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Muscular pre-conditioning using light-emitting diode therapy (LEDT) for high-intensity exercise: a randomized double-blind placebo-controlled trial with a single elite runner

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Abstract

Recently, low-level laser (light) therapy (LLLT) has been used to improve muscle performance. This study aimed to evaluate the effectiveness of near-infrared light-emitting diode therapy

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(LEDT) and its mechanisms of action to improve muscle performance in an elite athlete. The kinetics of oxygen uptake (VO_2), blood and urine markers of muscle damage (creatine kinase – CK and alanine) and fatigue (lactate) were analyzed. Additionally, some metabolic parameters were assessed in urine using proton nuclear magnetic resonance spectroscopy (^{1}H NMR). A LED cluster with 50 LEDs (λ = 850 nm; 50mW 15 s; 37.5 J) was applied on legs, arms and trunk muscles of a single runner athlete 5 min before a high-intense constant workload running exercise on treadmill. The athlete received either Placebo-1-LEDT; Placebo-2-LEDT; or Effective-LEDT in a randomized double-blind placebo-controlled trial with washout period of 7 d between each test. LEDT improved the speed of the muscular VO_2 adaptation (~−9 s), decreased O_2 deficit (~ −10 L), increased the VO_2 from the slow component phase (~+348 ml min^{-1}) and increased the time limit of exercise (~+589 s). LEDT decreased blood and urine markers of muscle damage and fatigue (CK, alanine and lactate levels). The results suggest that a muscular pre-conditioning regimen using LEDT before intense exercises could modulate metabolic and renal function to achieve better performance.

Keywords

Fatigue; LEDT; LLLT; muscle damage; NMR; oxygen uptake; photobiomodulation

Introduction

Low-level laser (light) therapy (LLLT), also known as photobiomodulation, has been known since 1967 and uses visible or near-infrared light to promote tissue healing, and reduce pain and inflammation (Chow, Johnson, Lopes-Martin, and Bjordal, 2009; Enwemeka et al, 2004; Huang, Sharma, Carroll, and Hamblin, 2011). LLLT can be delivered by different light sources such as laser diodes and LEDs (light-emitting diodes; Huang, Sharma, Carroll, and Hamblin, 2011). These light sources are different in monochromaticity and coherence since diode lasers are more coherent, have a tiny spectral bandwidth and promote less divergence of the light beams compared to the light emitted by LEDs (Huang, Chen, Carroll, and Hamblin, 2009). However, lasers and LEDs are considered to produce equivalent effects on the tissue if the dose of light delivered/applied is in accordance with the possible biphasic dose–response, previously reported (Enwemeka, 2005; Huang, Chen, Carroll, and Hamblin, 2009; Huang, Sharma, Carroll, and Hamblin, 2011); and light–tissue interaction depends on light absorption by specific structures in the cells that are known as chromophores (Karu, 2010; Karu and Kolyakov, 2005; Karu, Pyatibrat, and Afanasyeva, 2004; Karu, Pyatibrat, Kolyakov, and Afanasyeva, 2008).

Recently, LLLT and LED therapy (LEDT) have been used to promote better muscle performance when applied to muscles immediately before or after intense exercise (Borsa, Larkin, and True, 2013; Ferraresi, Hamblin, and Parizotto, 2012; Leal-Junior et al, 2013). LLLT and LEDT can stimulate mitochondrial metabolism promoting a higher energy supply in the cells, and especially in skeletal muscles cells (Ferraresi, Hamblin, and Parizotto, 2012; Ferraresi and Parizotto, 2013; Hayworth et al, 2010; Karu, 1999; Silveira et al, 2009). Because mitochondrial metabolism is affected by light therapy, it is expected that oxygen

consumption (VO_2) kinetics would also be affected since the mitochondria use oxygen as a final electron receptor in the electron transport chain (ETC).

Currently, only two studies (Da Silva Alves et al, 2014; De Marchi et al, 2012) have investigated the acute effects of light therapy on VO_2. However, these studies were limited to irradiation of only a few areas of the muscles involved in the exercise. In addition, these studies did not evaluate the kinetics of VO_2, a better way to assess the oxidative adaptation caused by exercise stimulus (Barstow, 1994). Thus, the analysis of VO_2 kinetics may show how efficient the process of oxygen delivery is (by the cardiorespiratory system) and oxygen utilization (by mitochondria). Among the current methods of analysis, exponential VO_2 data modeling seem to provide the best information about the VO_2 response at the onset of exercise (i.e. speed – time constant "τ" and magnitude of the steady state – amplitude "a"; Barstow, Casaburi, and Wasserman, 1993; Rossiter et al, 1999).

