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Abstract

PURPOSE: The randomized controlled OptiTrain trial showed beneficial effects on fatigue after a 16-week exercise intervention in patients with breast cancer undergoing adjuvant chemotherapy. We hypothesize that exercise alters systemic inflammation and that this partially mediates the beneficial effects of exercise on fatigue. .

METHODS: Two hundred and forty women scheduled for chemotherapy were randomized to 16 weeks of resistance and high-intensity interval training (RT-HIIT), moderate-intensity aerobic and high-intensity interval training (AT-HIIT) or usual care (UC). In the current mechanistic analyses, we included all participants with >60% attendance and a random selection of controls (RT-HIIT=30, AT-HIIT=27, UC=29). Fatigue (Piper Fatigue Scale) and ninety-two markers (e.g. IL-6, TNFα) were assessed at baseline and post-intervention. Mediation analyses were conducted to explore whether changes in inflammation markers mediated the effect of exercise on fatigue.

RESULTS: Overall, chemotherapy led to an increase in inflammation. The increase in IL-6 (pleiotropic cytokine) and CD8a (T-cell surface glycoprotein) was, however, significantly less pronounced following RT-HIIT compared to UC (-0.47 (95%CI -0.87;-0.07) and -0.28 (-0.57;0.004), respectively). Changes in IL-6 and CD8a significantly mediated the exercise effects on both general and physical fatigue by 32.0% and 27.7%, and 31.2% and 26.4%, respectively. No significant between-group differences in inflammatory markers at 16 weeks were found between AT-HIIT and UC.

CONCLUSION: This study is the first showing that supervised RT-HIIT partially counteracted the increase in inflammation during chemotherapy, i.e. IL-6 and soluble CD8a, which resulted in lower fatigue levels post-intervention. Exercise including both resistance and high-intensity aerobic training might be put forward as an effective treatment to reduce chemotherapy-induced inflammation and subsequent fatigue.

KEYWORDS: Exercise; Breast cancer; Fatigue; Chemotherapy; Inflammation; Mechanisms

Introduction

Fatigue is one of the most common and debilitating side effects of cancer and its treatment(1,2), with some studies reporting a prevalence as high as 70-99%(3). It typically increases during cancer treatment and can be experienced for up to 10 years after cancer diagnosis(4–6). Fatigue substantially interferes with daily life activities and as a consequence it impairs the overall quality of life (QoL) during and after cancer treatment(7,8).

The etiology of fatigue has not been fully elucidated and it may involve a variety of demographic, clinical, psychosocial, behavioral and biological factors(2). Proposed underlying mechanisms include mitochondrial dysfunction, hypothalamic-pituitary-adrenal axis dysfunction, anemia, circadian rhythm disruption, disturbance of monoamine pathways and chronic inflammation(9). To date, the mechanism that has gained most empirical attention and support is chronic inflammation.

Reviews show that elevated neutrophil and monocyte counts and higher levels of several inflammatory markers, including interleukin (IL)-6, IL-1β, IL-1ra, neopterin and C-reactive protein (CRP) are associated with fatigue in cancer survivors(2,9,10). In patients with breast cancer undergoing chemotherapy specifically, changes in IL-6 were positively correlated with changes in fatigue(11,12).

Growing evidence suggests that exercise is an effective intervention to reduce levels of fatigue in patients receiving adjuvant chemotherapy(13,14). It is hypothesized that this might be partially due to its anti-inflammatory effects. Three reviews showed that regular exercise after completion

of primary breast cancer treatment reduced the serum concentration of pro-inflammatory cytokines, including IL-2, IL-6, IL-8 and tumor necrosis factor (TNF)-α(15) and elevated levels of anti-inflammatory cytokines, including IL-10(9) and IL-1ra(16). Another study, including patients with breast, lung and colon cancer, found positive correlations between changes in cytokine concentrations (i.e. IL-10:IL-6, IL-10:IL-1β, IL-10:sTNFR1) after a 6-week exercise intervention during adjuvant chemotherapy(17). These positive correlations were significantly greater than the correlations observed in the control group, supporting the role of exercise in moderating therapy-induced inflammation.

