The use of Near-Infrared Spectroscopy to evaluate the effect of exercise on peripheral muscle oxygenation in patients with lower-extremity artery disease: a systematic review

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Abstract

Background: Near-Infrared Spectroscopy (NIRS) has been suggested as a new diagnostic tool in patients with lower-extremity artery disease (LEAD) patients. The aim of this systematic review was i) to summarize the impact of exercise therapy on lower-limb muscle oxygenation, evaluated by means of NIRS, in patients with LEAD and ii) to give an overview on NIRS instruments and methodology.

Methods: A systematic search was conducted in MEDLINE and Embase, from the earliest date available until 16th of March 2020, to identify peer-reviewed studies involving the use of NIRS in the evaluation of exercise training on muscle oxygenation in patients with LEAD. Primary outcomes were NIRS-derived variables during treadmill exercise. Effect sizes were calculated as standardized mean differences. Assessment of methodological quality was done using a combined checklist from the Cochrane bias and the quality assessment tool for before-after studies without control group.

Results: Eleven original trials were included involving 16 exercise groups and 4 control groups. Tissue saturation index (TSI) at rest remained unchanged following the exercise interventions. Yet, exercise training increased time-to-minimum TSI during exercise (range effect sizes: +0.172 to +0.927). In addition, exercise training led to a faster recovery to half and full TSI rest values in most intervention groups (range effect sizes: -0.046 to -0.558 and -0.269 to -0.665 respectively). Finally, NIRS data reproducibility and analytic methods were underreported in the included studies.

Conclusion: The available data suggests that exercise training improves deoxygenation and reoxygenation patterns, as measured with NIRS, in patients with LEAD. Whereas NIRS is a promising tool in the evaluation of LEAD, the low number of RCT's as well as large heterogeneity in NIRS assessment methods, outcome measures and instrumentation, warrants more research to better understand the role of muscle oxygenation associated with exercise-induced improvements in walking capacity.

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Introduction

Lower-extremity artery disease (LEAD) is characterized by a gradual atherosclerotic narrowing in the lower limbs. Although this obstruction in blood flow is often asymptomatic, 10-35% of LEAD patients present with typical symptoms during daily activities (1). The cramping-like pain, referred to as intermittent claudication (IC), is the cardinal symptom of LEAD and results from the oxygen supply-demand mismatch in muscles distal from the obstructed vessel (2). This mismatch has great clinical interest, as the combination of an increased local anaerobic state combined with physical inactivity might result in an acquired myopathy (3,4). This peripheral myopathy is characterized by histological and metabolic changes such as a muscle fiber type shift, reduced capillary density, higher lactate and acylcarnitine levels and dysregulated mitochondrial capacity (2–5). Additionally, increased blood viscosity, local endothelial and autonomic dysfunction further compromise optimal oxygen supply (2,5). Whether insufficient circulation is the main cause of peripheral myopathy is still under debate (6), yet the cascade is undoubtedly detrimental.

Following this, different diagnostic techniques have been introduced to evaluate the underlying etiology of LEAD and patient's response to therapy. Well-known evaluation methods vary in their ability to measure local hemodynamics (e.g., ankle-brachial index (ABI) or imaging methods such as magnetic resonance (MR)), local metabolism or tissue perfusion (e.g. muscle biopsies or transcutaneous oxygen pressure measurements). Yet, most methods are static or have limited penetration depth to study underlying changes in muscle perfusion during exercise (7). Therefore, non-invasive Near-Infrared Spectroscopy (NIRS) received considerable attention in vascular research starting from the early nineties (8), with its interest growing rapidly as new low-cost and portable devices are available with important clinical potential (9). In short, NIRS uses near-infrared light with different wavelengths (700-900 nm) to non-invasively transmit photons to the muscle of interest. As the absorption rate of the omitted photons is different for oxygenated and deoxygenated chromophores, receiving photodetectors can differentiate between both (8). Notably, NIRS dynamically reflects O₂ supply and demand during exercise in healthy and diseased populations (10). In LEAD studies, NIRS can be used as a diagnostic tool as it is associated with clinical measures of disease severity (e.g., ABI and recovery kinetics), and can evaluate the effects of exercise interventions (11,12).

Though NIRS offers unique opportunities to gain insight into the pathophysiological state of LEAD patients, it remains underused in today's clinical practice. Moreover, current reporting of NIRS methodology and derived outcome parameters is very heterogeneous and hinders widespread implementation. Therefore, we conducted a systematic review to summarize the literature to date

on the effect of exercise therapy on NIRS-derived outcomes in LEAD patients. We further aimed to provide a detailed summary on the NIRS instrumentation and assessment methods reported in current literature to guide future work in the field.

Methodology

We followed the guidelines of the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) and the Synthesis Without Meta-analysis (SWIM) (13,14), and prospectively registered with PROSPERO, the International Prospective Register of Systematic reviews (registration number: CRD42020175401).

Search strategy

A comprehensive search was conducted in two electronic databases: MEDLINE (Ovid) and Embase (Ovid), from their inception date until the 16th of March 2020, to identify relevant peer-reviewed journal articles (**Supplementary file S1**). Further, reference lists from the eligible papers were manually searched for additional studies. No language restrictions were imposed on the search.

Inclusion and exclusion criteria

Eligibility criteria included 1) prospective cohort studies, randomized and non-randomized controlled trials investigating the effect of an exercise intervention; 2) performed in humans aged ≥ 18 years with LEAD and IC (classified as Rutherford 1-3 or Fontaine 2a, 2b; 3) reporting mean and standard deviation, standard error (SEM) or median and interquartile range of at least one NIRS outcome parameter before and after the intervention and 4) published as a full-length publication in a peer-reviewed journal.

Study selection

The results from the initial search were imported into Rayyan software (15) for systematic screening by two independent reviewers (N.C. and P.C.). After removal of duplicates, title and abstract were screened for eligibility. In a second phase, we retrieved the full texts of the potentially relevant articles which were then reviewed by both reviewers. Disagreements were resolved by consulting with a third reviewer (V.C.).

Data extraction

A standardized Access Database file (Microsoft, Redmond, WA, USA) was used by both reviewers to independently extract data from the individual studies including publication details (year of publication, country of origin), study design, sample size, patient characteristics, exercise characteristics, NIRS instrumentation and assessment methodology as well as primary and secondary outcome measures.

Primary and secondary outcomes

Our main focus was on NIRS-derived parameters which could be assessed at rest, during a single bout of exercise or during recovery from exercise (**Figure 1**). For uniformity TSI (tissue saturation index: $[\text{oxygenated[heme]}/\text{Total[heme]}]$) is used as the reference term, including also StO₂ (muscle tissue oxygen saturation) or TOI (tissue oxygenation index). Although NIRS cannot discriminate between chromophores (hemoglobin and myoglobin), outcomes are reported as published (e.g., oxygenated hemoglobin (O₂Hb), deoxygenated hemoglobin (HHb) and total hemoglobin (tHb)) since intracellular myoglobin tends to stay constant during exercise (8,10). Arterial or venous occlusion experiments are considered as a separate category. Secondary outcomes included pain-free and maximal walking time or distance, 6-minute walking distance (6MWD), ABI and peak oxygen consumption (VO_{2peak}). In case of missing data, authors were contacted twice over a one-month period to request data.

Risk of bias

Study methods

Due to the different designs of the included studies, the Cochrane risk of bias tool (16) and the Quality assessment tool for before-after studies without control group (17) were combined to rate the methodological quality of included articles. This combined checklist was used by two independent reviewers (N.C. and P.C.) rating the following 12 items (rating: yes and no): objective clearly stated, eligibility criteria, representative study sample, participants enrolment, random sequence generator, control group, description of the intervention and consistency, assessors blinded to intervention, follow-up rate, statistical methods, complete outcome data and non-selective reporting. Disagreements between reviewers were resolved by discussion with a third reviewer (V.C.). The overall score for each article was obtained by summing the number of 'yes' answers.

NIRS methodology

A separate analysis of the quality of the NIRS assessment methods was performed by rating the following six items: NIRS device characteristics, clear description of anatomical position, reproducibility of positioning, adipose tissue thickness (ATT) measurement, description of the specified outcomes, analysis and reporting.

Statistics

Demographic data are presented as reported in the main article. Effect sizes of all included study groups were calculated by paired standardized difference of means using CMA (Comprehensive Meta-analysis V2.2.064). Standardized difference of means can be interpreted following Cohens' convention with the effect size being small (0.20), medium (0.50) or large (0.80) (18). To calculate the standardized effect sizes, we assumed paired data to be correlated with $r=0.5$. The decision to not

perform a meta-analysis was based on the diversity in study designs and methods, with a limited number of studies including a matched control group. Therefore, we opted to summarize effect sizes to discuss range and distribution for the most common outcomes. Furthermore, effects sizes were presented visually to discuss both direction of effect and heterogeneity. A conversion tool (19) was used to calculate mean and standard deviation for two studies (20,21) reporting median and interquartile range. If data was only available in figures, we extracted mean or standard deviation using Adobe Acrobat reader (Adobe Inc., San Jose, California, USA).

