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RESEARCHARTICLE

ADDITION OF STRENGTH TRAINING MODIFIES THE AEROBIC EXERCISE INFLAMMATORY RESPONSE IN HEART FAILURE PATIENTS – COMMENTS ON THE UNDERLYING PATHOPHYSIOLOGY

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Abstract

Aim: The present study investigates the effect of Strength Training addition in a High Intensity Interval Training (HIIT) program, on the inflammatory profile of chronic heart failure (CHF) patients.

Materials and Methods: Forty-six CHF patients were randomized into the two different exercise rehabilitation groups. A patient group (control) performed HIIT and another group performed Combined (HIIT and strength training) Exercise Training (COM) for a three-month period. Before and after rehabilitation, all patients performed a maximal cardiopulmonary exercise testing (CPET). Microcirculatory parameters were assessed by Near Infrared Spectroscopy; CRP, IL-2, IL-6, IL-10 and VEGF levels were measured in plasma.

Results: Cardiopulmonary parameters and microcirculatory indices improved after rehabilitation. CRP and the IL-6/IL-10 ratio decreased after rehabilitation. By linear regression analysis, a negative correlation was noted between IL-2, IL-6 and IL-10 changes (post-CPET values) and the VE/VCO_2 slope change, for the entire patient cohort. The correlation was separately maintained only in the COM group.

Conclusions: Overall, the results of the present study reinforce the view of an anti-inflammatory effect of exercise in CHF patients. The significant correlations demonstrated between the cytokine responses and the ventilatory response to exercise, particularly in the COM group, probably ensue from the special effect of strength training on peripheral muscle function.

Keywords: Heart failure, rehabilitation, inflammatory response, exercise training, strength training.

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INTRODUCTION

Chronic heart failure (CHF) is a clinical syndrome characterized by reduced ability of the heart to function as a pump and to provide adequate blood flow meeting the tissue needs.[1] CHF patients experience reduced exercise capacity, muscle weakness and early fatigue, resulting in limited daily physical activity. Pathophysiologically, decreased cardiac output is accompanied by disturbances of tissue microcirculation, endothelial dysfunction, disorders of oxidative metabolism and increased inflammatory response.[2,3]

The effect of exercise on the inflammatory response in CHF patients has been increasingly attracting the interest of medical researchers.[4,5] Most particularly, high intensity interval training (HIIT) is a form of exercise that has recently piqued the interest of researchers and rehabilitation professionals worldwide. The reason is its effectiveness compared to other, conventional forms of training that can be more time-consuming. Thus, it shows superior optimization of cardiovascular indices versus moderate-intensity, continuous training in post-infarction heart failure patients.[6] Overall, it seems to have comparable training effects in terms of muscle glycogen content, increased mitochondrial enzyme activity, and, in general, increased muscle oxidative capacity compared to endurance training, as it counterbalances the amount of time spent exercising with increase in exercise intensity.[7-10] However, there is no sufficient evidence on the specific inflammatory changes, induced by different rehabilitation programs; also, on whether these changes would be related to the change of certain, functional cardiopulmonary parameters or microcirculatory indices.

In this study, we aimed to investigate the effect of aerobic exercise alone or combined with strength training on the inflammatory profile of CHF patients, both at baseline and after a maximal cardiopulmonary exercise testing (CPET); also, to demonstrate possible associations, if any, between the inflammatory response with the corresponding changes of cardiopulmonary parameters assessed during CPET and microcirculatory indices derived by Near Infrared Spectroscopy (NIRS) application.

MATERIALS AND METHODS

Study population

The study was conducted in the Clinical Ergospirometry, Exercise & Rehabilitation Laboratory of the National and Kapodistrian University of Athens, at Evangelismos Hospital, Athens, Greece.

Patients with unstable angina, severe valvular heart disease, respiratory system disorders, orthopedic and neurological disorders, anemia and obesity [body mass index (BMI) ≥ 30 kg/m2] were excluded from the study (Figure 1). Forty-six stable CHF patients were enrolled. Data from thirty-four CHF patients were finally analyzed. Patients' New York Heart Association (NYHA) class was ≥ II and they had mildly-to-severely reduced left ventricular ejection fraction (LVEF) (maximum LVEF 43%). Medical history and clinical characteristics were recorded and hemodynamics (blood pressure, heart rate) were measured.

