Alma Mater Studiorum – Università di Bologna

DOTTORATO DI RICERCA IN

SCIENZA E CULTURA DEL BENESSERE E DEGLI STILI DI VITA

Ciclo 34

Settore Concorsuale: 06/N2 – SCIENZE DELL'ESERCIZIO FISICO E DELLO SPORT

Settore Scientifico Disciplinare: M-EDF/02 – METODI E DIDATTICHE DELLE ATTIVITA' SPORTIVE

EFFECT OF PULSED ELECTROMAGNETIC FIELDS (PEMFS) ON MUSCLE ACTIVITY, TISSUE OXYGENATION AND VO2 DURING EXERCISE.

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Esame finale anno 2022

Report paper in extenso

- **Trofè A**, Raffi M, Muehsam D, Meoni A, Campa F, Toselli S, Piras A. Effect of PEMF on Muscle Oxygenation during Cycling: A Single-Blind Controlled Pilot Study. Appl Sci. 2021;11(8):3624.
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- Piras A, **Trofè A**, Meoni A, Raffi M. Influence of radial optic flow stimulation on static postural balance in Parkinson's disease: A preliminary study. Hum Mov Sci. 2021 Nov 23;81
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- Mazzetti M, Raffi M, Di Michele R, Cortesi M, **Trofè A**, Piras A (2019) Lifetime exposure of swimming training: its effects on cardiac autonomic responses. Sport Sciences for Health Vol 15 (Suppl 1):105
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Abstract

The pulsed electromagnetic fields (PEMF) are a medical and non-invasive therapy used for clinical treatments. PEMF is successfully use in human for the treatment of bone disease, due to the piezoelectric effect that improve bone mass and density, by the stimulation of osteoblastogenesis, with modulation of calcium storages and mineral metabolism. PEMF enhance tissue oxygenation, microcirculation and angiogenesis, in rats and cells erythrocytes, in cells-free assay. Such responses could be caused by a modulation of nitric oxide signal and interaction between PEMF and Ca_{2+}/NO/cGMP/PKG signal. In humans, effects of PEMF on microcirculation are still discussed. PEMF improve blood flow velocity of smallest vein without changing their diameter. PEMF therapy helpful in patients with diabetes, due to increased microcirculation trough enhance capillary blood velocity and diameter. PEMF was able to affect calcium channels raising Ca2+ intracellular concentration, amplifying signal Ca2+ mediators and Ca-dependent pathways. Regarding physical activity, given the importance of muscle and pulmonary O_2 uptake and the crucial role played by mechanism of the muscular contraction, which are affected by Ca2+ channels and ions flux, in this research we investigated the influence of stimulation on muscular activity, tissue oxygenation and pulmonary VO_2, during exercise. We evaluated the effect of stimulation on different intensity of exercise, as heavy or moderate, different subjects, as a athlete or sedentary, and different sport activity, as a cycling or weightlifting. In athletes, we observed a tendency for a greater change and a faster kinetic of HHb concentration during stimulation. PEMF increased the velocity and the quantity of muscle O_2 available, leading to accelerate the HHb kinetics. Stimulation induced a bulk muscle O_2 availability and a greater muscle O_2 extraction, leading to a reduced time delay of the HHb slow component. This variations at the muscle level were not accompanied by changes of the pulmonary VO_2 on-kinetics on transition, with the stimulation that was not sufficiently enough to speed up phase II with the decrease in the slow component. This is due to the maximal cardiac output limits the muscle blood

flow and O_2 delivery to muscles, during a high intensity constant-load exercise, that involves large muscle mass, whereas PEMF might act only at the local level. In sedentary people, stimulation did not affect muscle oxygenation. A possible explanation for the lack of effect is related to the intensity of exercise and the type of muscle fibers recruited. In all our studies, stimulation increased the amplitude of muscle activity under different conditions, likely caused by the effect of PEMF on contraction mechanism of muscular fibers, by the change of membrane permeability and Ca^{2+} channel conduction. In athletes, we observed an increase of overall activity during warm-up. In sedentary people, stimulation increased the magnitude of muscle activity during moderate constant-load exercise and warm-up. In weightlifters, we observed a higher muscular basal tone activity, induced by stimulation during recovery phase. In athletes and weightlifters, stimulation caused an increase of blood lactate concentration during exercise, confirming a possible influence of stimulation on muscle activity and on glycolytic metabolism of type-II muscular fibers. Results of the present research show a possible application of PEMF in sport disciplines or physical activity. Our results suggest that PEMF application can stimulate the rate of muscle O_2 extraction and utilization, enhancing the muscle oxygenation. PEMF stimulation seems alike effective in both athletes and sedentary people, in order to raise the magnitude of muscular activity, in response to physical effort. PEMF could affect the energetic system inside muscular fibers, especially glycolytic metabolism of type II muscle fibers.

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Introduction

The pulsed electromagnetic fields (PEMF) are a medical and non-invasive therapy used for clinical treatments, approved for human use in 1979 by Food and Drug Administration (FDA).

PEMF and bone disease

PEMF therapy is successfully use in the treatment of bone disease, like osteoporosis (Zhu et al. 2017; Huang et al. 2008; T Wang et al. 2019; Yuan, Xin, and Jiang 2018), fracture (Hannemann et al. 2014; Chalidis et al. 2011; Daish et al. 2018) or arthritis (Iwasa and Reddi 2018; Tiantian Wang et al. 2019; Ross, Ang, and Almeida-Porada 2019). Due to the piezoelectric effect, PEMF improve bone mass and density, through the stimulation of osteoblastogenesis with modulation of calcium storages and mineral metabolism.

PEMF therapy was successfully combined with physical exercise, in the treatment of osteopenia or osteoporosis, as demonstrated by Ebid et al. (2021). In this study, 12 weeks of training (3 times for week, 60 min-session) combined with PEMF stimulation (3 times for week, 30 minutes at day) increased body mass density of the hip and lumbar spine, bone-formation and bone-resorption markers. The training protocol included flexibility, aerobic, strengthening, weight-bearing, balance exercises followed by whole-body vibration training. PEMF was applied to the whole body using a full-body mat three times per week for 12 weeks, showing that an exercise protocol which included PEMF was able to improve osteogenesis and osteoblast activity, prevent bone loss and increase fracture healing. Also, the PEMF stimulation increased the levels of biomarkers of osteoblast-associated bone formation, such as serum bone-specific alkaline phosphatase (BSAP), serum osteocalcin (OC), and serum carboxy-terminal pro-peptide of type I collagen (PINP), while decreasing the levels of serum C-terminal telopeptide (CTX), which was independent of BMD change. The positive effect of PEMF treatment on bone markers, was maintained for the following 6 months, with

increasing in bone mass density, enhancing bone formation, suppressing bone-resorption indicators, improving quality of life and reducing fall risk.

PEMF and tissue oxygenation

Others researchers showed that pulsed electromagnetic fields, improve tissue oxygenation (Muehsam et al. 2013), microcirculation (Bragin et al. 2015; Smith, Wong-Gibbons, and Maultsby 2004) and angiogenesis (Roland et al. 2000) in rats and cells erythrocytes, in cells-free assay. About tissue oxygenation and microcirculation, PEMF was able to enhance vascularization and increased diameter of arterioles in muscles of rats, after 2 minutes of a singular stimulation (Smith, Wong-Gibbons, and Maultsby 2004). In a cell-free preparation, 10 minutes of PEMF treatment was able to increase oxygen release up to 150 minutes after stimulation (Muehsam et al. 2013). Such responses could be caused by a modulation of nitric oxide (Diniz, Soejima, and Ito 2002) signal and interaction between PEMF and Ca_{2+}/NO/cGMP/PKG signal (Pall 2013). Moreover, Roland et al.(2000) showed angiogenesis effect after 12 weeks of PEMF stimulation with a significant increase in neovascularization in given rats. Angiogenesis would be generate from the prolongated stretch of vessel walls, in response to the release of nitric oxide and its vasodilatory effect.(Milkiewicz et al. 2001).

Despite that, effect of PEMF on microcirculation and the specific mechanism of interaction are still discussed (McKay, Prato, and Thomas 2007).

In humans, effects of pulsed electromagnetic field on blood circulation appear unclear. Kim et al. (2020) investigated the influence of 12 weeks of PEMF therapy on plasma Nitric Oxide (NO) in 23 subjects with mild to moderate metabolic syndrome. The authors showed that 16 minutes of stimulation, 3 times/day, were able to increase circulating plasma NO levels, at rest and at the end of submaximal exercise performed at moderate intensity. The increase in plasma NO bioavailability was more pronounced in subjects with existing hypertension. According to the authors, the observed

improvement in blood flow, following PEMF treatment, is mediated through calcium (Ca2+) / calmodulin (CaM)-dependent NO cascades. The potential mechanism is that PEMF enhances the binding of Ca2+ and calmodulin and then Ca2+CaM binds to e-NOS to release NO (Nelson, Zvirbulis, and Pilla 2013). Therefore, PEMF may increase NO synthesis activity (Noda et al. 2000) and consequently enhance NO-cyclic guanosine monophosphate (cGMP) cascades resulting in vasodilation (Miura, Takayama, and Okada 1993).

Stewart et al. (2020) investigated the effect of 12 weeks of PEMF therapy on blood pressure and vascular function in thirty hypertensive individuals. 16-min-PEMF treatment 3 times/day, significantly improved flow-mediated dilation absolute and normalized to hyperemia. Also, stimulation reduced blood pressure, mean, systolic and diastolic. Rikk et al. (2013), showed that PEMF treatment reduced systolic blood pressure in aging adults, but not diastolic or arterial stiffness, suggesting PEMF influence on peripheral resistance or microcirculation. Kwan et al. (2015) found PEMF therapy helpful in patients with diabetes, due to increased microcirculation trough enhance capillary blood velocity and diameter. Sun et al. (2016) showed that PEMF improved blood flow velocity of smallest vein without changing their diameter. In contrast, Biermann et al. (2020) did not find effect of PEMF treatment on cutaneous blood flow. In this study, 40min-PEMF stimulation was applied at one leg of 15 healthy volunteers, over 3 weeks in a 48-hour interval. PEMF had no influence on skin microcirculation in all outcome parameters, including flow, mixed venous O_2 saturation and relative venous Hb.

Nevertheless, the accurate interaction between pulsed electromagnetic fields and human cells and tissue seems unclear and needs investigations, as well as the stimulation parameters like time and frequency. It has been hypothesized that the different responses on PEMF therapy, depends on the biological tissue or dosage of stimulation of a specific electromagnetic signal (Smith, Wong-Gibbons, and Maultsby 2004).

PEMF and inflammation

A promising application for PEMF therapy concerns the treatment of acute and chronic tissue inflammation (Pesce et al. 2013; Vincenzi et al. 2013). In cellular culture of human skin, PEMF treatment was able to mediate gene expression involved in the acute phases of inflammations (Kubat, Moffett, and Fray 2015). The stimulation led to a reduction in cytokines pro-inflammation, like interleukin 1-beta, with increased of enzymes involved in the removal of reactive oxygen species, like superoxide dismutase 3 mRNAs and in the heme catabolism, like heme oxygenase 1 mRNA.

This might explain the positive influence of PEMF in the treatment of delayed onset muscle soreness (DOMS). Indeed, Jeon et al. (2015) investigated PEMF therapy on physiological manifestations associated on DOMS, like pain, soreness or muscle force generation, in order to assessed recovery after isometric exercise session. PEMF treatment on brachii biceps for ten minutes after training, reduced the severity of perceived symptoms of DOMS in the following days enacting quality of recovery. PEMF treatment also raised median frequency of muscle activation and reduced electromechanical delay during isometric contraction in the day after, suggesting opportunity to shortening recovery time. Despite that, no effect was found on peak of isometric force generation and more studies are necessary to confirm the positive influence of pulsed electromagnetic field to improve and accelerate recovery phase.

PEMF and physical exercise

Despite a lot of research and several uses for medical purpose, until now few other studies investigated the PEMF effect to physical activity. Galace de Freitas et al. (2014) suggested that the combination of exercise training and PEMF stimulation could be used to improve function, muscle strength and decrease pain, in patients with shoulder impingement syndrome. Parhampour et al. (2014) applied PEMF in association with six-weeks of resistance training program, in patients with severe hemophilia A and osteoporosis, in order to improve muscle strength, bone formation and joint function. Results showed that PEMF stimulation in association with resistance training, could be

more efficient than PEMF therapy alone, in order to improve bone formation due to increase level of serum bone-specific alkaline phosphatase. However, benefits deriving from the association of PEMF and training needs to be investigated further.

Grote et al. (2007) investigated short-term stimulation of pulsed electromagnetic field, on heart rate variability in the recovery phase after physical exercise, suggesting a possible influence on autonomic system. In this study twenty minutes of exposure on low frequency of PEMF accelerated recovery of heart rate variability, especially in very low frequency range, with more rapidly returned to initial sympathetic tone. Despite that, the basal autonomic tone seems to play a crucial role as well as the power of electromagnetic signal. Until now, this issue is still controversial, and more evidence are necessary, in order to determine the influence of pulsed electromagnetic field on autonomic system and recovery.

Tamulevicius et al. (2021) investigated the effect of acute PEMF treatment on aerobic performance in endurance athletes. Low frequency of PEMF stimulation was applied during 6 days of preseason training, in 14 male cross-country runners of the 2nd Division of the National Collegiate Athletic Association (NCAA). 8-minutes PEMF treatment was applied before and after each of the 6-training session, totalling 12 times. Acute PEMF therapy did not induce significant changes in almost all aerobic performance parameters, as absolute or relative VO_2peak, ventilation or maximum respiration rate. Despite that, stimulation induced significant variation in time for relative ventilatory threshold (VT), suggesting possible application of PEMF during short-term training, in order to elevate VT.

PEMF and Ca2+

Regarding sport and physical activity, a crucial role is played by the mechanism of muscular contraction, which are affected by Ca2+ channels and ions flux. About this, PEMF stimulation affects calcium channels raising Ca2+ intracellular concentration (Zhang et al. 2010; Kuan-Jung Li et al. 2006), amplifying signal Ca2+ mediators and

Ca-dependent pathways (Panagopoulos, Karabarbounis, and Margaritis 2002). Despite multiple mechanism, a possible explanation on how pulsed electromagnetic field affects biological system is in changes of membrane permeability (Ross et al. 2015) and ion channel conduction (Pakhomov et al. 2009). Further, it has been hypothesized an influence on phospholipids of plasma membrane that improves production of second messengers, with starting cascade of multiple intracellular signal transduction (Semenov, Xiao, and Pakhomov 2013; Tolstykh et al. 2013; Pilla et al. 2011).

Aims of the research

Until now, very few studies investigated the influence of PEMF stimulation during exercise or sport activity. Considering the importance of Ca2+ channels and ions flux on muscular contraction, investigating the PEMF stimulation during exercise, assessing interaction on muscular activity and influence on sport or physical activity, sounded very interesting.

Also, considering the influence of Pulsed electromagnetic fields on microcirculation, vascularization and tissue oxygenation seen above, and given the importance of oxygen uptake in sport activity (Bassett and Howley 2000), especially in aerobic disciplines (e.g., cycling) it is very useful to evaluated the effect of stimulation on muscle oxygenation and its influence on pulmonary oxygen during physical activity. Nevertheless, stimulation could be applied during exercise for multiple purposes, not only in sport disciplines. Indeed, an increase in muscle O_2 supply induced by PEMF treatment, could improve exercise in patients or older adults, whose physical activity are limited by an impairment in O_2 delivery and utilization. Also, PEMF therapy combined with light exercise could provide greater benefits in people with circulatory disease or pulmonary obstructions, in order to boost the results of training and increase their quality of life (Buekers et al. 2020).

Therefore, in this Ph.D. program, we investigated the PEMF stimulation during exercise in order to assess the influence on muscular activity and oxygen uptake. To investigate widely, we evaluated the effect of stimulation on different intensity of physical effort, as heavy or moderate, different subjects, as athlete or sedentary, and different sport activity, as a cycling or weightlifting. Based on our hypothesis, PEMF stimulation should result in higher O_2 muscle supply during exercise through increased O_2 release and uptake. We hypothesize that stimulation could improve muscular response, due to a greater amplitude of muscular activity, generated by enhancement of muscular contraction mechanisms.

The aim of the Study I was to assess how PEMF stimulation affects muscle activation, pulmunar O_2 consumption and muscle O_2 delivery and utilization, in semi-professional cyclists during a constant-load exercise at an intensity between heavy and severe. We sought to investigate the effect of PEMF stimulation during physical effort, on magnitude of the muscle activity, VO_2 and HHb kinetics. The O_2 uptake kinetics are an indicator of sport performance, reflecting physiological adaptions in response to exercise, providing very important insights for athletes. Indeed, a greater O_2 muscle supply during physical effort, improve the rate of oxidative metabolism and so, could improve the performance in sport disciplines, especially endurance (Burnley and Jones 2007). In addition, we evaluated blood lactate, in order to assess the influence of stimulation on glucose utilization and glycolytic metabolism, of type-II muscle fibers, strongly recruited when intensity of exercise exceed VT.

The aim of the Study II was to quantify the magnitude of stimulation on pulmunar oxygen uptake, muscle oxygenation and muscle activity in sedentary people, in order to evaluate how individual anthropometric features, could affect PEMF treatment. Specifically, the conductivity of the electromagnetic field signal inside the body is affected by the conductivity of body tissue that influenced by its water content. Skeletal muscles have high water content than fat mass, leading to greater conductivity of electromagnetic signal (Lepelaars 1996). In order to assess how exercise intensity

affects the effect of stimulation on muscular activation and energetic system, in this study we evaluated PEMF stimulation during a constant-load exercise, at moderate and fully aerobic intensity, with a poorly recruitment of type-II muscle fibers.

The aim of the Study III was to assess how PEMF affected the energetic system inside type-II muscular fibers, during a typically weightlifting exercise, as squat, performed by youngest weightlifters at heavy intensity, that fully involved fast-twitch fibers. We investigated the effect of stimulation, on magnitude of the muscle activity, blood lactate and heart rate variability (HRV), in order to clarify if short-term PEMF stimulation could influence autonomic system during exercise or recovery as showed by Grote et al. (2007). In their study, twenty minutes of stimulation improved recovery of HRV with more rapidly returned to initial sympathetic tone. Lastly, we evaluated effected of stimulation on rating of perceived exertion (RPE), in order to quantify if PEMF could affect the perception of fatigue.

Study 1. Effect of pulsed electromagnetic fields on muscle activity, pulmonary VO_2 and muscle oxygenation in semi-professional cyclists.

Design of the study

In this study we investigated the PEMF stimulation in youngest and semi-professional athletes who cycled during a constant-load exercise at an intensity between heavy and severe, in order to quantified how stimulation affects amplitude of muscular activity, VO_2 and HHb kinetics. The O_2 uptake kinetics represents an independent indicator of physical performance and reflects physiological adaptions in response to exercise, providing quantitative and qualitative insights for a typically subject. A greater O_2 muscle supply during exercise improve the rate of oxidative metabolism and so the physical performance.

There are different conditions in which muscle O_2 availability might be considered to be amongst the reasons responsible for relatively slow oxygen kinetics: for example, during physical effort where the muscle perfusion pressure is reduced, when muscle O_2 supply is deliberately restricted, and in older age and a variety of disease conditions (Jones and Poole 2005). The velocity and the rate of VO_2 increase after the onset is a determinant of sport performance and an indicator of well-done state of oxidative energetic system activity (Burnley and Jones 2007). A faster increase in VO_2 after the onset of physical activity indicates a greater muscle O_2 utilization, which is feature common to trained subject and moreover elite athletes, due to the lower mismatch between muscle O_2 delivery related to pulmonary VO_2 (Koppo, Bouckaert, and Jones 2004).

During physical activity, the O_2 uptake shows a typical kinetic related to the intensity of exercise. At the onset of exercise, there is a very short cardio-dynamic phase (first 10-20 seconds): on the first seconds of transition, a tight coupling between increases in blood flow and VO_2 occurs with unchanged muscle oxygenation (Grassi et al. 1996). More particular, the faster and higher raise in blood flow allows the raise of VO_2 with

a constant in muscle O_2 extraction. This phase is followed by a phase II, a primary component, and if the intensity is heavy above the ventilatory threshold, by a phase III, a slow component (Whipp and Wasserman 1972). During phase II, an increase in muscle oxygenation occurs due to higher muscle O_2 extraction, contributing to the raise of VO_2 along with augmented blood flow. This suggests an adequate muscle O_2 availability in relation to O_2 needs, showing the intrinsic slowness of oxidative metabolism to adapt to the metabolic request (O_2 needs) of heavy exercise, in support of the metabolic inertia hypothesis (Brad J. Behnke et al. 2002). This indicates that he muscle O_2 availability and the rate of adjustment of oxidative metabolism at the onset could determine the VO_2 kinetics on heavy exercise on-transition (MacDonald, Pedersen, and Hughson 1997). The phase II well reflects the gas exchange on muscles during exercise and best represents the athlete aerobic capacity (Poole et al. 1991).

When intensity of exercise is moderate under ventilatory threshold, the steady-state is usually reached in 2-3 minutes, whereas, when intensity is heavy (over ventilatory threshold), the kinetic shows an additional component (slow component) that delays the achievement of the steady-state. The slow component usually occurred after ~180 seconds of heavy exercise and presents an extra- O_2 consumption. It is characterized by a slower adaptation of aerobic energetic system to the metabolic request of the workload. His amplitude is affected by central and peripheral factors, such as blood O_2 transport and muscle O_2 utilization, but most of all, by the recruitment of type-II muscle fibers, which are less aerobic efficient than type-I, that demands more O_2 for a given workload intensity (Jones and Poole 2005).