Several studies have also reported the effects of light therapy on muscle performance during intense exercise (Borsa, Larkin, and True, 2013; Ferraresi, Hamblin, and Parizotto, 2012; Leal-Junior et al, 2013) but almost all of them have been limited to mainly analyzing markers of muscle fatigue (lactate) and muscle damage (creatine kinase – CK) only in blood (Leal-Junior et al, 2013). However, metabolic analysis based on proton nuclear magnetic resonance spectroscopy (^{1}H NMR) in urine can be more specific and can provide detailed information about energy metabolism (Enea et al, 2010; Pechlivanis et al, 2010). Furthermore, if combined, both analyses (urine and blood) could offer a "balanced" analysis of the production and removal processes of lactate and other metabolites from protein degradation that could show the degree of muscle damage.

Moreover, the majority of these previous studies involving light therapy and muscle performance only applied a limited number of irradiation points on muscles (Borsa, Larkin, and True, 2013; Leal-Junior et al, 2013). However, if the idea is to improve the performance of the entire muscle group, the number of irradiation points should cover all the muscle area involved in that specific exercise (Ferraresi et al, 2011; Ferraresi, Hamblin, and Parizotto, 2012; Ferraresi and Parizotto, 2013).

The present study investigated the acute effects of LEDT on muscular mitochondrial oxidation (through VO_2 kinetics analysis), blood markers of fatigue and damage associated with ^{1}H NMR metabolic analysis in urine. Muscle performance was assessed on a single elite runner who received a muscular pre-conditioning regimen using LEDT before high-intensity running exercises performed on a treadmill. As in previous studies, the muscular pre-conditioning was immediately applied before the exercises (Da Silva Alves et al, 2014; De Marchi et al, 2012), but for the current study the irradiation covered the entire muscle groups activated during the exercise (Ferraresi et al, 2011; Ferraresi, Hamblin, and Parizotto, 2012; Ferraresi and Parizotto, 2013).

Methods

One elite runner athlete, 28 years old, 1.80m height, 63.6 kg weight and 19.6 kg m^{-2} body mass index with 4 years of experience in high-level running competitions was enrolled in this single-subject randomized double-blind placebo-controlled trial.

The athlete was declared to be healthy without any type of skeletal muscle disorder, or neurological, metabolic, respiratory or cardiovascular disease. After explanation of the study purposes and procedures, he signed a consent form and was submitted to a protocol of exercise to measure his aerobic capacity, kinetics of VO_2, muscle performance and metabolic analysis of blood and urine. This study was conducted in compliance with the Declaration of Helsinki, approved by the Human Ethics Committee of the Federal University São Carlos (217/2012; Brazil) and registered at Clinical Trials.gov (NCT01770977).

Cardiopulmonary exercise test [CPX]: identifying the load target for the exercise

The CPX was performed on a treadmill (Master ATL, Inbramed, Porto Alegre, Brazil) using a ramping protocol consisting of 5 min of incremental increase in speed from 0.8 km h^{-1} to 18 km h^{-1}, followed by an incremental grade increase (0.5% each 30 s). This test was concluded when the athlete presented signs and/or symptoms of maximal exertion fatigue. The gas analyzer system was calibrated before the test following standard procedures (Balady et al, 2010). Ventilation and metabolic parameters were monitored and registered breath-by-breath (CPX-D/BreezeSuite 6.4.1, Medical Graphics, St Paul, MN). Electrocardiogram was continuously monitored (Active, Ecafix, Sao Paulo, Brazil) and heart rate (HR) was recorded by a digital telemetry system (Polar® S810i; Polar Electro Oy, Kempele, Finland). Blood pressure was assessed every 2 min. Using the ventilatory method, three independent evaluators determined the gas exchange threshold (GET) and the respiratory compensation point (RCP). The highest averaged VO_2 value observed at the last 30 s of exercise was considered the VO_2 peak. CPX was performed in a room with humidity between 35 and 40% and temperature between 24 and 25 °C (Table 1).

Constant workload exercise test [CWET]: running until voluntarily exhaustion

The CWET workload (speed and slope) was based on values of VO_2 acquired in the CPX test (previously described). A high workload corresponding to 95% of the VO_2 at RCP was employed (Whipp, 1994; Whipp and Casaburi, 1982). After 2 min of standing rest on the treadmill, the CWET was started with a single incremental adjustment to target workload (18.0 km h^{-1}, 4%) until the athlete presented signs and/or symptoms of exercise fatigue and decided to stop the exercise voluntarily. The monitoring and acquisition procedures of heart rate, ventilatory and metabolic parameters were similar to the CPX test.

CWET was performed four times in a room with humidity temperature controlled the same as the CPX test. The first CWET was carried out 2 d after the CPX test just to achieve familiarization with all procedures and methods and no data were acquired/analyzed since this was a simulated test. After 7 d, three further CWETs were performed: (1) placebo-1-LEDT; (2) placebo-2-LEDT; and (3) effective-LEDT. Among each CWET, an additional

period of 7 d was employed as washout period (Figure 1). CPX test and all CWETs were carried out always at the same period of the day (afternoon). Placebo or effective-LEDT was applied on body muscles in accordance with a randomized procedure.

