PLOS ONE

RESEARCH ARTICLE

Lactate production without hypoxia in skeletal muscle during electrical cycling: Crossover study of femoral venous-arterial differences in healthy volunteers

Jan Gojda[1,2☯*], **Petr Waldauf**[1☯], **Natália Hrušková**[3], **Barbora Blahutová**[3], **Adéla Krajčová**[1,2], **Tomáš Urban**[1], **Petr Tůma**[4], **Kamila Řasová**[3], **František Duška**[1]

1 Department of Anaesthesia and Intensive Care Medicine, Kralovske Vinohrady University Hospital and The Third Faculty of Medicine, Charles University, Prague, Czech Republic, **2** 2nd Department of Internal Medicine, Kralovske Vinohrady University Hospital and The Third Faculty of Medicine, Charles University, Prague, Czech Republic, **3** Department of Rehabilitation, Kralovske Vinohrady University Hospital and The Third Faculty of Medicine, Charles University, Prague, Czech Republic, **4** Department of Hygiene, The Third Faculty of Medicine, Charles University, Prague, Czech Republic

☯ These authors contributed equally to this work.
* jan.gojda@lf3.cuni.cz

Citation: Gojda J, Waldauf P, Hrušková N, Blahutová B, Krajčová A, Urban T, et al. (2019) Lactate production without hypoxia in skeletal muscle during electrical cycling: Crossover study of femoral venous-arterial differences in healthy volunteers. PLoS ONE 14(3): e0200228. https://doi.org/10.1371/journal.pone.0200228

Editor: Stephen E. Alway, University of Tennessee Health Science Center College of Graduate Health Sciences, UNITED STATES

Received: June 19, 2018

Accepted: February 11, 2019

Published: March 1, 2019

Data Availability Statement: All relevant data are within the paper and its Supporting Information file.

Funding: The study was supported by Ministry of Health of the Czech Rep. grant AZV 16-28663A (http://www.azvcr.cz/en). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

Background

Aim of the study was to compare metabolic response of leg skeletal muscle during functional electrical stimulation-driven unloaded cycling (FES) to that seen during volitional supine cycling.

Methods

Fourteen healthy volunteers were exposed in random order to supine cycling, either volitional (10-25-50 W, 10 min) or FES assisted (unloaded, 10 min) in a crossover design. Whole body and leg muscle metabolism were assessed by indirect calorimetry with concomitant repeated measurements of femoral venous-arterial differences of blood gases, glucose, lactate and amino acids.

Results

Unloaded FES cycling, but not volitional exercise, led to a significant increase in across-leg lactate production (from -1.1±2.1 to 5.5±7.4 mmol/min, $p<0.001$) and mild elevation of arterial lactate (from 1.8±0.7 to 2.5±0.8 mM). This occurred without widening of across-leg veno-arterial (VA) O_2 and CO_2 gaps. Femoral SvO_2 difference was directly proportional to VA difference of lactate ($R^2 = 0.60$, $p = 0.002$). Across-leg glucose uptake did not change with either type of exercise. Systemic oxygen consumption increased with FES cycling to similarly to 25W volitional exercise (138±29% resp. 124±23% of baseline). There was a net uptake of branched-chain amino acids and net release of Alanine from skeletal muscle, which were unaltered by either type of exercise.

Competing interests: The authors have declared that no competing interests exist.

Conclusions

Unloaded FES cycling, but not volitional exercise causes significant lactate production without hypoxia in skeletal muscle. This phenomenon can be significant in vulnerable patients' groups.

Introduction

Functional electrical stimulation-assisted cycling (FES cycling) is a method originally developed over 30 years ago for patients with spinal cord injury [1]. It uses computer-driven electrical pulses delivered by transcutaneous electrodes and directly activating muscle contractions, independently on functionality of the physiological pathway between upper motoneuron and the neuromuscular junctions. The method is now commercially available in the form of both stationary and mobile devices [2], used by patients with a wide range of conditions incl. spinal cord injury [3], stroke [4,5], and multiple sclerosis [6]. FES cycling was demonstrated to improve cardiovascular fitness, insulin sensitivity [7] bone density and muscle strength [2,8]. In recent years, FES-cycling has become particularly attractive for sedated critically ill patients. Early mobilization is the only intervention, which can partially prevent the development of intensive care unit-acquired weakness [9–14]—the major long-term consequence in the survivors of protracted critical illness [15,16]. Muscle atrophy [17,18] and dysfunction [18] occur very early in the critically ill and FES cycling can help to deliver exercise before the patient can co-operate with a physiotherapist [19].

Although FES cycling seems to be feasible in intensive care unit patients [19], before its effect on meaningful clinical outcomes can be tested in the critically ill and other vulnerable patients groups, important physiological questions need to be addressed. Metabolic efficacy (i.e. power output divided by metabolic cost) of the FES cycling is typically very low, around 5–10%, as compared to 25–40% in volitional cycling [20–22]). This is likely due to non-physiological pattern of muscle activation, where large muscle groups are activated simultaneously rather than small well-coordinated units [2,23]. Despite FES cycling increases cardiac output [24] and leg blood flow to the same extent [25] or even more [26] than volitional cycling and consequently oxygen delivery to the muscle should be normal, there are features suggesting early switch to anaerobic metabolism: early fatigue [23,27], rapid intramyocellular glycogen depletion [28], increase of respiratory quotient (RQ) >1 [20] and even a mild increase in arterial lactate levels [29]. Increased lactate production could be caused by microcirculation impairment during electrically stimulated asynchronous contraction [30] or by a mismatch between glycogenolysis activated by electrical stimulation [31] and pyruvate oxidation.

Nonetheless, a direct evidence of the presence of anaerobic metabolism in skeletal muscle during FES cycling is lacking. In addition, whilst the influence of volitional resistance exercise on amino acid metabolism has been extensively studied [32–36] there is no such data for FES cycling, although one study demonstrated activation of anabolic signalling in electrically stimulated gastrocnemius muscle in a rat [31]. These questions may be particularly relevant before FES-assisted exercise is introduced to critically ill patients, who are in profound protein catabolism and may be less able to clear lactate from systemic circulation.

In light of this we conducted a crossover study of volitional and FES supine cycling in healthy postprandial volunteers, where we combined indirect calorimetry with across-leg venous-arterial (VA) difference studies. We hypothesized that FES-cycling as compared to light volitional exercise would lead to increased production of lactate in correlation with

widening of VA-CO_2 gap (as the measure of anaerobic metabolism), and with increased amino-acid efflux from skeletal muscle during exercise.

Materials and methods

Study subjects

Our experimental group consisted of 14 young (31±8 years), non-obese (23.7±3.7kg/m^2) healthy volunteers (gender M/F = 11/3). University Hospital Kralovske Vinohrady's Research Ethics Board reviewed the protocol and approved the study. Prior to the enrolment, all subjects gave their written informed consent in accordance with the Declaration of Helsinki.

Overview of study design

The study was performed during two visits performed 1 week apart. Subjects were asked to attend the visit at 08:00 AM after an overnight fast. In between these visits, the subjects were advised to take their usual diet and avoid strenuous exercise. During the first visit, the volunteers underwent a physical examination and body composition measurement. After 30 min bed rest, their energy expenditure was measured using indirect calorimetry with a ventilated canopy system. Afterwards, in each subject's VO_{2MAX} was determined on a cycle ergometer with stepwise load by 25 W increments until exhaustion. During the second visit, subjects were given a standardized breakfast containing 70 g of carbohydrates, 10 g protein and 15 g of fat. Afterwards, femoral vein and radial artery were cannulated. After 30 min rest, the subjects were exposed in random order to one of two supine exercise protocols, separated by 3 hours rest. Both protocols begun with baseline measurements (AV difference studies and calorimetry) followed by 5 min of passive cycling. Then, the subjects either performed three 10 min cycles of volitional cycling (at 10, 25 and 50 W, respectively) separated by 5 min of passive cycling (Group A), or FES cycling (Group B). The exercise protocols are outlined in Fig 1.

