Exploration of the Physiological Effects of Exercise in Cardiovascular Diseases

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EXPLORATION OF THE PHYSIOLOGICAL EFFECTS OF EXERCISE IN CARDIOVASCULAR DISEASES

EDITED BY: Markos Klonizakis, Helena Lenasi and Ines Drenjančević
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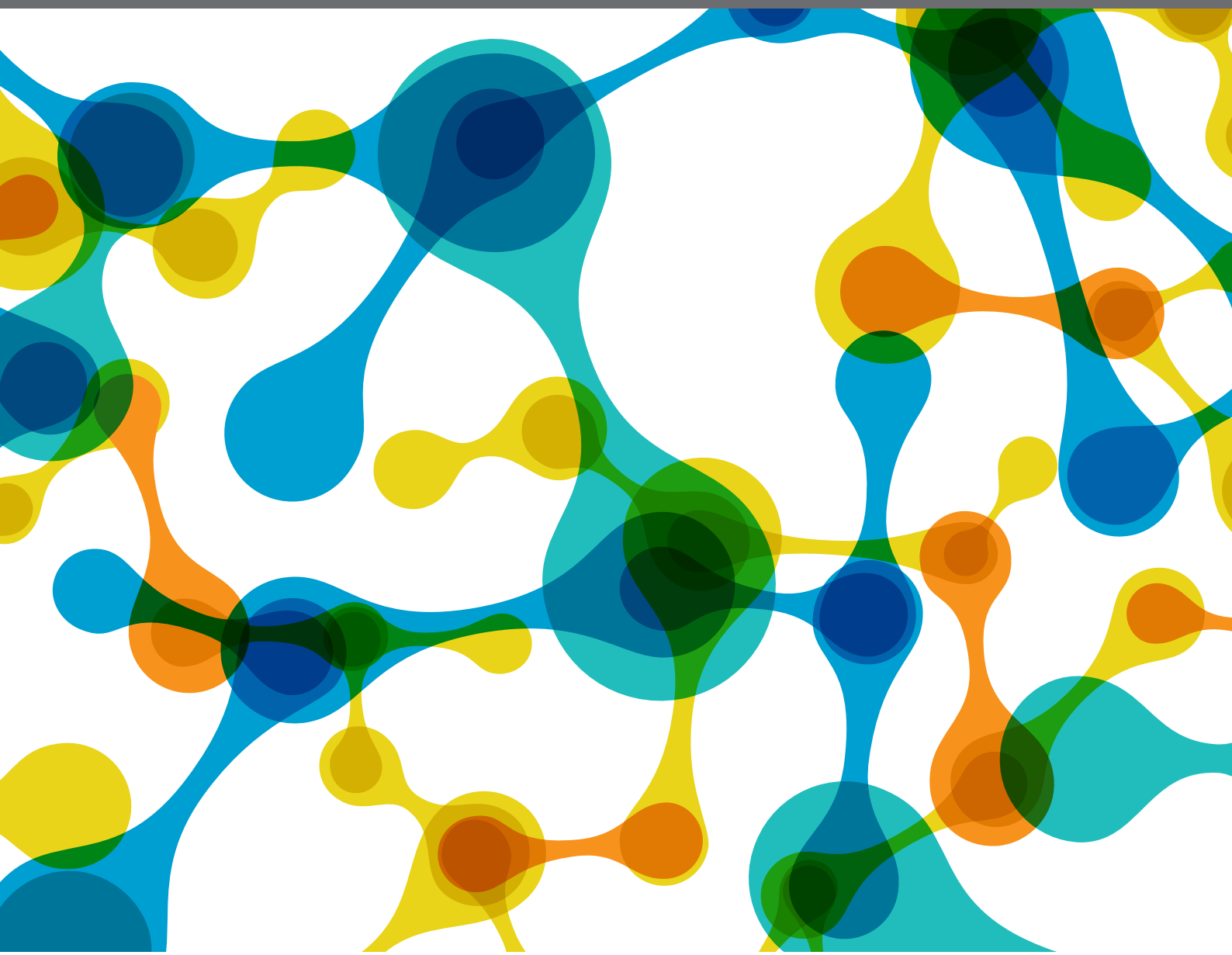

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EXPLORATION OF THE PHYSIOLOGICAL EFFECTS OF EXERCISE IN CARDIOVASCULAR DISEASES

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EDITORIAL
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Editorial: Exploration of the Physiological Effects of Exercise in Cardiovascular Diseases

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Editorial on the Research Topic

Exploration of the Physiological Effects of Exercise in Cardiovascular Diseases

With the cost of treatment for cardiovascular (CV) diseases increasing exponentially every year (Vandenberghe and Albrecht, 2019) it is important to find adjunct therapies to compliment established treatments. These should be sufficiently effective to either reverse or slow-down the progression of these diseases and conditions, not only enhancing treatment, but also improving patients' quality of life. Exercise has been earmarked as one of the main lifestyle components that could be introduced in therapeutic interventions, as it is usually easy to implement by facilitators and be followed by clinical populations (i.e., Klonizakis et al., 2018; Mitropoulos et al., 2020), offering also societal and quality of life benefits (Kesterton et al., 2019).

Nevertheless, exploring the physiological effects of exercise-based interventions is commonly neglected, with the main focus of studies being given to the interventions' therapeutic contribution. In addition, there is limited knowledge on the methods to either diagnose patients with some borderline diseases (or atypical symptoms) or to trace the efficiency of therapeutic approaches (in patients); to this end, new methods are emerging, helping to detect patients at risk or the response to exercise.

This Research Topic brings together contributions from researchers to advance our understanding as of how exercise affects the vascular physiology of clinical populations, allowing us to take valuable lessons and transfer the gained knowledge further.

Fanget et al. showed that establishing the force-velocity-power (FVP) relationship can support the assessment of the dynamic force production capacities in coronary artery disease (CAD) patients. In their study, mechanical parameters (e.g., theoretical maximum force, velocity, and the maximal power output) were determined during cycloergometer sprint sessions to estimate the FVP relationship slope. They suggest that the observed differences between patients and healthy individuals reflect loss of muscle mass, remodeling of motor units or a neuromuscular activation deficit, and potentially be of value for training adjustment and optimization.

A more invasive approach was applied by Chen et al. in a cohort of heart failure patients with preserved ejection fraction (HFpEF). This clinical group can be difficult to diagnose, as many patients remain asymptomatic at rest, but develop symptoms during exercise. Thus, it would be desirable to predict the response during exercise by using non-invasive measures. The authors showed that the stress echocardiography-derived E/e' ratio (e.g., *the ratio of early mitral inflow velocity to early diastolic tissue velocity*), which is a measure of diastolic function, can be a reliable predictor of abnormal exercise hemodynamics in HFpEF patients.

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Chronic obstructive disease (COPD) patients are another high-risk group, for whom the assessment of respiratory muscles function (at rest and during exercise) can yield useful information. Electromyography (EMG) of the diaphragm, coupled to simultaneous cardiac activity tracing by electrocardiogram (ECG) might represent an indirect measure of neural respiratory drive. As ECG tracings are prone to artifacts Dacha et al. developed a semi-automated protocol for ECG artifacts removal during transesophageal diaphragm EMG. Their findings suggest that the proposed semi-automated method can reliably be used to evaluate changes in EMG amplitudes over a wide range of minute ventilation recorded at rest and during exercise testing in COPD patients. In addition, compared to manual methods, the presented method is more time-efficient, and exhibits less inter-rater variability, and as such could be regarded as a reliable new standard for objective EMG amplitude analyses in future clinical and research settings.

Henni et al. have shown that transcutaneous oxymetry ($TcPO_2$) is a feasible and reliable, non-invasive clinical test to assess microvascular responsiveness in patients with thoracic outlet syndrome (TOS), regardless of the underlying etiology. Using the standardized Roos test, applied to challenge microcirculation, they showed that the $TcPO_2$ drop was correlated either with the clinical symptoms or with the ultrasonographic results allowing the detection of TOS in patients. In addition, $TcPO_2$ can be applied to track potential benefits of surgical or conservative (e.g., kinesotherapy) therapies.

Stupin et al. elucidated the benefits of n-3 polyunsaturated fatty acids (PUFAs) which have been confirmed in several studies as an effective dietary supplement to induce pleiotropic physiological effects, on CV, muscular and immune system, in healthy and in CV patients. Potential mechanisms of PUFAs action on CV system are presented, from their antithrombotic and anti-inflammatory effects, to their potential to improve endothelial (dys)function, respectively reducing the risk of CV events'. Moreover, PUFA's have also been suggested to modulate oxygen consumption during intense exercise which may be beneficial in increasing metabolic capacity, shifting the anaerobic threshold, and accordingly diminish the delayed-onset muscle soreness. Yet, the results of various studies are inconclusive with respect of the effects of PUFAs in men and women, in healthy and patients, and regarding their interaction with exercise. To this end, the paper might encourage researchers to perform additional mechanistic, epidemiologic and clinical studies on PUFAs.

The study by Vasić et al. represents a starting point for further research into optimal exercise modalities in CAD with a recent myocardial infarction or revascularization procedure, with water-based training likely emerging as a suitable exercise option. Endurance plus calisthenics exercise training in thermo-neutral water has been shown as safe, improving aerobic exercise capacity and vascular function in patients undergoing short-term residential cardiac rehabilitation after a recent CAD event.

A review paper by Guo et al. discusses the cardioprotective factors of exercise after myocardial infarction (MI). These factors are secreted by or enriched in the heart and execute their action in an autocrine or paracrine manner. The paper focuses on Growth Differentiation Factor 15 (GDF15), Exercise-induced Follistatin-Like1 (FSTL1), Non-coding RNAs (ncRNAs), Cardiac-derived miRNAs, longcRNAs and Cardiomyocytes secrete extracellular vesicles. All of these may be novel targets to study the mechanism of exercise-induced benefits, besides traditional signaling pathways.

Physical activity is an efficient strategy to delay development of obesity and insulin resistance, and thus the progression of obesity/diabetes-related cardiomyopathy. The study by Boardman et al. explored the effect of exercise on ischemic-tolerance when exercise was initiated after the development of obesity-mediated cardiomiopathy in high-fat fed mice.

The authors present the beneficial effects of exercise training with regard to improving the ischemic-tolerance in hearts with cardiomyopathy following obesity and insulin resistance. This study also emphasizes the exercise-induced improvement of cardiac energetics and mitochondrial function in obesity/diabetes.

Finally, Kambič et al. presented the results of assessment of the safety and efficacy of blood-flow restricted (BFR) resistance training in patients with stable CAD compared to usual care. Eight weeks of BFR resistance training did not show any unfavorable cardiovascular responses (such as hemodynamic alterations, anginal symptoms, or ventricular arrhythmias) but was associated with significant improvements in muscle strength and decrease in systolic blood pressure. Thus, it may be therefore provided as an additional exercise option to aerobic exercise to improve skeletal muscle functioning in patients with CAD.

In conclusion, the topic encompasses a heterogenous range of papers, that are relevant from the clinical point of view as they can broaden therapeutic strategies and challenge researchers to further study and elucidate unsolved questions. We look forward that this knowledge will be used in further, larger trials and translated to sustained clinical practice.

AUTHOR CONTRIBUTIONS

All authors contributed equally to the first draft of the manuscript, manuscript revision, and read and approved the submitted version. HL and ID share first authorship of this publication.

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ORIGINAL RESEARCH
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Microvascular Response to the Roos Test Has Excellent Feasibility and Good Reliability in Patients With Suspected Thoracic Outlet Syndrome

*Samir Henni[1,2], Jeanne Hersant[1], Myriam Ammi[3], Fatima-Ezzahra Mortaki[4], Jean Picquet[2,3], Mathieu Feuilloy[5] and Pierre Abraham[2,4]**

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Background: Exercise oximetry allows operator-independent recordings of microvascular blood flow impairments during exercise and can be used during upper arm provocative maneuvers.

Objective: To study the test-retest reliability of upper-limb oximetry during the Roos test in patients with suspected thoracic outlet syndrome (TOS).

Materials and Methods: Forty-two patients (28 men, 14 women; mean age: 40.8 years) were examined via transcutaneous oxygen pressure (TcpO2) recordings during two consecutive Roos tests in the standing position. The minimal decrease from rest of oxygen pressure (DROPmin) value was recorded after each maneuver was performed on both arms. The area under the receiver operating characteristic (ROC) curve defined the DROPmin diagnostic performance in the presence of symptoms during the tests. The Mann–Whitney *U*-test was used to compare the DROPmin in the symptomatic vs. asymptomatic arms. The test-retest reliability was analyzed with Bland-Altman representations. The results are presented as means ± standard deviations (SD) or medians [25–75 percentiles].

Results: The symptoms by history were different from the symptoms expressed during the Roos maneuvers in one-third of the patients. The DROPmin measurements were −19 [−36; −7] mmHg and −8 [−16; −5] mmHg in the symptomatic (n = 108) and asymptomatic (n = 60) arms, respectively. When TOS observed on ultrasound imaging was the endpoint, the area under the ROC curve (AUC) was 0.725 ± 0.058, with an optimal cutoff point of −15 mmHg. This value provided 67% sensitivity and 78% specificity for the presence TOS via ultrasound. When symptoms occurring during the test represented the endpoint, the AUC was 0.698 ± 0.04, with a cutoff point of −10 mmHg. This provided 62% sensitivity and 66% specificity for the presence of pain in the ipsilateral arm during the test. The test-retest reliability of DROPmin proved to be good but not perfect, partly because of unreliability of the provocation maneuvers.

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Conclusion: To the best of our knowledge, this study is the first to investigate microvascular responses during the Roos maneuver in patients with suspected TOS. The presence of symptoms was significantly associated with ischemia. TcpO2 facilitated the recording of both macrovascular and microvascular responses to the Roos test. The Roos maneuver should probably be performed at least twice in patients with suspected TOS.

Keywords: peripheral artery disease, exercise, provocative maneuvers, ischemia, microcirculation, oximetry

INTRODUCTION

Thoracic outlet syndrome (TOS) is one of the most controversial disorders in medicine, with multiple recent editorials having been published on the subject (Ahmad and Murthy, 2018; Burt, 2018; Illig, 2018). TOS comprises a variety of symptoms and physical findings resulting from the external compression of vessels and/or nerves as they pass through multiple narrow spaces between the thorax and the upper limb (Jubbal et al., 2018; Kaplan et al., 2018). As described by Demondion et al. (2006), the diagnosis is based on clinical findings, but making this diagnosis is often difficult. The use of imaging (radiography, ultrasonography, computed tomographic angiography, or magnetic resonance imagery) is required to determine the nature and location of the underlying structures causing the compression (Ammi et al., 2017).

Many provocative maneuvers have been proposed to induce dynamic compression of the neural or vascular structures. A provocative test was proposed by Roos in 1966, which is referred to as the "Roos test" or the "elevated arm stress test" (EAST). Beyond the reproduction of symptoms induced by the Roos test, an evaluation of perfusion via Doppler, pulse palpation, and/or observation of hand pallor are generally performed to detect arterial compression. However, coldness and color changes could also result from sympathetic vasoconstriction during neurogenic TOS (Sanders et al., 2007). In evaluating vasoconstrictive responses to adrenergic stimulation, the study of skin micro-circulation during provocative maneuvers could be of major interest in the study of TOS, at least for research purposes. To the best of our knowledge, such microvascular investigations have never been reported.

We aimed to evaluate the effects of the Roos (EAST) test on upper arm microcirculation using transcutaneous oxygen pressure (TcpO2) measurements. We hypothesized that this technique would be feasible and could account for both macrovascular and microvascular impairments. We also aimed at estimating the reliability of the responses observed in both unaffected patients and patients referred for suspected TOS. Last, we aimed to identify the preliminary results of preoperative versus postoperative evaluations.

MATERIALS AND METHODS

From March 2018 to September 2018, 42 patients were recruited into this prospective single-center study.

Ethical Standards

The patients were fully informed about the study and its procedures; all patients signed a written consent document. This research and all its procedures were performed in compliance with the principles outlined in the Declaration of Helsinki. The study was promoted by the University Hospital in Angers, approved by our institutional Ethics Committee, and was registered in ClinicalTrials.gov under Ref: NCT03355274.

Experimental Design

The study population comprised patients referred to the Department of Vascular Medicine for suspected TOS. All subjects were over 18 years of age and there was no maximum age limit set. Exclusion criteria were: pregnancy, any legal constraint, or current participation in another clinical trial. To date, one female patient was referred to us for a new evaluation after a prior surgery. Results from this observation are provided to illustrate the presence or absence of persistent ischemia in the surgically treated arm.

Initial Assessment

At the point of study inclusion, we recorded patient demographics and conditions including: age, sex, presence of one or more cardiovascular risk factors (hypertension, diabetes mellitus, dyslipidemia, active smoking), history of chest or shoulder surgery, and any ongoing treatments. We also measured weight, height, systolic and diastolic blood pressures, and evaluated positional microvascular responses to the Roos test using transcutaneous oximetry (TcpO2) as described below. Results from ultrasound investigations performed prior to referral were encoded as "presence of absence of positive TO" on each side, according to the patient's medical record. The technicians or physicians doing the TcpO2 test were blinded to these data.

Transcutaneous Oxygen Pressure Recordings

In brief, TcpO2 is a useful technique that measures the local skin oxygen partial pressure using electrochemical probes heated to 44.5°C to improve local perfusion and oxygen transcutaneous diffusion. TcpO2 changes, by calculating reductions in the rest of oxygen pressure (DROP) index, provide evidence of stimulation-induced regional blood flow impairments (Abraham et al., 2003, 2005; Bouye et al., 2004; Abraham, 2006; Henni et al., 2018). DROP is calculated as the limb level changes minus the changes at a reference chest electrode. DROP allows for removal of error

due to unpredictable transcutaneous gradients (Abraham et al., 2003; Grouiller et al., 2006; Blake et al., 2018; Henni et al., 2018). In the present study, we positioned one probe 5–7 cm distal to the elbow joint at the proximal and internal portion of each forearm and one probe on the chest (parasternal) for reference. After a minimum of 15 min of heating, we started a 1-Hz recording with a 30-s reference period, after which patients performed two consecutive Roos tests. The second test was started after the DROP values returned to zero and after a minimum recovery period of 1 min following the end of the first test. Provocative maneuvers were conducted in the standing position with the patient's back against a wall. The patients were required to raise their arms to 90 degrees of abduction, with the arms fully externally rotated and the elbows at 90 degrees of flexion. Further, we asked the patients to try to touch the wall behind them with their elbows and wrists, but not their back, to ensure that the arms were flexed slightly behind the frontal plane, as shown in **Figure 1**. This "surrender" or "candlestick" position with opening and closing of both hands was sustained in all patients to the point of maximum pain, or for a minimum of 2 min in the absence of pain. Patients were repeatedly asked to report any symptoms (pain, fatigue, numbness, or tingling) experienced during the Roos test on each arm. The minimum value of DROP (DROPmin) during or in the minute following the end of each Roos maneuver was recorded for each arm. Inherently, DROP is a negative value that decreases with the increase in the severity of ischemia (Abraham et al., 2005) and is expressed in millimeters of mercury (mmHg).

Statistical Analysis

The data are presented as numbers (percentages), medians [25, 75 percentiles], or means ± standard deviations (SD). A comparison of the DROPmin results between the symptomatic and asymptomatic arms was performed using the Wilcoxon rank test. A receiver operating characteristic (ROC) curve analysis comparing the DROPmin value to the presence/absence of symptoms was used to define the optimal cutoff point for the DROPmin values in the prediction of symptom occurrence. All statistical analyses were performed using SPSS (IBM SPSS statistics V15.0, Chicago, IL, United States). $P < 0.05$ was considered to be statistically significant. The area under the ROC curve (AUC) was used to determine whether DROP significantly predicted positive results on ultrasound imaging and/or the presence of symptoms during the Roos test. The optimal cutoff point was defined as the DROP value that resulted in the minimum distance to the 100% sensitivity / 100% specificity angle. The test-retest reliability of the DROP results observed for two consecutive Roos tests was analyzed using a Bland-Altman representation with logarithmic (10-log) transform in cases of heteroscedasticity, according to recommendations (Euser et al., 2008; Ludbrook, 2010). To create the 10-log transformation, DROPmin values were converted into absolute values (positive values). The absence of a decrease in DROP was encoded by −1 mmHg and not zero. Last, the test-retest reliability of TcpO2 was evaluated according to the similarity of symptoms between test 1 and test 2. Indeed, if discomfort or pain was present during one of the maneuvers, but absent during the other one, the TcpO2 difference was expected to have resulted from unsatisfactory maneuver reproduction, with the presence of compression during one test and the absence of compression during the other.

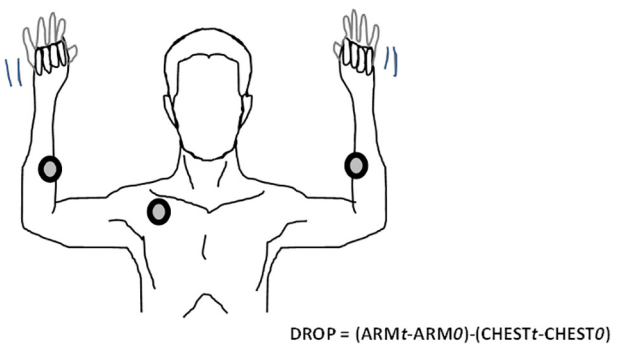

FIGURE 1 | Example of the Roos maneuver with TcpO2 recordings. The DROP calculation at time (t) is based on subtraction of the chest measurement from the arm measurements. Chest and arm changes are accounted for in the mean values measured over the 30 first seconds of recording "0." TcpO2, transcutaneous oxygen pressure; DROP, decrease from the rest of oxygen pressure.

RESULTS

The recruited patients included 28 men and 14 women, aged 40.8 ± 12.2 years old. The mean weight was 70.3 ± 16 kg and mean height was 164 ± 7 cm. Fifteen patients had one or more cardiovascular risk factors and 12 had a prior history of chest or shoulder surgery. Ongoing treatments included the use of lipid-lowering drugs ($n = 2$) and sartans ($n = 2$). Systolic and diastolic arm blood pressures were 126 ± 14 and 79 ± 11 mmHg, respectively. Among these patients, 15 reported unilateral symptoms on the right ($n = 7$) or left ($n = 8$) side and 27 reported bilateral symptoms. Among the patients reporting unilateral symptoms, 5 had previously undergone surgery for contralateral TOS. Among patients with unilateral symptoms, Roos maneuvers induced unilateral ($n = 8$) or bilateral ($n = 5$) pain. In two patients, the Roos maneuver did not reproduce the usual symptoms. Interestingly, among patients with unilateral symptoms, when the results were analyzed arm by arm, the symptoms were present in two consecutive tests in 8 patients, in neither of the tests in 5, and in one test only in 17 (in solely the first test in 8, and in solely the second test in 9). Of the 27 patients referred for bilateral symptoms, 2 had undergone previous unilateral ($n = 1$) or bilateral ($n = 1$) surgery for TOS. Among these patients, the Roos maneuvers induced unilateral ($n = 6$) or bilateral ($n = 20$) pain. In one patient, the Roos maneuvers did not reproduce the usual symptoms. Notably, when the 54 arms of the patients with bilateral symptoms were analyzed arm by arm, the symptoms were present during two consecutive tests in 31 patients, during neither test in 10, during solely the first test in 8, and during

solely the second test in 5. Of 42 patients, ultrasounds were positive for TOS on one or both sides in only 25 of them, as shown in **Table 1**.

TcpO2 showed excellent feasibility, with no technical failures or missing values. The chest TcpO2 value at rest was 69 ± 10 mmHg. TcpO2 at rest in the symptomatic (69 values) and asymptomatic (15 values) arms were 76 ± 12 and 74 ± 10 mmHg, respectively ($p > 0.05$). A typical example of a recording in a patient with TOS is presented in **Figure 2**. As shown, the Roos maneuvers were responsible for a sharp decrease in the right and left DROP values, although they were slightly decreased during the second maneuver on the left arm.

The lowest DROPmin values of the two tests were as follows: −12 [−21, −7] mmHg in the arm with an absence of TOS findings on ultrasound imaging and −27 [−50, −14] mmHg in the arms with ultrasound evidence of TOS ($p < 0.01$). When TOS identified on ultrasound imaging was the endpoint, the AUC was 0.725 ± 0.058 ($p < 0.001$ from random choice), with an optimal cutoff point of −15 mmHg providing 67% sensitivity and 78% specificity for the presence of TOS on ultrasound.

On average, the DROPmin values were as follows: −14 [−26, −6] mmHg for test 1 and −13 [−27, −7] mmHg for test 2 ($p > 0.05$) on the right side; −13 [−27, −7] mmHg for test and −11 [−29, −6] mmHg for test 2 on the left side. On an arm-by-arm basis, among the 168 available values (42 patients, 2 arms, 2 tests), the DROPmin values measured in the symptomatic (108 values) and asymptomatic (60 values) arms were −19 [−36, −7] mmHg and −8 [−16, −5] mmHg, respectively ($p < 0.001$). When the presence of symptoms during the test was the endpoint, the AUC was 0.698 ± 0.04 ($p < 0.001$ from random choice), with a cutoff point of −10 mmHg providing 62% sensitivity and 66% specificity for the presence of pain in the ipsilateral arm during the test.

For the entire test series, the average difference between the DROPmin results of test 2 vs. test 1 on a Bland-Altman representation showed a clear heteroscedastic distribution with a mean difference of 1.2 mmHg. **Figure 3** shows the 10-log transformed data, confirming good agreement between the two tests (despite outlier values), with a mean difference close to zero and reasonable limits of agreements (LA).

Finally, the patient presented in **Figure 2** had a second test prior to surgery (**Figure 4**, upper panel), confirming the results of the first test, and then had surgery on the right (dominant) side by an axillary approach. She was referred 3 months after surgery with complete relief of symptoms in the right arm and normalization of the DROPmin on the right side (**Figure 4**, lower panel). Because her left arm was only moderately symptomatic after surgery, she was unwilling to undergo a second operation.

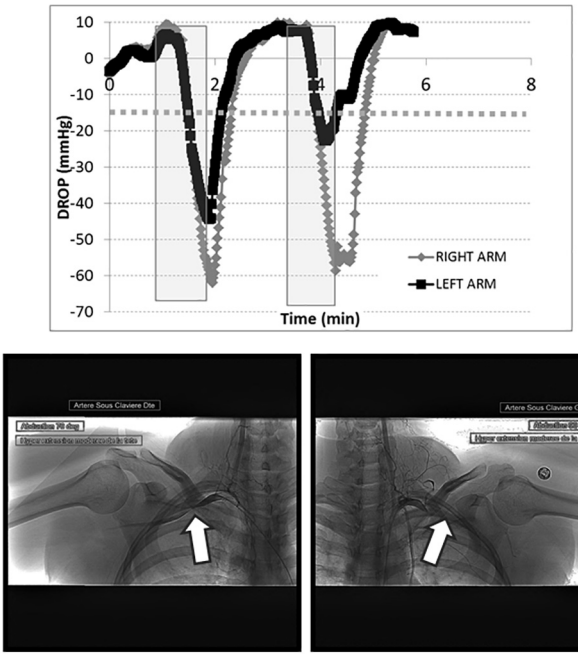

FIGURE 2 | Transcutaneous oxygen pressure recordings are expressed as DROP values (mmHg) during two consecutive Roos maneuvers (gray squares). The *x*-axis represents the time in minutes. As shown, a dramatic decrease in the DROP values occurs during the Roos maneuvers on both sides, below –15 mmHg (dotted line). The corresponding arteriography shows the right (left panel) and left (right panel) sides, confirming occlusion of the subclavian artery during abduction of the arms (white arrows). This arteriography is from a 31-year-old woman who suffered for more than 4 years from upper limb pain, mostly on the right (dominant) side. She previously worked as a hairdresser, but had to stop this activity because of the pain. The pain originated at the shoulder and extended to the hands, with some paresthesias in the fourth and fifth digits. During the clinical examination, the Wright and Adson positions quickly led to a loss of the radial pulses. The Tinel test was negative. Bilateral TOS was suspected. A routine chest X-ray showed no abnormality of the cervico-thoracic junction. A duplex-scan demonstrated dynamic arterial and venous obstructions. Objective signs of lower plexus nerve lesions on electromyogram favored a neurogenic-type outlet syndrome. We also performed a dynamic bilateral angiography via a 4-Fr femoral puncture. This confirmed arterial outlet syndrome with an occlusive costoclavicular grip and a significant dynamic stenosis in the pectoralis minor muscle tunnel. She was prescribed an extended period of kinesiotherapy. DROP, decrease from the rest of oxygen pressure; TOS, thoracic outlet syndrome.

TABLE 1 | Distribution of symptoms by history and results of ultrasound investigations in the studied population.

		Positive ultrasound results			
		None	Right	Left	Bilateral
Symptoms by history	Right	2	3	0	2
	Left	3	2	3	0
	Bilateral	12	2	4	9

DISCUSSION

To the best of our knowledge, this study is the first to investigate microvascular responses during the Roos test, one of the most largely used provocative maneuvers in the diagnosis of TOS. This study provides evidence that the technique is both feasible and reliable. Further, preliminary results suggest that it is sensitive to the changes observed after surgery.

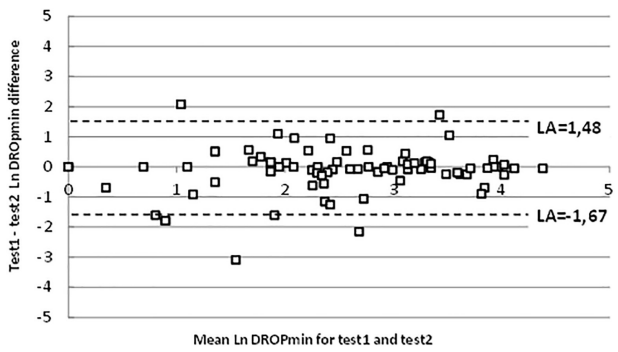

FIGURE 3 | Bland-Altman representation of test-retest reliability on logarithmic scales. "LA" are 95% limits of agreement.

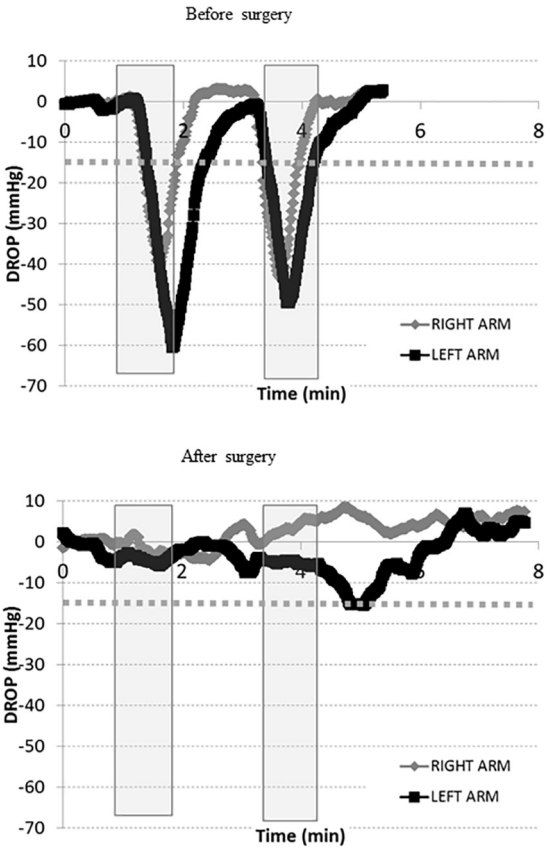

FIGURE 4 | Transcutaneous oxygen pressure recordings expressed as DROP values (mmHg) during two consecutive Roos maneuvers (gray squares). The *x*-axis represents time in minutes before and after surgery. The –15 mmHg limit is shown as a dotted line. These recordings were performed on the same patient described in **Figure 2** above. While she previously underwent an extended period of kinesiotherapy for several months without additional benefit, we proposed surgical treatment for her outlet syndrome. As shown, during the new pre-surgical visit (upper panel), the tests are positive on both sides. The surgery included complete resection of the right first rib (image 2) and pectoralis minor muscle resection through a Roos axillary approach. The patient rapidly recovered from surgery and expressed rapid pain relief. After surgery (lower panel), a moderate decrease in DROP is observed with test 2 on the left side, while test 1 was normal on both sides. The patient had no complaints of pain on the right side, but expressed moderate discomfort on the left side with test 2. DROP, decrease from the rest of oxygen pressure.

Positive tests suggesting the presence of arterial, and/or venous, and /or nerve compressions are frequently observed in the general population. Using photo-plethysmography (PPG) in 130 apparently unaffected individuals, arterial obstructions were identified in 78 of them (60%) (Gergoudis and Barnes, 1980). Abnormal ultrasound or arterial PPG findings on the EAST and Upper Limb Tension Test (ULTT) were observed in 69% of 64 high-performance string instrument musicians, in contrast to 15% of 22 control subjects (Adam et al., 2018). Arterial flow anomalies on Doppler ultrasound and PPG were identified in 13% of 100 limbs from 50 normal volunteers (Chen et al., 2014). In 98 dissections of the thoracic outlet from 50 unselected cadavers, Juvonen et al. (1995) observed 63% with abnormal anatomy and 10% (5/50) had bilaterally normal anatomy. Thus, most neurovascular compressions remain asymptomatic and the specificity of tests will never reach 100% if symptoms are used as gold-standard endpoints.

There are conflicting results about the respective prevalences of arterial (ATOS), venous (VTOS), and neurogenic (NTOS) types of TOS and "combined" / "disputed" types (Ferrante and Ferrante, 2017; Peek et al., 2018). Relative ATOS, VTOS, and NTOS prevalences seem to partially rely on how each form is defined. Restricting arterial TOS to embolic events and venous TOS to Paget-Schroetter thromboembolic complications, as proposed by Sanders et al. (2007), leads to the consideration that over 95% of all TOS are neurogenic and less than 1% are arterial. Other series have reported more balanced results, using less restrictive definitions for ATOS and VTOS with results ranging from 48.7 to 74% of NTOS, 22 to 44% VTOS, and 4 to 25% ATOS (Ann Freischlag, 2018; Henry et al., 2018; Likes et al., 2014). Finally, whatever the definitions used and the exact proportions of each TOS type, the diagnosis of compression has generally been based on non-invasive clinical signs and vascular investigations.

Clinical provocative tests are considered to be of limited sensitivity and specificity (Warrens and Heaton, 1987; Gillard et al., 2001), probably partially resulting from a high rate of positive results in apparently asymptomatic patients, as previously discussed (Nord et al., 2008); however, it may also possibly result from a low test-retest reliability of the provocative maneuvers (Warrens and Heaton, 1987; Nord et al., 2008). No single non-invasive technique seems to be optimal for diagnosing TOS. One of the most widely used and useful tests for diagnosing TOS is ultrasonography. Unfortunately, preoperative duplex ultrasounds are only 41% sensitive for the diagnosis of NTO (Orlando et al., 2016). Ultrasonography only has a sensitivity of 87% and specificity of 88%, with risks of false-positive and false-negative results (Gillard et al., 2001). False-positive tests are assumed to result from signal losses or, when compared to the presence of symptoms by history, from the possibility of compression in asymptomatic – assumed healthy – individuals. False-negative tests could result either from non-adapted maneuvers, insufficient practitioner experience, or

inadequate technical recording. Magnetic resonance imaging (MRI) sensitivity, specificity, and positive and negative predictive values have been reported to be 41, 33, 89, and 4%, respectively (Singh et al., 2014). Whatever the technique, assuming that coldness and color changes would not be caused by ischemia resulting from subclavian artery obstruction, but from an overactive sympathetic nervous system (Sanders et al., 2007), Doppler ultrasonography would not adequately detect neurally induced cutaneous vasoconstriction. Whether or not pallor and coldness are always of neural origin, the specific benefit of TcpO2 is that it can monitor both macro- and microvascular responses according to TcpO2 decreases at arm level. These responses reflect cutaneous flow changes from the eventual occlusion of proximal vessels and the eventual reduction in skin blood flow secondary to sympathetic nervous system-induced vasoconstriction. The specific interest in TcpO2 is that it is expected to decrease in cases of arterial compression (Babilas et al., 2008), secondary to neurally induced vasoconstriction (Provenzano et al., 2008) and isolated venous occlusion (Ostergren et al., 1983; Geis et al., 2008).

To the best of our knowledge, no other research has investigated upper limb TcpO2 during provocative tests to evaluate TOS. Transcutaneous oxygen pressure changes have been well-described at rest and during exercise (Abraham et al., 2003). During exercise, this measurement was shown to be both accurate and reliable in the evaluation of exercise-induced ischemia (Abraham et al., 2003, 2005; Bouye et al., 2004; Abraham, 2006; Henni et al., 2018). Although absolute TcpO2 has shown fair reliability (Rooke and Osmundson, 1989; Daviet et al., 2004), the calculation of the DROP index improved reliability in test–retest recordings (Bouye et al., 2004). In the present study, TcpO2 facilitated the measurement of regional blood flow impairments, regardless of the underlying mechanism, and showed satisfactory reliability. Importantly, the −15 mmHg cutoff point, using ultrasound results as a reference, is similar to the one identified from exercise TcpO2 measurements (Abraham et al., 2003; Grouiller et al., 2006; Audonnet et al., 2017; Henni et al., 2018). Determining the cutoff point is important because limb elevation physiologically decreases TcpO2 as hydrostatic pressure decreases (Dooley et al., 1997; Shah et al., 2008).

The present study had some limitations. First, we did not monitor the positions of the patients using three-dimensional cameras or accelerometers. This would be useful for excluding the ∼15% inconsistent test-retest results that relied on an imperfect reproducibility of carefully performed provocative maneuvers. Our opinion is that although provocative maneuvers were very carefully supervised, even non-measurable minor changes in position and intensity of shoulder muscle contractions could result in variable compressive effects. Similarly, we believe that the inconsistent test-retest results were not due to the technique itself, because TcpO2 changes during exercise-induced ischemia are highly reliable and operator-independent (Henni et al., 2018).

Second, we could not confirm the presence or absence of arterial compression using contrast-enhanced imaging in many of the patients. One reason for this was that most patients with positive tests were referred to physical therapy or declined surgery. Radiological imaging in such patients is not indicated. Further, admitting that pallor and coldness did not systematically result from arterial compression, but rather resulted from neurovascular vasoconstriction during nerve compression, indicated that contrast-enhanced imaging techniques should no longer be considered gold-standard techniques. This would be a limitation if our main goal was to evaluate the diagnostic performance of the TcpO2 technique compared to a gold standard. However, this was not our goal. We only intended to test the feasibility and reliability of the technique. Nevertheless, the postoperative results in one of our patients confirmed that DROPmin values improve after surgery.

Third, we only tested the Roos maneuver, although other tests have been proposed to facilitate the diagnosis of TOS. Our choice was deliberate here, and relates to the fact that the Roos maneuver is generally reported as the one that provides a greater number of positive results (Rayan and Jensen, 1995; Nord et al., 2008). Future studies should evaluate the Tcpo2 response to other tests.

Finally, TcpO2 is not considered a routine tool because of the cost of the device and the length of time required to reach stable values, specifically at arm level (Gorska et al., 2015; Chiang et al., 2018). Nevertheless, the diagnosis of TOS should be based on a holistic approach that includes the patient's history, clinical examination, and minimally invasive investigations. TcpO2 could provide additional non-invasive evidence of abnormal microvascular responses to provocative tests (at least for the Roos test) before radiological imaging is chosen in cases of inefficient physiotherapy.

CONCLUSION

In conclusion, to the best of our knowledge, this study is the first to examine microvascular responses during the Roos maneuver in patients with suspected TOS. However, additional studies with a larger number of patients are needed to evaluate the performance of TcpO2 with respect to making the diagnosis of TOS. These studies should compare the test results to a gold standard, analyze the test-retest reproducibility, evaluate the responses to other provocative maneuvers, and estimate its sensitivity to the treatment of neural and/or vascular compression of the outlet. The Roos maneuver should probably be performed at least twice in patients with suspected TOS because of the moderate test-retest reliability of symptoms induced by the Roos test, even during carefully supervised maneuvers. TcpO2 appears to be a promising non-operator-dependent investigative tool that allows for the simultaneous analysis of both arms during dynamic maneuvers in the standing position and investigations of the microvascular response to the Roos test. This might contribute to a holistic approach toward patients with suspected TOS.

AUTHOR CONTRIBUTIONS

SH, JH, MA, F-EM, JP, and PA participated in patient recruitment, data acquisition, data analysis, and patient treatment. SH and PA participated in the preparation of the

study and provided technical and administrative support for the project. JH, JP, and PA supervised the project. SH, JH, JP, and PA wrote the manuscript. MA and F-EM reviewed and critically revised the draft. All authors approved the final version of the manuscript.

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CLINICAL TRIAL
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Blood Flow Restriction Resistance Exercise Improves Muscle Strength and Hemodynamics, but Not Vascular Function in Coronary Artery Disease Patients: A Pilot Randomized Controlled Trial

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Resistance training may be associated with unfavorable cardiovascular responses (such as hemodynamic alterations, anginal symptoms or ventricular arrhythmias). In healthy adults, blood flow-restricted (BFR) resistance training improves muscle strength and hypertrophy improvements at lower loads with minimal systemic cardiovascular adverse responses. The aim of this study was to assess the safety and efficacy of BFR resistance training in patients with coronary artery disease (CAD) compared to usual care. Patients with stable CAD were randomized to either 8 weeks of supervised biweekly BFR resistance training (30–40% 1RM unilateral knee extension) or usual exercise routine. At baseline and after 8 weeks, patients underwent 1-RM knee extension tests, ultrasonographic appraisal of *vastus lateralis* (VL) muscle diameter and of systemic (brachial artery) flow-mediated dilation, and determination of markers of inflammation (CD40 ligand and tumor necrosis factor alfa), and fasting glucose and insulin levels for homeostatic model assessment (HOMA). A total of 24 patients [12 per group, mean age 60 ± 2 years, 6 (25%) women] were included. No training-related adverse events were recorded. At baseline groups significantly differ in age (mean difference: 8.7 years, $p < 0.001$), systolic blood pressure (mean difference: 12.17 mmHg, $p = 0.024$) and in metabolic control [insulin ($p = 0.014$) and HOMA IR ($p = 0.014$)]. BFR-resistance training significantly increased muscle strength (1-RM, +8.96 kg, $p < 0.001$), and decreased systolic blood pressure (−6.77 mmHg; $p = 0.030$), whereas VL diameter (+0.09 cm, $p = 0.096$), brachial artery flow-mediated vasodilation (+1.55%; $p = 0.079$) and insulin sensitivity (HOMA IR change of 1.15, $p = 0.079$) did not improve significantly. Blood flow restricted resistance training is safe and associated with significant improvements in muscle strength, and may be therefore provided as an additional exercise option to aerobic exercise to improve skeletal muscle functioning in patients with CAD.

Clinical Trial Registration: www.ClinicalTrials.gov, identifier: NCT03087292.

Keywords: blood flow restriction, resistance training, low-loads, coronary artery disease, cardiac rehabilitation

INTRODUCTION

Exercise training is a core component of the cardiac rehabilitation (Leon et al., 2005; Balady et al., 2007), with aerobic training recommended as the preferred modality (Bjarnason-Wehrens et al., 2004). Recommendations for resistance training have only recently and cautiously been put forward (Bjarnason-Wehrens et al., 2004). On the one hand, resistance training improves muscle strength, endurance and mass, bone density, and quality of life (Bjarnason-Wehrens et al., 2004; Williams et al., 2007; Wise and Patrick, 2011); while on the other hand, concerns have been raised over potentially unfavorable cardiovascular responses, such as blood pressure elevation, myocardial ischemia, and ventricular dysrhythmias (Haslam et al., 1988).

Current American Heart Association recommendations on resistance training in cardiovascular patients suggest lower loads [30% of one-repetition maximum (1-RM) for the upper limbs and 50–60% 1-RM for the lower limbs], as this would still improve muscle strength and endurance without excessive blood pressure elevation or other adverse cardiovascular events (Williams et al., 2007; Wise and Patrick, 2011). However, recent studies have shown some conflicting evidence against current resistance training guidelines in cardiovascular disease patients. One study has shown that moderate exercise loads (15 RM) induced greater hemodynamic response compared to higher exercise loads (4 RM) (Gjovaag et al., 2016), whereas others suggested longer sets may evoke higher hemodynamic drifts compared to shorter sets (Lamotte et al., 2010). Also, evidence suggests that higher training loads (>75% of 1-RM) are needed for optimal improvements in muscle hypertrophy and strength in healthy adults (American College of Sports Medicine [ACSM], 2009), whereas recommended loads for patients with coronary artery disease (CAD) are much lower (<30% 1-RM) (Bjarnason-Wehrens et al., 2004; Piepoli et al., 2011) and thus possibly insufficient to elicit increases in isometric strength and hypertrophy.

Blood flow restriction (BFR) exercise is a novel exercise modality in clinical settings, which induces muscle hypertrophy and strength with low to moderate training intensity through increased anabolic processes mediated by BFR (usually with cuff inflation) (Manini and Clark, 2009). BFR improves training adaptations (Loenneke and Pujol, 2009), such as muscle hypertrophy, muscle strength (Takarada et al., 2000; Madarame et al., 2008; Yasuda et al., 2014b), endurance (Kacin and Stražar, 2011) and acute hormonal responses (Pearson and Hussain, 2015), with minimal adverse cardiovascular or muscular effects (Manini and Clark, 2009). In healthy adults, BFR resistance exercise yields muscle hypertrophy and strength comparable to heavy-load resistance training (Hughes et al., 2017), using loads as low as 30% of 1-RM (Takarada et al., 2000). In addition to the improvement in muscle strength and hypertrophy, BFR resistance exercise was proven to be safe, with no significant differences following training in resting creatine kinase, interleukin-6, insulin-like growth factor 1 (IGF-1) or hemostatic markers (Fujita et al., 2007; Nakajima et al., 2007; Madarame et al., 2010; Karabulut et al., 2013; Patterson et al., 2013). Although most BFR research focused on muscular effects, it is important to note that resistance and aerobic BFR exercises may cause increases in heart rate and blood pressure that are greater than those observed with exercise performed at a similar intensity without BFR (Hackney et al., 2012). Since most BFR studies were conducted in healthy older adults (Karabulut et al., 2010, 2011; Yasuda et al., 2014b; Vechin et al., 2015) and musculoskeletal settings (ACL reconstruction, knee osteoarthritis) (Hughes et al., 2017), it is important to evaluate the cardiovascular response in individuals presenting with cardiovascular disease risk factors in a controlled settings (Hackney et al., 2012).

To date, only one study examined the acute effect of BFR resistance exercise in cardiovascular patients (Madarame et al., 2013). Apart from steadily increased heart rate, no adverse effects were reported during and after acute bouts of exercise, as the increase in haemostatic and inflammatory markers was independent of the exercise (Madarame et al., 2013). Chronic effects were observed in a recent trial in which decrease in brain natriuretic peptide and C-reactive protein were shown after 6 months of low intensity BFR aerobic cycling exercise in patients with chronic heart failure (Yasushi and Yudai, 2018), as the chronic impact of BFR resistance exercise in CAD patients still remains elusive. Therefore, we wanted to assess the impact of low-load BFR resistance training in patients with CAD on muscle strength and hypertrophy, vascular function, safety, cardiovascular responses, inflammatory markers, and insulin resistance.

MATERIALS AND METHODS

Participants

Patients with stable CAD were recruited from the Center for Preventive Cardiology, Department of Vascular Diseases, Division of Internal Medicine, University Medical Centre Ljubljana, or Coronary Club Ljubljana, both located in Slovenia.

Patients with documented CAD (>3 months after a myocardial infarction, percutaneous coronary intervention or/and coronary artery bypass grafting), aged between 18 to 75 years and physically active more than three times a week were included into the study. All participants were clinically stable and engaged in regular unsupervised physical activity (e.g., walking, cycling) after completion of cardiac rehabilitation, as assessed with short interview. Exclusion criteria were unstable or uncontrolled cardiovascular disease/event (unstable angina, recent myocardial infarction <3 months prior to inclusion, class III or IV heart failure, uncontrolled dysrhythmias, severe pulmonary hypertension, severe and/or symptomatic valve disease, acute myocarditis, endocarditis, or pericarditis, aortic syndrome or venous thromboembolism), acute systemic illness, uncontrolled hypertension (>180/110 mmHg), postural hypotension (≥20 mmHg drop in systolic blood pressure with symptoms of dizziness or light-headedness) (Williams et al., 2007; Wise and Patrick, 2011).

Study Design

Study was designed as randomized, open-label clinical trial aligned with the CONSORT guidelines (Schulz et al., 2010), adapted for two parallel groups (**Figure 1**). Participants were randomized into 2 groups (1 interventional and 1 control group) with ratio of 1:1 using adaptive (urn) randomization with sealed envelopes and randomization concealment from the recruiting investigator. Measurements were performed two times: at baseline and after the intervention period (8 weeks). Patients in both groups underwent clinical examination prior to inclusion and ultrasonographic assessment [systemic brachial vascular function and muscle thickness of m. *vastus lateralis* (VL)], muscle strength assessment and blood sample withdrawal at baseline and post-training intervention. The primary outcome of the study was change in muscle strength and hypertrophy from baseline to 8-week follow-up. The secondary outcome was change in vascular function. Other exploratory outcomes included cardiovascular markers, insulin resistance and inflammatory markers.

Measurements and exercise intervention were conducted at the Center for Preventive Cardiology, University Medical Centre Ljubljana, Slovenia. One week ahead of baseline measurement participants were familiarized with testing and training procedures. The intervention period lasted for 8 weeks of BFR resistance training period with baseline and post-training testing.

During the week ahead of study, all subject underwent full medical examination and a familiarization session. Each testing session was split to 2 days, with at least a day of rest between testing. On the first day, ultrasonographic images were taken to assess flow mediated dilatation (FMD) and muscle thickness, and unilateral 1-RM strength was assessed as well. During the second day, blood samples were collected before subjects performed 3 sets of 10 repetitions of BFR resistance exercise at an intensity of 30% of their previously achieved 1-RM leg extension. All subjects performed a standardized warm-up before each testing or training session as noted in previous section.

Prior to inclusion, all patients underwent a run-in phase aerobic exercise training for 4 weeks (3 times a week, 45 min of cycling, walking or combination thereof at 70–80% maximum heart rate). After the baseline measurements, the control group continued with usual care (aerobic exercise training), while in the intervention group BFR training was added on usual care.

Both groups were informed of the risk associated with the methods and procedures, and signed written consent prior to their inclusion. The protocol was approved by the Republic of Slovenia National Medical Ethics Committee and was registered on ClinicalTrials.gov (identifier: NCT03087292). The study was conducted in accordance with the Declaration of Helsinki and the American College of Sports Medicine guidelines for Use of Human Participants.

Exercise Intervention

Subjects in BFR resistance training group trained for 8 weeks, performing a total of 16 unilateral leg extension exercise sessions. During each week two exercise sessions were performed with 48 h of rest period in between. Each training sessions consisted of three sets of 8, 10, and 12 repetitions in first, second, and third set, respectively, with 45 s inter-set rest interval. Training intensity was initially set to 30% of 1-RM. At every next training session the number of repetitions was increased for at least two repetitions per set. Additionally, training load was increased every 2 weeks, from initial 30% 1-RM to 32.5% 1-RM in the third week, to 37.5% 1-RM in the fifth week and finally to 40% 1-RM in the seventh week. Furthermore, as the training intensity increased, the number of repetitions was lowered to those in first training session. A lifting cadence of 1 s:2 s (concentric: eccentric part) was used for both groups throughout the full range of motion. For exercise purposes, BFR was applied by compressing the medium part of each thigh separately using a pneumatic cuff (Riester, Jungingen, Germany). The cuff was 23 cm width and 42–50 cm in length. Before each session or test, the cuff was inflated between 15 and 20 mmHg greater than resting brachial systolic pressure (Manini and Clark, 2009), taking into consideration the width of the cuff (Hackney et al., 2012) and thigh circumference (Loenneke et al., 2012a). Pressure was maintained throughout the entire training session and was released at the end of last set. To assure safety of the patients, brachial blood pressure and heart rate were measured at the rest, after sets and 5 min after the end of each training session. Brachial systolic and diastolic pressures were monitored using automatic blood pressure monitor (Omron M6, Omron Healthcare, Inc., Vernon Hills, IL, United States) and heart rate was obtained using telemetry (Polar, Kempele, Finland). All measurement and training sessions were monitored by cardiologist and kinesiologist.