The randomized controlled OptiTrain trial showed beneficial effects on fatigue after a 16-week exercise intervention during adjuvant chemotherapy in patients with breast cancer(18). Here, we investigated the effects of exercise on inflammatory markers and whether the positive effects on fatigue were mediated by changes in inflammation. Additionally, we examined whether changes in inflammatory markers were correlated with changes in physiological outcomes. Finally, we aimed to identify groups of cytokines whose expression levels are correlated. We hypothesize that exercise alters systemic inflammation and that this partially mediates the beneficial effects of exercise on fatigue.

Methods

Setting and participants

The OptiTrain study is a randomized controlled exercise trial in women with breast cancer undergoing adjuvant chemotherapy (ClinicalTrials.gov, NCT02522260). A detailed description of the OptiTrain study design(19) and effects of the exercise intervention on fatigue, QoL, pain

and physical fitness(18,20) have been published previously. Patients were not involved in the design, conduct and interpretation of this study. Patients were involved in dissemination of the results.

The study was conducted at Karolinska University Hospital (Stockholm, Sweden) between March 2013 and July 2016. In short, 240 women with breast cancer were randomized to 16-weeks of resistance and high-intensity interval training (RT-HIIT, *n*=79), moderate-intensity aerobic and high-intensity interval training (AT-HIIT, *n*=80) or to usual care (UC, *n*=81). The OptiTrain study included women (aged 18-70 years) diagnosed with stage I-IIIa breast cancer, scheduled for adjuvant chemotherapy. Exclusion criteria were: advanced disease, heart or lung disease, cognitive dysfunction, other health problems that would prevent safe participation in the exercise testing or training as determined by their medical doctor, or not being able to understand the Swedish language. Ethical approval was obtained from the Regional Ethical Review Board in Stockholm Sweden (Dnr 2012/1347-31/1, 2012/1347-31/2, 2013/7632-32, and 2014/408-32) and all participants gave written informed consent prior to enrollment.

Intervention

Participants in the exercise intervention groups commenced the exercise training three days after the second chemotherapy session. Patients were asked to attend 60-min exercise sessions, twice-weekly, on non-consecutive weekdays for 16 weeks. Exercise sessions were supervised by an exercise physiologist or oncology nurse at the exercise clinic of Karolinska University Hospital. The exercise sessions of the AT-HIIT-group consisted of 20 min of moderate intensity aerobic exercise at a rate of perceived exertion (RPE) of 13-15, followed by 3x3 min bouts of high

intensity intermittent aerobic exercise at a RPE of 16-18 interspersed with 1 min low-intensity active recovery. The exercise sessions of the RT-HIIT-group consisted of 8 resistance exercises followed by the 3x3 min bouts of high intensity intermittent aerobic exercise. Participants performed 2-3 sets of 8–12 repetitions at an initial intensity of 70% of their estimated one repetition maximum (1-RM), progressing to 80% of 1-RM when more than 12 repetitions could be performed. The UC-group received information about physical activity but no supervised exercise training.

Outcome assessment

At baseline (i.e., before randomization) and post-intervention, participants visited Karolinska University Hospital for outcome assessment. The assessors of blood samples, but not the study investigators, were blinded to group allocation. Blood samples were drawn at both visits, in addition to performing physical measurements and completing questionnaires. At baseline, blood samples were drawn from the patients' PICC line in conjunction with receiving their second chemotherapy session (before chemotherapy infusion). The post-intervention blood samples were drawn three weeks following the sixth (last) round of chemotherapy and 48-72 hours after an exercise session.. Patients were asked to not eat or drink 3 hours before the blood draw. Samples were stored locally at -80ºC until analysis took place.

