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Peripheral adaptations to endurance training—Effect of active muscle mass

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Abstract

The purpose of this study was to evaluate whether adaptations to endurance training are affected by active muscle mass during training. Eleven healthy subjects performed 5 weeks of one-legged knee extension (1-KE) training with both legs, separately. During the 1-KE workouts for one of the legs, arm cycling was added (1L2A), while the other leg only performed 1-KE (1L). During the training sessions, the 1-KE power output and session duration were equal for both legs. Whole-body oxygen uptake (VO_2), adrenaline, and noradrenaline plasma concentrations were 112% ± 11%, 139% ± 144%, and 197% ± 101% higher during 1L2A than 1L, respectively. However, this did not affect the 1-KE training adaptations since submaximal O_2-cost, heart rate and blood lactate concentration, maximal $\dot{V}O_2$, and power output all improved equally in both legs. This was supported by similar capillarization and concentration of oxidative enzymes in the legs after the training period. The present study therefore indicates that the size of active muscle mass per se during exercise does not affect adaptations to endurance training.

KEYWORDS

arm cranking, muscle fiber, pulmonary oxygen uptake, single-leg, skeletal muscle, training adaptation

1 | INTRODUCTION

Whole-body endurance training potentially leads to both central and peripheral adaptations. The peripheral adaptations include muscular capillarization and increased oxidative capacity.[1] However, how the peripheral adaptations are regulated is not fully understood and there are contradicting findings regarding the importance of different factors such as high mass-specific skeletal muscular energy turnover and high local metabolic stress. For example, comparable endurance training studies (ie, similar subject and training characteristics) with one-legged exercise and whole-body exercises exhibit approximately the same skeletal muscular adaptations. Typically 20%-30% increased capillarization and 20%-40% increases in oxidative enzymes activity after 6-8 weeks of training are reported.[2-5] This happens though one-legged exercises clearly provide a higher available capacity for mass-specific skeletal muscular energy turnover than whole-body exercises.[6,7] On the other hand, when exercising with a large muscle mass, the sympathetic response is higher than when exercising with a small muscle mass.[8] This is potentially beneficial for muscular adaptations.

It has previously been shown that treatment with β-adrenergic blockers attenuates the acute increase in gene expression of the transcriptional coactivator peroxisome proliferator-activated receptor-ϒ coactivator (PGC-1α), known as a key regulator of mitochondrial biogenesis, after endurance exercise.[9,10] In addition, β-adrenergic blockers seem to hamper the mitochondrial adaptations to endurance training.[11,12] However, these studies are also accompanied by contradictory findings.[13-15]

Brandt et al[16] compared acute responses to matched cycling workloads (corresponding to 60% $\dot{V}O_2$max) for 60 minutes with or without the addition of 1-minute bouts of intermittent arm cycling every 5 minutes (~100 watts of additional workload). The addition of arm cycling resulted in a higher plasma adrenaline concentration. However, the PGC-1α mRNA response was similar. The same was the phosphorylation of upstream targets assumed to upregulate PGC-1α (ie, cAMP-response element binding, p38 mitogen-activated protein kinase [p38 MAPK], and AMP-activated protein kinase [AMPK]). Somewhat contrary to this finding did Fiorenza et al[17] observe a higher exercise-induced phosphorylation of p38 MAPK and Ca^{2+}/calmodulin-dependent protein kinase II (CAMKII) after speed endurance exercise compared with repeated sprints and high-volume continuous moderate-intensity cycling. The speed endurance protocol was associated with greater catecholamine response than the other two protocols, in addition to the most marked metabolic perturbations as evidenced by the greatest changes in muscle lactate and pH. However, the PGC-1α mRNA response was similar between speed endurance and continuous cycling, while it was lower after repeated sprint exercise.