Related to this topic, we sought to investigate the effect of PEMF stimulation on muscle activity during an exercise at the intensity above ventilatory threshold, in order to assessed how stimulation affects muscular response, especially when type-II muscle fibers are strongly involved. It is interesting to assess a possible link between changed in each component of O_2 kinetics and changed in amplitude of muscle activity induced by PEMF. Specifically, PEMF stimulation could enhanced muscle O_2 availability on

transition, improving a faster adaption of aerobic energetic system to the workload and a decrease in O_2 deficiency related to the metabolic demand. An augmented muscle O_2 uptake induced by PEMF, could enhance aerobic efficient of type-II muscle fibers, showing a different amplitude and time of appearance of the slow component of kinetics. Further, considering the influence of PEMF on Ca^{2+} channels and ions flux and the importance of these on muscle activity, stimulation could enhance muscular contraction process and preserve overall muscle activation during a severe intensity exercise.

Materials and Method

The study design was a single-blind, randomized controlled trial. Experiments were performed in 20 male semi-professional cyclists. Features of each athletes are shown in the table below. All subjects were volunteers, healthy, non-smokers and none of them were taking medications or supplements. None of the subjects reported physical deficit or muscular injury at the time of the study. All participants received a verbal explanation of experimental procedures, and informed consent was obtained before the beginning of recordings. The experimental protocol was approved by the Institutional Ethic Committee of the University of Bologna. The experiments were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Athletes	Age (years)	VO_2 max (ml/min/kg)	Workload (watt)	Weight (kg)	Height (cm)	BMI
A.D.R.	24	56,1	304	69,0	181	21,1
A.M.	15	55,3	310	68,0	182	20,5
D.P.	19	63,7	320	58,5	171	20,0
D.S	20	47,5	230	78,0	185	22,8
F.D	27	54,8	286	64,5	169	22,6
G.F	20	66,7	500	76,0	189	21,4
G.G.	34	36,9	281	89,0	180	27,5
G.P.	27	49,6	285	74,0	175	24,2
I.V.	19	62,0	352	70,0	180	21,6
J.B.	37	36,1	255	88,0	187	25,2
L.P	16	54,0	285	65,0	166	23,5
L.T.	20	64,4	340	70,0	183	20,9
M.B.	25	37,1	230	81,0	178	25,6
M.G.	18	69,5	340	58,0	183	17,3
M.L.	22	60,5	352	76,0	174	25,1
N.M.	19	66,6	362	64,0	180	19,8
P.L.	23	55,5	240	54,0	168	19,1
R.B	23	51,9	295	73,5	181	22,4
S.B	16	63,1	285	63,0	169	22,1
S.C.	21	42,4	290	90,0	180	27,8
Mean	22,3	54,7	307,1	71,5	178,1	22,5
SD	5,7	10,4	60,1	10,3	6,5	2,7
SEM	1,3	2,3	13,4	2,3	1,5	0,6

Table 1. Features of each participants of the study.

For the realization of this study, we recorded the surface electromyographic (EMG) activity from the Vastus Medialis (RVM) and Biceps Femoris caput longum (RBF) of the right leg. EMG data were recorder data sampling rate of 1000 Hz by a Free-EMG 1000 (BTS Bioengineering, Inc.). FREEEMG is a device with wireless miniaturized probes, for the dynamic analysis of muscle activity. The system communicates with a PC through the supplied USB 2.0 receivers, with wireless ieee802.15.4 data transmission. For signal acquisition, the probes (41,5×24,8x14mm) are directly attached to the electrodes and even signals be detected and captured. Each probe is equipped with internal memory to ensure uninterrupted recording in case of temporary connection loss.

Figure 1. Free-EMG 1000 BTS Bioengineering, Inc

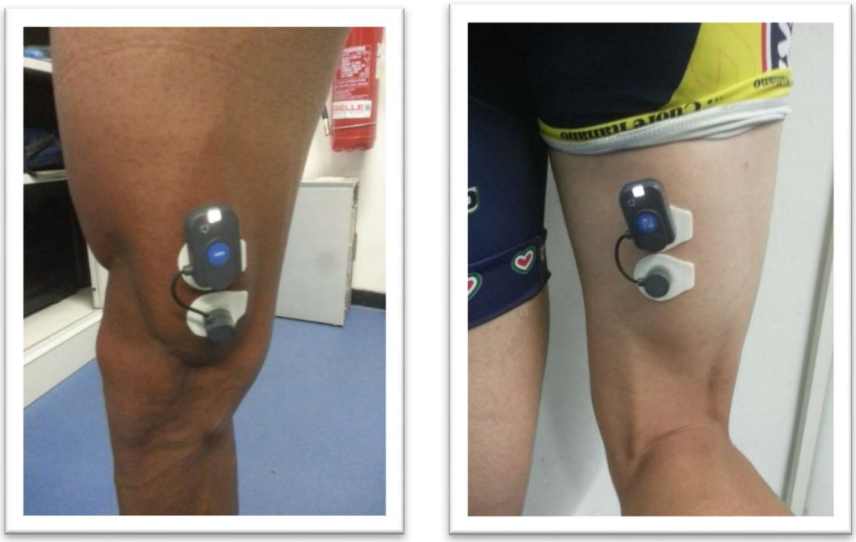

Figure 2. Electrode placement on the muscular belly of the RVM and RBF. To improve contact, the skin was shaved and cleaned with ethanol before placing the Ag/AgC1 disposable electrodes 32x32 mm with active area of 0.8 cm2 and inter-electrode distance of 2 cm used in bipolar configuration (RAM, s.r.l, Italy).

In order to stimulate the entire thigh, two PEMF loop-antenna devices (Torino II, Rio Grande Neurosciences, USA) were positioned on the right leg, at beginning and ending of the thigh. The PEMF waveform consisted of a pulse-burst modulated 27.12 MHz sinusoidal carrier, with 2 ms burst width repeated at 2 HZ, with peak magnetic field at the center of the loop 5±1 μT.

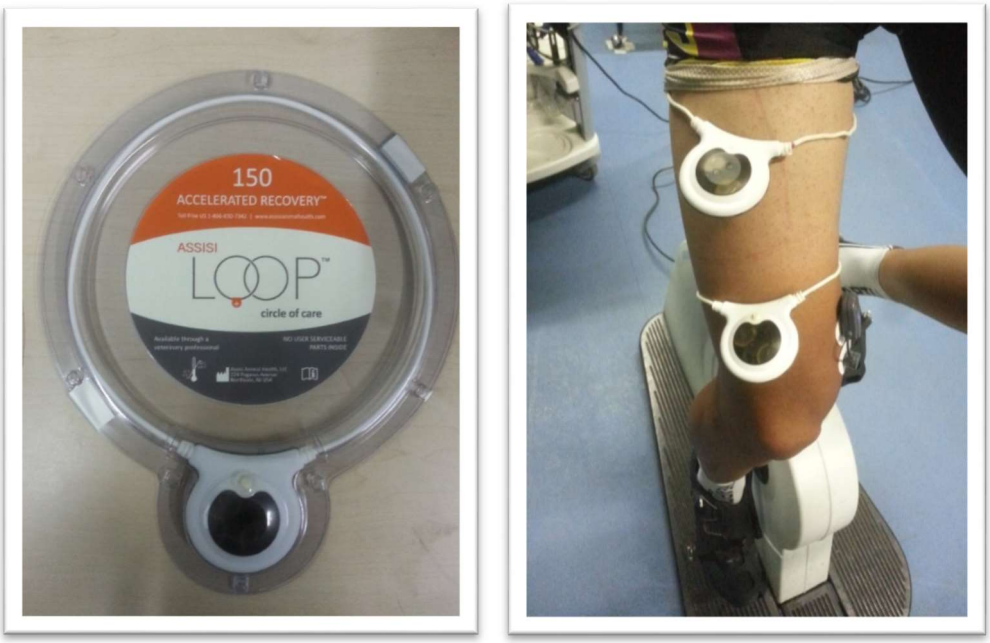

Figure 3. PEMF loop and its positioning on the right leg

To recorded muscle oxygenation, we used a near-infrared spectroscopy (NIRS, NIMO, Nirox s.r.l. Italy), a noninvasive and validated methods which used different light absorption of hemoglobin (Hb) and myoglobin related on their O_2 saturation (Mancini et al. 1994). In order to evaluate the effect of PEMF stimulation on muscle oxygenation during exercise, we recorded tissue oxygenation index (TOI) and deoxyhemoglobin (HHb) values with a continuous wave with a sampling rate set at 40 Hz. We collected both TOI and HHb, which are influenced by skin blood flow in the former, and reflect the rate of the muscle O_2 extraction and utilization in the latter (Grassi and Quaresima 2016).

During cycling, emitter and detector pair, was firmly located on the skin above the lower third of the right vastus lateralis (VL) muscle (about 12 cm upon the proximal border of patella and 3 cm lateral to the midline of the thigh), and held with two adhesive belt and a band of Velcro straps (Belardinelli et al. 1995). In order to prevent contamination from ambient light, the probe was covered with a black cloth and elastic strappings were placed around. Pen marks were made above the skin to indicate the

boundaries of the plastic spacer to check for any movements of the probe during cycling. Before starting trial, we measured skinfold thickness of VL by a caliper (Holtain), in order to do fat layer correction (Y. Yang et al. 2005).

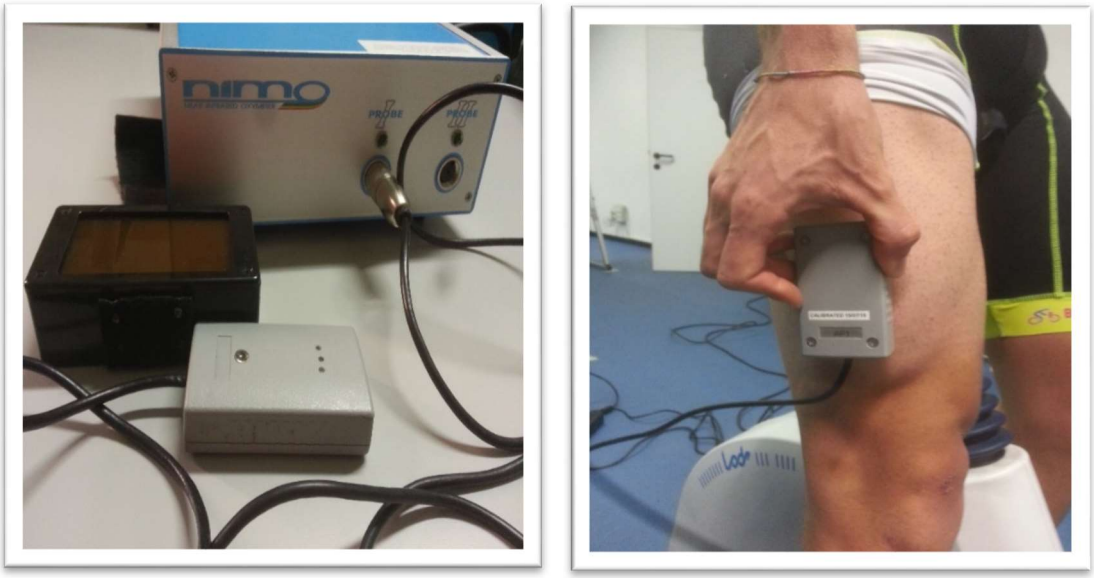

Figure 4. NIRS and positioning of the probe on the vastus lateralis of the right leg.

Experiments were performed on the same cycle-ergometer (H-300-R Lode, Exere Air Machine, Italy) under a standardized procedure, in a quiet room with a stable and comfortable temperature (22°C), at the same time of the day (9:00-12:00 AM) to avoid circadian influence (Piras, Cortesi, et al. 2019; Piras and Gatta 2017). Before starting each recording, athletes performed 10 minutes of free warm-up and 5 minutes of rest. Subjects were asked to avoid drinking caffeinated beverages before the experimental procedures and were instructed to avoid strenuous activity and alcohol in the 12h preceding the test. Expired gas and pulmonary O_2 uptake were collected with Quark b^2 breath-by-breath metabolic system (Cosmed, s.r.l. Rome, Italy). The system was calibrated immediately before each test in accordance with the manufacturer's guidelines: volume calibration was performed at different flow rates with a 3-L calibration syringe and calibration of gas analyzers was performed with a tank of

reference gas mixture (16.00% O_2, 5.00% CO_2), and ambient air (20.93% O_2 and 0.03% CO_2).

We also measured blood lactate concentration, before the beginning of each trial (baseline) and at the third minute of the constant-load exercise, in both experimental conditions. A blood sample was performed from the right ear lobe.

Figure 5. Lactate scout (EKF, Germany)

Athletes visited our laboratory five times, with three days between each visit, in which we performed different recordings. In the first day we recorded an incremental test and the maximum voluntary contraction (MVC) session. We first recorded the MVC of RVM and RBF, used to normalize electromyographic (EMG) data. As in previous studies (Piras et al. 2018; Raffi et al. 2017), we normalized the EMG activity to the peak of the MVC. Each subject performed for 5 seconds, an isometric contraction against a maximum load using isotonic machines (Exere Air Machine, Italy). They repeated the same procedure 3 times separated by 2 minutes of rest and we assume the maximum peak value, in order to determine the MVC of each muscle.

Then we recorded an incremental test on a cycle-ergometer, necessary to individualize workload for the succeeding recording sessions, through the detection of ventilatory

threshold (VT) and maximal oxygen consumption (VO_2 max). Each subjects cycled on an cycle-ergometer at 50 Watt for 5 minutes (warm-up), followed by workload at 80 watt and instantaneous increase of 20 Watt every 1 minute, at a cadence of 70 RPM, until the volitional exhaustion (Piras, Campa, et al. 2019; Campa et al. 2020). Heart rate was monitored by Polar Heart Rate Monitor (Polar Electro, Finland).

The maximal exercise test, lasted until attainment of oxygen uptake (VO_2) plateau or the attainment of at least two of the three additional criteria: (1) a plateau of heart rate despite an increased power, (2) inability to maintain the cycling cadence (i.e., dropped by >10 rpm), or (3) exercise cessation due to substantial fatigue. VO_2 plateau was defined as an increase in $VO_2 \leq 50$ ml/min during the last 30 seconds despite increased power (Yoon, Kravitz, and Robergs 2007). The highest VO_2 values reached during the exercise phase of the incremental test were considered as the maximal VO_2 uptake. The VT was calculated by comparing the V-slope and the ventilatory breakpoint, in accordance with Beaver et al. (Beaver, Wasserman, and Whipp 1986). Strong verbal encouragement was given during the test. The workload for each athlete was set to ~50% of the difference between power reached at ventilatory threshold (VT) and at VO_2 max (~50% Δ VT-VO_2 max).

After the initial session, the subjects came to our laboratory for four more recordings in which they performed 6-minutes of heavy constant-load exercise both during active (ON) and inactive (OFF) PEMF stimulation. The four sessions were performed in random order and the athletes have never been informed about the status of stimulation. The two circular 20 cm loops were positioned on the thigh in both experimental conditions (ON and OFF), because the stimulation was not perceived by the subjects at the cutaneous level (single blind trial). Athlete started the recording session with 1-minute of unloaded cycling, followed by an instantaneous increase of the individualized power, which was attained in ~3s. Each trial was ended automatically at the six minutes or intentionally, when athletes were unable to continued heavy physical effort and keep the individualized workload.

In each trial across both experimental conditions, data were collected during unloaded cycling, as we called “warm-up” for EMG data and “Baseline” for HHb, VO_2 and Lactate data, and during constant-load exercise, as we called “effort” for EMG data and “exercise” for HHb, VO_2 and Lactate data.

Figure 6. A typical athlete involved during the incremental test on the cycle-ergometer.

Data Analysis

The response of muscular activity over the entire trial was recorded by surface EMG and assessed measuring the root mean square (RMS) normalized to the peak of the MVC. We averaged the values of warm-up (unloaded cycling), phase of effort and muscular activity related to the time of exercise, for each condition (PEMF ON and PEMF OFF) in each muscle (RBF and RVM). The signals were positively rectified and band-pass filtered (Butterworth, 20–450 Hz) using SMART analyzer (BTS Bioengineering Inc.).

The software EMG easy report 6.03.8 (Merlo Bioengineering, Italy) was used for EMG traces, on data process and artefact removal (Vinti et al. 2018; Campanini et al. 2020; Mazzoli et al. 2018). First, we used a wavelet-based denoising filter, in order to reduce background noise and automatically remove of large and frequent artifacts (Merlo, Farina, and Merletti 2003). After detecting and removing specific PEMF artifacts on EMG traces, a consolidate process described below was applied (Mukhopadhyay and Ray 1998; Solnik et al. 2010; Merlo, Farina, and Merletti 2003). Starting from the raw signal, a peak emphasis operator, called Smoothed Non-Linear Energy Operator (SNEO) (Mukhopadhyay and Ray 1998) was applied. SNEO is similar to the Taeger-Kaiser, other operator frequently used with EMG signals (Solnik et al. 2010). Peaks positions and amplitudes were founded using thresholds of the minimum amplitude and distance between PEMF peaks. The position of unrecognized stimulus was found with linear interpolation of the values obtained in the previous point. Found positions of the artifacts, the parts of the signal 20ms before and 80 ms after peaks, were forced to zero. After that, the algorithm calculated the amplitude of the RMS limited at the signal of the muscle activity for each detected onset intervals. The activation intervals were calculated through specific algorithm (Merlo, Farina, and Merletti 2003) using a mean background noise level, as 10uV RMS.

Then, the values recorded were normalized to the peak of the MVC. The normalized RMS values were calculated in 100 ms bin from EMG signals using MATLAB (the MathWorks). For all variables recorded, we averaged the values of all subjects in each experimental condition (PEMF ON vs PEMF OFF). Then, we computed means and standard errors across groups. We compared mean values of muscular activity for warm-up, constant load-exercise (physical effort) and muscular activation related to the exercise duration (activity/time). A 2 (muscles, RVM and RBF) x 2 (conditions, PEMF ON and PEMF OFF) repeated measures ANOVA was performed on each parameter (warm-up, physical effort, activity/time) separately. Effect sizes were

calculated using partial eta squared (η^2_p), and means were considered significantly different at $p<0.05$.