Inflammatory markers

Plasma samples were analyzed using an immune-oncology multiplex proximity extension assay (Olink Bioscience, Uppsala Sweden) at the Clinical Biomarkers Facility, Science for Life Laboratory in Uppsala, Sweden. This panel measures inflammatory markers that are relevant for

key processes, such as apoptosis and chemotaxis. The quantification cycles were produced by the BioMark's Real-Time PCR Software. The quantification cycle values from an internal control (extension control) were subtracted from the measured quantification cycle value, an interplate control was corrected for, and a correction factor was subtracted to yield a normalized, log-2 transformed protein expression value. The value is a relative quantification, meaning that no comparison of absolute levels between different proteins can be made. Ninety-two inflammatory markers were assessed. Data was censored if values were below the detection limit. If the percentage of censored values was below 25%, all values below the detection limit were substituted by the detection limit divided by the square root of 2(21). Since some of the more relevant markers, specifically IFN-y, TNF-a, IL-4, IL-1b showed poor detection on the Olink platform, we tested these markers on the Merck Cytomag custom made platform. Fifteen inflammatory markers had more than 25% censored values and were therefore excluded from the analyses (i.e. CCL7, IL-1a, ADGRG1, FGF-2, IFN-β, NOS 3, IL-2, IL-5, IL-13, IL-21, IL-33, IL-35, CD28, SDF-1, IL12RB1).

Cancer-related fatigue

Cancer-related fatigue was self-assessed using the validated Swedish version of the Piper Fatigue Scale (PFS)(22). The PFS is a 22-item questionnaire and covers four dimensions of fatigue: behavioral/daily life, sensory/physical, cognitive, and affective/emotional meaning. Each item is composed of a scale from 0-10, with higher scores indicating higher levels of fatigue.

Physical (activity) measurements

Muscle strength was assessed using a hydraulic hand dynamometer and lower-limb muscle strength by using an isometric mid-thigh pull. Cardiovascular fitness, measured as predicted peak oxygen uptake (VO_{2peak}), was assessed by a submaximal exercise test on a cycle ergometer. Objectively measured physical activity was assessed at baseline by an accelerometer (GT3X ActiGraph® Corp, Pensacola, FL, USA).

Statistical analysis

For these secondary and mechanistic analyses, we only included all participants who attended ≥60% of all exercise sessions, since adherence is defined as successful if participants completed at least two-thirds of an exercise program(23). A random sample of controls was drawn. We selected all patients allocated to UC with available samples for both time points (i.e., baseline and post-intervention) and used the random sampling function in Excel. Descriptive statistics were used to summarize the baseline characteristics of the study population. To assess whether the effects of exercise on fatigue were mediated by changes in inflammation, we estimated a series of linear regression equations according to MacKinnon *(see figure, Supplemental Digital Content 1, a schematic representation of the mediation model, http://links.lww.com/MSS/C101)*(24). First, an ANCOVA was conducted to determine between-group differences in inflammatory markers, with post-intervention values of inflammatory markers as dependent variables (M_2), the randomization group as independent variable (X), and the baseline values of the inflammatory marker (M_1) and outcome fatigue (Y_1) as covariate. If the p-value of the effect of either RT-HIIT or AT-HIIT on the inflammatory marker was below 0.20, the second regression equation was used to assess mediation. Second, an ANCOVA was

conducted to determine between-group differences in the outcome fatigue, with post-intervention values of fatigue as a dependent variable (Y_2), the randomization group as an independent variable (X), and the baseline values of the inflammatory marker (M_1) and outcome fatigue (Y_1), and post-intervention levels of the inflammatory marker (M_2) as covariates. All models were adjusted for menopausal status and chemotherapy regimen (taxanes or non-taxane based). We did not adjust for multiple comparisons due to the exploratory nature of the current study.

The mediated effect of the intervention (X) on total and physical fatigue (Y_2) through inflammation marker M_2 was calculated. Due to non-normality of the mediated effect, resampling methods were used to construct confidence intervals around the mediated effect. The mediation effect was calculated by dividing the mediated effect by the total effect of exercise on fatigue.