Maximal Muscle Strength Measurement

BFR resistance training and unilateral isotonic leg extension strength measurements were performed on leg extension machine (Technogym, Cesena, Italy) following previous recommendations (Brown and Weir, 2001; Karabulut et al., 2011). Maximal strength was assessed by performing 1-RM testing at baseline (pre-training) and after (post-training) the completion of exercise intervention. The participants were familiarized with exercise testing protocol and were advised with proper lifting technique at least 3 days prior to testing. Patients were advised to perform the exercise while seated in an upright position with their back in permanent contact with the machine during the test, and with hands holding the handles of the machine. Before the measurement, participants were instructed to complete a warm up that included a 5–8 min of brisk walking (>4 km/h) on treadmill followed by two sets of static stretching for quadriceps muscles. During the test, participants were instructed to complete a warm-up set comprised of 8–10 repetitions for each leg at approximately 50% of their perceived maximal effort (1-RM). The weight was then increased progressively each set with simultaneously lowering number of repetitions until reaching the maximum weight that could be lifted for one repetition. Between each maximal effort there was a 2–3 min rest interval. Maximal muscle strength was determined within five attempts. Before and after measurement

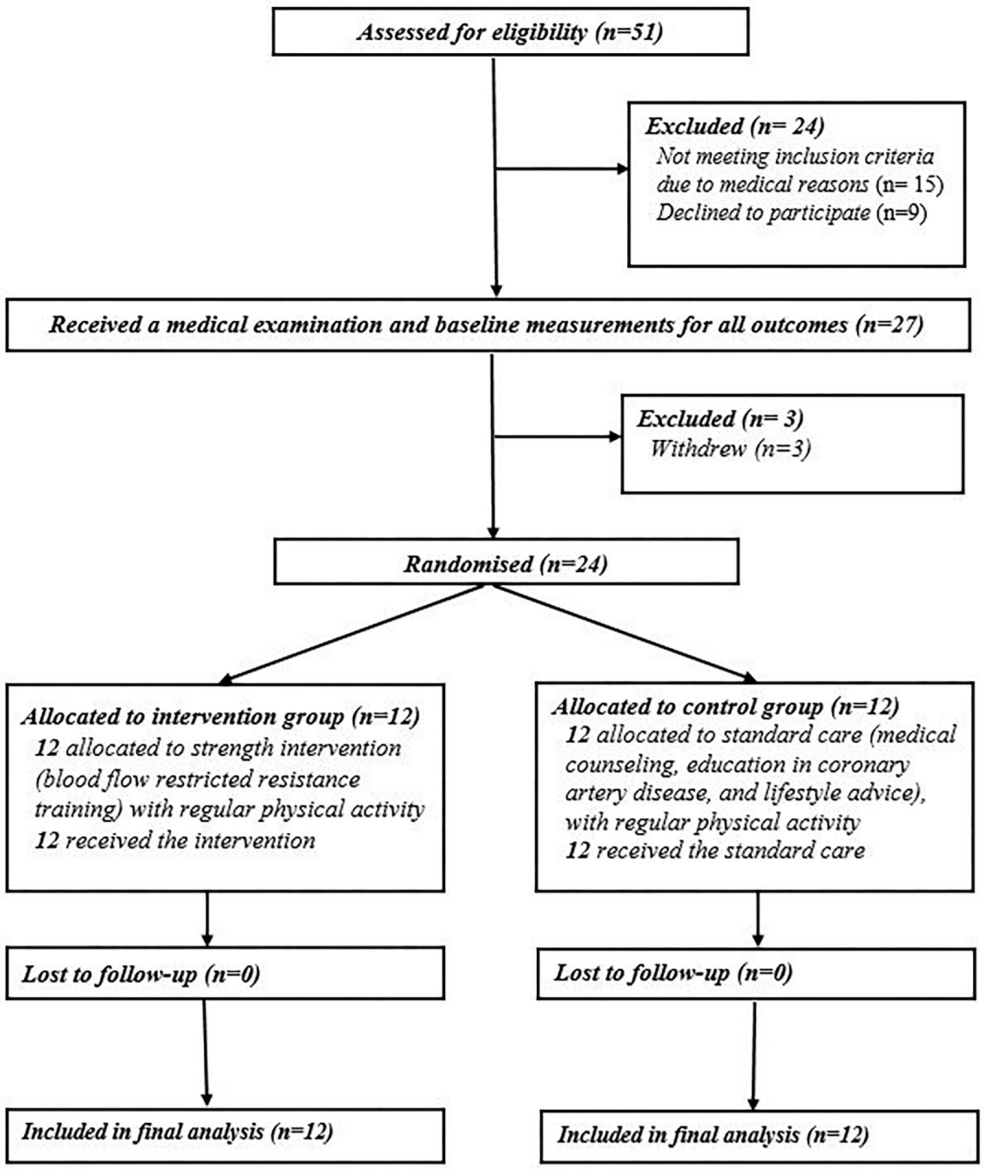

FIGURE 1 | Flow diagram of parallel randomization.

brachial blood pressure was obtained, while heart rate was monitored throughout the procedure.

Muscle Thickness and Vascular Function Measurement

Muscle thickness and vascular function was assessed with Aloka Prosound α7 ultrasound machine (Hitachi Healthcare Americas, Twinsburg, OH, United States) at baseline and after the completion of the study. Both measurements were performed at the same exact time of the day, mostly in the mornings.

Muscle thickness was assessed at rest, with subjects performing no physical activity at least a day before the testing. After applying hypoallergenic, watersoluble transmission gel, ultrasound transducer was placed on the surface of the skin. Muscle thicknesses of right VL were assessed. The measurement sites were determined at upper, midway and lower thirds between the greater trochanter and the lateral epicondyle of the knee. Those distances were measured with the subjects standing still with their knees fully extended. Three longitudinal images of the VL were recorded for each measurement, and the mean of the three values was used for further analysis (Martín-Hernández et al., 2013). Images were then independently analyzed by two experienced researchers and the mean of both was used as a final result.

Flow-mediated dilation was measured on the right brachial artery, approximately 5 cm above the antecubital fossa, according

to previously described guidelines (Corretti et al., 2002). The participants were instructed to lie in supine position. The artery was firstly visualized in the horizontal position on the screen, after which three measurements of the arterial diameter were obtained (d1). A cuff was then inflated below the antecubital fossa with the pressure of 50 mmHg above the systolic blood pressure. Ischemia was maintained for 4.5 min. Sixty seconds after the cuff deflation, three measurements of the arterial diameter were obtained again (d2). FMD was calculated with the following formula: [mean(d2) − mean(d1)]/mean(d1) and expressed in %.

Blood Markers Measurement

Blood samples were drawn from the antecubital vein using a 21-gauge needle; firstly, into a 4.5 mL vacuum tube containing 0.11 mol/L sodium citrate (9:1 v/v) (Becton Dickinson, Vacutainer System Europe, Heidelberg, Germany), followed by serum and K3-EDTA vacuum tubes (Laboratory Technic Burnik, Ljubljana, Slovenia). Plasma was prepared with 20-min centrifugation at 2000 × *g* and 15°C. Serum was prepared with 20-min centrifugation at 2000 × *g* and 20°C. Half milliliter aliquots of serum were snap frozen in liquid nitrogen and stored at ≤−70°C until analysis. Concentration of glucose was measured in fresh serum on the Fusion 5.1 biochemistry analyzer (Ortho Clinical Diagnostics, Rochester, NY, United States). Levels of CD40 ligand, insulin, TNF-α were measured in a thawed serum aliquote with the Luminex's xMAP® Technology utilizing magnetic beads coupled with specific antibodies, which allowed multiplexing. Analysis was performed according to manufacturer's instructions (R&D Systems, Abingdon, United Kingdom). Homeostatic model assessment (HOMA) was calculated using values plasma glucose levels and insulin levels via the HOMA Calculator[1].

Sample Size Calculation and Statistical Analysis

Sample size was calculated based on muscle strength as our primary outcome, as the loss of muscle strength in CAD patients is attributed to long term bed confinement, physical inactivity and in some cases also significant impairment in the cardiovascular disease itself (Bjarnason-Wehrens et al., 2004). The calculations suggested that 28 patients with CAD should be included in order to detect effect size value for muscle strength after BFR training larger 0.58 as described previously (Loenneke et al., 2012b), with an actual power of 0.95 at a level of statistical significance <0.05. Statistical power and sample size was calculated using G*Power statistical software (University of Düsseldorf, Germany).

Numeric variables were described as mean values and standard errors of mean, and categorical variables were described as numbers. Data were firstly screened for normality of distribution and homogeneity of variances and/or regression through Shapiro–Wilk's test, Levene's test and interaction between independent variables × covariate, respectively. Baseline and post-training differences between groups were determined with the Independent-Samples *t*-test for normally distributed variables and equal variances between groups, and Mann–Whitney *U* test was used for asymmetrically distributed variables and/or unequal variances between groups. Two-way analysis of variance (ANOVA) for repeated measurements was used to calculate main effects of group, time and group × time interaction. Within group effect of training intervention (baseline vs. post-training) was assessed with Paired-Samples *t*-test for normally distributed variables and with Wilcoxon's test for asymmetrically distributed variables. All data are displayed in the text, tables and figures. The data were analyzed using IBM SPSS Statistics v.21 statistical software package for Windows (SPSS Inc., Chicago, IL, United States). The level of significance was set *a priori* to an alpha of <0.05.

[1]https://www.dtu.ox.ac.uk/homacalculator/index.php

RESULTS

During the recruitment process, 51 volunteers agreed to participate in the study. After initial medical examination and measurements, 24 were included into the study (6 women, 18 men; age 60.5 ± 2.4 years). Among excluded participants, 15 were excluded due to medical exclusion criteria and 12 left the study prior to start due to personal reasons/preferences (**Figure 1**). Patients were randomly assigned to either BFR resistance training group (3 women, 9 men; age 64.9 ± 1.6 years) or the control (CON) group (3 women, 9 men; age 56.2 ± 2.5 years) (**Figure 2**).

Among 24 included patients, all have completed the study (**Figure 1**). The adherence rate remained complete (100%) throughout the exercise intervention in both groups. Despite some occasional reports of muscle pain at the end of training, no deaths, adverse effect of exercise, such as muscle damage, skeletal injuries, chest pain, shortness of breath, dizziness, palpitations, venous thrombosis, pulmonary embolism or rhabdomyolysis, were reported.

Baseline physical and clinical characteristics of the sample and both groups are displayed in **Table 1**. Overall, there were significant differences in mean age (BFR resistance training group 64.9 ± 1.6 years vs. CON group 56.2 ± 2.5 years) and systolic blood pressure (BFR resistance training group 129.67 ± 3.71 mmHg vs. CON group 117.50 ± 3.36 mmHg), otherwise there were no significant differences between groups in other physical and clinical variables.

Hemodynamic Response

Post-training responses of heart rate (RR), systolic and diastolic blood pressure following exercise intervention are shown in **Table 2**. At baseline there was a significantly lower systolic blood pressure in CON group ($p = 0.024$), with no significant differences in resting heart rate or diastolic blood pressure. After the training intervention, there was a significant main effect for group × time interaction ($p = 0.001$; $\eta^2 = 0.627$) and trend for group effect ($p = 0.074$) on systolic blood pressure. Contrary, no significant main effects of time, group or group × time interaction for resting heart rate and diastolic blood pressure were observed. Furthermore, there was a significant decrease in systolic blood pressure in BFR group after training intervention

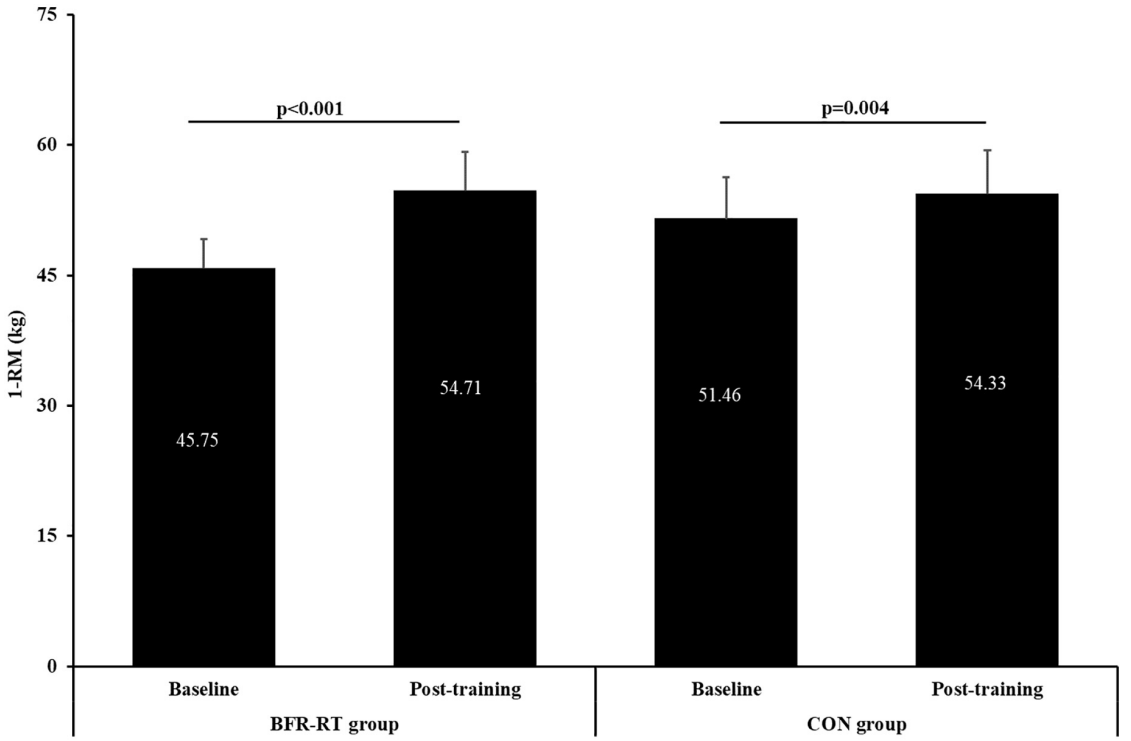

FIGURE 2 | Baseline and post-training values of one repetition maximum (1-RM) for both groups. BFR-RT group, blood flow restriction-resistance training group; CON group, control group. Values are presented as mean ± SE.

($p = 0.030$), whereas similar decrease was not observed in diastolic blood pressure and resting heart rate.

Acute hemodynamic responses to exercise are presented in **Table 3**. In both groups BFR exercise evoked significant increase in heart rate ($p < 0.001$) and systolic blood pressure at baseline and post-training exercise test, while diastolic blood pressure did not increase significantly. There was no significant difference between groups in hemodynamic change (at rest vs. post-last set).

Maximal Muscle Strength

At baseline and post-training there were no significant differences between groups in maximal muscle strength (**Figure 2**). After the training period, there was a significant main effect for time ($p < 0.001$, $\eta^2 = 0.769$) and group × time interaction ($p < 0.001$; $\eta^2 = 0.724$), but no for group ($p = 0.233$). Both groups significantly improved muscle strength post-training, with greater increase in the BFR resistance training group (16.37%, $p < 0.001$) than control group (5.29%, $p < 0.01$). Post-training leg strength increased for 8.96 and 2.88 kg in the BFR resistance training group and the control group, respectively.

Muscle Thickness

VL muscle thickness was assessed pre- and post-training period (**Table 4**). There was no between-group difference in muscle thickness observed at baseline nor after the completion of the study. After the training intervention, there was a significant main effect for time in lower third of VL ($p < 0.05$; $\eta^2 = 0.360$) alongside borderline significant time effect in midway third of VL ($p = 0.072$; $\eta^2 = 0.265$), whereas no significant group × time interaction was obtained in neither of all three thirds of VL. Additionally, a significant post-training decrease in muscle thickness of lower VT was observed in CON group ($p = 0.031$). On the contrary, improvements of muscle thickness on upper and midway thirds of VL in the BFR resistance training group showed a trend toward statistical significance (1.58–1.67 cm, $p = 0.096$ and 1.60–1.68 cm, $p = 0.082$, respectively).

Vascular Function

Two-way ANOVA showed no significant main effect for group ($p = 0.134$) or group × time interaction ($p = 0.28$), whereas a borderline significance was observed for time effect ($p = 0.092$; $\eta^2 = 0.236$). There were no significant differences between groups or pre- vs. post-training within group, although a trend toward improvement of FMD was obtained in the BFR resistance training group (6.48 ± 0.80% to 8.04 ± 0.98%, $p = 0.079$, respectively) (**Figure 3**).

Blood Markers

Baseline between group differences were observed in insulin levels and insulin resistance (HOMA IR; both biomarkers $p = 0.014$; **Table 4**). After the training intervention, there were significant main effects for time ($p = 0.036$; $\eta^2 = 0.340$) for CD40 ligand, significant main effect for group ($p = 0.005$; $\eta^2 = 0.306$) and group × time interaction ($p = 0.036$; $\eta^2 = 0.342$) for insulin

TABLE 1 | Baseline characteristics.

	Sample (*n* = 24)	BFR-RT group (*n* = 12)	CON group (*n* = 12)	*p*
Age (years)	60.5 (2.4)	64.9 (1.6)	56.2 (2.5)	<0.001
Female/male ratio (N)	18/6	9/3	9/3	1.000
Height (cm)	172.30 (2.42)	169.53 (1.87)	175.08 (2.71)	0.106
Weight (kg)	86.78 (3.53)	86.55 (3.76)	87.01 (3.45)	0.929
BMI (kg/m^2)	29.26 (1.11)	30.15 (1.25)	28.37 (0.94)	0.268
Systolic BP (mmHg)	123.58 (3.89)	129.67 (3.71)	117.50 (3.36)	0.024
Diastolic BP (mmHg)	80.04 (1.69)	81.92 (1.75)	78.17 (1.50)	0.118
Resting heart rate (bpm)	64.17 (3.15)	63.58 (2.68)	64.75 (3.67)	0.800
LVEF (%)	64.38 (1.43)	62.75 (1.69)	66.00 (2.28)	0.264
Post-surgery (years)	4.48 (0.83)	4.79 (1.30)	4.17 (1.09)	0.876
Myocardial infarction				
NSTEMI, N (%)	13 (54.2)	6 (50.0)	7 (58.3)	0.682
STEMI, N (%)	11 (45.8)	6 (50.0)	5 (41.7)	
Surgical intervention				
CABG, N (%)	5 (20.8)	2 (16.7)	3 (25.0)	1.000
PCI, N (%)	19 (79.2)	10 (83.3)	9 (75.0)	
Medications				
Aspirin, N (%)	24 (100.0)	12 (50.0)	12 (50.0)	/
Statin, N (%)	24 (100.0)	12 (50.0)	12 (50.0)	/
Beta blocker, N (%)	16 (66.7)	8 (50.0)	8 (50.0)	1.000
ACE/ARB, N (%)	17 (70.8)	8 (47.1)	9 (52.9)	1.000
Cardiovascular risk factors				
Arterial hypertension, N (%)	17 (70.8)	8 (47.1)	9 (52.9)	1.000
Hyperlipidemia, N (%)	23 (95.8)	11 (47.8)	12 (52.2)	1.000
Diabetes Mellitus, N (%)	5 (20.8)	3 (60.0)	2 (40.0)	1.000
Smoking				
Non-smoker, N (%)	7 (29.2)	3 (42.9)	4 (57.1)	
Smoker, N (%)	4 (16.6)	2 (50.0)	2 (50.0)	0.822
Ex-smoker, N (%)	13 (54.2)	7 (53.8)	6 (46.2)	

Data for continuous values are expressed as mean (±SE). BFR-RT, blood flow restriction-resistance training; CON, control group; BMI, body mass index; BP, blood pressure; LVEF, left ventricular ejection fraction; STEMI, ST, elevated myocardial infarction; NSTEMI, non ST-elevated myocardial infarction; CABG, coronary artery bypass graft; PCI, percutaneous coronary intervention.

TABLE 2 | Resting hemodynamics at baseline and after the intervention period.

Variable (unit)	Group	Baseline	Post-training	*p*
RR (bpm)	BFR-RT group	63.58 (2.68)	62.25 (1.88)	0.537
	CON group	64.75 (3.67)	63.67 (3.18)	0.729
Systolic BP (mmHg)	BFR-RT group	129.67 (3.71)	122.9 (2.74)	0.030
	CON group	117.50* (3.36)	120.08 (3.54)	0.306
Diastolic BP (mmHg)	BFR-RT group	81.92 (1.75)	79.67 (1.99)	0.133
	CON group	78.17 (1.50)	76.83 (3.15)	0.632

**Significantly different ($p < 0.05$) from BFR-RT group. Data are presented as mean (±SE). BFR-RT, blood flow restriction-resistance training; CON, control; RR, resting heart rate; BP, blood pressure.*

levels. In BFR group, training intervention led to a borderline significant decrease in insulin ($p = 0.077$), HOMA IR ($p = 0.079$) and CD40 ligand levels ($p = 0.052$), while a similar trend was additionally observed in the control group for CD40 ligand levels ($p = 0.060$).

DISCUSSION

To our knowledge, this is the first study to investigate the beyond-acute effects of BFR resistance training with low-loads (30–40% 1-RM) in CAD patients. Exercise training proved to be safe in patients, and was associated with increased muscle strength and a trend toward increased muscle thickness and altered inflammatory response.

Our findings confirmed that 8 weeks of BFR resistance training may improve muscle strength as appraised by unilateral leg extension 1-RM. Previous studies have shown similar increases in leg extension 1-RM strength in comparable age groups of healthy individuals (Karabulut et al., 2010, 2011; Yasuda et al., 2014a,b), with the exception of one study (Vechin et al., 2015). Also, the magnitude of strength gains in our study is consistent with previous reports (in the range between 15 and 30%). However, longer training intervention (Yasuda et al., 2014b) or higher frequency and repetitions (Karabulut et al., 2011) seem to provide additional strength gains. In addition to muscle strength, our study also showed a trend in increased muscle thickness (as observed in upper and midway section of muscle *VL*). Conversely, previous studies in healthy students (Abe et al., 2005; Fujita et al., 2008; Madarame et al., 2008) and older adults (Yasuda et al., 2014a,b; Vechin et al., 2015) reported a definite increase in muscle hypertrophy of *VL* after BFR resistance training, which may be due to higher intensity, volume or duration of exercise trainings, as well as (younger) age of participants in these studies.

Resistance training under BFR reduced resting systolic blood pressure after 8 weeks in intervention group. This may be a result of lowered hemodynamic stress for a given muscle force after resistance training and less evoked rate of heart rate-pressure product (HR times SBP, an indirect index of myocardial oxygen demand) (Williams et al., 2007). Conversely, 12 weeks of whole body resistance training at 60–80% of 1-RM without occlusion did not evoke any hemodynamic response at rest in CAD patients (Grafe et al., 2018). Thus it may be postulated that BFR using lower loads could promote better training adaptations compared to higher loads resistance training without occlusion (Hackney et al., 2012). Our acute hemodynamic response to exercise is in line with previous reports in healthy older men (Staunton et al., 2015) and women (Scott et al., 2018) using BFR-RT, despite lower increase in heart rate. This can be explained with the discrepancies between exercise modes. In both studies participants performed leg press exercise training under BFR, which involve much more muscle mass than unilateral knee extension and thus may lead to higher hemodynamic response. Also, longer time under occlusion may evoke higher

TABLE 3 | Acute hemodynamic exercise response at baseline and after the intervention period.

Variable (unit)	Group	Baseline		Post-training		Baseline Δ (*p*)	Post-training Δ (*p*)
		Pre exercise	Post-last set	Pre exercise	Post-last set		
RR (bpm)	BFR-RT group	63.58 (2.68)	81.75 (2.65)	62.09 (2.06)	83.46 (3.74)	18.17 (0.000)	21.36 (0.000)
	CON group	64.40 (4.39)	76.30 (3.97)	63.67 (3.19)	78.42 (4.07)	11.90 (0.000)	14.75 (0.000)
Systolic BP (mmHg)	BFR-RT group	131.46 (3.56)	143.14 (3.86)	122.37 (2.95)	142.82 (4.30)	11.68 (0.001)	20.45 (0.000)
	CON group	116.70 (3.98)	129.25 (2.11)	120.73 (3.82)	129.50 (5.80)	12.55 (0.014)	8.77 (0.035)
Diastolic BP (mmHg)	BFR-RT group	82.73 (1.70)	85.59 (2.16)	79.18 (2.12)	82.09 (2.31)	2.86 (0.112)	2.90 (0.217)
	CON group	77.50 (1.60)	80.25 (2.61)	77.09 (3.44)	78.64 (3.02)	2.75 (0.314)	1.55 (0.722)

Data are presented as mean (±SE). RR, resting heart rate; BP, blood pressure.

hemodynamic response, as both previous studies performed longer sets with at least 15 repetitions per set (Staunton et al., 2015; Scott et al., 2018).

The majority of previous studies in CAD have shown improvements in FMD after aerobic (Edwards et al., 2004; Blumenthal et al., 2005) or combined aerobic-and-resistance training (Vona et al., 2009; Anagnostakou et al., 2014). The vascular response, however, was higher with high intensity interval training (Ramos et al., 2015) or combined exercise training (Vona et al., 2009; Anagnostakou et al., 2014). Most clinical trials have shown improvements in FMD (Vona et al., 2009; Anagnostakou et al., 2014) after moderate resistance training, which is in line with our results. Apart from exercise type, the magnitude of effect is predominately associated with training duration, frequency and intensity, as longer interventions (>12 weeks), higher frequencies (3–4 times a week), higher intensities (>55% 1 RM) structured in whole body resistance regimens have the potential to provoke higher FMD response (Vona et al., 2009; Anagnostakou et al., 2014). Hence, discrepancies in training parameters may explain the modest FMD improvements in our study.

Inflammatory mediators appear to play a fundamental role in the initiation, progression, and eventual rupture of atherosclerotic plaques (Szmitko et al., 2003) and pathogenesis of cardiovascular diseases (Pedersen, 2017). Evidence suggests that increased TNF-α (Szmitko et al., 2003; Pedersen, 2017) and CD-40 ligand are linked to endothelial dysfunction and subsequent atherogenesis with late thrombotic complications (Szmitko et al., 2003). In contrast, physical activity can counteract with provoking anti-inflammatory effects by an inhibition of TNF-α (Pedersen, 2017) and CD-40 ligand levels (Bjornstad et al., 2008), although the latter mechanism was not proven following our BFR resistance training. The difference may occur as a result of short exercise duration (<15 min) and relatively limited muscle mass involved during the exercise (unilateral knee extension), as more pronounced systemic response between TNF-α and IL-6 were observed after longer strenuous exercise involving several large muscle groups (Pedersen, 2017). On the other hand, a 20-week combined endurance and resistance training decreased values of soluble CD-40 ligand in patients with chronic heart failure (Bjornstad et al., 2008).

BFR resistance training improved insulin sensitivity. Resistance training without BFR promotes a decrease in resting insulin levels and increase in insulin sensitivity (Williams et al., 2007), which is in line with our results. Similar favorable effects of resistance exercise with higher loads were also reported in patients with type 2 diabetes mellitus (Jorge et al., 2011; Kadoglou et al., 2013). Data from two previous study suggest that the decrease in HOMA IR can be time dependent, as there were no significant changes in HOMA IR after 12 weeks of resistance training in patients with type 2 diabetes mellitus of comparable age (Jorge et al., 2011). Contrary, a significant decrease in HOMA IR was obtained after 6 months of resistance training at the exercise intensity of 60–80% of 1-RM in patients with type 2 diabetes (Kadoglou et al., 2013). Furthermore, it is plausible that younger control group was more physically active prior to the documented coronary event, as this could explain their the significant lower baseline values of insulin and lower HOMA IR (Gayoso-Diz et al., 2011).

Our study has some limitations. Firstly, a relatively small sample size. This was reflected in a significant age-difference between intervention groups suggesting randomization failure,

TABLE 4 | Muscle thickness and blood markers at baseline and after the intervention period.

Variable (unit)		Baseline	Post-training	*p*
Upper third of VL diameter (cm)	BFR-RT group	1.58 (0.07)	1.67 (0.06)	0.096
	CON group	1.56 (0.05)	1.51 (0.07)	0.367
Midway third of VL diameter (cm)	BFR-RT group	1.60 (0.08)	1.68 (0.08)	0.082
	CON group	1.58 (0.05)	1.64 (0.06)	0.331
Lower third of VL diameter (cm)	BFR-RT group	1.64 (0.07)	1.57 (0.09)	0.429
	CON group	1.53 (0.07)	1.43 (0.08)	<0.05
Insulin (pg/mL)	BFR-RT group	1247 (132)	864 (180)	0.077
	CON group	655 (119)*	810 (253)	0.791
HOMA IR	BFR-RT group	4.01 (0.43)	2.86 (0.57)	0.079
	CON group	2.17 (0.40)*	2.38 (0.59)	0.733
CD40 ligand (pg/mL)	BFR-RT group	9116 (1100)	5735 (1221)	0.052
	CON group	7869 (1142)	5142 (784)	0.060
TNF-α (pg/mL)	BFR-RT group	5.8 (1.85)	4.9 (1.41)	0.581
	CON group	14.9 (6.41)	14.5 (4.99)	0.967

**Significantly different ($p < 0.05$) from BFR-RT group. Data are presented as mean (±SE). VL-m, vastus lateralis; HOMA, homeostatic model assessment; IR, insulin resistance; TNF-α, tumor necrosis factor alpha.*

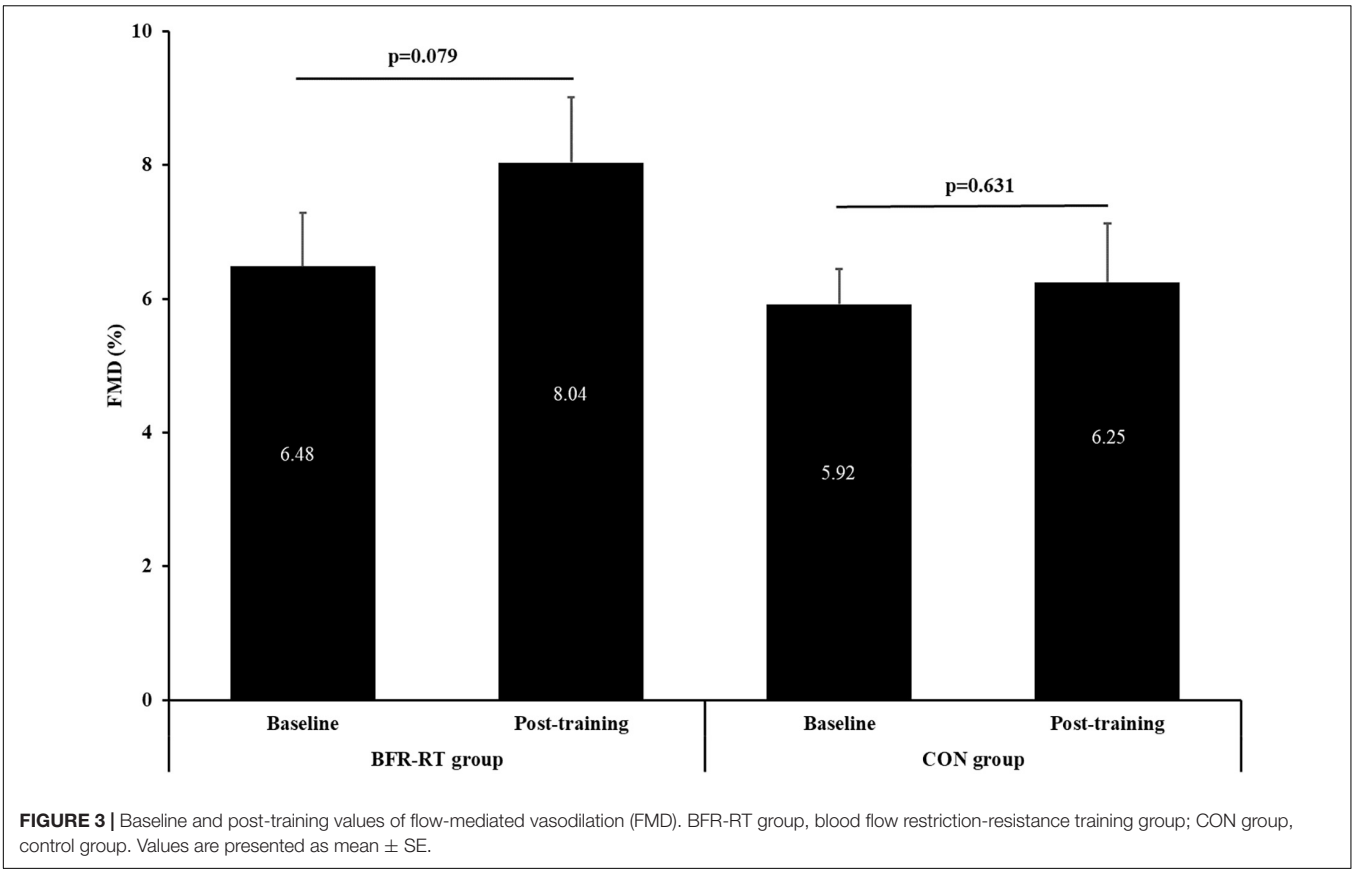

FIGURE 3 | Baseline and post-training values of flow-mediated vasodilation (FMD). BFR-RT group, blood flow restriction-resistance training group; CON group, control group. Values are presented as mean ± SE.

which possibly yielded pronounced between-group differences because of faster recovery in younger participants. Thus, our study was underpowered and should be regarded as pilot and hypothesis generating. Secondly, the allocation was not blind. The majority of subjects were physically active, which may have diminished the between-group differences and effects of our intervention itself. Moreover, as all patients (including control group) were made aware of positive effects of exercise on their strength and health in general, an additional increase in physical activity level during the study period cannot be ruled out. Thirdly, duration of the trial may have been too short to express sufficient responses. Since safety of BFR training has not been rigorously tested in clinical settings, and exact cardiovascular and coagulation responses were not clearly presented to this date, the duration of exercise intervention was chosen based on previous resistance training research in CAD patients. Nevertheless, longer exercise interventions (>12 weeks) might have provoked additional muscle gains and vascular function improvements. Lastly, virtually all patients were on secondary preventive medications, which may have confounded heart rate (beta-blockers), and blood pressure and vascular function (statins and ACE-inhibitors).

Our results have shown that BFR resistance training is efficient only in terms of improving muscle strength and blood pressure, whereas the effect on muscle size and biomarkers failed to reach statistical significance and needs to be addressed in larger and longer studies. Moreover, BFR resistance training seems to be potentially safe, with beneficial impact on hemodynamic responses, but not on vascular function. Therefore, it may be provided as an additional exercise option to safely and effectively improve skeletal muscle functioning and general health. Nonetheless, the current study reveals a need for future studies to evaluate the current findings on larger samples with longer training duration. Thus, such training effect on different health parameters can be exaggerated and later translated into early phase of cardiac rehabilitation.

ETHICS STATEMENT

The protocol was approved by the Republic of Slovenia National Medical Ethics Committee and was registered on ClinicalTrials.gov (Identifier: NCT03087292). The study was conducted in accordance with the Declaration of Helsinki and the American College of Sports Medicine guidelines for Use of Human Participants.

AUTHOR CONTRIBUTIONS

TK conceived the study design, recruited and consented the participants into the study, conducted the research,

analyzed the data, performed the statistical analysis, interpreted the data, drafted the manuscript, and was responsible for final content. MN conducted the research, analyzed and interpreted the data, and drafted the manuscript. KT conducted the research and drafted the manuscript. VS interpreted the data and drafted the manuscript. BJ conceived the study design, analyzed and interpreted the data, and drafted the manuscript. All authors read, critically reviewed, and approved the final version of the manuscript.

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frontiers in Physiology

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Comparison Between Manual and (Semi-)Automated Analyses of Esophageal Diaphragm Electromyography During Endurance Cycling in Patients With COPD

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Background: Electrocardiogram (ECG) contamination is present in diaphragm electromyography (EMGdi) recordings. Obtaining EMGdi without ECG contamination is crucial for EMG amplitude analysis. Manually selecting EMGdi in between QRS complexes has been most commonly applied in recent years (manual method). We developed a semi-automated analysis method based on Least Mean Square Adaptive Filtering combined with a synchronously recorded separate ECG channel to remove ECG artifacts from the EMGdi signals. We hypothesized that this approach would shorten analysis duration and might minimize the potential for inter-rater disagreement.

Aims: We aimed to evaluate agreement between the semi-automated method and the manual method and inter-rater reliability of the manual method.

Methods: Electromyography signals of seven patients with COPD were recorded using an esophageal catheter during an exercise test on a cycle ergometer. Four patients subsequently participated in an inspiratory muscle training (IMT) program for 8 weeks. After IMT, the tests were repeated. EMGdi/EMGdiMax as obtained either manually by the two assessors or retrieved from the semi-automated method were compared.

Results: Semi-automated EMGdi/EMGdiMax agreed well with values obtained by one of the two manual assessors (assessor 1) both at pre-intervention measurements (mean difference −0.5%, 95% CI: −19.6 to 18.6%) and for the pre/post IMT differences (mean difference 1.2%, 95% CI: −16.8 to 19.2%). Intra-class correlation coefficients between methods were 0.96 (95% CI: 0.94–0.97) at pre-intervention measurements and 0.78 (95% CI: 0.58–0.89) for pre/post IMT differences (both $p < 0.001$). EMGdi/EMGdiMax from assessor 2 was systematically lower than from assessor 1 and agreed less well with

the semi-automated method both at pre-intervention measurements (mean difference: 9.3%, 95% CI: −11.4 to 29.9%) and for pre/post IMT differences (mean difference 7.0%, 95% CI: −20.4 to 34.4%). Analysis duration of the semi-automated method was significantly shorter (29 ± 9 min) than the manual method (82 ± 20 min, $p < 0.001$).

Conclusion: The developed semi-automated method is more time efficient and will be less prone to inter-rater variability that was observed when applying the manual analysis method. It is, therefore, proposed as a new standard for objective EMGdi amplitude analyses in future studies.

Keywords: electromyography, electrocardiography, diaphragm electromyography, chronic obstructive pulmonary disease, respiratory muscle training

INTRODUCTION

Electromyography (EMG) is an assessment of muscle activation by recording the electrical activity of the muscle tissue. Assessments of diaphragm EMG (EMGdi) amplitude are frequently applied in both clinical and research settings, where they can serve as an indirect measure of neural respiratory drive (NRD) during different conditions such as resting breathing, exercise breathing, or during sleep (Luo and Moxham, 2005; Luo et al., 2011; Xiao et al., 2015; Steier et al., 2017). EMGdi can be recorded either via surface electrodes placed on the chest wall, with needle electrodes inserted into the costal diaphragm, or with an esophageal catheter equipped with EMG electrodes (Sinderby et al., 1998; Duiverman et al., 2004; Luo et al., 2008). The EMGdi recording contains artifacts from the power line, from movement, and from cardiac activity. Movement artifacts are associated with very low frequencies and can be easily removed by applying high pass filtering at 20 Hz. However, the cardiac activity artifacts, as detected by electrocardiogram (ECG), is more difficult to remove because of the overlapping bandwidth spectrum between ECG and EMGdi. The majority of the EMGdi signal is concentrated in the bandwidth between 20 and 250 Hz, while the bandwidth of the ECG frequency spectrum lies between 0 and 100 Hz (Schweitzer et al., 1979). It is crucial to obtain the EMGdi signal without the ECG contamination, to ensure the accuracy of the EMGdi signal (Levine et al., 1986; Zhou and Kuiken, 2006; Luo et al., 2008). Separating ECG from EMGdi is particularly challenging in EMG amplitude analyses, especially during exercise, since the EMGdi amplitude can be larger than the ECG. This makes it more difficult to identify ECG artifacts within the EMG signal.

One widely used method to obtain the EMGdi signal without ECG contamination is to manually select EMGdi data in between QRS complexes (Luo and Moxham, 2005; Luo et al., 2008; Jolley et al., 2009; Reilly et al., 2013; Schaeffer et al., 2014; Langer et al., 2018). By placing a separated time-synchronized ECG channel next to the EMG channel, the ECG channel is visually identifiable, thereby allowing to retrieve the EMGdi in between QRS complexes. However, there are some limitations to this method. First, this method is time-consuming, especially for recordings that contain many breathing cycles such as during exercise. Second, based on the experience in our research group, it might be subjective to inter-rater variability since the retrieved data can vary depending on the judgment of the assessor. Inter-rater variability could arise from the fact that several EMGdi parts are available to choose from in between QRS complexes during every inspiration. No specific instructions are currently available as to which interval should be preferably selected under these circumstances while selection of either ascending, descending, or peak intervals of the uncontaminated signal might result in vast differences in the recorded EMG amplitude. Selection width of the chosen interval while avoiding artifact to either side of the selected interval might be another factor that could explains the inter-rater variability of the obtained EMG amplitude for a given breath. A final limitation of the manual method is that EMGdi activity "buried" in the ECG signal cannot be selected. Depending on the location of the QRS complexes the data outside of contaminated area might not be the best representation of the actual EMG amplitude (e.g., the part containing the highest amplitude of the signal might not be available to select). This might be especially problematic during exercise, when several heartbeats typically occur during a single inspiration.

Several methods have been previously applied to automatically deduct or remove ECG artifacts from EMGdi signals. However, the majority of methods does not rely on ECG data from a separately collected ECG channel. These methods typically suffer from problems with frequency-overlapping, difficulties in waveform identification, and processing difficulties due to the sometimes smaller amplitude of the ECG signal in comparison to the EMG signal (e.g., during near maximal diaphragm activation throughout exercise hyperpnea) (Schweitzer et al., 1979; Zhou and Kuiken, 2006; Mak et al., 2010; Willigenburg et al., 2012). Bloch suggested using a separate and simultaneous recording of a time-synchronized ECG channel to avoid these problems (Bloch, 1983). For analysis, he proposed to initially use the amplitude threshold to identify the QRS complex of the ECG, followed by applying a least squares subtraction on the time domain to remove ECG artifacts (Bloch, 1983). This method introduced by Bloch has not been extensively evaluated or validated especially not for EMGdi recordings of resting and exercise breathing obtained with an esophageal catheter.

Up to now, there is no gold standard method available for removing ECG artifacts while analyzing EMGdi amplitude

data. From the reviewed methods above, manually selecting EMGdi in between QRS complexes has so far been the most applied method. This method will be mentioned onward as the "manual" method. Because of the shortcomings of the manual method, we were interested in developing and evaluating an alternative method that could potentially shorten the duration of the analysis and overcome several problems related to the expected inter-rater ambiguity that seems inherent to the somewhat subjective judgments that have to be made while applying the manual analysis method. Therefore, we developed a custom "semi-automated method" based on a Least Mean Square (LMS) Adaptive Filtering method (Bloch, 1983) combined with a synchronously recorded, separated ECG channel. We aimed to compare this "semi-automated method" with results obtained from the manual method. In addition, we also aimed to formally study the degree of inter-rater variability that can be expected when applying the manual analysis method. Responsiveness (i.e., the ability of a measure to detect change) is an important feature of assessment methods that needs to be evaluated separately from reliability and validity (Husted et al., 2000).The degree of agreement between methods was therefore evaluated both cross-sectionally (i.e., of data obtained at a single point in time) to evaluate validity and reliability as well as by comparing changes in activation observed after an intervention period between methods to evaluate and compare responsiveness.

Accordingly, the aims of this study were the following: (1) to investigate the inter-rater reliability of the manual method of EMGdi amplitude analysis and (2) to explore the agreement between the manual and the proposed semi-automated analysis method of EMGdi amplitude signals both cross-sectionally (to evaluate validity) and of changes in response to an intervention (to evaluate responsiveness).

MATERIALS AND METHODS

Study Design and Subjects

Clinically stable patients with moderate to severe COPD were included in this study. Data were retrieved from patients who had been enrolled in a clinical study (ClinicalTrials.gov Identifier: NCT03240640). The Ethical Committee Research of KU Leuven/UZ Leuven, Belgium approved the study (S58513). All participants signed written informed consent. EMGdi was recorded during a constant work rate cycle ergometer (CWR) test before and after 8 weeks of inspiratory muscle training (IMT). The EMGdi data were first analyzed using the manual method by two independent assessors. The same data were then analyzed again using the semi-automated method. Comparisons were made both between the results obtained by the two assessors using the manual analyzing method as well as between results obtained by both manual assessors and from the semi-automated method. The details of each analysis method are described below. Interim analysis of these data has been presented at ERS International Congress 2018 (Dacha et al., 2018).

Pulmonary Function and Respiratory Muscle Function Measurements

Pulmonary function testing (MasterScreen Body, CareFusion, Höchberg, Germany) was performed according to ERS guidelines (Miller et al., 2005; Wanger et al., 2005; Graham et al., 2017). Maximal inspiratory, expiratory mouth pressures (MIP and MEP) and transdiaphragmatic pressure (PdiMax) during sniff maneuver were assessed according to international guidelines (American Thoracic Society/European Respiratory Society [ATS/ERS], 2002).

EMG Recording and Analysis

Esophageal Catheter and Positioning

A multipair-esophageal electrode catheter (Yinghui Medical Equipment Technology Co., Ltd., Guangzhou, China) was used to assess EMGdi. The catheter is approximately 60 centimeters long, two millimeters in diameter, and is equipped with five EMG electrode pairs feeding five EMGdi channels. The catheter was inserted nasally and then swallowed by the patient. The positioning of the catheter was performed according to procedures established in previous studies (Luo and Moxham, 2005; Luo et al., 2011). In short, the patient was asked to perform several slow maximal inspiratory capacity (IC) maneuvers (an inspiration through the open mouth from the functional residual capacity to total lung capacity). The best position was determined as the location which the largest EMGdi amplitudes were recorded from the outer electrode pairs and the smallest from the middle pairs (**Figure 1**; Luo et al., 2001, 2011; Luo and Moxham, 2005). After positioning, the catheter was secured by taping one end onto the patient's nose.

EMGdi Sampling and Processing

The EMGdi signals were first amplified (Biomedical amplifier, Guangzhou, China), sampled at 2000 Hz by a data acquisition system (Micro1401-3, Cambridge Electronic Design Limited, Cambridge, United Kingdom) and then processed with a specific software package (Spike 2, Cambridge Electronic Design Limited, Cambridge, United Kingdom). During processing the raw EMGdi data were first high pass filtered at 20 Hz to minimize motion artifacts and then transformed into "Root Mean Square" (RMS). The EMGdi signals recorded during breathing were then normalized by presenting the recorded value relative to the signal obtained during maximal activation; EMGdi/EMGdiMax%. The highest EMGdi signal obtained from any of the five channels during each subsequent breath was retrieved for analyses. Maximal activation of the diaphragm was obtained during typical (i.e., fast) exercise IC maneuvers, either during resting breathing or during exercise breathing (Sinderby et al., 1998). The patients were asked to perform these IC maneuvers every minute during the three resting minutes preceding the cycling test, and every other minute during the cycling test. The largest RMS amplitude obtained during any of the recorded IC maneuvers was selected as EMGdiMax.

ECG Removal With the Manual Method

From the processed data, both assessors were instructed to perform the manual analysis of the EMGdi signals in agreement

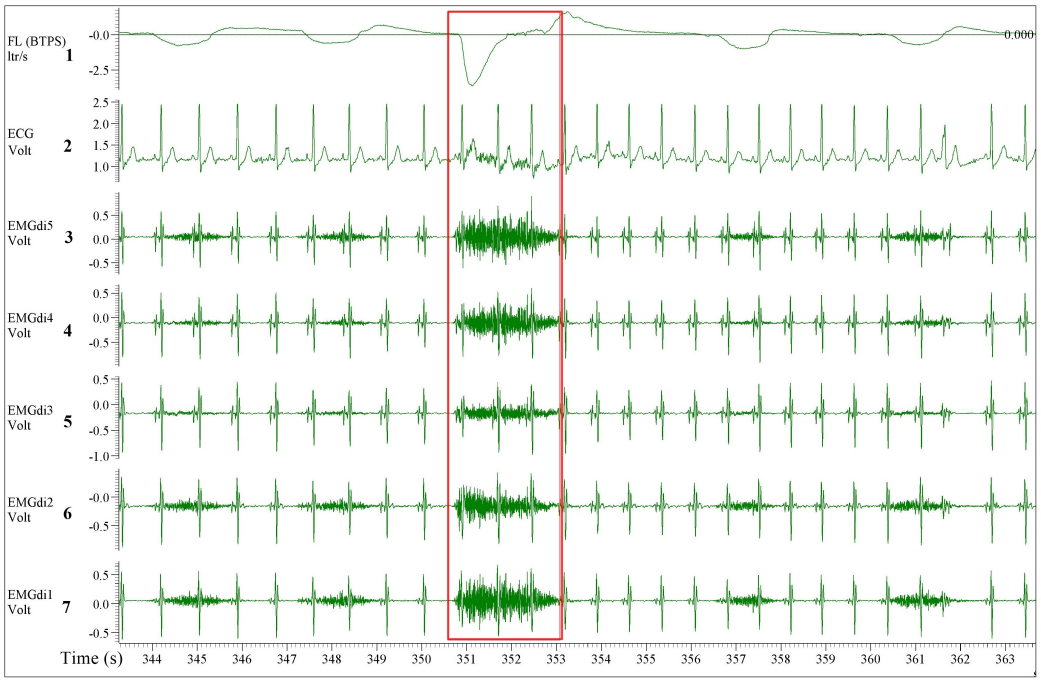

FIGURE 1 | A five diaphragm EMG (EMGdi) channel recording contains electrocardiogram (ECG) artifacts during resting breathing. From top to bottom the channels are as follow; Channel 1: respiratory flow (l/s; negative flow indicating the inspiratory cycle), Channel 2: ECG recording (volt), Channels 3–7: EMGdi recordings. The correct positioning of the catheter is shown when the largest EMGdi amplitudes are in the outer EMGdi channels (3 and 7), and the smallest amplitude is in the middle channel (5). The inspiratory capacity (IC) maneuver is highlighted in the red box indicated by the higher flow, which accompanies the maximal activation of the diaphragm (EMGdiMax).

with previously published methods. They were instructed to extract the EMGdi signals from segments of inspiratory cycles between QRS complexes (Luo et al., 2001, 2011; Luo and Moxham, 2005; Reilly et al., 2013); however, reflecting previously published methods, no instructions were given with regards to handling possible residual interference by P, T, or U waves (**Figure 2**). Thus, we cannot exclude the possibility of such interference within the manually derived EMGdi signal. Noteworthy, the values that have been extracted between QRS complexes in most previous literature is the peak RMS EMGdi signal of a given breath (Sinderby et al., 1996; Luo and Moxham, 2005; Reilly et al., 2011, 2012, 2013). However, as we were interested in measuring an estimate of the integral (i.e., mean) respiratory neural drive of the inspiratory cycle of a given breath, the mean value between QRS complexes that would represent the integral activation was used for analysis instead of the peak value (Langer et al., 2018). The time-synchronized flow and ECG channels were used as a guide for EMGdi selection. Five representative (preferably consecutive) breaths toward the end of each minute were selected. The choice of using five breaths toward the end of a given minute is based on in-house previous analysis that shows the mean value obtained from the last five breaths of a given minute being similar to the average of the values obtained from the last 30 s of the same minute. Breaths were disregarded in case they represented short sighs or included visible noise (e.g., from coughing) or if they were visibly different compared to surrounding breaths. The average of EMGdi of these five breaths was used as representative of diaphragm activation of each minute of the cycling test.

ECG Removal With the Semi-Automated Method

To perform semi-automated ECG exclusion using the newly developed algorithm several steps had to be executed. First, in the data acquisition software (Spike 2, Cambridge Electronic Design Limited, Cambridge, United Kingdom), the following recording channels were selected and exported at 1000 Hz into a text file using the export option from the data acquisition software: ECG, EMGdi (five channels),respiratory flow and volume, and a channel including event markers. These markers were manually inserted during the test to spot the transition from one condition to another during the test. The entire length of the data file, including the resting period before cycling, 1 min of unloaded cycling and all minutes of loaded cycling until symptom limitation were exported. The exported file was then imported into LABVIEW (National Instruments, Austin, TX, United States) software. The waveforms of the recorded channels was also visible in LABVIEW for inspection.