Results

Literature search

The original search yielded 440 studies from which, after removal of duplicates and exclusion of papers based on eligibility criteria, 11 original studies involving 12 full text papers remained (**Figure 2**). Two papers from Collins et al were included, involving the same population sample but two different exercise test protocols (i.e., constant and progressive work-rate). Demographic data for Collins et al was extracted from the original study (22,23). However, the NIRS sub study (23) was selected for quantitative analysis, since it contained more information regarding our research question. A request for missing data was sent to 9 authors of which 6 replied (23–28).

Study characteristics

Individual study characteristics are presented in **table 1**. Four studies were randomized controlled trials (RCT) (20,24,26,27), three were randomized trials (22,23,29,30), two were non-randomized controlled trials (21,31), and two applied a pre-post design without control group (25,28). Studies were published between 2009 and 2020. The majority (n=6) were conducted in the USA (20,22–24,26,28,30), while the remaining were performed in UK (21,27), Italy (31), Brazil (29) and Japan (25). A total of 666 participants (73% men) participated in the studies. Mean age of the participants was 68.4 years, with study means ranging between 64.3–71.8 years. In total 70 participants dropped out of the studies, leaving 596 available for analysis. All exercise intervention groups (n=16) and control groups (n=4) were analyzed as individual study groups. One non-randomized control group consisted of patients that underwent angioplasty (21). Three other control groups received either best medical treatment (20), advice on physical activity (27) or attention-control light resistance training (26). Comparison groups of healthy participants (n=2) were excluded from the analysis (30,31). Traditional treadmill walking (20,22,24,26,28–30) was the most frequently prescribed exercise modality (n=7). Three studies used alternative walking interventions with ankle loads (29), NIRS-guidance (30) or pole-striding (22). Two studies applied a home-based walking intervention involving two structured (26,31) and one self-paced (31) walking program. The remaining three studies used ergometer cycling (25), arm-cranking exercise (27) or a circuit training program (21). All exercise interventions

had a duration of 12 weeks (n=10) except for Manfredini who applied a 34-week intervention (31). Resting levels of TSI were reported in four studies (20,21,27,28), deoxygenation parameters during exercise in eight (20,21,23,25–29) and recovery outcomes following exercise in six studies (20,21,25,26,28,29) (**Figure 3**).

Synthesis of results

NIRS parameters at rest

In all studies resting TSI remained unchanged after the exercise interventions (20,21,27,28), usual care (20) or angioplasty (21) with effect sizes ranging between -0.191 and +0.272 (**Figure 4A**). Only Baker reported between group comparison and found no difference between the exercise and control group change scores (20). Muscle oxygen consumption (mVO_2) at rest, was assessed as HHb rate during venous occlusion (31) or by a combination of diffuse correlation spectroscopy (DCS) and frequency domain-NIRS measurements (20). Two study groups out of three observed a higher mVO_2 at rest (20,31) after structured walking training. This increase in mVO_2 was significantly higher after exercise training compared to usual care (20). These effects were explained by a higher dynamic blood flow and oxygen extraction fraction compared to usual care (20) or an increased mitochondrial capacity, noted by Murrow and colleagues after exercise (30). The latter effect was more pronounced in the traditional compared to a walking group receiving NIRS bio-feedback (Interaction-effect: $p=.003$) (30). Two other studies evaluated O_2Hb recovery after arterial occlusion (21,29) and found a significantly higher recovery rate in the angioplasty group but not in the exercise group (21). Furthermore, no interaction, time or group effects were observed after training on TSI recovery time, delta HHb and delta TSI following arterial occlusion at rest (29).

NIRS parameters during exercise

In all studies TTM was increased after the exercise intervention, with five out of seven trials reaching statistical significance (range effect sizes: +0.172, +0.927) (**Figure 4B**). Two RCT's (26,27) reported that changes in TTM were significantly larger compared to the control group (p -interaction: .025 and $<.001$). Similarly, Tew et al observed significantly higher TSI values at several time points during exercise after an arm-cranking intervention (27). Conversely, no changes in absolute deoxygenation were noted following seven exercise interventions (range effect sizes for delta TSI: -0.127, +0.350 and TSImin: -0.286 and 0.000). Though the lowest TSI value was unchanged, patients could sustain the low tissue oxygenation levels for a longer period after exercise training (time-to-resistance; time-effect $p <.001$) (29). Yet, a significant decrease in deoxygenation, which translates to higher TSI values during exertion, was observed only after revascularization (21) and best-medical treatment (20). Moreover, two studies reported the evolution of NIRS raw signals during exercise, and observed

improved maintenance of oxygenated hemoglobin during (sub)maximal exercise after walking training (24,31), without changes in deoxygenation (24) or total hemoglobin kinetics (31).

NIRS parameters during recovery

Two RCT's reported data on T50 (20,26) (i.e., time to recover 50% of resting TSI), and three studies (25,28,29) reported data for T100, defined as time to reach 100% of resting TSI, of which none included a control group (**Figure 4C**). Contrary to Baker (20), Gardner et al (26) found a faster recovery (T50) following either supervised or home-based exercise, which was significantly different from the control group ($p=.020$). As the training interventions were similar for both studies, methodological differences most likely explain this discrepancy. That is, Baker applied a frequency-domain technique to calculate half-time oxygen extraction fraction which resembled T50 (20). Although all studies showed a tendency towards an enhanced recovery following exercise training (25,28,29), assessed as T100 (range effect sizes: -0.665, -0.269), only the conventional training intervention group reached statistical significance (29). Similarly, Beckitt et al used T50 but defined it as half of the full recovery time. Following a circuit-based exercise training, they found enhanced recovery after a submaximal test similar to the group that received angioplasty treatment. Yet, enhanced recovery after maximal exertion was only seen after revascularization (21). Finally, relative reoxygenation, defined as time to reach 100% of resting TSI relative to test duration, was significantly improved following 12-weeks of walking, three times per week, with and without ankle weights (29).

Secondary outcomes

The majority of the studies reported significant improvements in pain-free (9/12 studies; 75%) and maximum walking capacity (14/16 studies; 87.5%) as well as 6MWD (24,26) after the exercise interventions. Similarly, three studies applying an RCT design confirmed enhanced maximal walking capacity following walking (20,26) and arm-cranking (27) exercise, respectively. However, a lack of improvement in maximal walking capacity during a progressive work-rate test was noted following unstructured exercise (home-based) (31) and after a pole-striding intervention (23). Further, only one study assessed exercise capacity and found a significantly improved VO_{2peak} following arm-cranking exercise compared to the control group (27). Whereas nine studies evaluated ABI, only one reported an improvement in the affected leg after 34-weeks of structured home-based walking intervention (31).

Detailed information on the effects of exercise training on individual study level can be found in **Supplementary files 3-5**.

Risk of bias assessment

The median quality of the included studies was 8 out of 12 (range 5-11) (**Supplementary table 6**). Specifically, the objective was clearly stated in nine articles, with two failing to provide a rationale

and/or clear hypothesis for their study. All studies gave a clear description on eligibility criteria, with the sample being representative for the IC population in eight. However, only two studies enrolled all eligible participants. Also, only four studies reported random sequence generation methods and four included an attention control group. Regarding the methodology, interventions were described in detail and delivered consistently in ten studies, but assessors were reported to be blinded in only two. Ten studies used adequate statistical methods to assess the significance of the changes in the outcomes, though four did not have sufficient participants at follow-up (dropout $\geq 20\%$). No incomplete outcome data were found, however, when comparing the results with the protocol, four studies failed to report all the pre-specified outcomes.

NIRS methodology assessment

Although all studies except one reported the used NIRS device (25), almost half of them failed to report various device characteristics (e.g., wavelengths, sample frequency) which are important when comparing different studies (**Supplementary table 2**). To obtain valid and reliable results, a precise description of the anatomical placement is needed (8). Yet, only eight studies described probe position in detail, of which only four took appropriate measures to ascertain reproducibility. The remaining studies provided only a vague description regarding probe positioning (e.g., calf muscle). Two studies reported calf adipose tissue thickness (ATT) (measured with MRI (20) and ultrasound (30) respectively), and only one excluded participants with calf ATT higher than 20mm (31). The use of raw or filtered data for analysis was not reported in any of the included articles. Regarding the NIRS parameters, nine studies described the reported variables in detail, and eight reported all the pre-specified outcomes.

