Scandinavian Journal of Medicine & Science in Sports

WILEY

ORIGINAL ARTICLE OPEN ACCESS

The Rate of Leg Fat Oxidation Is Not Attenuated During Incremental Intensity One-Leg Knee Extensor Exercise

J. W. Helge[1] | C. E. Shannon[2] | B. Stallknecht[1] | F. B. Stephens[3] | P. L. Greenhaff[4] | F. Dela[1,5,6]

[1]Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark | [2]School of Medicine, University College Dublin, Dublin, Ireland | [3]Sport and Health Sciences, The University of Exeter, Exeter, UK | [4]David Greenfield Human Physiology Unit, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, UK | [5]CopenAge, Copenhagen Center for Clinical Age Research, Department of Geriatrics, Bispebjerg-Frederiksberg University Hospital, Copenhagen, Denmark | [6]Department of Human Physiology and Biochemistry, Riga Stradiņš University, Riga, Latvia

Correspondence: J. W. Helge (jhelge@sund.ku.dk)

Received: 15 February 2024 | **Revised:** 16 September 2024 | **Accepted:** 17 September 2024

Funding: This work was supported by the University of Nottingham BBSRC Doctoral Training Programme. The Danish ministry of Culture and Committee on Sports Research.

Keywords: carnitine | exercise | metabolism | muscle | one-leg exercise

ABSTRACT

It is not clear if fat oxidation is attenuated at higher exercise intensities, when exercising with a small muscle mass, and therefore, we studied leg fat oxidation during graded one-leg exercise. Ten males (age: 27 ± 2 years, body mass: 82 ± 3 kg, BMI: 24 ± 1 kg m^{-2}, $\dot{V}O_{2max}$: 49 ± 2 mL min^{-1} kg^{-1}) performed one-leg exercise at 25% of maximal workload (W_{max}) for 30 min, followed by 120-min exercise at 55% W_{max} with the contralateral leg, and finally 30-min exercise at 85% W_{max} with the first leg. Blood was sampled from an artery and both femoral veins, and blood flow was determined using Doppler ultrasound. Muscle biopsies were obtained before and after 30 min at each workload. One-way RM ANOVA was applied to determine the impact of exercise intensity. Data are expressed as mean ± SEM. From rest through exercise average blood flow (0.4 ± 0.1, 2.1 ± 0.1, 2.6 ± 0.2, 3.7 ± 0.2 L min^{-1}) and oxygen uptake across the leg (0.03 ± 0.01, 0.23 ± 0.02, 0.35 ± 0.03, 0.53 ± 0.04 L min^{-1}) increased with exercise intensity ($p < 0.001$). Leg RQ (0.76 ± 0.04, 0.86 ± 0.02,0.87 ± 0.01, 0.92 ± 0.01, $p < 0.001$), leg plasma FA uptake (2 ± 2, 46 ± 8,83 ± 9, 114 ± 16 μmol min^{-1}; $p < 0.001$) and rate of leg fat oxidation (0.016 ± 0.005, 0.062 ± 0.012, 0.075 ± 0.011, 0.084 ± 0.018 g min^{-1}, $p < 0.007$) increased with exercise intensity. Muscle-free carnitine content was unchanged from rest at 25% W_{max} and decreased after 30 min exercise at 55% and 85% W_{max} (17.4 ± 1.6, 16.6 ± 0.7, 14.5 ± 1.2, 10.5 ± 1.0 mmol/kg dry muscle, respectively; $p < 0.006$). During incremental one-leg exercise, the rate of leg fat oxidation was not attenuated with increasing exercise intensity, probably due to an insufficient muscle metabolic stress response.

1 | Introduction

During exercise, the choice of endogenous and exogenous muscle substrate is primarily affected by exercise intensity and duration, substrate storage prior to exercise and state of training [1, 2]. When exercise intensity is increased, carbohydrate oxidation, derived from plasma glucose and muscle glycogen, increases [3–6]. In contrast, fat oxidation reaches its maximum at moderate whole-body exercise intensities ranging between 45% and 65% of maximal oxygen uptake [7, 8]. The precise mechanism behind the attenuated fat oxidation at higher exercise intensities is not fully resolved. One explanation could be a decreased plasma fatty acid (FA) uptake [5], which has been linked to a decreased arterial plasma FA concentration, caused by a high-intensity attenuated adipose tissue blood flow and/or an increased adipose tissue FA re-esterification [9, 10]. Other potential mechanisms are intramuscular and include a muscle lactic acid-induced decrease in intracellular pH leading to decreased mitochondrial long-chain FA uptake

[11], and/or a decreased free muscle carnitine content that limits long-chain FA uptake through attenuation of the CPT transport system [12, 13]. Finally, it has been suggested that decreased intramitochondrial Coenzyme A (CoASH) content induced through high carbohydrate-derived pyruvate dehydrogenase complex flux might attenuate mitochondrial FA oxidation through an effect on CPT2 and/or through a direct effect on the last step in the β-oxidation [12].

The studies referenced above applied whole-body exercise to investigate the mechanism behind the attenuation of fat oxidation at higher exercise intensities, where we, in a prior study, applied a one-leg kicking model and observed that fat oxidation was not attenuated during graded one-leg high-intensity exercise, thus implying that systemic limitations are contributing to the attenuation of whole-body fat oxidation during high-intensity exercise [14]. However, in our previous study, the one-leg exercise was performed with both legs working at the same time in two independently controlled ergometers, one leg at a moderate workload and the other at a high workload, which will elicit a higher neuroendocrine response than when using only the thigh muscle of one leg [14]. Therefore, we cannot exclude that cross-talk between the legs and/or higher sympathetic activation may have influenced fat oxidation at the higher workload [14]. The one-leg kicking exercise presents a unique model to study the local influence of exercise intensity since substrate and oxygen delivery for the contracting muscle is not restricted by central circulatory capacity [15]. The present study aimed to investigate the effect of contraction intensity per se on human muscle fat utilization studied directly by the leg balance technique and muscle biopsies. Our hypothesis was that muscle fat oxidation would not be attenuated at one-leg high-intensity exercise and that the concomitant changes in muscle metabolites would be somewhat limited and below the thresholds required to attenuate fat transport and oxidation.