Methods

Indirect calorimetry and body composition assessment. Resting energy expenditure and RQ were measured after overnight (12 h) fast and 30 min bedrest using canopy as a mixing chamber with 10 sec sampling (Quark RMR device, Cosmed, Italy). To determine peak oxygen uptake (VO_{2max}) exhaustive exercise test was performed in each subject on an electromagnetically braked bicycle ergometer Ergoline Ebike (Ergoline Gmbh, Germany). After 5 min warm-up period, a workload of 50W was initiated and increased by 25 W every minute continuously until fatigue despite the verbal encouragement. Oxygen uptake was measured using mask, breath-by-breath, 10 sec sampling period (Quark RMR device, Cosmed, Italy. ECG was monitored continuously. Gas analysers (container 5% CO_2, 16% O_2 and room air) and flow analyser were calibrated prior to each measurement. Body fat was assessed using bioimpedance analysis (NutriGuard 2000, Bodystat, Germany).

Cannulations. Femoral vein was cannulated 2–3 cm below inguinal ligament under ultrasound guidance. In order to avoid the admixture of blood from saphenous and pelvic veins [37], a single-lumen central venous catheter (B-Braun, Germany) was inserted retrogradely to the depth of 10–15 cm so that the tip was deep in the femoral muscular compartment. For arterial sampling, we used a 22 F catheter (BBraun, Germany) inserted into the radial artery.

Cycling protocols. For both volitional and FES cycling we used RT-300 bikes (Restorative Therapies Ltd., USA) and the exercise was performed in supine position. *Volitional cycling* consisted of three 10 min intervals of active cycling: 10W (13 revolutions/min, resistance 7 N/m), 25W (31 revolutions/minute, 7.6 N/m), 50W (35 revolutions/min, and resistance 13.4 N/

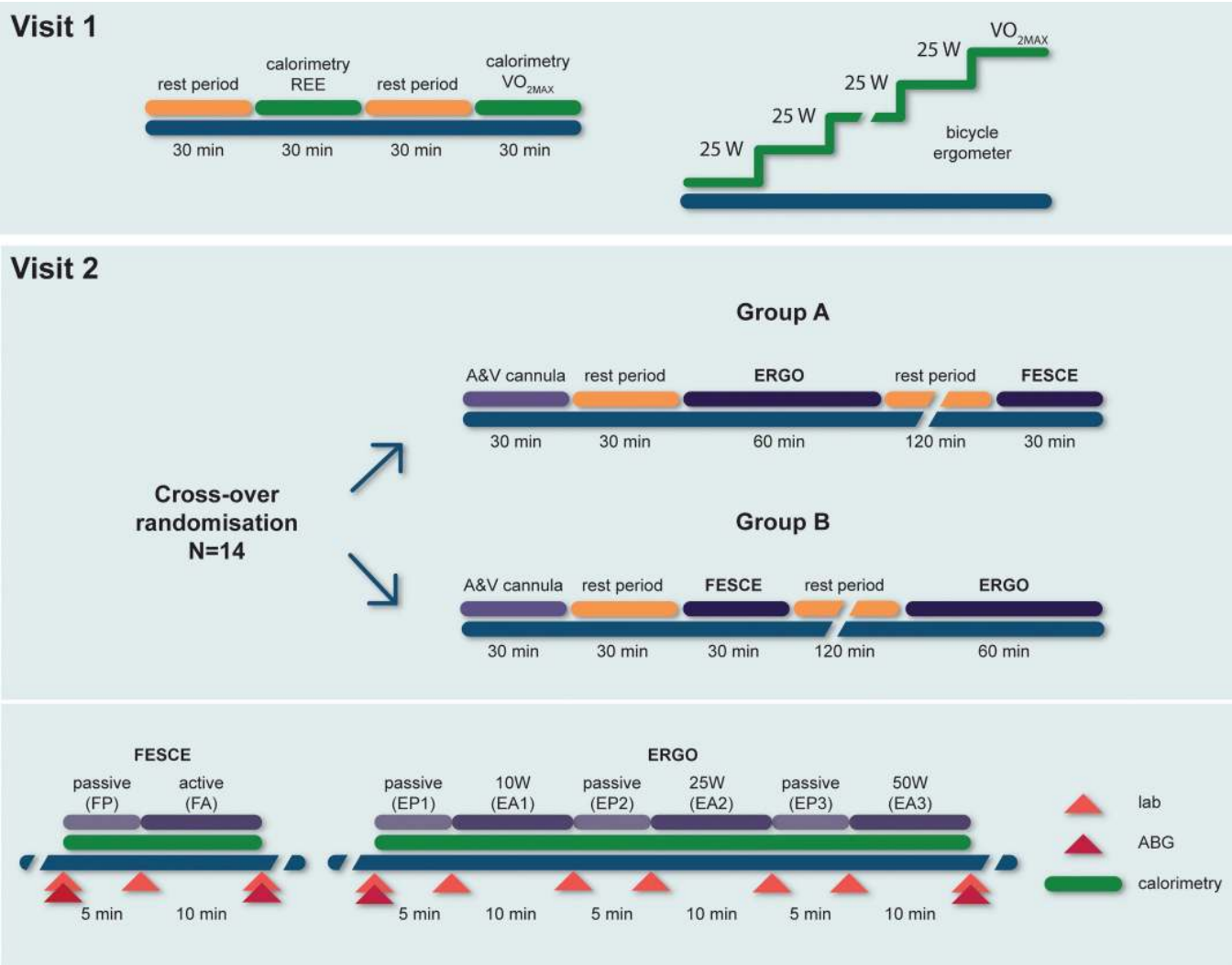

Fig 1. Overview of study design. Arrows designate arterial and venous blood sampling times. Note: ERGO = volitional cycling, FESCE = functional electrical stimulation cycling. Details of exercise are shown in the inlet at the bottom.

https://doi.org/10.1371/journal.pone.0200228.g001

m). These period were preceded (warm up) and separated by 5 min of passive cycling at 25 revolutions/min. *FES cycling*: Three pairs of transcutaneous electrodes (3 x 4", Restorative Therapies, Ltd., USA) electrodes were applied on each leg over quadriceps, hamstrings and gluteus maximus muscles, as per manufacturer's instructions. Prior to electrode placement, we measured the thickness of fat layer between the skin and muscle by ultrasound. After 5 min passive warm up (25 revolutions/min), the target speed was changed to 30 revolutions/min and stimulation gradually (1%/s) started to achieve 25 mA. Then, in each subject, the stimulation current was gradually increased to reach subjectively tolerated maximum. Oxygen uptake was measured continuously in both volitional and FES assisted cycling using mask breath-by-breath system (Quark RMR device, Cosmed, Italy). Gas analysers (container 5% CO_2, 16% O_2 and room air) and flow analyser were calibrated prior to each measurement.