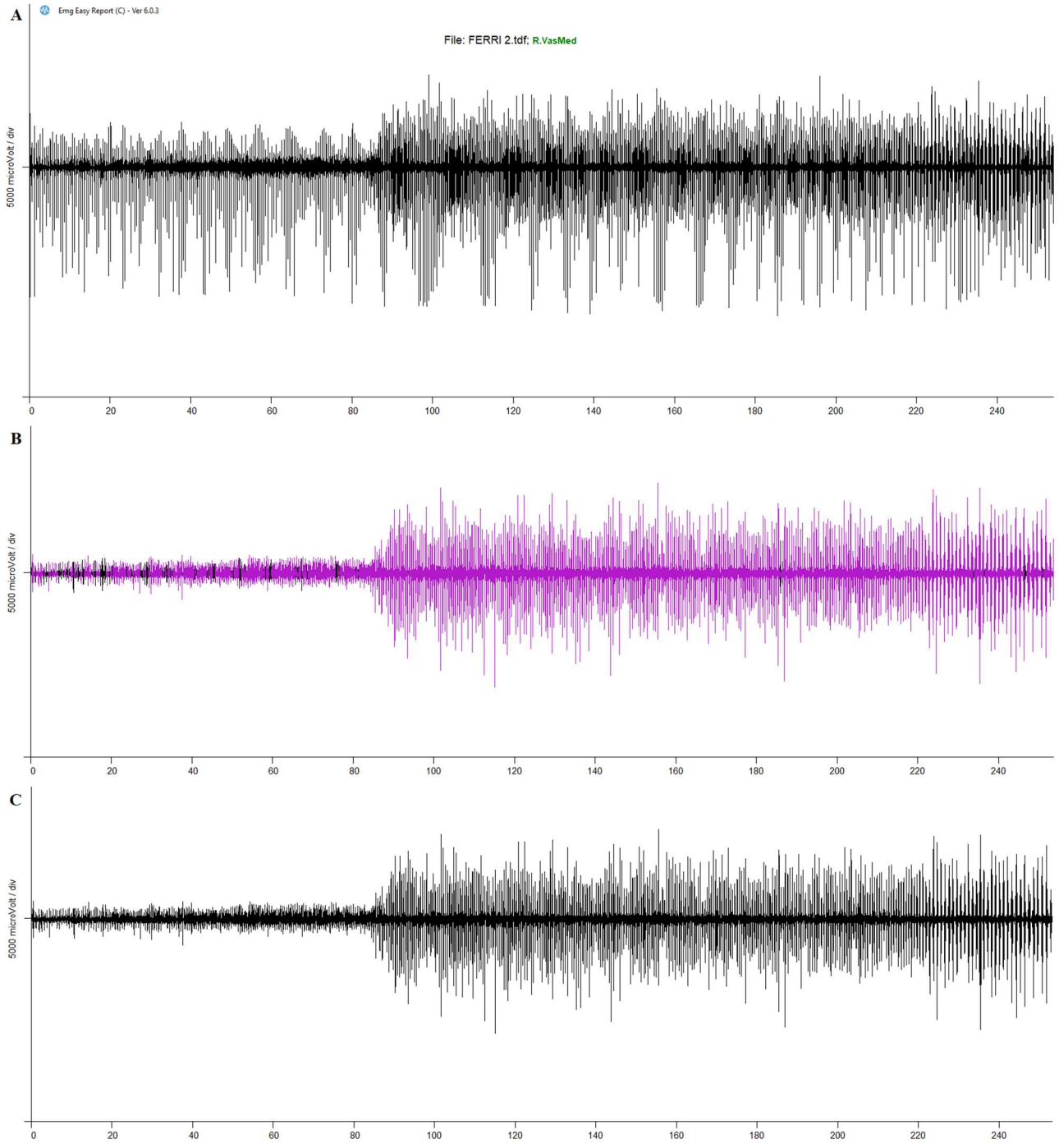

Figure 7. PEMF artefact removal, with Easy Report 6.03.8 (Merlo Bioengineering, Italy). Raw EMG trace (A) was processed by the software (B), using wavelet-based denoising filter and a specific algorithm, in order to return trace without PEMF artifacts (C)

Breath-by-breath VO_2 values obtained in the several repetitions of the same constant-load exercise (ON; OFF) were time aligned, interpolated on a second-by-second basis and then superimposed for each athlete. Then, we averaged VO_2 values every second and used it for kinetics analysis. The same procedure was followed for HHb values. Data collected during the first 20s of the on-transition (corresponding to the "cardio-dynamic phase") were excluded from the analysis. The baseline (unloaded cycling) of HHb and VO_2 was defined as the mean value measured 30 seconds before transition. In order to evaluate VO_2 and HHb on-kinetics, data were fitted by nonlinear regression functions (Grassi et al. 2003) :

$$VO_2(t) = VO_{2bas} + A_p * [\ 1 - e^{-(t - TDp)/\tau p}\ ] + A_s * [\ 1 - e^{-(t - TDs/\tau s)}\ ]$$

$$HHb(t) = HHb_{bas} + A_p * [\ 1 - e^{-(t - TDp)/\tau p}\ ] + A_s * [\ 1 - e^{-(t - TDs/\tau s)}\ ]$$

where $VO_2(t)$ is the pulmonary oxygen uptake during the entire trial; HHb(t) is the value of deoxyhemoglobin of vastus lateralis during the entire trial; VO_{2bas} is the pulmonary oxygen uptake at the baseline; HHb_{bas} is the value of deoxyhemoglobin of vastus lateralis at the baseline; A_p is the amplitude of the primary component, as the difference between VO_2 or HHb at the baseline with the VO_2 or HHb reached at the steady-state (as mean of the last 30 seconds); TD_p is the time delay of the primary component; τ_p is the time constant (tau) of the primary component; A_s is the amplitude of the slow component, as the difference between VO_2 or HHb reached at the steady-state with the VO_2 or HHb mean values of the last 30 seconds of exercise (as usually quantified by calculating the difference between 3 and 6 minute of constant load exercise). TD_s is the time delay of the slow component; τ_s is the time constant (tau) of the slow component.

We measured the magnitude and the percent contribution of the slow component to the total amplitude of the response. We also calculated the gain of VO_2, as the increase in VO_2 above baseline to the reached steady-state and corrected for individualized workload (WL), according to this equation (Buekers et al. 2020):

$$Gain = (VO_{2\,[150sec-180sec]} - VO_{2bas}) / WL$$

The model parameters were determined by least-squares nonlinear regression, in which the convergence criteria were satisfied by minimizing the sum of squared error. Although many powerful and dedicated software packages have been developed for regression analysis, we used the most widely distributed regression tool that is the Solver add-in bundled of Microsoft Excel (Brown 2006; Kemmer and Keller 2010; Walsh and Diamond 1995). The TOI values of the vastus lateralis during baseline, at 60 s, and at 180 s (±15 s) of the constant-load exercise were subsequently calculated. Blood lactate concentration was measured at the baseline and during exercise, in both experimental conditions. A blood sample was performed from the right ear lobe, before the starting of trial and at the third minute of the constant-load exercise.

Statistical analysis

All data are show as means ± SEM. Values were compared with Paired sample t-test with means considered significantly different at $p < 0.05$. To determine the magnitude of the stimulation effects, effect sizes (ES) were calculated as the mean difference standardized by the between subject standard deviation and interpreted according to the thresholds (Hopkins et al. 2009): <0.20; small, >0.20-0.60; moderate, >0.60-1.20; large, >1.20-2.00; very large,>2.00-4.00; extremely large, >4.00. Data were analyzed with SPSS v22.0 (IBM, New York, NY, USA).

Results

The figures shown below illustrate the mean values of EMG traces recorded during warm-up and during physical effort for each investigated muscle (RVM and RBF) across experimental conditions (PEMF ON and PEMF OFF). EMG activity over warm-up, during the entire constant-load exercise and related to the exercise duration were assessed, comparing the RMS normalized to the peak of the MVC.

Analysis showed significance for muscles main effect RVM and RBF ($F_{1,17}$=16.452; p< 0.001; η^2_p=0.141) and muscles for parameter main effect. as warm-up, physical effort and activity/time ratio ($F_{2,16}$=20.133; p< 0.001; η^2_p=0.287).

A greater amplitude on RVM in comparison to RBF, was found in both experimental conditions, during physical effort (PEMF OFF: RVM= 41.94 ±10.77; RBF= 26.73±13.78; PEMF ON: RVM=41.49±15.51; RBF= 26.60±13.61), when we related muscular activity to the exercise duration, as muscular activation-time ratio (PEMF OFF: RVM= 0.11±043; RBF=0,07±04; PEMF ON: RVM=0.11±0.47; RBF=0,07±0.04) and for mean muscle activation across parameter (RVM=16.57±0.77; RBF= 11.87±0.80).

Significant difference was found between condition during warm-up, with PEMF ON exhibited higher significant RMS value of both muscles with respect to PEMF OFF condition (RVM ON=9.90±0.77; RVM OFF=6.21±0.49; $t_{(16)}$ = -5.61; p<0.001; ES 0.57-moderate) (RBF ON=12.19±1.12; RBF OFF=5.48±0.47; $t_{(16)}$ = -6.29; p<0.001; ES 0.69-large). PEMFs stimulation caused a change of the muscular activation, with greater amplitude on PEMF ON condition.

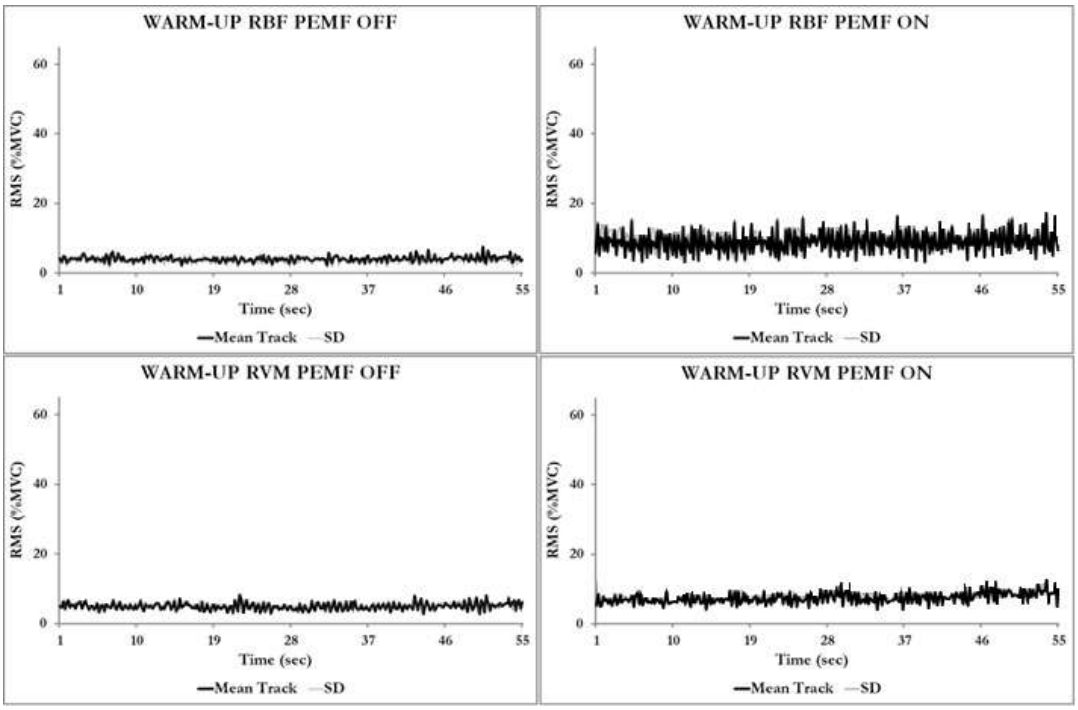

Figure 8. EMG traces during warm-up, for Right Vastus Medialis (RVM) and Right Biceps Femoris (RBF) in both experimental conditions (PEMF ON vs PEMF OFF). Black lines are the mean values, grey lines are the standard deviations.

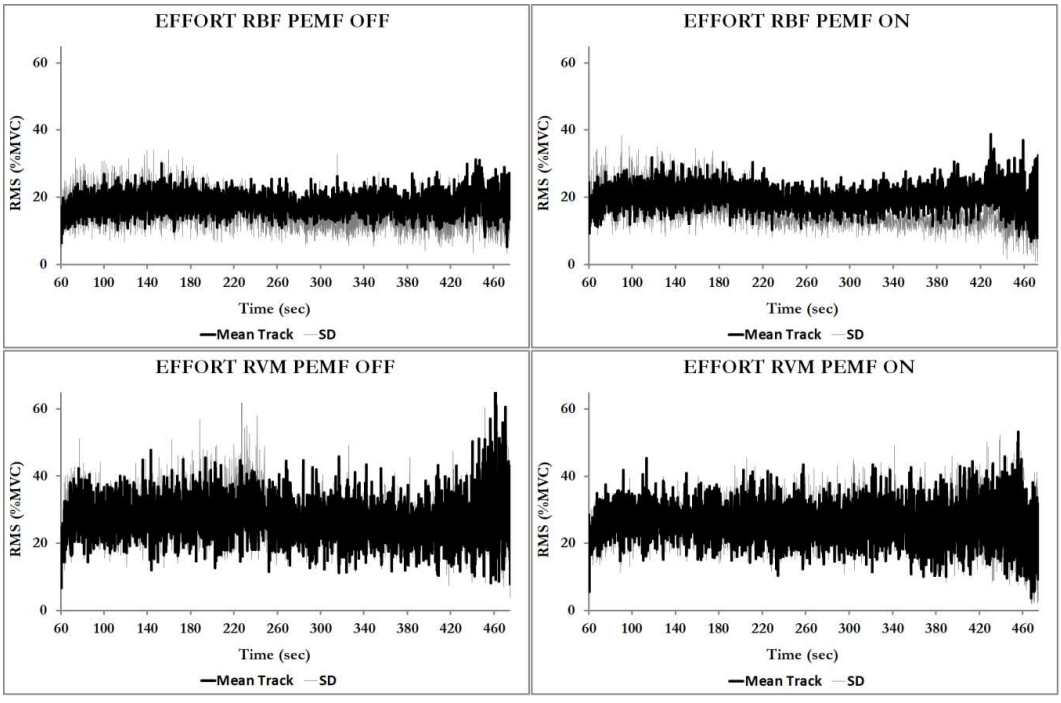

Figure 9. EMG traces during physical effort, for Right Vastus Medialis (RVM) and Right Biceps Femoris (RBF) in both experimental conditions (PEMF ON vs PEMF OFF). Black lines are the mean values, grey lines are the standard deviations.

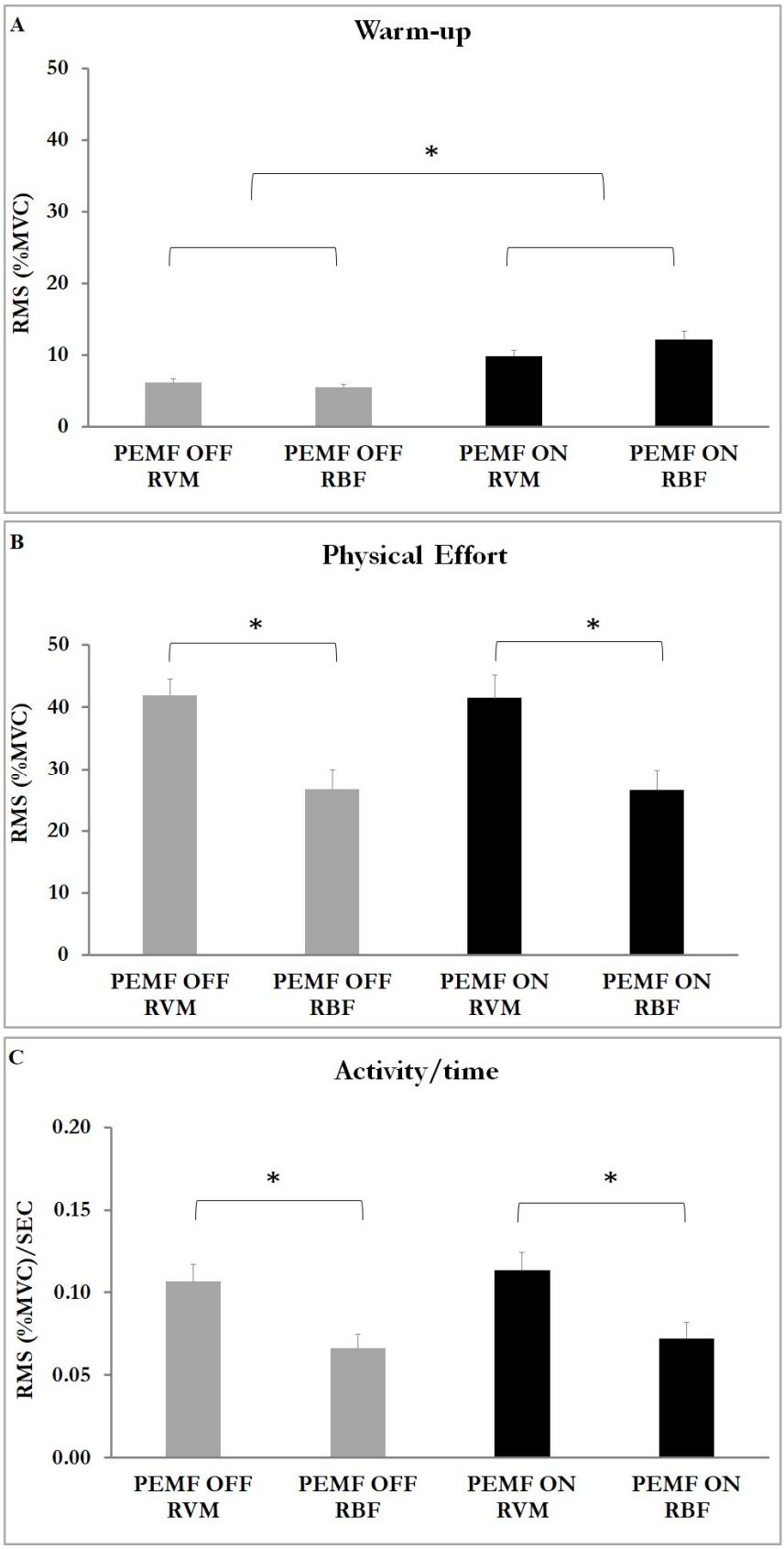

Figure 10. Histograms represent the root mean square (RMS) of the normalized EMG values (mean ± SEM) of both muscles (RVM; RBF) across conditions (ON; OFF). A. EMG activity at warm-up. B. EMG activity at phase of physical effort. C. EMG activity related to the time of exercise. Asterisks indicate significant differences at $p<0.05$.

For VO_2 kinetics, analysis have not shown any significant differences between conditions (ON vs. OFF). Tables below show the mean values for VO_2 kinetic recorded in both experimental conditions.

	Base (L/min)	Ss (L/min)	Ap (L/min)	TDp (sec)	τp (sec)	As (L/min)	TDs (sec)	τs (sec)	MRTp (sec)	MRTs (sec)	Sc (L/min)	Sc (%)	Gain (ml/min/kg)
OFF	0,93±0,0	3,45±0,1	2,52±0,1	19,15±1,5	19,55±1,5	0,54±0,0	107,26±5,9	93,78±15,8	38,70±1,8	201,04±15,6	0,24±0,0	6,24±0,9	8,88±0,2
ON	0,94±0,0	3,46±0,1	2,52±0,1	18,44±2,2	17,94±1,6	0,51±0,0	104,50±10,1	90,21±12,67	36,38±2,6	194,71±15,1	0,25±0,0	6,26±0,8	8,88±0,2

Table 2**.** Mean values (±SEM) of VO2 kinetics recorded during constant-load exercise in PEMF OFF and ON condition. Asterisk indicates significant differences ($p<0.05$). Legend: Base, baseline; Ss, steady-state; Ap, amplitude of primary component; TDp, time delay for primary component; τp, tau for primary component; As, amplitude of slow component; TDs, time delay for slow component; τs, tau for slow component; MRTp, mean response time for primary component; MRTs, mean response time for slow component; Sc, magnitude of slow component (Trofè et al. 2021).

VO_2 kinetics analysis for a typical subject is presented in figure below

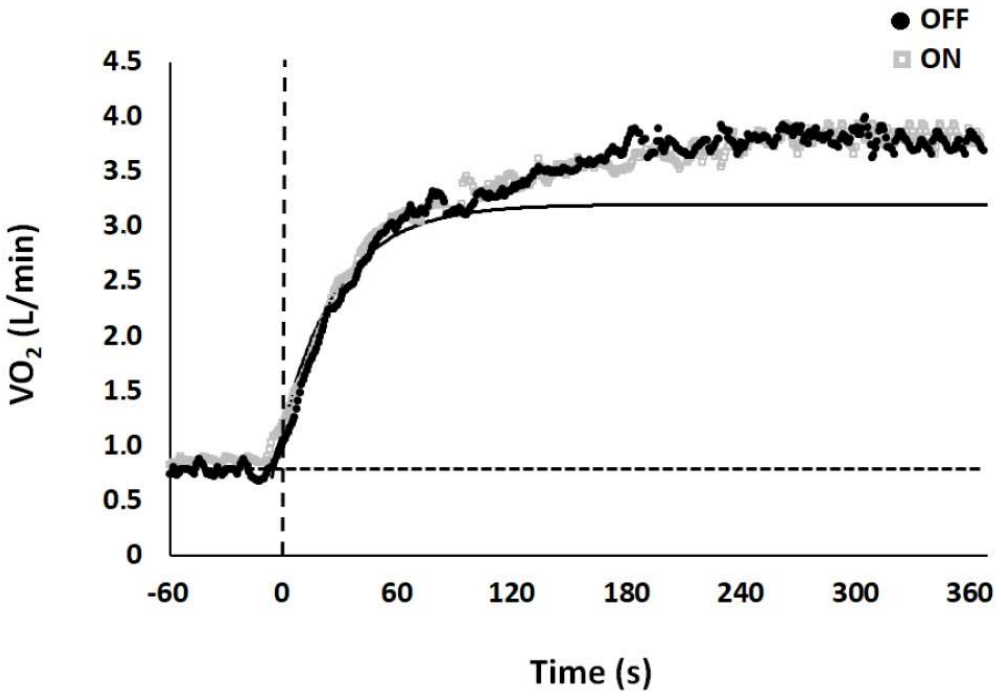

Figure 11. Model fit for VO2 kinetics between conditions, OFF (black dot) and ON (grey square) for a typical subject. Vertical dotted line at time 0 represents the transition from unloaded cycling (baseline) to constant load exercise. Horizontal dashed line represents the baseline. Data points are average values calculated each second (Trofè et al. 2021).

Tables below show the mean values for HHb kinetic recorded in both experimental conditions.We found that HHb tended to be higher (25.63 ± 4.1 vs. 23.21 ± 5.5 μM for ON and OFF, respectively, p = 0.062; d = 0.50) when subjects were stationary on the bike, just before the baseline started. Analysis showed a significant difference between mean values at steady-state ($t_{(17)}$ = −1.751; p = 0.049; ES = 0.17-small), for the amplitude ($t_{(17)}$ = −2.306; p = 0.017; ES = 0.24-moderate), TD ($t_{(17)}$ = 2.609; p = 0.009; ES = 0.33-moderate), τp ($t_{(17)}$ = 2.296; p = 0.017; ES = 0.28-moderate), and MRTp ($t_{(17)}$ = 3.531; p < 0.01; ES = 0.41-moderate) of the primary component, and for TD ($t_{(17)}$ = 1.760; p = 0.048; ES = 0.2-moderate) of the slow component.