To reduce the ECG content of the diaphragm EMG channels we used a method called "adapted filtering." The LMS Adaptive Filter is a pattern recognition algorithm, which is available in the LABVIEW software. This method is a filtering method in the frequency domain that aims to remove the ECG frequency content out of the total signal. The filter was tuned to comply with the minimum error and consequently delivered the best

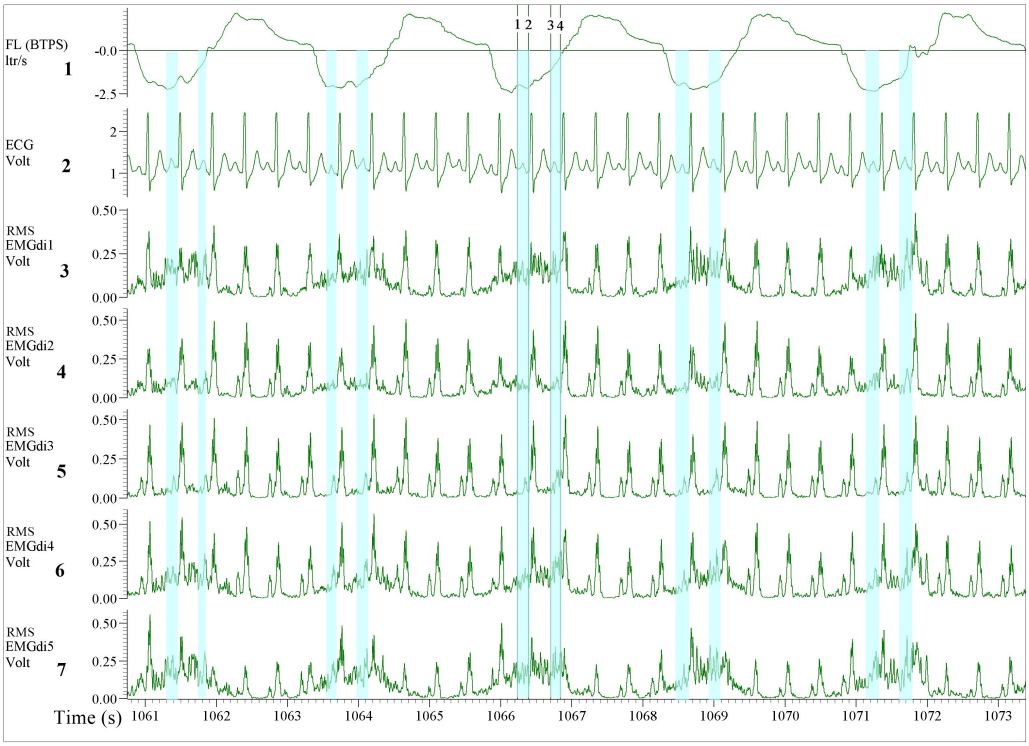

FIGURE 2 | Illustration of the diaphragm EMG (EMGdi) recordings toward the end of the (symptom limited) cycling test. Channel 1: respiratory flow (l/s; negative flow indicating the inspiratory cycle), Channel 2: electrocardiogram (ECG) recording (volt), Channels 3–7: RMS EMGdi recordings. For the data analysis using the manual method, the mean values of EMGdi in between QRS complexes during the inspiratory cycle were selected. The periods highlighted in light blue are the possible periods that could be chosen without ECG contamination in each inspiratory cycle. The average of EMGdi of five consecutive breaths was used as a representative value of EMGdi of that minute. Vertical cursors 1, 2, 3, and 4 were used as a tool during manual analysis to retrieve mean values of EMGdi in the selected period.

results to remove the ECG component from the recorded signal. We used a filter length of 70 and a step size of 0.01 as the most optimal coefficients for this analysis. A separate channel was used to record the ECG synchronously to tune the coefficients of the Finite Impulse Response (FIR) filter continuously. In this way, the removal was very precise, even though the heart rhythm was changing throughout the test. More detail concerning the LMS Adaptive Filtering can be found in this link http://www.ni.com/example/31220/en/.

The ECG filtering algorithm was pre-set in the LABVIEW software, the ECG channel was recognized automatically by the algorithm. After importing the data, the assessor selected the ECG exclusion option on all EMGdi channels. The algorithm then automatically ran and the assessor was notified when the "cleaned" EMGdi data were ready to be retrieved. These results were then saved in a separate text file. This text file containing the cleaned EMGdi data was then re-imported into the data acquisition software (Spike 2). The assessor then used a respiratory script application (commercially available upon purchase of the software) available in the data acquisition software (spike 2) to further process the data. The respiratory script automatically marks the inspiratory and the expiratory cycle of each breath throughout the selected recording interval based on the respiratory flow signal (i.e., based on zero-flow points). The mean of the integrated EMGdi signal (RMS) during every inspiratory cycle (marked periods) throughout the cycling test was then automatically calculated and exported to an excel sheet. The values of these mean integrated EMGdi signals of every breath could not be manipulated by the assessor. The assessor then identified the resting and exercise period of each test and each minute of the test was manually marked. The average of the mean of integrated RMS from every inspiratory cycle in each minute was then manually calculated and used as a representative diaphragm activation of each minute of the cycling test (**Figure 3**). In a similar way, IC maneuvers were manually identified and activation data retrieved. In summary, while the method involves some manual steps it is not possible to manually manipulate EMGdi amplitude signals within separate breaths. The method is therefore (in contrast to the manual method) not prone to inter rater variability. Differences in outcomes could only occur in case of not selecting appropriate minute intervals or IC maneuvers."

The reported results used for analysis were taken from one of the five channels that (on average) contained the largest EMGdi signals during IC maneuvers.

Exercise Testing

All patients underwent constant work rate (CWR) cycling tests consisting of 3 min of resting, 1 min of unloaded cycling and immediately followed by cycling against 75% of the

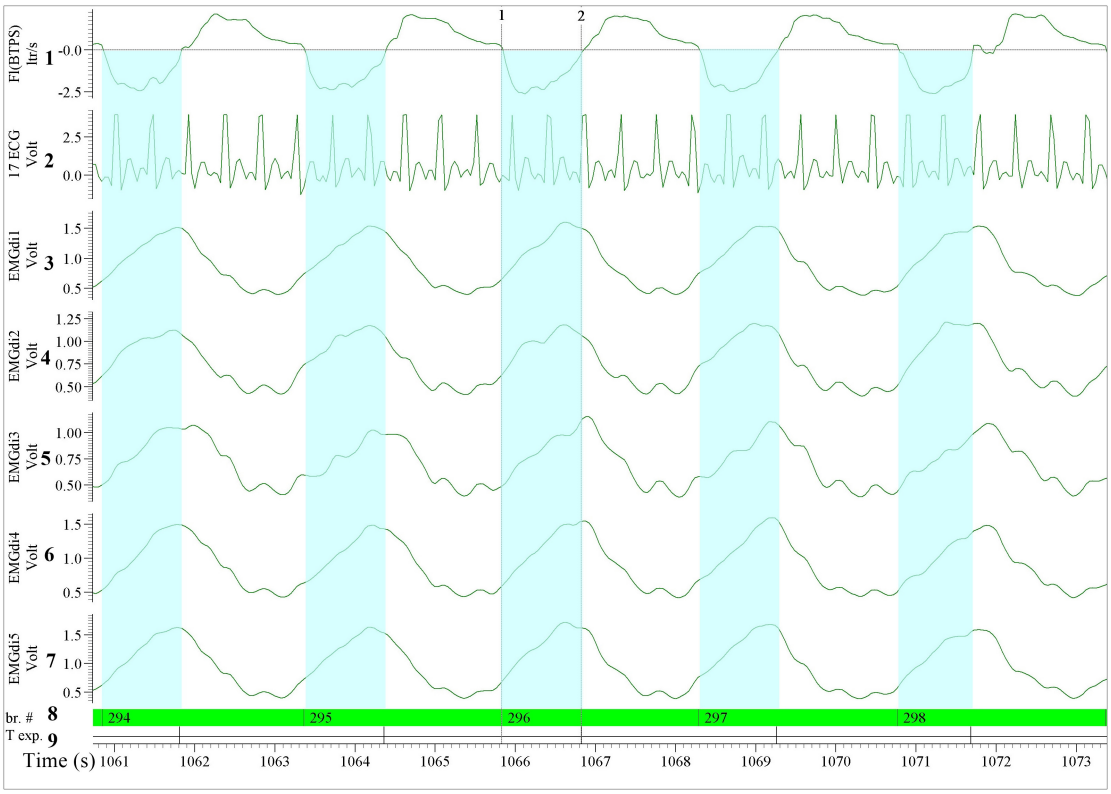

FIGURE 3 | Illustration of the diaphragm EMG (EMGdi) recordings toward the end of the (symptom limited) cycling test (period comparable to **Figure 2**). For the data analysis using the semi-automated method. Channel 1: respiratory flow (l/s; negative flow indicating the inspiratory cycle), Channel 2: ECG recording (volt); The absolute ECG values (volt) from this re-imported data after having been processed in LABVIEW using the semi-automatic algorithm were transformed into an abstract unit. Therefore, the signal appeared to be distorted and cannot be compared directly to the pre-processed ECG signal, Channels 3–7: the processed EMGdi data from our customized algorithm without ECG contamination in the diaphragmatic EMG signal (EMGdi). Channel 8: br.#, beginning of the inspiratory cycle and Channel 9: T exp., beginning of the expiratory cycle indicates the inspiratory and expiratory cycle of each breath which was marked automatically by the program. The EMGdi during a full inspiratory cycle can be selected (highlighted in light blue). The average of the mean EMGdi from every inspiratory cycle in each minute was analyzed. Vertical cursors 1 and 2 indicate a longer period that the value of EMGdi could be retrieved compare to the same breath in **Figure 2** that only shorter periods were available.

patient's peak work rate achieved during a maximal incremental cardiopulmonary exercise test (CPET) (American Thoracic Society/American College of Chest Physicians [ATS/ACCP], 2003) until symptom limitation. The tests were conducted on an electrically braked cycle ergometer (Ergometrics 900, Ergoline, Blitz, Germany) with detailed metabolic (SensorMedics Vs229d, Acertys Healthcare, Aartselaar, Belgium) and cardiopulmonary measurements (Cardiosoft, Acertys Healthcare, Aartselaar, Belgium). The respiratory flow signal was recorded during the exercise to be able to identify the respiratory cycle. ECG recordings were obtained via an impedance cardiography device (PhysioFlow, Manatec Biomedical, Folschviller, France) validated for COPD patients and recorded as a separate channel (Louvaris et al., 2019). Analog outputs of all variables (i.e., respiratory flow, five EMGdi and ECG) were collected in separate channels with a data acquisition unit (Micro1401-3, Cambridge Electronic Design Limited, Cambridge, United Kingdom). Data channels were synchronously collected by the same system and processed with the same acquisition software (Spike 2, Cambridge Electronic Design Limited, Cambridge, United Kingdom). The system was *a priori* checked for potential time delays between the different systems providing the signals of the different channels (e.g., ECG and EMGdi). No time delays were present and therefore, no additional post collection synchronization of data had to be performed.

Inspiratory Muscle Training (IMT)

Inspiratory muscle training was performed daily by four subjects using an electronic POWERbreathe®KH2 device (HaB International Ltd., Southam, United Kingdom) for 8 weeks, according to a previously published protocol (Langer et al., 2015). In short, the patients trained at the highest tolerable intensity, 30 breaths per session and two sessions per day. Progression of training intensity and MIP measurements were performed weekly.

Statistical Analyses

Comparisons of diaphragm activation at pre-measurement and pre/post IMT differences obtained from two assessors using

the manual signal processing method and with the semi-automated signal processing method were made. Pearson's correlation coefficient (r) was used to establish associations between measurements. The intra-class correlation coefficient (ICC) based on a mean-rating, absolute-agreement, 2-way mixed-effects model was used to quantify agreement between two assessors (inter-rater reliability) and between the two methods. Agreement of the results from two assessors and between the two methods was assessed by plotting mean differences between assessors or methods against average values (Bland-Altman plots) (Bland and Altman, 1986). Limits of agreement were defined as $\pm 1.96 \times$ standard deviation of the difference between the two methods, corresponding to 95% confidence intervals (CI). The interaction over time between the two assessors and the two methods was assessed using repeated measures ANOVA. Within rater Coefficient of Variation (CV) for the two raters was calculated from five representative breaths during resting and at the end of exercise. For the semi-automated method, the CV was also calculated at the same time points. Statistical analyses were performed using GraphPad Prism version 8 for Windows (GraphPad Software, La Jolla, CA, United States) and IBM SPSS Statistics 25.0 Desktop (IBM Corp., Armonk, NY, United States). Statistical significance was considered at $p < 0.05$. Data are presented as means ± SD.

RESULTS

Datasets supporting the conclusions of this manuscript are available on request. Characteristics of included patients are presented in **Table 1**. Patients exhibited moderate to severe airway obstruction with static hyperinflation, reduced exercise capacity, and inspiratory muscle strength. There are approximately four heartbeats during one inspiratory cycle both at rest and during exercise (**Table 1**).

TABLE 1 | Patient characteristics: pulmonary function, respiratory muscle strength, maximal, and endurance exercise capacity.

	All subjects (*n* = 7)
General characteristic	
Male:female	4:3
Age, years	66 ± 5
BMI, kg/m^2	25 ± 7
Pulmonary function	
FEV_1, L (%pred)	1.37 ± 0.57 (56 ± 31)
FEV_1/FVC, %	42 ± 15
IC, L (%pred)	1.96 ± 0.44 (76 ± 27)
FRC, L (%pred)	5.14 ± 1.85 (161 ± 42)
RV, L (%pred)	3.64 ± 1.48 (149 ± 57)
TLC, L (%pred)	7.10 ± 1.73 (122 ± 19)
D_LCO, mmol/min/Kpa (%pred)	4.63 ± 2.01 (59 ± 25)
Respiratory muscle strength	
MIP at RV, cmH_2O (%pred)	−77 ± 11 (85 ± 18)
Pdimax, cmH_2O	89 ± 19
MEP at TLC, cmH_2O (%pred)	167 ± 55 (99 ± 34)
Symptom-limited peak incremental cycling ergometer exercise test	
Cycling duration (minutes)	7.7 ± 1.5
Peak work rate, W (%pred)	82 ± 27 (64 ± 24)
VO_2, L/min (%pred)	1.40 ± 0.60 (74 ± 33)
HR, bpm (%pred HRmax)	118 ± 16 (76 ± 9)
Ventilation, L/min (%MVV)	44.6 ± 7.6 (88 ± 13)
Constant work rate cycling test (CWR cycling)	
Cycling work rate, W (%Wmax)	59 ± 20 (72 ± 3)
Cycling duration, min	8.0 ± 3.7
Resting HR, bpm (%pred HRmax)	80 ± 10 (52 ± 16)
HR at end exercise, bpm (%pred HRmax)	124 ± 17 (80 ± 10)
Resting BF, bpm	22 ± 8
BF at end exercise, bpm	32 ± 8
HR:BF at rest, per min	3.9 ± 1.1
HR:BF at end exercise, per min	4.1 ± 1.2
Ventilation, L/min (%MVV)	38.3 ± 8.4 (80 ± 11)
Resting Ti, s	1.1 ± 0.5
Ti at end exercise, s	0.8 ± 0.2
Resting Te, s	2.1 ± 0.7
% change from rest Ti	−25 ± 22
% change from rest Te	−36 ± 23

FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; IC, inspiratory capacity; FRC, functional residual capacity; RV, residual volume; TLC, total lung capacity; D_LCO, diffusing capacity of the lung for carbon monoxide; MIP at RV, maximal inspiratory mouth pressure at residual volume; PdiMax, maximal transdiaphragmatic pressure during sniff maneuver; MEP at TLC, maximal expiratory mouth pressure at total lung capacity; VO_2, oxygen consumption; HR, heart rate; MVV, maximal voluntary ventilation; BF, breathing frequency; Ti, inspiratory time; Te, expiratory time; %pred, %predicted.

Comparisons of EMGdi/EMGdiMax% Obtained by Either the Two Assessors or as Processed With the Semi-Automated Method From Data Collected During a Constant Work Rate Cycling Task

The intra-class correlation coefficients (ICC) between diaphragm activation signals obtained with the manual methods by two assessors at pre-measurement was 0.94, $p < 0.0001$, 95% CI: 0.17–0.98 (**Figure 4A**). The ICC between EMGdi signals from the semi-automated method and the results obtained by using the manual method from assessor 1 and 2 at pre-measurement were 0.96, $p < 0.0001$, 95% CI: 0.94–0.97 (**Figure 4B**) and 0.91, $p < 0.0001$, 95% CI: 0.60–0.97 (**Figure 4C**), respectively.

Bland – Altman plots for the agreement of EMGdi/EMGdiMax% for the above-mentioned comparisons are presented in **Figures 5A–C**. On average, the EMGdi/EMGdiMax% obtained from the manual method by assessor 2 resulted in lower values than those obtained from assessor 1 (average bias of the differences: −9.9%; CI: −22.9 to 3.0%, **Figure 5A**). The plot of agreement between EMGdi/EMGdiMax% values from the semi-automated method and results of the manual method obtained by assessor 1 showed that on average the values from the semi-automated method were very similar with the values obtained from the manual method by assessor 1 (average bias of the differences: −0.5%; CI: −19.6 to 18.6%, **Figure 5B**). The plot of agreement between the values from the semi-automated method and

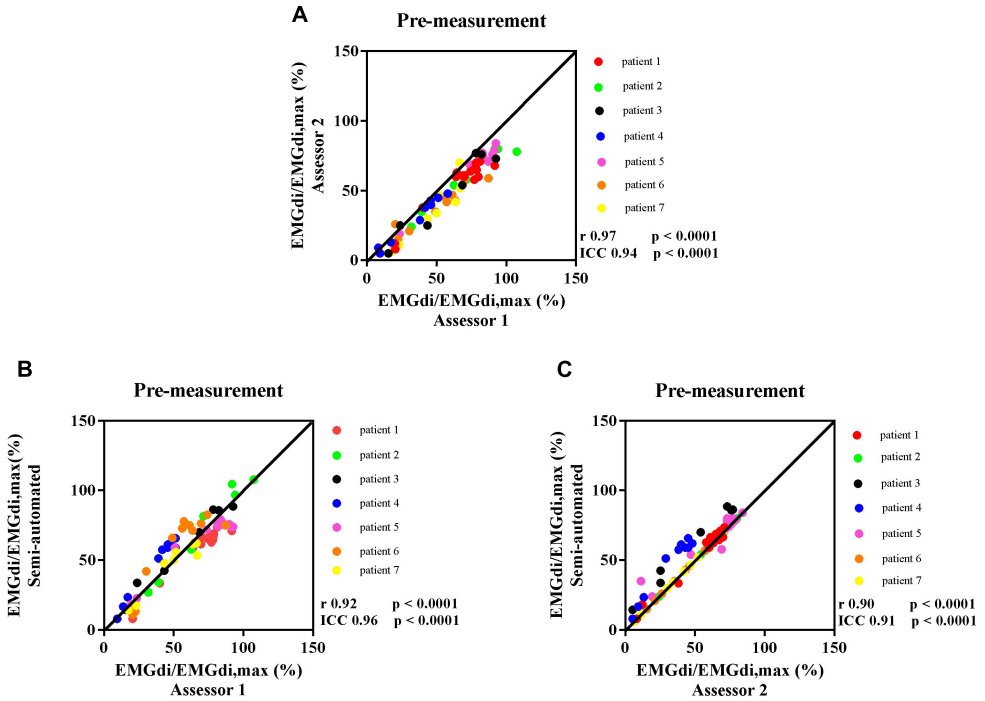

FIGURE 4 | The correlation between EMGdi/EMGdiMax% calculated from the manual method by two assessors during the pre-measurement CWR cycling test **(A)**; the semi-automated method and the manual method by assessor 1 **(B)**; and the semi-automated method and the manual method by assessor 2 **(C)**. Line of identity, linear regression coefficients, intra-class correlation coefficients (ICC), and significances are presented in each figure. Each of the data points represents the activation of diaphragm EMG (EMGdi/EMGdiMax%) of each patient in every minute during the pre-measurement CWR cycling test.

those obtained by the manual method from assessor 2 showed higher values from the semi-automated method (average bias of the differences: 9.3%; CI: −11.4 to 29.9% **Figure 5C**).

Average EMGdi/EMGdiMax% obtained from the manual method by two assessors and values obtained with the semi-automated method were plotted against time for each minute during CWR cycling are presented in **Figures 6A–C**. There were no significant method by time interactions observed neither between the values from two assessors (**Figure 6A**; $P = 0.24$), nor between values from the semi-automated method and assessor 1 (**Figure 6B**; $P = 0.30$), or the semi-automated method and assessor 2 (**Figure 6C**; $P = 0.11$).

Average absolute maximal activation values (obtained during IC maneuvers) obtained by assessor 1 and 2 with the manual analysis method of were 0.146 ± 0.062 volt and 0.150 ± 0.060 volt, respectively. No significant differences were found between the maximal activation values obtained by assessor 1 and 2 ($P = 0.25$).

The CV of assessor 1 was 22% at rest and 13% at the end of exercise at pre-measurement. At post-measurement, the CV was 21% at rest and 26% at end exercise. For assessor 2 the CV was 54% at rest and 11% at the end of exercise. At post-measurement, the CV was 28 and 20% at rest and end exercise, respectively. The CV calculated from the semi-automated were 13 and 11% at rest and end exercise, respectively, at pre-measurement. At post-measurement, the CV was 10 and 12% at rest and end exercise, respectively.

Comparisons of the Pre/Post Intervention Differences in EMGdi/EMGdiMax% Obtained by Either the Two Assessors or as Processed With the Semi-Automated Method From Data Collected During a Constant Work Rate Cycling Task

After 8 weeks of IMT, inspiratory muscle function was improved in four patients that had completed the IMT protocol [two men and two women, age 64 ± 4 years, BMI 25 ± 7 kg/m^2, FEV_1 1.56 ± 0.69 L (63 ± 41 %predicted)] who participated in the IMT intervention. Maximal inspiratory pressure (MIP) improved from −77 ± 15 cmH_2O (84 ± 16 %predicted) to −91 ± 25 cmH_2O (100 ± 30 %predicted). Maximal transdiaphragmatic pressure (Pdi) measured during maximal inspiratory sniff maneuvers improved from 93 ± 21 cmH_2O to 105 ± 24 cmH_2O. The average cycling duration was 8.4 ± 2.5 min at pre-measurement and 16.4 ± 7.8 min at post-measurement. The pre/post IMT differences in EMGdi/EMGdiMax% during cycling before and after IMT, were calculated. The correlations between the pre/post IMT differences in EMGdi/EMGdiMax% calculated from the manual method by two assessors and the

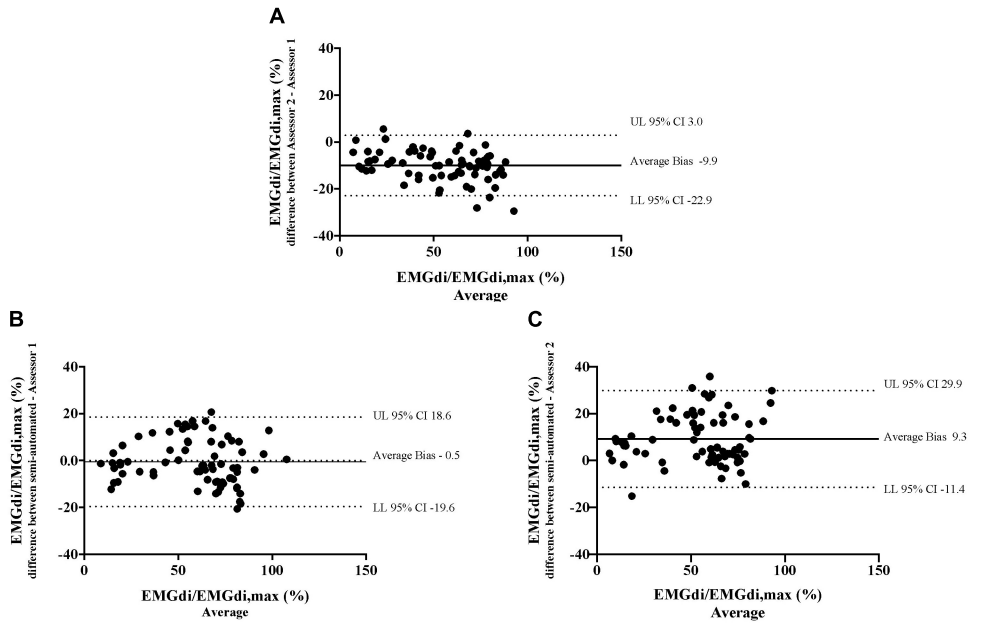

FIGURE 5 | The results from the manual method by two assessors and the semi-automated method are compared in seven patients in each minute during the pre-measurement CWR cycling test. Bland-Altman plots of EMGdi/EMGdiMax% calculated from the manual method by two assessors **(A)**; the semi-automated method and the manual method by assessor 1 **(B)**; the semi-automated method and the manual method by assessor 2 **(C)**.

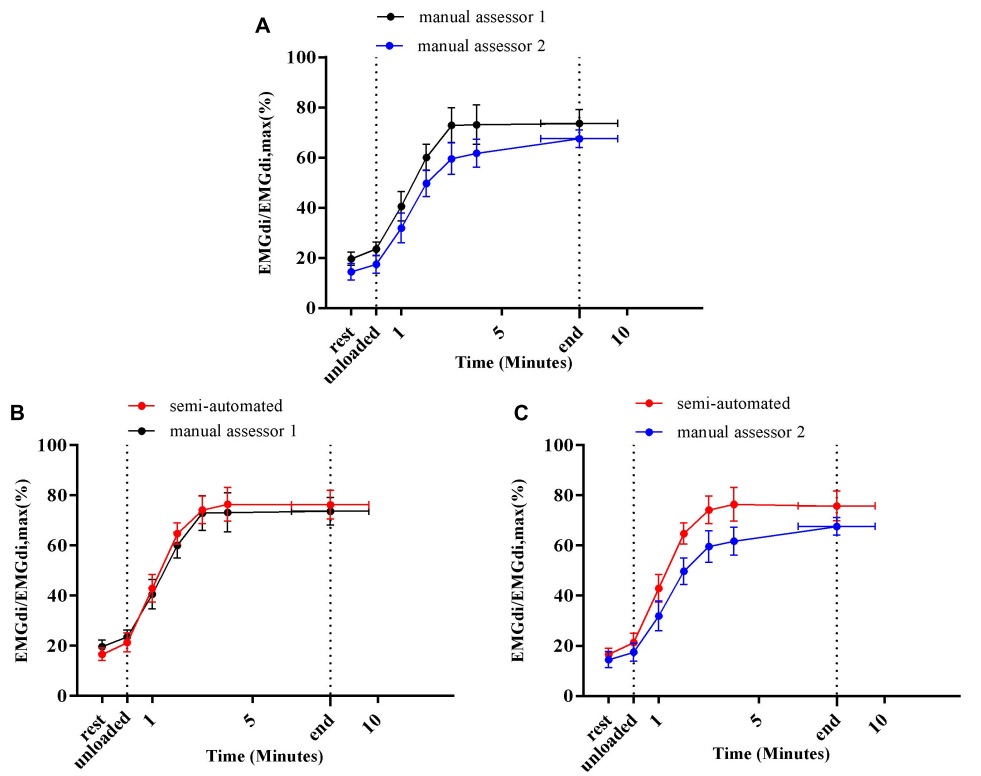

FIGURE 6 | Average diaphragm activation (EMGdi/EMGdiMax%) of seven patients during CWR cycling test at pre-measurement calculated from the manual method by two assessors **(A)**; the semi-automated method and the manual method by assessor 1 **(B)**; the semi-automated method and the manual method by assessor 2 **(C)**.

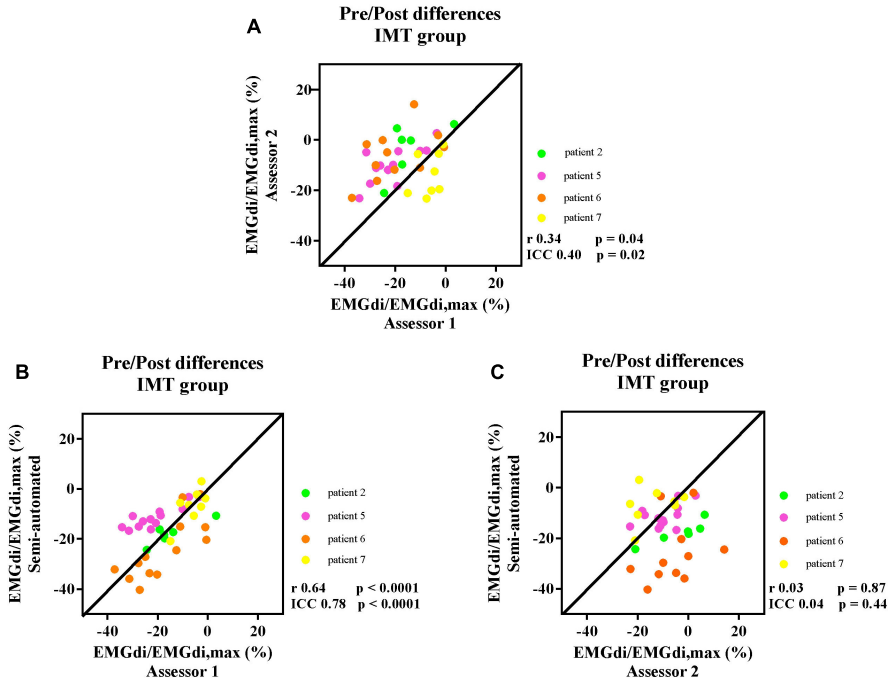

FIGURE 7 | The correlation between the pre/post difference in four participants EMGdi/EMGdiMax% calculated from the manual method by two assessors **(A)**; the semi-automated method and the manual method by assessor 1 **(B)**; the semi-automated method and the manual method by assessor 2 **(C)**. Line of identity, linear regression coefficients and intra-class correlation coefficients (ICC) and significances are presented in each figure. Each of the data points represents the pre/post differences of diaphragm EMG activation (EMGdi/EMGdiMax%) pre and post the intervention of each patient in every minute during CWR cycling test.

semi-automated method during the CWR cycling test before and after the intervention are presented in **Figures 7A–C**.

The ICC between the values of pre/post differences from assessor 1 and assessor 2 was 0.40, $P = 0.02$, 95% CI: −0.09 to 0.68 (**Figure 7A**). The ICC between the pre/post IMT differences from the semi-automated method and assessor 1 was 0.78, $p < 0.0001$, 95% CI: 0.58–0.89 (**Figure 7B**), while the ICC between the pre/post IMT differences from the semi-automated method assessor 2 was 0.04, $P = 0.44$, 95% CI: −0.58 to 0.46 (**Figure 7C**).

Bland – Altman plots for the agreements of pre/post IMT differences in EMGdi/EMGdiMax% calculated from two analyzing methods are presented in **Figures 8A–C**. On average, the pre/post IMT differences in EMGdi/EMGdiMax% obtained from the manual method from assessor 2 was lower than assessor 1 (average bias of differences: −8.2%; CI: −30.9 to 14.5%, **Figure 8A**). The pre/post IMT differences in EMGdi/EMGdiMax% obtained from the semi-automated method was on average similar to values obtained with the manual method by assessor 1 (average bias of differences: 1.2%; −16.8 to 19.2%, **Figure 8B**). The pre/post differences values from the semi-automated method are higher than the values from the manual method by assessor 2 (average bias of differences: 7.0%; CI: −20.4 to 34.4%, **Figure 8C**).

Average EMGdi/EMGdiMax% values obtained from the manual method by the two assessors and the semi-automated method were plotted against time for each minute during CWR cycling performed pre and post the IMT intervention period (**Figures 9A–C**). There were no significant method by time interactions observed between the values from two assessors (**Figure 9A**; $P = 0.29$), the semi-automated method and assessor 1 (**Figure 9B**; $P = 0.55$) and the semi-automated method and assessor 2 (**Figure 9C**; $P = 0.50$).

Average absolute maximal activation values (during IC maneuver) obtained with the manual analysis method by assessor 1 and 2 were 0.121 ± 0.075 volt and 0.124 ± 0.072 volt, respectively, at pre-measurement, and 0.158 ± 0.101 volt and 0.142 ± 0.92 volt, respectively, at post-measurement ($P = 0.62$ and $P = 0.20$, respectively).

Duration of Analysis Between Manual and Semi-Automated Method

The average duration of 11 CWR cycling tests (seven at pre-measurement and four at post measurement), including resting and unloaded cycling, was 13 ± 6 min (range 4.2 to 22.0 min). The analyzing time using the manual method was 82 ± 20 min (range 63 to 115 min) for assessor 1 and for the semi-automated method was 29 ± 9 min (range 18–49 min). Difference between methods 53 ± 15 min ($p < 0.0001$).

DISCUSSION

Main Findings

We validated a custom developed ECG removal method for EMG amplitude analysis against a commonly used manual approach. The main findings of this study are that the newly developed

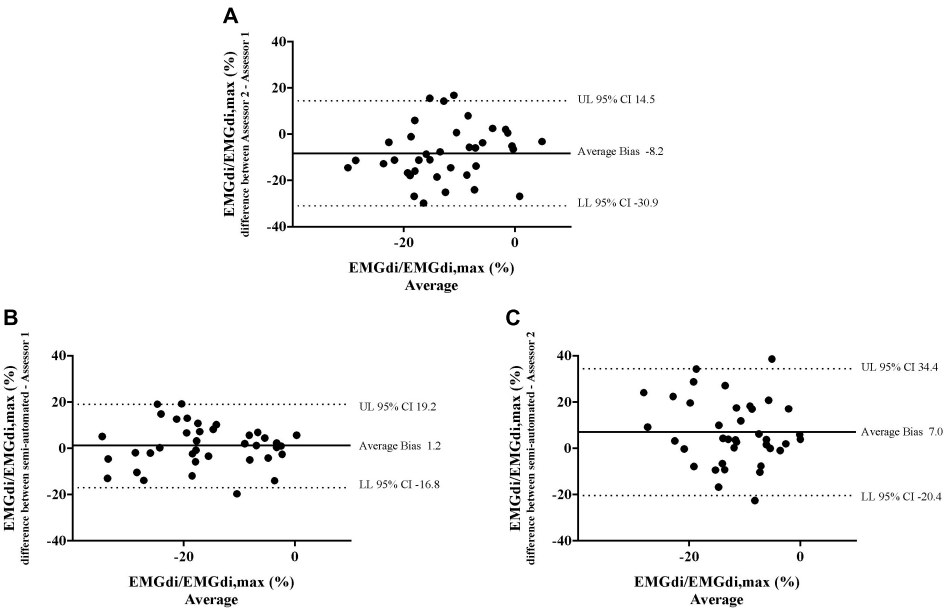

FIGURE 8 | The results from the manual method by two assessors and the semi-automated method are compared in four patients in each minute during CWR cycling test pre and post the intervention. Bland-Altman plots of the pre/post differences in EMGdi/EMGdiMax% calculated from the manual method by two assessors **(A)**; the semi-automated method and the manual method by assessor 1 **(B)**; the semi-automated method and the manual method by assessor 2 **(C)**.

semi-automated EMGdi analysis method is more time efficient and that it will be less prone to the inter-rater variability that was observed when the manual method was applied by two independent assessors. EMGdi amplitudes obtained with the semi-automated method agreed well with values obtained by one of the two manual assessors. The findings suggest that EMGdi analysis using the proposed semi-automated method can be used to evaluate changes in EMG amplitudes over a wide range of minute ventilations recorded at rest and during exercise in patients with COPD.

Inter-Rater Reliability of the Manual Method

Resting diaphragm activation (EMGdi/EMGdiMax%) values obtained by both assessors using the manual method ranged from 10 to 20% (**Figures 6A,B**, **9A,B**). During the CWR cycling exercise, this activation increased steeply at the beginning of the exercise and reached a plateau until the exercise was terminated by patients' symptom limitation (**Figures 6A,B**, **9A,B**). Similar patterns were observed in previous studies (Luo and Moxham, 2005; Luo et al., 2011; Langer et al., 2018). The observed differences of 8–9% in EMGdi amplitudes between raters are, however, substantial and might impact on the ability to detect differences in EMG amplitudes after interventions (**Figures 5A**, **8A**). Along these lines the pre/post IMT differences manually obtained by assessor 1 resulted in a reduction of approximately 20% of EMGdi/EMGdiMax at iso-time (**Figures 9A,B**), whereas analyses performed by assessor 2 resulted in a much smaller reduction of approximately only 10% (**Figures 9A,C**). In an attempt to explain these differences we looked into the manual analyses as performed by the two assessors in more detail.

Since EMGdiMax (volt) values obtained by the two assessors were similar, the differences in the EMGdi/EMGdiMax ratio between the two assessors must have originated from the selection of the EMGdi signal between QRS complexes of the tidal breaths. Retrospectively, we observed that in most cases there were several intervals between QRS complexes that assessors could select for their analyses (**Figure 2**). Upon closer inspection we further realized that assessor 1 systematically tended to choose the period that resulted in the "highest" EMGdi value of each inspiratory cycle (frequently occurring toward the very end of an inspiratory cycle), while assessor 2 always chose intervals that contained the "widest" available signal typically located more "centrally" within each inspiratory phase. This is illustrated in **Figure 2**. While assessor 1 systematically selected the period between cursor 3 to 4, assessor 2 tended to choose the period between cursor 1–2. We noticed that especially during pre-IMT assessments the amplitude of EMGdi was higher toward the end of each inspiratory cycle, indicating more pronounced diaphragm activation toward the end of the inspiratory cycle (**Figure 10A**). Since the given illustrative example occurred frequently during the tests the intervals selected by assessor 1 often resulted in higher values than the intervals chosen by assessor 2 (**Figures 5A**, **8A**).

As stated earlier the values of EMGdiMax were not significantly different between two assessors. This initially seems surprising given the different approaches taken by the two assessors as described above. It can be explained, however, based on the shorter inspiratory period (Ti) during the IC maneuvers (during which the EMGdiMax signals were obtained)

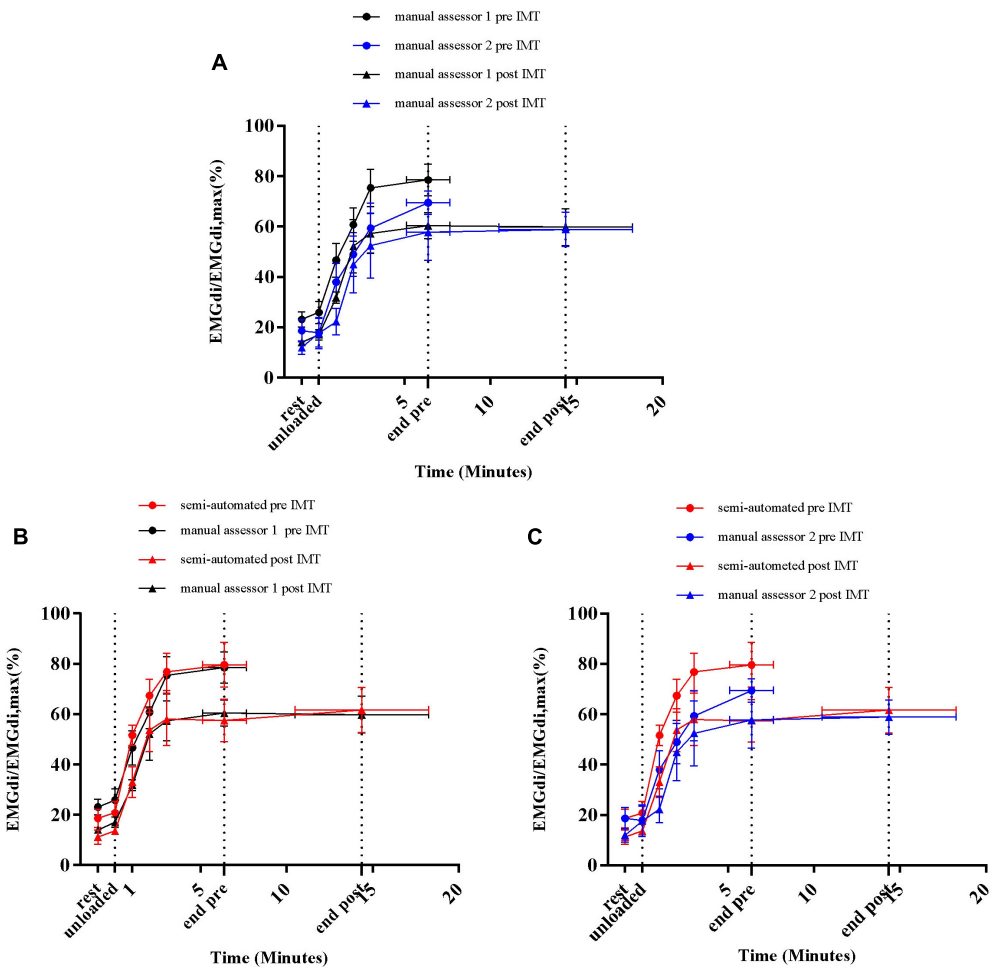

FIGURE 9 | Average diaphragm activation (EMGdi/EMGdiMax%) of four patients during CWR cycling test pre and post the intervention calculated from the manual method by two assessors **(A)**; the semi-automated method and the manual method by assessor 1 **(B)**; the semi-automated method and the manual method by assessor 2 **(C)**.

in comparison to the tidal breaths (during which the EMGdi intervals were selected). As illustrated in **Figure 11** during the short inspiratory periods of the IC maneuvers there was frequently only a single EMG interval between QRS complexes available to select. This can most likely explain the smaller differences in EMGdiMax values between assessors in comparison to EMGdi.

Interestingly there was also less disagreement between the results from assessor 1 and 2 during the post-intervention analyses in comparison to pre-intervention comparisons (**Figure 9A**). Despite both assessors treating the EMGdi data with the same approach as for the pre-intervention measurements (i.e., one rater looking for the highest while the other looked for the widest available signal interval), the values from assessor 2 were closer to the values from assessor 1. This finding can probably be explained by previously reported pre/post differences in EMG amplitude signal patterns over time, during muscular activation, in response to muscle training (Oliveira Ade and Goncalves, 2009). Known effects of muscle training, including inspiratory muscle training, are improvements in force output and motor learning, thereby decreasing muscle activation levels at iso-loads (Campos et al., 2002; Lay et al., 2002). In fact, higher EMGdiMax and decreased relative activation of the diaphragm (i.e., lower muscle activity) after training at iso-loads were previously reported by our group (Langer et al., 2018).

As shown in **Figure 10A** and as mentioned earlier, during pre-IMT assessments, the EMGdi signal from the diaphragm increased from the beginning toward the end of the inspiration. The EMGdi values (volt) between cursors 1–2 were always lower than those between cursors 3–4 (**Figure 10A**). The numbers marked in red are the values taken as a representative mean EMGdi of that breath (**Figures 10A,B**). After inspiratory muscle training, however, EMGdi values earlier during inspiration were less different from those toward the end of the inspiratory phase (**Figure 10B**). It therefore seems like patterns that had previously been observed after resistance training of peripheral muscles (reduced EMG/time slopes after training) (Oliveira Ade and Goncalves, 2009), were also detected in our diaphragm EMG

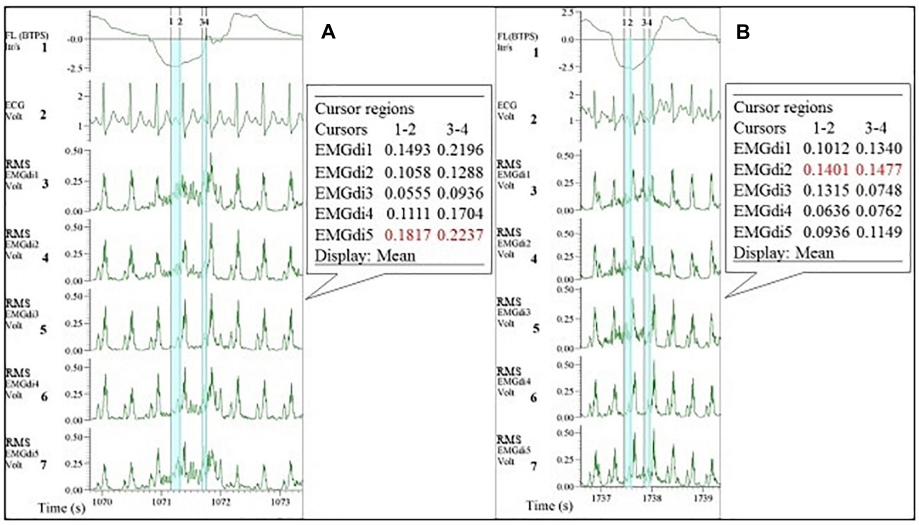

FIGURE 10 | Example of EMGdi recordings during cycling exercise toward the end of the (symptom limited) cycling test at the pre- **(A)** and post- **(B)** measurement for the analysis using the manual method. Channel 1: respiratory flow (l/s; negative flow indicating the inspiratory cycle), Channel 2: ECG recording (volt), Channels 3–7: diaphragm EMG (RMS EMGdi) recordings. The periods highlighted in light blue are the possible periods that could be chosen without ECG contamination in an inspiratory cycle. Numbers in cursors regions boxes show the mean EMGdi values between two vertical cursors. The numbers in red indicate that the differences in the mean values between cursors 1–2 are closer to the values between cursor 3–4 at post-measurement.

data. These findings can probably help to explain why inter-rater differences, despite of using similar approaches, were less pronounced after the resistance training period.

Comparisons Between Manual and Semi-Automated EMGdi Analyses

Data from the manual analyses of assessor 1 (who searched for the intervals containing the EMGdi signal with highest amplitude for every breath) resulted in good agreement with the results from the semi-automated method. We assume that the semi-automatically processed data are most representative of the "real" EMGdi values since the ECG contamination was eliminated from the signal. Based on these findings the analysis strategy of assessor 1 should probably be favored (i.e., selecting the EMGdi interval in between QRS complexes that provides the "highest" amplitude) should probably be favored above the approach taken by assessor 2. This is further supported by the fact that magnitude of pre-post intervention differences of both assessor 1 and from the semi-automated method are in line with findings from a previous study that assessed diaphragm activation during the CWR cycling test before and after a similar IMT intervention (Langer et al., 2018).Therefore, if the manual method should be used, we would recommend to manually select EMG parts between QRS complexes that result in the "highest" average EMGdi (i.e., selecting intervals toward the end of each inspiratory period). This strategy of manual analysis showed a good agreement with the semi-automated method on a group level, suggesting that both methods can be used interchangeably. The discussion onward will focus on the comparisons between the results of the semi-automated method and values obtained with the manual method from assessor 1.

Bland – Altman plots of both pre-intervention measurements and pre/post IMT differences in EMGdi/EMGdiMax% showed good overall agreement. Considering the differences, which scattered randomly above and below zero, it did not appear as if there were systematic over- or underestimations present, or that differences between methods became larger when activation was higher (i.e., at higher minute ventilation during cycling) (**Figures 5B**, **8B**).

Two factors probably contribute to the relatively wide (±20%) limits of agreement that were observed between methods. Firstly, during manual analyses QRS complexes occurring during the inspiratory cycles can cover major parts of the inspiratory EMG signal. These parts (which might contain the highest activation portion during a given inspiration) are consequently not available for analysis (**Figure 12**). This limited availability of EMGdi signal probably contributed to either over- or underestimation of the manual signal in contrast to the semi-automatically processed signal which could always take the full inspiratory period (i.e., from zero flow to zero flow) into consideration.

A second factor that probably contributes to the width of the limits of agreement between methods is the fact that the average EMGdi from the manual method is obtained from a representative sample of five consecutive breaths toward the end of each exercise minute. In contrast during the semi-automated method, all breaths performed during each minute are analyzed (Luo and Moxham, 2005). It might very well be that the sample of five breaths is not always a perfect representation of average EMGdi during a given minute of breathing resulting in between method differences on a minute-by-minute basis. The overall agreement of the EMGdi/EMGdiMax between two methods on a group level was, however, good, and no significant method by time interaction effects were observed (**Figures 6B**, **9B**).

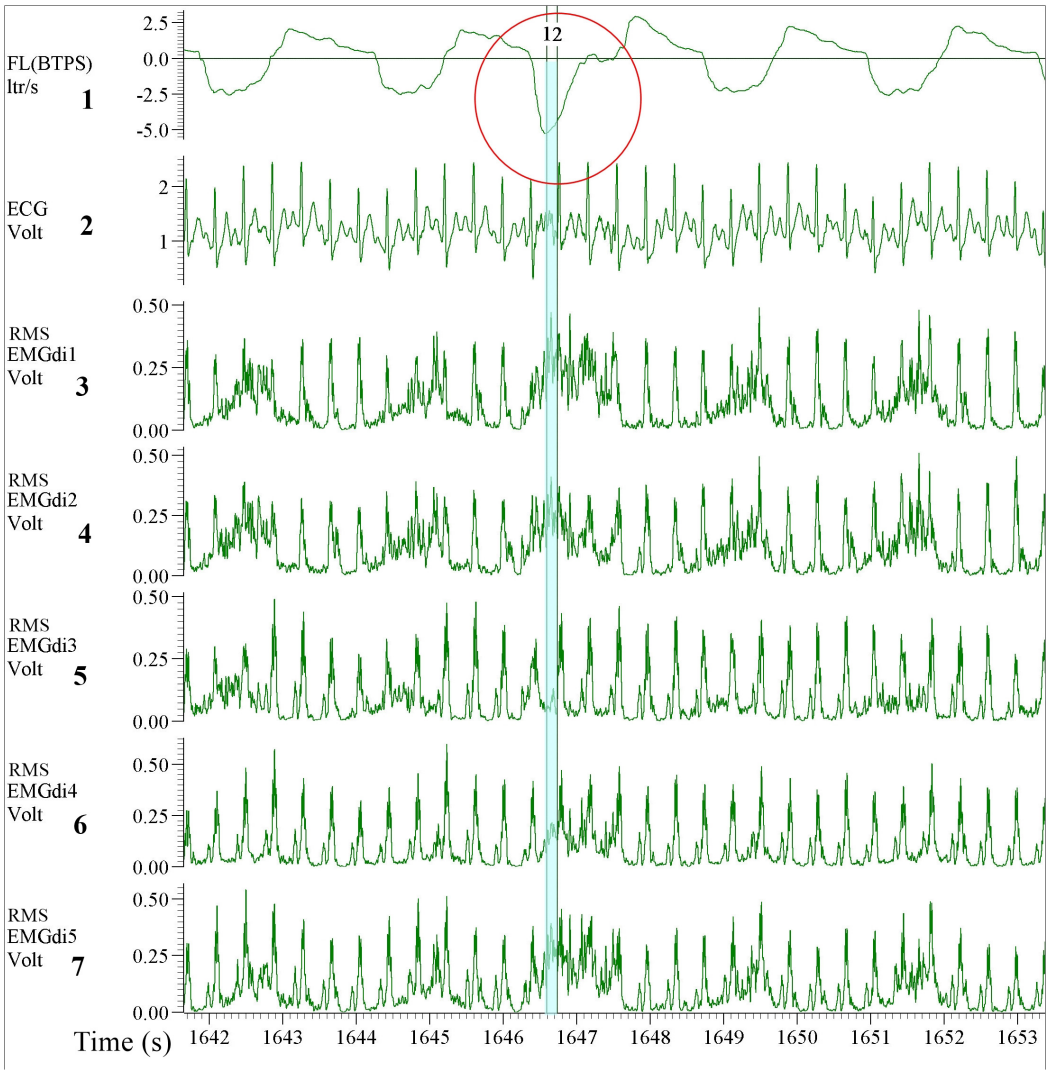

FIGURE 11 | Example of EMGdi recordings during a cycling exercise toward the end of the (symptom limited) cycling test for the analysis using the manual method. Channel 1: respiratory flow (l/s; negative flow indicating the inspiratory cycle), Channel 2: ECG recording (volt), Channels 3–7: Diaphragm EMG (RMS EMGdi) recordings. The IC maneuver is highlighted in the red circle indicated by the higher flow, which accompanies the maximal activation of the EMGdi. The periods highlighted in light blue are the periods that could be chosen without ECG contamination in an inspiratory cycle during IC maneuver. With the short inspiratory time during the IC maneuver, it left only one available (light blue) period that EMGdi could be retrieved.