2 | Materials and Methods

Ten healthy males (age: 27 ± 2 years [mean ± SEM], body mass: 82 ± 3 kg, height: 183 ± 2 cm, BMI: 24 ± 1 kg m^{-2}, $\dot{V}O_{2max}$: 49 ± 2 mL kg^{-1} min^{-1}) gave their written consent to participate in the study. The study was approved by the Ethics Committee for Medical Research in Copenhagen (KF 11-055/03). Descriptive data from the current study have been previously published in a separate paper with a different focus [16]. The subjects were regularly active training two or more times per week and using bicycling as their preferred mode of commuting transport in their daily life.

2.1 | Protocol

Prior to the experiment, subjects were accustomed to exercise on the knee extension ergometer, and maximal work capacity (W_{max}) was determined for each leg as described by Andersen and Saltin [17]. This measurement was used to determine the dominant/non-dominant leg for each subject. At least 5 days before the first experimental day subjects performed a maximal oxygen uptake ($\dot{V}O_{2max}$) test on a bicycle ergometer using a standard progressive exercise test. Two experiments were performed, on 1 day a one-leg kicking exercise with arteriovenous catheterization and muscle biopsies and on a separate day a whole-body FATmax test. Subjects were asked to refrain from vigorous physical activity for 2 days prior to both experimental days. On both days, subjects arrived in the morning after a 12 h fast and abstinence from coffee, tea, alcohol, and tobacco. During the experiments, subjects wore light clothes, and the room temperature was 23 ± 1°C.

On the first experimental day, after an initial short rest, a catheter (Arterial Cannula with FloSwitch, Becton Dickinson, UK) was inserted in the brachial or radial artery for blood sampling. Furthermore, catheters were placed in both femoral veins under local anesthesia by an aseptic technique, and the tips were advanced to ~2 cm below the inguinal ligament in the antegrade direction. The catheters were kept patent by intermittent flushing with sterile sodium citrate throughout the procedure. The protocol consisted of a 15 min resting period and three consecutive periods of one-legged knee extension exercise (Figure 1). Subjects first exercised with one leg for 30 min at 25% of maximal

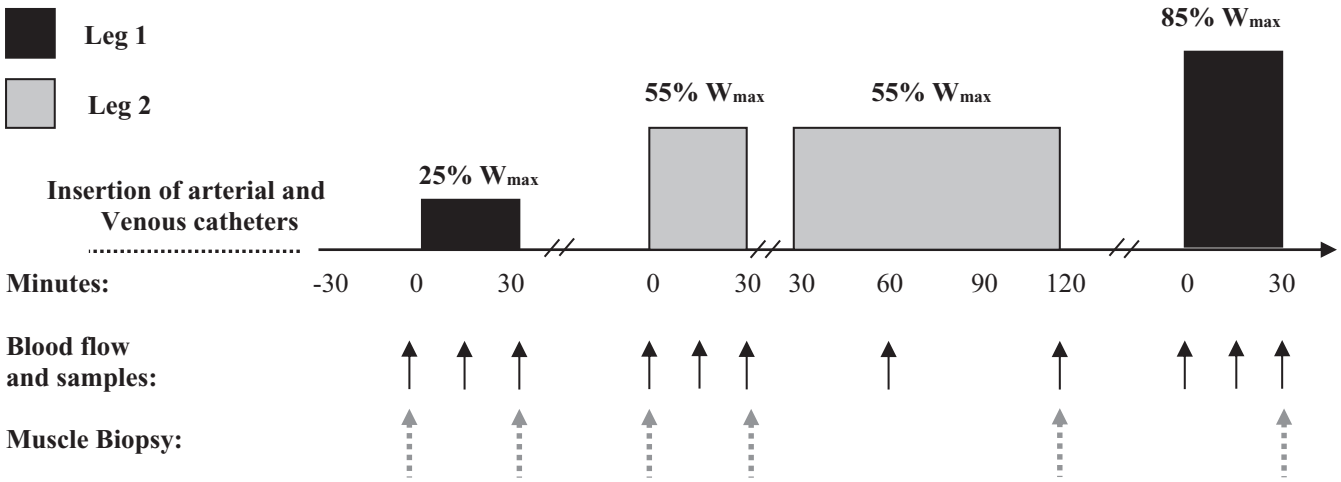

FIGURE 1 | Schematic diagram over the protocol of experimental Day 1. Ten males performed knee extension exercise with one leg at 25% W_{max} for 30 min while the other rested. After a 30-min rest, subjects exercised with the rested leg at 55% W_{max} for 30 + 90 min while the previously exercised leg rested. After another 30-min rest, subjects exercised with the first leg at 85% W_{max} for 30 min while the other leg was again resting.

work capacity (W_{max}), and after a 30 min rest, subjects exercised with the other leg for 30 min at 55% of W_{max} followed by a short break, to obtain a muscle biopsy, after which another 90 min of exercise was performed maintaining the same workload. After a 30 min rest, the subjects again exercised with the first leg for 30 min but now at 85% of W_{max} (Figure 1). The leg eligible for low-high respective moderate intensity was selected by randomized stratification, such that dominant and non-dominant legs were similarly represented. Both during the resting and the exercise periods, subjects were sitting in a chair with the torso strapped to the back of the chair. Subjects had free access to water throughout the experiment. Prior to each exercise bout and during exercise, blood flow was measured by the Doppler technique as previously described [18]. Whole-body pulmonary oxygen uptake and carbon dioxide excretion were measured regularly during exercise using an automated online system (Oxycon Champion, Erich Jaeger GmbH, Hoechberg, Germany).

Before and after each exercise period, a muscle biopsy was obtained from the vastus lateralis of the exercising leg. The muscle biopsy was performed under local analgesia (2–3 mL Lidocaine, 20 mg mL^{-1}) using the Bergström technique applied with suction. The incisions were made approximately 12–18 cm above the knee, and two samples were obtained from each incision, but in the opposite direction, distal or proximal to that of the first biopsy.

Thigh length, circumference, and skinfold measurements at three sites were used to determine thigh volume that was subsequently used to calculate quadriceps femoris muscle mass [19].