	Base (μM)	Ss (μM)	Ap (μM)	TDp (sec)	τp (sec)	As (μM)	TDs (sec)	τs (sec)	MRTp (sec)	MRTs (sec)	Sc (μM)	Sc (%)
OFF	33,34±4,2	60,70±4,1	27,36±2,3	7,80±1,1	8,10±1,2	8,95±1,8	97,56±13,3	111,44±17,1	15,90±1,6	209,00±17,1	1,99±0,4	2,77±0,4
ON	34,34±4,7	67,58±5,0*	33,40±3,3*	4,90±0,8*	5,66±0,7*	8,43±2,2	75,57±11,7*	137,85±19,4	10,56±1,1*	213,42±25,9	2,56±0,5	3,43±0,7

Table 3. Mean values (±SEM) of HHb kinetics recorded during constant-load exercise in PEMF OFF and ON condition. Asterisk indicates significant differences ($p<0.05$). Legend: Base, baseline; Ss, steady-state; Ap, amplitude of primary component; TDp, time delay for primary component; τp, tau for primary component; As, amplitude of slow component; TDs, time delay for slow component; τs, tau for slow component; MRTp, mean response time for primary component; MRTs, mean response time for slow component; Sc, magnitude of slow component (Trofè et al. 2021).

HHb kinetics analysis for a typical subject is presented in the figure below.

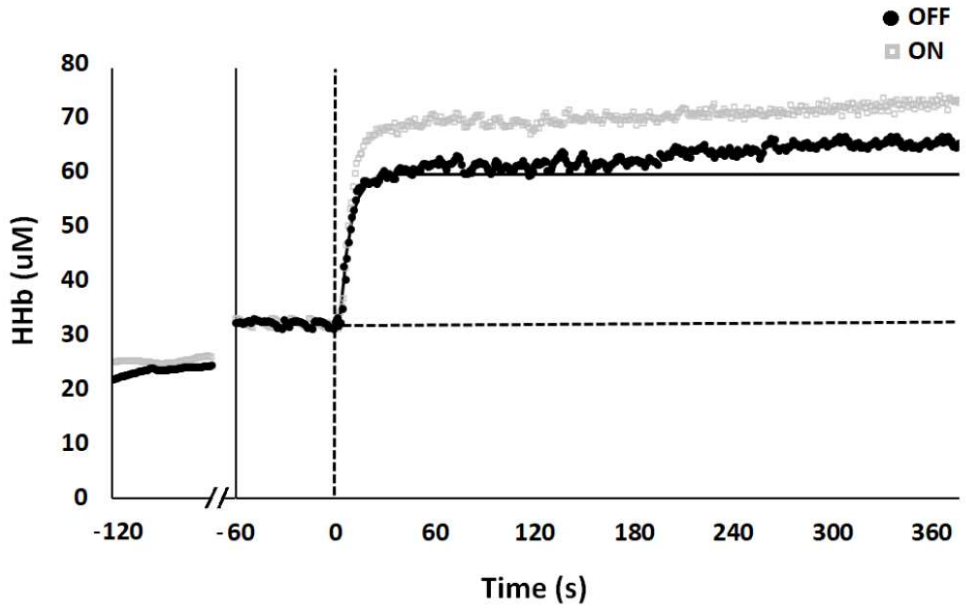

Figure 12. Model fit for HHb kinetics between conditions, OFF (black dot) and ON (grey square) for a typical subject. The first 60 s represent HHb values recorded when subject was stationary on the bike, just before baseline values (subsequent 60 s). Vertical dotted line at time 0 represents the transition from unloaded cycling (baseline) to constant load exercise. Horizontal dashed line represents baseline. Data points are average values calculated each second (Trofè et al. 2021).

There were no differences in TOI values between conditions ($p > 0.05$; figure below)

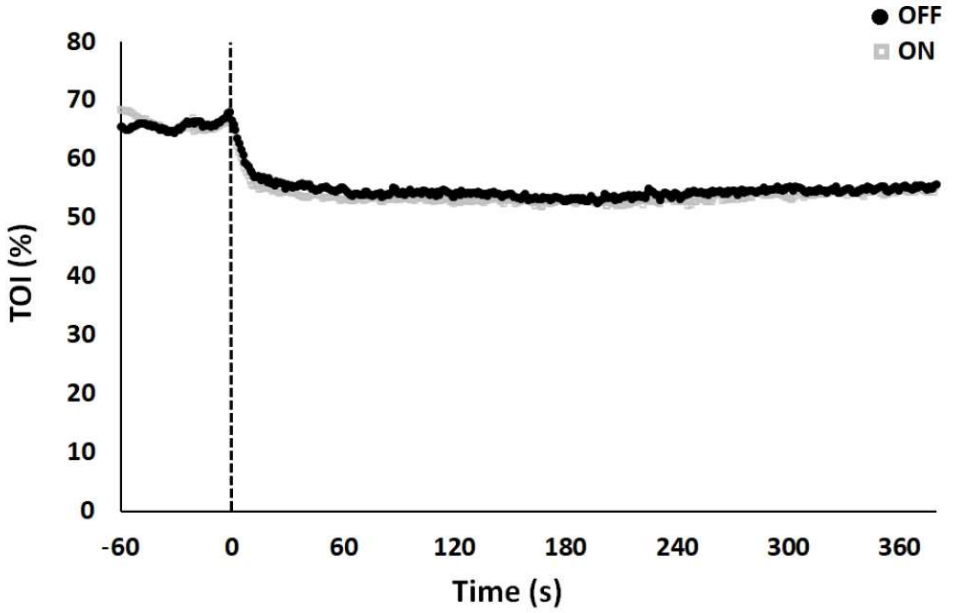

Figure 13. Vastus lateralis tissue oxygenation index (TOI) kinetics following acute PEMF stimulation (ON) and sham condition (OFF). Vertical dotted line at time 0 represents the transition from unloaded cycling (baseline at 0 W) to constant-load exercise. Data points are average values (%) calculated each second. (Trofè et al. 2021).

Finally, we measured lactate concentration (mmol/L) before the beginning of each trial (baseline) and at the third minute of the exercise. Analysis showed a significant difference between experimental conditions during the constant-load exercise. ($t_{(17)} = -5.14$; $p<0.001$; ES 0.50-moderate).When stimulation was active (PEMF ON) blood lactate was significantly higher compared to inactivate stimulation (PEMF OFF) (10.37±0.68 mmol/L vs 7.52±0.46 mmol/L).

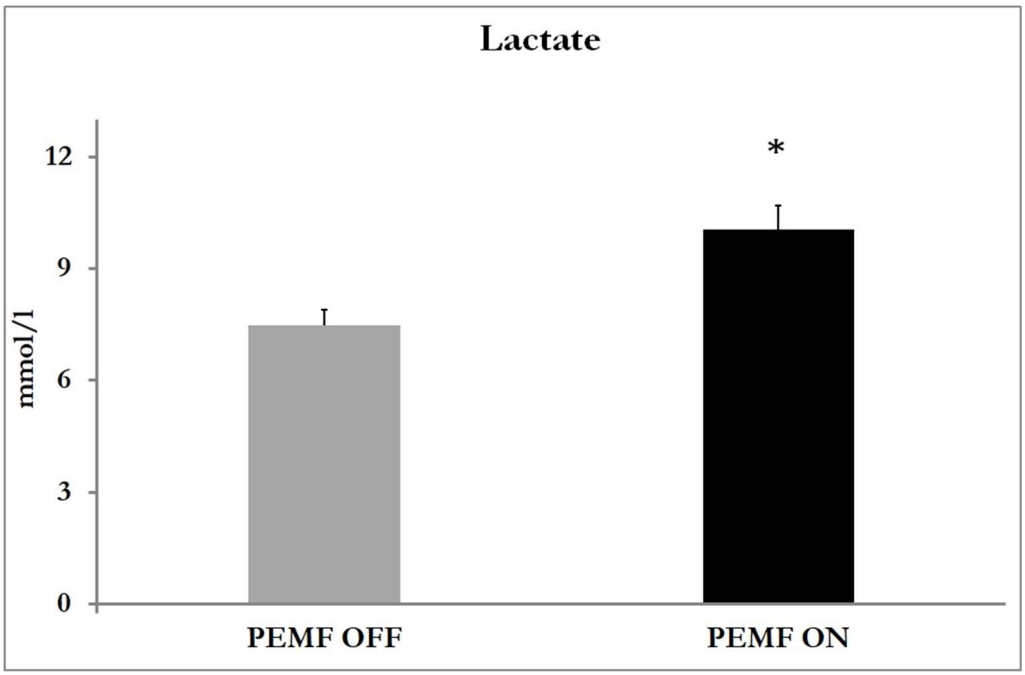

Figure 14. Blood Lactate concentrations measured at 3rd minutes of physical effort on both PEMF ON and OFF condition. Data are shown as mean values ± SEM. Asterisks indicate significant values at $p<0.05$ (Trofè et al. 2021).

Study 2. Effect of PEMF on muscle activity, pulmonary VO_2 and muscle oxygenation in sedentary young people

Design of the study

This study was designed based on the results obtained in the previous study. Indeed in study 1, PEMF stimulation increased muscle activity, for RVM and RBF, during warm-up. The increase in the amplitude of muscle activity, was probably caused by the effect of stimulation on contraction mechanism of muscular fibers. PEMF stimulation, likely caused a change of membrane permeability and Ca^{2+} channel conduction, enhancing Ca2+ intracellular concentration (Pakhomov et al. 2009; Ross et al. 2015; Zhang et al. 2010; Kuan-Jung Li et al. 2006), together to an amplified signal Ca2+ mediators and Ca-dependent pathways (Panagopoulos, Karabarbounis, and Margaritis 2002). Also, we noted a significantly higher activity for vastus medialis related to bicep femoris, but we were not surprised about this. Indeed, the main role of vastus medialis (VM) during cycling is well known, but the role of bicep femoris (BF) is still under discussion, and its activity is affected by fatigue, pedaling rate, coordination/activation timing (angle), training status, shoe-pedal interface and body position. Especially, largest BF activity seem to be related to increased fatigue in both vastus lateralis and medialis (Hug and Dorel 2009).

During exercise, we observed a higher blood lactate concentration during PEMF stimulation, without any negative effects on execution of exercise, seeing all cyclists completed 6-minutes trial. PEMF could affected glycolytic metabolism of type-II muscular fibers and the energetic system, especially glucose utilization and activity of metabolic enzymes, as succinate dehydrogenase and malate dehydrogenase (Sakurai et al. 2004; J. Yang et al. 2018). Also, a likely modulation of NO signal induced by PEMF, probably led to a change in contractile properties and metabolic and/or vascular control in fast-twitch human muscle fibers (Breese et al. 2013; Ferguson et al. 2013).

In the first study, type-II muscular fibers were strongly involved during exercise, because the intensity was severe, over ventilatory threshold, needing to a higher

recruitment of these fibers. In order to clarify if exercise intensity, linked to fibers recruitment, influenced effect of PEMF on muscle activity, in this second study we investigated stimulation during a moderate constant-load exercise, in order to evaluated PEMF at aerobic intensity, with a poorly recruitment of fast-twitch muscle fibers. Although, the increased muscle activation observed during warm-up, suggests that stimulation could be alike effective, even when only type-I muscle fibers are recruited.

We did not observe variations on pulmonary VO_2 kinetics during stimulation, for each of its components (phase II and slow component). We were not totally surprised, because PEMF might act only at the local level, whereas the maximal cardiac output limits the muscle blood flow and O_2 delivery to muscles, during a high intensity constant-load exercise that involves large muscle mass (i.e., cycling). Microvascular O_2 delivery as a constraint to muscle O_2 utilization and VO_2 kinetics is likely limited primarily by the rate of adjustment of O_2 delivery within the tissues. The fact that changes at the muscle level, were not accompanied by changes of the VO_2 on-kinetics on transition, is the proof that a metabolic mechanism independent of O_2, limits oxidative phosphorylation at the onset of exercise (Grassi 2001).

An accelerated oxygen release at muscle level, likely reflect the direct influence of a increased metabolism and/or the recruitment of type-II muscle fibers at the onset of severe exercise (DiMenna et al. 2010). VO_2 kinetics, seems to be linked to intracellular mechanisms controlling the rate of adjustment for oxidative phosphorylation and to a mismatch between tissue O_2 distribution utilization, that requires a progressively greater reliance on O_2 extraction for a given relative VO_2 (Murias et al. 2011).

We observed a change and a faster kinetic of HHb concentration during PEMF stimulation, leading to a reduced time delay of the HHb slow component. During exercise, stimulation increased the velocity and the quantity of muscle O_2 available, together enhanced muscle O_2 extraction. PEMF likely enhanced microcirculation and vascularization, through an increase in arteriole diameter (Smith, Wong-Gibbons, and Maultsby 2004). Also, we hypothesize an interaction between PEMF and

Ca_{2+}/NO/cGMP/PKG, leading to a modulation of nitric oxide (NO) signal, which facilitates the release of O_2 on muscle during contraction by enhancing vascularization (Diniz, Soejima, and Ito 2002; Pall 2013).

Moreover, it has been showed that NO supplementation (i.e., beetroot juice), speeds up muscle HHb kinetics during the transition from moderate to severe exercise intensity (Breese et al. 2013). We did not observe changes for tissue oxygenation index (TOI) during. Different studies have established the incidence of a good correlation between the HHb signal recorded with NIRS and the fractional O_2 extraction in animals and in exercising human muscles, and the concept is further strengthened only if the TOI remains constant. This is because TOI includes HbO_2, which is more significantly influenced by skin blood flow, occurring for thermoregulatory reasons due to exercise with respect to HHb (Grassi and Quaresima 2016). In order to quantify the magnitude of PEMF stimulation on pulmunar oxygen uptake and muscle oxygenation, in this second study we collected the mean value of VO_2, HHb and Hb during a moderate constant-load exercise. Also, we evaluated stimulation in a very different group from athletes, as sedentary people, in order to investigated how individual anthropometric features, especially fat mass, could affected PEMF treatment. Indeed, the conductivity of the electromagnetic field (EM) signal inside the body, is affected by the conductivity of specific tissue, that influenced by its water content. Tissues with high water content, like skeletal muscles, lead to higher conductivity of EM signal than fat mass (Lepelaars 1996).

Materials and Method

The study design was a single-blind, randomized controlled trial. Nine male sedentary young people participated at this study. Features of each participants are shown in the table below. All subjects were volunteers, healthy, non-smokers and none of them were taking medications or supplements. None of the subjects reported physical deficit or

muscular injury at the time of the study. All participants received a verbal explanation of experimental procedures, and informed consent was obtained before the beginning of recordings. The experimental protocol was approved by the Institutional Ethic Committee of the University of Bologna. The experiments were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Subjects	Age (years)	VO_2 max (ml/min/kg)	Workload (watt)	Weight (kg)	Height (cm)	BMI
L.B	32	33,9	108	76,0	175	24,8
E.M	30	31,9	113	72,0	180	22,2
A.T	34	33,2	100	77,0	177	24,6
S.B	34	33,3	108	87,0	178	27,5
M.S	23	34,4	88	62,0	170	21,5
A.A	20	33,9	85	67,5	173	22,6
S.P	25	39,5	105	64,0	164	23,8
E.B	35	30,5	83	79,0	173	26,4
S.I	32	35,3	106	74,0	168	26,2
Mean	29,4	34,0	99,6	73,2	173,1	24,4
SD	5,4	2,5	11,3	7,8	5,1	2,1
SEM	1,8	0,8	3,8	2,6	1,7	0,7

Table 4 Features of each participants of the study.

Experiments were performed on the same cycle-ergometer (H-300-R Lode, Exere Air Machine, Italy) as previous study, under a standardized procedure, in a quiet room with a stable and comfortable temperature (22°C), at the same time of the day (9:00-12:00 AM) to avoid circadian influence. Before starting each recording, subjects performed

10 minutes of free warm-up and 5 minutes of rest. Subjects were asked to avoid drinking caffeinated beverages before the experimental procedures and were instructed to avoid strenuous activity and alcohol in the 12h preceding the test. Pulmonary VO_2 and expired gas were collected with Quark b^2 breath-by-breath metabolic system (Cosmed, s.r.l. Rome, Italy) and the system was calibrated immediately before each recording in accordance with the manufacturer's guidelines. As in the previous study, we recorded EMG activity from the Vastus Medialis and Biceps Femoris caput longum of the right leg. EMG data were recorder data sampling rate of 250 Hz by a Free-EMG 1000 (BTS Bioengineering, Inc.). The same two circular 20 cm PEMF loop-antenna devices (Torino II, Rio Grande Neurosciences, USA) were positioned on the right leg in the same place of previous study, in order to stimulate the entire thigh.

Near-infrared spectroscopy (NIRS, NIMO, Nirox s.r.l. Italy) was used to recorded muscle oxygenation. To assess the influence of PEMF stimulation on muscle oxygenation, we recorded hemoglobin (Hb) and deoxyhemoglobin (HHb) values, with a continuous wave with a sampling rate set at 40 Hz. During trials, the probe was firmly placed in the same place as previous study, on the right vastus lateralis (VL) and held with two adhesive belt and a band of Velcro straps. Probe was covered with a black cloth, due to prevent contamination from ambient light and elastic strappings were placed around. Before starting each trial, we measured skinfold thickness of vastus lateralis by a caliper (Holtain), in order to do fat layer correction. Subjects came our laboratory five times, with three days between each visit, in which we performed different recordings. In the first day we recorded maximum voluntary contraction (MVC) session and an incremental test. MVC of RBF and RVM was used to normalize electromyographic data. EMG activity was normalized to the peak of the MVC.

As in previous study, each subject performed for 5 seconds, an isometric contraction against a maximum load using isotonic machines (Exere Air Machine, Italy). They repeated the same procedure 3 times separated by 2 minutes of rest and we assume the maximum peak value, due to determine the MVC of each muscle investigated. Then

we recorded an incremental test on a cycle-ergometer, necessary to individualize workload for the succeeding recording sessions, through the detection of maximal oxygen consumption (VO_2 max). Each subject cycled on a cycle-ergometer at 45 Watt for 5 minutes (warm-up), followed by workload at 60 watt and instantaneous increase of 15 Watt every 1 minute, at a cadence of 70 RPM, until the volitional exhaustion. Heart rate was monitored by Polar Heart Rate Monitor (Polar Electro, Finland). The same criteria as in the previous study, were used to determine test duration and the detection of VO_2 max. The workload for each athlete was set to ~50% of VO_2 max, in order to achieve a moderate and aerobic intensity of exercise, for the succeeding recording sessions. Then, subjects came to our laboratory for four more recordings in which they performed 30-minutes of moderate constant-load exercise, were PEMF stimulation was continuously active (phase ON) for one half of the exercise (15 minutes) and inactive (phase OFF) for the other half (15 minutes). Each subject performed four times the same trial, where stimulation was activated for two times in the first half of exercise (TRIAL ON-OFF) and for two in the second (TRIAL OFF-ON), in the random order and never been informed subjects about the status of stimulation.

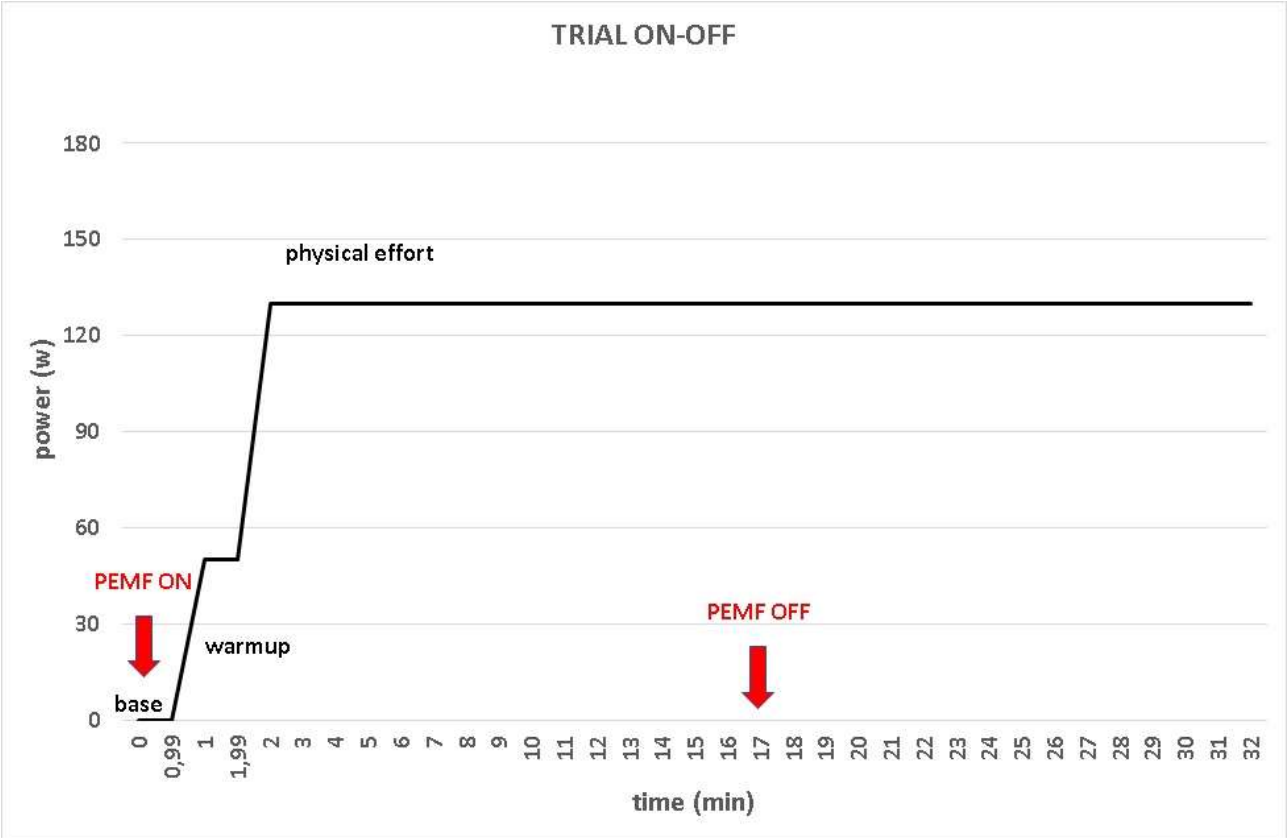

Figure 15. To facilitate the understanding of the trial, figure shows a typical ON-OFF trial: PEMF were activated at the beginning of the baseline (base) until the 17th minute (15th min of physical effort); at this time, PEMF were deactivated until the end. On the other way, in trial OFF-ON PEMF were inactivated from the beginning of the baseline (base) until the 17th minute (15th min of physical effort); at this time, PEMF were activated until the end.