The Pearson correlation coefficient was calculated to evaluate the linear relationship between physiological outcomes and inflammatory markers, and between changes in inflammatory markers. For the latter analyses, one-way hierarchical clustering was conducted using Pearson's correlation coefficient as a proximity distance matrix to identify groups of cytokines whose expression levels are correlated. A heatmap was created for all study arms using the 'heatmap' function in R or using GraphPad Prism. All analyses were performed using R version 3.5.1 or GraphPad Prism version 8.

Results

Participants

Overall, 30 of the patients allocated to RT-HIIT attended ≥60% of all exercise sessions, whereas 27 of the patients allocated to AT-HIIT attended ≥60% of all exercise sessions *(Figure 1)*. On average, the RT-HIIT group attended 79.5% (SD=20.3) of all exercise sessions, whereas the AT-group attended 82.1% (SD=17.4) of all exercise sessions.

Baseline characteristics for the patients included in the current analyses were comparable to the baseline characteristics of the patients included in the original OptiTrain study(18), except for physical activity levels. Women in the RT-HIIT group were significantly more active per day at a moderate-to-vigorous-intensity compared to women in the UC group. Women were primarily middle-aged and had on average a healthy BMI*(Table 1)*. The majority of women was postmenopausal (58%) and treated with anthracyclines alone (38%) or in combination with taxanes (57%).

Effects of exercise on inflammatory markers

Receiving adjuvant chemotherapy altered the plasma cytokine profile of patients with breast cancer *(Figure 2A)*. In general, chemotherapy led to an increase in pro-inflammatory cytokines in all groups, *(Figure 2A and* table, *Supplemental Digital Content 2, exercise effects on 92 different cytokines, http://links.lww.com/MSS/C102),* while other factors, such as EGF were reduced over the course of therapy. The increase in IL-6 and CD8a was significantly less pronounced following RT-HIIT compared to UC (-0.47 (95% CI -0.87;-0.07) and -0.28 (95% CI

-0.57;0.004), respectively) *(Figure 2B and Table 2)*. No significant differences in single inflammatory markers were found between AT-HIIT and UC at 16 weeks.

Mediation effects of inflammatory markers on fatigue

Compared to the original OptiTrain study, larger effects of the exercise intervention on fatigue were found at 16 weeks in this subgroup, especially for the AT-HIIT group compared to usual care (-1.59, 95% CI -2.94;-0.24) *(Table 3)*. The changes in IL-6 and CD8a significantly mediated the effects of RT-HIIT on both total and physical fatigue by 32.0% and 27.7%, and by 31.2% and 26.4%, respectively *(Table 4)*.

Correlations between physiological outcomes and inflammatory markers

Post-intervention, lower-body muscle strength significantly improved in both the RT-HIIT and AT-HIIT group compared to UC, whereas cardiorespiratory fitness significantly improved in the AT-HIIT group only and handgrip strength in the RT-HIIT group *(Table 3).* Correlations between changes in physiological outcomes and changes in inflammatory markers are shown in *Table 5*. A significant inverse correlation was found between change in cardiorespiratory fitness and change in serum levels of IFN-y (r = -0.33, p = 0.005). A significant positive correlation was found between change in handgrip strength and change in serum levels of CCL17 (r = 0.22, p = 0.045). No significant correlations between changes in BMI and lower limb muscle strength and changes in inflammatory markers were found.

Cluster analysis of cytokine correlations

Two major clusters, which are groups of inflammatory markers whose expression levels are correlated, were found within the RT-HIIT and usual care group *(Figure 2C).* The first cluster (cluster A) included anti-inflammatory markers: CASP8, CLL17, CD40L and EGF. The second cluster (cluster B) included pro-inflammatory markers: CD8A, ICOSLG, DCN, CXCL9, IL-6, FasL, TRAIL, CSF1, and MIC A/B. A positive correlation was observed between cytokines within each cluster. A negative correlation was observed between cytokines in cluster A and B in the usual care group, while weaker correlations between the two clusters were observed in the RT-HIIT group. The AT-HIIT group exhibited weaker correlations between inflammatory markers.