Whether differences in non-pharmacologically induced sympathetic response per se can influence skeletal muscular adaptations to endurance training in humans has not previously been reported. Abbiss et al[18] found higher improvement in markers of oxidative enzymes (ie, cytochrome *c* oxidase subunit II and IV protein concentration) after 3 weeks of one-legged cycling compared to bicycling, indicating that training with a small active muscle mass is more favorable for local muscular adaptations compared to whole-body exercise. However, because leg workload, and thus leg energy turnover, was higher during one-legged cycling (13%), such a study design is not appropriate for evaluating the importance of active muscle mass per se since not all confounding factors are taken into account. The study of MacInnis et al[19] compared training adaptations of work-matched one-legged moderate-intensity continuous and high-intensity interval cycling training. They concluded that the high-intensity interval protocol elicited superior mitochondrial adaptations, due to the greater increase in citrate synthase activity and mass-specific oxidative phosphorylation capacities than continuous exercise. The high-intensity exercise probably induced a higher catecholamine response due to the higher exercise intensity, but it probably also induced greater metabolic perturbations as seen in Fiorenza et al,[17] which makes it difficult to evaluate causality in the training adaptations.

The purpose of the present study was therefore to test whether local training adaptations are affected by the size of the active muscle mass per se during exercise. This was evaluated by adding arm cycling to one-legged knee extension (1-KE) for one of the legs only, while the other leg was kept as a control, performing ordinary 1-KE. Previously, it has been shown that O_2 delivery to the exercising leg in healthy subjects is maintained during combined arm cycling and 1-KE.[8,20] Therefore, with this model the legs can exercise with equal power output and energy demand. The maintained oxygen supply in this model should ensure similar metabolic stress in the exercising leg muscles even with the addition of arm cycling. However, whole-body energy turnover and catecholamine response will most likely increase with added arm cycling. We hypothesized that the addition of arm cycling to the 1-KE exercise would elicit greater training adaptations.

2 | METHODS

Eleven healthy subjects (two females) provided informed consent to participate in the study (Table 1). The project was approved by Norwegian Regional Committee for Medical Research Ethics and performed according to the Declaration of Helsinki. The subjects were familiarized to the testing and training protocol over a minimum of 4 days to exclude familiarization effects. They were instructed to maintain their usual physical activity level during the study and abstain from strenuous exercise 24 hours prior to the experimental trials.

2.1 | Training protocol

Each subject took part in 3- to 4-weekly training sessions for 5 weeks. The training consisted of dynamic 1-KE with a frequency of 60 contractions per min, performed separately on the subject's left and right legs using a custom-designed electromagnetic braked ergometer[21] that addressed external work to only the *quadriceps femoris* muscle.[22] One of the legs (randomized for left or right leg) was trained simultaneously with two-arm cycling (1L2A) (Excalibur Sport; Lode BV), while the arms were kept relaxed when the control leg was trained (1L; Figure 1). However, the workloads of the legs were equal. The two training exercises were performed in the same session with a 10-minute break between sessions to avoid any carry-over sympathetic response effect, as catecholamines have a half-life in plasma of ~2 minutes.[23] The training sessions therefore consisted of two 20- to 30-minutes bouts (gradually increased from 20-minutes bouts in week

TABLE 1 Subjects' characteristics

Age (years)	**27 ± 5**
Height (cm)	179 ± 8
Mass (kg)	80 ± 11
Body mass index	25 ± 2
$\dot{V}O_2$max (mL kg^{-1} min^{-1})	52 ± 6

Note: Values presented as mean ± standard deviation. $\dot{V}O_2$max, maximal oxygen uptake (pulmonary) (n = 11).