The two PEMF loops were positioned on the right leg in both experimental conditions, because the stimulation was not perceived by the subjects at the cutaneous level (single blind trial). Athlete started the recording session, with 1-minute standstill sitting on cycle-ergometer with extended right leg in order to recorded baseline values. Then, subjects cycled 1-minute of unloaded cycling, as the warm-up, followed by an instantaneous increase of the individualized workload, which was attained in ~3s. Subjects were instructed to keep cycling at a cadence of 70 RPM, for the entire duration of the trial. Each trial was ended automatically at the 30 minutes. In each trial (OFF-ON and ON-OFF), EMG, VO_2, Hb and HHb data were collected at the baseline (standstill sitting), on warm-up (unloaded cycling) and during constant-load exercise, in each of experimental conditions (phase ON and phase OFF).

We also measured basal lactate before the beginning of each recording (baseline) and lactate concentration during exercise, for each of experimental conditions (Phase ON and Phase OFF). A blood sample was performed from the right ear lobe, at the seventh minute and a half and at the twenty-second minute and a half, of constant-load exercise, corresponding to the middle of each experimental conditions phase.

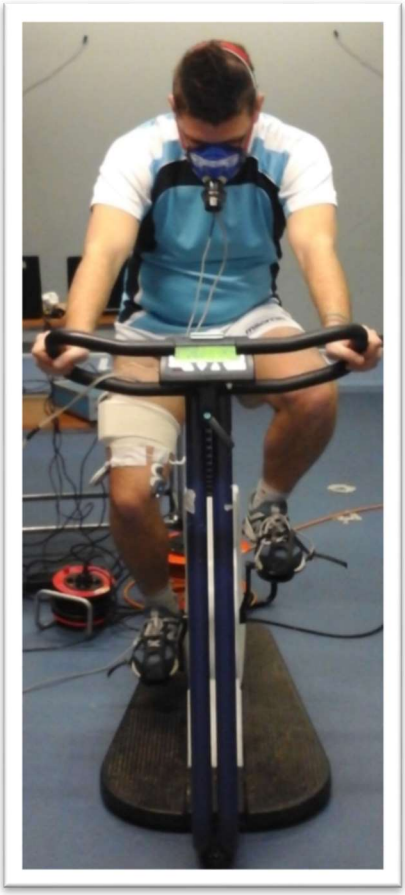

Figure 16. A typical subject involved in a constant-load exercise.

Data Analysis

The response of muscular activity over the entire trial was recorded by surface EMG and assessed measuring the root mean square (RMS) normalized to the peak of the MVC. We averaged the values of baseline, warm-up and exercise, for each condition (phase ON and phase OFF) in each muscle (RBF and RVM). The signals were

positively rectified and band-pass filtered (Butterworth, 20–450 Hz) using SMART analyzer (BTS Bioengineering Inc.). The software EMG easy report 6.03.8 (Merlo Bioengineering, Italy) was used for EMG traces, on data process and artefact removal (see description in study I).

Then, the values recorded were normalized to the peak of the MVC. The normalized RMS values were calculated in 100 ms bin from EMG signals using MATLAB (The MathWorks). For all variables recorded, we averaged the values of all subjects in each experimental condition (PEMF ON vs PEMF OFF). Then, we computed means and standard errors across groups. We compared mean values of muscular activity pre-exercise (baseline, warm-up) and on exercise, within trial and between trials. A 2 (muscles, RVM and RBF) x 2 (conditions, PEMF ON and PEMF OFF) repeated measures ANOVA was performed on each parameter (baseline, warm-up, constant-load exercise) separately. Effect sizes were calculated using partial eta squared (η^2_p), and means were considered significantly different at $p<0.05$.

Breath-by-breath VO_2 values obtained in the several repetitions of the same constant-load exercise were time aligned, interpolated on a second-by-second basis and then superimposed for each subject. Then, we averaged VO_2 values every second and used it for analysis.

The same procedure was followed for Hb and HHb values. The baseline and warm-up of Hb, HHb and VO_2, was defined as the mean value measured 30 seconds before transition phase. We measure lactate concentration at the baseline and during exercise, in both experimental conditions (phase ON and Phase OFF). A blood sample was performed from the right ear lobe before the beginning of the trial, at seventh minute and a half, and twenty-second minute and a half, of the constant-load exercise. For all variables recorded (Hb, HHb, VO_2, lactate and EMG activity for each investigated muscles), we averaged the values of all subjects, in each trial (ON-OFF and OFF-ON) and across experimental condition (phase ON vs phase OFF). Then, we computed

means and standard errors across groups. We compared mean values of all variables investigated for baseline, warm-up and exercise, in each experimental condition within trial and between trials.

Statistical analysis

All data are represented as mean ± SEM. Values were compared with paired sample t-test. Means were considered significantly different at $p < 0.05$. To determine the magnitude of the effect of PEMF stimulation, effect sizes (ES) were calculated as the mean difference standardized by the between subject standard deviation and interpreted according to the thresholds (Hopkins et al. 2009): <0.20; small, >0.20-0.60; moderate, >0.60-1.20; large, >1.20-2.00; very large,>2.00-4.00; extremely large, >4.00. Data were analyzed with SPSS v22.0 (IBM, New York, NY, USA).

Results

EMG activity were assessed comparing the RMS normalized to the peak of the MVC.

Analysis showed significance for muscles main effect RVM and RBF ($F_{1,7}$=187.214; p< 0.001; η^2_p=0.728), parameter main effect, as baseline, warm-up, exercise ($F_{4,4}$=14.082; p< 0.001; η^2_p=0.446), condition main effect, as PEMF ON vs PEMF OFF ($F_{1,7}$=33.987; p=0.014; η^2_p=0.327), muscles for parameter main effect ($F_{4,4}$=18.140; p< 0.001; η^2_p=0.509), muscles for condition main effect, as PEMF ON vs PEMF OFF ($F_{1,7}$=6.287; p=0.014; η^2_p=0.820).

A greater amplitude on RVM in comparison to RBF, was found during constant load exercise, in both experimental conditions, within and between trial (Trial OFF-ON: PEMF OFF: RVM= 13.92 ±1.33; RBF= 8.61±1.87; PEMF ON: RVM=21.77±1.53; RBF= 13.41±2.14); (Trial ON-OFF: PEMF OFF: RVM= 14.27 ±1.11; RBF= 8.22±1.52; PEMF ON: RVM=18.92±1.28; RBF= 9.82±1.15).

Analysis for muscle activity at the baseline, did not show any differences across conditions (OFF vs ON), for each investigated muscle (RVM and RBF). However, we noted that RVM activity tended to be higher when PEMF stimulation was active, (OFF=1.12±0.27 vs ON=2.34±0.69; p=0.058). Significant difference was found during warm-up, for each investigated muscle (RVM and RBF), between condition OFF vs ON, with PEMF ON exhibited higher significant RMS value of both muscles with respect to PEMF OFF condition (RVM ON=7.38±1.23; RVM OFF=3.84±0.53; $t_{(7)} = -2.32$; p=0.027; ES 0.55-moderate; RBF ON=9.77±0.98; RBF OFF=4.12±0.60; $t_{(7)} = -4.22$; p=0.002; ES 0.77-large).

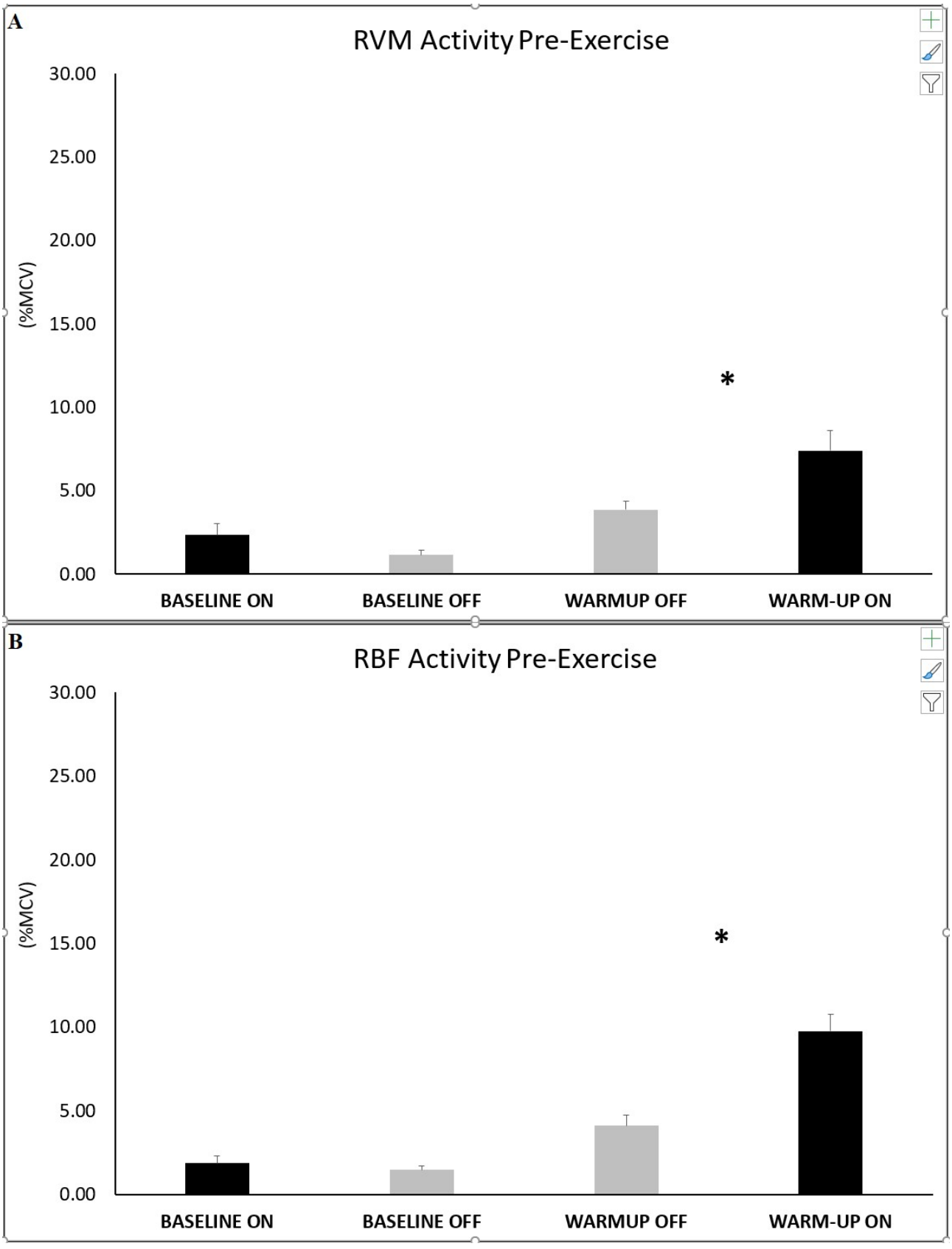

Figure 17. Histograms represent the root mean square (RMS) of the normalized EMG values (mean ± SEM) of the RVM (A) and RBF (B) at the baseline and during warm-up, across experimental conditions (ON vs OFF). Asterisks indicate significant differences at $p<0.05$.

Analysis during constant-load exercise within trials, showed significant differences for RVM activity across condition (PEMF OFF vs PEMF ON), in both trial (OFF-ON and ON-OFF). The amplitude of muscle activity was higher in phase ON related to phase OFF, in trial OFF-ON ($t_{(7)}$ =-16.10 p<0.001; ES=0.70-large) and trial ON-OFF ($t_{(7)}$ =-18.11 p<0.001; ES=0.57-moderate).

Analysis for RBF activity showed difference across condition (PEMF OFF vs PEMF ON) in trial OFF-ON. Stimulation caused a change of the muscular activation, with greater amplitude on PEMF ON condition ($t_{(7)}$ =-7.10 p<0.001; ES=0.39-moderate).

Analysis during exercise between trials, showed differences for RVM activity across conditions (phase ON vs phase OFF), in both first ($t_{(7)}$ =-8.61 p<0.001; ES=0.56-moderate) and second half ($t_{(7)}$ =-11.29 p<0.001; ES=0.70-large) of each trial. Muscle activity was higher in ON condition related to OFF.

Analysis for RBF activity showed differences across conditions (phase ON vs phase OFF), in the second half of the trial. Amplitude of muscle activity was higher in phase ON than OFF ($t_{(7)}$ =-2.88 p=0.012; ES=0.44-moderate), due to active PEMF stimulation.

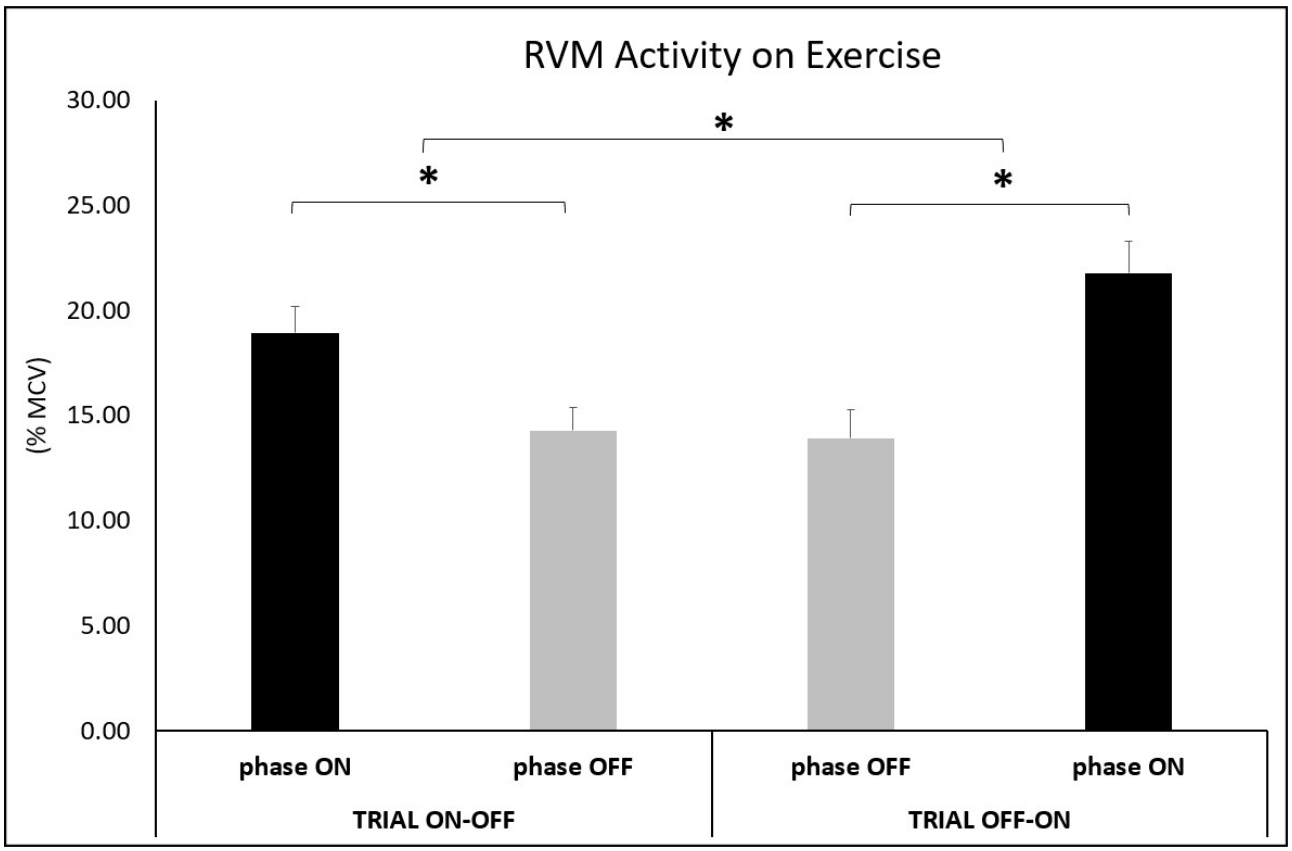

Figure 18. Histograms represent the root mean square (RMS) of the normalized EMG values of the RVM during constant-load exercise, across experimental conditions (phase ON vs phase OFF) within trial and between trials. Data are show mean ±SEM. Asterisks indicate significant differences at $p<0.05$.

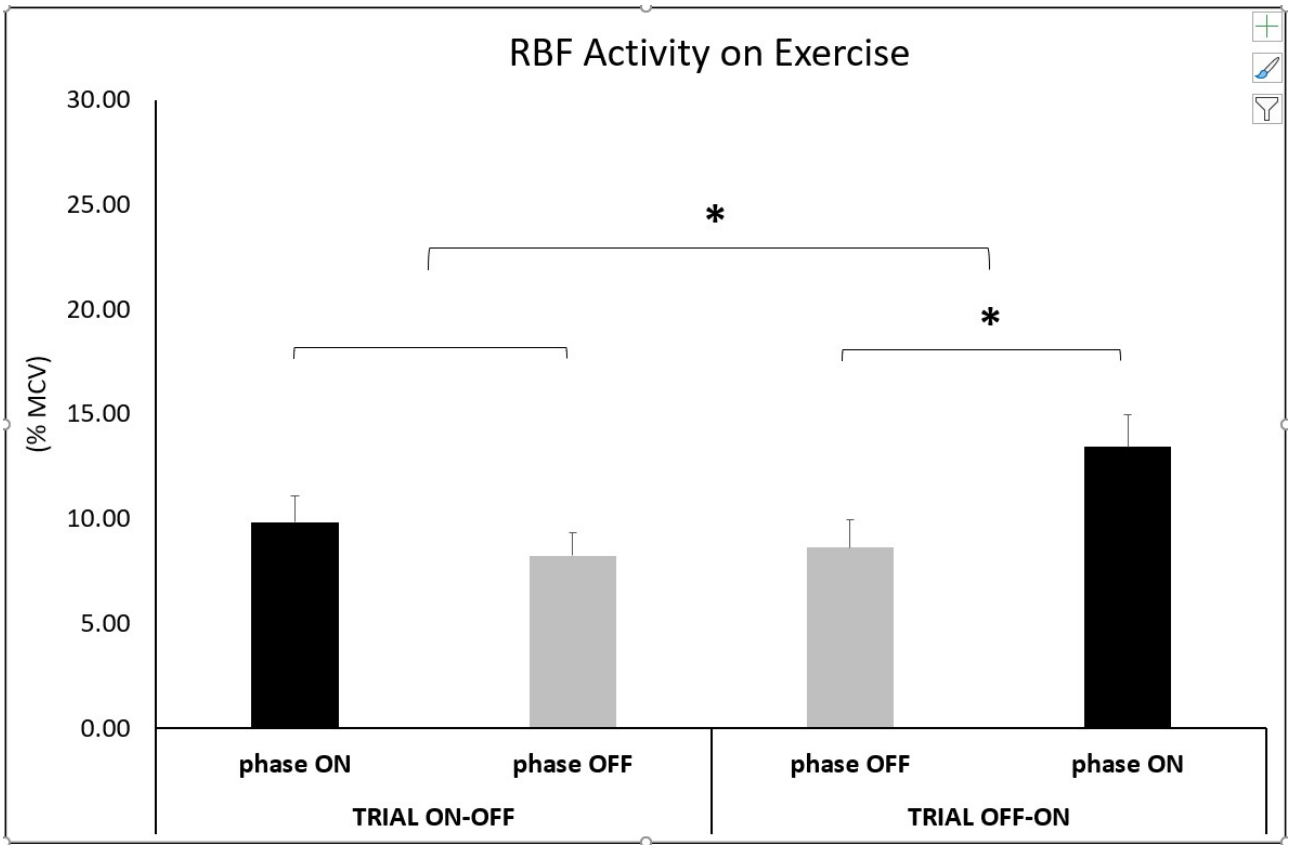

Figure 19. Histograms represent the root mean square (RMS) of the normalized EMG values of the RBF during constant-load exercise, across experimental conditions (phase ON vs phase OFF) within trial and between trials. Data are show mean ±SEM. Asterisks indicate significant differences at $p<0.05$

Analysis for VO_2, did not show any significant differences across conditions (ON vs OFF) at baseline and during warm-up.

During constant-load exercise, analysis showed significant differences across conditions (phase ON vs phase OFF) within each trial. Oxygen consummation was higher in the second half of exercise, independently from the experimental condition (Trial ON-OFF: Phase ON= 1511,39 ± 49,03 ml/min; Phase OFF= 1620 ± 58,20 ml/min; $t_{(7)}$=-7.08; p=0.000; ES=0.34-moderate); Trial OFF-ON: Phase OFF= 1525.39 ± 59.85 ml/min; Phase ON= 1664.83 ± 78.32 ml/min; $t_{(7)}$=-5.62; p=0.000; ES=0.33-moderate). Analysis between trials, did not show differences for oxygen uptake across experimental conditions.