Laboratory methods. Arterial and venous blood samples were analysed for blood gases, lactate and haemoglobin using POCT analyser Cobas b221 (Roche Diagnostics Limited, USA). For other analysis blood samples were centrifuged and frozen at -80˚C until analysed. Serum

creatine kinase and myoglobin was measured in a certified institutional laboratory (Cobas system, Roche Diagnostics Ltd., USA). Serum amino acid concentration in arterial/venous blood was analysed using capillary electrophoresis as described [38].

Calculations and statistics

Metabolic efficacy. Metabolic efficacy of volitional cycling was calculated as power output divided by the increase of energy expenditure [2]. Veno-arterial gap in the total content of carbon dioxide ($ctCO_2$ gap) was calculated according to equations used in ABL 900 Analyser (by Radiometer, Copenhagen, Denmark).

$$ctCO_2(B) = 9.286 \times 10^{-3} \times pCO_2 \times ctHb \times \left[1 + 10^{(pHEry - pKEry)}\right] + ctCO_2(P) \times \left(1 - \frac{ctHb}{21.0}\right)$$

where $ctCO_2$ (B) = CO_2 content in blood in mmol/L; $ctCO_2$ (P) = CO_2 content in plasma in mmol/L and equals to 0.23 x pCO2 + $cHCO_3^-$(P); pCO_2 is partial pressure in kPa, ctHb = haemoglobin content in mmol/L. $ctCO_2$(P). pH_{ERY} = estimated intracellular pH in red blood cells, which equals to 7.19+0.77 x (pH-7.4)+0.035 x (1-SO_2), where SO_2 is haemoglobin saturation with oxygen; and finally pK_{ERY} is a negative decadic logarithm of bicarbonate dissociation constant:

$$pK_{ERY} = 6.125 - \log\{1 + 10^{[pHEry - 7.84 - (0.06 \times SO_2)]}\}$$

Blood flow. In both FES and volitional cycling, leg oxygen uptake represents a relatively fixed proportion (76±8% and 78±9%, respectively) of whole-body oxygen uptake [39]. Therefore, an index of blood flow through the leg was calculated as whole-body oxygen consumption divided by the difference of oxygen content in arterial and femoral-venous blood. Blood oxygen content was calculated in mmol/L as 0.00983*pO_2 + SO_2[%]/100 * Hb *0.06206*(1-COHb [%]/100 –metHb[%], where SO_2 is saturation of haemoglobin with oxygen [%], Hb is haemoglobin [mmol/L], CO-Hb and met-Hb are fractions of carbonyl and methemoglobin, respectively, and pO_2 is partial pressure of oxygen [kPa].

Statistics. We used linear mixed effect model for 2x2 crossover design processed with software Stata 15 (Stata Corp., LLC, U.S.A.) [40,41]. The model consists of fixed and random part. In the fixed part, the model contained following parameters: (1) Sequence, i.e. order in which subject performed volitional and FES cycling protocols. Had this parameter been significant, a carry-over effect would have been present; (2) Period, basal vs. active, a parameter exploring the effect of the exercise, regardless whether volitional or FES; (3) Treatment, exploits the difference between volitional and FES cycling; and (4) Interaction Period#Treatment exploits whether FES cycling differs from volitional cycling during exercise period. Random part of the model contains subject number in order to take into account repeated measurements. Binary data are showed as frequency + %, continuous data as means ± SD. P value <0.05 was considered as significant. Whenever another test was used we specified this in the text. Sample size determination was performed prior commencement of the protocols with VA lactate difference as a primary outcome.

Results

Characteristics, tolerability and signs of muscle damage

All 14 subjects finished the protocol without adverse events; baseline (visit 1) calorimetry data are available for 13 subjects only due to a technical problem. Baseline characteristics are

outlined in Table 1. Sequence parameter of linear mixed effect model was not significant in any of analysed parameters (p = 0.14–0.94), so we assume no carry over effect from previous cycling protocol.

Maximum tolerated stimulation current of FES was 45±13 mA (range 25–67 mA). Although FES cycling caused a degree of discomfort, post-exercise serum myoglobin remained within reference range (<85 ng/mL) in all subjects (33±15 pg/mL, range 21–74). Nonetheless, there was a positive correlation between maximal stimulation current and post-exercise serum myoglobin (Spearman's $R^2 = 0.57$, p = 0.002).

Metabolic efficacy of volitional vs. FES cycling

Metabolic efficacy of volitional cycling was 39.2±5.6%. Unloaded FES cycling led to an increase of metabolic rate to 138±29% from baseline, which was comparable to the increase with 25 W volitional exercise (124±23%). See Fig 2. Energy gain from anaerobic glycolysis was negligible or negative for volitional cycling and 5.0±6.2 W for FES cycling.

Blood flow index

At rest before volitional and FES cycling, blood flow index was 6.6±2.4 vs. 6.3±3.4 (p = 0.57), and increased significantly (p<0.01) and similarly (p = 0.77) to 160% and 165% of baseline after volitional and FES exercise.

Exploring muscle metabolism during FES cycling

VA differences of both O_2 and CO_2 contents (ctO_2 and $ctCO_2$) tended to widen with volitional exercise (Fig 3A and 3B), whilst the opposite trend was seen for FES cycling. In line, there was no change in oxygen saturation of haemoglobin in femoral venous blood neither with volitional exercise (from 63.9±12.7% to 64.3±8.7%), whilst there was an increase after FES cycling (from 62.6±11.3 to 70.3±8.7%; p = 0.02). Across-leg respiratory exchange ratio (i.e. the ratio between VA differences of CO_2 and O_2 contents) although different at baseline (Fig 3C) tended to increase with volitional cycling, but this change was not significant. There was no change from baseline in across-leg glucose uptake of glucose (FES -5.5±3.9 to -5.9±3.6mmol/min; volitional -7.0±3.6 to -6.9±6.1mmol/min). Whole body RQ increased with FES cycling (0.88±0.02 to 0.95±0.02, p = 0.001, but did not change with volitional exercise (0.87±0.02 to 0.85±0.02, p = 0.55; See Fig 3D) and only FES cycling led to an increase in across-leg lactate VA differences and production (from -1.1±2.1 to 5.5±7.4 mmol/min, p<0.001 vs. from -0.9 ±1.1 to -0.4±1.2 mmol/min, p = 0.70 Fig 3E) with very high inter-individual variability (See

Table 1. Baseline characteristics of study subjects.

Parameter	Mean±SD	N
Age (years)	31±8	14
Sex (M/F)	11/3	14
BMI (kg/m^2)	23.7±3.7	14
Body fat (%)	14±6	14
REE (kcal/day)	1901±356	13
RQ at rest	0.90±0.10	13
VO_{2MAX} (ml/kg/min)	41±6	13

Note: BMI = body mass index, REE = resting energy expenditure, RQ = respiratory quotient, VO_{2max} = peak oxygen consumption. Baseline data from one subject are unavailable due to technical problem with the machine.