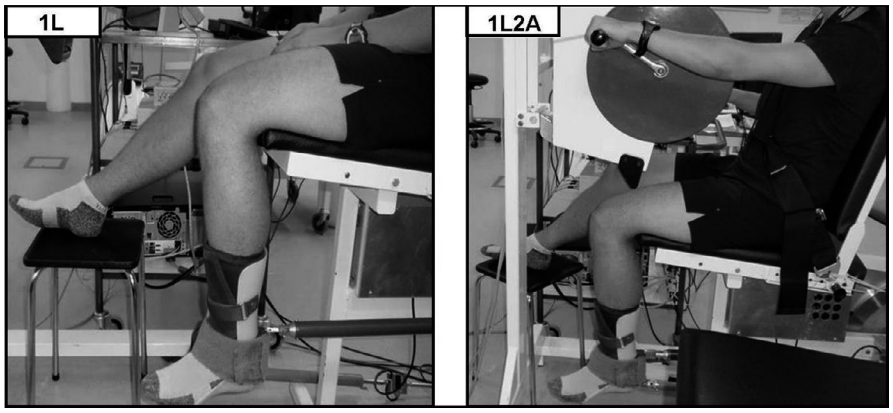

FIGURE 1 The training exercises 1L (left) and 1L2A (right)

1, 25-minutes bouts in weeks 2-3, and 30-minutes bouts in weeks 4-5), one being 1L and the other being 1L2A. The workloads were individually adjusted daily, with the aim of nearly exhausting the subjects at the end of the exercise bout, but always with the same power and duration for both legs during the same workout. This corresponded to an average 1-KE exercise intensity of 73% ± 3% of baseline peak power output (range, 67%-78%), while the arm cycling exercise intensity during 1L2A was 54% ± 5% (range, 45%-61%) of arm cranking peak power output. Start-up exercise was counterbalanced between 1L and 1L2A in the way that left and right legs were exercised first every second time.

During two separate workouts in the latter part of the training period, blood samples were taken immediately after finishing the first exercise bout of the workout to examine venous blood plasma concentrations of adrenaline and noradrenaline. The subjects arrived at the laboratory in the morning after an overnight fast or in the afternoon having fasted for 4 hours before training. Vacutainer® glass tubes (Becton Dickinson) with added EGTA-glutathione were used for the blood collection. The glass tubes were pretreated at the Section of Specialized Endocrinology (Clinic of Medicine, Oslo University Hospital) and later analyzed there. During exercise, measurements of pulmonary oxygen consumption ($\dot{V}O_2$), ventilation, and respiratory exchange ratio were carried out using an automated system (JAEGER Oxycon PRO™; Carefusion GmbH), which was calibrated according to the instruction manual. In addition, heart rate was monitored continuously with a Polar monitor (Polar Electro Oy) and a fingertip capillary blood sample was analyzed for lactate concentration after completing the exercise (YSI 1500 Sport; Yellow Springs Instruments).

2.2 | Exercise tests

Prior to and after the training period, a set of exercise tests including bicycling, 1-KE, and arm cycling were performed.

Maximal pulmonary oxygen uptake ($\dot{V}O_2$max) was tested on a bicycle ergometer (Excalibur Sport; Lode BV). A stepwise incremental protocol was used, and the initial power output was individually set to elicit exhaustion after approximately 6 minutes. Power output was increased by 20 or 25 watts (W) each minutes for women and men, respectively. Exhaustion was defined as when the subject could not maintain a cadence of 80 revolutions per min (RPM). $\dot{V}O_2$, ventilation, respiratory exchange ratio, and heart rate were measured continuously, while tests of capillary lactate concentration and ratings of perceived exertion[24] were completed afterward. Time to exhaustion was noted during all maximal tests (mm:ss).