Study design

Before the onset and after the completion of the rehabilitation program, all patients performed a ramp-incremental CPET on an electromagnetically braked cycle ergometer. Before and immediately after each CPET (corresponding to phases 1 and 2 for CPET performed before the rehabilitation program and to phases 3 and 4 for CPET performed after rehabilitation), blood samples were obtained for the determination of C-reactive protein (CRP), interleukin (IL-2), IL-6, IL-10 and vascular endothelial growth factor (VEGF) levels in plasma. After blood sample collection, NIRS measurements were performed on the contralateral upper limb, utilizing the vascular occlusion technique (VOT).[11, 12]

Stratification: After their initial CPET, patients were randomly assigned by age (cut-off point: 50 years) and peak VO2 (cut-off point: 16 ml/kg/min) in either the high-intensity interval training group (HIIT Group) or HIIT combined with muscle strength training group (COM Group).

Primary and secondary outcome: In this study, we aimed to

investigate the effect of High Intensity Interval Training (HIIT) alone or combined with strength training on the inflammatory profile of CHF patients, both at baseline and after a maximal CPET; also, to demonstrate possible associations, if any, between the inflammatory response with the corresponding changes of cardiopulmonary parameters assessed during CPET and microcirculatory indices derived by Near Infrared Spectroscopy. **Blinding:** Stratified randomization was performed by a researcher not involved in exercise sessions assessment, blood analysis and NIRS evaluation. It was a single-blinded, randomized controlled study.

Sample size: For power analysis: We conducted post-hoc power analysis using R package. Power was equal to 85%. (it is threshold).[13]

Cytokine levels

For the identification and quantification of CRP and cytokine levels, blood samples were drawn from each patient twice in each CPET, once at rest and once just after CPET. The procedure was repeated twice during the rehabilitation program (three-month duration), once at the initial CPET before rehabilitation and once at the final CPET after rehabilitation. Specifically, 3 ml of blood were drawn from a peripheral vein and placed in each Vacutainer vial with K2EDTA anticoagulant (BD, Mississauga, Ontario, Canada) for plasma collection. After centrifugation at 1500 g for 20 minutes at 4 ° C, the plasma of each sample was stored at -80 ° C until analysis. Cytokine levels were determined using the Human Soluble Protein Master Buffer Kit (BD, Mississauga, Ontario, Canada). Cytokine analysis was performed by flow cytometry with the appropriate commercial reagent packages (Cytometric Bead Array Human IL Flex Set, VEGF Flex Set) of the manufacturer (BD, Mississauga, Ontario, Canada). For the in vitro quantitative determination of CRP in human plasma, we used the Immonuturbidimetric corresponding instructions for use and analysis assay (Roche/Hitachi cobas c systems, Roche Diagnostics International Ltd). Immonuturbidimetry utilizes the traditional antigen–antibody reaction. Assessment of interleukin levels and VEGF was conducted via the BDTMCBA Human Soluble Protein Flex Set System.[14] BD CBA assays constitute a method of capturing a soluble analyte or set of analytes with beads of known size and fluorescence, facilitating the detection of sandwich complexes (capture bead + analyte + detection reagent) using flow cytometry. Four-color flow cytometry was performed with Navios (Beckman Coulter) flow cytometer. Values of cytokines are expressed as medians (25th - 75th percentiles) in pg/ml.

Near Infrared Spectroscopy (NIRS)

Near infrared spectrometer (Hutchinson, InSpectra Model 650, Hutchinson Technology, Hutchinson MN, USA) was used to continuously record the tissue oxygen saturation (StO_2) at the thenar eminence. VOT was accomplished by the application of a pneumatic cuff above the elbow, which was rapidly inflated with air 50mmHg above each patient's systolic blood pressure and deflated after 3 minutes. StO_2 was monitored during rest, ischemia and reperfusion phase. NIRS combined with VOT, in addition to measuring StO_2 at baseline, allows the assessment of dynamic indices related to 1. oxidative metabolism, i.e. oxygen consumption rate (OCR, StO_2 decrease slope) and 2. microvascular reactivity, i.e. reperfusion rate (RR, StO_2 increase slope) and reactive hyperemia (RH), which corresponds to the area above the baseline StO_2 value for the time interval between the first baseline recovery and when baseline reoccurs after peak hyperemic response.[11, 12] (Figure 2).

Exercise Program

Aerobic exercise was performed in the modality of high-intensity interval training (HIIT), based on a previously reported protocol. HIIT has attracted popularity in recent years, as a safe modality to induce at least similar benefits with continuous regimes in cardiac rehabilitation.[16, 17] The COM group performed the same aerobic interval workouts as the HIIT group followed by strength training which was prescribed based on a repetition maximum test (1RM) and included leg extension, leg curl and chest press exercises for the quadriceps, hamstring muscles, and chest muscles, respectively. Specifically, patients

of this group performed 3 sets of 10 to 12 repetitions at a gradually increasing intensity, from 60 to 75% of 1 Repetition maximum (1-RM) of knee extension, knee flexion and chest press exercises with 1-minute rest between sets. At the end of the aerobic interval training, HIIT group patients performed balance exercises instead of strength training, in order to attain the same exercise time with the COM group patients.