LED therapy (LEDT): randomization, blind procedures, placebo and therapy

Near-infrared LEDT was applied with an array of multi-diode containing 50 LEDs (850±20 nm) specially built for research by the Federal University of São Carlos and University of São Paulo (Figure 1). The features of each LED and parameters of LEDT are presented in Table 2. All parameters of this device for LEDT were calibrated using Thorlabs® (Dachau, Germany) optical meter model PM100D and photodiode power sensor model S130C. All parameters of LEDT used were based on literature reports (Borsa, Larkin, and True, 2013; Ferraresi, Hamblin, and Parizotto, 2012; Leal-Junior et al, 2013).

LEDT (placebo or effective) was applied on six main muscle groups (A, B, C, D, E and F) used during a run, as described in Table 2 and illustrated in Figure 1. All muscle groups received LEDT (placebo or effective) 5 min before each constant workload exercise test (CWET) in accordance with randomization procedures (Borsa, Larkin, and True, 2013; Ferraresi, Hamblin, and Parizotto, 2012; Leal-Junior et al, 2009; Leal-Junior et al, 2013). The randomization scheme was generated at Randomization.com (http://www.randomization.com) using balanced permutations in one block with three different therapies: (1) Placebo-1-LEDT; (2) Placebo-2-LEDT; and (3) Effective-LEDT. Thus, this study had the following order of treatments: 1) Placebo-1-LEDT; (2) Placebo-2-LEDT; and (3) Effective-LEDT (Figure 1). Neither subject nor evaluators knew if LEDT was effective or placebo during data collection and analysis. A hidden button for placebo or effective LEDT in the LED device was employed to ensure the double-blind procedures. This button was switched previously without the knowledge of either evaluator or the subject. In addition, this button was switched to “on” (effective LEDT) or “off” (Placebo) by the only researcher who just participated in the randomization procedure and LEDT application, having no access to data collection and analysis. As the light therapy used was infrared, nobody could identify if the LEDT was effective or placebo while the time display was on.

Analysis of VO_2 kinetics: data modeling

As the initial VO_2 phase is mainly attributed to the increase in cardiac output and pulmonary blood flow (Arena, Tevald, Peberdy, and Turner, 2004), the first 20 s were excluded from the VO_2 data (Whipp et al, 1982). Next, a bi-exponential model was employed to fit the VO_2 data for all CWETs where:

$$VO_2(t)=a_0+a_1\left(1-e^{\left(\frac{t-TD_1}{\tau_1}\right)}\right)+a_2\left(1-e^{\left(\frac{t-TD_2}{\tau_2}\right)}\right)$$

“t” is time; “a_0” is the mean VO_2 value during warm-up; “a_1” is muscle VO_2 amplitude; “a_2” is the “extra” VO_2 amplitude from the slow component phase (VO_2 amplitude increases in this phase mainly from the recruitment of type II muscle fibers with lower phosphorylation efficiency (Borrani et al, 2001)); “τ_1” is the speed of muscular VO_2

adjustment; "TD_1" is the time delay to start the muscular phase (Rossiter et al, 1999); and "TD_2" is time delay to start the slow component phase, which arises after some minutes of constant workload exercise above that of the volunteer's GET (Barstow, 1994; Gaesser and Poole, 1996; Whipp, 1994). Additionally, the total muscular oxygen deficit was calculated (O_{2def}=(τ+TD1) a_1; Whipp and Casaburi, 1982).

Blood lactate and creatine kinase analysis

Blood samples for lactate measurement were collected by puncturing the athlete's ear lobe using sterile lancets before and 5 min after each CWET test. The puncture site was cleaned with alcohol, dried and the first drop of blood was discarded. The blood collected (25 μL) was quickly transferred to Eppendorf tubes containing 50 μL of 1% sodium fluoride (NaF) and analyzed by electroenzymatic lactimeter (YSI 1500 – Yellow Springs, Ohio, USA; Simoes et al, 2013). Lactate was used to infer muscle fatigue.

CK was analyzed from 4.5mL of blood collected from the antecubital vein, before and 5 min after each CWET test. This blood was collected in heparinized tubes, centrifuged at 3000 × *g* for 10 min at 4 °C and the heparinized plasma was immediately pipetted into Eppendorf tubes and stored at −80 °C until analysis on the Reflotron Plus® biochemical analyzer (Roche, Mannheim, Germany; Hornery, Farrow, Mujika, and Young, 2007), using 30 μL of heparinized plasma in accordance with the manufacturer's guidelines.