On the second experimental day, subjects arrived at the laboratories following an overnight fast. After an initial 30 min of rest in a chair, a graded cycle ergometer exercise test was performed to determine maximal fat oxidation. The exercise test has been described previously [20]. In brief, exercise is performed at 60 W for 8 min, and after this, the workload is increased by 35 W every third min until the respiratory exchange ratio exceeded 1.0, which implies that estimations of fat oxidation become irrelevant due to non-steady state conditions. Pulmonary oxygen uptake and carbon dioxide excretion were measured regularly during exercise using an automated online system (Oxycon Champion, Erich Jaeger GmbH, Hoechberg, Germany). The heart rate was recorded continuously with a PE 3000 Sports Tester (Polar Electro, Finland).

2.2 | Sampling and Analyses

Blood was sampled immediately before and every 15 min during the first 30 min of the exercise periods and at 60 and 120 min during the 120 min exercise period. Prior to each blood sample (15–20 s), a cuff placed under the knee was inflated to suprasystolic pressure to minimize the contribution from the lower leg. Blood for determination of hormones, metabolites and substrates was sampled into iced tubes and immediately centrifuged. Blood for determination of insulin was stabilized with trasylol and ethylenediaminetetraacetic acid solution (EDTA) and kept at −20°C until analysis. Arterial plasma insulin concentrations were determined by a commercial ELISA (DakoCytomation, Great Britain). Blood for determination of epinephrine was stabilized with reduced glutathione and EDTA and kept at −80°C until analysis. Arterial plasma epinephrine concentrations were determined by a commercial radioimmunoassay (highly sensitive 2 CAT RIA, Labor Diagnostika Nord, Germany). Plasma concentrations of triacylglycerol (TG) were determined by a standard commercially available assay.

Blood oxygen saturation, hematocrit, PCO_2, PO_2, and pH were measured with the Astrup technique (ABL 30, Radiometer, Copenhagen, Denmark). Hemoglobin was determined spectrophotometrically (ABL 30, Radiometer, Copenhagen, Denmark).

The muscle biopsies were frozen in liquid nitrogen within 10–15 s of sampling and were stored at −80°C until further analysis. Before biochemical analysis, biopsy samples were freeze-dried and dissected free of connective tissue, visible fat, and blood using a stereomicroscope. Muscle glycogen concentration was determined in separate independent duplicate analyses as glucose residues after hydrolysis of the muscle sample in 1 M HCL at 100°C for 2 h [21]. Muscle TG content was determined as described previously [22]. Muscle ATP, lactate, carnitine, acetylcarnitine, creatine phosphate, and creatine content were analyzed as previously described [23–25].

2.3 | Calculations

Uptake and release of substrates and metabolites over the leg were calculated from arterial and venous differences multiplied by plasma or blood flow, according to the Fick principle. The oxygen and carbon dioxide content in the blood was calculated [26], and from this, the respiratory quotient (RQ) across the leg was calculated [22]. Indirect calorimetry calculations were performed according to the stoichiometric equations given by Frayn [27]. FA oxidation was determined by converting the rate of fat oxidation (g kg^{-1} min^{-1}) to its molar equivalent, assuming an average molecular weight of FAs to be 286 g mol^{-1} [27].

To determine maximal whole-body fat oxidation, the respiratory exchange ratio was averaged across the last 90 s of each exercise step in the graded exercise test and fat oxidation was calculated using indirect calorimetry as indicated above. Maximal fat oxidation and the workload eliciting this were determined by simple polynomial fitting of the individually measured data as previously described in full detail [28]. In order to assess differences in substrate utilization across the exercise intensities delta changes were calculated and evaluated.

2.4 | Statistical Analyses

SigmaPlot for Windows version 15.0 (Systat Inc., Delaware, Ca, USA) was used for the statistical analysis. All data are presented as means ± SEM. One-way analysis of variance with intensity as a factor was used to test for overall differences between exercise intensities (one-way RM ANOVA) and changes with time at each workload and across exercise intensities (two-way RM ANOVA) at the same workload. To further discriminate changes, a Student–Newman–Keuls test was used post hoc to locate the differences. A significance level of 0.05 in two-tailed testing was chosen a priori.

3 | Results

One-leg knee extension exercise workloads were applied to induce exercise at 25%, 55%, and 85% W_{max} (Table 1). From rest through 30 min of exercise, whole-body oxygen uptake, whole-body relative exercise intensity, minute ventilation, and heart rate increased ($p < 0.001$) as exercise intensity was increased (Table 1). The respiratory exchange ratio was higher ($p < 0.05$) at 85% than 55% W_{max} (Table 1). Femoral leg blood flow increased ($p < 0.001$) progressively from rest through 30 min of one-leg knee extension exercise at 25%, 55%, and 85% W_{max} (Table 1). From rest through 30 min of exercise, leg oxygen uptake ($p < 0.003$) and leg RQ ($p < 0.002$) increased with exercise intensity and resting values were significantly different ($p < 0.05$) from exercise values (Table 1). On a separate day, maximal fat oxidation determined by a progressive, graded whole-body cycle exercise test was observed at an average workload corresponding to 48.1 ± 1.8% of maximal oxygen uptake, and on an individual basis the range of FATmax varied from 40.5% to 57.8% of maximal oxygen uptake (Figure 2A,B). These data clearly demonstrate that the rate of whole-body fat oxidation declined during exercise as the intensity surpassed the FATmax exercise intensity.

3.1 | Blood Data

During 30 min of exercise at 25%, 55%, and 85% of W_{max} arterial plasma glucose concentration remained unchanged (Table 2). Arterial plasma glucose concentration was lower ($p < 0.05$) after 15 min of exercise at 85% W_{max} than at 25% and 55% W_{max} (Table 2). The leg glucose uptake increased (main effect, $p < 0.002$) with exercise intensity from rest through exercise at 85% W_{max} (Figure 3A). The arterial plasma lactate concentration was lower ($p < 0.05$) at 0 min before exercise at 85% W_{max} than before exercise at 25% and 55% W_{max} (Table 2). During the 30 min of exercise, arterial plasma lactate concentration was higher ($p < 0.05$) at 85% W_{max} than during exercise at 25% and 55% W_{max} (Table 2). The leg lactate release increased (main effect, $p < 0.002$) with exercise intensity from rest through exercise at 85% W_{max} (Table 1).