Data availability

The data associated with the present article are not publicly available but are available from the corresponding author on reasonable request.

Statistical Analysis

A repeated measures analysis of variance (ANOVA) was performed in order to estimate variable differences between phases. For non-parametric variables Friedman test was used. Parametric pairwise comparisons were conducted using paired t-test while non-parametric pairwise comparisons were evaluated via Wilcoxon Signed Ranks test. The method of multiple linear regression analysis was used to evaluate the association between different variables. Generalized estimating equations were used to evaluate OCR over phase and group. The level of statistical significance was set at $p < 0.05$ and 95% confidence interval (CI) was selected.

RESULTS

The patient groups were NYHA and BMI matched; also, their mean StO_2 and peak oxygen uptake (VO_{2peak}) values, prior to the rehabilitation program implementation, were not significantly different. Received medication did not alter during the 3-month rehabilitation program. Patient demographics and baseline CPET indices are shown in Table 1.

Following rehabilitation, a significant increase in LVEF (Mean=32.97, SD=8.33 vs. Mean=37.18, SD=8.73 %, p<0.001, 95% CI; -6.01 to -2.41) and VO_{2peak} (Mean=18.46, SD=4.46 vs. Mean=20.71, SD=6.20 ml/kg/min, p=0.011, 95% CI; -3.96 to -0.54) was observed, as well as a significant decrease in minute ventilation/carbon dioxide production (VE/VCO_2) slope (Mean=29.30, SD=5.54 vs. Mean=26.59, SD=6.61, p=0.019, 95% CI; 0.47 to 4.95), for the whole patient cohort. No significant differences were noted, regarding the above parameter changes, between the two patient groups.

Significant changes of NIRS indices and inflammatory markers are summarized in Table 2. No significant differences in inflammatory marker changes were established in between group comparisons.

Following rehabilitation, the change of OCR after CPET compared to baseline (phase 4 vs. 3) differed significantly in the two groups, i.e. OCR increased in the HIIT group while in the COM group OCR decreased (Mean=1.68, SD=3.15 vs. Mean=-1.15, SD=3.47 %/min, OCR change for the HIIT and COM group respectively, p=0.018, 95% CI; 0.51 to 5.14).

In a Generalized Estimating Equations model it was shown that the estimated average OCR value was 2.79 units (%/min) higher in the HIIT group (after adjustment for the OCR baseline value and the phase) (β=2.79, std. error=1.277, p=0.029, 95% CI; 0.28 to 5.29). Among the COM group subpopulation, no statistically significant differences in OCR were observed at the four assessment phases. The differences noticed in the whole patient cohort were due to the HIIT group effect. (Mean=11.60, SD=3.46 vs. Mean=12.30, SD=4.06 vs. Mean=14.74, SD=3.37 vs. Mean=16.42, SD=4.33 %/min, for the phases 1, 2, 3 and 4 respectively, p<0.001, for HIIT patients).

Furthermore, by linear regression analysis, the change of IL-2, IL-6 and IL-10 post-CPET values, following rehabilitation (phase 4 vs. 2), were negatively associated with the corresponding VE/VCO_2 changes (Table 3). In a further analysis this relationship was confirmed in the COM group but not in the HIIT group; in the COM group: for IL-2: β=-0.778, p<0.001, 95% CI; -0.88 to -0.68, for IL-6: β=-0.986, p=0.028, 95% CI; -1.85 to -0.12, for IL-10: β=-4.41, p<0.01, 95% CI; -4.97 to -3.85. In addition, for IL-2 and IL-10 post-CPET values, a similar association was established between their change following rehabilitation (phase 4 vs. 2) and the VO_{2peak} change: for IL-2: β= -0.157, p=0.044, 95% CI; -0.31 to -0.01, for IL-10: β=-0.977, p=0.031,

95% CI; -1.85 to -0.11.

DISCUSSION

In the present study, significant changes in the levels of certain inflammatory markers were observed after an acute exercise bout (CPET) as well as after exercise training. Specifically, CRP levels and the IL-6/IL-10 ratio, representing the pro-inflammatory/anti-inflammatory balance [18] decreased after the rehabilitation program, both before and after CPET (Figure 3). IL-10 increased after CPET, before and after training; in addition, IL-10 post-CPET value was higher after the rehabilitation program than its corresponding value before. IL-2 and IL-6 increased after CPET, but their values before and after training did not differ significantly. In particular, the mean values of IL-6 after rehabilitation, both before and after CPET, were lower compared to the pre-rehabilitation means (although not in a statistically significant level). Overall, our findings provide further corroborative evidence to support an anti-inflammatory effect of exercise and exercise training that has been previously shown in other studies.[5, 19]

Also, VEGF increased after CPET as well as after the rehabilitation program, in accordance with other studies, indicating a beneficial exercise effect i.e. promoting muscle angiogenesis.[20].

