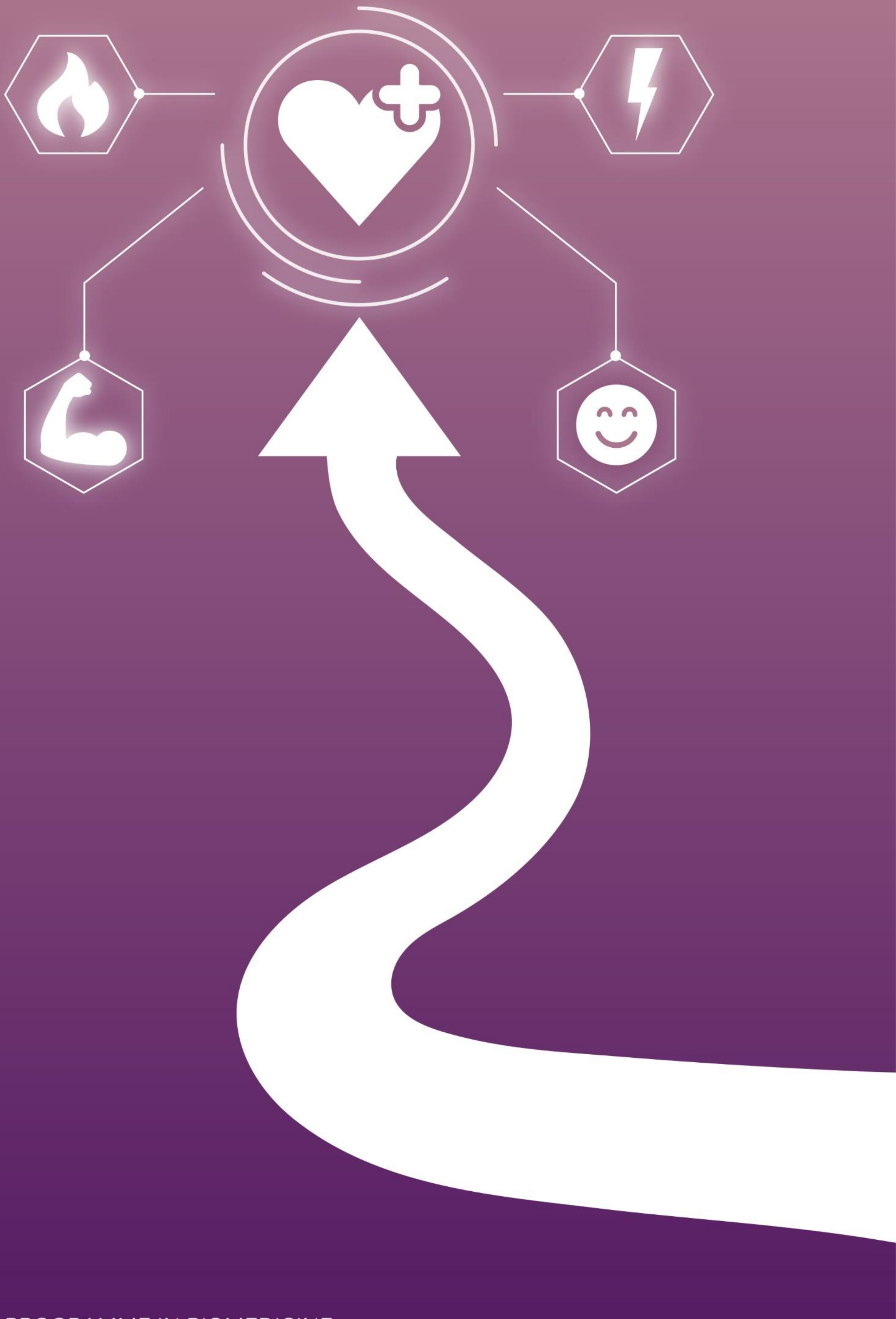

DOCTORAL PROGRAMME IN BIOMEDICINE

IMPACT OF WHOLE-BODY ELECTROMYOSTIMULATION ON ENERGY METABOLISM IN ADULTS

Unai Perez de Arrilucea Le Floc'h