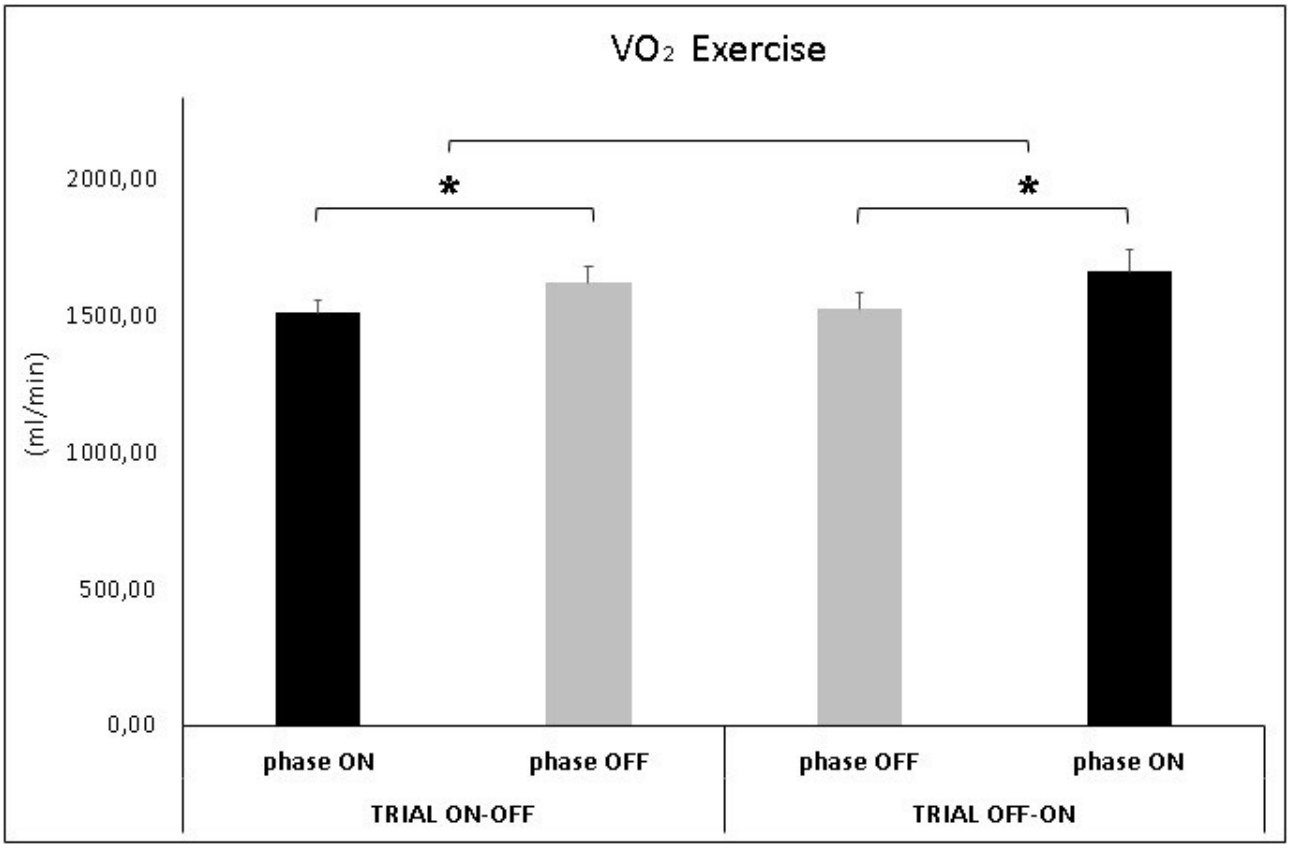

Figure 20. Histograms represent VO2 values during constant-load exercise, across experimental conditions (phase ON vs phase OFF) within trial and between trials. Data are show mean ±SEM. Asterisks indicate significant differences at $p<0.05$

Analysis of muscle oxygenation before constant-load exercise (baseline and warm-up), did not show differences for Hb and HHb values across experimental condition (ON vs OFF).

During exercise, analysis founded significant differences for Hb across conditions (phase ON vs phase OFF) within each trial. Hb value was higher in the second half of constant-load exercise, independently from the experimental condition (Trial ON-OFF: Phase ON= 75.64± 4.31 µM; Phase OFF= 85.95± 4.80 µM; $t_{(7)}$=-4.80 ; p=0.001; ES=0.37-moderate; Trial OFF-ON: Phase OFF= 75.83± 5.68 µM; Phase ON= 86.70 ± 6.18 µM; $t_{(7)}$=-5.81 ; p=0.000; ES= 0.31-moderate). Analysis between trials, did not show significant differences for Hb across experimental conditions.

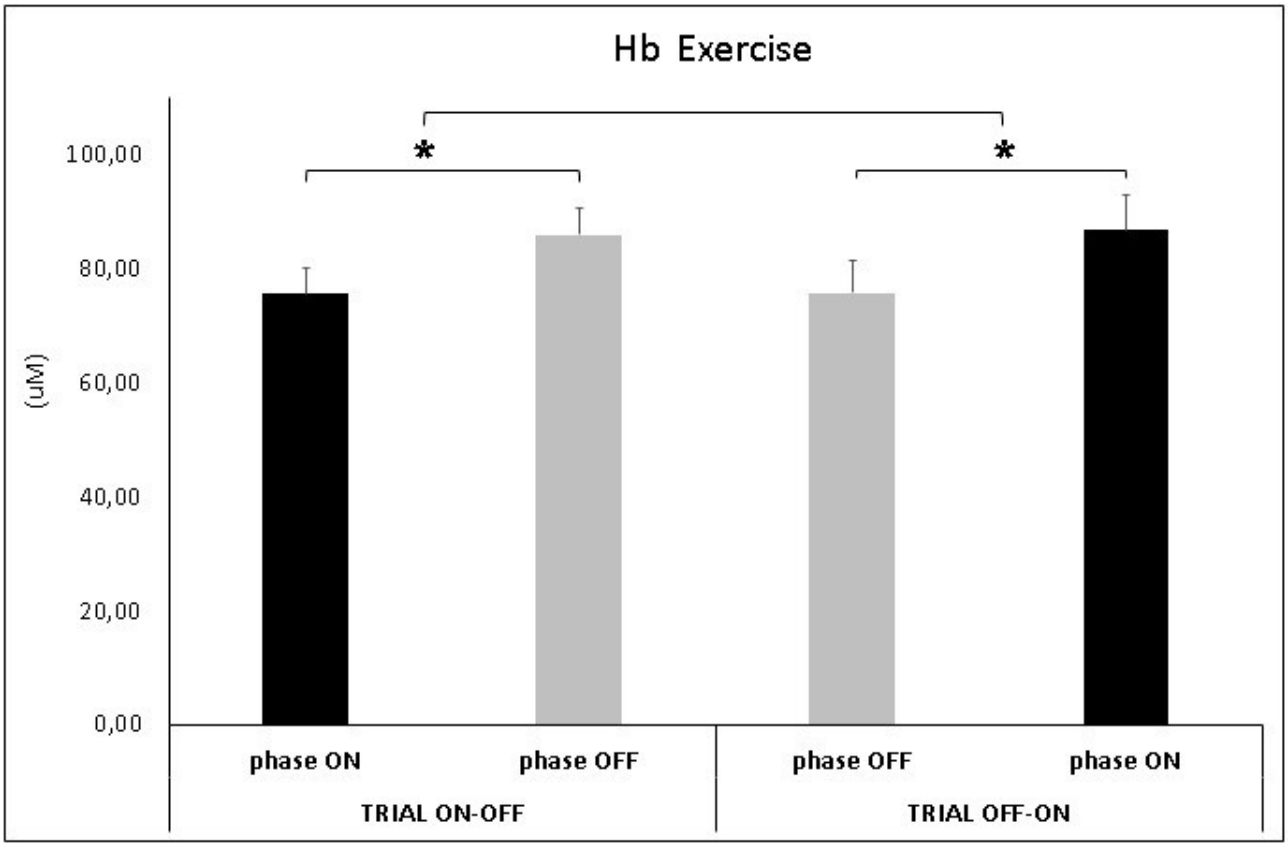

Figure 21. Histograms represent Hb values during constant-load exercise, across experimental conditions (phase ON vs phase OFF) within trial and between trials. Data are show mean ±SEM. Asterisks indicate significant differences at $p<0.05$

For HHb, analysis showed significant differences during constant-load exercise, within trial ON-OFF. HHb value was higher in phase OFF (33.29 ± 2.55 μM) related to phase ON (32.07 ± 2.44 μM) ($t_{(7)}$=-4.07; p=0.002; ES= 0.09-small). No significances were found in TRIAL OFF-ON, but we noted that HHb values tended to be higher in phase ON (33.96 ± 3.99 μM) related to phase OFF (32.36 ± 2.96 μM) (p=0.164). Analysis between trials, did not show any differences across experimental conditions.

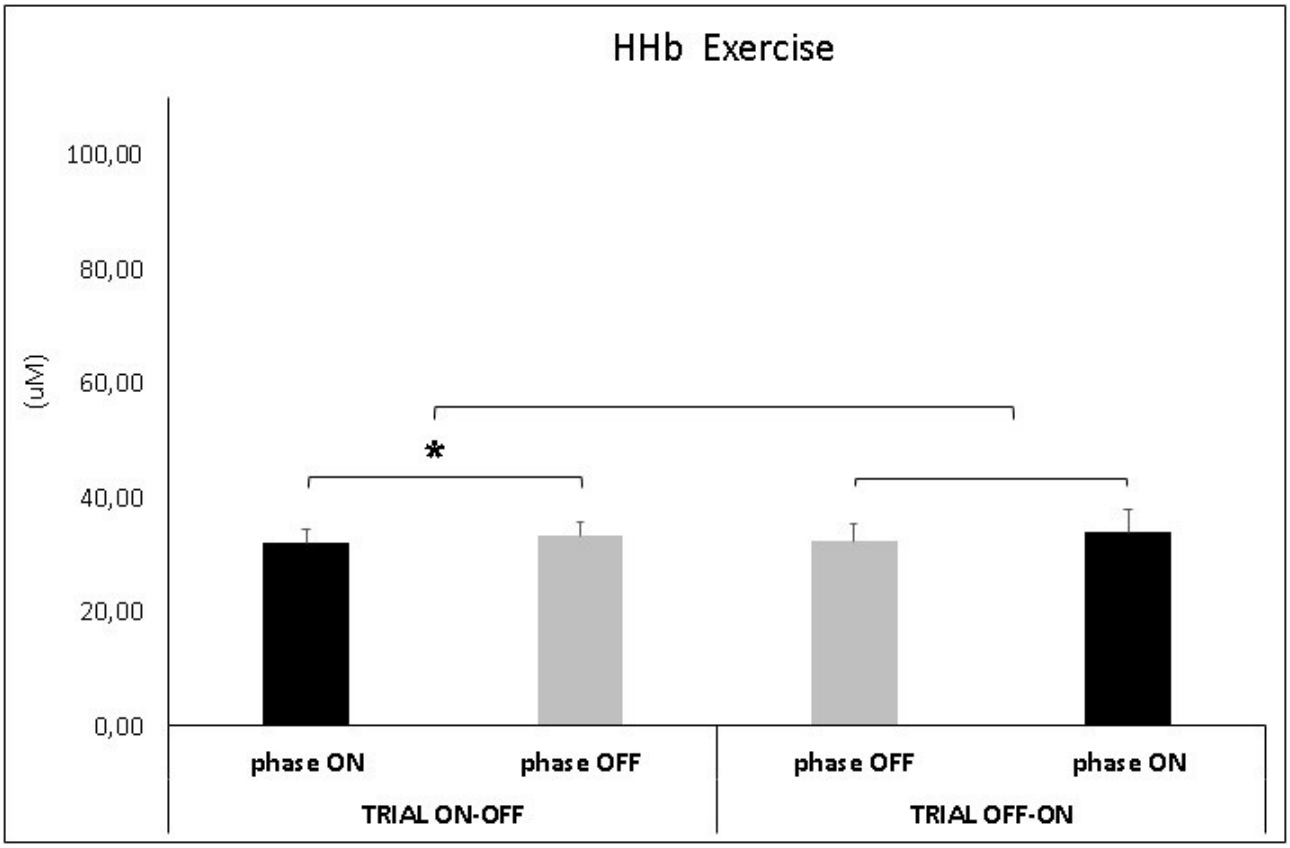

Figure 22. Histograms represent HHb values during constant-load exercise, across experimental conditions (phase ON vs phase OFF) within trial and between trials. Data are show mean ±SEM. Asterisks indicate significant differences at $p<0.05$

Analysis of blood lactate concentration within trial, did not show any significance across conditions (Phase OFF vs Phase ON). Analysis between trials, did not founded differences across experimental conditions. However, we noted that blood lactate in the second half of the trial, tended to be higher in ON condition than OFF (Phase OFF= 4.14±0.49 mmol/L; Phase ON= 4.71 ± 0.39 mmol/L; p=0.051).

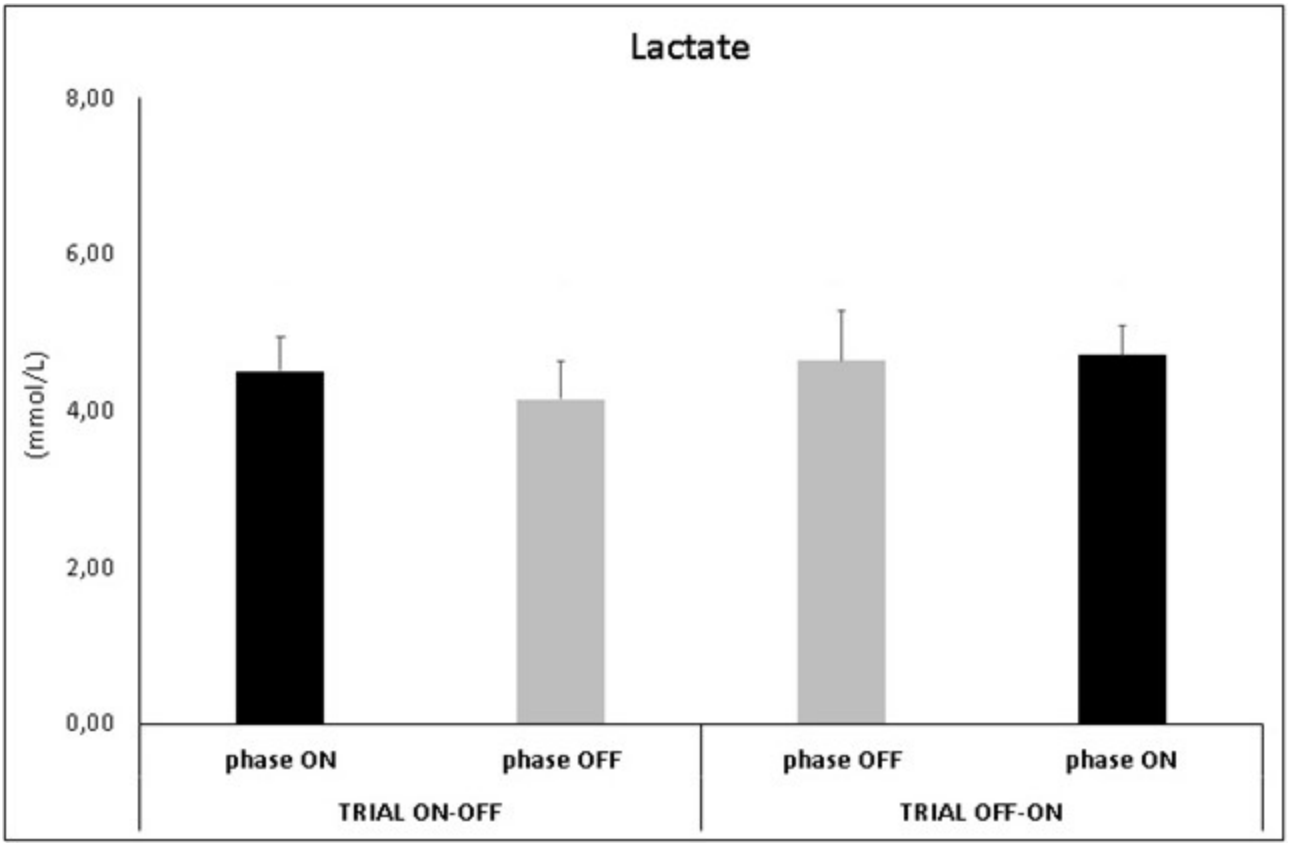

Figure 23. Histograms represent Lactate values during constant-load exercise, across experimental conditions (phase ON vs phase OFF) within trial and between trials. Data are show mean ±SEM. Asterisks indicate significant differences at $p<0.05$

Study 3. Effect of PEMF stimulation on muscle activity, blood lactate concentration and heart rate variability, during a squat.

Design of the study

This study was designed based on the results obtained in the previous studies. Indeed, PEMF stimulation increased the amplitude of muscle activity in both athletes and sedentary people, under different exercise intensity. Likely, PEMF stimulation increased magnitude activity of muscle fibers, in both type-I and type-II. On warm-up, in both semi-professional cyclist and sedentary people, during moderate constant-load exercise, in sedentary people, where physical effort was respectively light and moderate and fast-twitch muscle fibers were poorly recruited, PEMF stimulation strongly enhanced activity of type-I muscle fibers.

In athletes, during heavy constant-load exercise, where physical effort was severe and fast-twitch muscle fibers were massively recruited, PEMF stimulation likely boosted activity of type-II muscular fibers, as showed by an increase in blood lactate concentration.

These results confirm that the increase in the amplitude of the muscular response induced by PEMF, was likely caused by the effect of PEMF on contraction mechanism of muscular fibers through a change of membrane permeability and Ca^{2+} channel conduction, enhancing ion flux and cellular concentration (Pakhomov et al. 2009; Ross et al. 2015; Zhang et al. 2010; Kuan-Jung Li et al. 2006). Further, PEMF stimulation could amplify signal Ca2+ mediators and and Ca-dependent pathways (Panagopoulos, Karabarbounis, and Margaritis 2002), probably through changes in phospholipids of plasma membrane, that improve production of second messengers, with starting cascade of multiple intracellular signal transduction (Semenov, Xiao, and Pakhomov 2013; Tolstykh et al. 2013; Pilla et al. 2011).

Differently from athletes, in sedentary people PEMF stimulation did not raise blood lactate concentration during constant-load exercise, and this seems consistent with the

intensity of the exercise and the type of muscle fibers recruited. In fact, whereas in semi-professional cyclists exercise intensity was severe, needing to a higher recruitment of type-II muscle fibers, in sedentary people intensity was moderate and therefore, did not need a massive recruitment of fast-twitch muscle fibers. So this poor activation of type-II muscle fibers, did not allow PEMF to fully boosting their glycolytic metabolism, leading to increased blood lactate during constant-load exercise.

In addition to the influence on muscle contraction mechanisms, we hypothesized a potential mechanism of PEMF stimulation, in enhancing glycolytic metabolism of fast-twitch muscle fibers, together to the influence on nitric oxide signal, that led to a change in ccontractile properties and metabolic and/or vascular control in these muscle fibers (Breese et al. 2013; Ferguson et al. 2013). Indeed, it has been hypothesized that PEMF affected energetic metabolism, especially glucose utilization. In rats with streptozotocin-induced diabetic muscle atrophy, PEMF treatment affected metabolic enzymes in the quadriceps, with increased succinate dehydrogenase (SDH) and malate dehydrogenase activity (MDH), thus suggesting an increase in the metabolic capacity of muscle. Further, PEMF reduced blood glucose amount and increased serum insulin level. (J. Yang et al. 2018). In insulinoma cells, exposure to PEMF attenuated insulin secretion, suggesting effects on calcium channels and ions flux (Sakurai et al. 2004).

Thus, in order to evaluated how PEMF stimulation could affected the energetic system inside muscular fibers, especially in type-II, in this third study we investigated stimulation in weightlifters, during a heavy exercise that fully involved fast-twitch fibers. Also, considering that the greater blood lactate concentration induced by PEMF, did not affected execution and duration of exercise in athletes, we investigated effected of stimulation on rating of perceived exertion (RPE), in order to evaluated if a likely increase in blood lactate, affected the performance or increased fatigue.

Lastly, we proposed to evaluated effect of stimulation on heart rate variability (HRV) during exercise and recovery, to clarify if PEMF could affected autonomic system.

Grote et al. (2007) showed that twenty minutes of exposure on lower frequency of PEMF, accelerated recovery of HRV, with more rapidly returned to initial sympathetic tone, suggesting a possible influence of short-term PEMF stimulation on autonomic system. Despite that question is still controversial, and more evidence are yet necessary. Probably, the basal autonomic tone seems play a crucial role together to the power of electromagnetic signal.

Materials and Method

The study design was a single-blind, randomized controlled trial. Nine subjects, three females and six men participated at this study. All participants were active people, with extensive weight-lifting and squat experience. All subjects were volunteers, healthy, non-smokers and none of them were taking medications or supplements. None of the subjects reported physical deficit or muscular injury at the time of the study. All participants received a verbal explanation of experimental procedures, and informed consent was obtained before the beginning of recordings. The experimental protocol was approved by the Institutional Ethic Committee of the University of Bologna. The experiments were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Features of each participants are shown in the table below.