In addition, an improvement in exercise capacity indices was observed after exercise rehabilitation, as demonstrated in other studies;[21]microcirculatory indices improved as well. Particularly, faster RR might indicate increased microvascular reactivity in response to ischemic stimulus, following rehabilitation.[22] OCR increased only in the HIIT group. In the COM group, the mean OCR values were not significantly altered post-training, possibly due to a selective optimization of anaerobic mechanisms, i.e. glycolysis and creatine kinase reaction, and an improved mechanical efficiency of muscle work, i.e. reduced oxygen cost for a given workload, induced by addition of strength training.[23, 24]

Finally, significant correlations were demonstrated between the cytokine levels change and the ventilatory response to exercise (Table 3), which, separately, remained only in the COM group. Accounting for the emerged correlations, pertinent explanatory comments are provided below.

A. Regarding the interconnectionbetween the inflammatory and cardiopulmonary responses in exercise

In CHF patients, ergoreflex activation is enhanced, resulting in greater sympathetic nervous system (SNS) activation. Ergoreceptors, initiating the ergoreflex response, are stimulated by specific metabolic and mechanical stimuli, generated in CHF patients during exercise: 1. greater accumulation of metabolic products, e.g., extensive phosphocreatine (PCr) depletion with increased lactate levels and rapid drop in pH [25] (*increased metabolic stimulus*), 2. reduced total body mass (loss of peripheral muscle mass) is associated with ergoreflex overactivity since the same workload would correspond to a higher intensity per unit muscle volume [26](*increased mechanical stimulus*).

SNS and its neurotransmitters in turn, modulate the circulatory and ventilatory response to exercise; moreover, SNS activity directly affects inflammatory cytokine production.[27, 28] Exercise training reduces ergoreflex (and SNS) activation,[24] changing both cardiopulmonary and inflammatory response. Of note, changes in clinical indices of SNS activation (blood pressure, heart rate, ventilation) as a result of ergoreflex change,would be more pronounced after force training than after aerobic training; also, strength training mainly affects the ventilatory component of the ergoreflex response (compared to central hemodynamics).[29, 30] The presumed *interconnection(through SNS activation)between the inflammatory and cardiopulmonary responses in exercise*, could explain the correlation that emerged in our study (i.e., between cytokine levels and VE/VCO_2 alteration). The fact that this correlation was observed only in the group of patients who performed COM training is probably due to the greater effect of resistance training on muscle strength and mass,[30-32] and therefore to its specific effect on the mechanical stimuli that are generated during exercise: the greater increase in muscle mass by strength training allows the same amount of work to be performed at a lower intensity per unit muscle volume; aditionally, with increasing muscle strength, the same workload will represent a lower per-

centage of the maximum. Therefore, strength training can reduce the workload imposed on muscle fibers and, consequently, mechanical stress. A greater effect by strength training in ergoreflex/SNS activation could be especially supported in CHF patients, as it seems that the mechanoreflex becomes more intense during CHF while metaboreflex decreases.[29]

B. Regarding the specific correlations observed

More specifically, the noteworthy correlations that emerged in the present study are the following: the changes in the levels of certain cytokines (IL-2, IL-6, IL-10) between phases 2 and 4 (i.e. post-CPET, before vs. after the training program) were negatively correlated with the corresponding changes of VE/VCO_2 slope. In a separate group analysis this correlation remained only in the COM group; in the same group a negative correlation was also established between IL-2 and IL-10 levels change and VO_2peak change.

Therefore, a decrease in VE/VCO_2 slope (indicating improved exercise capacity) was associated with an increase in the inflammatory response after maximal exercise (increase in cytokine levels). However, at this point we should note that: 1. The relative IL-10 change was comparatively greater than that of IL-6 (and IL-2) per unit of VE/VCO_2 change (larger b-coefficient), which translates into an overall enhanced anti-inflammatory effect of this type of exercise training. 2. Especially for the COM group, the reduction in VE/VCO_2 slope may better reflect the improved muscle work performance than the increase in VO_{2peak}, i.e. the ability of muscles to perform more intense/prolonged exercise, which, in turn, can provoke an overall increased inflammatory response. A potential cause may be the fact that, in the COM group, muscle oxidative capacity (as assessed by OCR) did not significantly increase; as mentioned above, strength training mainly optimizes the performance of the anaerobic mechanisms.