Metabolic analysis based on proton nuclear magnetic resonance spectroscopy (^{1}H NMR) in urine

Urinary samples were collected before and 30±5 min after each placebo or effective intervention (Enea et al, 2010; Pechlivanis et al, 2010). The runner was instructed to discard the first urinary flow and to urinate about 10mL into a Falcon tube. Immediately this tube was frozen in liquid nitrogen and stored at −80 °C until analysis. In addition, during each intervention day, the athlete received general instructions to ingest the same amounts of food and water (Enea et al, 2010; Pechlivanis et al, 2010).

For ^{1}H NMR analysis, urine samples were thawed, and 1.0mL of urine was centrifuged (10 min at 10 000 × *g*) to eliminate cellular fragments and sediments. Afterwards 0.9mL of urine was added to 0.1mL of phosphate buffer solution in D_2O (1.5 M, pH = 7.00) containing 2mM of NaN_3 (sodium azide) and 0.1% of TSP-d_4 (sodium 3-trimethylsilyl [2,2,3,3-$^2D_4$] propionate), and pH was adjusted to 7.00±0.01 after a 20-minute delay with HCl or NaOH. At last, 0.6mL of urine was transferred to a 5-mm NMR tube (Enea et al, 2010).

^{1}H NMR analysis of urine samples were recorded on a Bruker AVANCE III spectrometer at a proton frequency of 600 MHz equipped with a 5mm TCI CryoProbe (z-gradient). The one-dimensional (1D) NOESY pulse sequence with water pre-saturation was used throughout. Spectra of CK and alanine (for muscle damage), creatinine (for energy metabolism) and glycine, dimethylamine (DMA) and trimethylamine *N*-oxide (TMAO; for renal function) were qualitatively and quantitatively assigned using Chenomx NMR suite 7.5 software, evaluation edition (Chenomx Inc., Edmonton, Canada), with reference to the literature (Wishart et al, 2009).

Statistical analysis

All results were presented in descriptive form (i.e. in absolute, percentage values and normalized per time of exercise performed (T_{lim})). Tables and graphs show all differences between each test. There is no statistical test performed since this study enrolled a single runner athlete.

Outcomes

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The parameters measured during CWET were: "a_0"; "a_1"; "a_2"; "τ_1"; "TD_1"; "TD_2"; time limit of exercise (T_{lim}); and deficit of oxygen (O_{2def}).

Placebo-1-LEDT and Placebo-2-LEDT had very similar results as shown in Figure 2, suggesting a very similar response to the same exercise and in accordance with the literature (Faisal, Beavers, and Hughson, 2010). Comparing both placebo-LEDT, Placebo-1-LEDT had approximately an additional of 200 ml min^{-1} of "extra" VO_2 amplitude in slow component ("a_2"; Figure 2) and Placebo-2-LEDT had higher "T_{lim}" of exercise (+144 s or +17%), lower "O_{2def}" (−4L or −2.8%) and "τ_1" (i.e. the speed of muscular VO_2 adjustment; −2 s or 5.9% faster).

On the other hand, effective-LEDT compared to Placebo-1-LEDT increased "T_{lim}" (+589 s or +70%), decreased "O_{2def}" (−12 L or −8.4%) and "τ_1" (−10 s or 29.4% more fast). Compared to Placebo-2-LEDT, effective-LEDT presented higher "T_{lim}" of exercise (+445 s or +45%), lower "O_{2def}" (−8L or −5.8%) and "τ_1" (−8 s or 25% more fast). In addition, effective-LEDT presented an earlier appearance of the slow component phase (lower "TD_2"), faster muscular oxidative adaptation (lower "τ" value), higher muscle VO_2 amplitude ("a_1") and the highest "extra" VO_2 amplitude in the slow component phase ("a_2"; Figure 2).

Placebo-1-LEDT, placebo-2-LEDT and effective LEDT presented 839, 983 and 1428 s for "T_{lim}"; 143, 139 and 131 L for "O_{2def}"; and 34, 32 and 24 s for "τ_1", respectively (Figure 3A, B and C, respectively).

Blood and urine analysis

Concentrations of each metabolite (in blood and/or urine through ^1H NMR analysis) are expressed in absolute rate, percentage of change and normalized per time of exercise (T_{lim}) of each CWET (Table 3).

Discussion

This preliminary study investigated the effects of muscular pre-conditioning using LEDT on the VO_2 kinetics and metabolic changes in blood and urine during high-intensity running exercise performed by an elite runner athlete. Several studies have used near-infrared LLLT or LEDT to improve muscle performance (Borsa, Larkin, and True, 2013; Ferraresi, Hamblin, and Parizotto, 2012; Leal-Junior et al, 2013). The main effects reported by these studies are: decreased muscle fatigue; lower blood lactate levels and less muscle damage

(blood CK levels); increased torque; workload; aerobic capacity; oxidative stress defense; and micro-circulation when applied to muscles (Borsa, Larkin, and True, 2013; Da Silva Alves et al, 2014; De Marchi et al, 2012; Ferraresi et al, 2011; Ferraresi, Hamblin, and Parizotto, 2012; Vieira et al, 2012).