https://doi.org/10.1371/journal.pone.0200228.t001

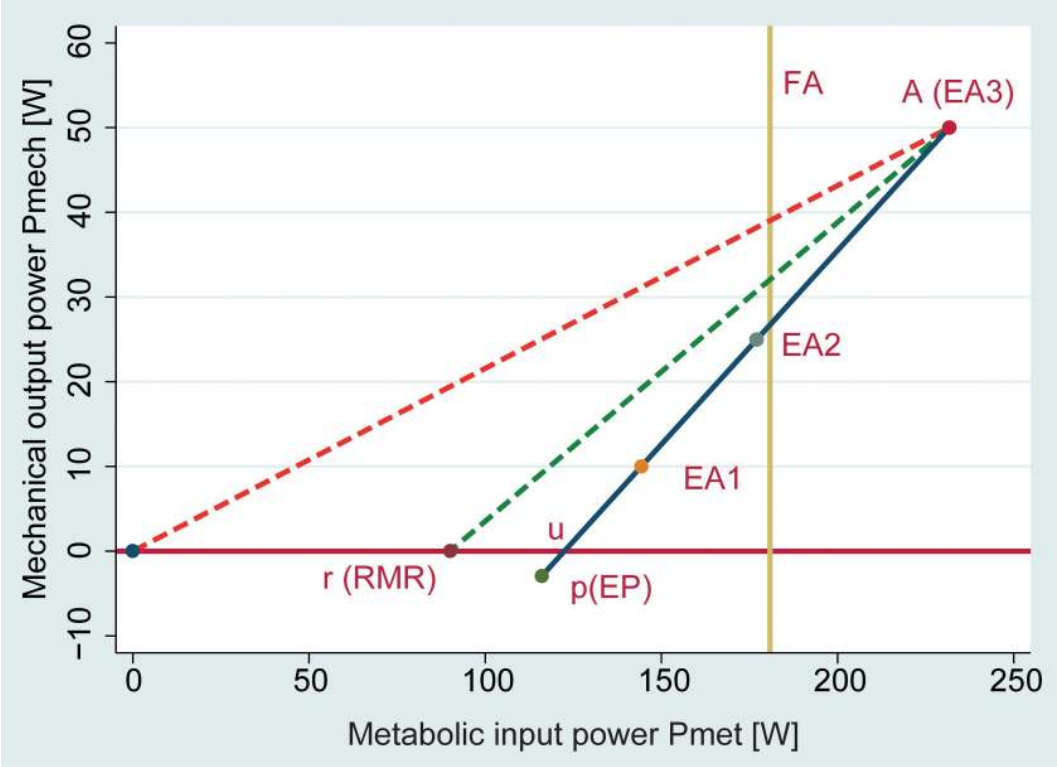

Fig 2. Hunt's diagram [2,22] outlining the efficacy of volitional exercise relative to metabolic cost of unloaded FES cycling (yellow line). Note: Metabolic efficiency is the gradient of the line joining the active cycling operating point (A) to one of the baseline conditions: u is unloaded cycling; r is rest, p is passive cycling.

https://doi.org/10.1371/journal.pone.0200228.g002

Fig 3F). Systemic arterial lactate levels remained normal after volitional cycling (from 1.6±0.6 mmol/l to 0.9±2.1 mmol/l, p = 0.887), and increased after FES cycling (from 1.6±0.7 mmol/l to 2.3±0.8 mmol/l, p<0.001).

Analysing lactate production

With FES cycling, there was a significant positive correlation between VA lactate difference and femoral venous haemoglobin saturation with oxygen (Spearman's R^2 = 0.6, p = 0.002, Fig 3G). Lactate producers had smaller veno-arterial difference in CO_2 content of the blood (R^2 = 0.3, p = 0.046, Fig 3H), effectively ruling out oxygen delivery problem. Subjects with femoral VA lactate difference >0.5 mmol/L ("lactate producers", n = 5, see Fig 3F) were compared with the rest of the group (n = 9) but no difference was found besides lactate having higher RQ at baseline (0.94±0.06 vs., 0.86±0.07, p = 0.034). Of note, stimulation current used during FES cycling was not different in lactate producers (42±10 vs. 44±16 mA, p = 0.87).

Amino acid metabolism

As expected in postprandial volunteers, at baseline resting skeletal muscle was taking up branched-chain amino acids (BCAAs) whilst producing Alanine (Ala). Skeletal muscle only produced Glutamine (Gln) at baseline in the volitional cycling group, otherwise the change was not significantly different from zero (Fig 4). Neither type of exercise led to a significant change of amino acid metabolism, but it is apparent from Fig 4 that with volitional cycling there was a trend to an increase in Ala production and a decrease of glutamine production,

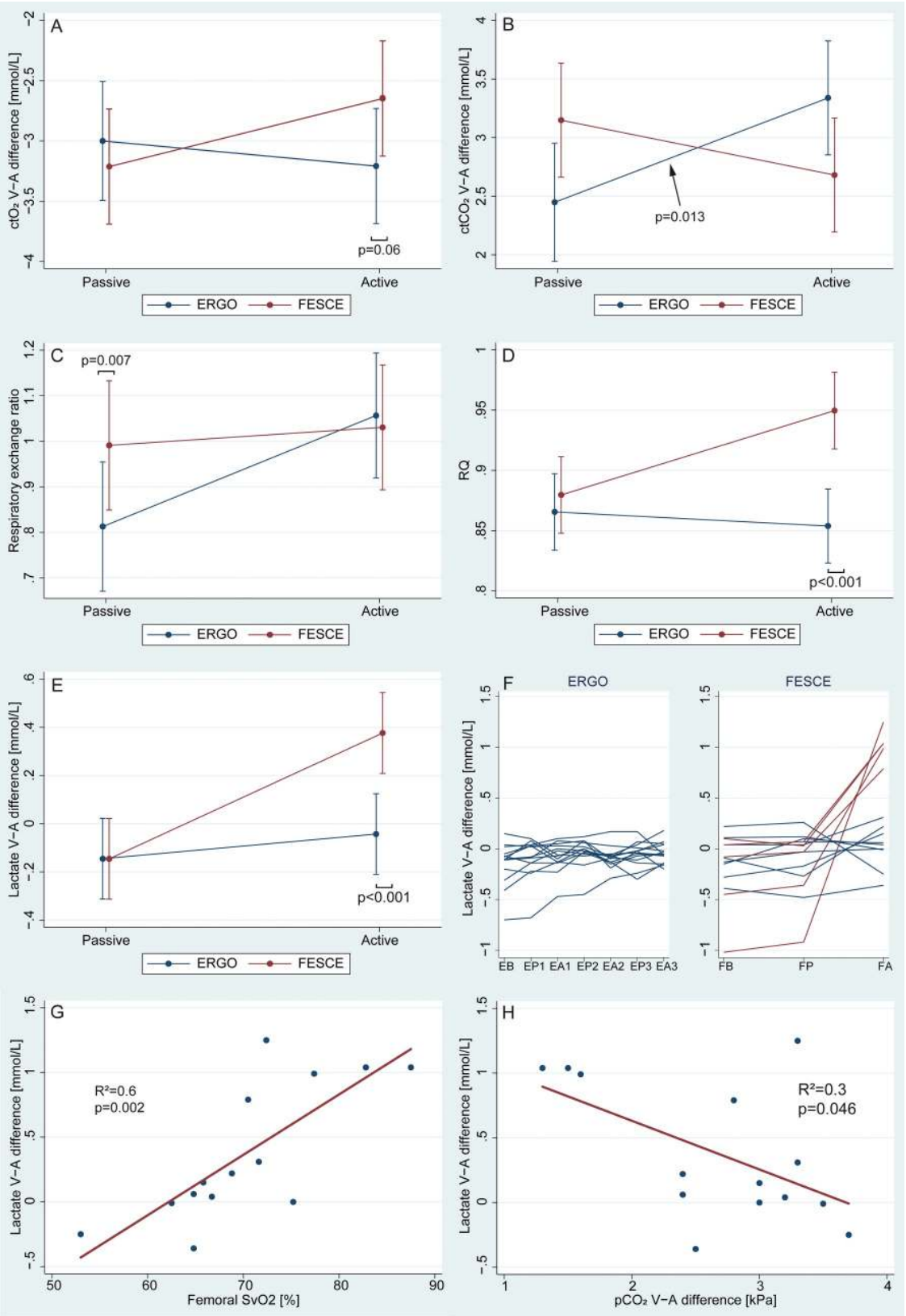
A
ctO₂ V−A difference [mmol/L]
p=0.06
Passive
Active
ERGO
FESCE
B
ctCO₂ V−A difference [mmol/L]
p=0.013
C
Respiratory exchange ratio
p=0.007
D
RQ
p<0.001
E
Lactate V−A difference [mmol/L]
p<0.001
F
ERGO
FESCE
EB EP1 EA1 EP2 EA2 EP3 EA3
FB FP FA
G
R²=0.6
p=0.002
Femoral SvO2 [%]
H
R²=0.3
p=0.046
pCO₂ V−A difference [kPa]