Discussion

The aim of this review was to systematically document the effect of exercise interventions on muscle adaptations measured by near-infrared spectroscopy after exercise interventions in LEAD patients and to provide an overview of current NIRS methodology. Although earlier reviews (11,12) have already addressed NIRS as a new method to evaluate and diagnose LEAD patients, this is the first comprehensive review investigating the effect of exercise therapy on NIRS outcomes.

Impact of exercise on NIRS parameters

Rest. Earlier studies reported comparable resting TSI in IC patients and healthy controls (9,12). Similarly, we found no change in resting TSI after training in IC patients. Moreover, an angioplasty treatment and an exercise group reported similar resting TSI (21). This could suggest that resting blood flow is usually sufficient for proper muscle oxygenation, with the underlying supply-demand mismatch occurring only during exercise and resembling the symptomatology of IC.

Exercise. A rapid maximal deoxygenation in the beginning of exercise was previously specified as the hallmark NIRS pattern in IC patients (9,10). Yet, our results did not establish any improvements in absolute measures of deoxygenation during treadmill testing. Whether deoxygenation during exercise is related to blood flow, as evaluated by resting ABI, remains unclear, with studies reporting contrasting associations ($r=-.56$, $p=.002$ (21) and $r=.105$, $p>.05$ (32)). Yet, time to reach maximal deoxygenation (TTM) substantially increased with training, meaning that patients could walk longer before maximal deoxygenation occurred. Similarly, O_2Hb was preserved during submaximal exercise without changes in tHb (24,31). Interestingly, Early deoxygenation is associated with maximal walking capacity (32,33) as suggested earlier by Fuglestad who found a strong $r=-.76$ correlation between deoxygenation at one minute into exercise and maximal walking distance (9). In line with these findings, Gardner et al found that the change in TTM correlated significantly with the change in maximal walking time ($r=.52$, $p<.001$ and $r=.43$, $p=.006$ for home-based and supervised groups, respectively) (26). As TTM commonly appears prior to initial pain experience, these moderate correlations might be explained by the objective and subjective mismatch in pain perception and perseverance following an exercise program (34).

Recovery. Concerning recovery, we noted a tendency towards faster T50 and T100 following training interventions. A faster reoxygenation has previously shown clinical relevance for symptom resolution and is associated with better ambulatory outcomes (9,32,34). However, only one study found statistically significant results, whereas two others observed trends towards enhanced recovery after submaximal exercise intensity (21) or recovery relative to total exercise time (29).

Underlying mechanisms of change using NIRS

Regarding resting muscle oxygen consumption, two studies (20,31) showed increased values after an exercise intervention. Yet, muscle oxygen consumption is composed of blood flow and arteriovenous oxygen extraction and therefore may involve various physiological adaptations (10). NIRS-derived outcomes during exercise present local alterations that are expected after 12-weeks of exercise, such as improved capillarization and microvascular hemodynamics (20,23,24,26,29), oxidative capacity (21,29,30) and oxygen extraction (20). In line with local adaptations involved, a recent study from Gardner reported associations between TTM and ambulatory function, independent of resting ABI and common health burdens (33). Noteworthy, Murrow et al evaluated mitochondrial oxidative capacity at recovery (30) using an exponential model to plot NIRS-derived O_2Hb following repeated arterial occlusions after an exercise bout. They showed a trend towards improved mitochondrial capacity after an exercise intervention, which is similar to the improvement seen in other clinical populations with underlying mitochondrial dysfunction (30,35).

Still, this topic is part of a historical debate in which both muscle oxidative capacity and macrocirculation are opposed to one another (6). Interestingly, the role of increased endothelial function and rheology has been suggested by the cross-transfer effect of arm-cranking exercise (27) with similar improvements in NIRS deoxygenation and recovery patterns. On the contrary, ABI analyses in all but one of the included samples did not reveal an augmented resting blood supply (20) which is in line with available evidence (36,37). However, Manfredini et al could discriminate two groups of responders after structured home-based exercise: 1) 20 patients that increased ABI, had a blunted decrease of O₂Hb during submaximal exercise and a lower heart-rate response, 2) 10 patients without ABI change increased their muscle oxygen consumption (31). These findings are confirmed by the study of Baker reporting both enhanced superficial blood flow and oxygen extraction levels during exercise (20). Collectively, redundancy of exercise responses and its physiological mechanisms may have an important role regarding increased walking capacity in IC patients (38).

Study biases and methodology

Proper reporting of NIRS instrumentation, testing and analysis is important as different technology and applied algorithms may impact the results and will hinder comparison among studies (8). In the included studies, reproducibility of study protocols in a pre-post setting was often insufficient with poor description of probe positioning (24,25,30) and without measures to ensure reproducibility for follow-up assessments (21,23,26,31). The latter is of utmost importance as reproducibility of the signals largely depends on a fixed probe location as oxygenation responses display large heterogeneity between different muscles, even within different regions of the same muscle (8). Additionally, the impact of adipose tissue thickness in the region of interest should be acknowledged when interpreting absolute concentrations of hemoglobin, as thicker adipose layer can reduce the penetration depth of NIRS emitted wavelengths (8). Though, the majority of included studies reported on TSI outcomes, which are less prone to measurement error (8). Furthermore, a lack of uniformity of NIRS parameters reported across the different studies was noted which makes comparisons among studies difficult. Therefore, we recommend that researchers and clinicians should try to address these issues when performing NIRS measurements during exercise tests. Some examples of common issues and their potential solutions are presented in **table 2**.

Limitations

The included studies had different methodologies and outcomes. To address this limitation, we calculated the sample effect sizes to present the direction of effect for most reported outcomes. The inclusion of non-randomized or non-controlled trials could introduce some bias, yet was pre-

specified and is in line with the exploratory nature of this review. In addition, it is becoming difficult to get ethical approval for controlled studies in LEAD as exercise is now a class IA recommendation in IC treatment (21). Yet, it should be acknowledged that these pre-post studies without randomized control group risk the regression to the mean. This phenomenon can be minimized by performing familiarization and multiple measurements at baseline, which were only performed in two non-controlled studies (22,28).

Conclusions

Evidence to date seems to suggest that exercise interventions may improve deoxygenation and reoxygenation patterns in patients with intermittent claudication, measured by NIRS applications during various exercise testing protocols. NIRS therefore appears to have potency as an evaluation tool of peripheral muscle oxygenation following exercise interventions. Yet, given the scarcity of data further research by means of larger robust RCT's is needed. New trials should focus on better methodologies, with harmonized NIRS outcomes and take into consideration the transparency and reproducibility.

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Conflict of interest

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Figure legends

Figure 1. Categorization of outcome data

Figure 2. Flow chart study inclusion

Figure 3. NIRS-derived outcomes in included studies

Figure 4A. NIRS outcomes: baseline

Figure 4B. NIRS outcomes: exercise

Figure 4C. NIRS outcomes: recovery

Figure legends

Figure 1. Typical pattern of NIRS-derived data during a treadmill walking test (unpublished data). Outcomes are categorized following the phase in the test: at rest, during exercise or de-oxygenation and during recovery or re-oxygenation.

Figure 2. Flow chart displaying the search's work flow

Figure 3. Distribution of the most common NIRS-derived muscle oxygenation variables in the included studies. Abbreviations: TSI rest (%): Tissue Saturation Index during resting conditions (%), mVO_2 : muscle oxygen consumption, TSImin (%): minimum TSI (%) during exercise, TTM: Time to reach TSImin (%), delta TSI: TSIrest – TSImin, T100: time to reach 100% of resting TSI, T50: time to reach 50% of resting TSI.

Figure 4A. Effect sizes on TSI and muscle oxygen consumption after intervention. Results in yellow and green reflect non-significant and significant ($p < .05$) effect sizes respectively.

Figure 4B. Effect sizes on time-to-minimum TSI (TTM), delta TSI and minimal TSI (TSImin) during exercise after intervention. Results in yellow and green reflect non-significant and significant ($p < .05$) effect sizes respectively. Data on Collins are from (23).

Figure 4C. Effect sizes of halftime (T50) and total recovery time (T100) after an intervention. Results in yellow and green reflect non-significant and significant ($p < .05$) effect sizes respectively.