Discussion

This study showed that chemotherapy led to a general increase in inflammation, however, the increase in IL-6 and CD8a was less pronounced following 16 weeks of RT-HIIT compared to usual care. Furthermore, we found that these two inflammatory markers partially mediated the previously proven beneficial effect of exercise on total and physical fatigue. We did not observe effects of AT-HIIT on inflammatory markers.

Our work extends current knowledge from prior studies on inflammation during cancer treatment, with studies reporting increased levels of IL-6 and decreased levels of IL-1RA in patients with breast cancer undergoing chemotherapy(11,25). Weekly paclitaxel has been shown to increase plasma levels of IL-10, whereas higher dose treatment, administered every 3-weeks, resulted in an up-regulation of plasma levels of IL-6 and IL-8(26). In contrast, circulating levels

of IL-6 have not been shown to increase following anthracycline-based treatment(27,28). Due to the wide variety of chemotherapy regimens in our study and the small study population, we were not able to examine effects of specific cytostatic therapies. As demonstrated by within-group differences in all groups, we observed a substantial increase in pro-inflammatory markers (e.g. IL-6, IL-12, IFN-y and TNF-a) and a decrease in anti-inflammatory markers (e.g. IL-10, EGF, ANG-1 and CASP-8), suggesting that induces an inflammatory environment.

Exercise has been shown to prevent or diminish inflammation in both healthy individuals(29,30) and patients with cancer(15,17,31,32). This study showed that RT-HIIT counteracted an increase in IL-6 and CD8A. This finding is not in line with results from two previous studies, which showed that combined resistance and aerobic training during adjuvant chemotherapy led to serum levels of IL-6 comparable to the usual care group(12) or to elevated levels of IL-6 in patients with breast cancer(17). In contrast to the aforementioned studies, we used a per-protocol analysis, and included patients who attended ≥60% of all exercise sessions and additionally, we implemented a more vigorous exercise program. Both aspects could have contributed to larger effects found in the current study. Other studies on the effects of exercise on inflammation had a different timing of the intervention period or included patients who received different treatment regimens. A recent meta-analysis showed that exercise, implemented after primary breast cancer treatment, reduced serum concentrations of IL-6, TNF-a and IL-8(15). Furthermore, Schmidt *et al.* (2015) observed that the increase in IL-6 during radiation therapy was counteracted by resistance exercise in women with breast cancer(31). The latter study also assessed the mediating role of IL-6 in the development of fatigue during radiation therapy and the moderation by resistance exercise. Schmidt and colleagues observed that IL-6 mediated the effect of exercise on

physical fatigue by 22%, which is in agreement with our observation that IL-6 mediated the effect by 27.7%.

RT-HIIT, but not AT-HIIT, was effective in moderating chemotherapy-induced inflammation, which can be explained by the underlying biology. It is generally known that resistance training primarily recruits type II muscle fibers compared to aerobic exercise, which mostly uses type I fibers(33). Evidence suggests that muscles express inflammatory markers in a fiber type specific manner(34), which might explain the different effects found for RT-HIIT and AT-HIIT on inflammatory markers compared to UC. During exercises with sufficient load, the skeletal muscle secretes myokines, such as IL-6(35). The rise in circulating IL-6 is responsible for the release of anti-inflammatory marker IL-10, which downregulates the expression of several pro-inflammatory markers including IL-6(16). Since this cascade of inflammatory cytokines is assumed to be less active following AT-HIIT, this might explain the smaller changes in concentrations of inflammatory markers found in this study arm. Indeed, there was a significant loss of IL-10 in both the UC and AT-HIIT groups and a trend towards a moderated IL-10 loss in the RT-HIIT group compared to UC, while there was no effect of AT-HIIT compared to UC. Future studies are needed to further explore potential mechanisms that can underpin the beneficial effects of RT-HIIT on inflammatory markers.