However, there is still no consensus about how all the parameters of near-infrared radiation such as wavelength, optical output, treatment time, energy, energy density, power, power density and number of radiation points should be optimized to increase muscle performance in exercise. Therefore, based on possible biphasic dose–response relationships previously reported (Huang, Chen, Carroll, and Hamblin, 2009; Huang, Sharma, Carroll, and Hamblin, 2011), the current study applied a similar total energy per muscle group (37.5 J) as has been previously reported. We used 50 radiation points to deliver the light to each muscle group in order to cover the entire muscular area employed during running exercise, and the optical output (50mW; Ferraresi, Hamblin, and Parizotto, 2012; Ferraresi et al, 2011; Vieira et al, 2012), treatment time (15 s; De Marchi et al, 2012) and the wavelength (Leal-Junior et al, 2009) were also described in the literature.

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The dotted lines plotted in Figure 3 demonstrated the expected oscillation or a progressive adaptation between each constant workload exercise test (even if this is not described in the literature; Faisal, Beavers, and Hughson, 2010). Note in each of these lines there is an abrupt break in linearity (or a tendency for a break) after effective-LEDT (Figure 3). These preliminary results may suggest an accelerated (here assessed by "τ_1"), and more efficient adenosine tri-phosphate (ATP) turnover from aerobic (by oxidative phosphorylation) and anaerobic alactic metabolism (Cr-P system) mainly in slow-twitch muscle fibers before the appearance of the slow component phase (Hepple, 2002). Furthermore, our results suggest a higher influence of aerobic ATP turnover in fast-twitch muscle fibers (glycolytic and oxidative) during the slow component phase (Jones et al, 2011) which probably produced an extended "T_{lim}" of exercise compared to Placebo-LEDT.

During the slow component phase there is a progressive increase in blood lactate levels due to progressive recruitment of fast-twitch fibers (Borrani et al, 2001) that produces metabolic acidosis (Barstow, 1994; Gaesser and Poole, 1996; Whipp, 1994). In this context, it is known that near-infrared LEDT can improve mitochondrial function, with possible mechanisms consisting of a lactate shuttle through mitochondrial monocarboxylate transporters and lactate oxidation to ATP synthesis during exercise (Ferraresi et al, 2011; Ferraresi and Parizotto, 2013; Vieira et al, 2012).

It has been reported that light therapy can promote an improvement of complex IV activity (cytochrome c oxidase; Karu, 2010) as well as the other complexes of the mitochondrial electric transport chain (ETC; Silveira et al, 2009) in non-injured skeletal muscles (Hayworth et al, 2010). Furthermore, some studies have reported better vasodilatation, microcirculation and tissue perfusion after effective-LEDT applied to the human body (Mak and Cheing, 2012). Thereby improved blood transport and oxygen delivery to muscle cells, together with increased mitochondrial metabolism, could modulate the VO_2 response during exercise, explaining the results of the VO_2 kinetics.

Blood lactate, blood creatine kinase and metabolic analysis based on ^{1}H NMR in urine

Lactate levels in blood normalized for time of exercise performed (T_{lim}) were much lower after effective-LEDT, suggesting a better clearance (lactate removal; De Marchi et al, 2012), or a smaller lactate production possibly by inhibition of lactate dehydrogenase (LDH) enzyme activity or increased lactate oxidation (consumption) in mitochondria (Ferraresi et al, 2011; Ferraresi, Hamblin, and Parizotto, 2012; Ferraresi and Parizotto, 2013). However, if NIR can improve vasodilatation and microcirculation (Mak and Cheing, 2012) and consequently provide better lactate clearance or removal, it is important to measure lactate concentration in urine (Enea et al, 2010; Pechlivanis et al, 2010) and blood analysis in order to show the real lactate balance during exercise.

We found effective-LEDT had the lowest levels of lactate in blood and urine normalized for time of exercise performed. These results may suggest that effects of light therapy could be better related to the decrease in lactate production (and/or increase in its consumption by mitochondria; Ferraresi et al, 2011; Ferraresi, Hamblin and Parizotto, 2012; Ferraresi and Parizotto, 2013) than to lactate removal or clearance as reported previously (De Marchi et al, 2012; Leal-Junior et al, 2013).