Table 1. Baseline characteristics of the included studies

Author Year Country	Design	Exercise group (E1)	Comparison group(s)	Sample size	Age	ABI	Sex	Test methods
		FITT-principle	Alternative exercise (E2) or control (C)	N included (N analyzed)			M/F (N)	
Baker et al 2017 USA	Randomized controlled trial	F: 3x/week	<i>Best medical treatment (C)</i>	T: 64 (64)	T: 66.5	T: 0.63	41/ 23	Progressive treadmill test 3.2 kph - 0% inclination + 2%/2 minutes
		I: Mild-moderate pain		E: 29 (29)	E: 66	E: 0.645		
		T: 60 min, 12 weeks						
		T: Treadmill walking		C: 35 (35)	C: 67	C: 0.65		
Beckitt et al 2012 UK	Controlled trial	F: 2x/week	<i>Angioplasty control (C)</i>	T: 56 (56)	T: 66.5	T: 0.70	39/ 17	Constant work 2.5 kph at 10% inclination Submaximal treadmill 100 seconds at submaximal intensity Arterial occlusion Acute hyperemic response
		I: Point of claudication		E: 42 (42)	E: 66	E: 0.69		
		T: 50 min, 12 weeks		C: 14 (14)	C: 68	C: 0.72		
		T: Circuit training: step- ups, toe-walking, heel raises, wobble board and resistance cycling						
Collins et al 2012a and Collins 2012b* USA	Randomized trial	F: 3x/week	F: 3x/week	T: 103 (95)	T: 69.7	T: 0.63	96/ 7	Constant work (a) 2.9 kph at 12% inclination Progressive work (b) Small increases in inclination every 30 seconds; 6 minutes speed was increased every 3 min; starting speed was 2.9 km/h and inclination 0% - protocol was designed to have a 1 MET (metabolic equivalent) every 3 minutes
		I: Low (25-44% VO _{2peak}) to moderate (45-59% VO _{2peak}) to high (60-84% VO _{2peak}) intensity	I: Low (25-44% VO _{2peak}) to moderate (45-59% VO _{2peak}) to high (60-84% VO _{2peak}) intensity	E: 52 (49)	E: 68.0	E: 0.65		
		T: 30-60 min, 24 weeks	T: 30-60 min, 24 weeks	C: 51 (46)	C: 71.4	C: 0.62		
		T: Walking <i>without</i> poles (treadmill and hospital corridors) (E1)	T: Walking <i>with</i> poles (outside and hospital corridors) (E2)					

Figoni et al 2009 USA	Pre-post trial	F: 5x/week of which 3x/center-based and 2x/week home-based	N/A	T: 21 (15)	T: 68.8	T: 0.59	21/ 0	Progressive work 3.2 kph - 0% inclination + 2%/2 minutes until a maximal inclination 14%
		I: Maximal claudication pain		E: 21 (15)	E: 68.8	E: 0.59		
		T: 12-week						
		T: (Treadmill) walking + calf exercises on ergometer						
Gardner et al 2014 USA	Randomized controlled trial	F: 3x/week	<i>Attention-control group (C): light resistance training (3x/week)</i>	T: 180 (180) <i>Intention-to-treat</i>	T: 65.7	T: 0.70	96/ 84	Progressive work 3.2 kph - 0% inclination + 2%/2 minutes
		I: Mild-moderate pain		E1: 60 (60)	E1: 67	E1: 0.68		
		T: 20-45 min, 12 weeks		E2: 60 (60)	E2: 65	E2: 0.68		
		T: 15-40 minutes, 12 weeks		C: 60 (60)	C: 65	C: 0.74		
		T: Home-based walking using a step monitor (E1)						
		T: Supervised treadmill walking (E2)						
Haga et al 2020 JAPAN	Pre-post trial	F: 3x/week	N/A	T: 19 (16)	T: 67	T: Left: 0.77 Right: 0.79	13/ 3	Constant work Speed between 2.4 and 3.6 kph, 0% inclination
		I: 70% maximal load		E: 19 (16)	E: 67	E: Left: 0.77 Right: 0.79		
		T: 40-60 min, 12 weeks						
		T: Ergometer cycling						
Manfredini et al	Non- randomized	F: 6x/week	F: 6x/week	T: 55 (45)	T: 71.3	T: 0.61	42/	Progressive work (NIRS measurements)

2012 ITALY	controlled trial	I: speed 20-30% lower than pain threshold (using metronome)	I: Self-selected pace up to moderate pain	E1: 36 (31)	E1: 71.9	E1: 0.59	13	1.5 kph + 0.1 kph/10 meter, 0% inclination Venous occlusion Muscle oxygen consumption
				E2: 19 (14)	E2: 70.3	E2: 0.67		
				C: 15 (15)	C: 38.3	C: 1.09		
		T: 20 minutes, 34 weeks	T: 20-30 minutes, 34 weeks					
		T: Structured home-based walking (E1)	T: Unstructured, free pace home-based walking (E2)					
		Healthy control group with unmodified active life-style (post-hoc) (C)						
Monteiro et al 2019 BRAZIL	Randomized trial	F: 3x/week	F: 3x/week	T: 40 (32)	T: 64.3	T: Left: 0.62 Right: 0.62	28/ 12	Constant work (1 minute of warm-up with progressive increase to 3.2 km/h and 10% inclination, at moderate-to-maximum claudication one-to-two minutes of active recovery at 2.0 km/h and 0% inclination) Arterial occlusion
		I: Maximum claudication	I: Maximum claudication	E1: 20 (16)	E1: 65.5	E1: Left: 0.61 Right: 0.62		
				E2: 20 (16)	E2: 63.1	E2: Left: 0.62 Right: 0.61		
		T: 30 minutes, 12 weeks	T: 30 minutes, 12 weeks					
		T: Treadmill walking (E1)	T: Modified treadmill walking using ankle weights (E2)					
Murrow et al 2019 USA	Randomized trial	F: 3x/week	F: 3x/week	T: 36 (18)	T: 71.8	T: 0.83	14/ 4	Progressive work 3.2 kph - 0% inclination + 2%/2 minutes Arterial occlusion Muscle oxygen consumption and muscle blood flow
		I: Claudication pain ≥6/10 scale	I: 15% reduction from TSI rest as a lower-level threshold	E1: 18 (10)	E1: 71.6	E1: 0.80		
		T: 40 minutes, 12 weeks	T: 40 minutes, 12 weeks	E2: 18 (8)	E2: 72.0	E2: 0.87		
		T: Treadmill walking (E1)	T: NIRS-guided treadmill walking	C: 20 (20)	C: 61.0	C: /		

			(E2)					
			<i>Age-matched control group (post-hoc)</i>					
Tew et al 2009 UK	Randomized controlled trial	F: 2x/week	<i>Control group with advice on PA (C)</i>	T: 57 (51)	T: 69.5	T: 0.68	51/ 0	Progressive work 3.2 kph - 0% +1% /minute
		I: 60-70% VO ₂ peak		E: 29 (27)	E: 69	E: 0.68		
		T: 20 minutes, 12 weeks		C: 28 (24)	C: 70	C: 0.69		
		T: Arm-cranking exercise						
Woessner et al 2018 USA	Randomized controlled trial	F: 3x/week	Similar exercise group <i>with</i> nitrate supplementation	T: 35 (24)	T: 69.7	T: 0.63	15/ 9	Progressive work 3.2 kph - 0% inclination + 2%/2 minutes
		I: Mild-moderate pain		E: 18 (13)	E: 71.5	E: 0.70		
		T: at least 30 minutes, 12 weeks		C: 17 (11)	C: 67.5	C: 0.55		
		T: Treadmill walking						

Note. Numbers in italics are data on analyzed numbers of participants. *Collins 2012b (23): Same training characteristics except that intervention duration was 12 weeks. Sample size: T: 85(79), E1: 40(36), E2:45(35). Age. T: 69.4, E1: 66.8, E2: 71.7. ABI. T: 0.63, E1: 0.63, E2: 0.62. Sex (79 ♂/6 ♀). Abbreviations: F: frequency, I: intensity, T: time, T: type, E: exercise group, C: control group, T: total sample.

Practical recommendations
<ul style="list-style-type: none"> • Report the technology used and the specific device characteristics: sample frequency, number of optodes, inter-optode distance and wavelength • Describe in detail the anatomical position of the probe placement and ensure methods for reproducibility for subsequent measurements and/or studies. • Adipose tissue thickness (ATT) should be measured at the site of interest and reported. Whenever possible, subject recruitment should be based on homogeneous ATT values or else a physiological calibration of the NIRS signals may be required. • Data from all raw NIRS signals (O₂Hb, HHb, tHb, diffHb) should be provided either in the main manuscript or in supplementary files. • Outcome variables should be clearly defined and replicable. In case of experimental variables or analyses, variables should be described in detail to avoid confusion. • The software used and the exact methods to analyze the data should be reported to ensure reproducibility and transparency. • Analyze and report changes from a timepoint of interest, amplitude of responses, and slopes. These are more insensitive to confounding factors such as adipose tissue thickness or random noise in the signal.

Table 2. Common issues in NIRS methodology and application found in the literature, and their potential solutions. For a more detailed list of recommendations we refer to the review written by Barstow (8).