In the present study, we also explored correlations between inflammatory markers and physiological outcomes, since the influence of exercise on the inflammatory pathway might be more pronounced in the patients showing physiological response. Of note, we found correlations between changes in lower muscle and handgrip strength and immunogenic markers including

CD8a. These results are confirmed by a recent pilot study from Narsale *et al.* (2019), who showed that naïve, memory and regulatory T-cells correlate with muscle strength and performance(36), suggesting that engagement in muscular strengthening activities might have beneficial effects on inflammatory markers. We did not find a correlation between changes in BMI and inflammatory markers, as suggested by earlier research(37). We speculate that BMI might not be a sufficiently sensitive parameter compared to changes in adipose tissue. Furthermore, it should be noted that we investigated correlations between changes in physiological outcomes and inflammatory markers over time. We assume that muscle strength is more likely to change over a 16-week time period compared to BMI, which might explain why we did not find any correlations between BMI and inflammatory markers. This study highlights that exercise not only influences markers via BMI based on previous research(38), but also via muscle strength(39). It can be speculated that as a result of the exercise-induced reduction of inflammation in blood, muscle strength will not be negatively affected by the catabolic effects of inflammation. Many studies examined the association between weight loss and inflammation and by putting this evidence and our findings into perspective, we suggest that it would be interesting to examine whether inflammation influences muscle strength by its effects on body composition in future studies.

Strengths and Limitations

The results of this study should be viewed in the context of several strengths and limitations. First, this study captured many key and non-key exercise-related inflammatory markers, which enables us to explore exercise effects on inflammation during adjuvant chemotherapy in greater detail. As a result, we hope that these results will guide future studies by helping them to decide

which inflammatory markers might be or might not be interesting to examine using more sensitive methods in the context of exercise during chemotherapy for breast cancer. Second, this is a randomized controlled trial, suggesting a causal relationship between the exercise interventions and inflammation. Limitations are that we only included women who attended more than 60% of all exercise sessions, thereby compromising randomization(40). Nevertheless, women included in the current analyses were comparable to the women in the original OptiTrain study with respect to all baseline characteristics, except for baseline physical activity levels. Patients in the intervention groups were more often moderately-to-vigorously-active compared to the usual care group. It is intuitive that patients who participated in physical activities prior to inclusion in the study would be more likely to adhere to the exercise program. Since this is a mechanistic study and our particular interest lies on the effects of performed exercise on inflammation, we did not adjust for this difference at baseline. Evidence suggests that exercise effects on inflammatory markers might be different for specific cytostatic therapies (taxanes vs. non-taxane based), however, we were not able to stratify our results for these two types of therapies due to the small sample size. Future studies are needed to further explore this. Although we captured many inflammatory markers, we missed a few interesting markers due to undetectable cytokine concentrations (e.g. IL-13, IL-1ra, IFN-β). Due to the exploratory nature of the current analyses, the probability of a type-I error was increased(41). Finally, cancer-related fatigue is a complex and multifactorial syndrome and therefore, additional potential mechanistic mediators should be investigated.

Clinical relevance

This present study helps to explain the beneficial effects of exercise on fatigue, and as a consequence this might enhance exercise promotion and adherence. Knowledge about the underlying mechanisms and the link between relevant inflammatory markers and physiological response may potentially move exercise training in cancer patients beyond a 'one size fits all' approach, since it provides us with extensive knowledge of the molecular effects on fatigue that are induced by different dosages, intensities and modes of exercise. Ultimately, the development of targeted interventions to ameliorate fatigue will improve the long-term QoL in the growing population of patients with cancer.

Conclusions

In conclusion, our study shows that chemotherapy induced an inflammatory environment in general. Resistance and high-intensity interval training concomitant to chemotherapy is suggested to be an effective intervention to reduce chemotherapy-induced inflammation and subsequent fatigue. The beneficial effect of exercise on fatigue seemed to be partially mediated by IL-6 and CD8a. Future studies are needed to confirm our findings and to assess the long-term effects of exercise on the inflammatory environment.