This suggests that both methods can be used interchangeably on a group level.

Degree of Variation

The CV were calculated from the manual analyses performed by two raters were mostly higher than the CV calculated from the semi-automated method. This lower degree of variability when using the semi-automated method will probably increase the ability to detect true differences between measurement conditions. The reduction in variability is most likely due to both the absence of noise within breaths as well as the fact that instead of a representative sample of five breaths all breaths of each minute were used for analyses.

Clinical Implications

The EMGdi/EMGdiMax ratio is currently being used as a surrogate of neural respiratory drive (NRD) and both magnitude as well as changes in NRD have been shown to be closely related to (changes in) dyspnea sensation (Jolley et al., 2015; Langer et al., 2018) which is an important symptom in patients with chronic obstructive and restrictive lung diseases (Parshall et al., 2012). It is essential to obtain correct values of diaphragm activation to be able to interpret the results linked with the patient's symptoms and also to reliably detect changes induced by different interventions. A reliable and objective method to process these data is beneficial for breathlessness management in patients with COPD both in research and clinical routine.

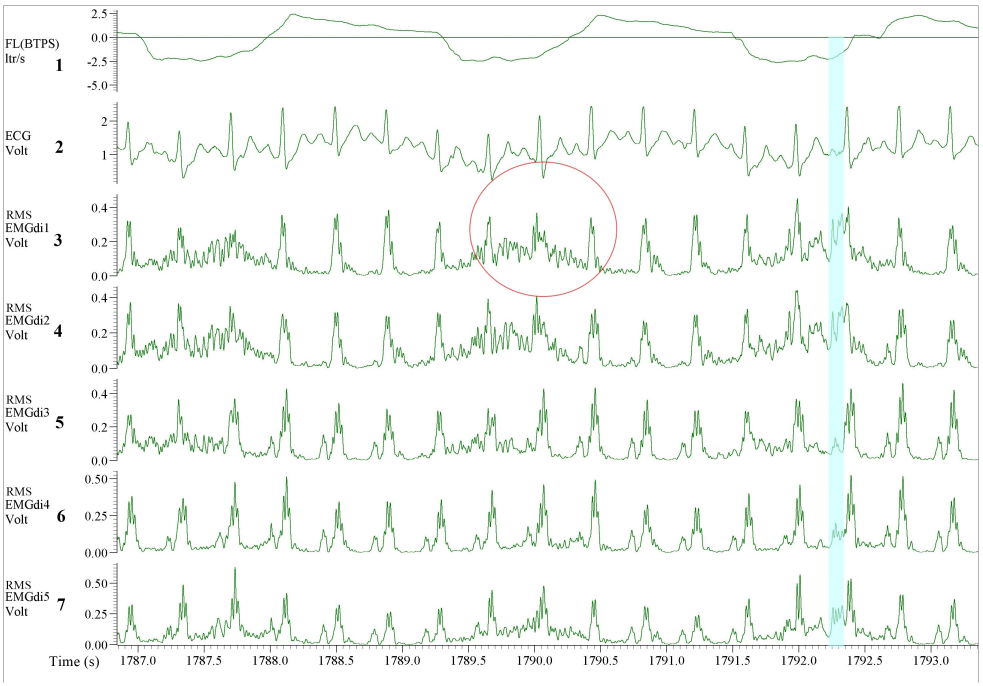

FIGURE 12 | Illustration of the EMGdi recordings during cycling exercise toward the end of the (symptom limited) cycling test when the inspiratory time (Ti) is shorter. Channel 1: respiratory flow (l/s; negative flow indicating the inspiratory cycle), Channel 2: ECG recording (volt), Channel 3–7: diaphragm EMG (RMS EMGdi) recordings. Several QRS complexes appear during the inspiratory cycle which is already a short period. The QRS complexes take up a large part during the inspiratory cycle resulting in less "clean" EMGdi to be selected. The red circle shows one inspiratory cycle when the QRS complex appears precisely at the peak of the amplitude of the EMGdi. The EMGdi buried under this QRS complex cannot be retrieved. Therefore, the assessor must choose the part outside of the QRS complex, which results in lower mean EMGdi value, thus underestimates the diaphragm activation. The blue highlight shows a small period of peak EMGdi. The mean EMGdi value of this period will be higher (average of high values in the short time) therefore the EMGdi being overestimated.

Strengths and Limitations

The semi-automated method was designed to overcome several shortcomings of the manual method. By automatically removing ECG artifacts throughout the recording, the analysis time is shortened by more than half. After the ECG artifact was removed the resulting "clean" EMGdi signal could be integrated over the full inspiratory cycle. This integration of EMG activity over the course of a contraction is a common practice for other skeletal muscles but was not possible with the manual EMGdi analysis methods available so far. In addition it facilitates the performance of breath-by-breath analyses which allows all data points to be considered. This is the first time that the inter-rater reliability of the often used "manual method" has been evaluated. We were for the first time able to identify several sources for inter-rater variability which should be eliminated by the more objective, semi-automated processing of full inspirations that have previously been cleaned of ECG artifacts by our newly developed method.

Limitations of our study are the relatively small sample size and the absence of an age-matched control group. The results would need to be confirmed in a larger sample of subjects performing different types of exercise tests resulting in a large variability of heart rate and ventilation responses. In addition, inclusion of a healthy age-matched control group and comparisons of findings with COPD patients would have allowed further investigations into the validity of the semi-automated method at rest and during exercise. Since our methods were only compared in a specific group of patients future studies might be required to further validate the use of the semi-automated EMGdi analysis in other populations. In our study EMGdi signals were evaluated only at the extremes of activation (i.e., resting breathing and close to maximal activation). The model/approach has not been tested over a range of intensities (and as such diaphragm activity). It would have been preferable to evaluate the responses over a broader range of minute ventilations and heart rate (e.g., during a stepwise maximal incremental exercise test). Further study is also needed with regard to responsiveness of the signals and reproducibility of findings both before and after different pharmacological and non-pharmacological interventions that are supposed to reduce respiratory effort and symptoms of breathlessness.

CONCLUSION

The semi-automated ECG artifact removal method for EMGdi analyses will be helpful to eliminate sources of inter-rater variability that were observed between different raters applying the manual method. Therefore the semi-automated method

offers a more objective approach for analyzing EMGdi data while at the same time requiring significantly less analyzing time. We propose this method as a new standard for objective EMGdi amplitude analyses in the future.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The Ethical Committee Research of KU Leuven/UZ Leuven, Belgium approved the study (S58513).

AUTHOR CONTRIBUTIONS

SD, RG, and DL contributed to the conception and design of the study. SD, ZL, and LoJ contributed to the acquisition. LuJ developed the analysis algorithm. SD and AR performed the data analysis. SD organized the database, performed the statistical analysis, and wrote the first draft of the manuscript. LuJ, AR, RG, and DL wrote sections of the manuscript. All authors contributed to the manuscript revision, and read and approved the submitted version.

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Short-Term Water- and Land-Based Exercise Training Comparably Improve Exercise Capacity and Vascular Function in Patients After a Recent Coronary Event: A Pilot Randomized Controlled Trial

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Background: We hypothesized that a 2-week twice daily aquatic endurance *plus* calisthenics exercise training program: (i) increases aerobic exercise capacity (peak oxygen uptake/$\dot{V}O_2$peak), (ii) improves endothelium-dependent flow-mediated vasodilation (FMD), and (iii) reduces circulating markers of low-grade inflammation and hemostasis, as compared to land-based endurance *plus* calisthenics exercise training or no exercise in patients undergoing short-term residential cardiac rehabilitation after a recent coronary artery disease (CAD) event.

Methods: Patients with a recent myocardial infarction or revascularization procedure were randomized into two interventional groups and a control group. The interventional groups underwent supervised aerobic endurance *plus* calisthenics exercise training either in thermo-neutral water or on land at moderate intensity (60–80% of the peak heart rate achieved during symptom-limited graded exercise testing) for 30 min twice daily for 2 weeks (i.e., 24 sessions). The control group was deferred from supervised exercise training for the 2-week duration of the intervention, but was advised low-to-moderate intensity physical activity at home while waiting. At baseline and after the intervention period, all participants underwent estimation of aerobic exercise capacity, brachial artery flow-mediated dilatation (FMD, measured ultrasonographically at rest and during reactive hyperemia after 4.5 min of forearm cuff inflation), markers of cardiac dysfunction (NT-proBNP), inflammation (hsCRP, IL-6, IL-8, IL-10), cell adhesion (ICAM, P-selectin), and hemostasis (fibrinogen, D-dimer).

Results: A total of 89 patients (mean age 59.9 ± 8.2 years, 77.5% males, $\dot{V}O_2$peak at baseline 14.8 ± 3.5 ml kg^{-1} min^{-1}) completed the study. Both exercise modalities were safe (no significant adverse events recorded) and associated with a significant improvement in $\dot{V}O_2$peak as compared to controls: age and baseline $\dot{V}O_2$peak-adjusted end-of-study $\dot{V}O_2$peak increased to 16.7 (95% CI 16.0–17.4) ml kg^{-1} min^{-1} with land-based training ($p < 0.001$ for change from baseline) and to

18.6 (95% CI 17.9–19.3) ml kg^{-1} min^{-1} with water-based training ($p < 0.001$ for change from baseline), but not in controls (14.9 ml kg^{-1} min^{-1}; 95% CI 14.2–15.6; $p = 0.775$ for change from baseline). FMD also increased in both intervention groups (from 5.5 to 8.8%, $p < 0.001$ with land-based, and from 7.2 to 9.2%, $p < 0.001$ with water-based training, respectively), as compared to controls (p for change 0.629). No significant changes were detected in biomarkers of inflammation, cell adhesion or hemostasis, whereas levels of NT-proBNP (marker of cardiac dysfunction) decreased in the water-based training group ($p = 0.07$ vs. controls).

Conclusion: Endurance *plus* calisthenics exercise training in thermo-neutral water is safe, and improves aerobic exercise capacity and vascular function in patients undergoing short-term residential cardiac rehabilitation after a recent CAD event.

Clinical Trial Registration: www.ClinicalTrials.gov, identifier NCT02831829.

Keywords: coronary artery disease, cardiac rehabilitation, aquatic exercise, myocardial infarction, exercise training

INTRODUCTION

Exercise-based cardiac rehabilitation remains a cornerstone of management and secondary prevention in patients with coronary artery disease (CAD) (Piepoli et al., 2010; Anderson et al., 2016). Acute coronary events – such as a recent myocardial infarction and/or coronary artery bypass grafting (CABG) procedure – may impair the ability of individuals to engage in exercise because of cardiac dysfunction, risks associated with the acute effects of exercise, post-procedure recovery, or immediate post-event psychological concerns (Anderson et al., 2016). In this respect, cardiac rehabilitation – either in outpatient settings or as an intensive short-term residential program – provides sufficient monitoring and reassurance to patients in the immediate aftermath of a recent CAD event, thus empowering them to confidently adopt long-term regular exercise and a healthy lifestyle (Mampuya, 2012; Menezes et al., 2014).

Aerobic exercise training on land (such as cycling, walking, jogging, or rowing) – either alone, or supplemented by non-weight bearing exercises (calisthenics) or low-weight resistance training – has been the most studied and therefore the most widely implemented (Bjarnason-Wehrens et al., 2010) exercise modality in cardiac rehabilitation programs. On the one hand, this exercise modality is safe in CAD patients, purportedly because it provides a regulated cardiac output increase to meet the perfusion demand of large exercising muscle groups, thus minimizing safety concerns over raised pre- and after-load in high-risk cardiac patients with low aerobic exercise capacity – such as those after a recent myocardial infarction or revascularization procedure (Balady et al., 2007). On the other hand, this exercise modality has been shown to mitigate risk factors and metabolic abnormalities (Casillas et al., 2007; Ismail et al., 2013), which contribute to CAD and its progression, as well as to improve endothelial dysfunction, which plays a central role in all stages of atherosclerosis. In patients with CAD, endothelium-dependent vascular dysfunction may be associated with impaired systemic (skeletal muscle) and coronary (myocardial) perfusion, resulting in exercise intolerance and myocardial ischemia at exertion, respectively (Bruning and Sturek, 2015). Moreover, endothelial dysfunction in CAD is associated with low-grade inflammation and increased monocyte adhesion, which promote atherosclerotic plaque build-up and rupture, as well as with increased hemostatic activity, which promotes coronary thrombosis (Kokkinos and Myers, 2010; Winzer et al., 2018). Conversely, land-based exercise training has been shown to revert or reduce these abnormalities. In a seminal study by Hambrecht et al. (2000), 4 weeks of in−hospital bicycle ergometer training improved endothelium−dependent arterial vasomotion in patients with CAD, likely through restoring the balance between synthesis and depletion of vasoactive and vasoprotective nitric oxide (NO) (Hambrecht et al., 2003). Mechanistically, this is largely due to a response to exercise-induced shear stress (Kokkinos and Myers, 2010; Bruning and Sturek, 2015). In addition, regular aerobic exercise training on land is associated with a reduction in low-grade inflammation [as assessed by high-sensitive C-reactive protein (hsCRP) levels], cell adhesion (as assessed by cell adhesion molecules, such as ICAM and P-selectin) and hemostatic activity (as assessed by markers of coagulation and fibrinolysis, such as fibrinogen and D-dimer) (Womack et al., 2003; Pedersen, 2017), suggesting an interplay between the effects of aerobic exercise on the endothelium, low-grade inflammation, cell adhesion, and hemostasis in patients with CAD (Kokkinos and Myers, 2010; Winzer et al., 2018).

In contrast to exercise on land, the implementation of aquatic exercises in cardiac rehabilitation programs remains debated (Lazar et al., 2013). Concerns have been traditionally raised over possible adverse cardiovascular effects of exercising in water – namely, an increased preload due to water immersion (i.e., hydrostatically driven raise in venous return and central venous pressure, possibly yielding ventricular dysfunction) (Meyer, 2006; Pendergast et al., 2015; Shah et al., 2017) and/or an increased afterload due to temperatures below thermo-neutrality (i.e., vasoconstriction in cold water associated with a risk of dysrhythmias) (Schmid et al., 2009). On the other hand,

the very same hemodynamic effects of thermo-neutral water immersion in patients with CAD have been associated with favorable improvements in cardiac performance and peripheral vascular reactivity after as little as 3 weeks of aquatic cardiac rehabilitation, suggesting pronounced exercise-induced shear stress as a possible mechanism (Mourot et al., 2010). Yet, previous research on the impact of aquatic exercise in patients with CAD is limited when compared with land-based modalities (Schmid et al., 2007, 2009; Volaklis et al., 2007; Laurent et al., 2009; Teffaha et al., 2011; Choi et al., 2015), and marred by small number of participants, selective patient inclusion [limited to stable CAD (Volaklis et al., 2007; Teffaha et al., 2011) or to patients achieving > 7 METs at exercise testing, (Tokmakidis et al., 2008) thus not reflecting the patient populations referred for cardiac rehabilitation in the immediate aftermath of a CAD event], and inferior study design [e.g., pre-post studies without comparator groups (Korzeniowska-Kubacka et al., 2016) or non-randomized patient separation] (Tokmakidis et al., 2008), with four notable exceptions. Volaklis et al., 2007 randomized 24 patients with stable CAD to 16 weeks of either water cycling *plus* water games, land cycling *plus* resistance training, or no exercise at all, and showed that water- and land-based protocols comparably improved exercise test time, muscle strength and blood lipid profiles. Conversely, Lee et al. (2017) randomized 60 older (>65 years old) patients with CAD and osteoarthritis to 24 weeks of either aqua walking or treadmill walking, and showed that both protocols comparably improved aerobic exercise capacity, but the improvements in body composition and lipid levels were significantly more pronounced with aqua walking. Teffaha et al. (2011) randomized 24 patients with CAD and 24 patients with heart failure to 3 weeks of cardiac rehabilitation, comprising land cycling *plus* calisthenics either on land or in water; both protocols were associated with significant increase in aerobic exercise capacity in patients with CAD. Similarly, Laurent et al. (2009) randomized 24 patients with CAD and 24 patients with heart failure to 3 weeks of cardiac rehabilitation, comprising land cycling *plus* gymnastics either on land or in water; both protocols improved aerobic exercise capacity in patients with CAD, but only water training was associated with increased levels of NO metabolites after the intervention period – indirectly suggesting an improvement in endothelial function with aquatic exercise. Given the specific hemodynamic responses to exercising in xiphoid-level water, with increased peripheral blood flow and enhanced shear stress yielding endothelial nitric oxide synthase up-regulation (Di Francescomarino et al., 2009; Ayme et al., 2014), improvements in endothelium-dependent vascular function may be hypothesized, and have indeed been confirmed with aquatic exercise in prehypertensive adults (Nualnim et al., 2012) and in patients with osteoarthritis (Alkatan et al., 2016), but not in patients with CAD. Thus, while several studies confirmed the relative cardiovascular safety of thermo-neutral water immersion in patients with CAD, only a limited number of trials assessed the efficacy of aquatic training modalities on aerobic exercise capacity, and none appraised the effect of such training on vascular function or explored its interplay with low-grade inflammation and hemostasis.

Therefore, we sought to compare the effect of a land- and a water-based exercise training program on aerobic exercise capacity and vascular function. In addition, we assumed that the improvements in endothelium-dependent vascular function would be accompanied by a reduction in markers of low-grade inflammation, endothelial adhesion and coagulation, given the association between inflammation and endothelial dysfunction, and the central role of endothelial integrity in promoting cell adhesion and coagulation. Hence, we hypothesized that a 2-week, twice-daily aquatic endurance *plus* calisthenics exercise training program would (i) increase aerobic exercise capacity (peak oxygen uptake/$\dot{V}O_2$peak), (ii) improve endothelium-dependent flow-mediated vasodilation (FMD), and (iii) reduce markers of low-grade inflammation, cell adhesion, and hemostasis as compared to land-based endurance *plus* calisthenics exercise training and to no exercise in patients undergoing short-term residential cardiac rehabilitation after a recent CAD event.

MATERIALS AND METHODS

Study Design, Setting, and Patients

The study was designed as a prospective, randomized, open-label clinical trial with three parallel groups (two intervention groups and one control group) (**Figure 1**).

The study was carried out at the Centre for Cardiac Rehabilitation in Šmarješke Toplice, Slovenia. The center provides residential cardiac rehabilitation for patients after a myocardial infarction or open-heart surgery, with a live-in 14-day program encompassing twice-daily supervised exercise training sessions, education, dietary and smoking cessation advice, medical supervision, and psychological counseling for individuals without access to outpatient rehabilitation services.

Patients after a recent CAD event [2–4 weeks after myocardial infarction, percutaneous coronary intervention (PCI) and/or coronary artery by-pass surgery (CABG)] with a left ventricular ejection fraction (LVEF) above 40% were invited to participate. Recruitment took place between May and October 2016. Patients with uncontrolled/decompensated valve diseases necessitating specific (surgical or percutaneous) management, patients after valve replacement, with uncontrolled dysrhythmias or presence of a permanent pacemaker, with contraindications to exercise, unable to perform exercise testing or to swim, with mental impairment, severe anemia, severe obstructive/restrictive lung disease, recent thromboembolic events, hepatic dysfunction, and/or age over 80 years were excluded.

Patients were randomized into (a) a water-based exercise training group, (b) a land-based exercise training group, or (c) a control group, using adaptive urn-randomization with sealed envelopes and allocation concealment from the recruiting investigator.

Before and after the intervention period (on Day 0 and Day 14), all participants underwent cardiopulmonary exercise testing, ultrasonographic assessment of FMD of the brachial artery, and blood sample collection. The primary outcomes were change from baseline of $\dot{V}O_2$peak and FMD. Exploratory

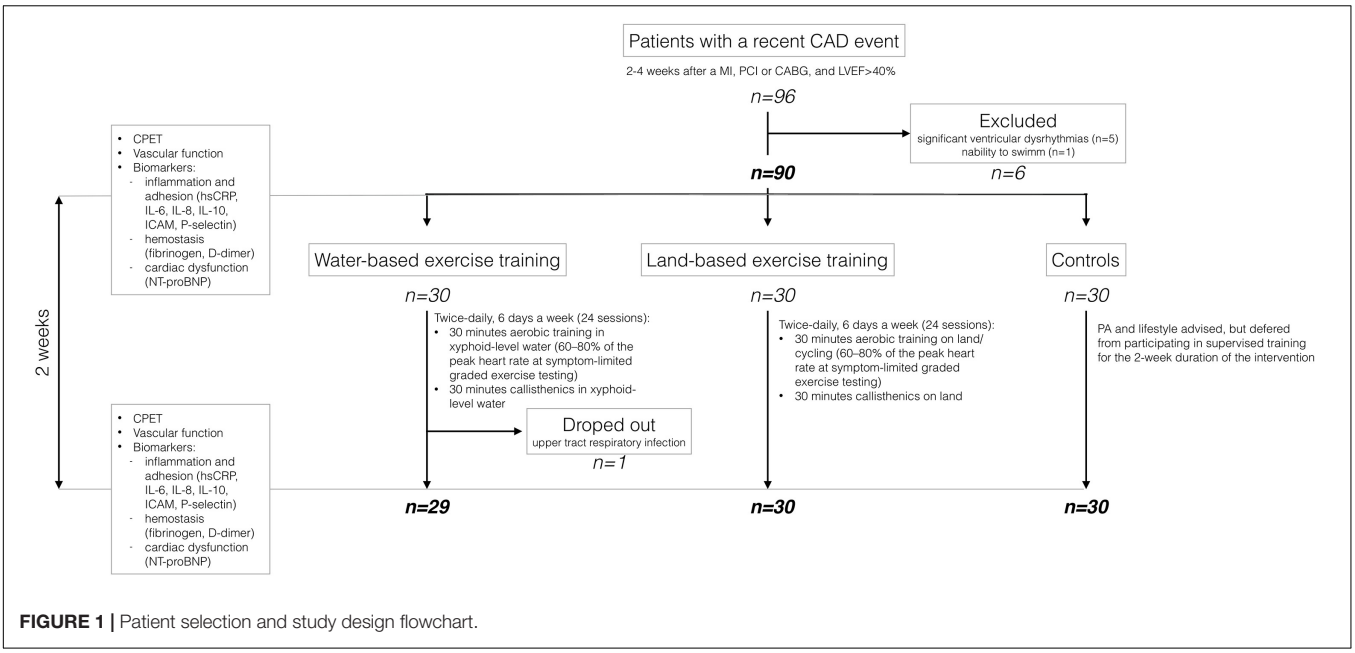

FIGURE 1 | Patient selection and study design flowchart.

outcomes included change from baseline of biomarkers of low-grade inflammation (hsCRP, IL6, IL8, IL10) and endothelial activation (ICAM, P-selectin), hemostasis (D-dimer, fibrinogen), and neurohormonal activity (NT-proBNP).

Written informed consent was obtained for each participant. The study complied with the World Medical Association Declaration of Helsinki on ethics in medical research and was approved by a local medical research ethics committee (0120-655/2016-2). This study is registered at ClinicalTrials.gov, number NCT02831829.

Intervention

The intervention was designed as either a water- or a land-based endurance *plus* calisthenics exercise program during 2-week residential cardiac rehabilitation. The exercise programs consisted of 30-min training sessions twice daily, 6 days a week (24 sessions in total).

Other aspects of rehabilitation (including lifestyle education and provision of a Mediterranean-style diet, medical supervision, psychological support) were identical for both intervention groups. The principles of treatment were not changed over the intervention period, but medication adjustment was allowed at the discretion of the treating cardiologist to ensure optimal control of risk factors.

Water-based exercise training comprised two daily training sessions in a heated swimming pool (32.8°C), with water depth at the xiphoid process level (1.5 m). The exercise program consisted of two 30-min sessions daily, namely aerobic endurance and calisthenics. *Aerobic endurance exercise* comprised 5 min of warm-up, 20 min of conditioning (water walking, side-stepping, cycling with arms) at 60–80% peak heart rate achieved during symptom limited graded exercise testing, and 5 min of cool-down. *Calisthenics* comprised 5 min of warm-up, 20 min of conditioning (engaging muscle groups of the upper and lower limbs, such as triceps extensions, triceps dips, modified leg press, leg abduction/adduction, wall push-ups at 60–80% peak heart rate), and 5 min of *cool-down.*

Land-based exercise training comprised two 30-min sessions daily, namely bicycle ergometer training (5 min warm-up, 20 min at 60–80% peak heart rate, and 5 min of *cool-down*) and calisthenics (5 min of *warm-up,* 20 min of exercises engaging muscle groups of the upper and lower limbs at 60–80% peak heart rate with a progressive increase in speed and the number of repetitions), and *cool-down*.

Patients in the control group were given lifestyle advice, and made aware of the beneficial effects of exercise and advised to engage in regular physical activities (i.e., usual daily activities, such as walking), but were asked to refrain from enrolling in a supervised exercise program for the duration of the intervention period (i.e., 2 weeks).

Assessment of Aerobic Exercise Capacity

Aerobic exercise capacity was determined by measuring $\dot{V}O_2$peak by cardiopulmonary bicycle exercise testing (CPET) using the cycle-ergometer Schiller CS-200 (Schiller A.G. Baar, Switzerland) with the Ganshorn Power Cube gas analysis unit (Ganshorn Deutschland GmbH). Calibration of primary sensors for flow, O_2 and CO_2 gas measurement were performed before each exercise test. All the participants underwent a symptom-limited exercise test. They were advised to adhere to normal medical regimes, avoid exercise and heavy meals on the day of testing. Resting data including ECG were monitored 3 min before starting the test. Participants were tested using a maximal incremental protocol: after 3 min of unloaded cycling ("0 W"), the work rate was continuously increased on the computer-controlled cycle ergometer in a ramp-like fashion to achieve the predicted maximal workload after 10 min. Predicted maximal workload

on the bicycle ergometer was estimated based on age, gender, and body surface area. The test was considered completed if the respiratory exchange ratio achieved was ≥1.1. During exercise, participants wore a mouthpiece connected with the gas analysis unit, thus measuring oxygen and carbon dioxide flow ($\dot{V}O_2$ and $\dot{V}CO_2$, respectively). ECG and heart rate were continuously monitored, and records were made every 2 min. Blood pressure was measured at rest and every 2 min during the test and cool-down period. Monitoring of the participants and the mentioned parameters continued for 6 min after test termination. There were no clinically relevant adverse effects during the exercise testing. To assess the reproducibility of exercise testing, 10 subjects were selected randomly and tested twice before the intervention started. The intra-class correlation coefficient (ICC for single measure) for $\dot{V}O_2$peak was 0.861, $p = 0.004$.

Assessment of Endothelial Function

Endothelial function was assessed by flow-mediated dilatation (FMD) of the right brachial artery with ultrasound scanning (Philips ultrasound system iE 33 with a high resolution linear-array vascular probe with a frequency of 10 MHz), under standardized conditions and in accordance with current recommendations (Flammer et al., 2012). The brachial artery was imaged 2–10 cm above the elbow fossa. To determine the endothelium-dependent vasodilatation, the forearm was tightened with the sphygmomanometer cuff until a pressure of 50 mmHg higher than the systolic pressure value was achieved. The grip was released after 4.5 min. Flow was measured within 15–20 s, and artery diameter 60–90 s after releasing the grip. After 15 min of rest, endothelium-independent vasodilatation was measured, induced by 0.4 mg of nitroglycerin (Nitrolingual spray®) upon sublingual spray application. The diameter of the brachial artery and the average velocity of blood flow were measured 3–4 min after dosing. FMD was expressed as percentage change from rest [(brachial artery diameter at peak hyperemia – diameter at rest) × 100/diameter at rest]. To assess the reproducibility of FMD, 10 subjects were selected randomly. The intra-class correlation coefficient for FMD was 0.855, $p = 0.004$.

Blood Markers

All patients had venous blood samples taken in the fasting state, in the morning, after 30 min of rest in the supine position, from the cubital vein into 4.5 mL vacuum tubes containing 0.11 mol/L sodium citrate (Becton Dickinson, Vacutainer System Europe, Germany). Plasma was prepared within 30 min with 20-min centrifugation at 2,000 × *g*. In fresh plasma, the concentrations of fibrinogen (Dade® Thrombin Reagent) and D-dimer (Innovance D-dimer, both Siemens Healthcare Diagnostics, Marburg, Germany) were determined on an automated coagulation analyzer CS2100i (Sysmex, Kobe, Japan). The remaining plasma was aliquoted, snap frozen in liquid nitrogen and stored at –75°C until analysis. In thawed plasma, NT-proBNP was determined on a Stratus® CS Acute Care™ analyzer based upon solid phase Radial Partition Immunoassay (RPIA) technology (Siemens Healthcare Diagnostics, Marburg, Germany). Plasma CRP, ICAM-1, IL-6, IL-8, IL-10, and P-selectin were measured with the xMAP® Technology utilizing magnetic beads coupled with specific antibodies (all R&D Systems, Minneapolis, United States) on a MagPix instrument (Luminex Corporation, Austin, United States).

Statistical Analysis

Data are presented as mean (standard deviation) for normally distributed continuous variables and as median (interquartile range) otherwise. Differences in the baseline characteristics of patients between groups were tested by ANOVA or Kruskal–Wallis test, as appropriate. Differences in the primary objective, end-of-study VO_2 max, between study groups were tested using ANCOVA, controlling for baseline VO_2 max and age of patients. ANCOVA with age and baseline measurement included as a covariate was used for all other normally distributed variables. *Post hoc* differences were tested by Sidak test. Variables, measured in percentages (FMD and NMD) were logarithmized prior the analysis. For the logarithmized FMD, the assumption of homogeneity of regression slopes was violated, therefore, between and within group ANOVA with age as a covariate was used to test the between-group differences. In all other non-normally distributed variables, the change from baseline for each patient was calculated and Kruskal–Wallis test was used to test the differences in change from baseline between the study groups. When statistically significant differences between groups were found, Mann–Whitney *U* test was used to test pairwise differences. The differences with $p < 0.05$ were treated as statistically significant. All analyses were done using the software SPSS, v. 21.

Sample size calculation suggests an 80% power at 0.05 significance level for the detection of a between-group difference (and assumed between-subject standard deviation) of 1 MET (3.5 ml kg^{-1} min^{-1}) with the inclusion of 90 patients (30 per group).

The differences with $p < 0.05$ were treated as statistically significant. All analyses were done using the software SPSS, v. 21.

RESULTS

Baseline Characteristics

Eighty-nine patients completed the study: 30 patients in the land-based training group, 29 in the water-based training group, and 30 in the control group; one participant dropped out during the course of the study due to upper respiratory tract infection (**Figure 1**). Mean age of the participants who completed the study was 59.9 ± 8.2, and 77.5% were male. All the participants had CAD, and 77 (86.5%) had suffered a myocardial infarction. Patients randomized to the land-based group were significantly older and achieved a lower $\dot{V}O_2$ peak at baseline (**Table 1**).

Aerobic Exercise Capacity

Both exercise modalities were associated with a statistically significant increase in $\dot{V}O_2$peak as compared with controls

TABLE 1 | Baseline characteristic of the patients who completed the study.

Variables	Patients who completed the study (*n* = 89)	Land-exercise group (I) (*n* = 30; 33.7%)	Water-exercise group (II) (*n* = 29; 32.6%)	Control group (III) (*n* = 30; 33.7%)	*P**
Age Mean(SD), years	59.9 (8.2)	62.4 (7.6)	56.7 (8.4)	60.6 (8.3)	0.026 (I–II)
Gender m/f, *n* (%)	69 (77.5)/20 (2.5)	21 (70)/9 (30)	24 (82.8)/5 (17.2)	24 (80)/6 (20)	0.464
					0.523
MI + PCI *n* (%)	60 (67.4)	19 (63.3)	17 (58.6)	24 (80)	
PCI *n* (%)	3 (3.4)	1 (3.3)	2 (6.9)	0 (0)	
CABG *n* (%)	9 (10.1)	3 (10)	3 (10.3)	3 (10)	
MI + CABG *n* (%)	17 (19.1)	7 (23.3)	7 (24.1)	3 (19)	
HBP *n* (%)	52 (58.4)	18 (60)	14 (48.3)	20 (66.7)	0.350
Dyslipidemia *n* (%)	65 (73)	19 (63.3)	22 (75.9)	24 (80)	0.318
Family history *n* (%)	53 (59.6)	19 (63.3)	18 (62.1)	16 (53.3)	0.692
Obesity *n* (%)	20 (22.5)	6 (20)	7 (24.1)	7 (23.3)	0.921
DM *n* (%)	15 (16.9)	3 (10)	5 (17.2)	7 (23.3)	0.385
Low physical activity level *n* (%)	44 (49.4)	17 (56.7)	15 (51.7)	12 (40)	0.415
Smoking status *n* (%)	48(53.9)	15 (59)	16 (55.2)	17 (53.9)	0.863
Aspirin *n* (%)	86 (96.6)	29 (96.7)	29 (100)	28 (93.3)	0.366
β blockers *n* (%)	76 (85.4)	26 (86.7)	24 (82.8)	26 (86.7)	0.887
Statins *n* (%)	79 (88.8)	26 (86.7)	26 (89.7)	27 (90)	0.906
ACE/ARB *n* (%)	72 (80.9)	24 (80)	22 (75.9)	26 (86.7)	0.566

*SD, standard deviation; IQR, interquartile range; CI, confidence interval; CAD, coronary artery disease; MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery by-pass graft; HBP, high blood pressure; DM, diabetes mellitus; ACE, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blockers. *Annotations for groups that differ as found by post hoc tests are provided in the brackets.*

(**Figure 2**). After controlling for baseline $\dot{V}O_2$peak and patients' age (ANCOVA), mean estimate end-of-study $\dot{V}O_2$peak increased by 15.3% (16.7 ml kg^{-1} min^{-1}; 95% CI 16.0–17.4 ml kg^{-1} min^{-1}; $p < 0.001$ for change from baseline) with land-based training, and by 27.4% (18.6 ml kg^{-1} min^{-1}; 95% CI 17.9–19.3 ml kg^{-1} min^{-1}; $p < 0.001$ for change from baseline) with water-based training, but not in controls (a 0.6% increase, i.e., 14.9 ml kg^{-1} min^{-1}, 95% CI 14.2–15.6; 14.9 ml kg^{-1} min^{-1}; $p = 0.775$ for change from baseline). The effect size (d) was moderate in the land-based group ($d = 0.61$), and large in the water-based group ($d = 1.02$). Time-to-exhaustion and peak workload also increased significantly in both intervention groups compared to controls (**Table 2**).

Vascular Function

End-of-study FMD increased from 5.5 to 8.8% ($p < 0.001$) in the land-based training group, and from 7.2 to 9.2% ($p < 0.001$) in the water-based training group; no significant change was observed in the control group ($p = 0.629$). NMD increased in both intervention groups (with larger increments in the land-based exercise group); the increase in the intervention groups was statistically significant in comparison to controls ($p < 0.001$). See **Table 2** and **Figure 2**.

Biomarkers

No significant changes were detected in biomarkers of low-grade inflammation, whereas levels of NT-proBNP and D-dimer decreased in the water-based training group ($p = 0.01$ for both; **Table 2**).

DISCUSSION

Supervised short-term exercise training – either water-based or land-based – is safe, and improves aerobic exercise capacity and vascular function in patients with CAD. Despite concerns about the safety and effectiveness of aquatic exercise in patients after a recent CAD event, our study in patients undergoing residential cardiac rehabilitation demonstrated that a 2-week, twice-daily water-based training program was not associated with adverse cardiovascular events, and improved aerobic exercise capacity (as determined by $\dot{V}O_2$peak) and endothelial function (as determined by FMD). To our knowledge, this is the largest study of water- vs. land-based training in patients after a recent CAD event, and the first to address vascular function in this context. Our results contribute to the growing body of evidence on the safety and effectiveness of aquatic exercise in cardiovascular patients, and suggest that aquatic exercise modalities may be a suitable option for cardiac rehabilitation of selected patients after a recent CAD event, such as those with concomitant musculoskeletal conditions, frailty or at risk of falls, or for those who might prefer aquatic exercise.

Aerobic Exercise Capacity

In our study, both land- and water-based training were associated with a significant increase in exercise capacity ($\dot{V}O_2$peak, time-to-exhaustion, and peak workload) as compared to controls. The magnitude of baseline-adjusted $\dot{V}O_2$peak increase – in the range of 2–4 ml kg^{-1} min^{-1}, roughly corresponding to 1 MET – was comparable in the two intervention groups, and represents a clinically significant achievement (Conraads et al., 2015). $\dot{V}O_2$peak improvements in our study were larger

TABLE 2 | Measurements for the three study groups at baseline and after training, and between group differences.

Variables	Land-exercise group (I)				Water-exercise group (II)				Control group (III)				*P**
	Baseline	After	Δ%	*p*	Baseline	After	Δ%	*p*	Baseline	After	Δ%	*p*	
VO_2 peak ml kg^{-1} min^{-1}	13.1 (2.8)	15.1 (3.2)	15.3	0.001	14.6 (3.3)	18.6 (3.9)	27.4	0.001	16.6 (3.6)	16.5 (3.8)	−0.6	0.775	0.001
VO_2 (L min^{-1})	1.07 (0.27)	1.25 (0.33)	16.8	<0.001	1.31 (0.34)	1.67 (0.41)	27.4	<0.001	1.47 (0.38)	1.45 (0.40)	−1.3	0.265	<0.001 (all)
TTE (sec)	605 (91)	710 (128)	17.3	<0.001	710 (123)	864 (158)	21.6	<0.001	796 (150)	800 (145)	0.5	0.493	<0.001 (all)
RER	1.10 (1.10–1.10)	1.12 (1.1–1.16)	1.8	0.001	1.10 (1.10–1.11)	1.15 (1.10–1.19)	4.5	0.001	1.10 (1.10–1.11)	1.10 (1.10–1.13)	0.0	0.026	0.026 (II vs. III)
Peak workload (Watt)	70 (15)	88 (21)	25.7	<0.001	88 (21)	113 (26)	28.4	<0.001	103 (25)	104 (24)	0.9	0.755	<0.001 (all)
FMD (%)	5.5 (3.1–8.6)	8.8 (5.3–11.4)	37.7	<0.001	7.2(4.0–8.7)	9.2 (7.4–12.9)	44.9	<0.001	7.0 (3.7–8.6)	6.4 (3.7–8.5)	−1.5	0.692	<0.001 (I–III, II–III)
NMD (%)	10.6 (7.0–13.3)	11.1 (9.0–14.3)	11.6	<0.001	10.5 (8.5–14.0)	11.4 (7.6–13.4)	5.4	0.042	10.9 (6.0–13.4)	11.6 (7.4–14.2)	2.9	0.579	0.013 (I–III)
Fibrinogen g L^{-1}	3.0 (3.0–3.4)	3.2 (3.0–3.5)	6.7	0.875	3.3 (3.0–3.9)	3.0 (2.7–3.8)	−9.1	0.248	3.0 (2.7–3.4)	3.0 (2.6–3.3)	0.0	0.791	0.565
D dimer μg L^{-1}	625 (280–1220)	465 (2701070)	−25.6	0.066	400 (270–810)	370 (260–590)	−7.5	0.001	310 (200–510)	270 (200–380)	−12.9	0.129	0.099
CRP mg/L	0.7 (0.4–1.7)	0.6 (0.4–2.0)	−9.1	0.734	0.9 (0.6–1.8)	0.5 (0.3–1.0)	−50.0	0.133	0.5 (0.4–1.3)	0.5 (0.3–2.0)	10.2	0.829	0.256
IL6 ng L^{-1}	10.1 (7.5–11.7)	10.9 (8.5–12.4)	7.9	0.211	9.6 (7.5–11.3)	9.3 (7.2–11.0)	−3.1	0.380	7.5 (6.9–9.6)	7.4 (6.2–9.9)	−1.3	0.041	0.090
IL8 ng L^{-1}	19.6 (16.4–21.3)	18.4 (16.4–22.9)	−6.1	0.755	17.8 (16.6–20.7)	19.2 (16.0–20.5)	7.9	0.927	19.5 (16.6–23.0)	19.0 (16.6−20.1)	−2.6	0.034	0.187
IL10 ng L^{-1}	17.6 (12.7–21.7)	17.2 (12.7–25.7)	−2.3	0.703	16.8 (12.7–25.7)	15.8 (10.6–21.0)	−6.0	0.710	14.7 (12.7–15.8)	14.2 (11.6–16.7)	−3.4	0.038	0.360
ICAM μg L^{-1}	372 (270–506)	362 (275– 567)	−2.7	0.642	523 (362–873)	514 (362–801)	−1.7	0.554	662 (507–806)	667 (520–787)	−0.8	0.325	0.515
Selectin mg L^{-1}	27.0 (6.8)	27.9 (7.9)	3.3	0.140	25.4 (4.9)	25.1 (4.9)	−1.2	0.626	27.8 (5.6)	26.9 (5.9)	−4.3	0.101	0.060
NT-pro-BNP ng L^{-1}	707 (401–1604)	669 (335–1360)	−5.4	0.074	396 (296–541)	Δ308 (187–394)	−1.2	0.001	111 (66–221)	111 (52–297)	−4.3	0.681	0.007 II–III
BMI kg m^{-2} median (IQR)	29 (26.5–31.6)	28.9 (26.9–30.7)	−0.3	0.104	30 (27.1–32.6)	29.1 (27.0–31.5)	−3.0	0.001	29.3 (26.8–30.9)	29.2 (26.8−32.0)	−0.3	0.144	0.082

Data are shown as mean and standard deviation (SD) or median and 25th percentile – 75th percentile. TTE, time-to-exhaustion; RER, respiratory exchange ratio; FMD, flow-mediated dilatation; NMD, nitroglycerin-mediated dilatation; CRP, C-reactive protein; IL, interleukin; ICAM, intercellular adhesion molecule; NT-proBNP, N-terminal prohormone B-type natriuretic peptide; BMI, body mass index. p, p between groups (ANCOVA).*

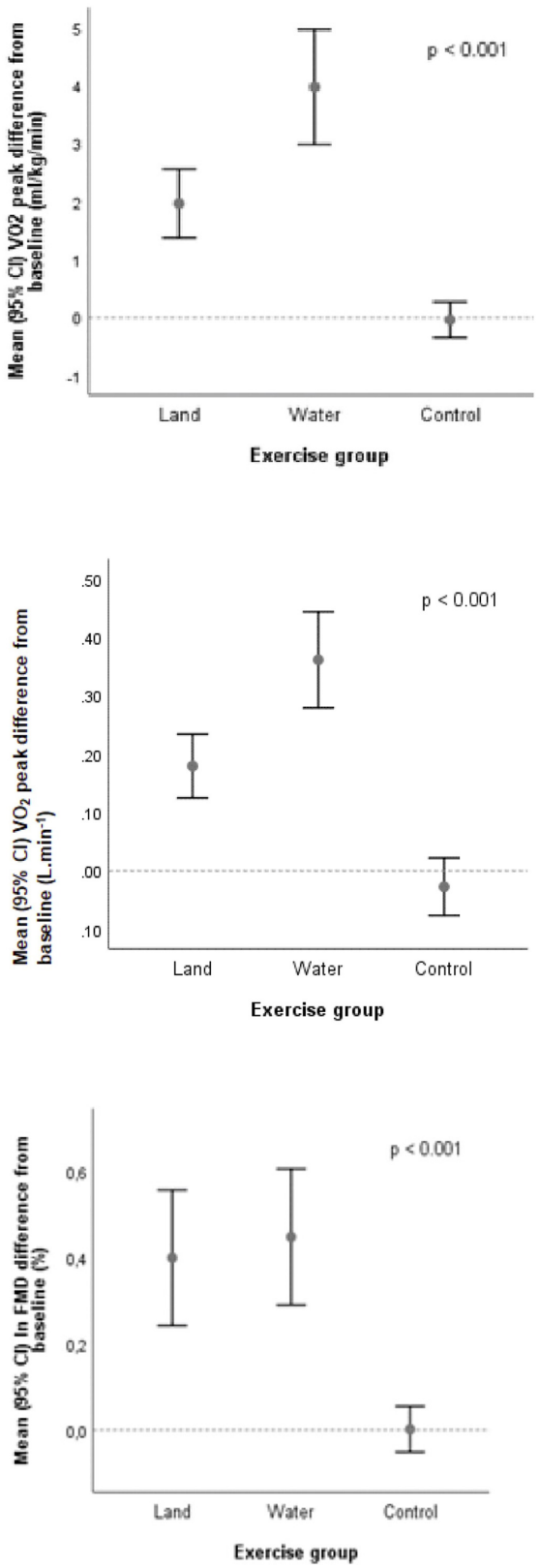

FIGURE 2 | Changes in aerobic exercise capacity ($\dot{V}O_2$peak) and flow-mediated dilatation (FMD) expressed on the logarithmic scale.

than those reported in previous randomized trials of short-term (3 weeks) aquatic rehabilitation (in the range of 2.0–2.4 ml kg^{-1} min^{-1}) (Laurent et al., 2009; Teffaha et al., 2011). Both Laurent et al. (2009) and Teffaha et al. (2011), however, enrolled more stable and younger CAD patients (mean age 52 and 54 years, respectively), which was reflected in higher baseline $\dot{V}O_2$peak (20 and 22 ml kg^{-1} min^{-1}, respectively) and therefore possibly disposed the study population to diminishing returns in aerobic exercise capacity improvements. In fact, the end-of-study $\dot{V}O_2$peak in our population (16.7 and 18.4 ml kg^{-1} min^{-1}, respectively) was still lower than that reported in studies with CAD patients (Casillas et al., 2007; Winzer et al., 2018), but nonetheless higher when compared to studies of water-based training in patients with chronic heart failure. This likely reflects the specifics of our patient population (i.e., after a recent myocardial infarction and/or revascularization procedure in the risk spectrum between stable CAD at one end, and clinically manifest cardiac dysfunction on the other); of note, our patient population represents the population of CAD patients traditionally referred for cardiac rehabilitation.

End-of-study $\dot{V}O_2$peak in our trial was also significantly higher in the aquatic exercise group ($p < 0.001$ after co-variance analysis adjusting for age and baseline capacity). Larger improvements with water- vs. land-based exercise training in CAD were observed previously (Teffaha et al., 2011; Lee et al., 2017). However, the magnitude of difference (2 ml kg^{-1} min^{-1}) was larger in our study when compared to previous reports (1 ml kg^{-1} min^{-1}) (Mourot et al., 2010; Teffaha et al., 2011), but the confidence intervals for our estimations were large and the study was underpowered to provide a definite conclusion as to whether differences in end-of-study $\dot{V}O_2$peak between aquatic and land-based training are indeed relevant. Methodologically, the difference favoring water-based over land-based exercise in our study may still be attributable to randomization failure (with patients in the land-based group being older and having lower baseline $\dot{V}O_2$peak). Alternatively, larger improvements may derive from either the specific physiology of water immersion or the higher intensity of aquatic exercise. In terms of specific physiology, water immersion is linked with hemodynamic and peripheral responses associated with improved myocardial efficiency and endurance (DiCarlo et al., 1991; Tei et al., 1995; Mourot et al., 2010). Previous studies (Teffaha et al., 2011; Lee et al., 2017) compared water- vs. land-based calisthenics on top of land-based endurance training (cycling), whereas our aquatic training protocol (both endurance and calisthenics) was entirely carried out in xiphoid-level water. In terms of the intensity, exercise prescription was based on peak heart rate achieved during symptom-limited graded exercise testing on a land bicycle ergometer. On the one hand, the hypothesized intensity may not be directly interchanged between aerobic exercise in water and cycling on ambient air. On the other hand, peak heart rate-derived intensity may not provide a precise measure of metabolic stress in comparison to, for instance, ventilatory thresholds, especially in patients with cardiovascular disease (Carvalho and Mezzani, 2010). The challenges of threshold appraisal in clinical practice, however, partially explain why the majority of

research – including ours – continues to favor the use of peak heart rate to prescribe exercise (Mann et al., 2013). Moreover, our intervention protocol did employ the aquatic heart rate adjustment (i.e., to a maximum rate 13% or 10 bpm lower than in land-based exercise) (Gabrielsen et al., 2000). In this respect, previous research yielded inconclusive results: some studies have corroborated the higher intensity (and thus the rationale for heart rate adjustment) of aquatic exercise (Lee et al., 2017), while others – including research in CAD populations exercising to up to 75% $\dot{V}O_2$peak – have not (Tokmakidis et al., 2008; Laurent et al., 2009; Teffaha et al., 2011). These inconclusive results may be reconciled by addressing the specific protocols of aquatic exercise, suggesting that the level of water may play an important role. In our study, deeper water (xiphoid process level) – which increases buoyancy and decreases resistance (Cider et al., 2003) – may have provided comparable intensity of aquatic exercise, but a significant hydrostatically induced hemodynamic response to immersion.

Vascular Function

Both water- and land-based exercise training improved vascular function (between 2 and 3 absolute percentage change improvements). Similar improvements have been reported with land-based aerobic training (Di Francescomarino et al., 2009), but not yet with aquatic exercise in patients with CAD. FMD – a marker of endothelial function and thus cardiovascular health (Flammer et al., 2012) – increased significantly after 2 weeks of exercise training in both intervention groups. While most previous studies employed exercise programs of longer duration, our intervention was relatively short-term and suggests that an increase in endothelial function can be detected as soon as 2 weeks after exercise training initiation; these results are in line with previous reports in both human trials (Tinken et al., 2008) and animal models (Graham and Rush, 2004). Improved endothelial function – as determined indirectly by increased levels of plasma nitric oxide metabolites – has been reported in water-based exercise training in patients with CAD (Conraads et al., 2015), while FMD increases have been documented in prehypertensive adults (Nualnim et al., 2012) and patients with osteoarthritis undergoing aquatic exercise training (Alkatan et al., 2016). Contrary to our findings, however, Alkatan et al. (2016) – in the only previous study comparing water-based training (swimming) with land-based training (cycling) – reported on larger improvements in endothelial function with the former (4 vs. 1% absolute percentage change improvement). Exercise type (swimming) and duration (8 weeks) may partly explain such differences; alternatively, Alkatan et al. (2016) enrolled cardiovascular disease-free individuals with osteoarthritis, whereas in our CAD population the vascular damage may have been too pronounced for a discernible difference between water- and land-based training to be detected.

Inflammation, Neurohormonal Activity, Hemostasis, and Endothelial Activation

Contrary to the increased aerobic exercise capacity and vascular function, neither inflammation nor endothelial activation markers improved. Atherosclerosis in general, and CAD in particular, are characterized by low-grade inflammation, which may be reduced with regular long-term exercise training (Nimmo et al., 2013). We hypothesized that improvements in endothelium-dependent vascular function would be accompanied by a reduction in the markers of low-grade inflammation, endothelial adhesion and coagulation, given the association between inflammation and endothelial dysfunction, and the central role of endothelial integrity in promoting cell adhesions and coagulation. However, 2 weeks of exercise training may have been too short to achieve such changes; aquatic exercise trials of longer duration in osteoarthritis (Alkatan et al., 2016) have reported improved vascular function and inflammation markers with water-based exercise. Adding to these observations, our study suggests that improvements in FMD – unparalleled by a reduction in the markers of inflammation (interleukins and hsCRP) and inflammation-induced endothelial activation (P-selectin and ICAM) – more likely derive from immediate exercise-induced hemodynamic changes rather than from a reversal in inflammation-caused vascular dysfunction. Alternatively, it is also possible that exercise-induced long-term changes in body mass, composition and metabolism might in the long run reverse low-grade chronic inflammation in cardiovascular disease; however, neither our 2-week exercise program nor longer 12-week trials (Mohammadi et al., 2018) achieved significant changes in the body mass index, and did not appraise potential body composition changes, which may be brought about by aquatic exercise.