Subject	Age (years)	Weight (kg)	Height (cm)	BMI	Workload 75%-1RM (kg)	Sex
V.C	29	52	164	19,3	45	F
C.L	33	66	161	25,5	45	F
A.B	26	65	170	22,5	48	F
R.S	20	76,5	174	25,3	70	M
R.C	30	77	178	24,3	95	M
L.L.B	29	95	178	30,0	100	M
R.C	29	85	170	29,4	80	M
M.E	30	83	188	23,5	75	M
A.P	40	72	173	24,1	70	M
Mean	29,6	74,6	172,9	24,9	69,8	
SD	5,3	12,7	8,1	3,3	20,6	
SEM	1,8	4,2	2,7	1,1	6,9	

Table 5. Features of each participants of the study.

Experiments were performed under a standardized procedure, in a quiet room with a stable and comfortable temperature (22°C), at the same time of the day (9:00-12:00 AM) to avoid circadian influence. Before starting each recording, subjects performed 10 minutes of free warm-up and 5 minutes of rest. Subjects performed personal exercises for the joint mobility and muscle flexibility, for lower and upper body, to getting ready for the squat. Subjects were asked to avoid drinking caffeinated beverages before the experimental procedures and were instructed to avoid strenuous activity and alcohol in the 12h preceding the test.

In order to stimulate the entire thigh, the two circular 20 cm PEMF loop-antenna devices of previous studies (Torino II, Rio Grande Neurosciences, USA), were positioned on the right leg, at beginning and ending of the thigh. As in the previous study, we recorded EMG activity from the Vastus Medialis and Biceps Femoris caput longum of the right leg. EMG data were recorder data sampling rate of 1000 Hz by a Free-EMG 1000 (BTS Bioengineering, Inc.). In order to removed PEMF artifacts on EMG traces, we utilized EMG easy report (Vinti et al. 2018; Campanini et al. 2020; Mazzoli et al. 2018) 6.03.7 (Merlobioengineering, Italy) using Wavelet filters, for automatically removal of large and frequent artifacts (Merlo, Farina, and Merletti 2003). The same two PEMF loop-antenna devices (Torino II, Rio Grande Neurosciences, USA) were positioned in the same place as previous study, on the right leg at beginning and ending of the thigh. We recorded EMG activity over the entire exercise and during the first 5 minutes of rest, for each of experimental conditions (ON and OFF).

In order to evaluated heart rate variability (HRV) as in a previous study (Grote et al. 2007), we used a validate (Giles, Draper, and Neil 2016; Caminal et al. 2018) portable heart rate monitor (V800, Polar, Finland) to recording R-R intervals, which is the time between successive heartbeats. We recorded heart rate variability before the beginning of each recording (baseline), over the entire phase of exercise and during the first 5 minutes of rest. Recording were performed in both activated (ON) and deactivated (OFF) PEMF stimulation.

We collected lactate concentration in PEMF ON and OFF conditions. We measured lactate before the beginning of each recorder (baseline), immediately at the end of exercise and at the end of rest, which lasted 15 minutes. A blood sample was performed from the right ear lobe. We also measured rating of perceived exertion (RPE) with the Borg CR-10 Scale (Arney et al. 2019) immediately at the end of exercise, for each of experimental conditions (ON and OFF).

Participants came to our laboratory five times, with three days between each visit, in which we performed different recordings. In the first day we recorded maximum voluntary contraction (MVC) of RBF and RVM to normalize electromyographic data. EMG activity was normalized to the peak of the MVC. Then, subjects performed a standardized one repetition maximum (1RM) test protocol, in order to individualize workload for the succeeding recording sessions. (Grgic et al. 2020; Seo et al. 2012)

After the initial session, the subjects came to our laboratory for four more times, in which they performed the same exercise, as a free-body squat, both during active (ON) and deactivated (OFF) PEMF stimulation. The sessions were performed in random order and the participants were never been informed about the status of stimulation. The two PEMF loops were positioned on the right leg in both experimental conditions (ON and OFF), because the stimulation was not perceived by the subjects at the cutaneous level (single blind trial). This study investigated the differences between activated (ON) and deactivated (OFF) PEMF stimulation during the execution of the squat. No constraints were given in the squat execution technique, thus all subjects were free to performed the exercise as they preferred. The only given indication was to maintain the same execution in the four experimental sessions. The exercise consisted in a 4 series of 10 repetitions (4x10) of squat with their 75% of 1RM, and 90 seconds of rest between series. At the end of the exercise, subjects sat on a chair for the entire recovery phase, which lasted 15 minutes.

In each trial across both experimental conditions, data were collected at the baseline, over entire exercise and during recovery phase. EMG activity for RVM and RBF were measured during exercise and in the first 5 minutes of the rest. Blood lactate was collected at the baseline, before started each recording, at the end of exercise and at the end of the rest.

A blood sample was performed from the right ear lobe. We recorded HRV at the baseline, during exercise and in the first 5 minutes of recovery phase. We also

measured the rating of perceived exertion (RPE) with the Borg CR-10 scale, immediately at the end of exercise.

Figure 24. A typical subject involved in a 1-RM test. All experiments were performed at the laboratory of exercise physiology (c/o Record, via del Pilastro 8, Bologna, Italy)

Data Analysis

The response of muscular activity over the entire trial was recorded by surface EMG and assessed measuring the root mean square (RMS) normalized to the peak of the MVC. We averaged the values of exercise and phase of recovery (5 minutes), for each condition (ON and OFF) in each investigated muscle (RBF and RVM). The signals were positively rectified and band-pass filtered (Butterworth, 20–450 Hz) using SMART analyzer (BTS Bioengineering Inc.).

The software EMG easy report 6.03.8 (Merlo Bioengineering, Italy) was used for EMG traces, on data process and artifact removal (see description in study I).

Then, the values recorded were normalized to the peak of the MVC. The normalized RMS values were calculated in 100 ms bin from EMG signals using MATLAB (The MathWorks). For all variables recorded, we averaged the values of all subjects in each experimental condition (PEMF ON vs PEMF OFF). Then, we computed means and standard errors across groups. We compared mean values of muscular activity, during exercise and recovery. A 2 (muscles, RVM and RBF) x 2 (conditions, PEMF ON and PEMF OFF) repeated measures ANOVA was performed on each parameter (exercise, recovery) separately. Effect sizes were calculated using partial eta squared ($\eta 2p$), and means were considered significantly different at $p<0.05$.

Heart rate variability was measured through R-R intervals, with portable heart rate monitor (V800, Polar, Finland). We collected values at the baseline before started recording, over the entire exercise and in the first 5 minutes of the rest, in each experimental condition. Blood lactate concentration was measured at the baseline, during exercise and at the rest in both experimental conditions (ON vs OFF).

A blood sample was performed from the right ear lobe, before the starting of trial, at the end of exercise and after 15 minutes, as the end of recovery phase. Rating of perceived exertion (RPE) was collected with the Borg CR-10 scale, at the end of exercise. For all variables recorded, we averaged the values of all subjects in each experimental condition (ON vs OFF). Then, we computed means and standard errors across groups. We compared mean values of all investigated variables for baseline, exercise and recovery in each experimental condition (ON vs OFF).

Statistical analysis

Data are represented as mean ± SEM. Values were compared with paired sample t-test. Means were considered significantly different at $p < 0.05$. To determine the magnitude of the effect of PEMF stimulation, effect sizes (ES) were calculated as the mean difference standardized by the between subject standard deviation and interpreted according to the thresholds (Hopkins et al. 2009): <0.20; small, >0.20-0.60; moderate,

>0.60-1.20; large, >1.20-2.00; very large,>2.00-4.00; extremely large, >4.00. Data were analyzed with SPSS v22.0 (IBM, New York, NY, USA).

Results

EMG activity were assessed comparing the RMS normalized to the peak of the MVC. Analysis for muscle activity during the phase of recovery (rest), showed differences across experimental conditions for RVM activity. During active (ON) PEMF stimulation, the amplitude of basal tone was significantly higher than deactivate (OFF) stimulation (OFF=0.40±0.09 vs ON=1.61±0.58; $t_{(7)}$ = -2.46; p=0.030; ES 0.46-moderate). Analysis for RBF activity did not show any significance across conditions (ON vs OFF). However, we noted that amplitude of basal tone, tended to be higher when PEMF stimulation was active (OFF=0.58±0.14 vs ON=1.54±0.69; p=0.084).

Analysis during exercise, showed significance for muscles main effect RVM and RBF ($F_{1,8}$= 301.898; p< 0.001; η2p=0.950), with a greater activity on RVM in comparison to RBF (mean: RVM= 37.29 ±1.40; RBF=11.32±0.38), in both experimental conditions (PEMF OFF: RVM=38,40±1.98; RBF=11.85±0.53; PEMF ON: RVM=36.19±1.98 ; RBF=10.80±0.53).

We did found any significant differences for condition main effect (PEMF ON vs PEMF OFF) for each investigated muscle (RVM and RBF).

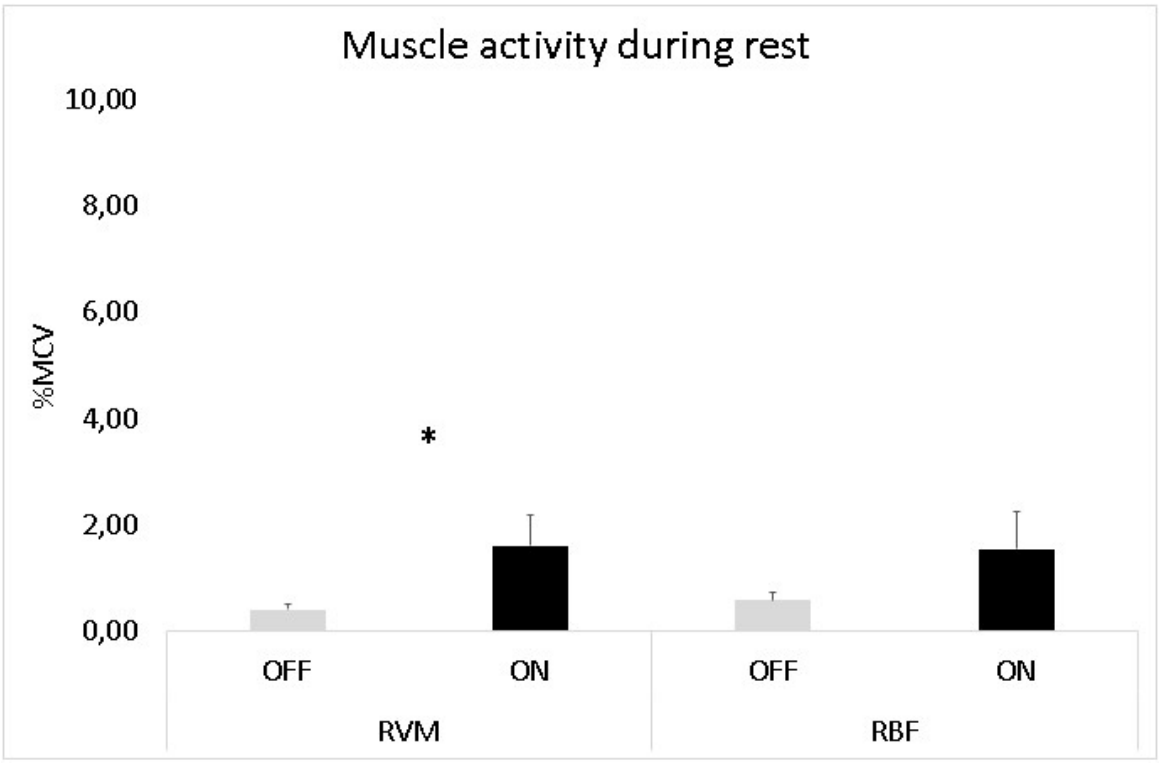

Figure 25. Histograms represent the root mean square (RMS) of the normalized EMG values (mean ± SEM) of the muscle activity for RVM and RBF at rest, across experimental conditions (ON vs OFF). Asterisks indicate significant differences at $p<0.05$

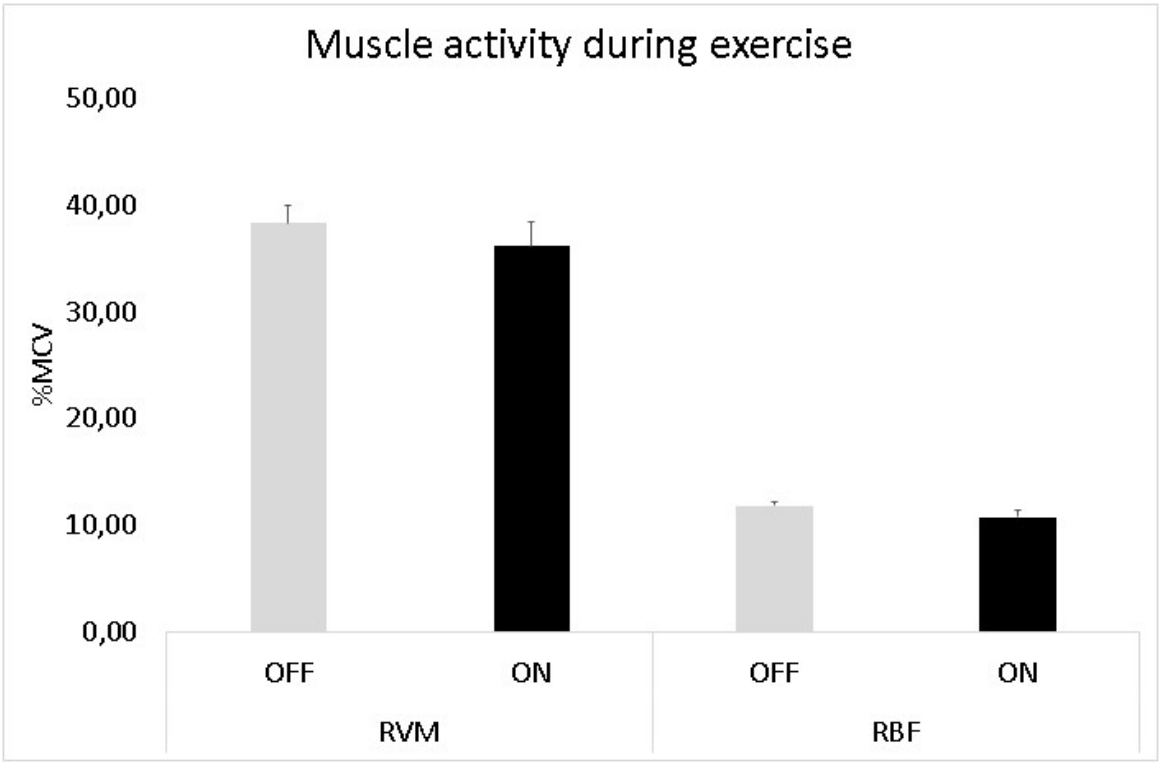

Figure 26. Histograms represent the root mean square (RMS) of the normalized EMG values (mean ± SEM) of the muscle activity for RVM and RBF during exercise, across experimental conditions (ON vs OFF). Asterisks indicate significant differences at $p<0.05$.

Analysis for heart rate variability (HRV), measured through R-R intervals, did not show any significant differences across experimental conditions (ON vs OFF), at baseline, on exercise and during the first 5 minutes of the phase of recovery (rest).

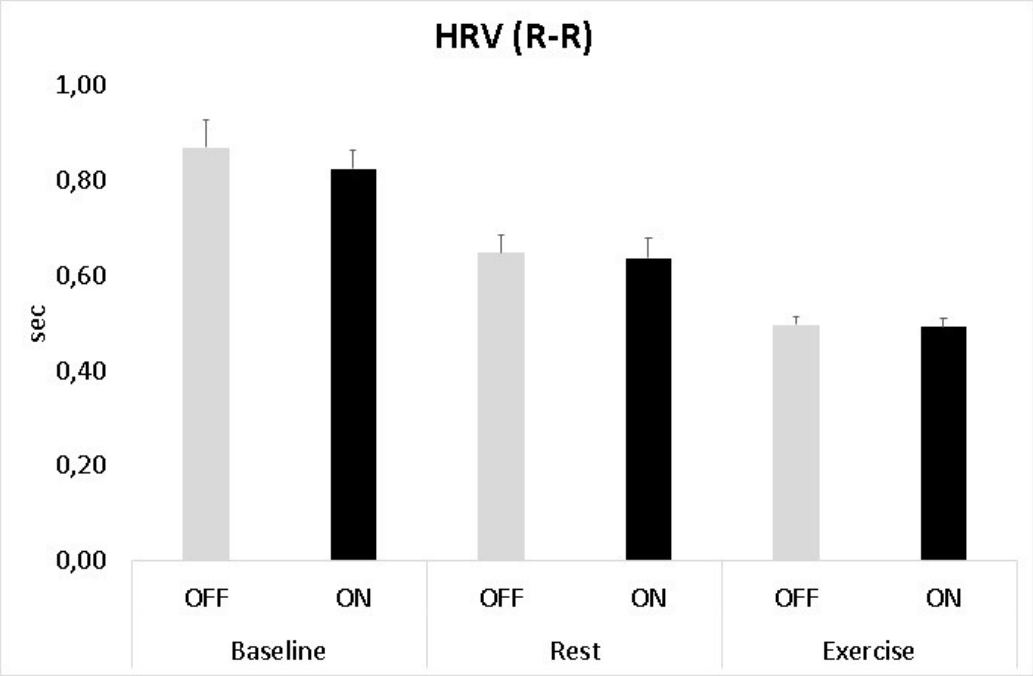

Figure 27. Histograms represent the values (mean ± SEM) of heart rate variability (HRV), measured through R-R intervals at baseline, on exercise and during the phase of recovery (rest), across experimental conditions (ON vs OFF). Asterisks indicate significant differences at $p<0.05$.

Analysis for blood lactate concentration, showed significant differences during exercise across experimental conditions (ON vs OFF). When PEMF stimulation was active (ON) lactate was higher than deactivate (OFF) stimulation (OFF=4.5±0.4 vs ON=5.0±0.4; $t_{(8)} = -2.03$; p=0.038; ES 0.22-moderate). No significances were found for recovery phase (rest) across conditions. However, we noted that when PEMF stimulation was active (ON), blood lactate at the end of rest (15 minutes) tended to be higher than deactivate (OFF) stimulation (OFF=3.9±0.4 vs ON=4.3±0.4; p=0.070).

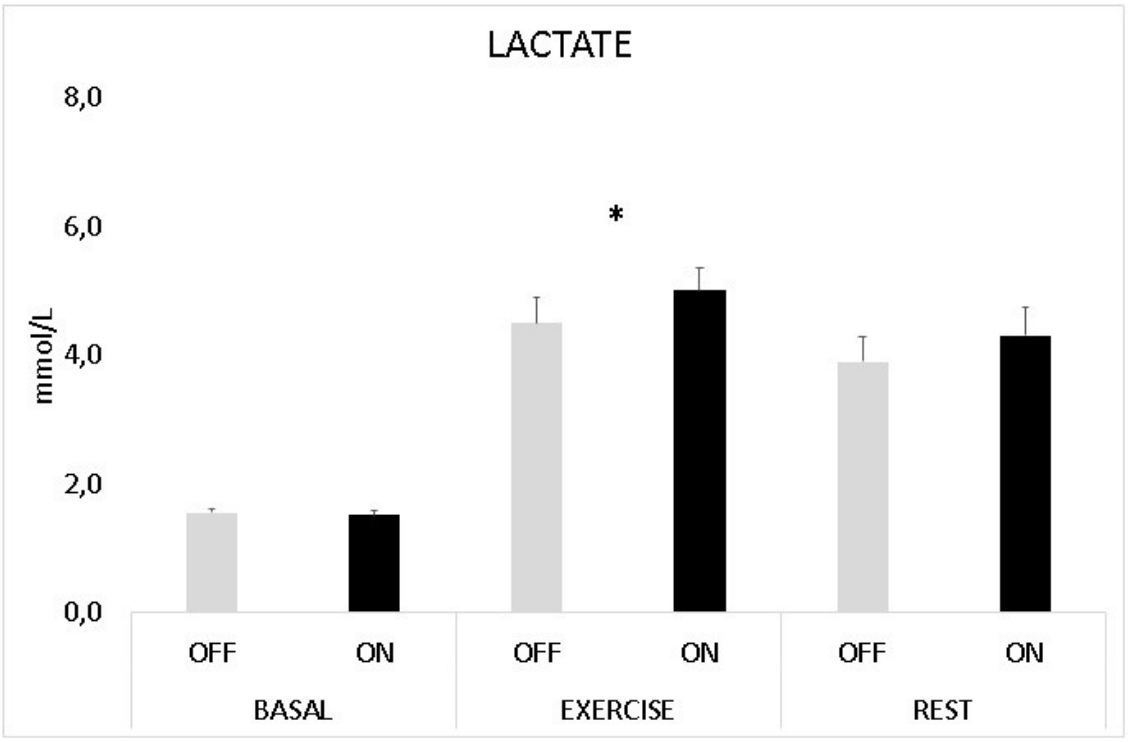

Figure 28. Histograms represent the values (mean ± SEM) of blood lactate at baseline, on exercise and during the phase of recovery (rest), across experimental conditions (ON vs OFF). Asterisks indicate significant differences at $p<0.05$.