Fig 3. Venous-arterial (VA) differences studies. Lactate VA difference is derived from multiplying femoral VA differences of concentrations and calculated leg blood flow. See text for further details. Linear regression was used in G and H. Note: ctO_2 and $ctCO_2$ = total blood content of oxygen and carbon dioxide; RQ = whole body respiratory quotient; SvO_2 = femoral venous saturation of haemoglobin with oxygen. ERGO = volitional cycling; FESCE = functional electrical stimulation-assisted cycling; Passive period vs Active FES/50W volitional period.

https://doi.org/10.1371/journal.pone.0200228.g003

whilst after FES cycling no such a trend was apparent (across-leg amino acid exchange remained unaffected). Uptake of BCAAs continued and did not change with either type of exercise ($p = 0.83$ and $p = 0.86$).

Discussion

The major finding of our study is that unloaded supine FES cycling leads to lactate production without signs of muscle hypoperfusion, as low blood flow through exercising limbs would have caused femoral venous haemoglobin desaturation (Esaki et al., 2005; Sun et al., 2016) and widening of VA-CO_2 gap [42], which were not observed in our subjects. Moreover, there was a significant positive correlation between across-leg lactate production and femoral venous oxygenation, suggesting that subjects producing lactate did so whilst extracting less oxygen from (and producing less CO_2 into) the local circulation. There was a marked interindividual variability in metabolic response to FES cycling: some subjects responded to FES similarly to volitional cycling, whilst others produced so much lactate that it elevated systemic (arterial) lactate concentrations well above the normal range. We have not found any convincing characteristics of the subjects producing lactate during FES, although they seemed to be oxidizing more carbohydrates at baseline. Notably there was no correlation between the amplitude of stimulation current used and the production of lactate.

Tissue dysoxia and femoral venous desaturations are known to accompany lactate production during high intensity volitional exercise (i.e. > approx. 60% $VO_{2\ MAX}$) [43, 44, 45], at which oxidative phosphorylation becomes oxygen dependent. At lower exercise intensities, there is a concomitant lactate production in fast twitch glycolytic muscle fibres and consumption in slow twitch fibres [46] and—as seen in our subjects—during a steady low intensity volitional exercise, skeletal muscle may become a net lactate consumer [47].

The most obvious explanation of FES-driven lactate production would be tissue dysoxia, occurring despite adequate flow of oxygenated blood through major vessels. Non-physiological asynchronous contractions of large muscle units activated by FES [2,23] could have caused an inhomogeneous perfusion at the level of microcirculation, with hypoxic regions and units with luxurious perfusion acting as functional AV shunts. The increase in whole-body RQ with FES cycling, would support the presence of some degree of anaerobic metabolism, but it could also be explained by impaired fatty acid oxidation with the preference of carbohydrate substrates [39] or by primary increased ventilation. The major argument against microcirculatory impairment and anaerobic lactate generation is the absence of widening of venous-arterial CO_2 gap. Carbon dioxide is produced also anaerobically and released from bicarbonate as the consequence of buffering acid load in hypoxic tissue, and because CO_2 diffuses rapidly even from poorly perfused tissue, VA-CO_2 gap is regarded as a very sensitive marker of tissue hypoxia caused by impaired microvascular flow [48]. Not only VA CO_2 gap was not widened after FES cycling, but in was inversely proportional to lactate production. Moreover, the 138±29% increase in the whole body oxygen consumption after FES-cycling observed by us and others [49] would also argue against major oxygen delivery problem.

Lactate production without tissue dysoxia may occur as a result of the dysbalance between pyruvate production from glycolysis and its conversion to acetyl-CoA and oxidation in tricarboxylic acid cycle [46,47]. Muscle contraction instantly triggers, via the increase in $Ca^{2+}{}_{[IC]}$,