Limitations

We have identified several limitations. Firstly, this was a single-center study involving a limited number of patients with a recent CAD event. The results can therefore not be extrapolated to other cardiovascular patients. Secondly, we assessed relevant but surrogate endpoints, and the clinical relevance of our findings should be confirmed in larger clinical trials. Also, our study focused on exercise capacity, vascular function and low-grade inflammation; whilst providing some insight into aquatic exercise in patients with CAD, our findings convey only limited inferences about the potential (patho)physiologic responses to water- vs. land-based exercise training in this patient population. Specific impacts of aquatic exercise in patients with CAD – such as on body composition and metabolism – should therefore be addressed in further studies. Thirdly, baseline between-group differences suggest randomization failure and have required statistical adjustment, which calls for our study to be regarded as pilot and hypothesis-generating. Fourthly, while both intervention groups underwent residential cardiac rehabilitation (controlling for some confounders, such as diet), the control group did not, which may have yielded overestimation of the effect of both interventions as compared to controls. Lastly, we chose a specific type (xiphoid-level endurance *plus* calisthenics training) and duration of exercise (2-week intervention), which can only address immediate physiological responses, but not sustainable effects of regular training.

CONCLUSION

Aquatic exercise is a safe and effective training modality for patients undergoing short-term residential cardiac rehabilitation after a recent CAD event. As compared to land-based exercise, endurance *plus* calisthenics exercise training in thermo-neutral water provides comparable improvements in exercise capacity and vascular function in patients with CAD. Our pilot study therefore represents a starting point for further research into optimal exercise modalities in CAD patients, with water-based training likely emerging as a suitable exercise option.

ETHICS STATEMENT

Patients who corresponded to the inclusion criteria were invited to participate in the study. A written informed consent was obtained for each participant. The study complied with the World Medical Association Declaration of Helsinki on ethics in medical research and was approved by the local medical research ethics committee (0120-655/2016-2).

AUTHOR CONTRIBUTIONS

DV contributed to drafting the work, acqusition, analysis, and interpretation of the data for the work, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. MN and MB substantially contributed to the conception of the work. BB substantially contributed to the design of the work. BJ contributed to the drafting the work, revising it critically for important intellectual content, and provided approval for the final manuscript.

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frontiers in Physiology

REVIEW
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The Physiological Effect of n-3 Polyunsaturated Fatty Acids (n-3 PUFAs) Intake and Exercise on Hemorheology, Microvascular Function, and Physical Performance in Health and Cardiovascular Diseases; Is There an Interaction of Exercise and Dietary n-3 PUFA Intake?

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Physical activity has a beneficial effect on systemic hemodynamics, physical strength, and cardiac function in cardiovascular (CV) patients. Potential beneficial effects of dietary intake of n-3 polyunsaturated fatty acids (n-3 PUFAs), such as α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid on hemorheology, vascular function, inflammation and potential to improve physical performance as well as other CV parameters are currently investigated. Recent meta-analysis suggests no effect of n-3 PUFA supplementation on CV function and outcomes of CV diseases. On the other hand, some studies support beneficial effects of n-3 PUFAs dietary intake on CV and muscular system, as well as on immune responses in healthy and in CV patients. Furthermore, the interaction of exercise and dietary n-3 PUFA intake is understudied. Supplementation of n-3 PUFAs has been shown to have antithrombotic effects (by decreasing blood viscosity, decreasing coagulation factor and PAI-1 levels and platelet aggregation/reactivity, enhancing fibrinolysis, but without effects on erythrocyte deformability). They decrease inflammation by decreasing IL-6, MCP-1, TNFα and hsCRP levels, expression of endothelial cell adhesion molecules

and significantly affect blood composition of fatty acids. Treatment with n-3 PUFAs enhances brachial artery blood flow and conductance during exercise and enhances microvascular post-occlusive hyperemic response in healthy humans, however, the effects are unknown in cardiovascular patients. Supplementation of n-3 PUFAs may improve anaerobic endurance and may modulate oxygen consumption during intense exercise, may increase metabolic capacity, enhance endurance capacity delaying the onset of fatigue, and improving muscle hypertrophy and neuromuscular function in humans and animal models. In addition, n-3 PUFAs have anti-inflammatory and anti-nociceptive effects and may attenuate delayed-onset muscle soreness and muscle stiffness, and preserve joint mobility. On the other hand, effects of n-3 PUFAs were variably observed in men and women and they vary depending on dietary protocol, type of supplementation and type of sports activity undertaken, both in healthy and cardiovascular patients. In this review we will discuss the physiological effects of n-3 PUFA intake and exercise on hemorheology, microvascular function, immunomodulation and inflammation and physical performance in healthy persons and in cardiovascular diseases; elucidating if there is an interaction of exercise and diet.

Keywords: n-3 PUFAs, exercise, cardiovascular, endothelium, inflammation, hemorheology, muscle, microcirculation

INTRODUCTION

It is well-accepted that physical activity has a beneficial effect on systemic hemodynamics, physical strength and cardiac function in cardiovascular (CV) patients (Joyner and Green, 2009; Smith et al., 2011c; Piepoli et al., 2016). The strength of such evidence is evident from the fact that regular physical activity and exercise are accepted as essential components for reducing the severity of CV risk factors and are incorporated in guidelines for primary and secondary cardiovascular disease (CVD) prevention by both European Society of Cardiology and American Heart Association (Smith et al., 2011c; Piepoli et al., 2016). Even though the effects of regular exercise are more difficult to apprehend in secondary than in primary CVDs prevention (studies performed as part of rehabilitation programs), the current position is that mild-to-moderate regular physical activity can be recommended to CVD patients with no exception (Smith et al., 2011c; Piepoli et al., 2016). In the last few decades, potential beneficial effects of dietary or supplementary daily intake of n-3 polyunsaturated fatty acids (n-3 PUFAs), such as α-linolenic fatty acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), on CV function and outcomes of CVDs (Auger et al., 2016), particularly hemorheology, vascular function, immunomodulation, inflammation and potential to improve muscular strength, have been investigated in different study populations (e.g., healthy, sedentary, athletes, CV patients). On the other hand, recent Cochrane review assessed the effects of intake of fish- and plant-based n-3 PUFA supplements, or in some cases enriched food or dietary advice (n-3 PUFAs doses ranged from 0.5 to >5 g/day) on CV events, adiposity, lipids, and all-cause mortality (Abdelhamid et al., 2018). That review evaluated 79 randomized controlled trials (RCTs) (with subsequent excluding of 25 RCTs), which included approx. 100,000 adults at different CV risks, mainly from high-income countries. Authors concluded that meta-analysis and sensitivity analyses in their review suggested little or no effect of increased DHA/EPA intake on all-cause mortality (high-quality evidence), CV mortality, CV events (high-quality evidence), coronary heart disease (CHD) mortality, stroke or arrhythmia. They may reduce CHD events; however, according to sensitivity analyses – this is small effect. All evidence was of moderate GRADE quality, except ones noted as high quality evidence. However, increased ALA may slightly reduce risk of CV events (low-quality evidence) and probably reduces risk of arrhythmia, while effects on stroke are unclear. Taken together, it seems that n-3 PUFAs and exercise *per se* may affect hemorheology and thrombotic as well as inflammatory status of the body and have protective effect, for example in atherosclerosis, which was not assessed in the aforementioned Cochrane review. Interaction of n-3 PUFA intake and life style (such as physical activity) was not included in the scope of the review by Abdelhamid et al. (2018).

This, in fact, brings some controversies in the field, since the consumption of such remedies is rather high in United States (Hopp and Shurtleff, 2018), while in EU countries the recommendations for daily n-3 PUFA intake are not always met in all population subgroups (Sioen et al., 2017). According to the 2012 National Health Interview Survey in the United States; 7.8% of adults (18.8 million) and 1.1% of children age 4 to 17 (664,000) had taken a fish oil supplement in the previous 30 days (Hopp and Shurtleff, 2018).

The aim of the present review was to summarize, in an orderly manner, current knowledge on the effect of exercise and dietary intake of n-3 PUFAs (in food stuff or in the form of supplements) on vascular function and physical performance in healthy persons and in CV patients, with particular attention paid

to hemorheology and coagulability, inflammation and vascular and muscular function.

To obtain a comprehensive review of current data, original research studies, narrative reviews and systematic reviews and meta-analyses were collected and analyzed. A search of PubMed database was performed by using the following search terms: omega-3 supplementation, n-3 PUFA supplementation, exercise, functional food, cardiovascular, healthy, vascular, endothelium, microcirculation, inflammation, hemorheology, and muscle (**Figure 1**). Only the English language literature pertaining to both humans and experimental animals with no time restriction were reviewed. Literature search algorithms and obtained results (number of articles) are described in **Figure 1**. From the literature search it is evident that a respective number of studies investigated the effect of n-3 PUFA supplementation on hemorheology, vascular/endothelial function/microcirculation, inflammation, and skeletomuscular system in both CV patients and healthy population (panel A). However, a significantly smaller number of studies dealt with the effect of n-3 PUFA supplementation in the form of functional food (panel C), or the potential combination intervention effect of n-3 PUFAs and regular exercise on mentioned parameters (panel B). Importantly, there is no available data (total of 5 research results) on the combined effect of n-3 PUFA supplementation in the form of functional foods and regular aerobic exercise on hemorheology, vascular/endothelial function/microcirculation and inflammation in both healthy population and CV patients (panel D).

METABOLISM OF POLYUNSATURATED FATTY ACIDS

EPA and DHA are present in phospholipids of the cell membrane, contributing to the n-3/n-6 ratio, and share the same enzymes with arachidonic acid (AA) in the eicosanoid-producing process (Wang et al., 2017; Gammone et al., 2018; Philipsen et al., 2018). EPA and DHA are metabolized into numerous eicosanoids and docosanoids, respectively, by cyclooxygenases (COX), lipoxygenase (LOX), and cytochrome P450 (**Figure 2**). Because n-3 PUFAs can compete for the same metabolic pathways against n-6 PUFAs, n-3 PUFAs supplementation may also affect the metabolism of AA, thereby shifting the profile of metabolites derived from AA (**Figure 2**) (Drenjančević et al., 2017).

The AA-derived prostaglandins and leukotrienes are potent pro-inflammatory mediators (Samuelsson et al., 1987), whereas the AA-derived metabolites lipoxins are potent anti-inflammatory and pro-resolving molecules (Serhan, 1994; Wallace and Fiorucci, 2003; McMahon and Godson, 2004). On the other hand, anti-inflammatory lipid mediators or specialized pro-resolving mediators (SPM), such as lipoxins, resolvins, protectins, and maresins, are endogenous n-3 PUFA-derived oxygenated metabolites of EPA, docosapentaenoic acid (DPA) and especially DHA. They are released upon specific stimuli and mediate cell signaling and cell-to-cell interactions. Mostly, they are responsible for regulating inflammation and specific immune responses (Calder, 2017). Addition of n-3 PUFA, particularly EPA and DHA supplements into the diet leads to their increased incorporation into cell membrane phospholipids at the expense of n-6 PUFAs, such as AA. EPA as a 20-carbon chain fatty acid competes directly with AA as a substrate for COX and LOX and leads to production of anti-inflammatory (less inflammatory) prostaglandins and leukotrienes (i.e., LTB3 and PGE3), while DHA, together with EPA, has been associated with production of counter-regulatory lipid mediators such as resolvins, protectins, and maresins (Calder, 2017; Molfino et al., 2017). Maresin 1 is biosynthesized via LOX by DHA to generate 14S-hydroperoxydocosa-4Z,7Z,10Z,12E,16Z,19Z hexaenoic acid, which undergoes further conversion via epoxidation in macrophages and is subsequently converted to 7R,14S-dihydroxydocosa-4Z,8Z,10E,12Z,16Z,19Z-hexaenoic acid, known as maresin 1 (MaR 1) (Serhan et al., 2012). Sun et al. (2017) recently showed that MaR 1 exhibited its protective effects, at least in part, via the Nrf-2-mediated heme oxygenase-1 (HO-1) signaling pathway. MaR 1 can directly modulate the inflammatory responses by affecting the function of already activated and clonally expanded Th1 and Th17 cells, and additionally influence immune responses by acting on their transcription factor-induced activation programs to prevent their generation from naïve CD4 + T cells. Furthermore, MaR 1 can enhance the differentiation of CD4 + T cells into Treg cells (Chiurchiù et al., 2016). They also showed that the protective effect of MaR 1 relied on its downstream antioxidative stress capabilities and and capability to maintain the balance between oxidative and antioxidative stress. MaR 1 significantly reduced reactive oxygen species, malondialdehyde, and 15-F2t-isoprostane generation and restored the activity of antioxidative enzymes (superoxide dismutase, glutathione peroxidase, catalase) (Sun et al., 2017).

Beside reduced production of potent pro-inflammatory AA-derived eicosanoids such as PGE2, anti-inflammatory action of EPA-derived eicosanoids is reflected in the fact that they have reduced affinity for eicosanoid receptors. For example, Wada et al. (2007) described that PGE_3 has 50 to 80% lower potency compared with PGE_2 toward the EP_1, EP_2, EP_3, and EP_4 receptors. In addition, increased EPA and DHA cellular content results in decreased expression of COX-2 enzyme (Jeromson et al., 2015; Gammone et al., 2018). **Figure 2** shows the metabolism of n-3 and n-6 PUFAs and the most important eicosanoids involved in the processes of inflammation, vascular reactivity, thrombosis, and similar.

EFFECTS OF n-3 PUFAs AND EXERCISE ON HEMORHEOLOGY AND COAGULABILITY

Supplementation or Dietary Intake of n-3 PUFAs Affects Hemorheology and Coagulability

Dietary n-3 PUFAs are associated with a hypocoagulable profile. In the Atherosclerosis Risk in Communities (ARIC) Study, four

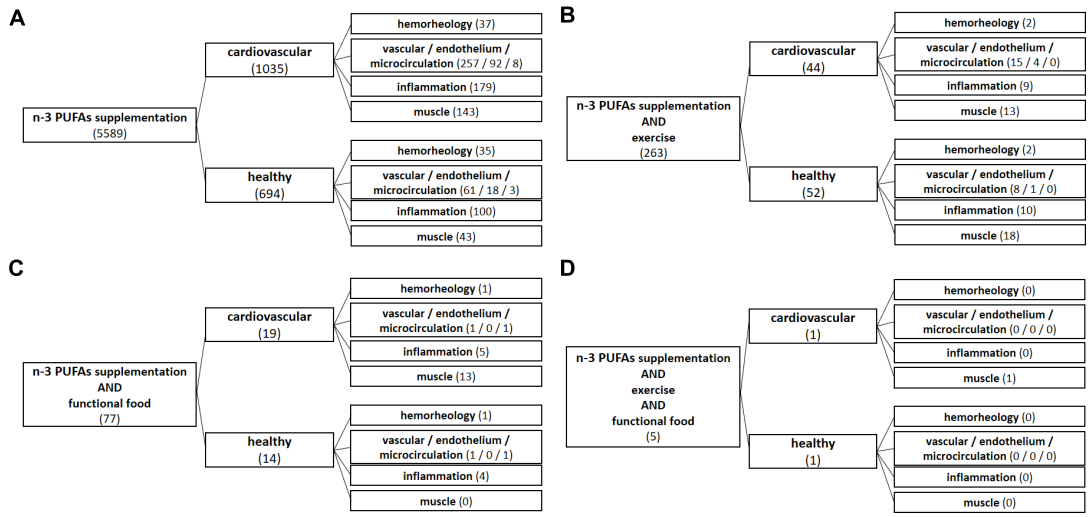

FIGURE 1 | Algorithms of literature search. From literature search it is evident that a respective number of studies investigated the effect of n-3 PUFA supplementation on hemorheology, vascular/endothelial function/microcirculation, inflammation, and skeletomuscular system in both cardiovascular patients and healthy population **(A)**. However, a significantly smaller number of studies dealt with the effect of n-3 PUFA supplementation in the form of functional food **(C)**, or the potential combined interaction effect of n-3 PUFAs and regular exercise on the mentioned parameters **(B)**. Importantly, there is no available data (a total of 5 search results) on the combined effect of n-3 PUFA supplementation in the form of functional foods and regular aerobic exercise on hemorheology, vascular/endothelial function/microcirculation and inflammation in both healthy population and CV patients **(D)**.

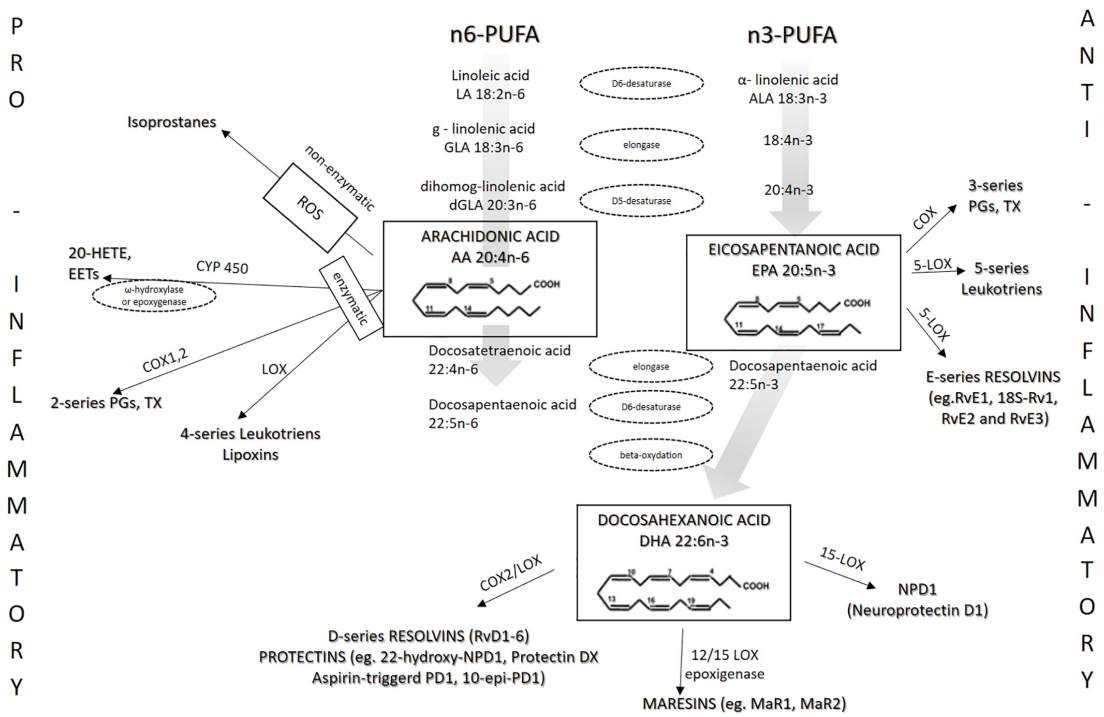

FIGURE 2 | Metabolism of n-3 and n-6 PUFAs and the most important eicosanoids. The present figure summarizes the metabolism of n-3 PUFAs and n-6 PUFAs by cyclooxygenases (COX), lipoxygenase (LOX), and cytochrome P450.

population-based samples amounting to over 15,000 participants, blacks and whites, have been studied (Shahar et al., 1993). This study assessed if dietary fatty acid ingestion leads to changes in blood concentrations of relevant coagulation factors. The investigators analyzed concentrations of fibrinogen, factor VII, factor (vWF), protein C, and antithrombin III. A food frequency questionnaire was used to document dietary intake. Multiple linear regression was used to assess cross-sectional

associations, with adjustments for age, race, gender, smoking and alcohol use, diabetes, body mass index, and field center. Dietary n-3 PUFAs have been shown to be negatively associated with concentrations of fibrinogen, factor VIII, and (vWF) (in black and white individuals) and are positively associated with protein C (in white subjects only). The ingestion of fish, which represented the predominant source of dietary n-3 PUFAs, was similarly related to the hemostatic profile. Namely, fish ingestion that was 1 serving higher per day showed an association with these predicted differences (95% confidence interval): factor VIII, −3.3% (−5.4, −1.3); fibrinogen, −2.9 mg/dL (−6.3, 0.5); vWF, −2.7% (−5.2, −0.1) (in black and white subjects); and protein C, +0.07 μg/mL (0.03, 0.11) (white subjects only). Other analyzed nutritional items were variably correlated with the levels of hemostatic factors. The results of the ARIC study with its associations that are population-based, albeit cross-sectional, imply that elevations in dietary intake of n-3 PUFAs from fish might lead to alterations of blood concentrations of several important coagulation factors. These associations are tied to the hypocoagulable profile (Shahar et al., 1993). Phang et al. (2014) performed a double-blinded, placebo-controlled randomized trial in 94 healthy adults, assessing platelet coagulation parameters such as factors I, II, V, VII, VIII, IX, X, vWF and endogenous thrombin potential, as well as platelet aggregation. They found that supplementation with EPA in healthy men and DHA in women leads to reduction in platelet aggregation. Similarly, plasma concentrations of factor II, factor V and vWF were reduced primarily in men with EPA supplementation, while reduction in platelet aggregation mediated by DHA in women was not associated with substantial changes in the assessed parameters of coagulation (Phang et al., 2014). This brings to light an interesting sex-specific difference in the antithrombotic effects of n-3 PUFAs.

The addition of n-3 PUFAs to simvastatin treatment in patients with combined hyperlipidemia lead to a reduction of the fraction of the free tissue factor pathway inhibitor in the fasting state. This n-3 PUFA addition also inhibited factor VII activation during post-prandial lipemia, as found by Nordøy et al. (2000) on a sample of 41 patients. A double-blind, cross-over study using olive oil as placebo, assessed the influence of a daily dosage of 6 g of fish oil on CV risk markers of 20 healthy young volunteers (10 men and 10 women). This study implicated a reduction in factor X (as a result of fish oil ingestion in women, compared to placebo) (Oosthuizen et al., 1994), but there are also inconsistencies in the findings, since some studies did not detect clear changes in the levels of coagulation factors (Vanschoonbeek et al., 2004) or platelet function with n-3 PUFA ingestion (Lee et al., 2006; Poreba et al., 2017).

A decrease in thromboxane A2 synthesis, which is an important platelet aggregation facilitator, contributes to the hypocoagulable state induced by n-3 PUFAs (Véricel et al., 2015; Lands, 2016; Jeansen et al., 2018). EPA and DHA are also antagonists of human platelet thromboxane A2 and prostaglandin H2 receptors (Véricel et al., 2015; Lands, 2016; Jeansen et al., 2018). In addition, n-3 PUFAs lead to a decrease in plasma viscosity in patients with hypolipoproteinemia by altering the protein pattern of the plasma (Ernst, 1989).

There is evidence that the n-3 PUFA ALA, which is converted to EPA and DHA, impairs formation of arterial thrombus, expression of tissue factor, and platelet activation in male C57Bl/6 mice who were fed a high-ALA diet for 2 weeks (Holy et al., 2010). Furthermore, there is also clinical evidence regarding the influence of n-3 PUFAs on clot properties. For instance, a study by Gajos et al. (2011) assessed the effects of n-3 PUFAs on top of dual antiplatelet therapy in stable coronary artery disease patients undergoing percutaneous coronary intervention. The addition of n-3 PUFAs (supplementation 1 g/day for 1 month) to standard therapy (30 treated subjects and 20 patients on placebo) lead to a decrease in thrombin formation and oxidative stress and favorably altered fibrin clot properties in the treated patients (Gajos et al., 2011).

Regarding the safety of n-3 PUFA consumption, when its antithrombotic effects are taken into consideration, it has been analyzed in various patient populations in clinical settings. An assessment of 8 clinical intervention studies (with over 600 treated individuals) that used nutritional supplements with fish oil as a source of n-3 PUFAs did not find any increased bleeding risk as a result of n-3 PUFA intake. Additionally, no significant changes in crucial parameters of coagulation (partial thromboplastin time and prothrombin time) were observed. Therefore, the authors concluded that n-3 PUFA intake seems to be safe even at a short-term dosage of up to 10 g/day of EPA + DHA or consumed for up to 52 weeks at higher than 1.5 g/day, in selected vulnerable and sensitive patient populations including individuals with gastrointestinal malignancies or intensive care patients (Jeansen et al., 2018). The potential protective antithrombotic effects of PUFAs are summarized in **Figure 3**.

Effects of Exercise on Hemorheology and Coagulability in CV Patients

Regarding the influence of exercise on platelet function in patients with CV disease, it is not entirely clear in which way exercise may influence platelets. In their systematic review, Hvas and Neergaard-Petersen analyzed a total of 18 studies from various databases (including PubMed, Embase, Scopus, and Cochrane Library). Of the 18 studies, 7 were with coronary artery disease patients, 5 with angina pectoris patients, 5 with hypertensive patients, and 2 with subjects who have peripheral artery disease (Hvas and Neergaard-Petersen, 2018). They found that conflicting results were reported, with certain studies reporting increased platelet aggregation and/or platelet activation, some studies found no difference, while several reported a reduction in platelet aggregation after exercise (compared with controls). Therefore, more conclusive research is needed to finally elucidate potential effects of exercise on platelet function.

Exercise *per se* has been related to beneficial changes of rheological characteristics of the blood. In a meta-analysis of available studies which investigate the effects of exercise on rheological characteristics of blood, Romain et al. (2011) concluded that regular exercise decreases hematocrit and red blood cell aggregation. However, the main criticism of Romain's

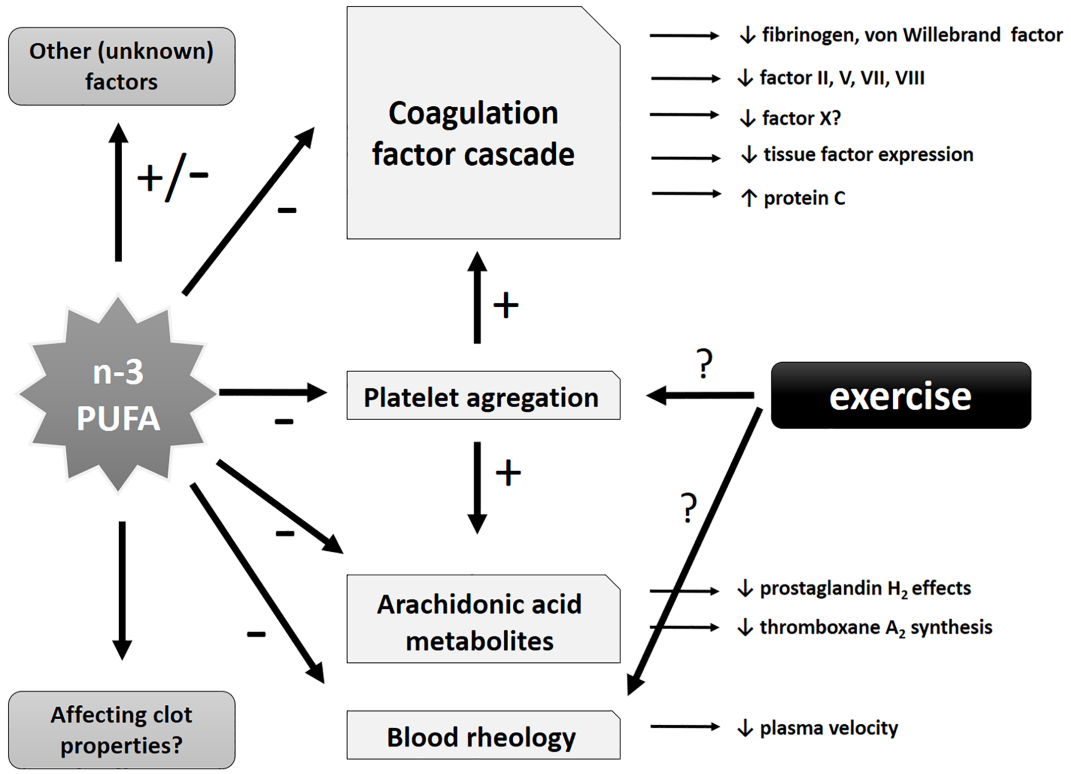

FIGURE 3 | Schematic representation of potential antithrombotic effects of n-3 PUFAs and exercise. Potential effects of n-3 PUFAs may include influence on specific coagulation factors of the coagulation cascade (with antithrombotic tendency), effects on arachidonic acid metabolites, blood rheology and (directly and indirectly) platelet aggregation (leading to a decrease in the latter). Other unknown or insufficiently investigated effects might also be involved.

analysis was the small number and a large heterogeneity of the available studies (methodology, study design, etc.) which hampered final conclusions (Romain et al., 2011), resulting in uncertainty of interpretation of data and necessity of future randomized controlled clinical studies for final conclusions.

Combined Interaction Effect of n-3 PUFA Consumption and Exercise on Hemorheology and Antithrombic Status of Blood in CV Patients

The search for combined interaction effect of n-3 PUFA consumption and exercise on hemorheology and coagulability of blood in CV patients rendered scarce results. It is reported that in previously sedentary, obese people (N = 22; 12 women, 10 men, BMI 26.6 ± 0.7 kg/m^2) 4 weeks of dietary and exercise protocols, exercise training [brisk walking and (or) jogging at 60% VO_2 maximum for 45 min/day, 5 days/week] has no interference or additive effects with 4 g/day of n-3 PUFA supplementation in attenuating post-prandial lipemia, but combined treatments may be additive in raising high-density lipoprotein (LDL) cholesterol (Thomas et al., 2007), which may help maintain antithrombotic and anti-atherosclerotic status of blood. Obviously, well-planned, controlled trial of combined dietary and exercise protocols focused on blood hemorheology could provide new evidence on interactions of interest.

THE EFFECT OF n-3 PUFAs AND EXERCISE ON VASCULAR FUNCTION IN HEALTHY SUBJECTS AND IN CARDIOVASCULAR PATIENTS

Since it is widely accepted that n-3 PUFA consumption reduces CV risk (Auger et al., 2016), numerous studies set their focus on investigating whether n-3 PUFA intake may prevent or delay the initial steps in the pathogenesis of various CV diseases, e.g., changes in endothelium and vascular function, in both healthy and diseased population. In particular, n-3 PUFAs may improve endothelial function by increasing the bioavailability of endothelial nitric oxide (NO) (through increasing its production by stimulating endothelial NO synthase gene and protein expression) (Lo et al., 2004; Gortan et al., 2013; Zhang et al., 2013) or by reducing the level of oxidative stress (through attenuating reactive oxygen species, which indirectly increases NO bioavailability) (Gortan et al., 2013; Zhang et al., 2013; Drenjančević et al., 2017). Additionally, vascular function could be improved by reducing inflammation (by affecting mediators of local endothelial and/or systemic inflammation) (Wang et al., 2011). All of these interactions are summarized in **Figure 4**.

While there is paucity of data on the effect of n-3 PUFAs on microvascular function in both healthy and diseased population (Pe et al., 2008; Stupin A. et al., 2018), there is large inconsistency

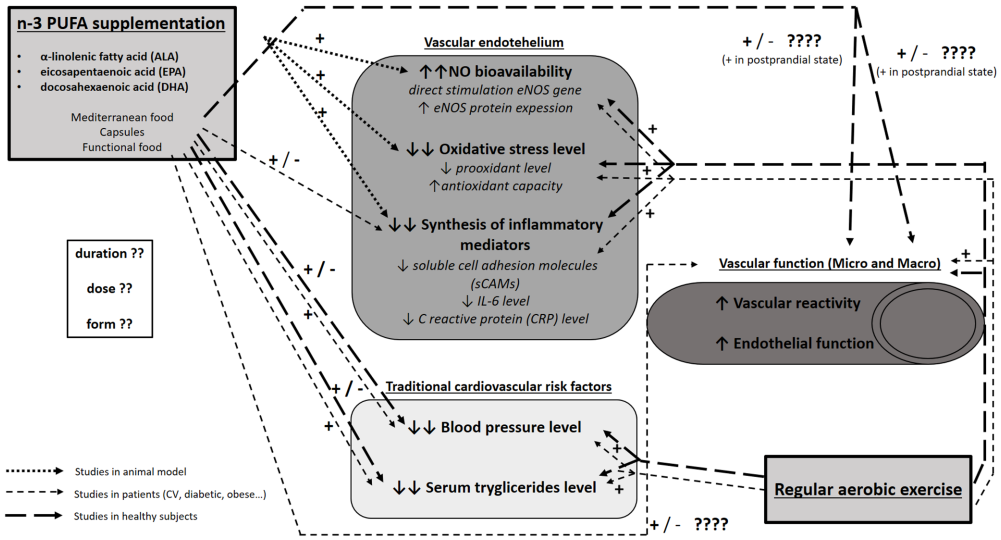

FIGURE 4 | Functional pathways and molecular mechanisms mediating interaction between n-3 PUFAs and exercise on the one side and endothelial function on the other. The present figure summarizes the results of studies conducted on experimental animals, healthy subjects, and patients (CV, diabetic, obese) on the effect of n-3 PUFA supplementation or regular exercise on endothelial function (NO bioavailability, oxidative stress level and inflammation), vascular function (functional vascular experiments in both macro- and micro-circulation), and traditional cardiovascular risk factors (e.g., blood pressure, serum triglyceride level). It is evident that studies on the effect of regular exercise on the abovementioned parameters provided more uniform results and yielded clear conclusions on the beneficial effect of regular exercise on CV health. On the other hand, studies on the effect on n-3 PUFAs on vascular and endothelial function, especially studies in patients and healthy subjects, provided very divergent and inconclusive results, evidently due to the heterogeneity in experimental design with the emphasis on different form, dose and duration of n-3 PUFA supplementation used in these studies. There is a paucity of data on the combined effect of n-3 PUFAs and regular exercise on CV function in general.

of functional vascular studies which evaluated the effects of n-3 PUFAs on macrovascular function, mainly assessed by flow-mediated dilation (FMD) of brachial artery as a gold standard method, in both healthy individuals and CV patients (Egert et al., 2014; Merino et al., 2014; Tousoulis et al., 2014; Yagi et al., 2015).

Beneficial Effect of n-3 PUFAs on Vascular Function in CV Patients

Studies in CV patients (e.g., coronary artery disease, metabolic syndrome, chronic heart failure) or individuals with increased CV risk (e.g., obesity, diabetes mellitus type 2, hyperlipidemia, cigarette smoking) strongly suggest that n-3 PUFA supplementation improves vascular and endothelial function (e.g., brachial artery FMD, venous occlusion strain-gauge plethysmography, microvascular endothelial cells) (Morgan et al., 2006; Egert et al., 2014; Yagi et al., 2015). For example, oral intake of 2 g of n-3 PUFAs per day for 12 weeks significantly improved FMD of brachial artery and pulse wave velocity in adults with metabolic syndrome (Tousoulis et al., 2014). A recent cross-over study in overweight individuals reported that a walnut-rich diet (23.1% energy from n-3 PUFAs) compared to an almond-rich diet significantly improved FMD of brachial artery and decreased soluble vascular cell adhesion molecule level (Bhardwaj et al., 2018). Furthermore, increased serum DHA level was a positive contributor to an increased FMD of brachial artery in 160 consecutive Japanese patients with coronary artery disease (Yagi et al., 2015). FMD of brachial artery was improved following intake of 1.8 g/day n-3 PUFA supplementation for 12 weeks in hypertensive patients with hypertriglyceridemia and high CV risk as demonstrated by interventional study (Casanova et al., 2016). Similar results were also reported in overweight dyslipidemic patients and type 2 diabetics (Egert et al., 2014; Tousoulis et al., 2014), along with decrease of soluble thrombodulin, the marker of vascular endothelial damage, in patients with the presence of one or more risk factors for atherosclerosis (Kawauchi et al., 2014). Kondo et al. (2014) reported that a 4-week diet period rich in n-3 PUFAs significantly improved reactive hyperemia measured by strain-gauge plethysmography in post-menopausal women with diabetes mellitus type 2. Furthermore, n-3 PUFA supplementation in patients with chronic heart failure improved endothelium-dependent vasodilation measured by venous occlusion strain-gauge plethysmography (Morgan et al., 2006). Beneficial effect of n-3 PUFAs on microvasculature was confirmed in the model of inflammatory bowel disease on a primary culture of human intestinal microvascular endothelial cells (Ibrahim et al., 2011).

Beneficial Effect of n-3 PUFAs on Vascular Function in Healthy Subjects

There is inconsistency in the results of studies investigating whether the same beneficial effect of n-3 PUFA consumption is also present in healthy population. Although the results of several studies demonstrated that n-3 PUFA supplementation improved brachial artery FMD in healthy individuals (Walser et al., 2006; Shah et al., 2007; Rizzaa et al., 2009), others failed to observe

such effect (Sanders et al., 2011; Skulas-Ray et al., 2011; Singhal et al., 2013). The most evidence for the benefits of n-3 PUFAs to vascular function in healthy individuals was observed in the post-prandial state, in both macrocirculation (using FMD) (Fahs et al., 2010; Miyoshi et al., 2014) and microcirculation (using LDF) (Armah et al., 2008; Pe et al., 2008). Supplementation of fish oil (rich in n-3 PUFAs) for 8 months improved endothelial function in normal healthy subjects (Khan et al., 2003). A recent study by our research group reported that young healthy subjects who consumed n-3 PUFA enriched eggs for 3 weeks (777 mg of n-3 PUFAs/day) had improved skin microvascular reactivity in response to vascular occlusion (Stupin A. et al., 2018). On the contrary, a single Mediterranean meal (rich in n-3 PUFAs; 2.29 g of n-3 PUFAs per meal) did not significantly alter FMD in healthy men, while a high-saturated fatty acid meal induced post-prandial endothelial dysfunction (Lacroix et al., 2016) and only n-3 PUFA supplementation higher than 1.8 g per day improved FMD in healthy adults (Sanders et al., 2011).

The results of two large meta-analyses (including both healthy individuals and CV patients) demonstrated that n-3 PUFA supplementation significantly increases FMD of brachial artery, but also showed that the health status of the study population, as well the dose of n-3 PUFA supplementation may modify the effect of n-3 PUFAs on vascular function (Wang et al., 2012; Xin et al., 2012). Furthermore, when the analysis included only double-blinded, placebo-controlled studies, the supplementation of n-3 PUFAs had no significant effect on vascular endothelial function (Wang et al., 2012). Consequently, the results of the abovementioned studies should still be interpreted cautiously, due to the large heterogeneity of these studies in terms of selection of participants, their age and health status, as well as in terms of the dose and form of n-3 PUFA supplementation used in the trials.

Beneficial Effects of Exercise on Vascular Function in CV Patients and Healthy Persons

Positive effects of moderate physical activity in preserving general health and preventing CV disease and age-related deterioration have been very well-established (Warburton and Bredin, 2017). Regular exercise significantly contributes to lowering arterial blood pressure or reducing blood lipid concentration (Joyner and Green, 2009). Functional vascular studies on the conductance and resistance arteries in CV patients showed decreased inflammation and endothelial dysfunction of the blood vessels (Hambrecht et al., 2003), while 4 weeks of exercise improved FMD of brachial artery in patients with stable coronary disease (Hambrecht et al., 2003).

Similarly, regular exercise has a beneficial effect on vascular function, even in healthy people. For example, athletes have better FMD of brachial artery compared to healthy sedentary individuals (Clarkson et al., 1999; Kasikcioglu et al., 2005). Several mechanisms may be involved in these positive effects of long-term regular physical activity, such as increased NO bioavailability, increased antioxidative defense in the vascular system and reduced levels of locally or systemically derived mediators of inflammation (Padilla et al., 2011). It has been demonstrated that the repeated increase in blood flow (e.g., vascular shear stress) during physical activity is probably a key mechanism that induces a positive adaptation of vascular function to regular physical activity by stimulating the NO-dependent vasodilatory pathway (Laughlin et al., 2008; Birk et al., 2012). Nevertheless, production of COX-derived vascular metabolites, including increased synthesis of prostacyclin, is involved in endothelial shear stress adaptations. Furthermore, increasing evidence suggests that increased shear stress is a signal that will reduce the level of endothelin 1, soluble cell adhesion molecules and various markers of endothelial activation (Himburg et al., 2007), all together contributing to improved macrovascular function.

Functional vascular studies on microcirculation of the skin showed that regular body activity lasting several weeks to several months resulted in the adaptation of skin microcirculation which is manifested by improved vasodilation-dependent endothelium in both healthy population and CV patients. The main evidence derives from vascular experiments studying the microcirculation response to the endothelium-dependent [acetylcholine (ACh)] and/or endothelium independent [sodium nitroprusside (SNP)] vasodilator. Kvernmo et al. (1998) demonstrated better response in terms of microvascular flow in the forearm skin following ACh administration in professional athletes compared with appropriate controls that had moderate body activity, while there was no difference in SNP response between these two experimental groups, suggesting that endothelial function was affected. Similarly, Stupin M. et al. (2018) reported that professional rowers had significantly better response of forearm skin microcirculation to vascular occlusion and ACh, but not SNP compared to sedentary healthy controls. Furthermore, Wang (2005) investigated endothelium-dependent and endothelial-independent vascular response in microcirculation of the skin in healthy sedentary individuals before and after 8 weeks of exercise. The results of this study have also shown a marked improvement in endothelium-dependent vascular response, but without any change in endothelium-independent response of skin microcirculation. Interestingly, in the further course of the study after 8 weeks without exercise such enhanced endothelium-dependent response was no longer present. In general, skin microvascular endothelial function was not different between adult and young active individuals (Black et al., 2008; Tew et al., 2010). On the other hand, older individuals practicing exercise have better endothelial vascular function in microcirculation of the skin compared to their sedentary peers (Black et al., 2008). Similar effects have been reported in patients with type 2 diabetes (Colberg et al., 2002) and chronic venous disease (Klonizakis et al., 2009).

Interactive Effects of n-3 PUFAs and Long-Term Exercise on Vascular Function in Humans

Taken together, it is evident that both n-3 PUFA supplementation and regular physical activity improve endothelial function in both macro- and microcirculation of healthy individuals, individuals

with increased CV risk and CV patients (Morgan et al., 2006; Joyner and Green, 2009; Egert et al., 2014). However, there is a paucity of data on the combination of these two divergent interventions on vascular function in these populations. The only available study at the moment of preparation of the manuscript is one on an experimental animal model by Barbeau et al. (2017), who reported that combining ALA and endurance exercise resulted in additional reduction of CV disease risk compared to individual interventions in the obese Zucker rat. However, there are many potentially common steps and outcomes that have been demonstrated to be modified by exercise and diet *per se*, as schematized in **Figure 4**. For example, arterial blood pressure and serum lipid concentration are both affected by n-3 PUFAs and exercise. Similarly, oxidative stress, inflammatory, and biomarkers of vascular function are affected independently by n-3 PUFAs and exercise. On the other hand, the interactive effect of n-3 PUFAs and exercise on functional responses of microcirculation is still unknown. Thus, it is tempting to speculate that these two stimuli which both individually result in improved endothelial function would have additive effect on endothelial function and may be a new challenging avenue of research.

IMMUNOMODULATORY AND ANTI-INFLAMMATORY PROPERTIES OF n-3 PUFAs AND EXERCISE IN ATHLETES AND CARDIOVASCULAR PATIENTS; MUSCLE SORENESS, MUSCLE STIFFNESS, AND JOINT MOBILITY

Immunomodulatory and Anti-inflammatory Properties of n-3 PUFAs

It is widely accepted that inflammation underlies many chronic non-communicable diseases, such as CV diseases, atherosclerosis, Alzheimer's disease, and cancer (Coussens and Werb, 2002; Libby, 2002; Weiner and Selkoe, 2002). In general, n-3 PUFAs are considered to have anti-inflammatory effects on various biological systems, including skeletal muscles, respiratory mucosa and CV system, all of which have an important role in exercise physiology (Biltagi et al., 2009; Tecklenburg-Lund et al., 2010; Mori, 2014; Jeromson et al., 2015; Gammone et al., 2018). However, our knowledge of immunomodulatory effects of n-3 PUFAs in humans is scarce (Chen et al., 2018; Dátilo et al., 2018; Phitak et al., 2018). Although much is known about the molecular basis of initiating signals and pro-inflammatory chemical mediators in inflammation (Samuelsson, 2012), it has only recently become interesting to explore endogenous stop signals such as lipid mediators. Most inflammatory reactions are self-limited, and, besides leukocytes withdrawal, the process of resolution includes a switch in synthesis of AA-derived pro-inflammatory mediators to anti-inflammatory pro-resolving n3-PUFA-derived mediators. Previous studies have demonstrated that n-3 PUFAs alleviate inflammation by affecting the production of inflammatory mediators such as eicosanoids, reactive oxygen species and cytokines [i.e., tumor necrosis factor α (TNFα), interleukin 1β and interleukin 6 (IL-6)], responsible for release of other inflammatory factors and acute-phase proteins from the liver (Northoff and Berg, 1991; Calder, 2006; Ambrozova et al., 2010; Jouris et al., 2011).

Increased consumption of EPA and DHA has been linked to decreased serum levels of hsCRP in a heterogeneous set of clinical trials (Reinders et al., 2012; Kubota et al., 2015). For example, one interesting observation is that increased n-3 PUFA intake has increased apolipoprotein A1 and decreased high-sensitivity C reactive protein (hsCRP) concentration and serum concentration of soluble cell adhesion molecules and other pro-inflammatory factors in patients at intermediate-high CV risk, resulting in improved peripheral vasoactivity (Merino et al., 2014). In a cross-sectional study on 992 patients with stable coronary artery disease, a multivariable linear regression model demonstrated that levels of n-3 PUFAs (DHA + EPA) were inversely and independently associated with CRP and IL-6 concentration (Farzaneh-Far et al., 2009). Regarding other inflammatory parameters that were assessed, results are mixed and dependent on the extent of obesity, as beneficial effects were only observed in severely, but not moderately obese individuals (Itariu et al., 2012; Kratz et al., 2013). Furthermore, n-3 PUFAs affect the life and function of lymphocyte subpopulation; administration of n-3 PUFAs decreases lymphocyte proliferation, and alters neutrophil and natural killer (NK) cell function (Varming et al., 1995; Thies et al., 2001a,b).

Transcription factor nuclear factor κB (NF-κB) has a central role in the initiation of an immune response by inducing transcription of genes encoding various cytokines (i.e., IL-1, IL-6, TNFα), chemokines, cell adhesion molecules, anti-apoptotic factors, cell cycle regulators (i.e., cyclin D), and other cell-type specific mediators of inflammation (i.e., inducible NO synthase, COX2) (Afonina et al., 2017). EPA and DHA are natural agonists of transcription factor peroxisome proliferator-activated receptor γ (PPAR-γ), whose activation potently inhibits cytokine-induced NF-κB transcriptional activity in skeletal muscles in a time- and dose-dependent manner, as evidenced by significantly reduced levels of TNFα, IL-8, intercellular adhesion molecule 1 and chemokine (C-X-C motif) ligand 1 (Grygiel-Górniak, 2014).

Immunomodulatory and Anti-inflammatory Properties of Exercise

Prolonged and exhaustive exercise, but not moderate exercise, leads to a transient increase in frequencies in peripheral blood lymphocytes during the exercise, possibly due to the mobilization of CD4 + T cells, CD8 + T cells, CD19 + B cells, CD16 + NK cells, and CD56 + NK cells from the peripheral lymphoid organs, which is followed by a significant decline in total blood lymphocyte numbers immediately after cessation of exercise. Some of these changes have been attributed to elevated levels of catecholamines during intense training. Furthermore, heavy training loads inhibit NK and B cells activity, and promote Th2 response characterized by Th2 cells and Treg expansion. Levels of pro-inflammatory cytokines and chemokines (i.e., IL-1, IL-6, TNF-α, MIP-1, IL-8) rise during intense training and

some remain elevated for hours after the acute intense bout of training. In the humoral immune response, reduced antibody production and secretion of antibodies to the mucosa have been observed (Pedersen, 2000; Shaw et al., 2018). These changes could lead to defective immune responses and make athletes prone to infection.

The effect of exercise on NF-κB transcriptional activity is dependent on the type and intensity of physical activity. An acute bout of exercise activates myocardial NF-κB and increases toll-like receptor 4 signaling leading to inflammation (Balan and Locke, 2011; Cristi-Montero et al., 2012; Vella et al., 2012), while moderate exercise reduces NF-κB signaling and activates the SIRT1-AMPK-PGC1α axis, resulting in decreased inflammation and reduced muscle loss (Liu and Chang, 2018). All of this has been summarized in **Figure 5**.

Combined Interaction Effect of n-3 PUFAs and Exercise on Immunomodulation

It is interesting that n-3 PUFAs from fish oil have been found to reduce serum cortisol levels and the production of TNF-α and IL-8 after strenuous exercise. Fish oil supplementation has no effect on lymphocytosis observed during strenuous exercise; however, it prevented the post-exercise decrease in CD8 + T cells (Peres et al., 2018). Four weeks of n-3 PUFA supplementation attenuates the increase in serum inflammatory markers during a period of 1 to 4 days after eccentric exercise (DiLorenzo et al., 2014). DHA supplementation reduces exercise-induced muscle soreness and stiffness, which could be beneficial for improving tolerance to new and/or strenuous exercise programs and achieving better training adaptations (DiLorenzo et al., 2014; Corder et al., 2016). Furthermore, n-3 PUFAs exhibit an antinociceptive effect by binding to the receptors in nervous tissue (Nakamoto et al., 2011). Additional mechanisms of n-3 PUFA-mediated pain control include decreased pro-inflammatory cytokine secretion (Zaloga and Marik, 2001) and/or blockage of cationic channels (Xiao et al., 1995). Other possible mechanisms of decreased nociception include direct stimulation of opioid receptors or alteration of plasma levels of endogenous opioid peptides (Nakamoto et al., 2011). Based on these findings, it seems plausible that n-3 PUFA supplementation could be useful for preventing inflammation and delayed-onset muscle soreness (DOMS) resulting from exhausting exercise (Corder et al., 2016).

In athletes, n-3 PUFAs have potentially beneficial effects on the immune system and inflammation, in terms of alleviation of the observed post-exercise immunosuppression and anti-inflammatory effects that reduce exercise-induced DOMS and muscle stiffness. The consumption of omega 3 fatty acids modulates Th1/Th2 balance and leads to an increase in the plasma levels of ALA, EPA and DHA, and proportionally to the reduction in the levels of AA. Such system leads to a decrease in the production of inflammatory lipid mediators which provide a high-level immune response after exhaustive training. Consumption of fish-oil supplementation at a dose of 1.8 g/day during 6 weeks significantly reduced prostaglandin E2, interferon-γ, and TNFα concentration in elite swimmers (Andrade et al., 2007). Consumption of 1.6 g/day of n-3 PUFAs combined with a single bout of endurance exercise increased the level of IL-2 production and NK cell cytotoxic activity 3 h after the exercise in healthy men, suggesting interactive effects of n-3 PUFAs and exercise on the native immune system (Benquet et al., 1994; Kawabata et al., 2014; Da Boit et al., 2017). Reduced pro-inflammatory production of cytokines, decreased neutrophil function, and NK cell cytotoxicity is established in cases of a single bout of high-intensity or long-duration endurance exercise (Gleeson et al., 2011; Gammone et al., 2018).

Increased oxidative stress induced by exercise is accompanied by a reduced immune response and decreased resistance to infection (Sen, 1995) and makes athletes prone to upper respiratory tract infections. Furthermore, exercise-induced oxidative stress and inflammation, if prolonged, might give rise to systemic inflammation, insulin resistance, and diabetes mellitus type 2 (Bogdanovskaya et al., 2016). At that point, n-3 PUFAs may become important mediators in the prevention of infection (Gray et al., 2012). This is supported by the findings that 6 weeks of consumption of 3 g/day of fish oil (double-blind, randomized, placebo-controlled trial) increased post-exercise production of IL-2 in peripheral blood mononuclear cells and NK cell activity (Gray et al., 2012). In another study, n-3 PUFA dietary supplementation reduced bronchial inflammation by altering immune cell composition in the sputum and reduced pro-inflammatory eicosanoids and IL-1 and TNFα production (Schubert et al., 2009). In addition, activated muscles secrete myokines and cytokines such as IL-6 and TNFα which, under some circumstances and at low levels, have been shown to have anti-inflammatory effects (Bruunsgaard, 2005).