Regarding specifically IL-6, which is expected to increase with increasing exercise workload,[4] it has been traditionally considered an essential pro-inflammatory agent; however, its increase during exercise may induce an anti-inflammatory response as it inhibits the expression of pro-inflammatory cytokines and stimulates the production of anti-inflammatory cytokines, including IL-10.[33] Moreover, IL-6 is considered an energy sensor: it is produced by contracting muscles (myokine) to ensure supply of metabolic substrates in case of augmented exercise intensity/duration and the subsequent depletion of energy stores that may ensue; during exercise, contraction-induced IL-6 augments fat oxidation and muscle glucose uptake; it also increases hepatic glucose production.[33, 34]

IL-6 is also involved in the mechanism of muscle repair/hypertrophy, which is activated after myotrauma that usually occurs during strength training.[35]

In the same group, the opposite correlation observed (compared to the one mentioned above for VE/VCO_2 slope) between IL-2 and IL-10 change with the change in VO_{2peak} (decrease in cytokine levels with increase in VO_{2peak}) and the lack of an association of IL-6 with VO_{2peak} may be due to: a) the reduced oxidative stress that accompanies an increase in aerobic capacity,[36] resulting in a reduced inflammatory response,[37] and b) the increased ability of peripheral muscles to oxidize their metabolic substrates;[38] thus the dependence of skeletal muscles on extramuscular energy stores -which would necessitate an IL-6 release for the mobilization of energy substrates from adipose tissue and liver- is mitigated. It is also possible that the increasing VO_{2peak} signifying enhanced aerobic metabolism of the slow twitch fibers may be followed by decrease of the ergoreflex response in this patient group.[29]

Limitations

The small number of patients and the lack of measurement of the change in muscle strength/mass after the rehabilitation program in the two patient groups, which would strengthen the interpretive approach of our study results. Nevertheless, the important and particularly interesting results of the present study are certainly a basis for the design of other studies in the direction of further clarification of the pathophysiology of this complex disease.

CONCLUSIONS

The results of the present study reinforce the view of the anti-inflammatory effect of exercise in CHF patients. Particularly important were the observed correlations between the differentiation of the inflammatory response (after CPET, i.e. phase 4 vs. phase 2 difference), and the changes in the corresponding cardiopulmonary parameters (VE/VCO_2 slope, VO_{2peak}). Specifically, negative correlations were noted between IL-2, IL-6 and IL-10 changes and the VE/VCO_2 slope, in the entire patient cohort, while further analysis revealed that the correlations were maintained only in the COM group; these correlations most probably derive from the special effect of this type of exercise (addition of strength training) on peripheral muscle function.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of Evangelismos Hospital (approval number:447/13-12-2018).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study or their next of kin.

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ANNEX

FIGURE 1. Flow chart describing the process of the randomized clinical trial. HIIT group, High-intensity Interval Training group; COM group, Combined Exercise group.