The arterial plasma FA concentration was higher ($p < 0.05$) at 0 min before exercise at 85% W_{max} than at 25% and 55% W_{max} (Table 2). During each 30 min of exercise arterial plasma FA concentration was higher ($p < 0.05$) at 85% of W_{max} than at 25% and 55% W_{max} (Table 2). The leg plasma FA uptake increased ($p < 0.001$) with exercise intensity from rest through exercise to 85% W_{max} (Figure 4a). The arterial plasma glycerol concentration was higher ($p < 0.05$) at 0 min before exercise at 85% W_{max} than at 25% and 55% W_{max} (Table 2). During the 30 min of exercise, arterial plasma glycerol concentration increased ($p < 0.05$) at all workloads, and it was higher ($p < 0.05$) at 85% W_{max} than during exercise at 25% and 55% W_{max} (Table 2).

Arterial plasma TG concentration was similar prior to exercise and remained unchanged through the 30 min of exercise at each exercise intensity (Table 2). The arterial plasma TG concentration after 15 min of exercise at 85% W_{max} was lower ($p < 0.05$) than at 25% and 55% W_{max}. The arterial plasma insulin concentration during exercise was lower ($p < 0.05$) than at rest at 55% and 85% W_{max} (Table 2). The arterial epinephrine concentration remained unchanged at 25% and 55% of W_{max} but did increase ($p < 0.05$) significantly from rest (~20%) after 30 min exercise at 85% W_{max} (Table 2).

3.2 | Muscle Data

Quadriceps muscle mass was similar for the two legs, 3.11 ± 0.09 and 3.14 ± 0.10 kg. Prior to the exercise at 25%, 55%, and 85% W_{max} muscle glycogen content was similar 386 ± 14, 401 ± 18, and 400 ± 16 mmol kg^{-1} dm. (dry mass), respectively. Muscle glycogen breakdown during exercise increased significantly with exercise intensity increased ($p = 0.006$, one-way RM ANOVA; Figure 3B). However, post hoc analysis revealed net glycogenolysis during exercise was not different between the 25% and 55% W_{max} workloads.

TABLE 1 | Workload, whole-body cardiopulmonary variables and leg flow, leg oxygen uptake, and leg RQ at rest and during 30 min of one-leg kicking exercise at different exercise intensities.

Intensity	Rest	25% W_{max}	55% W_{max}	85% W_{max}	Main effect
Workload watt	—	13.2 ± 0.6^{a}	26.4 ± 0.7^{b}	43.8 ± 2.0^{c}	($p < 0.001$)
Heart rate, bpm	69 ± 1^{a}	81 ± 3^{b}	84 ± 4^{c}	107 ± 4^{d}	($p < 0.001$)
Whole-body $\dot{V}O_2$, ml min^{-1}	NA	610 ± 32^{a}	740 ± 40^{b}	1084 ± 53^{c}	($p < 0.001$)
Whole-body Rel. load, %	NA	15.4 ± 0.9^{a}	18.6 ± 1.1^{b}	27.2 ± 1.2^{c}	($p < 0.001$)
V_E, l min^{-1}	NA	17.8 ± 0.7^{a}	18.9 ± 1^{a}	31.4 ± 2.0^{b}	($p < 0.001$)
RER	NA	0.83 ± 0.02^{ab}	0.81 ± 0.01^{a}	0.86 ± 0.02^{b}	($p < 0.02$)
Leg RQ	0.76 ± 0.04^{a}	0.86 ± 0.02^{b}	0.87 ± 0.01^{b}	0.91 ± 0.01^{b}	($p < 0.003$)
Leg blood flow, l min^{-1}	0.4 ± 0.1^{a}	2.1 ± 0.1^{b}	2.6 ± 0.2^{c}	3.7 ± 0.2^{d}	($p < 0.001$)
Leg $\dot{V}O_2$ Uptake mL min^{-1}	34 ± 4^{a}	232 ± 17^{b}	347 ± 30^{b}	526 ± 37^{b}	($p < 0.002$)
Leg lactate release mmol min^{-1}	0.01 ± 0.01^{a}	0.16 ± 0.05^{a}	0.22 ± 0.06^{a}	1.77 ± 0.50^{b}	($p < 0.002$)

Note: Data were tested by one-way repeated ANOVA analysis. The whole-body relative load is the work expressed as % of maximal oxygen uptake. A difference in letter (example; a vs. b) denotes significant difference between values, e.g., exercise intensity levels ($p < 0.05$). Two letters indicate that the value is similar to the values that has either of the individual letters (ab similar to both a and b). Values are means ± SEM.

Nonetheless, at 85% W_{max} net glycogenolysis was greater than at both 25% and 55% W_{max}. Prior to the three exercise bouts, muscle TG stores were identical being 38 ± 4, 34 ± 4, and 35 ± 2 mmol kg^{-1} dm at 55% and 85% W_{max}, respectively. No changes were observed in muscle TG content after each 30-min exercise bout at any of the exercise intensities. The rate of leg fat oxidation during the 30 min was progressively greater ($p < 0.007$, one-way RM ANOVA) going from rest to exercise at 25%, 55% and 85% W_{max} (Figure 4B).

Muscle ATP, creatine phosphate, and creatine content remained unchanged across the different exercise intensities (Table 3). Muscle lactate content was significantly increased ($p < 0.05$, two-way RM ANOVA) above the resting state after exercise at all three exercise loads (Table 3). Muscle-free carnitine remained unchanged from rest during exercise at 25% W_{max}, but was significantly reduced ($p < 0.05$) step-wise at 55% and 85% W_{max}. Muscle acetylcarnitine was not significantly changed, except for an increase ($p < 0.05$) from 0 to 30 min during exercise at 55% W_{max}. Muscle total carnitine content remained unchanged with exercise intensity and time (Table 3, Figure 3C).