Unfortunately, there is a lack of similar data in CV patients, e.g., combined effect of n-3 PUFAs and exercise on immune status in CV patients has not been studied yet.

n-3 PUFAs ENHANCE ENDURANCE CAPACITY DURING EXERCISE IN ANIMAL MODELS AND IN HUMANS

Numerous studies show that n-3 PUFAs may have effects on skeletal muscle metabolism (You et al., 2010; Smith et al., 2015), indicating that n-3 PUFAs regulate signaling pathways related to growth and hypertrophy (Smith et al., 2011a), while animal studies demonstrated that n-3 PUFAs diminished skeletal muscle protein breakdown (Whitehouse et al., 2001). Gingras et al. (2007) demonstrated that food enriched in menhaden oil enhances the activation of anabolic signaling proteins in bovine muscle, and muscle protein synthesis is increased as well (Smith et al., 2011a). Consumption n-3 PUFAs may increase some pathways involved in modulation of mitochondrial activity and extracellular matrix organization and decrease the inhibition of mTOR, which is the key anabolic regulator (Yoshino et al., 2016). Animal studies suggest that transcriptional changes in muscle are responsible for anabolic effects of n-3 PUFAs, such as Akt/FOXO, TLR4, and NOD signaling (Castillero et al., 2009; Liu et al., 2013) and enhance the gene expression of key modulators of mitochondrial function (Baillie et al., 1999; Philp et al., 2015).

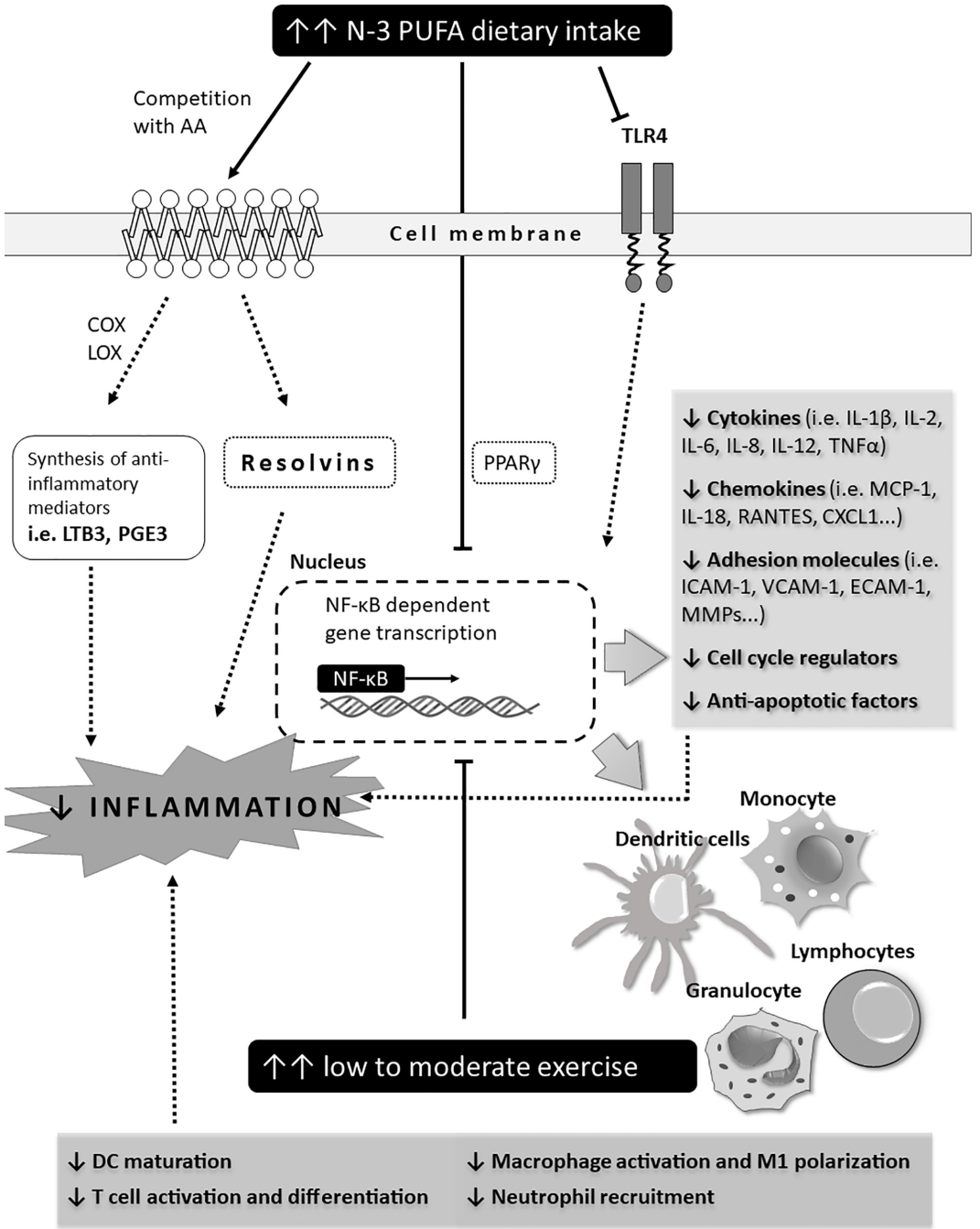

FIGURE 5 | Schematic representation of potential immune mechanisms affected by n-3 PUFAs and exercise in CV patients (outcomes). The effect of exercise and n-3 PUFAs on NF-κB transcriptional activity. Effect of exercise is dependent on the type and intensity level of physical activity. An acute bout of exercise activates myocardial NF-κB and increases toll-like receptor 4 signaling leading to inflammation while moderate exercise reduces NF-κB signaling and activates the SIRT1-AMPK-PGC1α axis, resulting in decreased inflammation and reduced muscle loss.

Results of an vitro study show a general amelioration in energy metabolism of skeletal muscle after n-3 PUFA intake (Hessvik et al., 2010), and a recent animal study on mice has showed that n-3 PUFAs inhibit metabolic dysfunction in skeletal muscle, since a high saturated fat intake increases accumulation of lipids in red muscle, which in turn increases mediators of lipolysis, oxidation, and thermogenesis (e.g., fatty acid transporter genes Fatp4 and Fat/Cd36, fatty acid storage-related gene Dgat1 and Hsl were increased) and suppresses lipogenic genes (Philp et al., 2015).

Furthermore, n-3 PUFAs can modify the membrane fluidity of proteins and lipids within the cell membrane, reducing oxygen consumption and thus improving endurance capacity

(Philpott et al., 2019). Different studies on healthy animals (for example birds – semipalmated sandpipers Calidris pusilla or rats) showed that n-3 PUFA supplementation supports recovery of muscle damage caused by exercise and changes muscle lipid composition (Ayre and Hulbert, 1997; Ruf et al., 2006; Maillet and Weber, 2007; Nagahuedi et al., 2009). Similarly, n-3 PUFAs prevent metabolic dysfunction in mice skeletal muscle by limiting the accumulation of intramyocellular lipids in type I muscle fibers (Philp et al., 2015).

It seems that enhanced aerobic capacity is the result of significant exploitation of n-3 PUFAs, which have similar effects in mammalian cells *in vitro* and *in vivo* (Nagahuedi et al., 2009). Certain animals, like migratory birds, salmons and rats, when consuming n-3 PUFAs enriched foods exhibited increased endurance and a large increase in aerobic capacity ("natural doping") (Ayre and Hulbert, 1997; McKenzie et al., 1998; Guglielmo et al., 2002; Maillet and Weber, 2007). Natural doping is mediated by the incorporation of n-3 PUFAs into the membrane phospholipids and their binding to nuclear receptors which are necessary for regulation of genes which control lipid metabolism. In addition to that, n-3 PUFAs participate in oxygen delivery to skeletal muscle and increase exercise performance (Bruckner et al., 1987). Daily supplementation of n-3 PUFAs in older people, in combination with physical activity, resulted in a significant increase in muscle strength (Rodacki et al., 2012; Smith et al., 2015). Moreover, it has been demonstrated that consumption of 3 g/day of n-3 PUFAs decreases eccentric exercise-induced soreness as a marker of inflammation and minimizes the severe DOMS that results from strenuous strength exercise (Jouris et al., 2011). Supplementation of n-3 PUFAs decreased post-exercise soreness in healthy adults (Jouris et al., 2011) manifested as decreased blood markers of muscle damage and inflammatory markers (Mickleborough et al., 2015).

Combined Effect of n-3 PUFAs and Exercise on Cardiac Function and Endurance Capacity in Healthy Humans and CV Patients

There is a lack of well-controlled studies on the interaction of n-3 PUFA intake and exercise, particularly in CV patients. In regard to healthy humans, an increase in anaerobic endurance capacity (but not in 20 m sprint performance), explosive leg power, and 1RM knee extensor strength was observed in competitive soccer players who took n-3 PUFA supplementation during 4 weeks of training, suggesting an interaction of n-3 PUFA intake with exercise that requires further study (Gravina et al., 2017). Also, there is a certain level of interaction of dietary n-3 PUFA intake and the level of concomitant habitual exercise in healthy adult subjects, benefiting cardiac autonomic control, measured as heart rate variability (Harbaugh et al., 2013).

Fish oil-enriched diet might cause changes in some systemic hemodynamic parameters and cardiac function (e.g., mean aortic pressure and heart rate) related to stress (moderate exercise), suggesting increased production of endothelium-derived relaxing factor (Lortet and Verger, 1995). For example, rats that were consuming fish oil had decreased vascular resistance (Demaison et al., 2000). Also, n-3 PUFAs exhibited several potential anti-arrhythmic effects in animal models (Den Ruijter et al., 2007). Furthermore, a cross-sectional study in 992 patients with stable coronary artery disease demonstrated that levels of n-3 PUFAs (DHA + EPA) were strongly associated with heart rate recovery, exercise capacity, and exercise time. Increased levels of n-3 PUFAs were also associated with decreased risk of impaired heart rate recovery and exercise time in these patients (Moyers et al., 2011).

EFFECTS OF n-3 PUFAs ON THE SKELETOMUSCULAR SYSTEM IN HEALTHY PEOPLE

Skeletal muscle is a plastic tissue, sensitive to changes in dietary lipids, capable of adapting to diet and physical activity, with a high level of ability to alter its phenotype, depending on prior nutritional status of the muscle. As the main component of cellular membranes, n-3 PUFAs participate in enzyme regulation and act as signaling molecules (Jeromson et al., 2015).

Increased physical activity, such as intense training, competition situations in elite athletes and rehabilitation physiotherapy (e.g., in CV patients), is followed by muscle microtrauma, oxidative stress, inflammation, neutrophilia, dehydration, lactic acid accumulation, fatigue of central nervous tissue, nutrient stores catabolism, and soreness. Muscle stem cells, tissue satellite cells, are important for the process of growth and recovery of skeletal muscles, reacting to the regeneration process due to mechanical stress induced by exercise (Hawley et al., 2014; Tachtsis et al., 2018). Potential consequences of reducing inflammation after exercise would be faster recovery time, pain reduction, minimization of post-exercise pain, facilitation of exercise training in individuals ranging from patients who are starting exercise programs, medical treatments (physical therapy, cardiac rehabilitation), or athletes undergoing heavy conditioning (Hawley et al., 2014; Tachtsis et al., 2018). Anti-inflammatory effects of n-3 PUFAs could promote muscle stem cell responsiveness to injury by attenuating systemic inflammation (Apolinário et al., 2015; Mackey et al., 2016).

Athletes' diet may contain n-3 PUFAs considering that they may have an impact on muscle remodeling, muscle recovery, and immune surveillance; however, a small number of studies were conducted in elite athletes. Studies in young and middle-aged (Smith et al., 2011b) or older adults (Smith et al., 2011a) have demonstrated that 8 weeks of n-3 PUFA supplementation (1.86 g of EPA and 1.50 g of DHA) increased muscle protein synthesis rates by sensitizing skeletal muscle to potent anabolic stimuli, such as amino acids and insulin. This effect of n-3 PUFAs includes direct incorporation of n-3 PUFAs into the muscle phospholipid membrane (Smith et al., 2011b; McGlory et al., 2014). Another interesting finding is that n-3 PUFAs may have an effect on preventing and faster healing of slighter soft-tissue injuries caused by exercise (Calder, 2012). Direct incorporation of n-3 PUFAs in the muscle cell membrane (McGlory et al., 2014) and their ability to modify the structural

integrity of the cell membrane, together with anti-inflammatory properties of n-3 PUFAs, indicate a protective role of n-3 PUFAs in reducing the effect of eccentric muscle strain on muscle damage (Calder, 2012). Several examples confirm that speculation. Patients with rotator cuff-related shoulder pain taking 1.53 g EPA and 1.04 g DHA for 8 weeks exhibited improvements in disability and pain after 3 months (Sandford et al., 2018). The double-blind study of Lewis and Sandford has shown significant pain reduction after 32 days of n-3 PUFA supplementation and administration of antioxidant pills in recreational athletes with tendinopathies (Lewis and Sandford, 2009). Moderate beneficial effect of n-3 PUFAs (551 mg EPA and 551 mg DHA) on muscle soreness with improved explosive power was observed in elite rugby players (Black et al., 2018). In healthy elderly women, a diet containing n-3 PUFAs improved dynamic explosive strength capacity in resistance training (Edholm et al., 2017). However, not all examples had positive effects. For example, there was no effect of n-3 PUFAs on strength, power and speed improvement, although there was an increment in anaerobic endurance capacity in soccer players (Gravina et al., 2017).

The effect of EPA and DHA on muscle mass in humans is limited. While Smith and colleagues reported increment in muscle protein synthesis after 1.86 g of EPA and 1.5 g of DHA per day for 8 weeks (Smith et al., 2011b), and increment in tight muscle mass in a group of elderly persons (Smith et al., 2015),the same effect was not observed in young athletes. In summary, positive effects of n-3 PUFAs on muscle mass are demonstrated in sedentary people, while effects in trained subjects are still unclear. Improvement in muscle function was also observed by using electromyography and measuring electrical mechanical delay in sedentary and trained people (Rodacki et al., 2012; Lewis et al., 2015). The use of EPA and DHA can have an effect after training on neuromuscular adaptation by implementing n-3 PUFAs in muscle and nerve cells (Bourre, 1989), resulting in improvement of acetylcholine sensitivity and fluidity of the membrane (Lauritzen et al., 2001; Rodacki et al., 2012; Da Boit et al., 2017).

Most of the previous studies investigated the effect of n-3 PUFAs on inflammatory processes and muscle metabolism during exercise, and only a few evaluated their effect on exercise performance (Armstrong et al., 1991; Balvers et al., 2010; Jouris et al., 2011; Mickleborough et al., 2015; Da Boit et al., 2017). For example, for people starting with new exercise programs in order to improve tolerance to new and/or stressful exercises, for those who are performing more intense exercises to reduce pain and stiffness of the muscles, and thus for greater adaptability to training and preparation for competition, supplementation of DHA in diet is justified (Corder et al., 2016).

Data obtained through studies on humans about n-3 PUFA supplementation and its effects in exercise response are still inconclusive, possibly due to the differences in definitions of heterogeneity in study designs; setting, mode, intensity, and duration of exercise; definition of population; different types, dosage, and duration of n-3 PUFA supplementation; the timing of measurements and selections of biomarkers; individual responsiveness and adherence to exercise and dietary protocols, and variation in disease substrates (Mickleborough et al., 2015; Calvo et al., 2017; Da Boit et al., 2017; Kones et al., 2017).

THE EFFECT OF n-3 PUFA SUPPLEMENTATION ON PHYSICAL PERFORMANCE IN CARDIOVASCULAR PATIENTS

A long-term CV effect of exercise training is a decrease in heart rate and heart rate variability due to increased vagal tone (Routledge et al., 2010). Obesity is a known CV risk factor. Since, it was established that dietary intake enriched with fish oil containing n-3 PUFAs may extend life expectancy, many theses about their anti-obesity properties have arisen. Moreover, studies in animal models consistently report that n-3 PUFAs reduce fat mass, particularly in the retroperitoneal and epididymal regions. However, such effects in humans are still under debate. Moreover, despite the thesis on the anti-obesity effect of n-3 PUFAs, human studies on this issue did not demonstrate their positive effects on adiposity and body composition with certainty, but instead they reported that n-3 PUFAs may not aid weight loss (Albracht-Schulte et al., 2018). Moreover, completely contradictory results were brought by a recent study which demonstrated that a higher n-3/n-6 PUFAs ratio in healthy middle-aged women led to adiposity (increased waist circumference) and higher levels of triglycerides, glucose and insulin (Torres-Castillo et al., 2018). Another study showed that consumption of n-3 PUFAs is not associated with total body fat and body fat distribution in the same group of women (Muka et al., 2017). Large meta-analysis from 2018 on the effect of n-3 PUFAs on primary and secondary prevention of CV diseases, which included a total of 7100 both healthy participants and participants with existing illness from 12 trials, reported that increasing n-3 PUFA intake probably causes slight weight gain (Abdelhamid et al., 2018).

Dietary n-3 PUFA intake combined with lifestyle modifications (including increase in regular exercise) leads to improvement of the clinical signs of peripheral vascular disease (claudication) in these patients (N = 24 male patients). Also, significant changes in lipid lipoprotein composition, specifically decreased LDL cholesterol, were observed (Nestares et al., 2003). In patients with non-ischemic dilated cardiomyopathy (N = 133, randomized to experimental and placebo group), intake of n-3 PUFAs at a dose of 2 g/day for 12 months increased the left ventricular systolic function and cardiac functional capacity, compared to placebo treatment, which might reduce hospitalizations for heart failure (Nodari et al., 2011). However, there is a lack of data on the combined effect of n-3 PUFAS and exercise on physical performance in CV patients. One may only speculate that they may have positive additive effects, particularly in CV patients that develop cachectic conditions.

SUMMARY AND CONCLUSION

This manuscript aimed to provide a comprehensive review of less studied effects of diet and lifestyle on hemorheology,

inflammation, and vascular function in healthy persons and CV patients. Since n-3 PUFA supplements are widely used in the general population and prescribed to CV patients, the focus of this review was on n-3 PUFAs effects complementary or interactive with physical activity. It turned out that the field is inconsistent, due to a wide spectrum of conducted studies claiming opposite results (from little or no effect on CV parameters to beneficial effects on micro and macrovascular function, inflammation and potentially prevention of thrombosis and atherosclerosis. Considering that CV and metabolic diseases have underlying low-grade inflammation at the level of endothelium, it is of particular interest to evaluate all lifestyle factors that can affect these conditions. In this sense, n-3 PUFAs and exercise appear to be good candidates. Major limitations in analysis were the following: heterogeneous study approach, including (a) human and animal studies using transgenic mice or inbred animals subjected to specific challenge; (b) age and health status of the studied population (obese, suffering of CV, autoimmune, inflammatory disorders, focus on primary or secondary prevention of CV events); (c) acute or chronic and moderate or extreme physical activity; (d) dose, composition, and duration of n-3 PUFA supplementation. Dietary intake of n-3 PUFAs is mainly by supplements and very seldom there are studies on functional (enriched) food involved. Nevertheless, results suggest that there is an open space for evaluating the impact of consumption of n-3 PUFAs and exercise on characteristics of blood composition and hemorheology, antithrombic effects, and microvascular function. In the field of functional food in particular, there is a lack of RCT obtained data. Hence, more translational controlled clinical studies with defined experimental protocol are necessary for better understanding of the effects of n-3 PUFA supplementation in health and disease.

AUTHOR CONTRIBUTIONS

All authors wrote and revised the manuscript, edited the figures, and approved the submitted version of the manuscript.

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frontiers in Physiology

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3-Weeks of Exercise Training Increases Ischemic-Tolerance in Hearts From High-Fat Diet Fed Mice

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Physical activity is an efficient strategy to delay development of obesity and insulin resistance, and thus the progression of obesity/diabetes-related cardiomyopathy. In support of this, experimental studies using animal models of obesity show that chronic exercise prevents the development of obesity-induced cardiac dysfunction (cardiomyopathy). Whether exercise also improves the tolerance to ischemia-reperfusion in these models is less clear, and may depend on the type of exercise procedure as well as time of initiation. We have previously shown a reduction in ischemic-injury in diet-induced obese mice, when the exercise was started prior to the development of cardiac dysfunction in this model. In the present study, we aimed to explore the effect of exercise on ischemic-tolerance when exercise was initiated after the development obesity-mediated. Male C57BL/6J mice were fed a high-fat diet (HFD) for 20–22 weeks, where they were subjected to high-intensity interval training (HIT) during the last 3 weeks of the feeding period. Sedentary HFD fed and chow fed mice served as controls. Left-ventricular (LV) post-ischemic functional recovery and infarct size were measured in isolated perfused hearts. We also assessed the effect of 3-week HIT on mitochondrial function and myocardial oxygen consumption (MVO_2). Sedentary HFD fed mice developed marked obesity and insulin resistance, and demonstrated reduced post-ischemic cardiac functional recovery and increased infarct size. Three weeks of HIT did not induce cardiac hypertrophy and only had a mild effect on obesity and insulin resistance. Despite this, HIT improved post-ischemic LV functional recovery and reduced infarct size. This increase in ischemic-tolerance was accompanied by an improved mitochondrial function as well as reduced MVO_2. The present study highlights the beneficial effects of exercise training with regard to improving the ischemic-tolerance in hearts with cardiomyopathy following obesity and insulin resistance. This study also emphasizes the exercise-induced improvement of cardiac energetics and mitochondrial function in obesity/diabetes.

Keywords: obesity, exercise, mice, Langendorff, mitochondria, MVO_2, infarct size, functional recovery

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INTRODUCTION

Cardiovascular disease is a major cause of morbidity and mortality in type 2 diabetic patients (Kannel and McGee, 1979). Obese and diabetic patients are also at risk of developing a specific cardiomyopathy. The pathogenesis of this cardiomyopathy is multifactorial and complex, including fibrosis, inflammation, mitochondrial dysfunction, altered substrate utilization, oxidative stress and altered Ca^{2+} handling (Bugger and Abel, 2014; Jia et al., 2018). An early hallmark is also elevated myocardial oxygen consumption (MVO_2), leading to cardiac inefficiency (Boudina et al., 2007; Wright et al., 2009; Cole et al., 2011; Hafstad et al., 2013; Lund et al., 2015). Diabetes also increases the risk of acute myocardial infarction as well as death following infarction (Haffner et al., 1998). In accordance, experimental studies using animal models of obesity, insulin resistance and/or diabetes generally show less tolerance to ischemic-reperfusion injury. Although the pathophysiological mechanisms contributing to ischemic injury in normal hearts has been subject to a comprehensive investigation, the underlying mechanisms leading to the higher ischemic susceptibility in the diabetic heart are not well known. As increased oxygen consumption will be particularly disadvantageous under conditions of limited oxygen availability, there is reason to suggest that the obesity/diabetes-induced increase in MVO_2 contributes to a higher susceptibility to ischemic-reperfusion injury.

Exercise training is considered a key element in the management of type 2 diabetes (Colberg et al., 2010; Zanuso et al., 2010) as well as in the prevention and treatment of cardiovascular diseases (Myers et al., 2002). In support of this, experimental studies have shown that chronic exercise not only reduces obesity and insulin resistance (Zanuso et al., 2010) but also prevents or ameliorates the development of cardiac dysfunction (Bidasee et al., 2008; Lu et al., 2017). Accordingly, we have previously found reduced obesity and insulin resistance, accompanied by preserved cardiac function and decreased infarct size after 10 weeks of high-intensity exercise training (HIT) in mice fed a high-fat diet (HFD) for 18 weeks (Lund et al., 2015). As the exercise procedure was initiated prior to the development of cardiac dysfunction, its effect on the heart could have been due to delayed progression and severity of obesity/diabetes-related cardiomyopathy. In the present study, we aimed to explore the cardiac effect of exercise training with regard to improving ischemic-tolerance in a model with obesity-mediated cardiomyopathy.

MATERIALS AND METHODS

Animals and Exercise Protocol

C57BL/6J male mice (5–6 weeks) were purchased from Charles River Laboratories (Germany). Obesity and insulin resistance were induced by feeding the mice a (HFD 58V8, TestDiet, United Kingdom, 60% of calories from fat) for 20–22 weeks. All mice received chow and drinking water *ad libitum* and were housed at 23°C on a reversed light-dark cycle. Due to the nocturnal nature of mice, all exercise training occurred during their dark cycle. During the last 3 weeks of the feeding regime, the mice were assigned to maintain a sedentary lifestyle (HFD_{SED}) or high-intensity interval training (HFD_{HIT}) by treadmill running 5 days/week as previously described (Hafstad et al., 2011, 2013). The exercise protocol consisted of 10 bouts of 4-min, high-intensity, treadmill running at 25° inclination, corresponding to 85–90% of VO_{2max}, interspersed by 2 min active rest (Hafstad et al., 2011, 2013). Aerobic capacity, determined as VO_{2max}, was assessed before and after the 3–wk exercise protocol, using a treadmill in a metabolic chamber (Columbus Instruments, Columbus, OH) (Hafstad et al., 2011). Blood was collected from the saphenous vein following a 4-h fasting period. Blood glucose concentration was measured with a glucometer (FreeStyle Lite, Alameda, CA, United States), and plasma insulin was analyzed using commercial kits from DRG Diagnostics (Marburg, Germany). Animal experiments were approved by the Norwegian National Animal Research Authority (FDU ID 3698), which conforms to the National Institute of Health guidelines (NIH publication No. 85-23, revised 1996) and European Directive 2010/63/EU.

Assessment of Left Ventricular Susceptibility to Ischemic Injury

Isolated perfused hearts were subjected to ischemia-reperfusion, and post-ischemic recovery of left ventricular (LV) function was assessed using an intra-ventricular fluid-filled balloon where a vent was also inserted into the LV, through the apex. The volume of the balloon was adjusted to give an end-diastolic pressure of 5–10 mmHg. The hearts were perfused in a recirculating mode, with a modified Krebs-Henseleit bicarbonate buffer supplemented with 5 mM glucose and 0.4 mM palmitate prebound to 3% BSA. After 20 min stabilization and 25 min global ischemia, post-ischemic functional recovery was measured over a 60 min period. Reperfusion was continued for an additional 40 min to allow for determination of infarcted tissue. At the end of reperfusion, hearts were frozen at −20°C, prior to slicing and staining using a 1% 2,3,5-triphenyl-2H-tetrazolium chloride solution. Infarct size was determined using ImageJ software (National Institutes of Health, Bethesda, MD, United States). Fiber-optic oxygen probes (FOXY-AL300; Ocean Optics, Duiven, Netherlands) were used to assess PO_2 in the perfusion buffer above the aortic cannula and in the pulmonary artery. Coronary flow and the arterial-venous difference of PO_2 was used to determine myocardial oxygen consumption (MVO_2) as previously described (Lund et al., 2015; Boardman et al., 2017). In order to assess a work-independent (unloaded) MVO_2, the hearts were subjected to an unloaded perfusion condition to minimize the workload (Boardman et al., 2009).

Mitochondrial Respiration

Cardiac mitochondria were isolated from hearts harvested prior to the ischemic insult. Briefly, tissue from the left ventricle was homogenized and trypsinized (5 mg/mL) in isolation buffer (250 mM sucrose, 0.5 mM EDTA, 10 mM Tris; pH 7.4). After a further homogenization, and differential centrifugation,

mitochondrial pellets were suspended in respiration buffer containing 0.5 mM EGTA, 3 mM $MgCl_2$, 60 mM K-lactobionate, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM sucrose, 1 g/L BSA, 280 U/mL catalase, 20 mM histidine, 20 μM vitamin E succinate, 3 mM glutathione, 1 μM leupeptin, 2 mM glutamate, 2 mM malate, and 2 mM ATP; pH 7.1. Mitochondria were kept on ice for 1 h prior to mitochondrial respiration experiments. Oxygen consumption was measured using an oxygraph (O2-k, Oroboros Instruments, Austria), where pyruvate (5 mM) and malate (2 mM) or palmitoyl-CoA (25 μM), L-carnitine (5 mM), and malate (2 mM) served as substrates. V_0 was defined as the respiration in the presence of substrates before ADP was added. An oxidative phosphorylation state (V_{max}) was defined as the respiration peak after adding 100 μmol/L ADP. Respiration rates were adjusted to protein content (Bradford Protein Assay Kit). The respiratory coupling ratio (RCR) was calculated as V_{max}/V_0.

Statistical Analysis

All data are presented as mean ± standard error of means. Numbers of observations are presented as "n." Differences between three groups were analyzed using one-way ANOVA with multiple comparisons (Holm-Sidak method as *post hoc* test). Where the normality test failed (Shapiro–Wilk test), a Mann–Whitney rank-sum test was performed.

RESULTS

The Effect of HFD and 3-Week HIT on Obesity, Insulin Resistance and Aerobic Capacity

Sedentary mice fed an obesogenic diet for 20–22 weeks (HFD_{SED}) developed obesity as indicated by elevated bodyweight and perirenal fat mass when compared to chow fed control (CON) mice (**Table 1**). Diet-induced obesity was accompanied by a marked insulin resistance (HOMA-IR), as well as reduced aerobic capacity (VO_{2max}).

As expected, subjecting obese mice to 3-week HIT (HFD_{HIT}) resulted in increased aerobic capacity and a small but significant lowering of body weight and perirenal fat mass (**Table 1**). HIT also reduced insulin resistance (HOMA-IR), mainly due to a reduction in circulating insulin levels. It should be noted that 3-week of HIT did not induce cardiac hypertrophy, which was supported by unaltered gene expression of hypertrophic markers (data not shown).

The Effect of HFD and 3-Week HIT on Post-ischemic Functional Recovery and Infarct Size

Previous reports from our group, have demonstrated delayed LV relaxation (increased Tau), increased end-diastolic pressure and an elevated end-diastolic pressure-volume relationship (Pedersen et al., 2018) in this mouse model following LV pressure-volume analysis. In the present study, intraventricular pressure was measured using a fluid-filled balloon in Langendorff perfused hearts (**Table 2**). However, this perfusion mode does not allow for the reliable determination of diastolic function (Pedersen et al., 2018). Although this represents a clear limitation of the present study, it should be noted that we have, in this model, repeatedly documented a diastolic dysfunction at identical age/feeding duration that is relevant for this study (18–20 week on obesogenic diets).

The Langendorff perfusion mode is well suited to examine changes in tolerance to ischemia-reperfusion. Functional recovery was followed during the first 60 min of reperfusion after a period of global ischemia. Hearts from HFD_{SED} mice showed impaired recovery of the rate-pressure-product (RPP, given as % of the pre-ischemic value) when compared to hearts from CON mice (**Figure 1**). As heart rate was not

TABLE 1 | Animal characteristics of control mice (CON) and HFD-fed obese mice subjected to 3 weeks of high-intensity (HFD_{HIT}) exercise training or a sedentary (HFD_{SED}) lifestyle.

	CON	HFD_{SED}	HFD_{HIT}
N	20	20	13
Body weight (g)	31.5 ± 0.6*	47.6 ± 0.7	43.2 ± 1.2*
Tibia length (mm)	18.2 ± 0.1	18.2 ± 0.1	18.2 ± 0.1
Perirenal fat mass (g)	0.3 ± 0.1*	1.5 ± 0.1	1.3 ± 0.1*
Blood glucose $_{fasted}$ (mmol/L)	6.3 ± 0.3	7.5 ± 0.2	6.0 ± 0.2*
Plasma insulin $_{fasted}$ (μg/L)	0.8 ± 0.3*	3.2 ± 0.3	2.3 ± 0.4
HOMA-IR	5.5 ± 0.1*	26.6 ± 2.6	15.2 ± 2.6*
VO_{2max} (mL/min/kg)	48.8 ± 0.7*	43.2 ± 0.5	47.4 ± 0.3*
Heart weight (mg wwt)	143 ± 4	150 ± 3	148 ± 3
Heart weight/tibia length	7.81 ± 0.32	8.12 ± 0.25	8.26 ± 0.17

*Data are means ± SE. Aerobic capacity (VO_{2max}) was measured in 10 and 8 animals, respectively. Homeostatic model assessment of insulin resistance; HOMA-IR. *$p < 0.05$ vs. HFD_{SED}.*

TABLE 2 | Steady-state parameters of LV function obtained in isolated Langendorff perfused hearts from control mice (CON) and HFD-fed obese mice subjected to 3 weeks of high-intensity exercise training (HFD_{HIT}) or a sedentary lifestyle (HFD_{SED}).

	CON	HFD_{SED}	HFD_{HIT}
N	13	16	16
Coronary flow (mL/min)	3.2 ± 0.1	3.7 ± 0.2	3.6 ± 0.2
Heart rate (bpm)	315 ± 10	307 ± 9	293 ± 9
LV max-systolic pressure (mmHg)	137 ± 8	151 ± 10	168 ± 12
LV end-diastolic pressure (mmHg)	9.4 ± 0.7	9.9 ± 0.9	10.2 ± 0.5
LV developed pressure (mmHg)	128 ± 8	141 ± 11	158 ± 11
dP/dt_{max} (mmHg/sec)	4624 ± 384	5603 ± 407	6559 ± 376
dP/dt_{min} (mmHg/sec)	−3394 ± 177	−3818 ± 281	−4339 ± 203
RPP (mmHg*bpm)	40163 ± 2536	43218 ± 3430	45850 ± 2929

*Data are means ± SE. These data include left ventricular (LV) functional assessment in hearts subjected to ischemia reperfusion (included in **Figure 1**) and hearts used for isolation of mitochondria (included in **Figure 3**). dP/dt_{max} and dP/dt_{min}; maximum positive and negative first-time derivative of LV pressure. RPP; rate-pressure-product (developed pressure × heart rate). End-diastolic pressure was adjusted to be between 5 and 10 mmHg. *$p < 0.05$ vs. HFD_{SED}.*

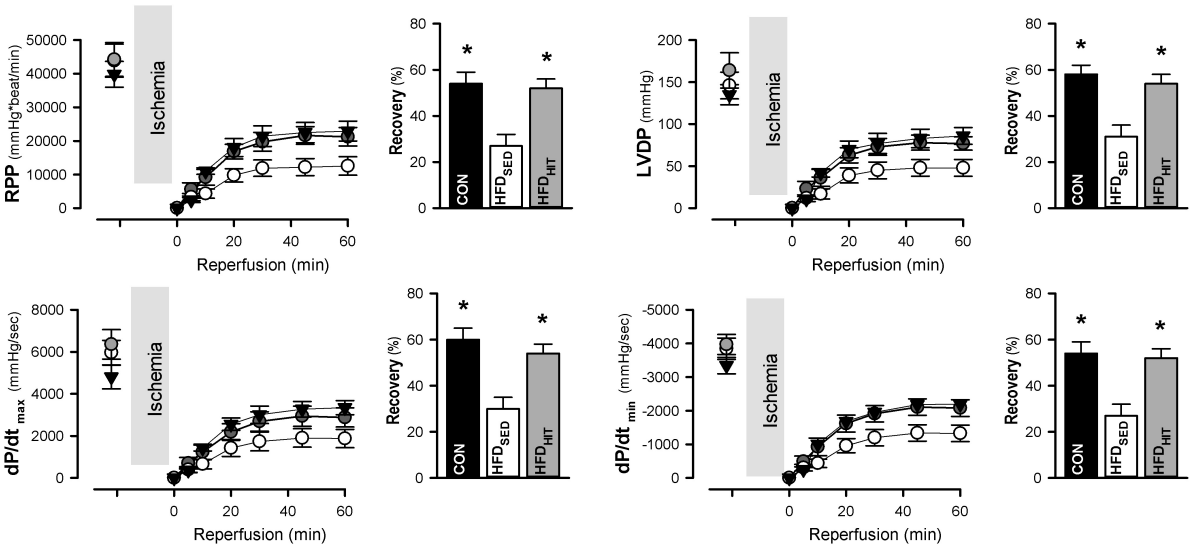

FIGURE 1 | Rate-pressure product (RPP), LV developed pressure (LVDP), and the maximum and minimum of the pressure derivative (dP/dt_{max}, dP/dt_{min}) in isolated perfused hearts from control mice (CON, $n = 8$) and HFD fed mice subjected to a sedentary lifestyle (HFD_{SED}, $n = 10$) or to 3 weeks of high-intensity exercise training (HFD_{HIT}, $n = 6$). Bars indicate the calculated post-ischemic recovery (% of pre-ischemic values) of these parameters. Data are means ± SE. $^*p < 0.05$ vs. HFD_{SED}.

different between the groups, the reduced recovery of RPP was primarily due to the reduced recovery of LV developed pressure (**Figure 1**). Likewise, we also found a significantly impaired recovery of both the maximum and minimum of the pressure derivative (dP/dt_{max} and dP/dt_{min}) indicating increased post-ischemic stunning in HFD_{SED} hearts (**Figure 1**). Three weeks of exercise training was found to significantly improve functional recovery not only of RPP, but also LV developed pressure, as well as dP/dt_{max} and dP/dt_{min} (**Figure 1**). HR was not altered by exercise, which emphasized that exercise increased LV contraction by improving both contractility and relaxation.

Impaired functional recovery of post-ischemic function in HFD_{SED} hearts was accompanied by increased infarct size when compared to CON (**Figure 2**). In addition, 3-week HIT was found to reduce ischemia-reperfusion induced cell death (i.e., infarct size) in hearts from HFD mice.

The Effect of HFD and 3-Week HIT on MVO_2 and Mitochondrial Respiration

This study confirmed that HFD_{SED} hearts demonstrate higher oxygen consumption (MVO_2) compared to CON hearts when perfused in an unloaded state (Lund et al., 2015). Further, we examined mitochondrial respiration in isolated mitochondria from hearts perfused for 30 min, but not subjected to ischemia-reperfusion. The ADP-dependent oxidative phosphorylation state (V_{max}) in mitochondria from HFD_{SED} hearts was reduced when compared to CON hearts, both when using pyruvate as well as palmitoyl-CoA as respiratory substrates (**Figure 3**). As ADP-independent respiration (V_0) was also lower in these hearts, the rate-dependent RCR remained the same in CON and HFD_{SED} (pyruvate: 5.7 ± 0.3 and 5.6 ± 0.3, palmitoyl-CoA: 5.3 ± 0.1 and 5.2 ± 0.2, respectively).

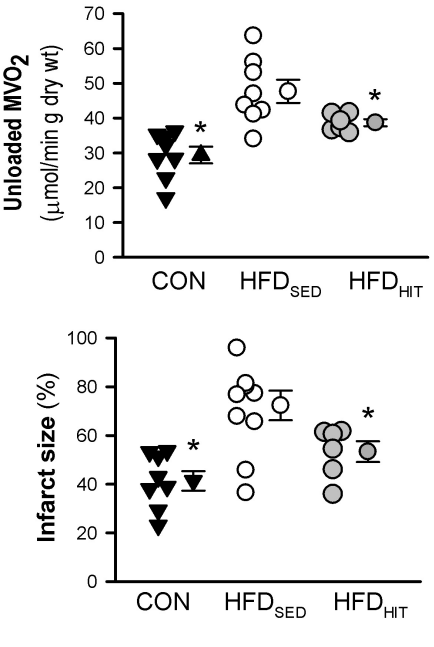

FIGURE 2 | Unloaded MVO_2 and infarct size was measured in isolated perfused hearts from control mice (CON, $n = 8$) and HFD fed mice subjected to a sedentary lifestyle (HFD_{SED}, $n = 10$) or 3 weeks of high-intensity exercise training (HFD_{HIT}, $n = 6$). The unloaded MVO_2 was obtained by deflating the balloon in the LV for a short period during baseline conditions and pacing the hearts at 7 Hz. Data are means ± SE. $^*p < 0.05$ vs. HFD_{SED}.

We have previously demonstrated that 10 weeks HIT reduced unloaded MVO_2 (Lund et al., 2015). The present study shows

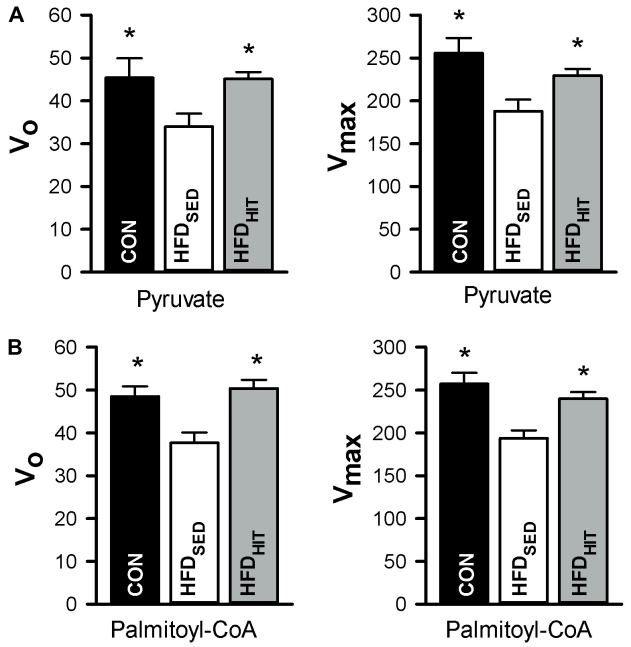

FIGURE 3 | Respiration in isolated mitochondria (nmol O_2/min/mg protein) from hearts of control mice (CON, $n = 5$) mice and HFD-fed obese mice subjected to a sedentary lifestyle (HFD_{SED}, $n = 7$) or 3 weeks of high-intensity exercise training (HFD_{HIT}, $n = 7$). The respiratory medium contained pyruvate (5 mM) and malate (2 mM) **(A,B)** or palmitoyl-CoA (25 μM), L-carnitine (5 mM) and malate (2 mM). V_0 respiration, the respiratory state before ADP is added. V_{max}, the respiration peak after adding 100 μmol/L ADP. Data are means ± SE. $^*p < 0.05$ vs. HFD_{SED}.

that 3 weeks of HIT was sufficient to induce this effect (**Figure 2**). In isolated cardiac mitochondria, 3-week HIT was also shown to increase oxidative phosphorylation (V_{max}) under both substrate conditions (**Figure 3**). Interestingly, exercise also significantly increased V_0, indicating an exercise-mediated induction of a mitochondrial proton-leak. Due to a concomitant increase in both the ADP-dependent and -independent O_2 consumption, we did not find exercise to alter RCR (pyruvate: 5.1 ± 0.3, palmitoyl-CoA: 4.9 ± 0.2). Finally, although 10 weeks of HIT was previously shown to increase CS activity in cardiac tissue in normal hearts (Hafstad et al., 2013), 3-week HIT was not sufficient to alter CS activity in cardiac tissue from HFD mice (10.6 ± 1.0 vs. 12.3 ± 0.4 IU/g wwt, HFD_{SED} vs. HFD_{HIT}, respectively).

DISCUSSION

We have previously shown in diet-induced obese mice, that exercise training initiated prior to changes in cardiac function, prevented the subsequent development of dysfunction (Hafstad et al., 2013; Lund et al., 2015) as well as exacerbated infarction (Lund et al., 2015). As this effect could have been due to delayed progression of the cardiomyopathy (because of reduced obesity and insulin resistance), we have now explored the effect of exercise on cardiac ischemic-tolerance in mice with obesity-mediated cardiomyopathy. We found that a shorter duration of exercise, initiated at the end of the feeding protocol, improved ischemic-tolerance, despite having only mild effects on co-morbidities. We also found that this effect was associated with an exercise-mediated reduction in myocardial oxygen consumption (MVO_2) and improved cardiac mitochondrial respiration.

In accordance with previous studies (Lund et al., 2015; Pedersen et al., 2018), feeding C57BL/6J mice an HFD for more than 18 weeks led to obesity, insulin resistance and low aerobic capacity (VO_{2max}). These mice also develop impaired LV function, primarily manifested as a diastolic dysfunction (Lund et al., 2015; Pedersen et al., 2018). A hallmark of obesity/diabetes related cardiomyopathy is mechanical inefficiency (i.e., the ratio between cardiac work and MVO_2) (Cole et al., 2011; Hafstad et al., 2013; Lund et al., 2015). Cardiac inefficiency occurs prior to development of dysfunction (Wright et al., 2009; Hafstad et al., 2013), and is due to a higher oxygen cost for non-mechanical processes (Hafstad et al., 2013; Lund et al., 2015). Accordingly, in the present study, hearts from the sedentary HFD fed mice, showed a higher unloaded MVO_2. As a high O_2 consumption is particularly detrimental under conditions of limited O_2 availability, this O_2 wasting can contribute to render these hearts more susceptible to an ischemic-injury. In support of this, the present study, as well as a range of other studies (Yi et al., 2011; Pons et al., 2013; Littlejohns et al., 2014; Lund et al., 2015), have reported a decreased tolerance to myocardial ischemia-reperfusion in models of obesity/diabetes.

Chronic exercise training improves obesity and insulin resistance (Colberg et al., 2010; Qiu et al., 2014), and may thus delay or ameliorate the severity of diabetic cardiomyopathy. In accordance, experimental studies from our lab have demonstrated that 10 weeks of exercise training of diet-induced obese mice delayed the progression of obesity, reduced insulin resistance (Hafstad et al., 2013; Lund et al., 2015), prevented the development of LV dysfunction (Hafstad et al., 2013; Lund et al., 2015) and decreased cardiac susceptibility to ischemic-injury (Lund et al., 2015). Notably, in these studies, exercise was started prior to the development of dysfunction (i.e., halfway through the 18-week feeding protocol). The cardiac effects could therefore be due to a postponed development of cardiomyopathy. In the present study, we wanted to examine the effect of shorter exercise period, which did not prevent the development of cardiomyopathy. Therefore, mice were fed HFD for 20–22 weeks, and subjected to HIT during the final 3 weeks of the feeding period.

Compared to the longer exercise protocol (10-week HIT) (Lund et al., 2015), the systemic effects of 3-week HIT were, as expected, less marked. Accordingly, while 10-week HIT reduced perirenal fat by approximately 36–44% (Lund et al., 2015; Boardman et al., 2017), the corresponding reduction by 3-week HIT was only 11%. Similarly, 3-week HIT also resulted in a lesser decrease in HOMA (30 vs. 60%), and a smaller increase in VO_{2max} (7 vs. 20%). Finally, in contrast to 10-week, 3-week HIT was not sufficient to develop cardiac hypertrophy.

We have assessed ischemic susceptibility in hearts perfused in Langendorff perfusion mode with an intraventricular balloon. As this perfusion mode cannot detect changes in diastolic function in mouse hearts (Lund et al., 2015; Pedersen et al., 2018), the present study could not confirm the diastolic dysfunction that has been shown to develop in this model (Lund et al., 2015; Pedersen et al., 2018). Nor can the present study determine whether or not 3-week HIT ameliorated LV diastolic dysfunction. This study, however, clearly demonstrated that 3-week HIT improved post-ischemic functional recovery as well as reduced infarct size. Thus, despite a less marked modification of the associated co-morbidities, these hearts show improved ischemic-tolerance. This is also supported by Pons et al. (2013), who demonstrated that 4 weeks of exercise in *ob/ob* mice, reduced infarct size without altering hyperglycemia, hypercholesterolemia, hyperinsulinemia, fat mass or body weight.

While the mechanisms leading to exercise-induced cardioprotection in normal hearts have been widely studied, fewer studies have examined this in models of insulin resistance and/or type 2 diabetes. The present study supports that exercise can decrease myocardial O_2 wasting found in diabetic cardiomyopathy. The increased O_2-consumption and decreased efficiency are an early and consistent finding in these hearts (Wright et al., 2009; Hafstad et al., 2013). Although the mechanisms for O_2 wasting are not fully understood, several of the reported pathophysiological changes in these hearts can impair mechanoenergetics, by altering ATP production and/or utilization. These changes include elevated fatty acid supply and/or utilization (Belke et al., 2000; Aasum et al., 2003), structural remodeling (i.e., myocardial stiffness) (How et al., 2006), impaired mitochondrial function and oxidative stress (Fauconnier et al., 2007; Anderson et al., 2009), as well as altered Ca^{2+} handling (Stolen et al., 2009; Epp et al., 2013). Importantly, several of these processes, can also be altered by exercise training (Hafstad et al., 2015). As a major part of the work-independent O_2 consumption is linked to maintainance of Ca^{2+} homeostasis, exercise-mediated effects on myocardial Ca^{2+} transport (Stolen et al., 2009; Epp et al., 2013) can lead to decreased MVO_2 (Hafstad et al., 2013). Exercise can also reduce oxidative stress [by increasing antioxidant capacity (Muthusamy et al., 2012)], and improve the redox state (Fisher-Wellman et al., 2013). As we found an exercise-induced mitochondrial proton leak [in the present study, and previously (Hafstad et al., 2013)], it could be suggested that obesity-mediated redox modification of the Ca^{2+} handling proteins is prevented by exercise due to leak-induced decrease in mitochondrial membrane potential.

CONCLUSION

The present study highlights the beneficial effects of exercise training with regard to improving the ischemic-tolerance in hearts with obesity-induced cardiomyopathy. This study also emphasizes exercise-induced improvement of cardiac energetics and mitochondrial function.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the corresponding author, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

This study was carried out in accordance with the recommendation of the National Institute of Health guidelines (NID publication No. 85–23, revised 1996) and European Directive 2010/63/EU. All protocols involving animals in the present study were approved by the Norwegian National Animal Research Authority.

AUTHOR CONTRIBUTIONS

NB, AH, and EA conceived and designed the research, and edited and revised the manuscript. NB, AH, JL, and LR performed the experiments. NB, LR, and EA analyzed the data. NB and EA interpreted the results of the experiments, prepared figures, and drafted the manuscript. NB, AH, JL, LR, and EA approved the final version of the manuscript.

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Stress Echocardiography-Derived E/e' Predicts Abnormal Exercise Hemodynamics in Heart Failure With Preserved Ejection Fraction

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Background: The correlation between echocardiographic parameters and hemodynamics data in patients with heart failure with preserved ejection fraction (HFpEF) is unclear. It is important to find a non-invasive echocardiographic parameter for predicting exercise pulmonary capillary wedge pressure (PCWP).

Aim: This study sought to determine the correlation between echocardiographic parameters and hemodynamics data at rest and during exercise in HFpEF patients.

Methods and Results: This study was a cross-sectional cohort exploratory analysis of baseline data from the ILO-HOPE trial. A total of 34 HFpEF patients were enrolled. The average age was 70 ± 12 years, and most (74%) were women. The patients underwent invasive cardiac catheterization and expired gas analysis at rest and during exercise. Echocardiography including tissue Doppler imaging was performed, and global longitudinal strain and other novel diastolic function indexes were analyzed at rest and during exercise. At rest, no significant correlation was noted between resting PCWP and echocardiographic parameters. However, a significant correlation was observed between post-exercise PCWP and stress E/e′ (septal, lateral, and mean) ratio ($p = 0.003$, 0.031, 0.012). Moreover, post-exercise ΔPCWP showed a good correlation with stress E/e′ (septal, lateral, and mean; all $p \leq 0.001$) and global longitudinal strain (GLS) during exercise ($p = 0.03$). After multivariate regression analysis with adjustment for possible confounding factors including age and sex, there was still a significant correlation between post-exercise ΔPCWP and E/e′ ($r = 0.62$, $p < 0.001$ for E/e'_{mean}).

Conclusion: Only stress echocardiography derived tissue Doppler E/e′ ratio is closely correlated with abnormal exercise hemodynamics (PCWP and post-exercise ΔPCWP) in HFpEF. This echocardiographic marker is substantially more sensitive than other novel echocardiographic parameters during exercise, and may have significant diagnostic utility for ambulatory HFpEF patients with dyspnea.

Clinical Trial Registration: https://www.clinicaltrials.gov, identifier NCT03620526.

Keywords: HFpEF, stress Doppler echocardiography, hemodynamics, GLS, tissue Doppler and strain echocardiography

INTRODUCTION

Heart failure with preserved ejection fraction (HFpEF) is diagnosed using three criteria: signs and symptoms of heart failure, left ventricular (LV) ejection fraction (LVEF) > 50%, and objective evidence of diastolic dysfunction including elevated levels of natriuretic peptide and echocardiographically relevant structural heart disease or abnormal diastolic parameters (Ponikowski et al., 2016). The prevalence of HFpEF is higher than that of heart failure with reduced ejection fraction (HFrEF), and increases significantly with age. HFpEF accounts for 50% of heart failure cases in the community (Dunlay et al., 2017). Physiologically, heart failure can be defined as an imbalance between the cardiac output and metabolic demands, which generally results in increased LV filling pressure (LVFP). However, the diagnosis of HFpEF is sometimes difficult owing to non-specific symptoms, non-significantly elevated natriuretic peptide, and the absence of diastolic dysfunction on resting Doppler echocardiography (Nagueh Sherif et al., 2017). Invasive cardiac catheterization for direct hemodynamic measurements can help solve the problem: elevation of the mean pulmonary capillary wedge pressure (PCWP) could be the evidence of HFpEF (Paulus et al., 2007). Further, patients with suspected early HFpEF with normal LVFP at rest can demonstrate a steep increase in PCWP during exercise in hemodynamic stress testing. The response in a stress hemodynamics study indicates whether the symptoms are of cardiac origin (Kitzman et al., 1991; Maeder et al., 2010).