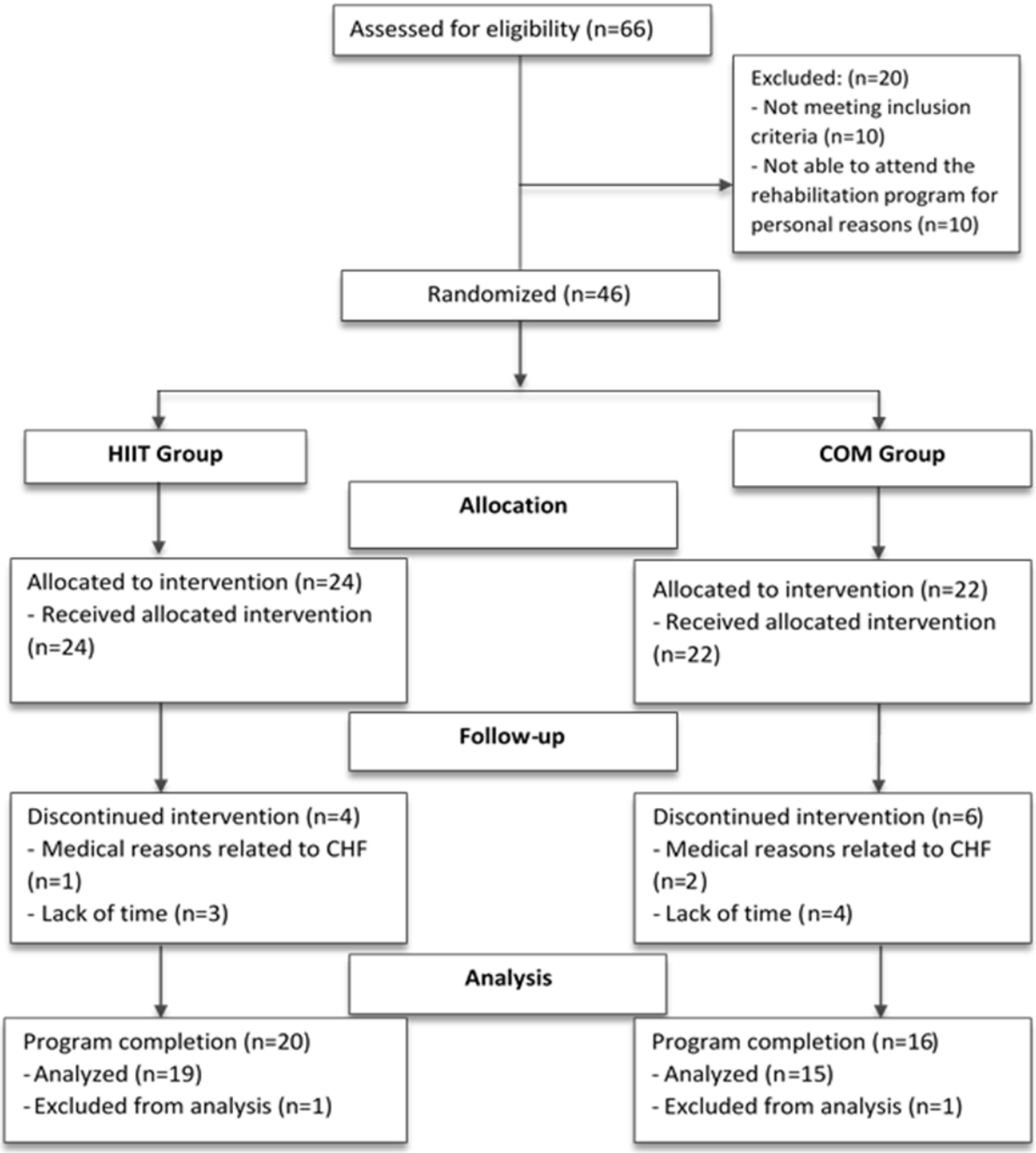

FIGURE 2. Near infrared spectrometry analysis with InSpectra Analysis Program, Version 4.01, Hutchinson Technology. O_2 saturation monitoring during rest, vascular occlusion technique and reperfusion phase.

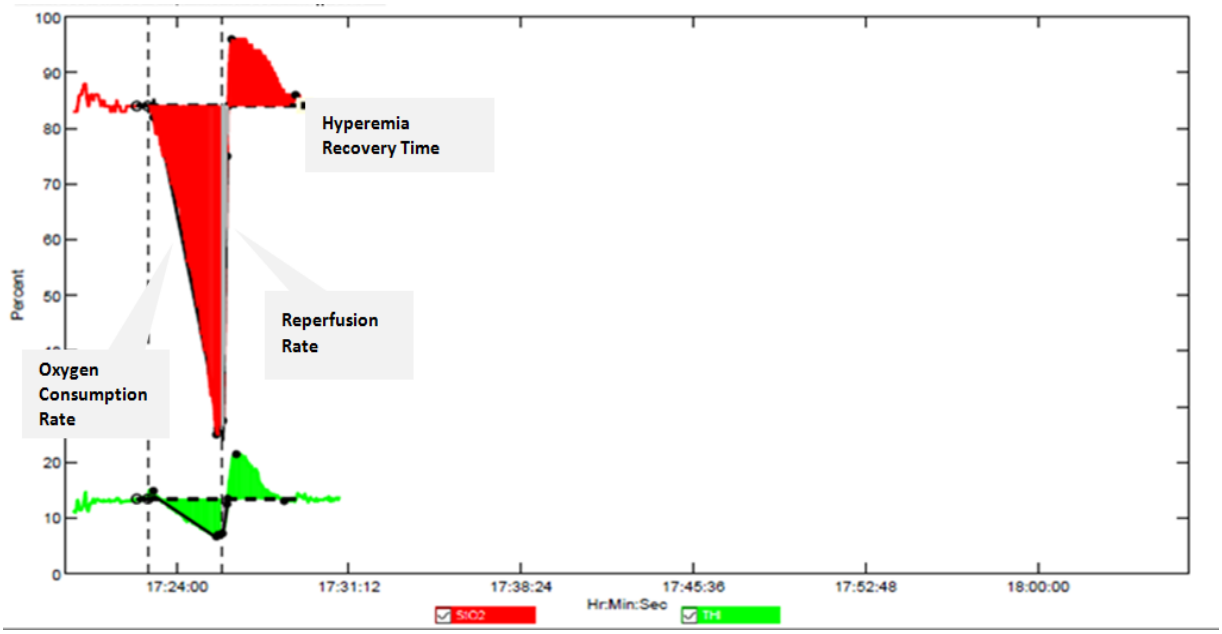

TABLE 1. Demographic characteristics for patients with CHF enrolled in the cardiac rehabilitation program, as well as for each exercise training group. No differences were observed between the 2 groups ($p > 0.05$).