Figure 1. Typical pattern of NIRS-derived data during a treadmill walking test (unpublished data). Outcomes are categorized following the phase in the test: at rest, during exercise or de-oxygenation and during recovery or re-oxygenation.

Figure 2. Flow chart displaying the search's work flow

Figure 3. Distribution of the most common NIRS-derived muscle oxygenation variables in the included studies. Abbreviations: TSl rest (%): Tissue Saturation Index during resting conditions (%), mVO₂: muscle oxygen consumption, TSlmin (%): minimum TSl (%) during exercise, TTM: Time to reach TSlmin (%), delta TSl: TSlrest – TSlmin, T100: time to reach 100% of resting TSl, T50: time to reach 50% of resting TSl.

Figure 4A. Rest

Figure 4A. Effect sizes on TSI and muscle oxygen consumption after intervention. Results in yellow and green reflect non-significant and significant ($p < .05$) effect sizes respectively.

Figure 4B. Exercise

Figure 4B. Effect sizes on time-to-minimum TSI (TTM), delta TSI and minimal TSI (TSImin) during exercise after intervention. Results in yellow and green reflect non-significant and significant ($p < .05$) effect sizes respectively. Data on Collins are from (23).

Figure 4C. Recovery

Figure 4C. Effect sizes of halftime (T50) and total recovery time (T100) after an intervention. Results in yellow and green reflect non-significant and significant ($p < .05$) effect sizes respectively.

Search strategy

1) Medline (Pubmed)

((((((((((intermittent claudication[Title/Abstract]) OR (lower extremity artery disease[Title/Abstract])) OR (peripheral artery disease[Title/Abstract])) OR (peripheral vascular disease[Title/Abstract])) OR (peripheral arterial disease[Title/Abstract])) OR (peripheral obliterative arteriopathy[Title/Abstract])) OR (arterial occlusive disease[Title/Abstract])) AND (((((((((((exercise[Title/Abstract]) OR (walking[Title/Abstract])) OR (rehabilitation[Title/Abstract])) OR (physical activity[Title/Abstract])) OR (endurance[Title/Abstract])) OR (aerobic[Title/Abstract])) OR (strength[Title/Abstract])) OR (resistance[Title/Abstract])) OR (training[Title/Abstract])) OR (cycling[Title/Abstract])) OR (swimming[Title/Abstract])) AND (((((((near-infrared spectroscopy) OR (near infrared spectroscopy)) OR (NIRS)) OR (muscle oxygenation)) OR (oximetry)) OR (spectroscopy)) (363 HITS)

2) Embase

#1 'lower extremity artery disease'/exp OR 'lower extremity artery disease'

#2 'intermittent claudication'/exp OR 'intermittent claudication'

#3 'peripheral occlusive artery disease'/exp OR 'peripheral occlusive artery disease'

#4: #1 OR #2 OR #3 (173814 HITS)

#5 exercise:ab,ti OR rehabilitation OR training:ab,ti OR swimming:ab,ti OR cycling:ab,ti OR walking:ab,ti OR 'physical activity':ab,ti OR strength:ab,ti OR resistance:ab,ti OR aerobic:ab,ti OR endurance:ab,ti (2403529 HITS)

#6 'near infrared spectroscopy' OR oximetry:ab,ti OR 'muscle oxygenation' OR 'spectroscopy' OR nirs(637974 HITS)

#7: #4 AND #5 AND #6

#8: #7 AND 'human'/de AND [embase]/lim NOT [medline]/lim (77 HITS)

TOTAL HITS: 440 HITS

Supplementary Table 2: Overview of NIRS devices in the included studies.

DEVICE	TECHNIQUE	CHANNELS	WAVELENGTHS	COMPANY	STUDIES	WEBSITE
INSPECTRA 325	Multi-distance CW	/	3	Hutchinson, USA	4 (22, 23, 26, 28)	www.hutchinson.tdk.com
NIRO 300	Multi-distance CW	3	4	Hamamatsu, Japan	2 (21, 27)	www.hamamatsu.com
OXYMON MK III	Multi-distance CW	2	3	Artinis, Netherlands	2 (30, 31)	www.artinis.com
PORTAMON	Multi-distance CW	1	3	Artinis, Netherlands	2 (24, 29)	www.artinis.com
FOIRE-3000		24	3	Shimadzu, Kyoto, Japan	1 (25)	
CUSTOM-BUILT FD-NIRS	Frequency-domain	/	3	/	1 (20)	/

Abbreviations: CW: continuous wave. Channels: the number of transmitters - receiver combinations.

Supplementary Table 3. NIRS outcomes, results, comments and statistics.

Author	At Rest	During Exercise	Recovery	Other outcomes	Comments	Applied Statistics
Baker et al 2017	E1: TSI = C: TSI =	E1: Δ TSI $\uparrow$ C: Δ TSI $\downarrow$ <i>In between group difference</i> <i>(p < .001)</i>	E1: T50 (T_R) = C: T50 (T_R) =	Frequency-domain outcomes (NIRS): Oxygen extraction fraction (OEF) Ratio: exercise $\uparrow$ and control $\downarrow$ Total hemoglobin/myoglobin count (THC) At rest: both in exercise and control = Delta hemoglobin/myoglobin count (ΔTHC) During Exercise: both in exercise and control =	-Data was converted from original median & IQR but no changes were noted in statistical significance values reported - T_R was used as the outcome of interest regarding recovery and approaches TSI T50 -Flow measurements using DCS (diffuse correlation spectroscopy) did not show any blood flow changes at rest (F_o). A significant in between group difference was noted when comparing ratio (F_{ex}/F_o) change scores, in favor of the exercise group. -Combining NIRS and DCS measurements, a dynamic outcome for local muscle oxygen consumption (rVO₂) was significantly altered in favor of the exercise group.	-Wilcoxon signed rank test of ratios from both baseline and follow-up tests -TSI rest was the same at baseline and follow-up in both the E1 (p > .05) and C (p > .05). - Δ TSI was shown to be significantly different in-between groups (p < .001) but no group-specific data was provided
Beckitt et al 2012	E1: TSI = Angioplasty C: TSI =	E1: during both maximal and submaximal tests: Δ TSI = <i>Angioplasty C:</i> Δ TSI = (Maximal test) Δ TSI $\downarrow$ (Submaximal test)	E1: T50 = (Maximal) E1: T50 $\downarrow$ (Submaximal) <i>Angioplasty C: T50 $\downarrow$</i>	Arterial occlusion O₂Hb recovery rate (after ischemia reperfusion): E1: = Angioplasty: $\uparrow$	-Data was converted from median & IQR	-Wilcoxon signed rank test -TSI rest was the same at baseline and follow-up in both the E1 (p > .5) and C (p > .3). - Δ TSI had a statistical tendency (p= .10 and p= .02) to decrease during maximal and submaximal exercise in the angioplasty control-group. -T50 was significantly lower (p= .02) after a submaximal effort and a tendency to decrease after maximal effort in the exercise group (p > .10). In the angioplasty control-group T50 decreased significantly after both (sub)maximal effort. Although data was only provided for the submaximal test (p= .01)

Collins et al 2012 (a and b)	No data	During progressive treadmill test: E1: TTM ↑ E2: TTM =	No data	During Constant-work rate test: Isotime TSI: level of TSI recorded at a time consistent at baseline and follow-up Both in E1 and E2 =	-Data was provided in two separate manuscripts on the NIRS response during a progressive, graded treadmill test and a constant work-rate test. Time-point taken were 12-weeks and 24-weeks respectively.	-In the manuscript covering the progressive treadmill test (Collins et al, 2012 b) the authors used paired t-test in an intention-to-treat sample. In the manuscript covering the constant work-rate test (Collins et al, 2012a) the authors used both ANOVA/ANCOVA models -For progressive test a TTM improvement has been found in the traditional walking group only (p= .00 vs p= .22). Data on the constant-work rate test lacks independent pre-post comparison but our analysis of ES suggests a similar trend (ES: p= .003 vs p= .155). A time-effect for both groups is reported to be significant (p < .001).
Figoni et al 2009	No data	E1: TSI _{min} =	E1: T100 =	TSI exercise: the mean TSI during exercise E1: ↓ TSI exercise area: area over the StO ₂ curve during exercise, %minutes E1: ↓	-The authors report that TSI exercise and TSI exercise area were significantly lower after the exercise intervention (p= .01 respectively)	-Paired t-test -No significant changes were found for minimum TSI value during exercise after the intervention (p= .24). Recovery time showed a statistical tendency (p= .04) after correcting for multiple testing.
Gardner et al 2014	No data	Both in E1 and E2: TTM ↑ C: TTM =	Both in E1 and E2: T50 ↓ C: T50 =		-Significant different responses on exercise and recovery parameters after both home-based and supervised exercise interventions compared to an attention-controlled group.	-Paired t-test (with ITT to substitute missing values) -Statistically significant increase in time to minimum TSI with faster recovery (T50) for both exercise groups (p < .001). No changes in the control group (P not reported). ANOVA for change scores (p= .025 and p= .020 respectively)
Haga et al 2020	No data	E1: ΔTSI =	E1: T100 =			-Paired t-test -ΔTSI was presented for both the left and right leg without significant changes after the exercise intervention (p= .847 and p= .947) -T100 was statistically unaltered with p= .144 after exercise