On a separate day, the subjects underwent 1-KE and arm cycling tests. Initially, three 5-minutes submaximal 1-KE workloads were completed (at 20, 30, and 40 W). Thereafter, a maximal 1-KE stepwise incremental protocol of 5 W increase per min, starting at 20 W, was conducted on the same leg until exhaustion, which was defined as the inability to maintain the contraction frequency (60 RPM). After resting for 90 minutes, the tests were repeated with the subjects' contralateral legs. Finally, they did a stepwise incremental arm cycling protocol, starting at 30 or 40 W, and increased by 10 or 15 W every minute until exhaustion (RPM < 60), for women and men, respectively. $\dot{V}O_2$, ventilation, respiratory exchange ratio, and heart rate were measured continuously during all the tests, while measurements of capillary lactate concentration and ratings of perceived exertion were completed immediately after exhaustion. During maximal 1-KE, some subjects exhibited a more elevated $\dot{V}O_2$ at exhaustion than expected from the increases in workload, despite the subjects being fastened tightly with a four-point seat belt and trained to keep their non-exercising extremities as relaxed as possible. In accordance with Andersen et al,[22] we therefore corrected $\dot{V}O_2$max by extrapolating the $\dot{V}O_2$/power output relationship from submaximal values for all subjects. All the tests were conducted in the same environment with the same protocol, equipment, and test personnel.

2.3 | Biopsy

After the training period, muscle biopsies of the mid-portion of the *vastus lateralis* muscle were taken from the subjects'

1L- and 1L2A-trained legs under local anesthesia, as described in detail elsewhere.[25]

2.3.1 | Histochemical analysis

Initially, 8-μm cross sections from the biopsies were incubated for 30 minutes in BSA blocking solution (10% BSA in PBS, diluted 1:6 in deionized water; Thermo Fisher Scientific, Inc). Subsequently, they were incubated with primary antibodies against muscle fiber membrane (rabbit anti-dystrophin, diluted 1:500; Abcam plc®), myosin heavy chain type II (mouse anti-SC71, diluted 1:100; DSHB), and capillaries (mouse anti-CD31, diluted 1:100; Dako). Anti-dystrophin and anti-SC71 were incubated at room temperature for 60 minutes, while anti-CD31 was incubated overnight at 4ºC, followed by incubation with appropriate secondary antibodies (Alexa 488 or 494; Biotium Inc) for 60 minutes at room temperature. Between stages, sections were washed for 3 × 10 minutes in 0.05% PBS-t solution. The stained sections were afterward visualized using a high-resolution camera (DP72; Olympus Corp.) mounted on a microscope (BX61; Olympus Corp.) with a fluorescent light source (X-Cite 120PCQ; EXFO Photonic Solutions Inc). Fiber type distribution, fiber cross-sectional area, and capillaries were identified using TEMA software (CheckVision). Capillarization was expressed as capillaries around each fiber (CAF), and CAF was related to fiber area (CAFA) for type I and type II (IIa and IIx gathered) fibers, in addition to capillary density (capillaries per mm^2) and capillaries per fiber (C/F).

2.3.2 | Protein analysis

Muscle samples were homogenized in 1 mL tissue protein extraction reagent (T-PER®, Thermo Fisher Scientific, Inc) and 10 μL Calbiochem® Protease Inhibitor Cocktail Set IV (Merck Millipore) per ~50 mg muscle tissue. The lysate was centrifuged at 10 000 g for 10 minutes at 4ºC. Protein concentration was assessed with a commercial kit (Bio-Rad *RC/DC*™ Protein Assay kit; Bio-Rad Laboratories, Inc), a filter photometer (Expert 96; ASYS Hitech GmbH) and the software provided (Kim version 5.45.0.1; Daniel Kittrich).