CK has been used as a “gold standard” to measure muscle damage after intense exercise and frequently is related to higher oxidative stress (De Marchi et al, 2012). Near-infrared radiation seems to modulate oxidative stress and prevent muscle damage if applied before intense exercises (De Marchi et al, 2012; Leal- Junior et al, 2009). In accordance with these previous studies, the CK level in blood normalized for time of exercise had much lower levels in effective-LEDT CWET, than in Placebo. Alanine was quantified in urine to show the renal clearance function of amino acids released from damaged muscle. CK levels in blood are associated with muscle damage, and aminoacids from protein breakdown are released into the bloodstream and excreted in urine (Enea et al, 2010). The increased amount of alanine in the urine after high-intensity exercise suggests that muscle damage occurred (Enea et al, 2010). In effective-LEDT, the amounts of alanine in urine normalized for time of exercise was lower than in Placebo-2-LEDT, which had the second higher “T_{lim}” of exercise, suggesting a protective effect of effective-LEDT against muscle damage as reported previously (Borsa, Larkin, and True, 2013; Ferraresi, Hamblin, and Parizotto, 2012; Leal-Junior et al, 2009, 2013).

The other important metabolite identified in the urine was creatinine. This metabolite is frequently associated with renal dysfunction in seriously ill patients suffering increased protein breakdown (catabolism process; Bairaktari et al, 2002). However, the creatinine in the urine of healthy people subjected to intense exercise suggests that phosphocreatine is being biochemically broken down (Pechlivanis et al, 2010). Phosphocreatine (associated with higher cellular energy states) is produced by the re-phosphorylation of creatine by ATP; therefore, if more mitochondrial ATP is available (LEDT effect; Ferraresi et al, 2011; Pechlivanis et al, 2010) a higher energy state in muscles will be expected, which in turn could explain the apparently faster ATP turnover rate during the beginning of exercise. Thus, creatinine production and its excretion were decreased only in effective-LEDT, suggesting better alactic energy metabolism.

Other important metabolites identified in urine were glycine, DMA and TMAO, all of which are related to renal function (Bairaktari et al, 2002). High levels of lactic acidosis may cause acute renal dysfunction through tubular acidosis (Bairaktari et al, 2002). In agreement with the literature, the concentration of glycine normalized for "T_{lim}" of exercise in Placebo-1-LEDT and Placebo-2-LEDT were sharply decreased, suggesting reversible renal dysfunction (Bairaktari et al, 2002). Additionally, glycine was increased in effective-LEDT suggesting a minor renal dysfunction. On the other hand, increased excretion of DMA and TMAO suggested renal dysfunction (Bairaktari et al, 2002; Enea et al, 2010). DMA was excreted in both placebo interventions, and TMAO was more excreted in Placebo-2-LEDT, suggesting worse renal function.

Our study design aimed to have 2 d of placebo therapy in order to demonstrate the expected small oscillation between repeated exercise tests, unlike other studies that performed just one test during cross-over procedures (Da Silva Alves et al, 2014). This strategy, for our understanding, should be understood as a valuable caution to warrant that all changes in performance and VO_2 kinetics possibly is attributable to the effective LEDT and should be greater than the normal oscillations between repeated exercise tests, as demonstrated in both days of placebo therapy.

However, the main possible concern about our results is the order of the therapies, once that effective LEDT was randomized to the last day of exercise test. At this point, it is very important to remark that due to our strategy of performing 2 d of exercise testing using placebo, the expected changes in VO_2 response for a next placebo visit was plotted (Figure 3 – dotted lines), although being hard to measure the behavior of this oscillation (but for sure not higher than ~−9 s in $\Delta\tau$ – Figure 3(C); Faisal, Beavers, and Hughson, 2010).

Regarding this same figure, it is essential to note an abrupt inflection of the response when effective LEDT was applied before the exercise test (even considering this normal oscillation or the already discussed "learning" influences), showing superior responses. In other words, we were extremely careful to warrant that the LEDT effects should be greater than the small oscillation between tests, that are normal physiological responses and does not mean better or worse performances. In addition, the elite runner enrolled in this study had 4 years of running experience. Exercises like those performed in this study are part of the participant's daily training program. Therefore, the "learning" effect to running is unlikely, although this is not conclusive from this pilot study.

As an elite athlete the VO_2 adjustment is optimized close to its maximum and the small changes observed ($\Delta\tau = -2$ s – Figure 3C) between the placebo visits is due to small oscillations expected despite the error inherent to the modeling process (from intra-breath noise); and that there is insignificant influences of the circadian rhythm on VO_2 kinetics (Faisal, Beavers, and Hughson, 2010).

The present study has shown potential effects of LEDT on performance and VO_2 kinetics during intense and constant workload exercises (that simulate endurance training protocols) associated with metabolic analysis in blood and urine using ^{1}H NMR analysis. However, generalization of our findings should be avoided because this pilot study enrolled just one

single elite runner participant. We encourage more researchers to reproduce this study with more athletes to corroborate our findings, and to investigate other muscle adaptations such as gene expression and muscular VO_2.