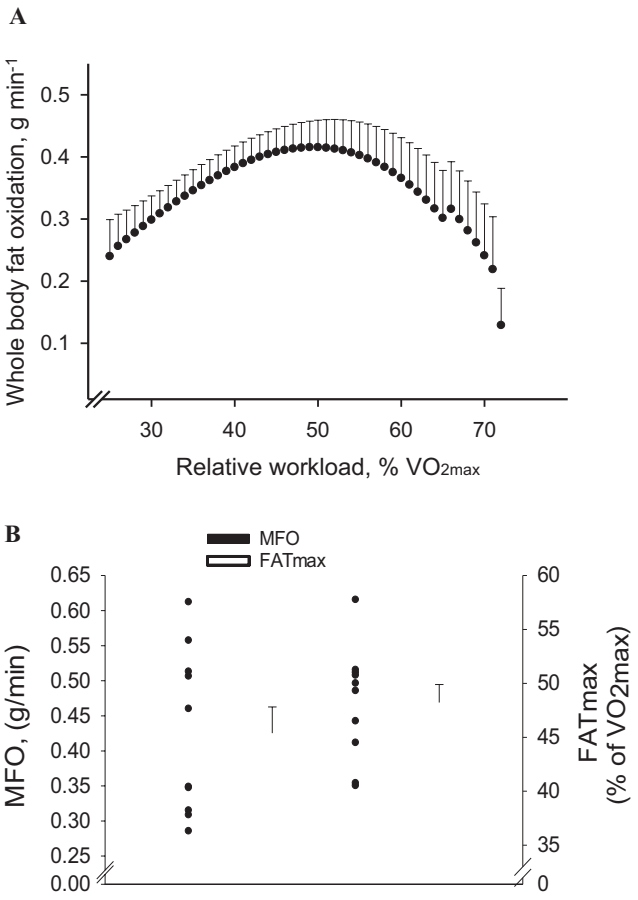

FIGURE 2 | Whole-body maximal fat oxidation (MFO) as a function of exercise intensity during a graded cycle ergometer exercise test using the FATmax protocol [20]. In subfigure (A), data points are based on an average of the mathematically modeled individual curves where fat oxidation is depicted as a function of relative exercise intensity ($N = 10$, $N = 9$ after 65% relative load) and subfigure (B) shows average and individual values for MFO and FATmax for all 10 subjects. Values are means ± SEM.

4 | All Data From the Prolonged Exercise at Moderate Intensity

Femoral leg blood flow remained constant at 2.6 ± 0.2 L blood per min during the 120 min of exercise at 55% W_{max}. Through the exercise at 55% W_{max}, leg oxygen uptake remained constant, whereas leg RQ decreased ($p < 0.05$, one-way RM ANOVA) from 0.87 ± 0.01 to 0.83 ± 0.01 at the end of the exercise. During exercise at 55% W_{max}, arterial plasma glucose and plasma lactate were decreased ($p < 0.05$) during the last 60 min of exercise (Table 2). Leg glucose uptake and lactate release remained unchanged through the 120 min of exercise. After the 120 min of exercise, muscle glycogen content was decreased to 314 ± 28 mmol kg^{-1} dm and across the last 90 min of exercise, muscle glycogen breakdown rate was decreased to 0.6 mmol kg^{-1} dm min$^{-1,}$ approximately 30% of the breakdown rate during the initial 30 min. Muscle TG stores remained unchanged at 35 ± 2 mmol kg^{-1} dm after 120 min. The rate of leg fat oxidation during the last 90 min at 55% W_{max} was increased ($p < 0.05$) to 0.086 ± 0.018 g min^{-1}.

The arterial plasma FA concentration was only increased ($p < 0.05$) after 120 min of exercise (Table 2). The leg plasma FA uptake increased through exercise to 147 ± 25 μmol min^{-1} after 120 min. The arterial plasma glycerol concentration increased through the exercise at 55% W_{max} (Table 2). Arterial plasma TG was lower after 60 and 120 min of exercise than at 0 min, whereas the arterial plasma epinephrine remained unchanged (Table 2).

5 | Discussion

The major finding of the present study was that the rate of leg fat oxidation during one-leg knee extensor exercise is not attenuated during high-intensity exercise compared to low and moderate-intensity exercise. However, the rate of whole-body fat oxidation decreases during high-intensity exercise when whole-body exercise was performed by the same volunteers. The most likely explanation for this difference between exercise modalities is that the magnitude of muscle metabolic stress during the one-leg exercise protocol was insufficient to limit muscle fat oxidation.

In a prior study of one-leg kicking exercise, similar fat oxidation across high, moderate, and low exercise intensity was observed, but this was using a design where moderate and high-intensity one-leg exercise was performed simultaneously, such that an interaction between the legs could not be excluded [14]. In the present study, where the exercise was performed only with one leg, we confirm this finding and observe a main effect of exercise intensity and if anything that the rate of leg fat oxidation increases with exercise intensity. This observation is in clear contrast to whole-body exercise, where the rate of fat oxidation peaks between 45% and 55% of maximal oxygen uptake [3, 5, 20, 29], in line with the whole-body fat oxidation peaking at 48 ± 2% for the subjects in this study. Dean and colleagues had young trained men perform one-leg kicking exercise for 60 min at 60% W_{max}, 10 min at 80% W_{max} and then exercise until exhaustion at 100% W_{max}, with a 10-min break between each bout [30]. With this approach, fat oxidation measured by whole-body

TABLE 2 | Arterial plasma substrate concentrations during one-leg kicking exercise at different exercise intensities.

Intensity	Time	Glucose mmol l^{-1}	Lactate mmol l^{-1}	FFA μmol l^{-1}	TG mmol l^{-1}	Glycerol mmol l^{-1}	Insulin pmol L^{-1}	Epinephrine nmol l^{-1}
Rest	Rest	5.4 ± 0.2	0.8 ± 0.1	354 ± 48	5.4 ± 0.2	34 ± 5	45 ± 6	1.1 ± 0.3
25% W_{max}	15 min	5.4 ± 0.1	0.8 ± 0.1	314 ± 41	5.4 ± 0.1	46 ± 5	41 ± 8	0.8 ± 0.3
	30 min	5.4 ± 0.2	0.8 ± 0.1	328 ± 39	5.4 ± 0.2	49 ± 4[a]	37 ± 5	0.9 ± 0.2
55% W_{max}	0 min	5.5 ± 0.2	0.8 ± 0.1	366 ± 43	5.5 ± 0.2	39 ± 2	NA	NA
	15 min	5.5 ± 0.2	1.0 ± 0.2	322 ± 44	5.5 ± 0.2	52 ± 5[a]	35 ± 5[a]	0.8 ± 0.1
	30 min	5.4 ± 0.1	0.8 ± 0.1	376 ± 51	5.4 ± 0.1	60 ± 5[a]	31 ± 4[a]	0.8 ± 0.1
	60 min	5.3 ± 0.1[a]	0.7 ± 0.1[b]	420 ± 33	5.3 ± 0.1[a]	62 ± 4[a]	30 ± 5[a]	0.7 ± 0.1
	120 min	5.2 ± 0.1[a,b]	0.6 ± 0.1[b]	676 ± 74[c]	5.2 ± 0.1[a,b]	87 ± 10[a]	25 ± 3[a]	0.8 ± 0.1
85% W_{max}	0 min	5.3 ± 0.2	0.6 ± 0.1[d]	655 ± 68d[c]	5.3 ± 0.2	58 ± 2[d]	NA	NA
	15 min	5.2 ± 0.1[d]	2.2 ± 0.4[a,d]	474 ± 35[d]	5.2 ± 0.1[d]	83 ± 5[c]	29 ± 5[a]	1.1 ± 0.3
	30 min	5.2 ± 0.1	1.9 ± 0.3[a,d]	781 ± 97[b,d]	5.2 ± 0.1	121 ± 14[a,b,d]	31 ± 5[a]	1.3 ± 0.1[a]