Although a hemodynamics study through cardiac catheterization remains the gold standard, it is impractical to perform invasive assessments on every patient suspected to have HFpEF, especially during exercise. Previously, the most commonly measured parameter for estimating LVFP was the ratio of early mitral inflow velocity to early diastolic tissue velocity (E/e′). However, only a few studies have evaluated the correlation between exercise E/e′ and invasively measured LVFP. Burgess et al. (2006) found a correlation between E/e′ and LVFP during exercise. Talreja et al. (2007) also found that exercise E/e′ was associated with increased PCWP (> 20 mmHg). Moreover, an abnormal response was defined as exercise E/e′ > 15 on Doppler stress echocardiography based on further outcome studies (Holland et al., 2010; Shim et al., 2011). However, some recent studies suggested that E/e′ does not reflect the LVFP increase during exercise (Maeder et al., 2010; Choi et al., 2016). Therefore, whether non-invasive measurement of E/e′ with stress Doppler echocardiography can represent increased LVFP remains unclear. In this study, we analyzed all patients from the ILO-HOPE trial to determine the relationship between echocardiographic parameters, including traditional tissue Doppler and novel strain parameters, and hemodynamics data at rest and during exercise. We aimed to find applicable echocardiographic parameters to predict abnormal exercise hemodynamics and validate the role of stress echocardiography, which may refine the diagnosis of early HFpEF.

MATERIALS AND METHODS

Study Participants and Study Design

The study is a subgroup analysis of ILO-HOPE trial to determine the association between echocardiographic parameters and hemodynamics data. ILO-HOPE is a prospective, randomized, double-blind, placebo-controlled trial conducted to evaluate the efficacy of iloprost inhalation in improving exercise hemodynamics in HFpEF patients. However, we performed the analysis before iloprost inhalation to avoid the interference. All the patients were enrolled from cardiovascular outpatient clinics with high suspicion for HFpEF. According to the 2016 European Society of Cardiology heart failure guidelines, the American Heart Association, and our previous studies (Wu et al., 2010, 2011, 2015, 2017; Ponikowski et al., 2016), HFpEF is diagnosed according to the following criteria: (i) presence of typical symptoms and signs of heart failure, (ii) LVEF > 50%, (iii) elevated N-terminal pro-B-type natriuretic peptide (NT-proBNP) level (at least > 125 pg/mL), and (iv) echocardiographic structural [left atrial volume index > 34 mL/m^2 or LV mass index ≥ 115 g/m^2 (men) and ≥ 95 g/m^2 (women)] or functional [E/e′ ≥ 13 and mean e′ (septal and lateral wall) < 9 cm/s] changes. After confirming the diagnosis of HFpEF, subjects were hospitalized for cardiac catheterization (left heart for coronary artery evaluation and right heart for hemodynamics data acquisition) and subsequent standardized exercise protocol. Informed consent was obtained before enrolling in the clinical trial. Patients with chronic renal failure (creatinine > 250 μmol/L), significant liver disease, significant coronary artery disease (coronary artery stenosis ≥ 70% without intervention, or a positive stress test), secondary

hypertension, pericardial disease, significant valvular heart disease (> mild stenosis, > moderate regurgitation), cancer, cor pulmonale, congenital heart disease, left-to-right shunt, myocardial infarction within 60 days, high-output heart failure, long-term use of phosphodiesterase 5 inhibitors, or chronic atrial fibrillation were excluded.

In this subgroup analysis, we evaluated the correlation between echocardiographic parameters and hemodynamics data in different phase first (at rest and during exercise). We also performed correlation study between resting echocardiographic parameters and exercising hemodynamics in order to determine whether resting echocardiography can predict hemodynamic response during exercise.

Standardized Exercise Protocol and Hemodynamics Data Acquisition

Cardiac catheterization for hemodynamics recording with simultaneous expired gas analysis was performed at rest and during supine exercise at a 20-W constant workload for 6 min on an electromagnetic braked cycle ergometer (Ergometrics ER800; Ergoline GmbH, Bitz, Germany), as previously described (Borlaug et al., 2015). Arterial and venous blood samples were obtained, and hemodynamic and expired gas data were acquired at rest and during exercise. Right heart catheterization through the right internal jugular vein was performed. The pressure kit transducers were zeroed at mid-axilla. Right atrial pressure, pulmonary artery (PA) pressure, and PCWP were recorded at end-expiration phase by using a 7-Fr Swan-Ganz catheter and high-fidelity micromanometer-tipped catheters (Biosensors International, Singapore) advanced through the lumen of a 7-Fr sheath (Terumo, Tokyo, Japan) in the right internal jugular vein. The mean right atrial pressure and PCWP were measured at mid A-wave. Arterial blood pressure (BP) was continuously measured using a 6-Fr catheter (Terumo) through the radial artery.

Oxygen uptake (VO_2) data were obtained from expired gas analysis with a computerized breath-by-breath metabolic system (MetaMax 3B; Cortex Biophysik GmbH, Germany) and averaged from the 60 s preceding arterial and mixed venous blood sampling (Talreja et al., 2007). Ventilatory efficiency was checked using the ventilatory equivalent for carbon dioxide (VE/VCO_2).

CO and stroke volume were calculated using the direct Fick method and heart rate data. Pulmonary vascular resistance (PVR), PA compliance (stroke volume/PA pulse pressure), and systemic vascular resistance were also obtained using standard formulas. LV systolic performance was assessed according to LV stroke work calculated using the standard formula.

Two-Dimensional and Tissue Doppler Echocardiography

An echocardiographic ultrasound system (IE33; Philips, Andover, MA, United States) was used for echocardiographic examinations at rest and during exercise. Transthoracic echocardiographic images were acquired in the fundamental imaging mode. Each patient also underwent two-dimensional imaging, Doppler echocardiography, and tissue Doppler ultrasonography. LV dimensions and LVEF (M-mode) were measured in the parasternal long-axis view at rest according to the American Society of Echocardiography guidelines (Lang et al., 2005). Left atrial volume index was measured using the biplane area-length method (Lang et al., 2015). Early (E) and late (A) diastolic transmitral velocities and deceleration time were obtained using Doppler echocardiography at rest and during exercise. Peak early diastolic annular velocity was also measured at the septal (e'_{septal}) and lateral ($e'_{lateral}$) mitral annulus on tissue Doppler echocardiography at rest and during exercise. With respect to right heart function, the tricuspid regurgitation peak gradient, tricuspid annular plane systolic excursion (M-mode), and tricuspid annular systolic velocity were measured using echocardiography.

Speckle Tracking

Echocardiographic images were analyzed offline with commercially available software (QLAB Software version 10, Cardiac Motion/Mechanics Quantification; Philips) for speckle tracking. The endocardium border was automatically detected after manually defining the points of the LV basal myocardium and LV apex. Manual adjustment was done if needed. Systolic global longitudinal strain (GLS) was calculated from the magnitude of peak longitudinal strain of 17 ventricular segments (acquired from apical four-chamber, three-chamber, and two-chamber views) according to the American Society of Echocardiography/European Association of Echocardiography consensus statement (Mor-Avi et al., 2011). During offline strain analysis, 10 patients were excluded due to inadequate image acquisition, especially during exercise. All strain analysis was conducted by two experienced cardiologists (Z-WC and C-YH) who were familiar with strain analysis. Intraobserver and interobserver reproducibility was evaluated in 15 randomly selected subjects. The coefficients of variation for GLS were 3.1 and 5.5% for intraobserver and interobserver reproducibility, respectively.

Statistical Analysis

The results are expressed as mean ± standard deviation or *n* (%). Within-group differences of echocardiographic parameters and hemodynamics data between rest and exercise were assessed using paired Student's *t*-test. Pearson's correlation tests were performed to determine correlations between PCWP and echocardiographic parameters at rest and during exercise. The correlation between PCWP and NT-proBNP level was non-parametrically analyzed by Spearman's correlation test. The change of PCWP from the rest to exercise state was recorded as ΔPCWP. The correlation between ΔPCWP and stress echocardiographic parameters was also checked. Significant determinants found in the Pearson's correlation test ($p \leq 0.05$) were then examined using multivariate linear regression with adjustment for age and sex. All statistical analyses were performed using SPSS for Windows version 25.0 (SPSS Inc.,

Chicago, IL, United States). A value of $p \leq 0.05$ was considered statistically significant.

RESULTS

Thirty-four patients were enrolled in ILO-HOPE trial between January and August 2018. The baseline characteristics, including age, sex, body mass index, comorbidities, medications, and laboratory values, are summarized in **Table 1**. The average age was 70 ± 12 years, and 74% were women. Concerning comorbidities, 24 (71%) patients had hypertension, six (18%) had coronary artery disease, and 13 (38%) had diabetes. The median NT-proBNP level was 242 pg/mL.

Echocardiographic Parameters at Rest and During Exercise

Echocardiographic parameters measured at rest and during exercise are listed in **Table 2A**. The subjects had significantly higher mitral E velocity, higher mitral A velocity, shorter deceleration time, higher peak early diastolic annular velocity (septal or lateral mitral annulus), and higher E/e′ ratio during exercise than at rest. The mitral E/A ratio showed no significant difference between rest and exercise. From the strain echocardiography analysis, higher GLS magnitude and higher early diastolic strain rate (SR_e) were noted in the exercise stage. In right-heart-related parameters, higher tricuspid regurgitation peak gradient and tricuspid annular systolic velocity were detected during exercise. The tricuspid annular plane systolic excursion was similar between the exercise and rest stages.

TABLE 1 | Baseline characteristics of HFpEF patients.

	HFpEF (*N* = 34)
Age, years	70 ± 12
Women (%)	25 (74)
Body mass index, kg/m^2	26.1 ± 4.5
Comorbidities	
Coronary disease (%)	6 (18)
Hypertension (%)	24 (71)
Diabetes (%)	13 (38)
Medications	
ACEI or ARB (%)	19 (56)
Beta-blocker (%)	22 (65)
CCB (%)	11 (32)
Statin (%)	10 (29)
Diuretic (%)	15 (44)
Nitrate (%)	4 (12)
Laboratory values	
Hemoglobin, g/dL	12.4 ± 1.5
Creatinine, mg/dL	1.0 ± 0.7
NT-proBNP, pg/mL	242 (195)

Values are mean ± standard deviation, median (interquartile range), or n (%). ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; CCB = calcium channel blocker; NT-proBNP = N-terminal pro-B-type natriuretic peptide.

TABLE 2A | Rest and stress echocardiographic parameters in HFpEF patients (*N* = 34).

	Rest	Stress	*p*-value
LVEDD, mm	47.00 ± 4.47		
LV ejection fraction, %	68.29 ± 7.83		
Left atrial volume index, mL/m^2	34.15 ± 8.65		
Mitral E velocity, cm/s	85.64 ± 22.97	108.99 ± 31.35	**< 0.001**
Mitral A velocity, cm/s	89.96 ± 24.45	100.29 ± 28.51	**< 0.001**
Mitral E/A ratio	1.04 ± 0.57	1.18 ± 0.59	0.143
Deceleration time, ms	190.00 ± 49.48	140.29 ± 33.62	**0.006**
Tissue Doppler echocardiography			
e'_{septal}, cm/s	6.68 ± 2.03	7.90 ± 2.57	**< 0.001**
$e'_{lateral}$, cm/s	8.73 ± 2.55	9.80 ± 2.66	**< 0.001**
e'_{mean}, cm/s	7.70 ± 2.18	8.85 ± 2.39	**< 0.001**
E/e'_{septal}	13.40 ± 3.79	14.85 ± 6.31	**< 0.001**
$E/e'_{lateral}$	10.09 ± 2.41	12.52 ± 7.97	**0.011**
E/e'_{mean}	11.44 ± 2.77	13.37 ± 6.88	**0.001**
Strain echocardiography			
GLS,%	−17.33 ± 1.97	−18.39 ± 2.39	**0.009**
AP2 L. strain,%	−17.70 ± 1.98	−18.77 ± 2.88	**0.038**
AP3 L. strain,%	−17.23 ± 2.75	−17.88 ± 2.73	0.248
AP4 L. strain,%	−17.53 ± 2.25	−18.50 ± 2.16	**0.002**
SR_{IVR}, 1/s	0.28 ± 0.11	0.30 ± 0.10	0.396
E/SR_{IVR}, cm	335.76 ± 127.33	384.91 ± 158.59	0.194
SR_e, 1/s	0.77 ± 0.18	0.98 ± 0.22	**< 0.001**
E/SR_e, cm	111.68 ± 30.45	113.47 ± 42.54	0.779
Right heart function parameters			
TRPG, mmHg	27.17 ± 9.18	41.83 ± 10.84	**< 0.001**
TAPSE, cm	2.29 ± 0.45	2.76 ± 1.99	0.281
TAS′, cm/s	13.18 ± 2.92	14.80 ± 4.46	**< 0.001**

Values are mean ± standard deviation. LVEDD = left ventricular end diastolic dimension; mitral E/A ratio = ratio of peak early (E) to peak late (A) diastolic transmitral velocities; e′septal/lateral/mean = peak early diastolic annular velocity measured at the septal/lateral mitral annulus and their mean; E/e′septal/lateral/mean = ratio of E to e′septal/lateral/mean; GLS = global longitudinal strain; AP2/AP3/AP4 L. strain = longitudinal strain in apical two-chamber/three-chamber/four-chamber view; SR_{IVR} = strain rate during isovolumetric relaxation; E/SR_{IVR} = ratio of E to SR_{IVR}; SR_e = early diastolic strain rate; E/SR_e = ratio of E to SR_e; TRPG = tricuspid regurgitation peak gradient; TAPSE = tricuspid annular plane systolic excursion; TAS′ = tricuspid annular systolic velocity. The bold font character means statistically significance ($p < 0.05$).

Hemodynamics Data at Rest and During Exercise

Resting and exercise hemodynamic changes were recorded (**Table 2B**). At rest, the subjects had elevated BP (systolic BP = 170 ± 23 mmHg, mean BP = 108 ± 13 mmHg), elevated PCWP (18 ± 7 mmHg), mildly increased PVR (1.02 ± 0.94 mmHg/L/min), and normal CO (5.3 ± 2.2 L/min). During exercise, all subjects had significantly increased heart rate, BP, PA pressure, PCWP, LV stroke work, and cardiac output. Concerning metabolic factors, both VO_2 and CaO_2–CvO_2 significantly increased during exercise. However, PVR and PA compliance presented a downtrend after exercise but without statistical significance.

TABLE 2B | Baseline and exercise hemodynamics in HFpEF patients (N = 34).

	Rest	20-W exercise
Vital signs		
Heart rate, beats/min	69 ± 10	102 ± 23†
Systolic BP, mmHg	170 ± 23	185 ± 45‡
Diastolic BP, mmHg	77 ± 12	81 ± 13‡
Mean BP, mmHg	108 ± 13	118 ± 16†
Central pressures		
RA, mmHg	9 ± 4	15 ± 6†
PA systolic, mmHg	34 ± 11	55 ± 15†
PA mean, mmHg	22 ± 7	37 ± 11†
PCWP, mmHg	18 ± 7	29 ± 9†
Vascular and ventricular function		
PVR, mmHg/L/min	1.02 ± 0.94	0.96 ± 1.00
PA compliance, mL/mmHg	5.1 ± 2.8	4.2 ± 2.8
SVR, DSC	1699 ± 614	969 ± 372†
LVSW, g/beat	95 ± 43	113 ± 40‡
Integrated function and metabolism		
VO_2, mL/min	218 ± 79	572 ± 131†
CaO_2–CvO_2, mL/dL	4.3 ± 0.8	6.5 ± 1.8†
CO, L/min	5.3 ± 2.2	9.5 ± 4.0†
Stroke volume, mL	78 ± 36	96 ± 37‡

*Values are mean ± standard deviation. *Columns show rest and exercise hemodynamics. All between-group comparisons at rest and during exercise are p = not significant. †p < 0.0001 versus baseline, within-subject change. ‡p ≦ 0.05 versus baseline, within-subject change. BP = blood pressure; CaO_2–CvO_2 = arteriovenous O_2 content difference; CO = cardiac output; DSC = dyne/s · cm^5; LVSW = left ventricular stroke work; PA = pulmonary artery; PCWP = pulmonary capillary wedge pressure; PVR = pulmonary vascular resistance; RA = right atrial; SVR = systemic vascular resistance; VO_2 = oxygen consumption; W = watts.*

Correlation Between PCWP and Echocardiographic Parameters

At rest, no echocardiographic parameters, including tissue Doppler and strain echocardiography, correlated well with PCWP (**Table 3A**). Among the clinical parameters, only NT-proBNP showed a significant correlation with resting PCWP (p = 0.028) (**Table 3B**). During exercise, mitral E velocity, mitral E/A ratio, deceleration time, and $E/e'_{septal/lateral/mean}$ revealed significant correlations with exercising PCWP (**Table 3A**). Moreover, stress echocardiographic parameters, including mitral E velocity, mitral E/A ratio, deceleration time, and $E/e'_{septal/lateral/mean}$, showed an even better correlation with post-exercise ΔPCWP (**Table 3A**). GLS also showed a significant correlation (p = 0.03) with ΔPCWP. These significant parameters remained independent factors after multivariate linear regression analysis with adjustment for age and sex (**Table 4**). The correlation between exercise E/e'_{septal} and post-exercise PCWP/ΔPCWP is plotted in **Figures 1A,B**.

DISCUSSION

To our knowledge, this is the first study to examine and compare the correlation between LV diastolic echocardiographic parameters, including traditional tissue Doppler and novel strain analysis, and PCWP in HFpEF patients. No resting echocardiographic correlated significantly with resting PCWP, while some stress echocardiographic transmitral E wave-derived parameters (mitral E velocity, mitral E/A ratio, deceleration time, and $E/e'_{septal/lateral/mean}$) correlated well with PCWP during exercise. We also found that exercise E/e′ correlated better with PCWP increase than post-exercise PCWP, which emphasizes the importance of diastolic stress echocardiography. Diastolic stress echocardiography was applied to detect impaired LV diastolic function reserve during exercise (Lancellotti et al., 2016). It is of value in the diagnosis of HFpEF in patients with symptoms of breathlessness and poor exercise capacity. Recent guidelines suggested that HFpEF can be diagnosed on the basis of symptoms, preserved ejection fraction, and objective evidence of echocardiographic diastolic dysfunction (Ponikowski et al., 2016). However, HFpEF symptoms often occur during exercise because LVFP can be normal at rest and only increase during exercise, which, in turn, leads to dyspnea and effort intolerance (Holland et al., 2010). Further, even with the newest recommendations for LV diastolic function evaluation, some patients are still classified as indeterminate (Nagueh et al., 2016). The recommended modality is supine bicycle, which allows Doppler recordings and diastolic function assessment throughout the test. Normal hemodynamic changes in HFpEF patients included elevations in LV end-diastolic pressure (LVEDP), PCWP, and PA systolic pressure, which occur in parallel to each other. To non-invasively estimate hemodynamic changes, it is important to validate the correlation between echocardiographic parameters and hemodynamics data, especially PCWP and LVEDP.

Among the diastolic echocardiographic parameters, E/e′ was the most established parameter that is correlated with LVFP. However, the correlation was validated and more reliable in HFrEF (Ommen et al., 2000; Ritzema et al., 2011). Moreover, the existing studies are relatively few and showed only a moderate correlation. A systematic review published in 2016 disclosed that there is insufficient evidence supporting the estimation of LVFP with E/e′, and that the diagnostic accuracy of E/e′ is limited (Sharifov et al., 2016). The pooled correlation coefficient between E/e′ and invasively measured LVFP was 0.56 (Nauta et al., 2018). Moreover, previous studies had small sample sizes and included a wide variety of cardiac diseases, which are not specific for HFpEF patients. Echocardiography and invasive hemodynamics measurements were not always performed simultaneously. Otherwise, very few studies reported the correlations between invasive hemodynamics parameters and other echocardiographic parameters. From the multicentre EACVI Euro-filling study in 2017 (Lancellotti et al., 2017), only mitral E velocity (p = 0.003), mitral E/A ratio (p = 0.01), deceleration time (p = 0.0005), and $E/e'_{lateral}$ (p = 0.03) significantly correlated with invasive LVEDP (estimated using PCWP) in the subgroup analysis of patients with LVEF > 50%. Both E/e'_{septal} and E/e'_{mean} did not correlate well with invasive LVEDP. Further analysis showed no significant difference with regard to percentage in different cut-off of diastolic parameters ($e'_{septal} < 7$ cm/s, $e'_{lateral} < 10$ cm/s, $E/e'_{septal} \geq 15$, $E/e'_{lateral} \geq 13$, $E/e'_{mean} \geq 14$, left atrial volume

TABLE 3A | Correlation between rest/post-exercise PCWP/ΔPCWP and echocardiographic parameters.

	Rest PCWP		Post-exercise PCWP		ΔPCWP	
Rest echocardiographic parameters	**Pearson correlation coefficient**	***p*-value**	**Pearson correlation coefficient**	***p*-value**	**Pearson correlation coefficient**	***p*-value**
LVEDD	0.086	0.634				
LV ejection fraction	−0.302	0.087				
Left atrial volume index	−0.093	0.608				
Mitral E velocity	0.221	0.209			0.032	0.859
Mitral E/A ratio	0.224	0.211			−0.135	0.454
Deceleration time	−0.257	0.143			−0.175	0.322
e'_{septal}	0.313	0.071			−0.235	0.181
$e'_{lateral}$	0.096	0.590			−0.148	0.404
e'_{mean}	0.202	0.252			−0.196	0.266
E/e'_{septal}	−0.061	0.732			0.351	**0.042**
$E/e'_{lateral}$	0.078	0.662			0.180	0.309
E/e'_{mean}	−0.004	0.981			0.274	0.117
GLS	0.186	0.384			0.189	0.378
SR_{IVR}	0.025	0.907			−0.108	0.615
E/SR_{IVR}	0.099	0.645			0.262	0.217
SR_e	0.226	0.287			−0.058	0.788
E/SR_e	0.086	0.689			0.215	0.312
TRPG	0.195	0.268			−0.061	0.732
TAPSE	−0.092	0.603			−0.075	0.674
TAS′	−0.161	0.363			−0.089	0.617
Stress echocardiographic parameters						
Mitral E velocity			0.469	**0.005**	0.532	**0.001**
Mitral E/A ratio			0.559	**0.001**	0.673	**<0.001**
Deceleration time			−0.380	**0.026**	−0.525	**0.001**
e'_{septal}			−0.031	0.860	−0.216	0.219
$e'_{lateral}$			−0.010	0.953	−0.252	0.151
e'_{mean}			−0.023	0.898	−0.257	0.142
E/e'_{septal}			0.493	**0.003**	0.684	**<0.001**
$E/e'_{lateral}$			0.371	**0.031**	0.546	**0.001**
E/e'_{mean}			0.425	**0.012**	0.620	**<0.001**
GLS			0.377	0.069	0.443	**0.030**
SR_{IVR}			0.097	0.654	0.278	0.188
E/SR_{IVR}			0.273	0.197	0.207	0.333
SR_e			0.194	0.364	0.151	0.482
E/SR_e			0.337	0.107	0.369	0.076
TRPG			0.236	0.179	0.130	0.465
TAPSE			−0.065	0.716	0.136	0.444
TAS′			−0.295	0.101	−0.267	0.139

LVEDD = left ventricular end diastolic dimension; mitral E/A ratio = ratio of the peak early (E) to peak late (A) diastolic transmitral velocities; e′septal/lateral/mean = peak early diastolic annular velocity measured at the septal/lateral mitral annulus and their mean; E/e′septal/lateral/mean = ratio of E to e′septal/lateral/mean; GLS = global longitudinal strain; SR_{IVR} = strain rate during isovolumetric relaxation; E/SR_{IVR} = ratio of E to SR_{IVR}; SR_e = early diastolic strain rate; E/SR_e = ratio of E to SR_e; TRPG = tricuspid regurgitation peak gradient; TAPSE = tricuspid annular plane systolic excursion; TAS′ = tricuspid annular systolic velocity. The bold font character means statistically significance ($p < 0.05$).

index $\geq$ 34 mL/m^2, tricuspid regurgitation velocity $\geq$ 2.8 m/s) between LVEDP $\geq$ 15 and $<$ 15 mmHg. The current study population was entirely composed of HFpEF patients. The correlation between main diastolic echocardiographic parameters and PCWP at rest was even poorer in our analysis. Although novel strain echocardiography parameters were also analyzed, the GLS, strain rate (either in isovolumetric relaxation or early diastolic phase), and ratio of mitral E velocity to strain rate all showed no significant correlation to PCWP at rest.

Although diastolic stress echocardiography may help in the diagnosis of HFpEF, the correlation between exercise E/e′ and invasively measured LVFP remains inconclusive. Some studies

TABLE 3B | Correlation between rest/post-exercise PCWP and clinical parameters.

Clinical parameters	Rest PCWP		Post-exercise PCWP	
	Pearson correlation coefficient	*p*-value	Pearson correlation coefficient	*p*-value
Age	−0.088	0.622	−0.042	0.812
Sex	0.300	0.085	0.151	0.395
Body mass index	0.192	0.277	0.059	0.740
Comorbidities				
Coronary disease	0.155	0.383	0.241	0.169
Hypertension	−0.052	0.772	−0.056	0.754
Diabetes	0.263	0.133	0.180	0.307
Medications				
ACEI or ARB	0.190	0.282	0.218	0.216
Beta-blocker	0.149	0.401	0.118	0.505
CCB	−0.163	0.356	−0.006	0.975
Statin	0.216	0.221	0.284	0.103
Diuretic	0.022	0.900	−0.041	0.818
Nitrate	0.122	0.492	0.036	0.842
Laboratory values				
Hemoglobin	−0.124	0.486	−0.122	0.492
Creatinine	0.272	0.120	0.231	0.188
NT-proBNP*	0.483	**0.004**	0.333	0.054

**The correlation between PCWP and NT-proBNP level was non-parametrically analyzed by Spearman's correlation test. ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; CCB = calcium channel blocker; NT-proBNP = N-terminal pro-B-type natriuretic peptide. The bold font character means statistically significance (p < 0.05).*

found a good correlation between exercise E/e′ and LVFP (Burgess et al., 2006; Talreja et al., 2007; Obokata et al., 2017) and even that exercise E/e′ was an independent predictor of outcomes (Holland et al., 2010; Shim et al., 2011; Takagi et al., 2014; Kosmala et al., 2018a,b), but some did not (Maeder et al., 2010; Choi et al., 2016). In their 2017 systematic review, Oleg et al. concluded that the evidence for the usefulness of E/e′ in estimating LVFP during exercise remains limited (Sharifov and Gupta, 2017). Our study provided comprehensive measurements of resting and exercising echocardiographic parameters, as well as simultaneous invasive hemodynamics studies at rest and exercise in our cohort of purely HFpEF patients. From our analysis, mitral E velocity, mitral E/A ratio, deceleration time, and $E/e'_{septal/lateral/mean}$ showed a good correlation with PCWP during exercise.

It had been known that transmitral Doppler E wave is proportionate to the difference between left atrium (LA) pressure and LV diastolic pressure, which was influenced by the rate of myocardial relaxation. Otherwise, tissue Doppler e' velocity is a measure of LV myocardial relaxation in early diastole and relatively load independent (Agmon et al., 2000). As a result, it can be inferred that transmitral E wave-derived parameters show some correlation with PCWP, and combination of E and e' (E/e') may be a better predictor. However, from our result, we found these transmitral E wave-derived parameters (mitral E velocity, mitral E/A ratio, deceleration time, and $E/e'_{septal/lateral/mean}$) only correlated with PCWP significantly when exercising but not at rest. It may be speculated that the correlation between these transmitral E wave-derived parameters and PCWP only exists significantly in condition of elevated LVFP and impaired myocardial relaxation (such as HFrEF or HFpEF when exercising).

Moreover, we found that only resting E/e'_{septal} correlated with increased PCWP during exercise (**Table 3A**). However, in the exercise stage, echocardiographic parameters including mitral E velocity, mitral E/A ratio, deceleration time, and $E/e'_{septal/lateral/mean}$ showed a much better correlation with ΔPCWP (**Table 3A**). These result indicated that the severity of diastolic dysfunction or impaired myocardial relaxation during exercise may influence the change of PCWP more rather than PCWP during exercise. Further, the significant correlations remained after multivariate regression analysis with adjustment for possible confounding factors including age and sex. Dorfs et al. (2014) demonstrated that PCWP increase was associated with increased mortality despite a normal resting PCWP. Reddy et al. (2018) also reported that increased PCWP was associated with reduced exercise capacity. Otherwise, $\Delta E/e'_{septal/lateral/mean}$ also correlated well with ΔPCWP (**Supplementary Table S1**). All these findings emphasize the importance of diastolic

TABLE 4 | Multivariate regression analysis with post-exercise PCWP and ΔPCWP as the dependent variable (adjusted for age and sex) (*N* = 34).

Variable	Post-exercise PCWP			Post-exercise ΔPCWP		
	β (95% CI)	Adjusted R^2	*p*-value	β (95% CI)	Adjusted R^2	*p*-Value
Mitral E velocity, cm/s	0.137 (0.044–0.231)	0.195	**0.005**	0.094 (0.040–0.148)	0.261	**0.001**
Mitral E/A ratio	8.857 (3.956–13.757)	0.289	**0.001**	6.482 (3.824-9.140)	0.434	**<0.001**
Deceleration time, ms	−0.104 (−0.195 to −0.013)	0.118	**0.026**	−0.087 (−0.138 to −0.036)	0.253	**0.001**
E/e'_{septal}	0.718 (0.262–1.174)	0.220	**0.003**	0.603 (0.372–0.834)	0.451	**<0.001**
$E/e'_{lateral}$	0.427 (0.042–0.813)	0.110	**0.031**	0.381 (0.170–0.591)	0.276	**0.001**
E/e'_{mean}	0.568 (0.133–1.002)	0.155	**0.012**	0.501 (0.273–0.729)	0.365	**<0.001**
GLS, %	–	–	**–**	1.147 (0.122–2.171)	0.160	**0.030**

Mitral E/A ratio = ratio of the peak early (E) to peak late (A) diastolic transmitral velocities; e'septal/lateral/mean = peak early diastolic annular velocity measured at the septal/lateral mitral annulus and their mean; E/e'septal/lateral/mean = ratio of E to e'septal/lateral/mean; GLS = global longitudinal strain. The bold font character means statistically significance (p < 0.05).

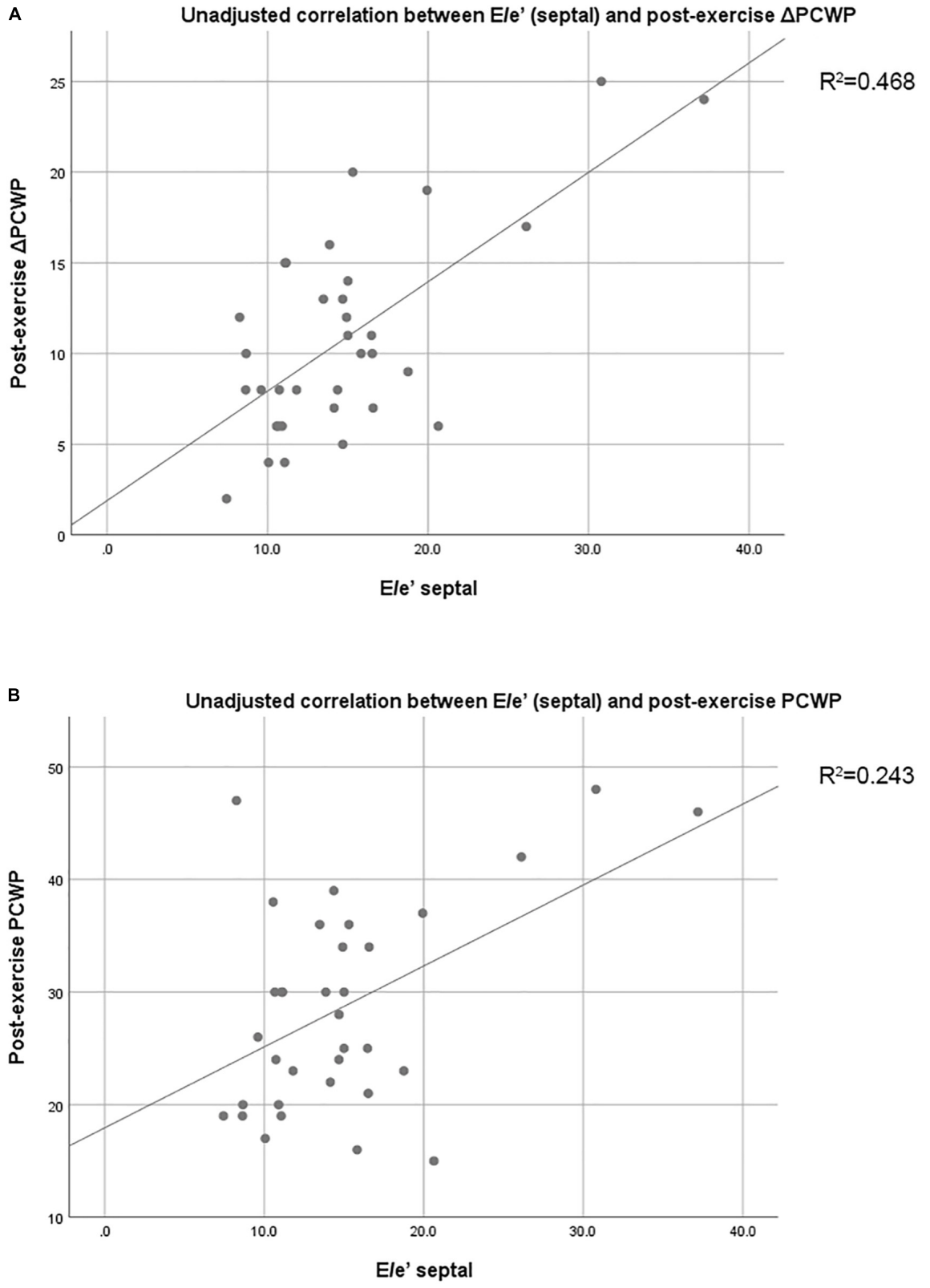

FIGURE 1 | (A) Unadjusted correlation between E/e'_{septal} and post-exercise PCWP. **(B)** Unadjusted correlation between E/e'_{septal} and post-exercise ΔPCWP. PCWP, pulmonary capillary wedge pressure; Δ, change; E/e'_{septal}, ratio of peak early diastolic transmitral velocity to peak early diastolic annular velocity measured at the septal mitral annulus.

stress echocardiography. On the basis of current evidence, we recommend diastolic stress echocardiography as a diagnostic tool for patients suspected of having HFpEF, especially those with a normal or indeterminate resting diastology.

We also performed strain analysis through two-dimensional echocardiographic speckle tracking. Strain is the measurement of myocardium deformation, whereas the strain rate is the speed of myocardial deformity. In previous studies, HFpEF patients had a lower magnitude of GLS and decreased strain rate despite preserved LVEF compared with normal controls (Kraigher-Krainer et al., 2014; Tabassian et al., 2018). Moreover, GLS is associated with reduced exercise capacity in HFpEF patients (Hasselberg et al., 2015). Wang et al. (2007) showed that E/SR_{IVR} best correlated with PCWP, especially when E/e′ ranged from 8 to 15. Magoon et al. (2018) also found that E/SR_e had a better correlation with PCWP than E/e'_{septal} in patients undergoing coronary artery bypass grafting with preserved ejection fraction. Meanwhile, Ebrahimi et al. (2019) reported that SR_{IVR} was a better index for predicting PCWP intra-operatively in patients undergoing coronary artery bypass grafting. However, their study population all had coronary artery disease, and the authors performed the measurements after general anesthesia induction. In our study, although the novel parameter GLS significantly correlated with ΔPCWP during exercise ($p = 0.03$), other diastolic strain-based indices showed a poor correlation with PCWP, either at rest or during exercise. In summary, strain echocardiography has better sensitivity to detect subclinical impairment of systolic function or subtle diastolic dysfunction (Chen et al., 2018), E/e' has better correlation with ΔPCWP during exercise.

At last, the BP response to exercise is an important diagnostic parameter. In healthy subjects, systolic BP rise according to the increasing workload. However, diastolic BP usually remained unchanged or decrease slightly (O'Brien et al., 2002). In baseline characteristics of HFpEF patients, we found that the diastolic pressure increased significantly after 20-W exercise (**Table 2B**). These suggested the HFpEF patient in our study have stiff arteries. Chantler et al. (2008a) investigated the influence of arterial system on left ventricle performance. This interaction is called arterial–ventricular coupling, which could be indexed by the ratio of effective arterial elastance to LV end-systolic elastance (E_A/E_{LV}). During exercise, E_{LV} increased disproportionately to make sure the sufficient cardiac performance to meet the needs of the body. Borlaug et al. (2006) found that HFpEF patient had a threefold smaller increase in E_{LV} during upright bicycle exercise, compared with hypertensive patients with LV hypertrophy. As a result, the change of E_A/E_{LV} during exercise may also be blunted. Otherwise, it can be inferred that these effects have contributed to the exercise intolerance in HFpEF patients (Chantler et al., 2008b), which could be reflected by increased LVFP during exercise and subsequent abnormal stress echocardiographic parameters.

Clinical Implication

Invasive hemodynamic measurements can help solve the confusion in diagnosing HFpEF. The mean PCWP confirms the diagnosis of HFpEF (Paulus et al., 2007), and hemodynamic stress testing could be considered in "gray cases" of patients with early HFpEF with normal filling pressure at rest. In such cases, a steep increase in PCWP during exercise is a typical hemodynamic response in HFpEF, indicating that the dyspnea on exertion is of cardiac origin (Kitzman et al., 1991). Moreover, HFpEF patients usually experience hemodynamic derangement especially during exercise, presenting as a higher LVFP (PCWP). HFpEF is an increasingly recognized cause of pulmonary hypertension due to its emerging epidemic. Some recent studies have shown that the exercise PCWP level is highly associated with the symptoms and life quality of HFpEF patients (Obokata et al., 2018), and more clinical trials have investigated exercise PCWP as a primary outcome (Borlaug et al., 2015). Theoretically, it is not possible to perform invasive exercise hemodynamic testing in every patient. Despite the increasing number of emerging diastolic function echocardiographic parameters, our study suggested exercise E/e' to non-invasively estimate the possible hemodynamic response. By performing echocardiography during standardized exercise tests, the risk and outcomes may be predicted, consequently allowing treatment plan adjustments for HFpEF patients.

Study Limitations

The main limitation of our study is the relatively small sample size. For this reason, some echocardiographic parameters, including strain echocardiography-derived parameters, might not correlate well with PCWP. Moreover, though some parameters correlated significantly, statistical type II error might exist. Second, this study is a subgroup analysis from ILO-HOPE trial. All patient recruitment and exclusion criteria were designed for ILO-HOPE trial. For example, the patients with chronic atrial fibrillation were excluded, and they are not uncommon in HFpEF populations. However, we believe that these selection criteria can also be applied appropriately in our subgroup analysis to evaluate the correlation between echocardiographic parameters and hemodynamics data for most HFpEF patients. Third, some medication may influence the strain analysis (especially beta-blockers), reduce preload, and alleviate LVFP (ACEI or ARB, diuretics, and nitrate). However, the improvement of hemodynamics change is parallel to echocardiographic parameter. Our main finding may not be affected. Fourth, our current study measured echocardiographic data and cardiac performance at rest and under limited levels of exercise but not maximal-effort exercises. As a result, the correlation between hemodynamics data and echocardiographic parameters was unknown at peak exercise. However, it would be difficult for patients to do peak exercises repeatedly in one single test and usually HFpEF patients perform low level of exercises in their daily life, especially the elderly. Fifth, our cross-sectional study cannot infer causality. Also, the coefficient of determination (adjusted R^2) in correlation between E/e'(septal) and post-exercise ΔPCWP is only 0.468. The strength of correlation might be from few patients in the population. Further large-scale studies are required to evaluate

the capacity of exercise E/e' to predict ΔPCWP during exercise in HFpEF patients.

CONCLUSION

E/e′ showed a significant correlation with both exercise PCWP and ΔPCWP even after adjustment for age and sex. Nevertheless, novel strain rate indices showed no association with PCWP and ΔPCWP, whereas GLS correlated with ΔPCWP. As exercise PCWP and ΔPCWP reflect the symptoms of HFpEF patients, exercise E/e′ may further refine the diagnosis of HFpEF. Our study results emphasize the clinical value of diastolic stress echocardiography.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The study was performed in accordance with the Declaration of Helsinki and was approved by the institutional review board of the National Taiwan University Hospital (Clinical trial number: 201704075MIND). All patients provided their written informed consent prior to participation in the study.

AUTHOR CONTRIBUTIONS

C-KW, Z-WC, and L-YL designed the whole study, and analyzed and interpreted the data. C-KW and Z-WC wrote the manuscript. S-YC was also responsible for measurement of oxygen uptake and the computerized breath-by-breath metabolic system. C-KW and L-YL recruited the patients and were also in charge of the whole program. C-YH and Z-WC performed cardiac catheterization and echocardiography for the patients. All the authors critically reviewed the manuscript for important intellectual content.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2019.01470/full#supplementary-material

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Dynamic Force Production Capacities Between Coronary Artery Disease Patients vs. Healthy Participants on a Cycle Ergometer

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Background: The force-velocity-power (FVP) profile is used to describe dynamic force production capacities, which is of great interest in training high performance athletes. However, FVP may serve a new additional tool for cardiac rehabilitation (CR) of coronary artery disease (CAD) patients. The aim of this study was to compare the FVP profile between two populations: CAD patients vs. healthy participants (HP).

Methods: Twenty-four CAD patients (55.8 ± 7.1 y) and 24 HP (52.4 ± 14.8 y) performed two sprints of 8 s on a Monark cycle ergometer with a resistance corresponding to 0.4 N/kg × body mass for men and 0.3 N/kg × body mass for women. The theoretical maximal force (F_0) and velocity (V_0), the slope of the force-velocity relationship (S_{fv}) and the maximal mechanical power output (P_{max}) were determined.

Results: The P_{max} (CAD: 6.86 ± 2.26 W.kg^{-1} vs. HP: 9.78 ± 4.08 W.kg^{-1}, p = 0.003), V_0 (CAD: 5.10 ± 0.82 m.s^{-1} vs. HP: 5.79 ± 0.97 m.s^{-1}, p = 0.010), and F_0 (CAD: 1.35 ± 0.38 N.kg^{-1} vs. HP: 1.65 ± 0.51 N.kg^{-1}, p = 0.039) were significantly higher in HP than in CAD. No significant difference appeared in S_{fv} (CAD: −0.27 ± 0.07 N.kg^{-1}.m.s^{-1} vs. HS: −0.28 ± 0.07 N.kg^{-1}.m.s^{-1}, p = 0.541).

Conclusion: The lower maximal power in CAD patients was related to both a lower V_0 and F_0. Physical inactivity, sedentary time and high cardiovascular disease (CVD) risk may explain this difference of force production at both high and low velocities between the two groups.

Keywords: force-velocity-power relationship, cardiac rehabilitation, physical activity, acute coronary syndrome, cycle sprint, exercise physiology, health

INTRODUCTION

After an acute coronary syndrome, a cardiac rehabilitation (CR) program is essential to restore or increase physical capacities and reduces cardiovascular disease (CVD) risk (Pavy et al., 2012; Iliou et al., 2015; Price et al., 2016). The objective for active subjects is to regain their place in society and for older persons is to maintain their independence (Pavy et al., 2012; Iliou et al., 2015). It is necessary to adapt the content of CR sessions to optimize aerobic and anaerobic performance along with quality of life (Price et al., 2016).

Specifically, the improvement of maximum power output of the neuromuscular system is one of the objectives sought in CR. Muscle power (P), which is the product of force (F), and velocity (V), is essential to enhance anaerobic performance (Cronin and Sleivert, 2005; Morin and Samozino, 2016). Maximal power capacities depend on force production abilities over the entire spectrum of contraction velocities, which can be well described by the force-velocity (FV) relationship (Morin and Samozino, 2016). The orientation of this FV relationship toward rather maximal force at low velocities (i.e., force capacity) or force at high velocities (i.e., velocity capacity) is well characterized by its slope, which refers to the FV profile (Giroux et al., 2016).

Several studies have been carried out on the force-velocity-power (FVP) relationship and sport performance in top athletes (Samozino et al., 2014a,b; Giroux et al., 2016; Morin and Samozino, 2016). This FVP profile can be evaluated on ballistic push-offs and sprint movements (on treadmill or cycle ergometer) (Seck et al., 1995; Morin et al., 2010). Different profiles may be determined according to the type of physical activity and sometimes even the athlete's position (e.g., toward force capacity for forward players and toward velocity for back players in rugby) (Morin and Samozino, 2016). The analysis of the FVP profile highlights the weaknesses in force production capacity of each athlete. A specific training oriented in force or in velocity should be adapted according to whether the athlete wants to maintain his/her specificity or tip the balance of the FV profile that presents a deleterious imbalance for his/her future performances (i.e., change the slope of the right of the FV profile toward an optimal slope) (Jiménez-Reyes et al., 2016; Morin and Samozino, 2016).

Optimizing the exercise training program is constantly sought in rehabilitation among patients always younger with coronary artery disease (CAD) (Price et al., 2016). Indeed, the CAD prevalence rose from an estimated 290 cases per 100,000 for those 40–44 years of age to 11,203 cases per 100,000 at 1990–2015 (Roth et al., 2017). Therefore, it might be interesting to talk about performance even in patients and to use the FVP relationship to more precisely identify this loss of muscle force production capacity in patients suffering from cardiovascular impairment as well a loss of functional capacities of their neuromuscular system. Usual rehabilitation sessions are based on the results of functional explorations performed in aerobic (cardiorespiratory exercise test) and resistance (static and dynamic quadriceps test) at the beginning of the rehabilitation cycle. Instead of using the results of a muscle test, we could rely on the results of the initial FVP. So far, the measurement of muscle strength production capacities has been determined by isometric and dynamic leg extension test. The FVP profile would allow, through a simple and rapid assessment, to target the weakest qualities in patients in order to individualize the training for each person.

The aim of the present study was to analyze and compare the force production capacities through the mechanical variables of the FVP relationship obtained during pedaling between CAD patients and healthy participants (HP). We hypothesized that CAD patients present lower maximal power than HP, with notably a lower maximal force production at low velocity; but without previous studies, we had no evidence to suggest the impact of FV profile (i.e., whether one would be more affected than the other).

MATERIALS AND METHODS

Participants

Coronary artery disease patients volunteered to participate in this study at the beginning of the CR. The inclusion criteria were the following: (a) over 18 years of age; (b) received medical treatment and percutaneous coronary intervention (angioplasty with stent implantation) or surgical revascularisation (coronary artery bypass grafting); and (c) maximal aerobic power superior to 60 W for women and 80 W for men (Borjesson et al., 2011). They received a measurement of their maximal oxygen uptake ($\dot{V}O_2max$) during an ergocycle stress test before and after a CR program. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institution's human research committee. The study was registered in the National Institutes of Health ClinicalTrials.gov database.

HP were 18 years old as patients, they were free from any kind of CVD, performing regular physical exercise and were volunteers to participate. The ethics committee (*IRBN372016/CHUSTE*) and the national commission for informatics and liberties (*CNIL165853*) approved the cohort study for pedaling testing.

History of sport was assessed by only two questions: (1) did you do sports (CAD patients) or (2) do you practice sports (HP)? If yes, which sport? Sport was quantified in weekly metabolic equivalent of task (MET) and expressed in h/week (i.e., intensity in MET × duration × frequency), using the compendium of sports, and ranged from 6 (vigorous intensity = sport) to 18 MET (running at 17 km/h) (Ainsworth et al., 2000).

Experimental Protocol

At the beginning of each experiment, saddle height was adjusted and toe clips were well fastened to avoid losing the pedals. After a 5-min warm-up, participants performed two maximal 8-s duration sprints, separated by a 2-min rest period, against friction loads of 0.4 and 0.3 $N.kg^{-1}$ body mass for men and women, respectively (**Figure 1**). We performed pre-tests to determine these appropriate loads. They had to remain seated during the test. For each trial, the participants had to pedal as fast as possible during all the sprint. For this, the experimenter (MF) encouraged vigorously each participant throughout the sprint. We retained the data of the best sprint (i.e., the one with the highest maximal power).

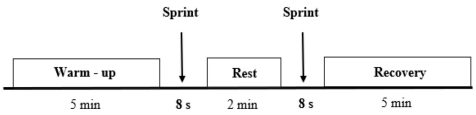

FIGURE 1 | Protocol diagram.

FIGURE 2 | Friction loaded Monark cycle ergometer. A, Strain gauge; B, Belt; C, Optical encoder; D, flywheel.

Material

A friction-loaded cycle ergometer (Monark, Vansbro, Sweden) was used (**Figure 2**). All features of the ergometer were detailed in previous studies (Arsac et al., 1996; Morin and Belli, 2004). The apparatus was instrumented with a strain gauge (FGP Instrumentation, FN 3030 type, Les Cloyes Sous Bois, France) to measure the friction force applied by the tension of the belt and an optical encoder (Hengstler type RI 32.0, 100 pts/turn, Aldingen, Germany) to measure the flywheel displacement. The inertia was determined from the linear relationship obtained by free deceleration of the flywheel. Data were sampled at 200 Hz and recorded in LabVIEW software. Data were filtered with a 4th order low pass Butterworth filter at 30 Hz. Angular velocity and pedaling frequency were calculated from filtered displacement.

F-V Relationship

The power output (P in watts) produced at each instant during the sprint was computed as follows (Morin and Belli, 2004):

$$P = (F_{\text{frict}} + F_{\text{inert}}) \times V, \quad (1)$$

where F_{frict} was the friction force, F_{inert} the inertial force (computed from the flywheel inertia and acceleration) and V the flywheel linear velocity. Instantaneous flywheel linear velocity was calculated from the flywheel displacement. The force ($F = F_{\text{frict}} + F_{\text{inert}}$), power ($P$) and velocity ($V$) variables corresponded to mechanical outputs at the flywheel (Jiménez-Reyes et al., 2016).

The F, V, and P values were averaged for each pedal downstroke, which were defined between two successive minimal values of instantaneous power (Samozino et al., 2007). The lower limb force production capacities can be described by the negative linear relationship (F-V) and the second order polynomial relationship (P-V). From these two relationships, a few parameters, which reflected the mechanical limits of the neuromuscular system, can be determined (**Figure 3**; Driss et al., 2002; Morin and Samozino, 2016): the theoretical maximum force (F_0) which could be developed at zero velocity (intercept value on the y-axis); the theoretical maximum velocity (V_0) until which force could be produced (intercept value on the x-axis); and the maximum power output (P_{max}), corresponding to the maximum power that an individual is able to develop and the slope of F-V relationship (S_{fv}) which can be computed as follows:

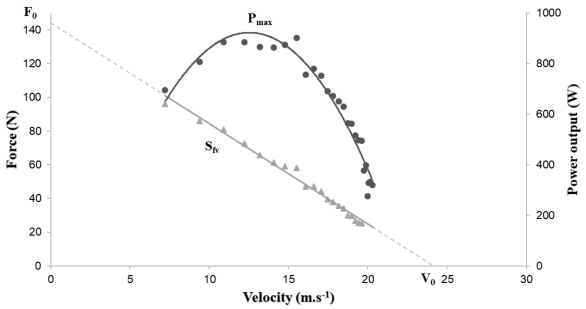

FIGURE 3 | Graphical representation of power-force-velocity relationship.

$$P_{\text{max}} = (F_0 \times V_0)/4, \quad (2)$$

$$S_{\text{fv}} = -F_0/V_0, \quad (3)$$

Statistical Analysis

All data were expressed as mean ± standard deviation (SD). After checking distribution of normality with the Shapiro–Wilk test, Student's t-tests for independent groups were used to detect differences in F_0, V_0, S_{fv}, and P_{max} between the two populations. For all analyses, statistical significance was defined as $p < 0.05$. Cohen's d was also computed to indicate the effect size, which corresponded to the difference between two means divided by the pooled SD. We interpreted the data obtained in this way: <0.2 was trivial, 0.2–0.5 a weak effect, 0.5–0.8 a medium effect, and >0.8 a strong effect (Parker and Hagan-Burke, 2007).