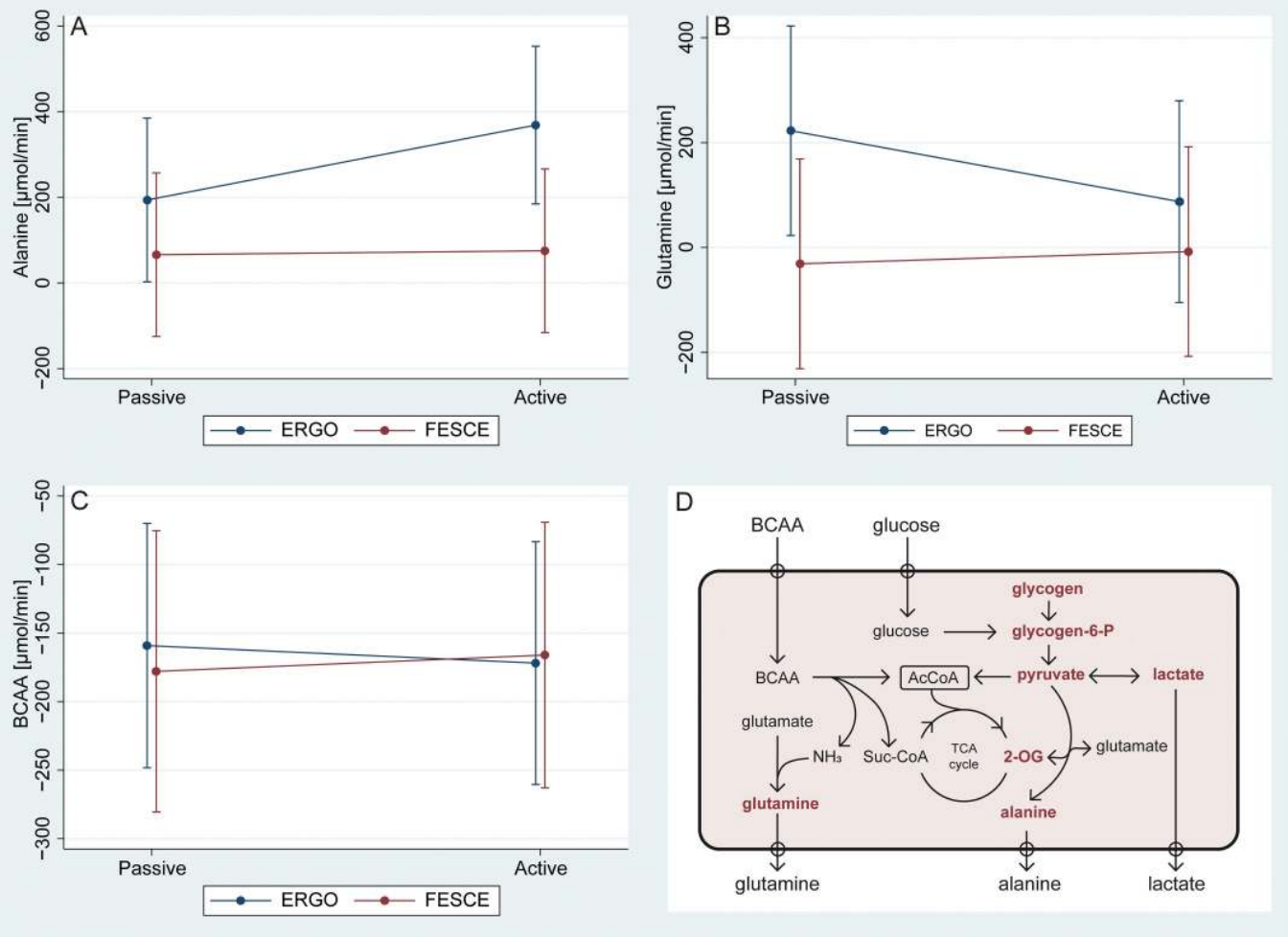

Fig 4. Amino acid metabolism during volitional and FES cycling. Values are derived from multiplying femoral VA differences of concentrations and calculated leg blood flow. Note: BCAA = branched-chain amino acids (i.e. the sum of Valine, Leucine, and Isoleucine); ERGO = volitional cycling; FESCE = functional electrical stimulation-assisted cycling; Passive period vs Active FES/50W volitional period. TCA = tricarboxylic acid cycle, 2-OG = 2-oxoglutarate.

https://doi.org/10.1371/journal.pone.0200228.g004

glycogenolysis and glycolysis, producing pyruvate. Sudden increase in cytosolic pyruvate concentration shifts the near-equilibrium reaction: *Pyruvate* + *Glutamate* ↔ *Alanine* + *2-oxoglutarate*, rightwards. Alanine is increasingly released during exercise and 2-oxoglutarate is believed to increase the functional capacity of tricarboxylic acid cycle [50] allowing for increase in oxidative ATP production. BCAAs uptake in skeletal muscle continues or even increases during exercise, providing carbons for oxidative pathways and nitrogen for Alanine and Glutamine formation (Fig 4D). Although non-significant, we have observed some trends to these responses after volitional cycling, but no rearrangement at all of amino acid metabolism was seen with FES exercise. Glycolytic compartment is known to respond much faster compared to oxidative phosphorylation and a rapid increase in cytosolic pyruvate concentration could lead to lactate release from cells even in the absence of tissue hypoxia [46]. Moreover, FES cycling compared to volitional exercise is known to activate glycogenolysis and glycolysis disproportionally faster than oxidative pathways [20,39]. In light of this, our data are consistent with aerobic lactate generation due to a dysbalance between pyruvate generation from glycogenolysis and glycolysis and its oxidation in citric acid cycle. Indeed, skeletal muscle is not a

metabolically homogenous tissue [47] and FES may preferentially trigger muscle contraction in glycolytic fast twitch fibres, whilst lactate oxidizing slow fibres may have been less sensitive to electrical stimulation. The sensitivity of different muscle fibres to external stimulation is unknown and remains to be studied, but a higher sensitivity of fast twitch fibres would be in keeping with the finding, that a long-term external electrical stimulation of a denervated muscle restores its mass and contractile power, but not fatigability [51].

From clinical point of view we found important the absence of venous haemoglobin desaturation during FES-cycling as decreased central venous saturation impairs systemic oxygenation in patients with a degree of intrapulmonary shunt. Mild lactic acidosis could be of concern in patients with impaired lactate clearance (e.g. liver failure). Unloaded FES cycling led to VO_2 response comparable to 25W volitional exercise, which would represent a very significant exercise load for critically ill patients, who tend to have even higher metabolic cost for a given power output [52] and only tolerated cycling at 3–6 W in one study [52]. Lastly, although the absence of laboratory signs of muscle damage and amino acid release is reassuring, the positive association of post-exercise serum myoglobin with stimulation current amplitude suggest a risk of muscle damage from the use of stimulation currents above 70mA, which are often needed to elicit visible contractions in sedated critically ill patient, perhaps due to their impaired muscle excitability [16].

The major weakness of our study is that we have not used direct measurements of leg blood flow and tissue oxygenation. We only use indirect indices, which prevents us from drawing any conclusions about the influence of FESCE on blood flow, which might have been altered, eg. by altered function of muscle pump. However, effects of FES exercise on leg blood flow are known [17,25] and the main finding of the study, i.e. lactate production without evidence of tissue hypoxia, can be supported by across-leg VA differences alone. Muscle tissue oxygen concentrations are known to be closely reflected by femoral venous oxygen content [43,53].

In conclusion, we have demonstrated that 10 min of supine FES cycling in healthy volunteers leads to production of lactate without features suggestive oxygen consumption/delivery mismatch, which are known to accompany lactate production during high intensity voluntary exercise [42,43]. Despite a significant increase in systemic oxygen consumption (proportional to 25W of volitional exercise) and unaltered across-leg glucose uptake with FES cycling, we have not observed the rearrangement of amino acid metabolism towards anaplerosis.

Supporting information

S1 Table. Dataset spreadsheet.
(XLSX)

Acknowledgments

The authors thank to Jana Potočková, Šárka Gregorová, Šárka Vosalová for technical assistance and to all healthy volunteers, who decided to participate in this study.

Author Contributions

Conceptualization: Jan Gojda, Kamila Řasová, František Duška.

Data curation: Petr Waldauf, František Duška.

Formal analysis: Jan Gojda, Petr Tůma, Kamila Řasová, František Duška.

Funding acquisition: František Duška.

Investigation: Jan Gojda, Petr Waldauf, Natália Hrušková, Barbora Blahutová, Adéla Krajčová, Tomáš Urban, Petr Tůma.

Methodology: Jan Gojda, Petr Waldauf, Barbora Blahutová, Tomáš Urban, Petr Tůma, František Duška.

Project administration: Jan Gojda, Natália Hrušková, Barbora Blahutová.

Resources: Kamila Řasová, František Duška.

Software: Petr Waldauf, Natália Hrušková, Barbora Blahutová, Adéla Krajčová, Petr Tůma.

Supervision: Kamila Řasová, František Duška.

Validation: Jan Gojda, František Duška.

Visualization: Petr Waldauf.

Writing – original draft: Jan Gojda, František Duška.

Writing – review & editing: Jan Gojda, Petr Waldauf, Adéla Krajčová, Tomáš Urban, Kamila Řasová, František Duška.