Manfredini et al 2012	No data	No data	No data	Deoxygenation (O₂Hb AUC) and total hemoglobin (tHb AUC) E1: O ₂ Hb AUC ↑, tHb AUC = E2: O ₂ Hb AUC =, tHb AUC = Venous occlusion Muscle oxygen consumption (mVO₂): E1: ↑ E2: =	-Healthy control group with unmodified active lifestyle -Muscle oxygen consumption was assessed after venous occlusion and was significantly increased in the structured exercise group only (p= .03, with p not reported in the self-paced and control group respectively).	-Paired t-test -Data was provided on Area Under Curve (AUC) for total hemoglobin and oxygen saturated hemoglobin during submaximal exercise (1.7-3.0 km/h), with significant changes in AUC O ₂ Hb in the structured exercise group (p= .006) but not in the self-paced or control group (p not reported). Baseline AUC for O ₂ Hb was significantly higher in the healthy control group (p < .001)
Monteiro et al 2019	No data	E1: TTM ↑ E1: ΔTSI = E2: TTM = E2: ΔTSI =	Both in E1 and E2: T100 ↓	Arterial occlusion ΔHHb Both in E1 and E2: = ΔTSI Both in E1 and E2: = T100 Both in E1 and E2: = Treadmill exercise ΔHHb Both in E1 and E2: = Time of resistance Both in E1 and E2: ↑ Deoxygenation rate (ΔTSI/sec) Both in E1 and E2: ↓ Re-oxygenation rate (ΔTSI/min) Both in E1 and E2: = Relative re-oxygenation rate Both in E1 and E2: ↑ Waking economy (m/ΔHHb) Both in E1 and E2: = Waking economy (m/ΔTSI) Both in E1 and E2: ↑	-The authors report different outcomes during exercise with no change in ΔHHb (deoxygenated haemoglobin) but an increased time of resistance (after reaching lowest TSI values) in both groups (situation-effect: p= .001 and pre-post for both groups p < .05). -Reoxygenation rate (ΔTSI/exercise + recovery time) was not altered after exercise. Yet, when corrected for total walking time (relative reoxygenation rate) the authors found a significantly shorter recovery time in both groups (situation-effect: p= .017 and pre-post for both groups p < .05)	-Linear mixed models and between and post treatment statistical test are described -TTM was significantly different after the intervention for both groups (situation-effect: p= .01) with a significant between group-difference (interaction-effect: p= .020). TTM significantly increased in the conventional group alone (p < .05) -ΔTSI did not change after the intervention in both groups (situation-effect: p= .709) -Recovery was significantly faster after both interventions (situation-effect: p= .002 and pre-post for both groups p < .05)

Murrow et al 2019	No data	No data	No data	Arterial occlusion Muscle oxygen consumption (mVO₂) E1: ↑ E2: = O₂Hb recovery half-time E1: = E1: =	-The authors limited NIRS assessment to arterial occlusion maneuvers -O ₂ Hb (oxygenated hemoglobin) recovery half-time was not significantly altered after exercise in both groups (interaction-effect: p= .90) -Muscle oxygen consumption was increased (interaction-effect: p= .003) the conventional group alone (p= .004) compared to the NIRS-trained group (p= .183) with p-values derived from the calculated effect sizes.	-Mixed effect model
Tew et al 2009	E1: TSI = C: TSI =	E1: TTM ↑ E1: TSI _{min} = C: TTM = C: TSI _{min} =	No data	Isotime TSI% (30, 60, 120, 180 and 240 seconds) E1: ↑ (for 30, 60, 120, 180 seconds) C: = (for 30, 60, 120, 180 seconds)	-TSI was significantly higher after intervention on all time-points except for the 240 seconds mark (p < .05). In comparison, controls did have a higher TSI at 240 seconds alone.	-ANCOVA and paired t-test -TTM was significantly increased in the intervention group alone (p< .05) with an interaction-effect of p < .001 . -No changes were noted in TSI _{min} (interaction-effect: p= .186) (pre-post p-values not reported)
Woessner et al 2018	No data	No data	No data	Deoxygenation characteristics E1: = Oxygenation characteristics (parameter a, parameter b in $f=a*x/(b+x)$) E1: ↑	-The authors examined the oxygenated and deoxygenated hemoglobin response (fitted to an exponential decay and single rectangular hyperbola respectively) during exercise and observed a significant increase in O ₂ Hb-maintenance following exercise intervention in the placebo-controlled nitrate group.	-Chi-square test between groups

Supplementary table 4A. NIRS Effect sizes at rest.

At rest	Sample size	Effect size (SMD)	Confidence intervals (95%)	Pre Mean $\pm$ SD	Post Mean $\pm$ SD	Group P-value	Group comparison P-value	Measurement method
<i>Tissue Saturation Index</i>								
Tew E1	27	0.272	(-0.113, +0.656)	47.9 $\pm$ 9.0	50.8 $\pm$ 11.8	NR	NR	NR
Tew C	24	-0.122	(-0.523, +0.280)	46.0 $\pm$ 6.3	45.2 $\pm$ 6.8	NR		NR
Baker E1	29	0.259	(-0.111, +0.630)	59.7 $\pm$ 20.3*	64.3 $\pm$ 14.0*	> .05	> .05	Standing
Baker C	35	0.217	(-0.119, +0.552)	55.3 $\pm$ 20.1*	59.3 $\pm$ 16.2*	> .05		Standing
Beckitt C	14	0.073	(-0.452, +0.579)	55.3 $\pm$ 12.4*	56.3 $\pm$ 14.8*	> .3	NR	Standing
Beckitt E1	42	0.030	(-0.273, +0.332)	55.7 $\pm$ 11.5*	56.0 $\pm$ 10.7*	> .5		Standing
Figoni E1	15	-0.191	(-0.702, +0.320)	84.0 $\pm$ 14.0	81.0 $\pm$ 17.0	NR	NA	Supine
<i>Muscle oxygen consumption (ml/100g/min in Manfredini et al, in Baker et al units are not reported)</i>								
Baker E1	29	1.069	(+0.612, +1.525)	2.406 $\pm$ 0.920*	3.343 $\pm$ 0.826*	NR	< .001	DCS/FD-NIRS
Baker C	35	-0.150	(-0.483, +0.183)	2.583 $\pm$ 0.973	2.403 $\pm$ 1.344	NR		DCS/FD-NIRS
Manfredini E1	31	0.370	(+0.006, +0.733)	0.044 $\pm$ 0.028	0.054 $\pm$ 0.026	.03	NR	Venous occlusion
Manfredini E2	14	0.169	(-0.358, +0.697)	0.040 $\pm$ 0.022	0.044 $\pm$ 0.025	NS		Venous occlusion
<i>Mitochondrial function</i>								
Murrow E1	10	1.166	(+0.362, +1.969)	0.87 $\pm$ 0.51	1.46 $\pm$ 0.51	NR	.003	Arterial occlusion
Murrow E2	8	0.499	(-0.236, +1.234)	1.07 $\pm$ 0.48	1.31 $\pm$ 0.48	NR		Arterial occlusion

Abbreviations: SMD: Standardized Mean Difference, E: exercise group, C: control group, NR (not reported), NA (not applicable) and NS (not significant)/ *Converted from median and IQR. Group P-value and Group Comparison P-value are presented as reported in their main manuscripts. Statistically significant values ($p < .05$) are expressed in bold.

Supplementary Table 4B. NIRS effect sizes during exercise.