Demographic characteristics	**All patients**	**HIIT Group**	**COM Group**
Number of patients (N)	34	19	15
Gender (*Males / Females)*	28 / 6	16 / 3	12 / 3
Age (years)[a]	56.5 ± 10	55 ± 11	57 ± 9
BMI (kg/m^2)[a]	26.9 ± 2.9	27.2 ± 2.8	26.6 ± 3
NYHA stage (class II / III)	25 / 9	14 / 5	11 / 4
LVEF before rehabilitation (%)[b]	32.5 (29.5 - 40)	35 (30 - 43)	30 (25 - 35)
CHF aetiology [n (%)]C			
Dilated cardiomyopathy	7 (20)	5 (24)	7 (30)
Ischemic	20 (60)	11 (52)	13 (57)
Other (valvulopathy, etc)	7 (20)	5 (24)	3 (13)
Medicaltreatment [n (%)]			
Angiotensin- receptor blockers	5 (14.7)	4 (21.1)	1 (6.7)
Beta-blockers	33 (97.1)	19 (100)	14 (93.3)
Aldosterone antagonists	24 (70.6)	14 (73.7)	10 (66.7)
Diuretics	21 (61.8)	12 (63.2)	9 (60)
Vasodilators	2 (5.9)	1 (5.3)	1 (6.7)
Amiodarone	4 (11.8)	3 (15.8)	1 (6.7)
Ca channel blockers	-	-	-
Digoxin	2 (5.9)	-	2 (13.3)
Cardiopulmonary exercise testing indices before rehabilitation			
VO_{2peak} (ml/kg/min)[a]	18.5 ± 4.5	18.6 ± 4.9	18.3 ± 3.9
VO_{2peak} predicted (%)[a]	64.5 ± 16	63.9 ± 19	65.3 ± 12.2
Peak WR (watts)[a]	98.3 ± 37.6	102.5 ± 40.4	93 ± 34.4
AT (ml/kg/min)	11.8 ± 2.6	12.3 ± 2.5	11.2 ± 2.6
VE/VCO_2 slope	29.3 ± 5.5	28.8 ± 6.6	30 ± 4
Microcirculatory indices and inflammatory markers/cytokines before rehabilitation.			

CRP (mg/L)[a]	0.36 ± 0.51	0.24 ± 0.20	0.51 ± 0.71
IL-2 (pg/ml) [a]	36.86 ± 2.17	36.68 ± 0.67	37.07 ± 3.23
IL-6 (pg/ml) [a]	21.95 ± 15.02	21.89 ± 15.36	22.02 ± 15.12
IL-10 (pg/ml) [a]	24.80 ± 1.54	24.78 ± 1.70	24.82 ± 1.36
VEGF (pg/ml) [a]	22.48 ± 24.26	25.78 ± 30.86	18.31 ± 11.38

HIIT group, High-intensity Interval Training group; COM group, Combined Exercise group; BMI, Body Mass Index; NYHA, New York Heart Association; CHF, Chronic Heart Failure; VO_2, Oxygen uptake; CPET, Cardiopulmonary Exercise Testing; LVEF, Left Ventricular Ejection Fraction; WR, Work Rate; AT, Anaerobic Threshold; VE/VCO_2, slope of the ventilatory equivalent for carbon dioxide production; CRP, C-Reactive Protein; IL, Interleukin; VEGF, Vascular Endothelial Growth Factor.

Notes: a Values are expressed as mean ± SD, b Values are expressed as median (25th - 75th percentiles).

TABLE 2. Microcirculatory indices and inflammatory markers/cytokines change, before and after exercise rehabilitation.