RESULTS

Twenty-four CAD patients and 24 HP aged 55.8 (± 7.1) y and 52.4 (± 14.8) y, respectively, participated in this investigation. Overweight (mean BMI > 25 kg/m^2) concerned 54% of the CAD patients and they had significantly higher BMI than HP (27.5 ± 5.4 vs. 24.4 ± 3.4 kg/m^2, $p < 0.05$). In addition, these patients were physically inactive (5.3 ± 6.8 vs. 39.7 ± 42.0 MET-h/week, $p < 0.001$). Descriptive characteristics of participants are presented in **Table 1**.

TABLE 1 | Morphological characteristics of participants.

Variable	CAD Patients *n* = 24 (*7 Females/17 Males*)	Healthy Participants *n* = 24 (*8 Females/16 Males*)	*p*-value
Age (y)	55.8 ± 7.1	52.4 ± 14.8	0.319
Body mass (kg)	**81.5 ± 17.1***	**71.9 ± 13.6**	**0.036**
Height (cm)	172 ± 9	171 ± 10	0.864
BMI (kg/m^2)	**27.5 ± 5.4***	**24.4 ± 3.4**	**0.020**
PA (MET-h/week)	**5.3 ± 6.8*****	**39.7 ± 42.0**	**0.000**

*BMI, body mass index; PA, physical activity; CAD, coronary artery disease; MET, metabolic equivalent of task. Values presented as means ± SD. Statistical difference between two groups *($p < 0.05$), ***($p < 0.001$).*

TABLE 2 | Mechanical performance sprint variables.

Variable	CAD Patients (*n* = 24)	Healthy Participants (*n* = 24)	*p*-value	Cohen's *d*
F_0 (N)	106.84 ± 27.78	117.14 ± 43.43	0.333	0.283
F_0 (N.kg^{-1})	**1.35 ± 0.38***	**1.63 ± 0.51**	**0.039**	**0.642**
V_0 (m.s^{-1})	**5.10 ± 0.82***	**5.79 ± 0.97**	**0.010**	**0.722**
V_0 (rad.s^{-1})	**19.6 ± 3.16***	**22.3 ± 3.73**	**0.010**	**0.726**
S_{fv} (N.kg^{-1}.m.s^{-1})	−0.27 ± 0.07	−0.28 ± 0.07	0.541	0.142
P_{max} (W)	**543.47 ± 170.36***	**709.88 ± 328.14**	**0.032**	**0.612**
P_{max} (W.kg^{-1})	**6.86 ± 2.26****	**9.78 ± 4.08**	**0.003**	**0.816**

*F_0, theoretical maximum force; V_0, theoretical maximum velocity; S_{fv}, Slope of linear force-velocity relationship; P_{max}, maximal power output; CAD, coronary artery disease. Values presented as means ± SD. Statistical difference between two groups *($p < 0.05$), **($p < 0.01$).*

The average of mechanical parameters of the FVP for each group are reported in **Table 2**. We noted a significant difference for P_{max}, V_0, and F_0. A smaller in P_{max} (−29.8%, $p = 0.003$), V_0 (−11.9%, $p = 0.010$), and F_0 (−18.2%, $p = 0.039$) were observed in CAD patients compared to HP. However, no statistical difference was observed between the two populations for S_{fv} ($p = 0.557$) of FVP (**Figure 4**).

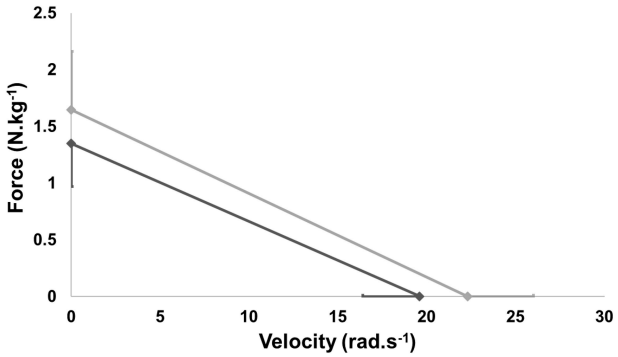

FIGURE 4 | Mean force-velocity profile of healthy subjects and coronary patients.

DISCUSSION

This study was the first to examine the mechanical parameters of FVP in CAD patients. The main findings of this research were that (i) P_{max}, V_0, and F_0 were significantly lower in CAD patients than in HP, while (ii) S_{fv} was similar for these two populations.

Decrease in P_{max}, F_0 and V_0 on CAD Patients

Considering power output as the product of force and velocity (eq.1), the decline of maximum power was induced by a reduction of both force production capacities at high (V_0) and low (F_0) velocities. The values presented in the literature are mostly based on high performance athletes, which leads to higher results than those reported in our study (Vandewalle et al., 1987; Dorel et al., 2005). Dorel et al. (2005) and Vandewalle et al. (1987) measured peak power (P_{max}) of elite cyclists who performed short maximal sprints (about 5–6 s) on a Monark cycle ergometer. The findings of these two studies were, respectively 19.3 ± 1.3 and 16.8 ± 1.23 W.kg^{-1}, i.e., it was almost two and three times the maximum power developed, respectively, by HP and CAD patients.

This difference in force and velocity between the two groups can be explained by different factors. First, CAD patients were significantly fatter than HP and therefore had a higher BMI. In addition, we have no significant difference between the BMI in CAD patients before and after CR (Before CR: 27.6 ± 5.5 kg/m^2 and after CR: 26.6 ± 5.0 kg/m^2, $p = 0.44$). Secondly, prior to their acute coronary syndrome, patients were physically inactive. They spent on average 5 MET-h/week, which was very low knowing that 1 MET represented the metabolism at rest (3.5 ml O2/kg/min) (Jetté et al., 1990). Besides, it was recommended to walk on average 10,000 steps/d and to practice at least 150 min of physical activity weekly (Le Masurier et al., 2003) which represents an average dose of 7.5 MET-h/week (Hupin et al., 2015). We noted a significant difference on $\dot{V}O_2$max

in CAD patients before and at the end of CR (before CR: 22.70 ± 5.20 ml/min/kg and after CR: 25.82 ± 5.78 ml/min/kg, p = 0.0018). Thanks to CR, CAD patients improved their $\dot{V}O_2$max by 15%. Compared to the reference values for people of the same age [i.e., 38.4 ml/min/kg (Wilmore et al., 2008)] this confirms that CAD patients are deconditioned before the CR program. Thirdly, patients often had more sedentary behavior before cardiovascular event. These risk behavioral factors, combined with CVD risk factors such as smoking and poor diet, significantly increase cardiovascular morbidity and mortality (Gbd 2013 Risk Factors Collaborators, 2015). We can suppose that high CVD risk and an excess fat mass prevented them from contracting at high velocities.

This difference of V_0 might be due to a remodeling of the motor units toward a slower typology (Power et al., 2014). Other physiological factors could be involved, such as an increase internal resistance produced by connective tissue (Valour et al., 2003) an increase in percentage of type I fibres (Thom et al., 2007) and selective atrophy of type II fibers (Häkkinen et al., 1996). This last assumption was in accordance with (Hautier et al., 1998), who showed an important relationship between maximal power and the relative area of fast twitch fibers. Indeed, most HP practiced explosive sports such as football and tennis, which requested fast twitch fibers and anaerobic metabolism.

The variation in F_0 might be caused by sarcopenia, the loss of muscle mass in patients (Häkkinen et al., 1996). In addition, most of the patients had sedentary behavior (i.e., overweight and physically inactive), which led to higher a percentage of fat tissue compared to HP. Thirdly, they might also have a neuromuscular activation deficit or the decrease in the effectiveness of the transmission of the voluntary command to the muscle could explain the decrease in force (Morse et al., 2004).

In addition, all CAD patients (except one) were receiving beta-blocker medical treatment (none among HP). These medications have the effect of decreasing heart rate and blood pressure (Goldberger et al., 2015). Beta-blockers would change neuromuscular recruitment strategy, which would explain the impaired maximal sprint performance (Hunter et al., 2002; Fisher et al., 2010). Moreover, statin therapy demonstrated a benefit in CAD patients to reduce CVD risk (Shepherd et al., 1995); however, they had deleterious effects on skeletal muscle, ranging from muscle complaints (which explained the withdrawal of statin in 2 CAD patients) to myositis (Mikus et al., 2013). Finally, the treatment of CAD patients (statin, beta-blockers), the disease and low physical activity had negative effects on muscle function.

Similar Values of Slope

No significant difference was observed for the S_{fv} variable between the two groups. The P_{max} impacted both force production at high and low velocities. The current results differed with previous studies which indicate higher F_0 values (Driss et al., 1998; Giroux et al., 2016). Indeed, Driss et al. (1998) assessed mechanical properties of FVP in male volleyball players during short maximal sprint (about 6 s) on a Monark cycle ergometer and they reported a F_0 value almost two times higher than ours.

Limitation

As physical activity was evaluated solely through a few questions, which is an approximate measure of the quantity of physical exercise, the main limitation of this study regards the objectivity of physical activity assessment. For future studies, more precise tools such as actimeters should be used.

Perspectives

This study could be continued by a randomized study with a larger number of participants to assess the impact of training in force or velocity production force capacities according to the initial FVP of the patients. Indeed, evidence may be emerging that high-intensity strength training is more effective to increase acutely myofibrillar protein synthesis, cause neural adaptations and, in the long term, increase muscle strength, when compared to low-intensity strength training (Hansen et al., 2019). Also, studies report that cardiovascular demand is lower in high-intensity than low-intensity resistance exercises, thus potentially pointing toward sufficient medical safety of a simple sprint on cycle ergometer for the cardiovascular system (Bjarnason-Wehrens, 2019). The *F-V* (deficit in force or velocity) imbalance initially observed from an evaluation of the FVP (sprint on cycle ergometer) would be optimized thanks to an adapted training program. We hypothesize that *F-V* profile could be used in CR in CAD patients, as an additional and novel tool, to induce a *F-V* balance adapted through personalized sessions (**Supplementary Material**).

CONCLUSION

The lower maximal power in CAD patients was related to both a lower V_0 and F_0. Physical inactivity, sedentary time and high CVD risk may explain this difference of force production at both high and low velocities between the two groups.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the French Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MF and DH contributed to the conception and design of the work. JR, PS, and TB contributed to the analysis and interpretation of the data. MF drafted the manuscript. JR, PS, J-BM, RT, FR, JL, and DH critically revised the manuscript. All authors gave final approval and agreed to be accountable for all aspects of work ensuring integrity and accuracy.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2019.01639/full#supplementary-material

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frontiers in Physiology

REVIEW
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Novel Mechanisms of Exercise-Induced Cardioprotective Factors in Myocardial Infarction

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Exercise training has been reported to ameliorate heart dysfunction in both humans and animals after myocardial infarction (MI). Exercise-induced cardioprotective factors have been implicated in mediating cardiac repair under pathological conditions. These protective factors secreted by or enriched in the heart could exert cardioprotective functions in an autocrine or paracrine manner. Extracellular vesicles, especially exosomes, contain key molecules and play an essential role in cell-to-cell communication via delivery of various factors, which may be a novel target to study the mechanism of exercise-induced benefits, besides traditional signaling pathways. This review is designed to demonstrate the function and underlying protective mechanism of exercise-induced cardioprotective factors in MI, with an aim to offer more potential therapeutic targets for MI.

Keywords: exercise, cardioprotective factors, exosomes, extracellular vesicles, myocardial infarction

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INTRODUCTION

Myocardial infarction (MI) is a serious result of cardiovascular disease and the leading cause of mortality and morbidity (Andersson et al., 2018). Approaches to inhibit the pathological process of MI are essential for its treatment and prognosis. To date, well-documented evidences have proved that appropriate exercise training can alleviate MI, which reduces mortality and improves the net clinical benefit in patients with MI (Anderson et al., 2016; Lear et al., 2017; Moholdt et al., 2018). Therefore, exercise training has been widely recommended as a therapeutic strategy for MI.

Various factors, including multiple polypeptides, nucleic acids, and similar substances are induced during exercise, which, in part, exert protective biological effects against several diseases (Whitham et al., 2018). Parts of cardioprotective factors are largely secreted by or enriched in the heart, which facilitate direct communication between the myocardium and other organs, and induce repair of cardiac injury under pathologic conditions (Shimano et al., 2012). Recently, it has been reported that the level of some cardioprotective factors is significantly increased during exercise training (Sanchis-Gomar et al., 2016; Whitham et al., 2018). Discovery and characterization of exercise-induced cardioprotective factors are of great interest because they may lead to a better understanding of the alterations in cell-to-cell communication in MI and help identify new therapeutic targets.

However, the secretory mechanisms of exercise-induced cardioprotective factors and their network regulation in MI are complex. Extracellular vesicles (EVs) released by cells are composed of a lipid bilayer enclosing soluble cytosolic material and nuclear components, which could act

in an autocrine or paracrine manner to play a role in intercellular communication (Kowal et al., 2014). EVs divide into apoptotic bodies, microvesicles, and exosomes (EXs) depending on their size (Kowal et al., 2014). EVs, especially the most widely studied EXs, can be induced by exercise to facilitate the exchange of peptides, non-coding RNAs (ncRNAs), mRNA, DNA, and metabolites between cells and tissues, and are considered to play a key role in intercellular communication under physiological and pathological conditions, such as MI (Kowal et al., 2014; Davidson et al., 2017).

Intriguingly, circulatory EVs/EXs content varies in an intensity-dependent manner in response to exercise training (Bei et al., 2017). The heart is the main endocrine organ affected during exercise, and some cardiac-derived protective factors can be induced by exercise and carried by EVs/EXs to ameliorate the pathological processes of MI (Ogawa and de Bold, 2014; Sanchis-Gomar et al., 2016). Thus, this review summarizes the protective role and underlying mechanism of cardiac-derived protective factors that can be induced by exercise in MI, with an aim to demonstrate their potential as therapeutic targets (**Figure 1**).

EXERCISE-INDUCED PEPTIDES AND MI

Certain cardiac-derived peptides exert multiple biological functions under pathophysiological states. It has been reported that exercise can induce the secretion of various cardioprotective factors, which exert protective effects in MI (Safdar et al., 2016) (**Table 1**).

Growth Differentiation Factor 15 (GDF15) and Follistatin-Like1 (FSTL1)

Growth differentiation factor 15 (GDF15), also known as macrophage inhibitory cytokine 1, is a polypeptide molecule that could be synthesized and secreted by cardiomyocytes (Shimano et al., 2012; Emmerson et al., 2017). GDF15 expression is induced under various pathophysiological states and significantly increased during MI onset (Khan et al., 2009). It has also been found that higher levels of GDF-15 in patients with acute coronary syndrome are associated with raised risks of spontaneous MI, as well as cardiovascular and total mortality (Hagström et al., 2016). Thus, GDF15 could serve as both a biomarker and predictor for the prognosis of MI.

Moreover, GDF15 expression can be induced by exercise. A recent study reported that plasma GDF15 levels in healthy male individuals significantly increased from 215 pg/mL at rest to 295 pg/mL at the end of the exercise bout, and further increased to about 350 pg/mL at the end of recovery (Kleinert et al., 2018). More interestingly, GDF15 from femoral artery and femoral vein has been, respectively, tested before, during, and after exercise and found no difference. This finding suggests that exercise-induced GDF15 may not be secreted by the skeletal muscle (Kleinert et al., 2018), but possibly be derived from other organs, especially the cardiac muscle.

Growth differentiation factor 15 has been found to play important roles in regulating the pathophysiological process of MI via its vital anti-inflammatory function (Kempf et al., 2011). Excessive inflammatory cell recruitment to the infarcted area after MI aggravates myocardial remodeling and leads to fatal complications. Kempf et al. (2011) reported that GDF15-deficient mice showed a significant increase in the recruitment of polymorphonuclear leukocytes (PMNs) to the infarcted myocardium and an increase in the incidence of cardiac rupture. Conversely, infusion of recombinant GDF15 in MI mice repressed PMN recruitment by directly inhibiting chemokine signaling and integrin activation, indicating that GDF15 protects against fatal cardiac rupture after MI though controlling inflammatory cell recruitment. Rainer et al. (2014) further showed that suppression of cardiomyocyte-specific transforming growth factor (TGF)-β promoted GDF15 expression, which inhibited neutrophil integrin activation and heart remodeling after infarction (Rainer et al., 2014). Although GDF15 is increasing after MI and its higher level can predict a worse prognosis, it may be a promising exercise-induced therapeutic target for MI.

FSTL1, a member of the follistatin family, is abundantly expressed in the skeletal and cardiac muscle, and has been shown to mediate multiple aspects of MI (Wei et al., 2015; Shen et al., 2019). Maruyama et al. (2016) found that FSTL1 expression significantly increased after MI, and inhibiting its expression increased mortality due to cardiac rupture in the acute phase of MI. Moreover, FSTL1 deficiency-induced cardiac rupture was mainly associated with attenuation of the migratory and proliferative capabilities of cardiac fibroblasts and reduction of extracellular matrix proteins mediated via the extracellular signal-regulated kinase (ERK)1/2 pathway (Maruyama et al., 2016). Shen et al. (2019) showed that FSTL1 expression declined dramatically in hypoxia-induced mesenchymal stem cells (MSCs), while overexpression of FSTL1 significantly prolonged MSC retention after implantation in the ischemic myocardium, thereby preserving heart function after MI by limiting scar formation, reducing inflammatory response, and enhancing neovascularization. These findings suggest that FSTL1 might serve as a key therapeutic target for MI.

Exercise training has been shown to induce FSTL1 expression (Xi et al., 2016; Kon et al., 2019a). In healthy individuals, 60 min of cycling dramatically increased the serum FSTL1 level by 22%, with a concentration increase from 16.9 ng/mL (pre-exercise) to 20.1 ng/mL (immediately after exercise), which further increased to 21.9 ng/mL at 30 min after completing the exercise [23]. More interestingly, exercise training further increases ischemia-induced FSTL1 expression. A previous study showed that FSTL1 expression in MI mice was 1.96-fold higher than that in control mice, and it further increased by 4.04-fold after intermittent aerobic exercise (Xi et al., 2016). In addition, exercise-induced FSTL1 has been shown to improve cardiac remodeling and prognosis after MI via promoting angiogenesis and reducing cardiac fibrosis (Xi et al., 2016).

Interestingly, it was previously detected by a luciferase-based reporter gene assay that FSTL1 as an upstream regulator of GDF-15. It has been found that treatment with FSTL1 activated GDF15 production in cultured cardiomyocytes. More importantly, transgenic production of FSTL1 stimulated GDF15 production

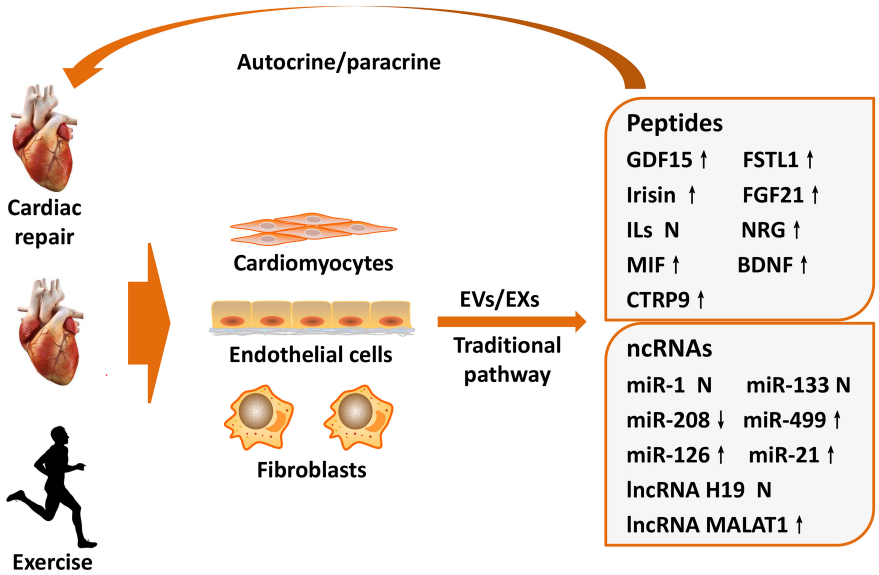

FIGURE 1 | Exercise-induced cardioprotective factors on cardiac repair after MI. The heart is an endocrine organ that produces and releases cardioprotective factors during exercise training. Cardioprotective factors mainly derived from cardiomyocytes, endothelial cells, and cardiac fibroblasts, which not only secreted by traditional signaling pathways but also delivered via EVs/EXs that changed components during exercise. Subsequently, these factors exert important regulatory functions for cardiac repair after MI in autocrine or paracrine manners, which may partially explain exercise-induced beneficial mechanism of MI. BDNF, brain-derived neurotrophic factor; CTRP9, C1q/TNF-related protein 9; EVs, extracellular vesicles; EXs, exosomes; FSTL1, follistatin-like 1; FGF21, fibroblast growth factor 21; GDF15, growth differentiation factor 15; ILs, interleukins; lncRNA, long non-coding RNA; MI, myocardial infarction; MIF, migration inhibitory factor; miR, microRNA; MALAT1, metastasis associated lung adenocarcinoma transcript 1; NRG, neuregulin; ncRNAs, non-coding RNAs. ↑ means upregulate, ↓ means downregulate, N means not clarified or controversial.

in the murine heart, whereas cardiomyocyte selective deletion of FSTL1 decreased production of GDF15 in cardiomyocytes, suggests that these proteins function as components of an interactive network (Widera et al., 2012). Moreover, the circulating concentration of FSTL1 has been shown to be dependently related to GDF15 and act as a biomarker to predict cardiovascular mortality in patients with acute coronary syndrome (Widera et al., 2012). Overall, the co-expression of FSTL1 and GDF15 provides an explanation for the mechanism of exercise-induced cardioprotection in MI, at least in part.

Irisin and Fibroblast Growth Factor 21 (FGF21)

Irisin, a new type of muscle factor highly expressed in the myocardium, has been considered as a novel exercise-induced cardioprotective factors (Tsuchiya et al., 2015; Wang H. et al., 2017). Irisin levels have been shown to be significantly induced by various exercise types. Plasma irisin levels were increased by 65% in mice after 3 weeks of free wheel running exercise, while circulating irisin levels were increased by twofold in healthy adult humans after 10 weeks of supervised endurance exercise training compared to the non-exercising group (Bostrom et al., 2012). More interestingly, the effects of different exercise duration and models on irisin levels are distinct. Irisin levels were shown to increase significantly after acute strenuous exercise and a 30 min bout of intensive exercise in children and young adults, but remained unchanged after 6 weeks of chronic exercise training (Loffler et al., 2015). Endurance exercise significantly increased irisin levels, which began to decrease after 2 h of exercise (Nygaard et al., 2015).

Recently, irisin has been found to protect against the pathologic process of MI via its anti-apoptotic, pro-angiogenetic, and cardiac regenerative functions (Wang H. et al., 2017; Liao et al., 2019; Zhao et al., 2019). Wang H. et al., 2017 showed that irisin treatment induced remarkable improvements in ventricular functional recovery and reduction of infarct size in the Langendorff perfused ischemia/reperfusion (I/R) injury heart of mice via suppressing the opening of mitochondrial permeability transition pore, which results in mitochondrial swelling, and protecting mitochondrial function to reduce cardiomyocyte apoptosis. Liao et al. (2019) reported that treatment with irisin for 2 weeks significantly reduced infarct size and fibrosis in MI mice, significantly increased angiogenesis in the ischemic area, and decreased cardiomyocyte apoptosis via activating the ERK signaling pathway. Besides, it has been recently reported that irisin can induce cardiac regeneration and functional improvement of MI mice via promoting the function of cardiac progenitor cells (CPCs) (Zhao et al., 2019). Thus, irisin is a key exercise-induced cardioprotective factor exerting multiple functions in MI.

FGF21 has been found to ameliorate the pathological progression of MI (Joki et al., 2015; Hu S. et al., 2018; Tang et al., 2018). It has been shown that injection of MI mice with recombinant interleukin (IL)-22 in the 1st week after acute MI effectively prevents left ventricular

TABLE 1 | Exercise-induced peptides mediate MI.

Names	Secreted cells	Functions	Main modulation mechanisms	References
GDF15	Cardiomyocytes	Inhibit inflammation	Repress PMN recruitment by directly inhibiting chemokine signaling and integrin activation	Kempf et al., 2011
FSTL1	Fibroblasts, cardiomyocytes	Inhibit inflammation and cardiac fibrosis, promote angiogenesis	Attenuate the migratory and proliferative capabilities of cardiac fibroblasts and expression of extracellular matrix proteins	Maruyama et al., 2016
Irisin	Cardiomyocytes	Reduce apoptosis; induce cardiac regeneration	Suppress the opening of mitochondrial permeability transition and protect mitochondria function; promote the function of cardiac progenitor cells	Wang H. et al., 2017; Zhao et al., 2019
FGF21	Cardiomyocytes, cardiac endothelial cells	Attenuate ventricular remodeling and myocyte apoptosis, increase capillary density	Decrease pro-inflammatory cytokines levels in an adiponectin-dependent manner and decrease miR-145-mediated autophagy	Joki et al., 2015; Hu S. et al., 2018
IL-33	Cardiac fibroblasts	Inhibit apoptosis and inflammation, reduce cardiac fibrosis	Suppress macrophage infiltration and production of inflammatory cytokines, inhibit NF-κB and p38 MAPK pathways, induce M2 macrophage polarization	Yin et al., 2014; Li et al., 2019
NRG	Cardiac endothelial cells	Attenuate apoptosis	Attenuate endoplasmic reticulum stress by activating PI3K/AKT pathway	Fang et al., 2017
MIF	Cardiomyocytes, cardiac fibroblasts	Promote cardiomyocytes survival and regulate inflammation	Promoted CSCs survival, proliferation and endothelial differentiation, activate PI3K/AKT/mTOR and AMPK pathways	Cui et al., 2016
BDNF	Endothelial cells, myocardial cells	Promote angiogenesis, inhibit inflammation and ventricle remodeling	Targeting its functional receptor, tyrosine receptor kinase B	Wang et al., 2018
CTRP9	Cardiac endothelial cells	Attenuate cardiomyocyte death	Activation of ERK/MMP-9 and ERK/Nrf2 signaling, upregulation of anti-oxidative proteins	Yan et al., 2017

BDNF, brain-derived neurotrophic factor; CTRP9, C1q/tumor necrosis factor-related protein-9; CSCs, cardiac stem cells; ERK, extracellular signal-regulated kinase; FSTL1, follistatin-like 1; FGF21, fibroblast growth factor 21; GDF15, growth differentiation factor 15; IL, interleukin; MI, myocardial infarction; MIF, migration inhibitory factor; miR, microRNA; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; MMP, matrix metalloproteinases; NRG, neuregulin; NF-κB, nuclear factor-κB; PMN, polymorphonuclear leukocytes; PI3K/AKT, phosphatidylinositol-3-kinase/protein kinase B.

(LV) dysfunction and attenuates ventricular remodeling via markedly increasing FGF21 expression in a signal transducer and activator of transcription (STAT)3-dependent manner, indicating that FGF21 might be a promising therapeutic target for MI treatment (Tang et al., 2018). Another recent study showed that increased FGF21 expression can ameliorate cardiac remodeling in MI mice via increasing capillary density around the infarct area and reducing cardiomyocyte apoptosis together with decreasing pro-inflammatory cytokine level in an adiponectin-dependent manner (Joki et al., 2015). Besides, FGF21 has been reported to protect against I/R-injury via decreasing miR-145-mediated autophagy (Hu S. et al., 2018).

Circulating FGF21 levels have been shown to significantly increase with exercise training (Geng et al., 2019). In healthy subjects, the level of FGF21 increases from 276.8 to 460.8 ng/L after 2 weeks of physical activity (Cuevas-Ramos et al., 2012). Another random cross-sectional study showed that 5 weeks of endurance exercise significantly increased circulating FGF21 levels in the elderly, and decreased their liver fat content (Taniguchi et al., 2016). It has also been speculated that exercise may induce cardiac-specific FGF21 expression via the sirtuin1 (Sirt1)/peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) signaling pathway (Guo et al., 2016). Thus, FGF21 is considered as an important exercise-induced cardioprotective factor for MI treatment.

It has been widely considered that irisin is widely expressed in cardiac and skeletal muscle, while FGF21 is also abundantly exist in the liver, adipose tissue, skeletal and cardiac muscle to mediate lipid metabolism. A previous study identified that exercise-induced irisin secretion could interact with FGF21 to exert biological functions, suggesting that they may regulate muscle–adipose crosstalk during exercise (Lee et al., 2014). Thus, their regulatory network may be a way to explain

muscle–adipose or organ-organ crosstalk during exercise training, and thereby indicating further mechanisms of exercise-induced benefits in MI.

IL Family

Several members of the IL family have been identified as cardioprotective factors, some of which are induced by exercise. IL-33, a cytokine belonging to the IL-1 family, is abundantly expressed in the heart, and attenuates inflammatory response and acts as the specific ligand for soluble ST2 (Shimano et al., 2012; Chen et al., 2018). IL-33 is mainly secreted by cardiac fibroblasts, exerting its function in a paracrine manner to exchange information with cardiomyocytes, and thereby playing an important role in the pathophysiological process of MI (Chen et al., 2018).

It has been reported that IL-33/ST2 pathway activation results in inhibition of apoptosis and inflammation, reduction of cardiac fibrosis, and improvement of cardiac function (Chen et al., 2018). Yin et al. (2014) found that IL-33 effectively suppressed macrophage infiltration and production of inflammatory cytokines in the myocardium after MI, and injection of recombinant IL-33 in MI mice reduced infarct size, attenuated cardiac remodeling, and improved cardiac function by inhibiting nuclear factor-κB (NF-κB) and p38 mitogen-activated protein kinase (MAPK) signaling pathways. Besides, Li et al. (2019) showed that IL-33 reduced infarct area and prevented the progression of fibrosis by inducing M2 macrophage polarization in MI mice model via activating the Janus kinase (JAK)/STAT pathway. Thus, IL-33 exerts anti-inflammatory effects in MI.

Various factors, including exercise training, have be shown to stimulate IL-33 expression. Further, it has been demonstrated that long-term medium-intensity exercise not only decreases the expression of pro-inflammatory factors, like Toll-like receptor 4 (TLR4), NF-κB, and IL-18, but also significantly increases the level of the anti-inflammatory factor IL-33 in patients with diabetes mellitus (Liu et al., 2015). Hence, IL-33 is considered as an exercise-induced cardioprotective factor that exerts important protective functions in MI.

Besides, IL-6 and IL-1β have also been identified as cardioprotective factors, as they aggravate MI. Distinctly, exercise training has been shown to reduce their expression, which may be a potential approach to ameliorate MI (Pedersen, 2017). Although not all IL family members are widely expressed in the heart and induced by exercise, they exert vital functions to regulate the course of MI. Several members of this family have been identified as exercise-induced cardioprotective factors, and may serve as promising therapeutic targets for MI.

Other Exercise-Induced Polypeptides

Neuregulin (NRG) is expressed in human cardiac endothelial cells and acts as an endothelial cell-derived molecule that exerts cardioprotective effects (Hedhli et al., 2011). NRG has been found to protect against cardiomyocyte apoptosis induced by hypoxia-reoxygenation, and in an *in vivo* study it was shown to reduce infarct size and cardiomyocyte apoptosis after myocardial I/R-injury (Hedhli et al., 2011). Furthermore, the cardioprotective effect of NRG was shown to be mediated via attenuation of endoplasmic reticulum stress and cardiomyocyte apoptosis by activating the phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) signaling pathway under I/R states (Fang et al., 2017). More interestingly, exercise can upregulate NGR and its ligand expression to promote cardiac repair, indicating that NRG is an exercise-induced cardioprotective factor and serves as a promising therapeutic target for MI (Cai et al., 2016).

Migration inhibitory factor (MIF) is a macrophage factor that regulates inflammation and immunity, and is secreted by cardiomyocytes and cardiac fibroblasts to promote cardiomyocyte survival and regulate inflammation after MI (Voss et al., 2019). Besides, it has been reported that MIF promotes cardiac stem cell survival, proliferation, and endothelial differentiation by targeting its receptor CD74 via activation of the PI3K/AKT/mammalian target of rapamycin (mTOR) and adenosine monophosphate-activated protein kinase (AMPK) signaling pathways. This finding suggests a potential therapeutic role of MIF in the treatment of MI (Cui et al., 2016). Additionally, MIF expression can also be induced by exercise, and it has been identified as an exercise-induced cardioprotective factor that protects against MI (Chang et al., 2019).

Brain-derived neurotrophic factor (BDNF), widely expressed in various non-neural tissues, such as vascular endothelial cells and myocardial cells, plays a protective role in MI via targeting its functional receptor, tyrosine receptor kinase B (Zhang et al., 2019). Recent studies have found that BDNF can promote angiogenesis, inhibit inflammatory response, and attenuate cardiac remodeling, thereby improving cardiac function and prognosis after MI (Wang et al., 2018). Well-documented evidences have shown that BDNF expression is significantly induced by exercise training, and thereby it can be considered as a beneficial exercise-induced cardioprotective factor (Wang et al., 2018; Zhang et al., 2019).

C1q/TNF-related protein-9 (CTRP9) is a novel cardioprotective factor primarily secreted by the adipose tissue and cardiac endothelial cells (Zhao et al., 2018). CTRP9 has been shown to enhance adipose-derived mesenchymal stem cell (ADSC) proliferation and survival after implantation to MI mice. Further, CTRP9 stimulates ADSC migration and attenuates cardiomyocyte cell death after MI via binding with N-cadherin, activation of ERK/MMP-9 and ERK/Nrf2 signaling, and upregulation/secretion of anti-oxidative proteins (Yan et al., 2017). Besides, CTRP9 expression is also induced by exercise, and a single bout of high intensity interval training is sufficient to stimulate CTRP9 secretion in healthy men (Kon et al., 2019b). Thus, CTRP9 has been identified as a novel exercise-induced cardioprotective factor exerting potential therapeutic effects in MI.

EXERCISE-REGULATED NCRNAS AND MI

Non-coding RNAs, which do not code for proteins but functionally regulate protein expression, have been identified as critical regulators of cell function and important candidates that protect against MI (Guo et al., 2017). MicroRNAs (miRNAs,

miRs) are a class of short-chain ncRNAs that have been reported to regulate MI via negatively modulating their target genes (Chistiakov et al., 2016). Cardiac-derived miRNAs induced by exercise may explain the exercise-induced beneficial effects on MI (Gomes et al., 2014) (**Table 2**).

miR-1 and miR-133

miR-1 and miR-133 are highly expressed in cardiomyocytes and play an important role in regulating myocardial autonomy, conduction, contraction, and myocyte differentiation and proliferation (Chistiakov et al., 2016). Previous studies have reported that miR-1 and miR-133 expression is regulated by exercise. It was shown that miR-1 and miR-133 expression significantly increased in marathon athletes after exercise (Gomes et al., 2014). However, another study demonstrated that miR-1 and miR-133 expression was significantly downregulated after exercise (Pietrangelo et al., 2015). These paradoxical results may be attributed to individual variations and differences in exercise duration. More interestingly, both miR-1 and miR-133 have been shown to exert important regulatory functions in MI (Duan et al., 2018; Yu et al., 2019).

While the regulatory role of miR-1 in MI remains controversial, studies suggest that transplantation of stem cells with miR-1 overexpression exerts important protective effects after MI. It has been reported that transplantation of embryonic stem cells with miR-1 overexpression can significantly reduce apoptosis and improve cardiac function via phosphatase and tensin homolog deleted on chromosome 10 (PTEN)/AKT pathway activation (Glass and Singla, 2011). Additionally, transplantation of MSCs overexpressing miR-1 into the infarcted myocardium of mice was shown to improve cell survival rate, promote cardiomyogenic differentiation, and improve cardiac function, indicating that miR-1 has a protective effect on MI (Huang et al., 2013). However, the direct function of miR-1 in MI requires further investigation.

miR-133 plays a cardioprotective function in MI. Li et al. (2015) found that overexpression of exogenous miR-133 significantly attenuated cardiomyocyte apoptosis, both *in vitro* and *in vivo*, in a hypoxia-reoxygenation injury model via inhibition of death associated protein kinase 2 (DAPK2). Izarra et al. (2014) further reported that miR-133 can attenuate myocardiocyte apoptosis through inhibiting the expression of pro-apoptotic genes, including caspase-9, apoptotic protease activating factor, DAPK2, Bcl2-like 11, and Bcl-2-modifying factor. In an MI rat model, it was reported that overexpression of miR-133 significantly promotes angiogenesis and cardiomyocyte proliferation, and inhibits cardiac hypertrophy and fibrosis, thereby improving cardiac function (Izarra et al., 2014). Another study showed that transplantation of miR-133-overexpressing MSCs in ischemic area in MI rat model markedly improved cardiac function (Chen et al., 2017). Therefore, overexpression of miR-133 may be an important therapeutic strategy for MI treatment.

miR-208 and miR-499

The cardiac-enriched miRNAs, miR-208 and miR-499, are mainly involved in modulation of differentiation and development of cardiac fibroblasts and cardiomyocytes, and play a vital role in maintaining normal cardiac function (Chistiakov et al., 2016). It has been shown that the expression of miR-208 is significantly downregulated, while that of miR-499 is markedly upregulated after exercise training, which may partly explain the benefits of exercise (Baggish et al., 2014; Soci et al., 2016).

miR-208 promotes cardiomyocyte apoptosis and cardiac remodeling after MI. Yan et al. (2016) found that inhibition of miR-208 expression in neonatal rat cardiomyocytes under hypoxia condition could relieve cardiomyocyte injury. Bian et al. (2015) showed that overexpression of miR-208 markedly aggravated I/R-induced myocardial injury in rats and promoted hypoxia-induced cardiomyocyte apoptosis *in vitro*, while knockdown of miR-208 suppressed cardiomyocyte apoptosis. Further, they found that miR-208 induced apoptosis via its target gene Ets1. The expression of miR-208 is directly correlated with β-MHC levels and cardiac collagen capacity. Therefore, inhibiting miR-208 expression may be a potential therapeutic approach for MI.

miR-499 has important regulatory effects on the pathological process of MI. Wang et al. (2014) found that miR-499 protected cardiomyocytes against H_2O_2-induced apoptosis and overexpression of miR-499 in rat cardiomyocytes increased cell survival rate by inhibiting the mitochondrial apoptosis pathway and pro-apoptotic gene expression. The pro-apoptotic gene Dyrk2 promoted apoptosis by increasing the level of p53 phosphorylation, whereas miR-499 significantly inhibited Dyrk2 expression, preventing the transfer of activated p53 to the mitochondria and inhibiting apoptosis. miR-499 has also been shown to suppress calcineurin-mediated dephosphorylation of dynamin-related protein-1 (Drp1) to inhibit cardiomyocyte apoptosis, thereby reducing Drp1 accumulation in the mitochondria and mitochondrial fission (Wang et al., 2011). Furthermore, Shi et al. (2019) found that miR-499 reduced H9C2 cell injury by inhibiting the expression of Sox6, and suppressed apoptosis by increasing Bcl-2 and decreasing Bax and caspase-3 expression under hypoxia-reoxygenation condition. Therefore, miR-499 can target multiple genes to protect against apoptosis after MI.

Other Cardiac-Derived miRNAs

miR-126, mainly expressed in endothelial cells, significantly promotes angiogenesis around the infarct area after MI (Jiang et al., 2014). The expression of cardiac-enriched miR-126 is significantly changed after MI, exerting pro-angiogenetic effects to maintain vascular wall integrity by negatively regulating its target gene Spred1 (Guo et al., 2018). Exercise can increase miR-126 expression. It has been reported that miR-126 expression was upregulated by 2.1- and 4.6-fold in healthy subjects after a maximal symptom-limited exercise test and 4 h of cycling, respectively (Uhlemann et al., 2014). Thus, miR-126 is an important exercise-induced factor that protects against MI mainly by promoting angiogenesis.

miR-21, a miRNA transcribed by RNA polymerase II, is widely expressed in endothelial cells and exerts protective functions after

TABLE 2 | Cardiac-enriched ncRNAs regulate MI.

ncRNAs	Regulated by exercise	Functions	Main modulation mechanisms	References
miR-1	Controversial	Inhibit apoptosis, promote cardiomyogenic differentiation	Activate PTEN/AKT pathway and improve transplanted MSC survival rate	Glass and Singla, 2011; Huang et al., 2013
miR-133	Controversial	Attenuate cardiomyocyte apoptosis and cardiac fibrosis, promote angiogenesis and cardiomyocytes proliferation	Inhibit pro-apoptotic genes DAPK2 expression, improve transplanted MSC survival rate	Izarra et al., 2014; Li et al., 2015; Chen et al., 2017
miR-208	Downregulate	Aggravate cardiac fibrosis, promote apoptosis	Induce apoptosis through regulation of target gene Ets1, directly proportional to β-MHC and cardiac collagen capacity	Bian et al., 2015
miR-499	Upregulate	Inhibit cell apoptosis	Inhibit mitochondrial apoptosis pathway, inhibit pro-apoptotic gene Dyrk2, reduce the dephosphorylation of Drp1 and the accumulation of Drp1 in mitochondria	Wang et al., 2011, 2014
miR-126	Upregulate	Promote angiogenesis, maintain vascular wall integrity	Inhibit its target gene Spred1	Guo et al., 2018
miR-21	Upregulate	Inhibit fibrosis, inflammation, and apoptosis	Inhibit its target gene Jagged1, inhibit KBTBD7, p38 and NF-κB pathway and TNF-α induced apoptosis	Wang Z.H. et al., 2017; Yang et al., 2018; Zhou X.L. et al., 2018
lncRNA H19	Normalized H19 gene methylation	Reduce necrosis and cardiac remodeling, enhance angiogenesis, activate autophagy	Target to miR-103/107, miR-139, and miR-675-5p, respectively	Wang et al., 2015; Gong et al., 2017; Huang P. et al., 2019
lncRNA MALAT1	Upregulate	Promote angiogenesis, cells proliferation and autophagy, inhibit apoptosis, increase cardiac fibrosis	Sponge miR-558, miR-145, and miR-200a-3p, respectively	Guo et al., 2019; Huang S. et al., 2019; Sun and Zhang, 2019

DAPK2, death associated protein kinase 2; Drp1, dynamin-related protein-1; KBTBD7, kelch repeat and BTB (POZ) domain containing 7; lncRNA, long non-coding RNA; MI, myocardial infarction; miR, microRNA; MALAT1, metastasis associated lung adenocarcinoma transcript 1; MSC, mesenchymal stem cell; NF-κB, nuclear factor-κB; PTEN, phosphatase and tensin homolog deleted on chromosome 10; TNF-α, tumor necrosis factor-α.

MI. A previous study demonstrated that miR-21 exerts anti-fibrotic effects via promoting cardiac fibroblast-to-myofibroblast transformation by regulating its target gene Jagged1 (Zhou X.L. et al., 2018). Another study showed that miR-21 attenuated inflammation, cardiac dysfunction, and maladaptive remodeling post-MI through targeting kelch repeat and BTB (POZ) domain containing 7 and inhibiting p38 and NF-κB pathway activation (Yang et al., 2018). Intriguingly, a recent study showed that the serum level of miR-21 was upregulated in elderly patients with acute MI, and that miR-21 suppressed TNF-α-induced apoptosis in human cardiomyocytes via stimulating the activation of JNK/p38/caspase-3 signaling pathway (Wang Z.H. et al., 2017). Additionally, miR-21 expression has been shown to be regulated by exercise, indicating a novel mechanism underlying exercise-induced benefits in MI (Wahl et al., 2016).

Long ncRNAs (lncRNAs)

lncRNAs are transcripts longer than 200 nucleotides that regulate various biological processes by interacting with multiple transcription factors (Wang et al., 2015). Recently, it has been identified that lncRNAs play a crucial regulatory function in MI. The lncRNAs H19 and metastasis associated lung adenocarcinoma transcript 1 (MALAT1) have been widely studied, and their levels are shown to be regulated by exercise. Exercise-induced lncRNA H19 expression has been shown to involve in epigenetic modifications (Xu et al., 2017). lncRNA MALAT1 expression can be induced by swimming via inhibition of apoptotic functions, thereby protecting hippocampal neurons against ischemic diseases (Shang et al., 2018).

The lncRNA H19 has been reported to play multiple roles after MI. Wang et al. (2015) reported that lncRNA H19 directly binds to miR-103/107 and reduces its expression, which promotes Fas-associated protein with death domain expression, and participates in H_2O_2-induced necrosis in H9C2 cells. This finding revealed that lncRNA H19 prevents cardiomyocyte necrosis in MI. Furthermore, lncRNA H19 was shown to alleviate myocardial cell injury through reduction of miR-139 expression and further upregulation of its target gene Sox8 via PI3K/AKT/mTOR and MAPK pathway activation (Gong et al., 2017). Additionally, lncRNA H19 has been shown to enhance angiogenesis, prevent cardiomyocyte apoptosis, and improve cardiac function after MI via targeting miR-675-5p (Huang P. et al., 2019). Besides, overexpression of lncRNA H19 in mice was shown to reduce infarct size and improve cardiac

function via activating autophagy (Zhou M. et al., 2018). These findings demonstrate that lncRNA H19 exerts essential protective functions in MI.

lncRNA MALAT1 is highly expressed in vascular endothelial cells and has a complex function in MI (Li L. et al., 2018). It was initially reported that genetic deletion or pharmacological inhibition of MALAT1 reduced vascular growth *in vivo*, suggesting that MALAT1 promoted angiogenesis in ischemic diseases (Michalik et al., 2014). Besides, it has been shown that MALAT1 protects against cardiomyocyte apoptosis after MI by sponging miR-558, thereby inducing Unc-51-like autophagy-activating kinase 1-dependent protective autophagy (Guo et al., 2019). However, a recent study reported that MALAT1 promoted cardiac fibrosis and deteriorated cardiac function post-MI by increasing TGF-β1 activity and inhibiting miR-145 expression (Huang S. et al., 2019). Additionally, MALAT1 has been shown to increase cell apoptosis in MI via acting as a competing endogenous RNA to sponge miR-200a-3p (Sun and Zhang, 2019). Thus, lncRNA MALAT1 exerts critical regulatory functions in various biological processes during MI, including angiogenesis, cell proliferation, apoptosis, and cardiac fibrosis. Nevertheless, whether it plays a protective or detrimental role in MI requires further investigation.

NOVEL SECRETORY MECHANISMS OF EXERCISE-INDUCED CARDIOPROTECTIVE FACTORS IN MI

Recent researchers have identified EVs as an important cell-to-cell communication way (Doroudgar and Glembotski, 2011). EVs, secreted by cells in the form of vesicles containing various signaling molecules, play a role in cell-to-cell communication under pathophysiological conditions (Safdar et al., 2016). As previously described, EXs are one of the most widely studied EVs. Generally, EXs act in an endocrine, autocrine, or paracrine manner to exert their biological functions (Safdar et al., 2016).

The biogenesis and release of EXs is related to complex regulatory factors. As previously described, EXs biogenesis begins within the endosome system and is matured in an endosomal sorting complex required for transport (ESCRT)-dependent or independent manner (Colombo et al., 2014). Molecular regulators implicated in exosome release include multiple factors, especially Soluble NSF Attachment Protein Receptor (SNARE) and Rabs GTPases (Colombo et al., 2014). Thus, EXs carries not only secreted factors but also a cluster of scaffold proteins, which may be used to derive their source cells. It has been found that EXs originated from cardiomyocytes mainly express caveolin-3 and Troponin T, from cardiac fibroblast express CD90.2, and from endothelial cells express CD31 (Loyer et al., 2018).

At present, many kinds of cells have been shown to secrete EXs, including various stem cells, endothelial cells, cardiofibroblasts, and cardiomyocytes (Barile et al., 2017). EXs derived from different cells exhibit different functions. A recent study reported that cardiomyocytes release EVs under ischemic stress; and cardiomyocytes as the main source of bioactive EXs in coronary serum (Li H. et al., 2018). Interestingly, certain cells exert various biological functions by releasing EXs with different components, which varies under different culture conditions and stimuli (Gallet et al., 2017; Ribeiro-Rodrigues et al., 2017; Huang P. et al., 2019).

Previous studies have shown that EVs/EXs stimulated by ischemia exert important cardioprotective functions after MI via changing their contents (Gallet et al., 2017; Ribeiro-Rodrigues et al., 2017; Gao et al., 2018). Ribeiro-Rodrigues et al. (2017) found that EXs secreted by cardiomyocytes under ischemic conditions contained high levels of matrix metalloproteinases (MMP), miR-222, and miR-143, and stimulated the formation of new functional vessels following MI. Barile et al. (2014) reported that infarcted hearts injected with EVs from CPCs showed reduced cardiomyocyte apoptosis, enhanced angiogenesis, and improved LV ejection fraction compared with those injected with control medium, and this effect was induced by the changed levels of miR-210, miR-132, and miR-146a-3p in EVs. Li H. et al., 2018 also found that, compared to EXs from healthy controls, EXs from patients with myocardial ischemia enhanced endothelial cell proliferation, migration, and tube formation via downregulation of miR-939-5p, and thereby increased endothelial nitric oxide production, eventually promoting angiogenesis.

More interestingly, exercise can induce various organs to release EVs/EXs that are enriched in various peptides, ncRNAs, and other substances. The release of EXs was shown to significantly increase immediately after cycling exercise and then decline again within 90 min at rest, while release of EXs was moderate but appeared more sustained after treadmill running. Moreover, release of EXs into the circulation has been shown to be initiated in the aerobic phase, suggesting that it is independent of the metabolic changes during exercise (Fruhbeis et al., 2015). Further, in another study, after a 1 h bout of cycling exercise in healthy humans, an increase in the circulation of over 300 proteins was observed, and most of them were secreted by EXs, suggesting that exercise exerts systemic biological effects (Whitham et al., 2018). Thus, EXs may provide a new approach to explain the multiple protective mechanisms of exercise in MI.

Additionally, it has been found that exercise can induce cardiomyocytes to release EVs/EXs, which exert a cardioprotective function in MI. A recent study showed that the serum level of EVs was increased by about 1.85-fold in mice after 3 weeks of swimming (Bei et al., 2017). Furthermore, intramyocardial injection of EVs induced by exercise exerted additional anti-apoptotic effects on H_2O_2-treated H9C2 cardiomyocytes compared to exercise alone. The protective effects on acute ischemia in mice were mediated by the activation of the ERK1/2 and HSP27 pathways (Bei et al., 2017). Thus, exercise-induced EVs/EXs released by cardiomyocytes exert important protective functions in MI.

Cardioprotective factors carried by EXs have been found to regulate MI pathology (Hu M. et al., 2018). In an MI mice model, it was reported that the level of cardiac-enriched miRNAs, such as miR-1 and miR-499 was predominantly increased in circulating EXs, subsequently mediating functional crosstalk between the ischemic heart and bone marrow for repair of cardiac injury (Cheng et al., 2019). Recently, the exosomal lncRNA H19 derived from MSCs has been reported to mediate the cardioprotective

effects in infarcted hearts via promoting endothelial cell function and angiogenesis (Huang P. et al., 2019). Besides, polypeptide molecules, like MIF and BNDF can also be delivered by EXs to exert biological functions (Suire et al., 2017; Amosse et al., 2018). Thus, EXs may serve as important vehicles to deliver exercise-induced cardioprotective factors and as mediators of intercellular communication in MI, which may be a possible mechanism underlying exercise-induced benefits.

In summary, exercise has beneficial effects on MI, but its mechanisms are complex and need to be fully elucidated. It has been found that exercise can induce the secretion of a variety of polypeptide molecules, ncRNAs, and other substances derived from the myocardium and other organs. Several recent studies have reported that EVs/EXs are enriched in a large number of cardioprotective factors, and their secretion is regulated by exercise. This review summarizes the effects of newly discovered exercise-induced cardiogenic peptides and ncRNAs on MI and their potential mechanisms, thus aiming to provide a new theoretical basis for the application of exercise training in the clinical treatment of MI.

AUTHOR CONTRIBUTIONS

YG drafted the manuscript. JC and HQ checked and revised the manuscript. All authors read and approved the final manuscript.

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