Exercise	Sample size	Effect size (SMD)	Confidence interval	Pre Mean $\pm$ SD	Post Mean $\pm$ SD	Group P-value Manuscript	Group comparison P-value	Testing protocol
<i>TTM</i>								
Monteiro E1	16	0.927	(+0.341, +1.513)	150 $\pm$ 91.9	772 $\pm$ 712	< .05	.020	Constant work-rate
Monteiro E2	16	0.297	(-0.204, +0.798)	104 $\pm$ 89.6	140.6 $\pm$ 140.6	NR		Constant work-rate
Collins E1	40	0.704	(+0.358, +1.050)	255 $\pm$ 249.6	476.4 $\pm$ 353.4	.000	.002	Progressive work
Collins E2	45	0.172	(-0.122, +0.467)	221.4 $\pm$ 184.2	258 $\pm$ 232.2	.22		Progressive work
Gardner E1	60	0.577	(+0.303, +0.850)	195 $\pm$ 247	341 $\pm$ 259	< .001	.025	Progressive work
Gardner E2	60	0.579	(+0.306, +0.852)	166 $\pm$ 196	308 $\pm$ 275	< .001		Progressive work
Gardner C	60	0.103	(-0.151, +0.356)	245 $\pm$ 283	272 $\pm$ 236	NR	< .001	Progressive work
Tew E1	27	0.418	(+0.025, +0.811)	268 $\pm$ 305	410 $\pm$ 366	.05		Progressive work
Tew C	24	-0.083	(-0.483, +0.318)	497 $\pm$ 372	466 $\pm$ 379	NR		Progressive work
<i>delta TSI</i>								
Baker E1	29	0.350	(-0.025, +0.725)	12.3 $\pm$ 8.58*	16.00 $\pm$ 11.70*	> .05	< .001	Progressive work
Baker C	35	-0.593	(-0.952, -0.234)	14.0 $\pm$ 6.18*	10.3 $\pm$ 6.18*	> .05		Progressive work
Beckitt E1	42	0.113	(-0.191, +0.417)	38.3 $\pm$ 8.44*	39.3 $\pm$ 9.21*	> .3	NR	Constant work-rate
Beckitt C	14	-0.690	(-1.273, -0.107)	38.0 $\pm$ 9.88	31.7 $\pm$ 8.24	.046		Constant work-rate
Monteiro E1	16	0.026	(-0.464, +0.516)	19.1 $\pm$ 8.40	18 $\pm$ 8.80	NR	.575	Constant work-rate
Monteiro E2	16	-0.127	(-0.619, +0.365)	21.1 $\pm$ 8.17	21.3 $\pm$ 8.50	NR		Constant work-rate
Haga E1 (Left)	16	0.008	(-0.482, +0.498)	17.7 $\pm$ 12.8	17.8 $\pm$ 13.3	0.947	NA	Constant work-rate
<i>TSImin</i>								
Tew E1	27	0.000	(-0.377, +0.377)	39 $\pm$ 14	39 $\pm$ 15	NR	.186	Progressive work
Tew C	24	-0.285	(-0.693, +0.123)	38 $\pm$ 10	35 $\pm$ 11	NR		Progressive work
Figoni E1	15	-0.286	(-0.802, +0.231)	11 $\pm$ 14	7 $\pm$ 14	.24	NA	Progressive work

Abbreviations: SMD: Standardized Mean Difference, E: exercise group, C: control group, NR (not reported), NA (not applicable) and NS (not significant)/ *Converted from median and IQR. Group P-value and Group Comparison P-value are presented as reported in their main manuscripts. Statistically significant values ($p < .05$) are expressed in bold. **Collins 2012a**: similar results on TTM as Collins 2012b for a constant rate treadmill test (only time-effect presented with $p = .05$).

Supplementary table 4C. NIRS effect sizes at recovery.

Recovery	Sample size	Effect size (SMD)	Confidence intervals	Pre Mean ± SD	Post Mean ± SD	Group P-value	Group comparison P-value	Testing protocol
T50								
Gardner E2	60	-0.558	(-0.830, -0.286)	165 ± 147	94 ± 76	< .001	.020	Progressive work
Gardner E1	60	-0.477	(-0.744, -0.210)	168 ± 184	92 ± 94	< .001		Progressive work
Gardner C	60	-0.027	(-0.280, +0.226)	145 ± 149	141 ± 145	NR		Progressive work
Baker C	35	-0.101	(-0.485, +0.283)	108.7 ± 86.6*	100 ± 85.0*	> .05		Progressive work
Baker E1	29	-0.046	(-0.410, +0.318)	95.3 ± 61.6*	92.7 ± 53.8*	> .05		Progressive work
T100								
Monteiro E1	16	-0.665	(-1.207, -0.124)	720.7 ± 921.8	155.1 ± 168.5	< .05	.601	Constant work-rate
Monteiro E2	16	-0.457	(-0.972, +0.058)	637.8 ± 974.5	223.8 ± 157.6	< .05		Constant work-rate
Haga E1	16	-0.424	(-0.935, +0.088)	200.1 ± 151.6	143.1 ± 104.8	.144	NA	Constant work-rate
Figoni E1	15	-0.269	(-0.784, +0.246)	444 ± 403.2	343.8 ± 330.6	.04	NA	Progressive work

Abbreviations: SMD: Standardized Mean Difference, E: exercise group, C: control group, NR (not reported), NA (not applicable) and NS (not significant)/ *Converted from median and IQR. Group P-value and Group Comparison P-value are presented as reported in their main manuscripts. Statistically significant values ($p < .05$) are expressed in bold.

Supplementary Table 5. Secondary outcomes effect sizes.

Secondary outcomes	Sample size (n)	Effect size (SMD)	Confidence interval (95%)	Pre Mean ± SD	Post Mean ± SD	Group P-value	Group comparison P-value	Testing protocol
Pain Free Walking distance (meter)								
Beckitt E1	42	1.880	(+1.377, +2.383)	57.7 ± 30.7*	119.3 ± 34.5*	.01	NR	Constant work-rate
Beckitt C	14	1.513	(+0.746, +2.280)	62.3 ± 35.4*	161.7 ± 75.8*	> .3		Constant work-rate
Haga E1	16	0.411	(-0.100, +0.921)	160 ± 128	228 ± 187	.034	NA	Constant work-rate
Manfredini E1	31	0.769	(+0.368, +1.169)	89.2 ± 46.7	127.7 ± 52.9	.0001	NR	Progressive work
Manfredini E2	14	0.291	(-0.244, +0.826)	103.1 ± 64.1	123.2 ± 73.1	NS	.035	Progressive work
Tew E1	27	0.718	(+0.295, +1.141)	147 ± 125	255 ± 167	< .05		Progressive work
Tew C	24	0.083	(-0.317, +0.484)	177 ± 160	192 ± 195	NR		Progressive work
Pain Free Walking time (seconds)								
Murrow E1	10	1.475	(+0.580, +2.371)	150 ± 170.8	402 ± 170.8	< .01	.97†	Progressive work
Murrow E2	8	1.107	(+0.227, +1.987)	216 ± 169.7	414 ± 186.7	< .01		Progressive work
Collins (a) E1	49	0.624	(+0.318, +0.930)	252 ± 162	408 ± 288	< .001	NR	Constant work-rate
Collins (a) E2	46	0.473	(+0.169, +0.778)	222 ± 120	558 ± 762	< .001		Constant work-rate
Collins (b) E1	40	0.336	(+0.018, +0.655)	237 ± 178.2	313.8 ± 257.4	.034	.38	Progressive work
Collins (b) E2	45	0.204	(-0.092, +0.499)	199.8 ± 165	238.8 ± 210	.15		Progressive work
Gardner E1	60	0.487	(+0.220, +0.755)	195 ± 171	300 ± 242	< .001	< .001	Progressive work
Gardner E2	60	0.672	(+0.392, +0.952)	193 ± 150	363 ± 292	< .001		Progressive work
Gardner C	60	0.098	(-0.156, +0.351)	205 ± 167	222 ± 180	NR		Progressive work
Woessner E1	13	1.033	(+0.360, +1.706)	/	Δ59.2 ± 57.3	< .05	NA	Progressive work
Maximal Walking distance (meter)								
Beckitt E1	42	2.240	(+1.673, +2.806)	124.3 ± 35.5*	233 ± 55.3*	.002	NR	Constant work-rate
Beckitt C	14	1.381	(+0.649, +2.113)	133.7 ± 47.8*	323.3 ± 154.8*	.001		Constant work-rate
Manfredini E1	31	0.572	(+0.192, +0.952)	140 ± 60.1	173.2 ± 55.7	.001	NR	Progressive work
Manfredini E2	14	-0.098	(-0.623, +0.428)	147.3 ± 82.5	139.8 ± 69.7	NR		Progressive work
Haga E1	16	0.646	(+0.107, +1.185)	453 ± 345	702 ± 416	.006	NA	Constant work-rate
Monteiro E1	16	1.708	(+0.940, +2.477)	184 ± 156.4	1669.6 ± 937.2	NR††	.444	Constant work-rate
Monteiro E2	16	1.110	(+0.487, +1.733)	174 ± 141.9	1154.5 ± 945.9	NR††		Constant work-rate
Tew E1	27	0.561	(+0.155, +0.967)	496 ± 250	661 ± 324	< .05	.011	Progressive work