2.3.3 | Western blotting

Equal amounts of protein (35 μg) per sample were separated on Mini-PROTEAN® TGX Stain-Free™ gels (Bio-Rad Laboratories, Inc) at 200 volts for ~30 minutes and subsequently transferred to polyvinylidene difluoride membranes (Bio-Rad Laboratories, Inc) through blotting. Membranes were blocked with 5% nonfat milk solution (in Tris-buffered saline with 0.1% Tween-20; TBS-t) for 120 minutes. Afterward, primary antibodies for citrate synthase (rabbit anti-CS, diluted 1:2 000; Abcam plc®), cytochrome c oxidase subunit 4 (mouse anti-COX4, diluted 1:1 000; Abcam plc®), and hydroxyacyl-coenzyme A dehydrogenase (rabbit anti-HADH; diluted 1:8 000; Abcam plc®) were incubated overnight at 4ºC. Membranes were then washed for 15 minutes with TBS-t and 3 × 5 minutes with Tris-buffered saline, followed by incubation with secondary antibodies to anti-mouse (goat anti-mouse, diluted 1:30 000; Thermo Fisher Scientific, Inc) or anti-rabbit (goat anti-rabbit, diluted 1:3 000; Cell Signaling) for 60 minutes at room temperature. All antibodies were diluted in a 1% fat-free skimmed milk and 0.05% TBS-t solution. After a new washing round and incubation with SuperSignal® West Dura Extended Duration Substrate (Thermo Fisher Scientific, Inc), the proteins were visualized by chemiluminescence and quantified by densitometry (ChemiDoc™ MP; Bio-Rad Laboratories, Inc). All densitometry values were expressed relative to the total amount of protein (Bio-Rad stain-free method). All samples were run as duplicates, and mean values were used for statistical analyses. The 1L-trained leg was set as the control and 100%.

2.4 | Statistical analysis

Power calculations were performed to estimate the sample size needed to obtain an average increase of 0.2 L min^{-1} in 1KE $\dot{V}O_2$max in 1L and an additional 0.075 ± 0.075 L min^{-1} in 1L2A. A sample size of 11 for each training modality should be sufficient to detect differences between legs (α level of 0.05). Variables with pre- and post-measurements for 1L- and 1L2A-trained legs were tested for mean differences with two-way repeated measures ANOVA with time (pre and post) and leg (1L and 1L2A) as within factors. When significant main effects were indicated, Fisher's least significant difference *post-hoc* tests were used to compare means. Differences and changes for the other variables studied were evaluated using paired two-tailed Student's *t* test (level of significance, $P < 0.05$). All results are presented as mean ± standard deviation.

3 | RESULTS

Before training, the two legs were similar concerning all variables measured at that time point (Table 3 and Figure 2).

3.1 | Training

The participants performed 17.3 ± 1.7 training sessions with 1L and 1L2A. Power output and amount of work were the same in both legs (in average, 35 ± 8 W and 50 ± 17 kJ per training session, respectively). Acute systemic responses, as evaluated by cardiorespiratory variables, capillary blood lactate concentrations, and venous blood catecholamine concentrations, were higher during IL2A workouts than in 1L workouts (Table 2).