	Pre Rehabilitation Program		***Post Rehabilitation program***			
	Pre CPET (Phase 1)	***Post CPET (Phase 2)***	***Pre CPET (Phase 3)***	***Post CPET (Phase 4)***	***P***	***Pairwise Comparisons***
BSL StO_2 (%)[b]	80.00 (6.25)	79.00 (7.25)	80.00 (5.25)	79.50 (9.25)	0.428	*None*
OCR(%/min)[a]	12.64±4.32	13.27± 3.96	15.53± 3.73	15.97± 4.10	**<0.001**	***Phase 1 vs. 3***(*p*<0.001), ***Phase 2 vs. 4*** (*p*<0.001)
RR (%/sec)[a]	4.02± 1.13	3.95± 1.21	4.86± 1.12	4.44± 1.50	**0.011**	***Phase 1 vs. 3*** (*p*=0.003)
HRT (sec)[a]	145.44± 36.61	134.47± 35.95	144.44± 31.88	139.56± 26.91	0.425	*None*
CRP (mg/L)[b]	0.20 (0.40)	0.20 (0.33)	0.10 (0.20)	0.15 (0.23)	**<0.001**	***Phase 1 vs. 3***(*p*<0.001), ***Phase 2 vs. 4*** (*p*=0.007)
IL-2 (pg/ml)[b]	36.24 (0.84)	36.65 (1.35)	36.28 (1.15)	36.98 (1.48)	**0.003**	***Phase 1 vs. 2*** (*p*=0.040), ***Phase 3 vs. 4*** (*p*=0.002)
IL-6 (pg/ml)[b]	17.20 (10.22)	18.61 (4.41)	15.08 (8.76)	16.94 (5.79)	**0.002**	***Phase 3 vs. 4*** (*p*=0.017)
IL-10 (pg/ml)[b]	24.50 (2.41)	28.34 (3.36)	24.83 (5.32)	29.25 (2.02)	**<0.001**	***Phase 1 vs. 2*** (*p*<0.001), ***Phase 2 vs. 4*** (*p*=0.028), ***Phase 3 vs. 4*** (*p*=0.003)
VEGF(pg/ml)[b]	14.31 (7.39)	21.58 (11.12)	23.21 (30.11)	26.64 (24.14)	**<0.001**	***Phase 1 vs. 2*** (*p*=0.001), ***Phase 1 vs. 3*** (*p*<0.001), ***Phase 2 vs. 4*** (*p*=0.045)
IL-6/IL-10[b]	0.68 (0.39)	0.66 (0.11)	0.58 (0.20)	0.57 (0.15)	**0.004**	***Phase 1 vs. 3*** (*p*=0.039), ***Phase 2 vs. 4*** (*p*=0.010)

CPET, cardiopulmonary exercise testing; BSL StO_2, Baseline Tissue Oxygen Saturation; OCR, Oxygen Consumption Rate; RR, Reperfusion Rate; HRT, Hyperemia Recovery Time; CRP, C-Reactive Protein; IL, Interleukin; VEGF, Vascular Endothelial Growth Factor. Notes: Significance level at 0.05. [a]Values referred as mean ± standard deviation (SD). p-values calculated using repeated measures of analysis of variance (ANOVA). Pairwise comparisons calculated using paired t-test. [b]Values referred as median and interquartile range (IQR). p-values calculated using Friedman test. Pairwise comparisons calculated using Wilcoxon Signed Ranks test.

TABLE 3. Associations between post-CPET interleukin (IL)-2, -6, -10 changes and VE/VCO2 slope change, before and after exercise rehabilitation.

Model		B	t•	P	F/P‡	R^2
1	(Constant)	-0.346	-0.589	0.560	*p*<0.001	0.591
(IL-2)	VO_{2peak} pre vs. post	-0.084	-0.819	0.419		
	VE/VCO_2 post vs. pre	**-0.523**	**-6.652**	**<0.001**		
2	(Constant)	-1.726	-0.964	0.343	*p*=0.034	0.196
(IL-6)	VO_{2peak} pre vs. post	-0.116	-0.371	0.714		
	VE/VCO_2 post vs. pre	**-0.654**	**-2.731**	**0.010**		
3	(Constant)	-1.868	-0.586	0.562	*p*<0.001	0.604
(IL-10)	VO_{2peak} pre vs. post	-0.357	-0.639	0.528		
	VE/VCO_2 post vs. pre	**-2.922**	**-6.855**	**<0.001**		

CPET, cardiopulmonary exercise testing; IL, Interleukin (pg/ml); VO_{2peak}, Oxygen uptake at peak exercise (ml/kg/min);VE/VCO_2, slope of the ventilatory equivalent for carbon dioxide production. **Notes**:Dependent variables: Differences between phase 4 vs. 2 of IL-2, IL-6, IL-10. Independent variables: VO_{2peak}, VE/VCO_2 post vs. pre rehabilitation program. Unstandardized coefficient (β). ‡P-value for the F-test of overall significance test of the model. †R^2: Goodness-of-fit measure for linear regression models. • t: t statistic of coefficient.

FIGURE 3. C- Reactive Protein and IL-6/IL-10 ratio estimated means for each exercise group among the four phases (IL, interleukin).