Analysis for Rating of perceived exertion (RPE) collected with the Borg CR-10 at the end of exercise, did not show any significant differences, across experimental conditions (ON vs OFF).

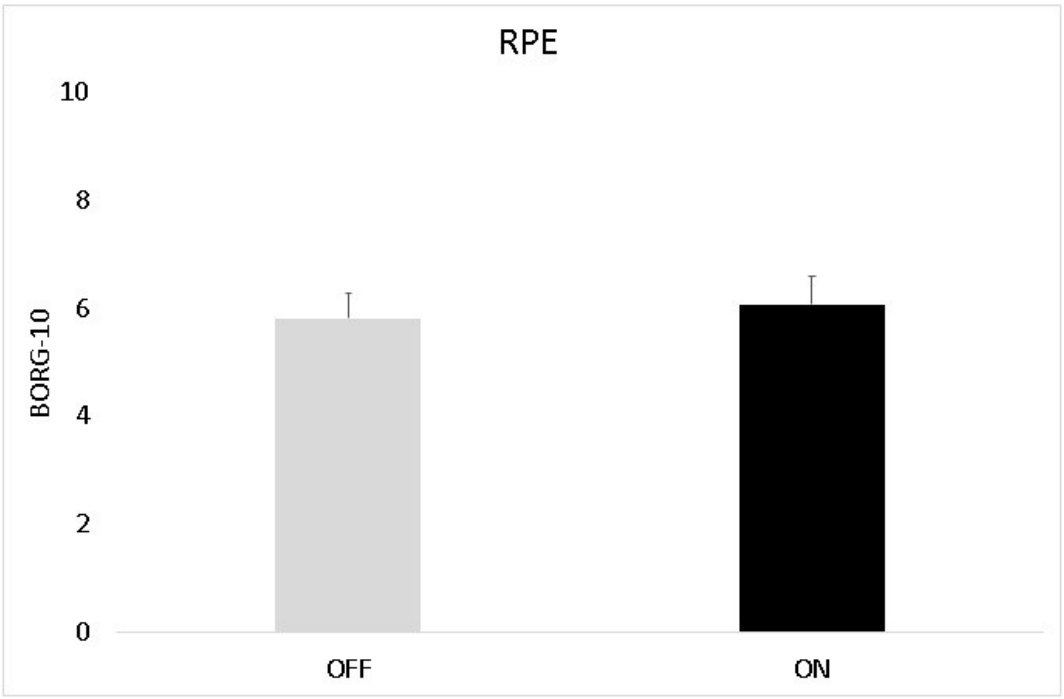

Figure 29. Histograms represent the values (mean ± SEM) of Rating of perceived exertion collected with the Borg CR-10, across experimental conditions (ON vs OFF). Asterisks indicate significant differences at $p<0.05$

Discussion

Effect of PEMF on muscle oxygenation

In semi-professional athletes, we observed a tendency for a greater change and a faster kinetic of HHb concentration during active PEMF stimulation. During heavy constant-load exercise, PEMF increased the velocity and the quantity of muscle O_2 available, leading to accelerate the HHb kinetics. Stimulation induced a bulk muscle O_2 availability and a greater muscle O_2 extraction, leading to a reduced time delay of the HHb slow component. PEMF stimulation enhanced microcirculation and vascularization probably through an increase in arteriole diameter (Smith, Wong-Gibbons, and Maultsby 2004). The amplitude of the HHb primary components is related to muscle O_2 release and homogeneity of perfusion. It has been hypothesized that the interaction between stimulation and Ca_{2+}/NO/cGMP/PKG could lead to a modulation of nitric oxide (NO) signal which facilitates the release of O_2 on muscle during contraction by enhancing vascularization (Diniz, Soejima, and Ito 2002; Pall 2013). As postulated by Fick's law of diffusion, the oxygen pressure in the microvasculature (i.e., muscle PO_2mv) is the driving force for blood–myocyte O_2 transfer. During metabolic demand, muscle PO_2mv on-kinetics is determined by the dynamic matching between O_2 delivery and utilization (i.e., QO_2/VO_2 ratio) (B J Behnke et al. 2001).

Changes in nitric oxide levels affect muscle PO_2mv during transitions of metabolic demand, suggesting that increased NO-mediated function could, at least in part, increase muscle microvascular oxygenation in trainers (Hirai et al. 2012). Cocksedge et al. (2020) founded that supplement rich of NO_3^- (i.e., beetroot juice), did not affected exercise tolerance, TOI and VO_2 kinetics, when severe intensity exercise occurred in normoxia as in this study (instead of hypoxia, e.g., exercise in altitude).

In sedentary people, PEMF stimulation did not affect muscle oxygenation during warm-up or moderate constant-load exercise. Analysis did not find differences in HHb

values during warm-up and during exercise, despite we found significant effect within trial ON-OFF with higher values in OFF condition. However, such effect was small (ES= 0.09-small) and it appeared to be more related to the fatigue of physical effort of keeping the workload until the end. Within trial OFF-ON, we noted a tendency for a greater HHb values even in the second half of the trial, in ON condition (p=0.164) and analysis between trials did not show significance across conditions.

Also, analysis for Hb during exercise seemed to confirm that the changes in muscle oxygenation, were more related to the fatigue of physical effort, than to experimental condition. Indeed, analysis within trials showed that Hb values were significant higher ever in the second half of the trial, independently from condition. Analysis between trials, did not show differences across experimental conditions, as well as for Hb values during warm-up.

A possible explanation for the lack of effect of PEMF stimulation in sedentary people, could be the different conductivity of the electromagnetic (EM) field signal inside the body. As showed by Lepelaars (1996), the conductivity of tissue at high frequencies of stimulation is affected by its water content. Tissues with high water content, like skeletal muscles, lead to higher conductivity of EM signal than fat mass. Also, the dispersion of signal lead to a retardation and attenuation of electromagnetic fields. We hypothesize that the higher fat mass of sedentary people (BMI 24.4±0.7) than athletes (BMI 22.05± 0.6), could affected the conductivity of PEMF signal inside thigh, both during warm-up and exercise, decreasing the effect of stimulation. Another possible explanation, is related to the intensity of exercise and the type of muscle fibers recruited. Based on previous hypothesis, the interaction between PEMF and Ca_{2+}/NO/cGMP/PKG led to a modulation of NO signal, that enhanced vascularization and facilitated the release of O_2 on muscle. Breese et al. (2013) showed that NO_3^- changes contractile properties and metabolic and/or vascular control in type II muscle fibers.

Nitric oxide supplementation (i.e., beetroot juice), could affected metabolic and/or vascular control in fast-twitch human muscle fibers, in accordance with previous research in rodents (Ferguson et al. 2013). NO supplementation during severe intensity exercise, seems to be related to the level of muscle deoxygenation sustained during such intensity. Moreover, it has been showed that NO supplementation (i.e., beetroot juice), speeds up muscle HHb kinetics during the transition from moderate to severe exercise intensity (Breese et al. 2013). The exercise conducted at severe but not moderate intensity, leads to a higher recruitment of type II muscle fibers, as it was for athletes but not for sedentary people in our studies. Probably, the effect of PEMF stimulation on muscle oxygenation, could be affected by exercise intensity (maybe heavy or more), linked to muscle fibers recruited (maybe type-II).

Effect of PEMF on VO_2

PEMF did not affect both the pulmonary VO_2 kinetics in athletes and pulmonary oxygen uptake values in sedentary people. In athletes, the variations at the muscle level were not accompanied by changes of the VO_2 on-kinetics on transition, with the stimulation that was not sufficiently enough to speed up phase II with the decrease in the slow component. This is due to the maximal cardiac output limits the muscle blood flow and O_2 delivery to muscles, during a high intensity constant-load exercise, that involves large muscle mass (i.e., cycling), whereas PEMF might act only at the local level.

The absence of faster kinetics for VO_2 under conditions where O_2 transport has been increased, is proof that a metabolic mechanism independent of O_2, limits oxidative phosphorylation at the onset of exercise (Grassi 2001). Also, in isolated canine muscle, the removal of a potential oxygen diffusion limitation within the periphery, or a faster modification of blood flow, did not accelerate the VO_2 kinetic response as showed by Grassi et al. (1998). Since we observed an accelerated oxygen release at muscle level,

the reason for the slower primary phase of VO_2 on-kinetics, likely reflect the direct influence of an increased metabolism and/or the recruitment of higher order muscle fibers (type II) at the onset of severe exercise (DiMenna et al. 2010). These fibers, have a higher oxygen cost of contraction and a slower VO_2 on-kinetics, related to the type I muscle fibers smaller and more oxidative.

This could also explain the slow VO_2 response. These data support that a slower VO_2 kinetics, may be linked not only to intracellular mechanisms controlling the rate of adjustment for oxidative phosphorylation, but also related to a mismatch between tissue O_2 distribution utilization, that requires a progressively greater reliance on O_2 extraction for a given relative VO_2 (Murias et al. 2011). According to Murias et al. (2011), Our results suggest that when τs is less than 20 s, oxygen delivery and utilization are well matched, so additional provision of O_2 may not result in more quickly VO_2 kinetics. Indeed, it has been showed that when the rate of adjustment in pulmonary VO_2 on-kinetics was reduced to become similar to the rate of adjustment of HHb on-kinetics, no further speeding of VO_2 kinetics was found with further training (Murias, Kowalchuk, and Peterson 2010). Further, these values support a role for microvascular O_2 delivery as a constraint to muscle O_2 utilization, meaning that there could be a point beyond which VO_2 kinetics is limited primarily by the rate of adjustment of O_2 delivery within the tissues. Moreover, an acceleration of the time course of the matching of muscle O2 delivery to O2 utilization, can speed the rate of adjustment when time constant is longer than 20 s. However, when time constant is less than 20 s, it would appear that the adjustment of VO2 is primarily governed by intracellular mechanisms (Murias et al. 2011).

PEMF stimulation could modulate NO signal, and nitric oxide represents an important component of the metabolic inertia to the VO_2 dynamics during severe intensity exercise. It is not yet clear which specific mechanism by nitric oxide contributes to the metabolic inertia at exercise onset. However, an in vitro study showed that nitric oxide competes with oxygen at mitochondrial level (Wilkerson, Campbell, and Jones 2004).

We hypothesize that intracellular features could slow the VO_2 on-kinetics to the level detected in the present study, although our results cannot discriminate between metabolic and O_2 delivery factors which reduce VO_2 on-kinetics.

Effect of PEMF on blood lactate

In athletes and weightlifters, PEMF stimulation caused an increase of blood lactate concentration during exercise, confirming a possible influence of stimulation on muscle activity and on glycolytic metabolism of type-II muscular fibers (Sakurai et al. 2004). This effect could be caused by the change of membrane permeability and Ca^{2+} channel conduction, enhancing ion flux and cellular concentration (Ross et al. 2015) that improve contraction mechanisms during exercise, together to the likely influence of PEMF to NO signal, that probably led to a change in contractile properties and metabolic and/or vascular control in type II muscle fibers, as discussed above. In addition to contraction mechanisms, it is possible to hypothesize that PEMF stimulation affected the energetic system inside muscular fiber, especially glucose utilization. In rats with streptozotocin-induced diabetic muscle atrophy (J. Yang et al. 2018), chronic PEMF treatment affected metabolic enzymes in the quadriceps, with increased succinate dehydrogenase (SDH) and malate dehydrogenase activity (MDH), thus suggesting an increase in the metabolic capacity of muscle.

Further, it has been found that PEMF treatment reduced blood glucose amount and increased serum insulin level. In insulinoma cells, exposure to PEMF attenuated insulin secretion, suggesting effects on calcium channels and ions flux (Sakurai et al. 2004). The high values of lactate recorded on exercise during PEMF stimulation, in both cyclist and weightlifters, suggest a potential mechanism of microstimulation in enhancing contraction mechanism of type-II muscular fibers and boosting their glycolytic metabolism. In both cyclist and in weightlifters, the higher value of blood lactate concentration induced by stimulation, had no negative effects on execution of exercise. Indeed, all cyclists completed the 6-minutes of the trial and weightlifters

showed no difference in rating of perceived exertion (RPE). Lactate production is essential to protect muscle from metabolic acidosis and delay muscle fatigue during heavy physical exercise, preventing the impairment of exercise performance (Robergs, Ghiasvand, and Parker 2004).

Lactate consumes two protons and by definition, retards acidosis by facilitating proton removal from muscles. Lactate is crucial to prevent pyruvate accumulation and supply muscles production of cytosolic NAD^+ based on continued ATP regeneration from glycolysis (Robergs, Ghiasvand, and Parker 2004). The high-intensity exercise used in both athletes and weightlifters' study, with high increases in power during physical effort, led to a faster reduction in intramuscular pH, suggesting that PEMF stimulation promoted type II fiber metabolism and lactate production to delay metabolic acidosis.

In sedentary people, we did not find differences between the experimental conditions and this result, seems to be consistent with the intensity of the exercise and the type of muscle fibers recruited. In fact, the intensity was moderate and therefore did not need a massive recruitment of type-II muscle fibers. So, this poor activation, did not allow PEMF to fully enhanced their glycolytic metabolism, increasing blood lactate as observed in the other two studies, where exercise intensity was severe, needing a massive involvement of fast-twitch muscle fibers.

Effect of PEMF on muscle activity

In all our studies, PEMF stimulation increased the amplitude of muscle activity under different conditions. In semi-professional cyclists, we observed an increase of overall activity for each investigated muscle during warm-up. In sedentary people, we founded a greater muscle activity during warm-up in ON condition for each investigated muscle. During moderate constant-load exercise, PEMF stimulation increased the magnitude of muscle activity of both vastus medialis and bicep femoris. In

weightlifters, we observed a higher muscular basal tone activity for RVM and RBF, induced by PEMF stimulation during recovery phase.

A tendency for a higher muscular basal tone during PEMF stimulation, was also noted in sedentary people for RVM activity, when subjects standstill sitting on cycle-ergometer with extended right leg (baseline). The increase in the magnitude of the muscular response induced by stimulation was likely caused by the effect of PEMF on contraction mechanism of muscular fibers, both type-I and II. PEMF stimulation increased amplitude of muscle fibers activity, according to exercise intensity and muscle fibers recruited. During light physical effort, as warm-up in our studies, or during moderate constant-load exercise, stimulation increased overall activity of type-I muscle fibers. PEMF enhanced basal tone activity of these fibers, during the stationary recovery or the standstill sitting. During heavy physical effort, as severe constant load exercise in semi-professional cyclists, PEMF stimulation likely boosted the glycolytic metabolism of type-II muscle fibers. A possible explanation for this effect, arises from the change of membrane permeability and Ca^{2+} channel conduction, enhancing ion flux and cellular concentration (Pakhomov et al. 2009; Ross et al. 2015), leading to improve contraction mechanisms.

The mechanism of muscular contraction, who are affected by Ca2+ channels and ions flux and PEMF stimulation, could affected calcium channels and raise Ca2+ intracellular concentration (Zhang et al. 2010; Kuan-Jung Li et al. 2006). Also, the role of stimulation to amplified signal Ca2+ mediators and Ca2+-dependent pathways has been showed in several studies (Panagopoulos, Karabarbounis, and Margaritis 2002). It was suppose an influence of PEMF to phospholipids of plasma membrane, that improve production of second messengers with starting cascade of multiple intracellular signal transduction (Semenov, Xiao, and Pakhomov 2013; Tolstykh et al. 2013; Pilla et al. 2011). In weightlifters, we did not observed differences for muscle activity during exercise for each investigated muscle. Probably, the higher muscle activation during squat covered the effect of stimulation.

In fact, RVM and RBF were always in tension without a complete released phase (as in cycling), in order to keep the weight of the barbell on the back and ensured balance and stability, controlling postural oscillations and avoided imbalances. Or the dosage of stimulation was not sufficiently to increase even over the amplitude of muscle activity during exercise. Despite this, the higher blood lactate concentration recorded on exercise during stimulation indicate an effect of PEMF on muscle activity, especially on contraction mechanism and glycolytic metabolism of type-II muscular fibers strongly involved during exercise. In athletes, the analysis of muscle activity of RVM and RBF during exercise showed a significantly higher activity for vastus medialis related to bicep femoris.

This result is not surprising given that the effective role of vastus medialis during cycling is well-known, but the role of bicep femoris is still under discussion. The magnitude of the bicep femoris is more affected by fatigue, pedaling rate, coordination/activation timing (angle), training status, shoe-pedal interface and body position (Hug and Dorel 2009). Bicep femoris, is a bi-articular muscle involved in knee flexion and hip extension and seems to be more important for energy transfer between joints during cycling rather than to supply main force (Hug and Dorel 2009). One of the largest activity and an earliest activation of bicep femoris seem to be related to increased fatigue in both vastus lateralis and medialis as a consequence of modified coordination and activation patterns (Hug and Dorel 2009). In the present study protocol, the workload was instantaneous and strongly near to maximal causing an immediate and large response of the main muscles of cycling, such as vastus medialis, causing a rapid increment of its muscular activity. Thus, the bicep femoris increased its activity later, upon arrival of fatigue in the vastus medialis.

Conclusion

Until now, no studies have investigated the application of pulsed electromagnetic fields on pulmonary O_2 uptake, muscle oxygenation, and muscular activity during physical exercise.

Results of the present research show a possible application of PEMF in sport disciplines or physical activity, in order to enhance the muscle oxygenation and the amplitude of muscular activity, in response to physical effort, especially related to the time of exercise. Based on the present results, stimulation affect both type-I and type-II muscular fibers, therefore could be used in both moderate and severe exercise, as well as during light physical effort, in order to increase overall muscular response.

PEMF stimulation on muscle activity, seems alike effective in both athletes and sedentary people. Also, the augmented basal tone in stationary recovery or standstill sitting, in addition to the higher muscle activity observed during warm-up, suggest a possible application of PEMF on preparatory activity before training or competition in different sports (e.g., performance of jump or sprint), in order to raise the magnitude of muscular activation. And more, PEMF stimulation could also be applied in support to older-age people or subjects with a breakable muscle-joint structure, in order to increase overall muscular response during light exercise.

PEMF applied during severe exercise enhanced muscle oxygenation through an increase of muscle O_2 supply, suggesting potential applications during severe training. Indeed, stimulation, could be employed during hard work-out sessions, in order to *i)* augment O_2 availability and fast-twitch fibers activity, *ii)* enhancing performance through a better aerobic efficiency, *iii)* improving muscular response, and *iv)* increasing the benefits of training program.

In sport activity, an increase in muscle O_2 availability during work-out session induced by PEMF, could enhance training programs, especially of endurance disciplines. Also,

PEMF stimulation could be employed to augment the amplitude of muscular activity during hard work-out sessions to improve muscular response to heavy workload and increase the benefits of the exercise program. And more, PEMF could be used during warm-up, in order to raise the amplitude of muscular responses during the preparatory activity of different performance like jump, shot or sprint. Or also, an augmented muscle O_2 uptake after work-out could improve recovery phase and shorten recovery time between training session.

Our results suggest that PEMF application can stimulate the rate of muscle O_2 extraction and utilization. These changes at the muscle level were not matched by an improvement in the predictable estimators of endurance performance, such as changes in the VO_2 on-kinetics (speeding up of the primary phase and decrease in the slow component) or the lower pulmunar oxygen uptake for the same workload. In fact, the effect of PEMF on blood circulation are still unclear and further investigation is needed, such as the accurate parameters of stimulation (e.g. time and frequency) and exercise protocols. To our knowledge, this is the first study that assessed PEMF stimulation on pulmonary O_2 uptake, during physical exercise.

Differently from Grote et al. (2007), in our study PEMF stimulation did not affect HRV on exercise and during recovery phase. Probably, the different frequency of used stimulation (higher in our study) led to a different result confirming that the influence of PEMF on HRV and on autonomic system could be more affected by the power of electromagnetic signal, as well as the individual basal autonomic tone.
PEMF could be used during physical activity for multiple purposes, not only in sport activity. It could be important to investigate PEMF treatment in patients and older adults, whose daily mobility and life quality are limited by an impairment in O_2 delivery and utilization. These people could have greater benefits from therapeutic strategies that increase their O_2 availability, a valuable aim for forthcoming investigations. Indeed, an increase in muscle O_2 availability and amplitude of muscular

activity induced by PEMF stimulation, could improve rehabilitation programs after injuries or hospitalizations. Also, stimulation could aid during exercise at low aerobic intensity, people with cardiocirculatory or metabolic disease, like metabolic syndrome or pulmonary obstructions, in order to encourage the adherence to physical activity programs.

Nevertheless, PEMF stimulation could facilitate exercise in people with fragile muscles and joints (e.g., osteoporosis, metabolic syndrome, post-operative rehabilitation), in order to boost the results of training program, even a worthy goal for future studies.

This research suggests that PEMF could affect the energetic system inside muscular fibers, especially glycolytic metabolism of type II muscle fibers. Likely, stimulation could influence glucose utilization, through an augment in SDH and MDH activity, increasing in the metabolic capacity of muscle. It is also possible that PEMF treatment could affect blood glucose and insulin secretion suggesting a possible investigation in diabetic people during an adapted training program.

To the best of our knowledge, this is the first research investigating PEMF stimulation during heavy and moderate exercise in both athletes and sedentary people. We believe that these first observations could open new horizons in the field of sport or physical activity. Further studies are necessary to elucidate the stimulation parameters and exercise protocol necessary to make more effective and efficient PEMF stimulation on human subjects.

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