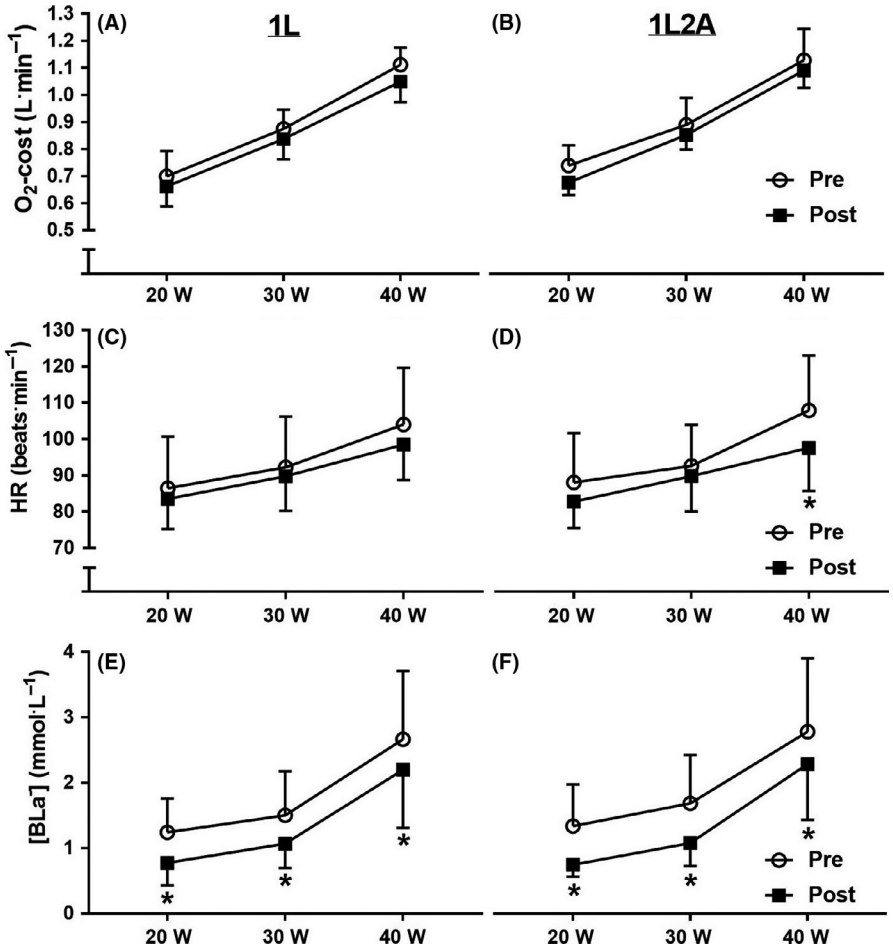

FIGURE 2 Submaximal 1-KE pre- and post-training values for O_2-cost (A and B), HR (heart rate; C and D), and [BLa^-] (capillary lactate concentration; E and F) for 1L and 1L2A. *, Significant different from pre-value ($P < 0.05$)

3.2 | Training effects

1-KE $\dot{V}O_2$max increased with training only for 1L2A ($P < 0.05$; Table 3). However, there was no interaction effect between the legs (1L2A vs 1L, $P = 0.52$). The same was true for 1KE $\dot{V}$Emax (interaction effect between 1L2A and 1L, $P = 0.33$). Peak power output was improved for both legs ($P < 0.05$), but in a similar manner ($P = 0.61$).

Capillary lactate concentration was reduced with training (−21% ± 16%); $P < 0.05$), but no interaction effect was found between the two legs ($P = 0.18$, Figure 2). Submaximal 1-KE O_2-cost (−6% ± 10%; $P = 0.07$) and heart rate (−6% ± 9%; $P = 0.05$) only tended to decrease with training.

$\dot{V}O_2$max during ordinary cycling and arm cycling improved during the training period by 3 ± 3% (from 52.4 ± 6.3 to 54.2 ± 6.7 mL kg^{-1} min^{-1}) and 8% ± 8% (from 2310 ± 632 to 2482 ± 631 mL min^{-1}), respectively ($P < 0.05$ for both).

3.3 | Muscle fiber characteristics (after training)

Differences in muscle characteristics between the two legs were evaluated from biopsies taken after the training period. There were no differences in relative proportions of fiber types, cross-sectional area, capillarization, or oxidative enzymes between the two legs (Table 4 and Figure 3).

TABLE 2 Acute physiological responses to the training workouts

	1L	1L2A
Power output, leg (W)	36 ± 9	36 ± 9
Power output, arms (W)	–	74 ± 18
NA (pmol L^{-1})	4465 ± 1866	12445 ± 3905[a]
A (pmol L^{-1})	1341 ± 837	2462 ± 1161[a]
[BLa^-]	2.7 ± 0.8	4.7 ± 1.2[a]
HR (beats min^{-1})	108 ± 21	157 ± 15[a]
$\dot{V}O_2$ (L min^{-1})	1.1 ± 0.3	2.3 ± 0.5[a]
$\dot{V}$E (L min^{-1})	33 ± 8	74 ± 14[a]
RER	0.93 ± 0.06	0.96 ± 0.04
RPE	14.4 ± 1.6	14.4 ± 1.2

Note: Variables tested during an exercise session in the latter part of the training period (training session #13 out of 17; n = 9). Values are means ± standard deviation. Noradrenaline (NA) and adrenaline (A) concentrations in venous blood plasma.