References

1. Glaser RM. Physiologic aspects of spinal cord injury and functional neuromuscular stimulation. Cent Nerv Syst Trauma. 1986; 3: 49–62. Available: http://www.ncbi.nlm.nih.gov/pubmed/3524868 PMID: 3524868
2. Hunt KJ, Fang J, Saengsuwan J, Grob M, Laubacher M. On the efficiency of FES cycling: a framework and systematic review. Technol Health Care. 2012; 20: 395–422. https://doi.org/10.3233/THC-2012-0689 PMID: 23079945
3. Szecsi J, Schiller M. FES-propelled cycling of SCI subjects with highly spastic leg musculature. NeuroRehabilitation. 2009; 24: 243–53. https://doi.org/10.3233/NRE-2009-0475 PMID: 19458432
4. Lo H-C, Hsu Y-C, Hsueh Y-H, Yeh C-Y. Cycling exercise with functional electrical stimulation improves postural control in stroke patients. Gait Posture. 2012; 35: 506–10. https://doi.org/10.1016/j.gaitpost.2011.11.017 PMID: 22153770
5. Peri E, Ambrosini E, Pedrocchi A, Ferrigno G, Nava C, Longoni V, et al. Can FES-Augmented Active Cycling Training Improve Locomotion in Post-Acute Elderly Stroke Patients? Eur J Transl Myol. PAGEPress; 2016; 26: 6063. https://doi.org/10.4081/ejtm.2016.6063 PMID: 27990234
6. Szecsi J, Schlick C, Schiller M, Pöllmann W, Koenig N, Straube A. Functional electrical stimulation-assisted cycling of patients with multiple sclerosis: Biomechanical and functional outcome–A pilot study. J Rehabil Med. 2009; 41: 674–680. https://doi.org/10.2340/16501977-0397 PMID: 19565162
7. Mohr T, Dela F, Handberg A, Biering-Sørensen F, Galbo H, Kjaer M. Insulin action and long-term electrically induced training in individuals with spinal cord injuries. Med Sci Sports Exerc. 2001; 33: 1247–52. Available: http://www.ncbi.nlm.nih.gov/pubmed/11474322 PMID: 11474322
8. Young W. Electrical Stimulation and Motor Recovery. Cell Transplant. 2015; 24: 429–446. https://doi.org/10.3727/096368915X686904 PMID: 25646771
9. Morris PE, Herridge MS. Early intensive care unit mobility: future directions. Crit Care Clin. Elsevier; 2007; 23: 97–110. https://doi.org/10.1016/j.ccc.2006.11.010 PMID: 17307119
10. Schweickert WD, Kress JP. Implementing Early Mobilization Interventions in Mechanically Ventilated Patients in the ICU. Chest. 2011; 140: 1612–1617. https://doi.org/10.1378/chest.10-2829 PMID: 22147819
11. Choong K, Koo KKY, Clark H, Chu R, Thabane L, Burns KEA, et al. Early Mobilization in Critically Ill Children. Crit Care Med. 2013; 41: 1745–1753. https://doi.org/10.1097/CCM.0b013e318287f592 PMID: 23507722
12. TEAM Study Investigators, Hodgson C, Bellomo R, Berney S, Bailey M, Buhr H, et al. Early mobilization and recovery in mechanically ventilated patients in the ICU: a bi-national, multi-centre, prospective cohort study. Crit Care. 2015; 19: 81. https://doi.org/10.1186/s13054-015-0765-4 PMID: 25715872
13. Pawlik AJ. Early Mobilization in the Management of Critical Illness. Crit Care Nurs Clin North Am. 2012; 24: 481–490. https://doi.org/10.1016/j.ccell.2012.05.003 PMID: 22920471

14. Friedrich O, Reid MB, Van den Berghe G, Vanhorebeek I, Hermans G, Rich MM, et al. The Sick and the Weak: Neuropathies/Myopathies in the Critically Ill. Physiol Rev. American Physiological Society; 2015; 95: 1025–109. https://doi.org/10.1152/physrev.00028.2014 PMID: 26133937

15. Herridge MS, Tansey CM, Matté A, Tomlinson G, Diaz-Granados N, Cooper A, et al. Functional Disability 5 Years after Acute Respiratory Distress Syndrome. N Engl J Med. 2011; 364: 1293–1304. https://doi.org/10.1056/NEJMoa1011802 PMID: 21470008

16. Kress JP, Hall JB. ICU-Acquired Weakness and Recovery from Critical Illness. N Engl J Med. 2014; 370: 1626–1635. https://doi.org/10.1056/NEJMra1209390 PMID: 24758618

17. Levine S, Nguyen T, Taylor N, Friscia ME, Budak MT, Rothenberg P, et al. Rapid Disuse Atrophy of Diaphragm Fibers in Mechanically Ventilated Humans. N Engl J Med. 2008; 358: 1327–1335. https://doi.org/10.1056/NEJMoa070447 PMID: 18367735

18. Parry SM, Puthucheary ZA. The impact of extended bed rest on the musculoskeletal system in the critical care environment. Extrem Physiol Med. BioMed Central; 2015; 4: 16. https://doi.org/10.1186/s13728-015-0036-7 PMID: 26457181

19. Parry SM, Berney S, Warrillow S, El-Ansary D, Bryant AL, Hart N, et al. Functional electrical stimulation with cycling in the critically ill: A pilot case-matched control study. J Crit Care. 2014; 29: 695.e1–695.e7. https://doi.org/10.1016/j.jcrc.2014.03.017 PMID: 24768534

20. Duffell LD, de N. Donaldson N, Newham DJ. Why is the Metabolic Efficiency of FES Cycling Low? IEEE Trans Neural Syst Rehabil Eng. 2009; 17: 263–269. https://doi.org/10.1109/TNSRE.2009.2016199 PMID: 19258202

21. Hunt KJ, Hosmann D, Grob M, Saengsuwan J. Metabolic efficiency of volitional and electrically stimulated cycling in able-bodied subjects. Med Eng Phys. 2013; 35: 919–925. https://doi.org/10.1016/j.medengphy.2012.08.023 PMID: 23253953

22. Hunt KJ, Ferrario C, Grant S, Stone B, McLean AN, Fraser MH, et al. Comparison of stimulation patterns for FES-cycling using measures of oxygen cost and stimulation cost. Med Eng Phys. Elsevier; 2006; 28: 710–8. https://doi.org/10.1016/j.medengphy.2005.10.006 PMID: 16298543

23. Downey RJ, Merad M, Gonzalez EJ, Dixon WE. The Time-Varying Nature of Electromechanical Delay and Muscle Control Effectiveness in Response to Stimulation-Induced Fatigue. IEEE Trans Neural Syst Rehabil Eng. 2017; 25: 1397–1408. https://doi.org/10.1109/TNSRE.2016.2626471 PMID: 27845664

24. Kjaer M, Perko G, Secher NH, Boushel R, Beyer N, Pollack S, et al. Cardiovascular and ventilatory responses to electrically induced cycling with complete epidural anaesthesia in humans. Acta Physiol Scand. Blackwell Publishing Ltd; 1994; 151: 199–207. https://doi.org/10.1111/j.1748-1716.1994.tb09738.x PMID: 7942055

25. Kim CK, Strange S, Bangsbo J, Saltin B. Skeletal muscle perfusion in electrically induced dynamic exercise in humans. Acta Physiol Scand. Blackwell Publishing Ltd; 1995; 153: 279–287. https://doi.org/10.1111/j.1748-1716.1995.tb09864.x PMID: 7625181

26. Scremin OU, Cuevas-Trisan RL, Scremin AM, Brown C V, Mandelkern MA. Functional electrical stimulation effect on skeletal muscle blood flow measured with H2(15)O positron emission tomography. Arch Phys Med Rehabil. 1998; 79: 641–6. Available: http://www.ncbi.nlm.nih.gov/pubmed/9630142 PMID: 9630142

27. Tepavac D, Schwirtlich L. Detection and prediction of FES-induced fatigue. J Electromyogr Kinesiol. 1997; 7: 39–50. Available: http://www.ncbi.nlm.nih.gov/pubmed/20719690 PMID: 20719690

28. Kim CK, Bangsbo J, Strange S, Karpakka J, Saltin B. Metabolic response and muscle glycogen depletion pattern during prolonged electrically induced dynamic exercise in man. Scand J Rehabil Med. 1995; 27: 51–8. Available: http://www.ncbi.nlm.nih.gov/pubmed/7792551 PMID: 7792551