Note: Data were tested by two-way repeated ANOVA analysis. The 60 and 120 min value at 55% W_{max} are only tested against the 0, 15, and 30 min values at 55% W_{max}. Symbols indicate significant differences.
Abbreviations: FFA: free fatty acids, TG: triacylglycerol.
[a]$p < 0.05$ versus rest.
[b]$p < 0.05$ versus 15 min.
[c]$p < 0.05$ versus 60 min.
[d]$p < 0.05$ versus same time point other workloads. Values are means ± SEM.

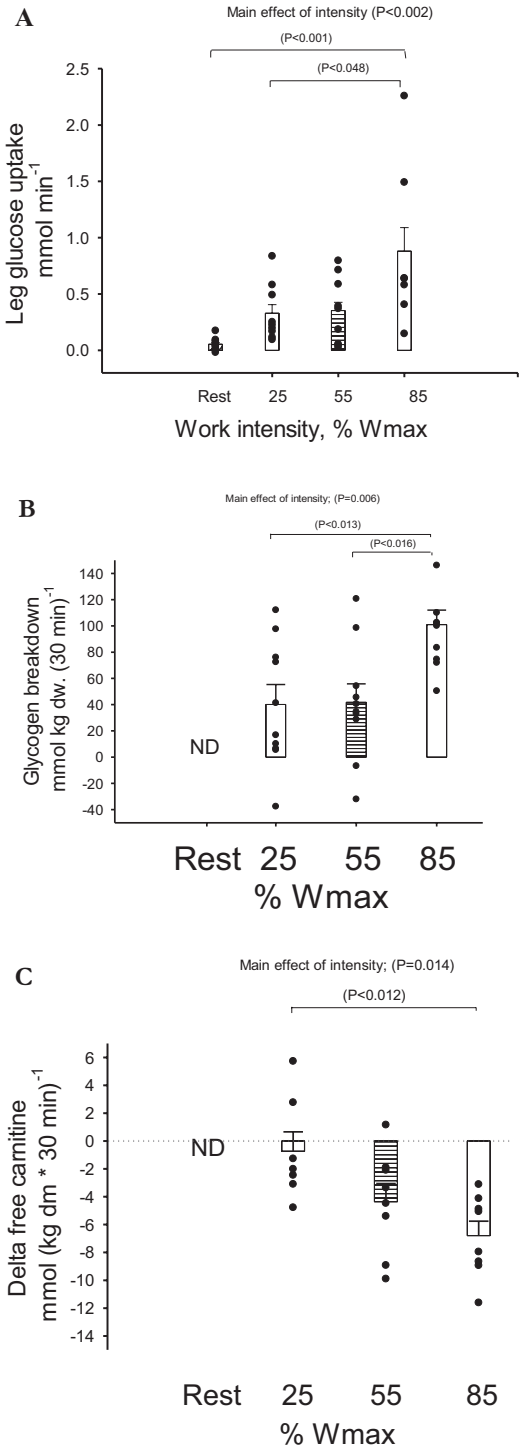

FIGURE 3 | Leg glucose uptake (A), muscle (vastus lateralis) glycogen breakdown (B and C) the change from rest in free muscle carnitine during 30 min of one-leg knee extension exercise at 25%, 55%, and 85% W_{max}. Individual values are indicated by the black circles ($N = 7–10$). Post hoc testing was performed to discriminate changes in workloads. Values are Means ± SEM. ND, not determined.

indirect calorimetry was similar at one-leg kicking exercise at 60% and 80% W_{max} but lower at 100% W_{max}. Overall, these findings support that the rate of fat oxidation is not attenuated during high-intensity single-leg exercise, whereas at maximal exercise intensity fat oxidation is attenuated [30].

The workloads selected in this study were similar to a prior study [14], and the aim was to reflect a similar muscle oxygen utilization per kg muscle mass as whole-body exercise. The estimation and comparison of muscle oxygen utilization between one-leg kicking and whole-body cycling exercise require several assumptions [14], and overall, the muscle oxygen uptake in this study at 85% W_{max} resembles the oxygen utilization when performing whole-body cycle exercise around 80%–85% of $\dot{V}O_{2max}$.