Abbreviations: [BLa^-], capillary lactate concentration; HR, heart rate; RER, respiratory exchange ratio; RPE, rating of perceived exertion; $\dot{V}$E, ventilation; $\dot{V}O_2$, oxygen consumption (pulmonary).

[#]*P*-value <0.05 between exercises.

TABLE 3 Values achieved during maximal 1-KE

Exercise	VO_2max (L min^{-1})	VEmax (L min^{-1})	HRmax (beats min^{-1})	[BLa$^-$] (mmol L^{-1})	Peak power (W)
1-KE max					
1L pre	1.25 ± 0.26	62 ± 18	150 ± 21	3.8 ± 1.1	49 ± 12
1L post	1.31 ± 0.33	69 ± 22	151 ± 22	3.9 ± 0.8	55 ± 12[a]
Change (%)	4 ± 9	11 ± 17	1 ± 10	8 ± 28	14 ± 7
1L2A pre	1.20 ± 0.24	57 ± 15	149 ± 19	3.7 ± 0.9	48 ± 12
1L2A post	1.29 ± 0.24[a]	69 ± 24[a]	147 ± 16	3.9 ± 0.8	55 ± 13[a]
Change (%)	8 ± 10	21 ± 22	−1 ± 9	11 ± 26	16 ± 9

Note: Values are means ± standard deviation.
Abbreviations: [BLa$^-$], capillary lactate concentration; HR, heart rate; VE, ventilation; VO_2, oxygen uptake (pulmonary); W, watt.
**P*-Value <0.05 between pre- and post-values (n = 11).

4 | DISCUSSION

The present study shows that 5 weeks of 1-KE training with or without added arm training led to the same training adaptations demonstrated by similar improvements in 1-KE VO_2max and peak power output. In addition did the O_2-cost, heart rate, and capillary lactate concentration during submaximal 1-KE change in a similar manner, and capillarization and concentration of oxidative enzymes in the legs were matching after the training period. This occurred though whole-body aerobic energy turnover, blood lactate concentration, and catecholamine responses were different during the training workouts, which implies that the difference in systemic variables per se did not affect the training adaptations to endurance training.

There are not many studies comparing adaptations after training periods with exercises engaging different active muscle mass. To the field of exploring which factors that are important for training adaptations, the present study indicates that systemic variables such as catecholamine response, capillary lactate levels, and whole-body aerobic energy turnover per se during exercise are probably not mandatory for muscular adaptations. It is therefore reasonably to assume that local evoked factors are of greater importance. Theoretically, these local factors could include the mass-specific energy turnover, either per se or in concert with metabolic stress. Metabolic stress with intramuscular metabolite accumulation of, that is, lactate, inorganic phosphate, and hydrogen ions, is associated with activation of metabolic pathways such as AMPK, CAMKII, p38 MAPK, and PGC-1α that is important for muscular training adaptations.[26] This approach fits well with the previous findings of similar muscular adaptations after training with a small active muscle mass compared to whole-body exercises [2-5] and with the finding of superior muscular adaptations in training protocols developing the highest metabolic stress, although the protocols were work-matched.[23] Blood flow occlusion during 1-KE training, and therefore restricted O_2 availability, has been shown to enhance the

TABLE 4 Muscle fiber characteristics

	1L	1L2A
Fiber type, fiber area, and capillarization		
Fiber type 1 (%)	45 ± 11	40 ± 8
CSA (μm^2) fiber type 1	5075 ± 1209	4251 ± 1238
CAF fiber type 1	5.6 ± 1.1	5.2 ± 1.1
CAFA fiber type 1	1.19 ± 0.23	1.31 ± 0.19
CSA (μm^2) fiber type 2	5347 ± 1433	4936 ± 1642
CAF fiber type 2	5.2 ± 0.8	5.1 ± 1.0
CAFA fiber type 2	1.05 ± 0.27	1.10 ± 0.22
CD (capillaries·mm^2)	494 ± 120	540 ± 100
C/F	2.49 ± 0.47	2.43 ± 0.53

Note: Values are means ± standard deviation.
Abbreviations: C/F, capillaries per fiber (n = 8); CAF, average number of capillaries around fiber; CAFA, CAF related to CSA; CD, capillary density; CSA, cross-sectional area.