Conclusion

Light-emitting diode therapy (LEDT) using a multi-diode array of LEDs with near-infrared wavelength and the dose applied in this study possibly can improve VO_2 kinetics; increase time of exercise; decrease muscle damage, fatigue and renal dysfunction during intense running exercise performed on a treadmill.

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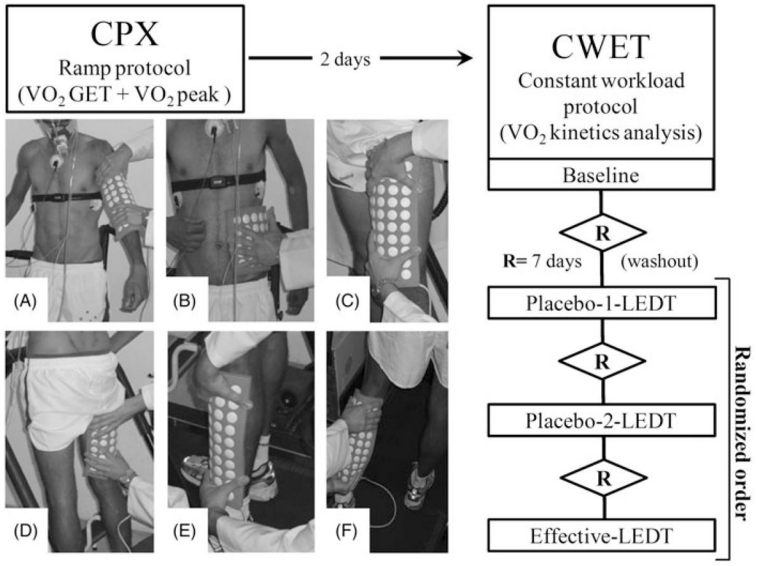

Figure 1.

Workflow of the study procedures and muscle groups irradiated by light-emitting diode therapy (LEDT): (A) biceps brachii and triceps brachii; (B) external oblique and latissimus dorsi; (C) femoral quadriceps; (D) hamstrings; (E) tibialis anterior and peroneus longus; (F) gastrocnemius and soleus. The order of each therapy (Placebo-1-LEDT, Placebo-2-LEDT and effective-LEDT) was randomized. The sequence of LEDT application was not randomized and had the following sequence: F, E, D, C, B, A.

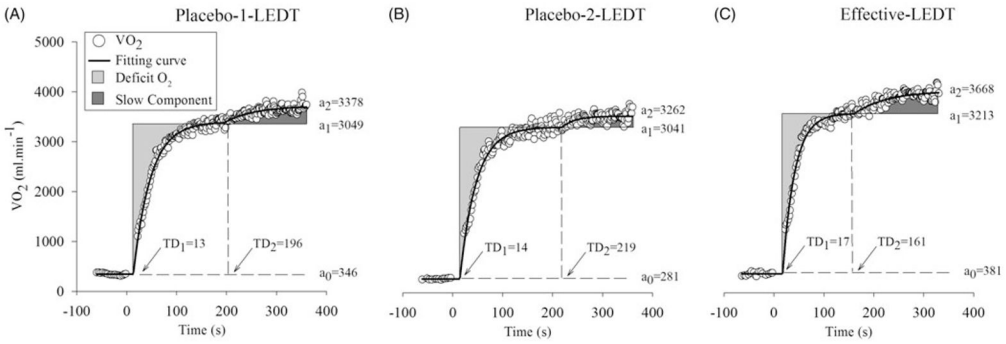

Figure 2.

Values of VO_2; a_0, a_1 and a_2; TD_1 and TD_2 during constant workload exercise tests (CWETs): Placebo-1-LEDT, Placebo-2-LEDT and Effective-LEDT. Abbreviations: VO_2 (oxygen uptake); a_0 (mean VO_2 value during warm-up), a_1 (muscle VO_2 amplitude in muscular phase); a_2 ("extra" VO_2 amplitude in slow component phase); TD_1 (time delay to start muscular phase); TD_2 (time delay to start the slow component phase); LEDT (light-emitting diode therapy).

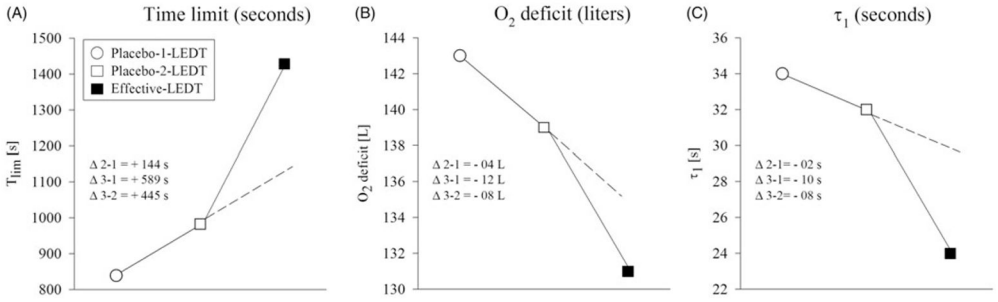

Figure 3.