An interesting aspect of this study is to understand why the rate of fat oxidation was not attenuated at the higher exercise intensity during one-leg exercise. Currently, two of the primary mechanisms thought to attenuate fat oxidation during whole-body high-intensity exercise are a marked decline in muscle-free carnitine concentration as it becomes acetylated with increasing muscle carbohydrate flux and/or a lactic acidosis-induced decline in muscle pH, both of which are thought to attenuate CPT transport capacity and thus limit mitochondrial fat transport and oxidation [13]. Van Loon et al. [5] reported a 55% decrease of muscle-free carnitine content at 75 W_{max} (72 ± 2% $\dot{V}O_{2max}$) and a concomitant 35% decrease in the rate of FA oxidation. Similarly, Roepstorff et al. [31] found a 50% decrease in muscle-free carnitine content and a parallel decrease in fat oxidation at 55% $\dot{V}O_{2max}$. In the present study, muscle-free carnitine content declined by 39% at 85% W_{max} and importantly remained higher than the Km of CPT1 for carnitine (0.5 mM). In line with this, the rate of fat oxidation was not attenuated. Moreover, acetylcarnitine accumulation was not significantly increased, reflecting only modest activation of the pyruvate dehydrogenase complex and carbohydrate oxidation. In line with this Gibala and colleagues reported that estimations of TCA cycle flux actually exceed PDC flux during one-leg exercise, thus ameliorating the likelihood that PDC flux will attenuate TCA flux, as it has been proposed for whole-body exercise [32]. In the present study, muscle pH was not measured, but a muscle lactate concentration of 13.5 ± 2.9 mmol kg^{-1} dm and rate of leg lactate release of 1.8 ± 0.5 mmol min^{-1} at 85% W_{max} strongly point to muscle lactic acidosis being unlikely to have attenuated muscle carnitine palmitoyl transferase (CPT) activity [11, 33]. In short, a decrease of free carnitine content and increase in muscle lactic acidosis were insufficient to attenuate the rate of fat oxidation at the highest exercise intensity in the present single-leg exercise study. Furthermore, as the muscle (phosphocreatine) PCr did not change from rest, this indicates that the ATP demand of contraction was met wholly by mitochondrial energy production, suggesting that the metabolic stress of one-leg kicking exercise was limited even at 85% W_{max}. This observation of low muscle metabolic stress at 85% W_{max} is somewhat conflicting former studies where for example Gibala and Saltin [34] found decreased muscle PCr (~30%) and increased muscle lactate (8–34 mmol kg^{-1} dw.) using the one-leg model exercising at 70% W_{max} for 15 min. Also, Jones, Wilkerson, and Fulford [35] applying NMR found a marked reduction (60%) in muscle PCr at 80% of peak work rate in a custom-built NMR one-leg exercise model. However, it is difficult to compare the workload in this supine exercise model with the one-leg kicking model, and this may explain the difference in the observed muscle metabolic stress. When the one-leg kicking model was initially applied, Saltin and colleagues observed that compared to similar whole-body exercise there is a "super perfusion" of the muscle, which would favor oxidative metabolism and attenuate changes

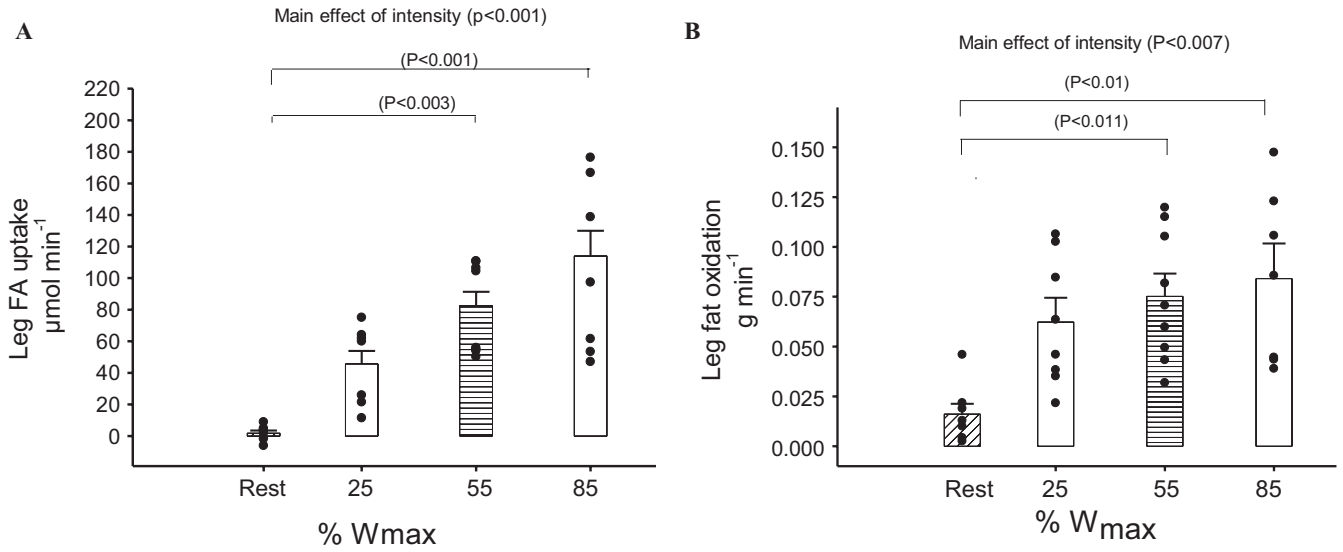

FIGURE 4 | Leg FA uptake (A) and leg fat oxidation (B) during 30 min of one-leg knee extension exercise at 25% and 85% W_{max} and 120 min at 55% W_{max}. Individual values are indicated by the black circles ($N = 7–10$). Values are Means ± SEM.

TABLE 3 | Muscle metabolites measured in biopsies obtained before and after the exercise loads ($N = 8$).

Intensity	Time	ATP mmol (kg dm)$^{-1}$	Lactate mmol (kg dm)$^{-1}$	Free carnitine mmol (kg dm)$^{-1}$	Acetyl carnitine mmol (kg dm)$^{-1}$	Total carnitine mmol (kg dm)$^{-1}$	Creatine phosphate mmol (kg dm)$^{-1}$	Plas creatine mmol (kg dm)$^{-1}$
25% W_{max}	0 min	25.7 ± 1.0	4.8 ± 0.6	17.4 ± 1.6	3.1 ± 1.7	20.5 ± 1.7	88.6 ± 2.0	54.0 ± 2.1
	30 min	26.0 ± 1.1	7.1 ± 1.2[a]	16.6 ± 0.7	4.3 ± 0.8	20.9 ± 1.2	87.0 ± 3.8	54.6 ± 2.6
55% W_{max}	0 min	25.9 ± 0.8	4.4 ± 0.4	18.9 ± 1.0	0.8 ± 0.2	19.7 ± 1.0	85.5 ± 2.4	56.0 ± 1.6
	30 min	26.2 ± 0.9	8.3 ± 2.2[a]	14.5 ± 1.2[a]	6.1 ± 1.9[d]	20.6 ± 1.6	87.8 ± 3.0	53.8 ± 3.2
	120 min	25.3 ± 0.9	5.3 ± 1.2	13.9 ± 1.5[c]	5.6 ± 2.1	19.5 ± 1.7	85.7 ± 2.1	56.5 ± 1.9
85% W_{max}	0 min	25.7 ± 0.6	5.2 ± 0.7	17.3 ± 1.0	2.3 ± 0.8	19.6 ± 0.9	87.5 ± 2.5	54.0 ± 2.2
	30 min	25.9 ± 0.7	13.5 ± 2.9[a]	10.5 ± 1.0[a,b]	8.1 ± 1.8	18.6 ± 1.6	80.9 ± 4.1	60.6 ± 2.9