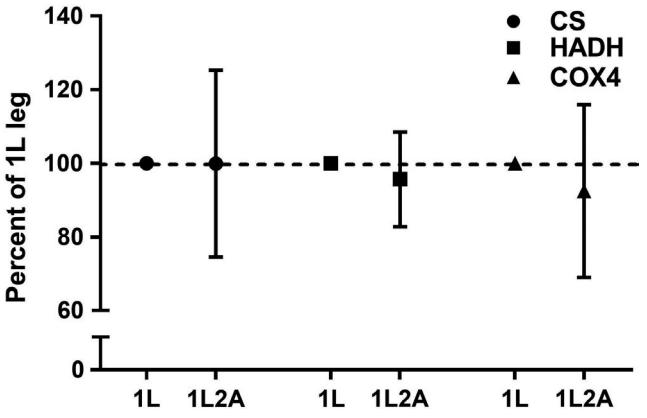

FIGURE 3 Relative concentrations of oxidative enzymes. 1L leg set to 100%. CS, citrate synthase; HADH, hydroxyacyl-coenzyme A dehydrogenase; COX4, cytochrome C oxidase subunit 4. Error bars are 1 standard deviation (n = 10)

acute training stimulus in the form of greater mRNA levels of PGC-1α and vascular endothelial growth factor.[27,28] The insufficient muscular oxygen availability during exercise, causing local metabolic stress, is therefore probably highly significant in the oxidative and angiogenic improvements seen after endurance training. Lack of differences in training adaptation indicators between legs in the present study may therefore be explained by equal metabolic stress during training. This is supported by equal leg deoxy- and total hemoglobin signals in the present study, measured by near-infrared spectroscopy in four out of 11 subjects (unpublished data). This indicates preserved blood flow in both legs, which also other studies have confirmed with invasive measures.[8,20]

However, in the present study it also may be questioned whether the difference in systemic variables between the 1L and 1L2A training exercises was sufficient to provide a differentiation in training response. This implies also that an increase in circulating catecholamines of 2-3 times more than that achieved during 1L is required for local training adaptations to take place. On the other hand, exercise protocols with even higher systemic response than during 1L2A would probably challenge oxygen delivery to the exercising leg due to blood flow restrictions, as first shown by Secher et al,[29] and thus threaten our purpose of achieving equal muscular metabolic stress in the legs. This makes the importance of differences in systemic variables per se on training adaptations difficult to investigate. However, in studies manipulating the amount of sympathetic response pharmacologically by administering β-adrenergic blockers, there are conflicting results in both acute responses to exercise and adaptations to training.[9-15] Although the protocols in most cases are workload- or intensity-matched, there is no consensus, most likely because of differences in the degree of adrenergic blockade and dissimilarities in cardiovascular responses, together with the relative exercise capacities of subjects treated with β-blockers and control subjects.[30] It is also important to note that this study has some limitations. The lack of pre-training biopsies prevents investigation of muscular adaptations. In addition can the contralateral training protocol used not evaluate cross-transfer effects between the legs. The short break (10 minutes) between training modalities was enough to decrease catecholamine levels, but other factors, for example, different metabolites and cytokines, could still be upregulated and potentially interfere with training adaptations on the contralateral leg.

In conclusion, one-leg training with equal amounts of work and power output resulted in similar training responses in the legs regardless of whether arm cycling was added to the training or not. This indicates that local muscular training adaptations take place to the same extent in the presence of differences in systemic variables.

5 | PERSPECTIVES

How peripheral adaptations to endurance training are stimulated is still far from fully understood. Further studies are necessary to evaluate the different mandatory factors contribution and importance. Knowledge in this area is valuable when planning effective training protocols not only for elite athletes, but also for patient groups with different phenotypes and needs.

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