Tew C	24	0.091	(-0.310, +0.492)	600 ± 300	626 ± 266	NR		Progressive work
<i>Maximal Walking time (seconds)</i>								
Baker E1	29	0.439	(+0.058, +0.820)	493 ± 332.2*	718 ± 590.3*	NR†	.001	Progressive work
Baker C	35	0.297	(-0.042, +0.635)	416.7 ± 216.4*	503.7 ± 333.9*	NR†		Progressive work
Collins (a) E1	49	0.842	(+0.516, +1.168)	478.8 ± 216	1266 ± 1024.2	NR	NR	Constant work-rate
Collins (a) E2	46	0.684	(+0.363, +1.005)	443.4 ± 173.4	901.2 ± 739.2	NR		Constant work-rate
Collins (b) E1	40	0.703	(+0.357, +1.049)	480.6 ± 240.6	678 ± 308.4	.000	.002	Progressive work
Collins (b) E2	45	0.104	(-0.188, +0.397)	465.6 ± 240.6	469.8 ± 334.2	.92		Progressive work
Figoni E1	15	1.000	(+0.380, +1.620)	431.4 ± 237.6	676.2 ± 251.4	.000	NA	Progressive work
Murrow E1	10	0.738	(+0.039, +1.437)	508.2 ± 182.1	642.6 ± 182.1	NR	< .05	Progressive work
Murrow E2	8	0.757	(-0.029, +1.542)	522 ± 181.6	659.4 ± 181.6	NR		Progressive work
Gardner E1	60	0.345	(+0.085, +0.605)	380 ± 274	490 ± 350	< .001		Progressive work
Gardner E2	60	0.710	(+0.427, +0.993)	356 ± 222	547 ± 299	< .001	< .001	Progressive work
Gardner C	60	0.088	(-0.165, +0.342)	464 ± 237	486 ± 260	NR		Progressive work
Woessner	13	1.153	(+0.452, +1.855)	/	Δ238.7 ± 207.0	< .01	NA	Progressive work
<i>6 Minute Walking Time</i>								
Gardner E1	60	0.386	(+0.124, +0.649)	328 ± 108	372 ± 119	< .001		
Gardner E2	60	0.165	(-0.089, +0.420)	326 ± 94	341 ± 87	< .05	.028	
Gardner C	60	0.052	(-0.201, +0.305)	376 ± 73	380 ± 81	NR		
Woessner E1	13	2.033	(+1.081, +2.985)	/	Δ24.6 ± 12.1	< .05	NA	
<i>Peak oxygen consumption (ml/kg/min)</i>								
Tew E1	27	0.322	(-0.065, +0.708)	17.2 ± 2.7	18.2 ± 3.4	< .05	.038	
Tew C	24	-0.120	(-0.521, +0.282)	18.6 ± 5.1	18.0 ± 4.9	NS		
<i>Ankle Brachial Index</i>								
Baker E1	29	0.055	(-0.309, +0.420)	0.65 ± 0.17*	0.66 ± 0.19*	> .05	> .05	Baseline E1>E2
Baker C	35	-0.072	(-0.404, +0.260)	0.61 ± 0.16*	0.60 ± 0.09*	> .05		
Manfredini E1	31	0.638	(+0.252, +1.024)	0.59 ± 0.13	0.68 ± 0.15	< .0001	NR	
Manfredini E2	14	0.273	(-0.261, +0.806)	0.67 ± 0.11	0.70 ± 0.11	NR		
Murrow E1	10	0.000	(-0.620, +0.620)	0.80 ± 0.24	0.80 ± 0.24	NR		
Murrow E2	8	0.318	(-0.392, +1.028)	0.87 ± 0.22	0.94 ± 0.22	NR	.52	
Tew E1	27	0.231	(-0.151, +0.613)	0.68 ± 0.13	0.71 ± 0.13	NR		
Tew C	24	0.000	(-0.400, +0.400)	0.69 ± 0.12	0.69 ± 0.15	NR	NR	
Monteiro E1 Right	16	0.107	(-0.384, +0.598)	0.63 ± 0.17	0.65 ± 0.20	NR	NR	

Monteiro E2 Right	16	0.059	(-0.432, +0.549)	0.62 ± 0.18	0.63 ± 0.16	NR		.070
Monteiro E1 Left	16	0.303	(-0.198, +0.804)	0.63 ± 0.17	0.68 ± 0.16	NR		
Monteiro E2 Left	16	-0.060	(-0.550, +0.431)	0.63 ± 0.18	0.62 ± 0.15	NR	NR	.101
Haga E1 Right	16	-0.102	(-0.594, +0.389)	0.79 ± 0.19	0.77 ± 0.20	.189	NA	
Haga E2 Left	16	-0.050	(-0.540, +0.440)	0.77 ± 0.21	0.76 ± 0.19	.621	NA	
Woessner E1	13	0.290	(-0.265, +0.845)	0.70 ± 0.19	0.76 ± 0.22	NR	NA	

Abbreviations: SMD: Standardized Mean Difference, E: exercise group, C: control group, NR (not reported), NA (not applicable) and NS (not significant) / *Converted from median and IQR /† (Murrow et al report a different p-value in the text compared to the table: p=.97 vs p<.05). Group P-value and Group Comparison P-value are presented as reported in their main manuscripts. Statistically significant values (p< .05) are expressed in bold.

Supplementary table 6A. Risk of bias assessment – study level

Study	Objective clearly stated	Eligibility clearly described	Representative study sample	All eligible participants enrolled	Random sequence generation	Control group	Clear description of intervention and delivered consistently	Assessors blinded to intervention	Sufficient follow-up rate	Statistical methods to examine pre-post with p-values	Complete outcome data	Non-selective reporting	Overall score
Baker et al 2017	YES	YES	YES	NO	NO	YES	YES	NO	YES	YES	YES	YES	9
Beckitt et al 2012	NO	YES	NO	NO	NO	NO	YES	NO	YES	YES	YES	NO	5
Collins et al 2012 (a,b)	YES	YES	YES	NO	YES	NO	YES	NO	NO	YES	YES	NO	7
Figoni et al 2009	YES	YES	NO	NO	NO	NO	YES	NO	NO	YES	YES	YES	6
Gardner et al 2014	YES	YES	YES	YES	YES	YES	YES	NO	YES	YES	YES	NO	10
Haga et al 2020	NO	YES	NO	NO	NO	NO	YES	NO	YES	NO	YES	YES	5
Manfredini et al 2012	YES	YES	YES	NO	NO	NO	YES	NO	YES	YES	YES	YES	8
Monteiro et al 2019	YES	YES	YES	YES	YES	NO	YES	YES	YES	YES	YES	YES	11
Murrow et al 2019	YES	YES	YES	NO	NO	NO	NO	NO	NO	YES	YES	YES	6
Tew et al 2009	YES	YES	NO	NO	YES	YES	YES	NO	YES	YES	YES	YES	9
Woessner et al 2018	YES	YES	YES	NO	NO	YES	YES	YES	NO	YES	YES	NO	8

The overall score for each article is calculated by summing the number of ‘yes’ answers.

Supplementary Table 6B. Risk of bias assessment – NIRS specific

Study	NIRS device characteristics				Anatomical description	Reproducibility	ATT measurement	Analysis		
	Manufacturer	Sampling frequency	Wavelengths	Optodes				Filter	Variables defined	All outcomes reported
Baker et al 2017	YES	YES	YES	YES	YES	NO	YES	NO	YES	YES
Beckitt et al 2012	YES	YES	YES**	YES	YES	YES	NO	NO	YES	NO
Collins et al 2012 (a,b)	YES	YES	NO	NO	YES	YES	NO	NO	YES	NO
Figoni et al 2009	YES	YES	YES**	NO*	YES	YES	NO	NO	YES	YES
Gardner et al 2014	YES	YES	NO	NO*	YES	NO	NO	NO	NO	NO
Haga et al 2020	NO	NO	NO	NO	NO	NO	NO	NO	NO	YES
Manfredini et al 2012	YES	NO	YES	YES	YES	YES	NO***	NO	YES	YES
Monteiro et al 2019	YES	YES	NO	NO	YES	NO	NO	NO	YES	YES
Murrow et al 2019	YES	YES	YES	YES	NO	NO	YES	NO	YES	YES
Tew et al 2009	YES	YES	NO	NO	YES	NO	NO	NO	YES	YES
Woessner et al 2018	YES	NO	YES	NO	NO	NO	NO	NO	YES	YES

Abbreviations: ATT: Adipose Tissue Thickness. *Optodes: only mentioning the used probe, **General statements rather than specific wavelength information, ***Not mentioned in the methods although present in the exclusion criteria

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	4
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4-5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	S file 1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4-5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4-5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5-6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	/
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	6
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	S file 6
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	6-7
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	7-9
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	9
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	/
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	9-11
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	12
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	13

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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