29. Glaser RM. Physiology of Functional Electrical Stimulation-Induced Exercise: Basic Science Perspective. Neurorehabil Neural Repair. Sage PublicationsSage CA: Thousand Oaks, CA; 1991; 5: 49–61. https://doi.org/10.1177/136140969100500106

30. Gater DR, McDowell SM, Abbas JJ. Electrical Stimulation: A Societal Perspective. Assist Technol. Taylor & Francis Group; 2000; 12: 85–91. https://doi.org/10.1080/10400435.2000.10132012 PMID: 11067581

31. Tsutaki A, Ogasawara R, Kobayashi K, Lee K, Kouzaki K, Nakazato K. Effect of intermittent low-frequency electrical stimulation on the rat gastrocnemius muscle. Biomed Res Int. Hindawi; 2013; 2013: 480620. https://doi.org/10.1155/2013/480620 PMID: 23936807

32. Dreyer HC, Fujita S, Cadenas JG, Chinkes DL, Volpi E, Rasmussen BB. Resistance exercise increases AMPK activity and reduces 4E-BP1 phosphorylation and protein synthesis in human skeletal muscle. J Physiol. Wiley-Blackwell; 2006; 576: 613–24. https://doi.org/10.1113/jphysiol.2006.113175 PMID: 16873412

33. Hulston CJ, Wolsk E, Grondhal TS, Yfanti C, Van Hall G. Protein Intake Does Not Increase Vastus Lateralis Muscle Protein Synthesis during Cycling. Med Sci Sport Exerc. 2011; 43: 1635–1642. https://doi.org/10.1249/MSS.0b013e31821661ab PMID: 21364482

34. Biolo G, Maggi SP, Williams BD, Tipton KD, Wolfe RR. Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. Am J Physiol Metab. 1995; 268: E514–E520. https://doi.org/10.1152/ajpendo.1995.268.3.E514 PMID: 7900797

35. Holm L, van Hall G, Rose AJ, Miller BF, Doessing S, Richter EA, et al. Contraction intensity and feeding affect collagen and myofibrillar protein synthesis rates differently in human skeletal muscle. Am J Physiol Metab. 2010; 298: E257–E269. https://doi.org/10.1152/ajpendo.00609.2009 PMID: 19903866

36. Dideriksen K, Reitelseder S, Holm L. Influence of Amino Acids, Dietary Protein, and Physical Activity on Muscle Mass Development in Humans. Nutrients. 2013; 5: 852–876. https://doi.org/10.3390/nu5030852 PMID: 23486194

37. van Hall G, González-Alonso J, Sacchetti M, Saltin B. Skeletal muscle substrate metabolism during exercise: methodological considerations. Proc Nutr Soc. 1999; 58: 899–912. Available: http://www.ncbi.nlm.nih.gov/pubmed/10817157 PMID: 10817157

38. Tůma P. Rapid determination of globin chains in red blood cells by capillary electrophoresis using INST-Coated fused-silica capillary. J Sep Sci. 2014; 37: 1026–1032. Available: http://dx.doi.org/10.1002/jssc.201400044 PMID: 24677638

39. Kjær M, Dela F, Sørensen FB, Secher NH, Bangsbo J, Mohr T, et al. Fatty acid kinetics and carbohydrate metabolism during electrical exercise in spinal cord-injured humans. Am J Physiol Integr Comp Physiol. American Physiological SocietyBethesda, MD; 2001; 281: R1492–R1498. https://doi.org/10.1152/ajpregu.2001.281.5.R1492 PMID: 11641120

40. Jones B, Kenward MG. Chapter 5: Analysis of continuous data. In: Design and analysis of cross-over trials [Internet]. Available: http://researchonline.lshtm.ac.uk/2537976/

41. Soulele K, Macheras P, Silvestro L, Rizea Savu S, Karalis V. Population pharmacokinetics of fluticasone propionate/salmeterol using two different dry powder inhalers. Eur J Pharm Sci. 2015; 80: 33–42. https://doi.org/10.1016/j.ejps.2015.08.009 PMID: 26296862

42. Vallet B, Teboul J-L, Cain S, Curtis S. Venoarterial CO(2) difference during regional ischemic or hypoxic hypoxia. J Appl Physiol. 2000; 89: 1317–1321. https://doi.org/10.1152/jappl.2000.89.4.1317 PMID: 11007564

43. Sun Y, Ferguson BS, Rogatzki MJ, McDonald JR, Gladden LB. Muscle Near-Infrared Spectroscopy Signals versus Venous Blood Hemoglobin Oxygen Saturation in Skeletal Muscle. Med Sci Sport Exerc. 2016; 48: 2013–2020. https://doi.org/10.1249/MSS.0000000000001001 PMID: 27635772

44. Esaki K, Hamaoka T, Rådegran G, Boushel R, Hansen J, Katsumura T, et al. Association between regional quadriceps oxygenation and blood oxygen saturation during normoxic one-legged dynamic knee extension. Eur J Appl Physiol. 2005; 95: 361–370. https://doi.org/10.1007/s00421-005-0008-5 PMID: 16096839

45. Gladden LB. Lactate metabolism: a new paradigm for the third millennium. J Physiol. Wiley-Blackwell; 2004; 558: 5–30. https://doi.org/10.1113/jphysiol.2003.058701 PMID: 15131240

46. Gladden LB. Lactate metabolism: a new paradigm for the third millennium. J Physiol. Wiley-Blackwell; 2004; 558: 5–30. https://doi.org/10.1113/jphysiol.2003.058701 PMID: 15131240

47. Brooks GA. Intra- and extra-cellular lactate shuttles. Med Sci Sports Exerc. 2000; 32: 790–9. Available: http://www.ncbi.nlm.nih.gov/pubmed/10776898 PMID: 10776898

48. Mallat J, Lemyze M, Meddour M, Pepy F, Gasan G, Barrailler S, et al. Ratios of central venous-to-arterial carbon dioxide content or tension to arteriovenous oxygen content are better markers of global anaerobic metabolism than lactate in septic shock patients. Ann Intensive Care. Springer; 2016; 6: 10. https://doi.org/10.1186/s13613-016-0110-3 PMID: 26842697

49. Hettinga DM, Andrews BJ. Oxygen consumption during functional electrical stimulation-assisted exercise in persons with spinal cord injury: implications for fitness and health. Sports Med. 2008; 38: 825–38. Available: http://www.ncbi.nlm.nih.gov/pubmed/18803435 PMID: 18803435

50. Wagenmakers AJ. Muscle amino acid metabolism at rest and during exercise: role in human physiology and metabolism. Exerc Sport Sci Rev. 1998; 26: 287–314. Available: http://www.ncbi.nlm.nih.gov/pubmed/9696993 PMID: 9696993

51. Ashley Z, Sutherland H, Russold MF, Lanmüller H, Mayr W, Jarvis JC, et al. Therapeutic stimulation of denervated muscles: The influence of pattern. Muscle Nerve. 2008; 38: 875–886. https://doi.org/10.1002/mus.21020 PMID: 18563723

52. Hickmann CE, Roeseler J, Castanares-Zapatero D, Herrera EI, Mongodin A, Laterre P-F. Energy expenditure in the critically ill performing early physical therapy. Intensive Care Med. 2014; 40: 548–555. https://doi.org/10.1007/s00134-014-3218-7 PMID: 24477456

53. Mathewson KW, Haykowsky MJ, Thompson RB. Feasibility and reproducibility of measurement of whole muscle blood flow, oxygen extraction, and VO_2 with dynamic exercise using MRI. Magn Reson Med. 2015; 74: 1640–1651. https://doi.org/10.1002/mrm.25564 PMID: 25533515