Note: Data were tested by two-way repeated ANOVA analysis. The values obtained after 120 min exercise at 55% W_{max} were only compared to values obtained at the 0 and 30 min at this exercise workload. Symbols indicate significant differences.
[a] $p < 0.05$ 0 min versus 30 min.
[b] $p < 0.05$ 85% W_{max} versus (25% and 55% W_{max}).
[c] $p < 0.05$ 120 min versus 0 min.
[d] $p < 0.05$ within 55% W_{max} (0 versus 30 min). Values are means ± SEM.

in muscle AMP and ADP [15, 36]. In a review, Jeppesen and Kiens proposed that the lower epinephrine response during one-leg kicking, compared to similar whole-body exercise, would lead to a lower muscle glycogen breakdown and thus a lower glycolytic flux in the muscle and a higher fat oxidation [37]. In the present study, glycogen breakdown across the 30-min exercise at 85% W_{max} was 83 ± 14 mmol kg dm, whereas in the study by van Loon et al. the breakdown across 30 min at 75% W_{max} (72 ± 2% $\dot{V}O_{2max}$) was 186 mmol kg dm, which suggest that indeed muscle glycogen breakdown was lower during exercise at comparable mechanical workloads. Interestingly, we observed muscle glycogen breakdown to be 165 ± 25 mmol kg dm during 35 min of exercise at 85% W_{max} [14], but this was with a study design where both legs simultaneously performed one-legged exercise, and the epinephrine response was 160% greater (rest, 0.73 ± 0.15–1.9 ± 0.6 nmol l^{-1}) compared to 20% greater from rest to exercise in the present study (rest, 1.1 ± 0.3–1.3 ± 0.1 nmol l^{-1}). Overall, this indicates that the muscle glycogen degradation is indeed lower during one-leg kicking exercise compared to whole-body exercise at a similar workload, and this in combination with the higher blood perfusion capacity during one-leg exercise, facilitates that the ability to oxidize fat is not attenuated until near maximal exercise loads are achieved. The present observation that the rate of muscle fat oxidation was not limited during one-leg high-intensity exercise, but clearly was limited during whole-body high-intensity exercise performed by the same volunteers on a separate day, clearly points to systemic factors and/or an interaction between systemic factors and contracting muscle being key to the regulation and attenuation of the rate of fat oxidation during high-intensity exercise. Further

studies are needed to fully elucidate the influence of the interaction between systemic substrate availability and local muscle regulatory mechanisms controlling fat oxidation in skeletal muscle.

5.1 | Limitations

In the present study, we applied a one-leg kicking model, and it is necessary to discuss the possible limitations of this model. We elected to do the invasive parts of the experiments on one experimental day to avoid arterio-venous catheterization on two or more separate days and to avoid potential differences in diet and physical activity patterns in the days leading up to the experiments. With this selected design, the leg that performed high-intensity exercise had previously also performed low-intensity exercise. Despite the prior low-intensity exercise, muscle glycogen stores were similar before the low and high exercise intensity. Another concern is the higher arterial plasma FA concentration during high intensity compared to low and moderate intensity exercise. The higher plasma FA concentration was probably induced both by the prolonged exercise and the extended duration of the fasting state. In this model where super perfusion is possible, an increase in both leg blood flow and arterial substrate concentration will increase delivery. It is well known that FA delivery influences plasma FA uptake [38], which may explain part of the higher plasma FA uptake and oxidation. However, Romijn and colleagues observed that with a very high plasma FFA concentration induced by intravenous lipid heparin infusion the decline in fat oxidation at whole-body high-intensity exercise was not offset [9]. We observed that fat oxidation during whole-body exercise peaked at 48% of $\dot{V}O_{2max}$ and that fat oxidation increased with 120 min of prolonged exercise, thus demonstrating the normal expected effects of exercise on fat oxidation in the subjects included in the study.

In conclusion, we have demonstrated that the rate of muscle fat oxidation is not attenuated during exercise of increasing intensity when single-leg exercise is performed, and this can be explained by the magnitude of muscle metabolic stress being less than that seen during cycle or two-legged exercise where the rate of fat oxidation is known to decline. Furthermore, the lower muscle glycogen breakdown during single-leg exercise due to lower sympathetic activation may contribute to the observed maintenance of fat oxidation rates. The present findings imply that the attenuation of fat oxidation during high-intensity whole-body exercise is due to systemic factors and/or possibly interaction between systemic factors and muscle intermediary metabolism.

5.2 | Perspective

In this study, we extend and confirm findings from a prior study [14] and demonstrate that for exercise with a limited muscle mass, here the one-leg kicking model, fat oxidation is not attenuated during high-intensity exercise. Based on the features of the one-leg model, differences in the ability to enhance leg blood flow versus increasing the arterio-venous difference is likely to be key to this finding. Further work utilizing these exercise models may allow us to tease out the specific mechanism that explains the attenuation of fat oxidation at high-intensity whole-body exercise.

Author Contributions

J.W.H., F.D., and B.S. designed the research. J.W.H., B.S., F.D., performed the study. J.W.H. and C.E.S. performed the analysis. J.W.H. and C.E.S. performed the statistical analysis. J.W.H. made graphs and tables. All authors contributed to the interpretation of the data. J.W.H. wrote the first draft of the manuscript and all authors read, contributed to drafts, and approved the final manuscript.

Acknowledgments

We highly appreciate the expert technical assistance of Thomas Beck, Jeppe Bach, and Regitze Kraunsøe. The study was supported by The Danish Ministry of Culture Committee on Sports Research and the University of Nottingham BBSRC Doctoral Training Programme.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Compliance

The present manuscript is in compliance with the statistics policy and the human ethics policy outlined in Scand. J. Sports Med. The contact details of the Data Protection Officer of University of Copenhagen is dpo@adm.ku.dk.

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