Comparisons between constant workload exercise tests (CWET): Placebo-1-LEDT, Placebo-2-LEDT and effective-LEDT. Abbreviations: T_{lim} (time limit of exercise); "τ_1" (speed of muscular VO_2 adjustment); L (liters); LEDT (light-emitting diode therapy). Note in this figure that there are dotted lines plotted to demonstrate a tendency or linearity for the expected oscillation or small adaptation after multiple constant workload exercise tests. However, after effective-LEDT, there was a clearly break in this tendency or linearity, suggesting beneficial effects of LEDT on VO_2 kinetics.

Table 1

Results of functional parameters at gas exchange threshold (GET) and peak of cardiopulmonary exercise test (CPX) performed on treadmill ramp protocol to set the workload for all constant workload exercise tests (CWETs).

CPX	GET	Peak
Time (min)	9	17.00
VO_2 (mL $kg^{-1}.min^{-1}$)	48.6	67.60
VO_2 (L min^{-1})	2.96	4.40
VCO_2 (L min^{-1})	2.54	4.48
HR (bpm)	154	182

HR, heart rate; VO_2, oxygen uptake; VCO_2, carbon dioxide output.

Table 2

Parameters of light-emitting diode (LED) therapy (LEDT) and regions of LEDT irradiation on body before constant workload exercise tests (CWETs).

Number of LEDs: 50
Wavelength: 850±20 nm (infrared)
Frequency: continuous output
Optical output: 50mW
LED spot size: 0.2 cm^2
Power density: 250mW cm^{-2}
Treatment time over each muscle group: 15 s
Energy per diode at 15 s: 0.75 J
Energy density per diode at 15 s: 3.75 J cm^{-2}
Number of irradiation points per muscle group: 50
Total energy delivered per muscle group: 37.5 J
Muscle groups irradiated before CWETs: (A) biceps brachii and triceps brachii; (B) external oblique and latissimus dorsi; (C) femoral quadriceps; (D) hamstrings; (E) tibialis anterior and peroneus longus; (F) gastrocnemius and soleus
Total energy delivered on body: 450 J
Total power output: 2500mW
Application mode: device held coupled in skin contact

Table 3

Results of blood creatine kinase (U L^{-1}) and blood lactate (mmol L^{-1}), and metabolic analyses based on ^{1}H NMR (mM) in urine.

	Placebo1-LEDT				Placebo2-LEDT				Effective-LEDT			
Concentration	**Before**	**After**	**Δ%**	**Δ%/T_{lim} (839 s) × 10^{-2}**	**Before**	**After**	**Δ%**	**Δ%/T_{lim} (983 s) × 10^{-2}**	**Before**	**After**	**Δ%**	**Δ%/T_{lim} (1428 s) × 10^{-2}**
CK (blood)	105	156	48.57	5.78	113.00	169.00	49.56	5.04	110.00	149.00	35.45	2.48
Lactate (blood)	1.50	10.20	580	69.12	0.84	9.69	1053	107.12	1.74	9.60	451.72	31.63
Lactate (urine)	0.32	39.70	12 306	1466.74	1.20	52.20	4250	432.34	0.63	41.10	6423	449.84
Creatinine (urine)	10.80	11.80	9.26	1.10	61.50	70.00	13.82	1.40	20.90	19.70	−5.74	−0.40
Alanine (urine)	0.31	0.54	74.19	8.84	1.59	2.37	49.06	4.98	0.711	1.14	60.34	4.22
Glycine (urine)	0.84	0.81	−3.57	−0.42	3.69	3.22	−12.74	−1.29	1.11	1.37	23.42	1.64
DMA (urine)	0.33	0.39	18.18	2.16	2.05	2.38	16.10	1.63	0.80	0.73	−8.75	−0.61
TMAO (urine)	0.19	0.13	−31.58	−3.76	5.43	4.77	−12.15	−1.23	0.35	0.27	−22.86	−1.60

Data were expressed as absolute concentration, percentage changes and normalized per time of exercise performed (T_{lim}) value and percentage. LEDT, light-emitting diode therapy; CK, creatine kinase; ^{1}H NMR, proton nuclear magnetic resonance; DMA, dimethylamine; TMAO, trimethylamine *N*-oxide; mM, milimolar; Δ%, delta in percentage, T_{lim}, time limit of exercise.