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Conflict of interest statement

The authors declare no conflict of interest and have read and approved the manuscript. The authors acknowledge that the result of the present study do not constitute endorsement by the American College of Sports Medicine. The results are presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation.

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Figure Captions

Figure 1. Flowchart of patients participating in the OptiTrain study and included in the current analyses.

Figure 2. Panel A: Heatmap of changes in inflammatory marker expression following chemotherapy in exercising (RT-HIIT and AT-HIIT) and usual care (UC) groups. Colors indicate log2 fold-changes in post- versus pre-chemotherapy expression values (red – increased, blue – decreased, white – unchanged). **Panel B:** Heatmap of significantly-altered inflammatory marker expression following chemotherapy in exercising (RT-HIIT and AT-HIIT) and usual care (UC) groups. Colors indicate log2 fold-changes in post- versus pre-chemotherapy expression values (red – increased, blue – decreased, white – unchanged). Comparisons were made using ANCOVA and *p* values<0.2 were deemed significant. **Panel C:** Hierarchical cluster analysis heat-maps showing nearest-neighbor correlations of inflammatory markers in the RT-HIIT, AT-HIIT and the control (UC) group. Positive correlations are represented in graded shades of blue, whereas negative correlations are represented in graded shades of red. Cluster A and B positively correlated within the RT-HIIT and usual care group. All correlations were weaker in the AT-HIIT group.

List of Supplemental Digital Content

Supplemental Digital Content 1. pdf

Supplemental Digital Content 2. Docx

Figure 1

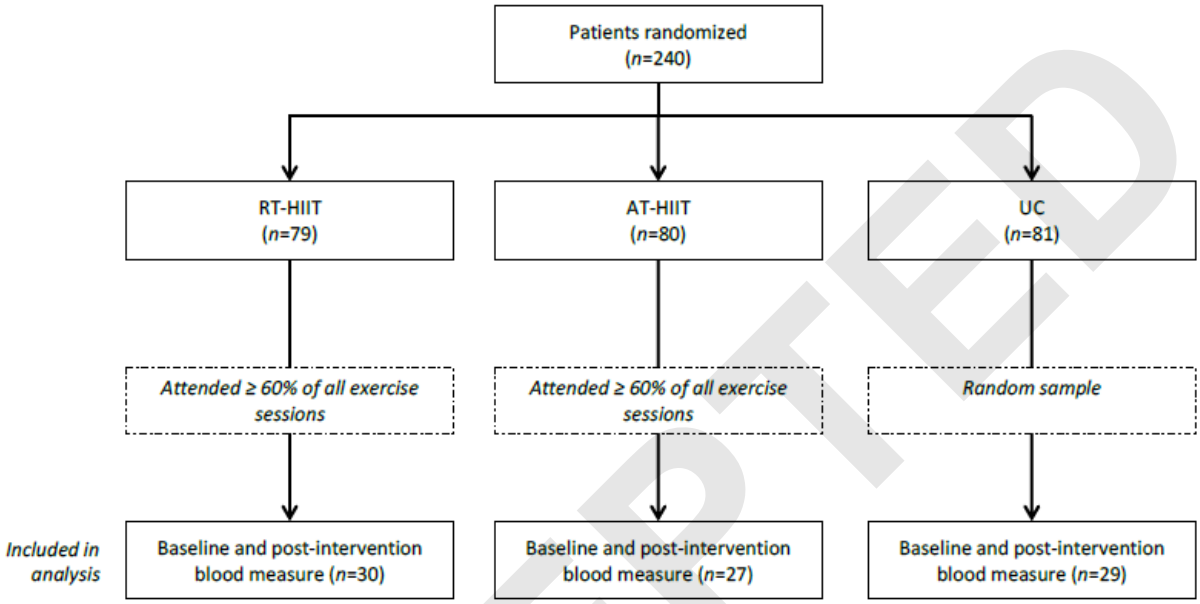


Figure 2A

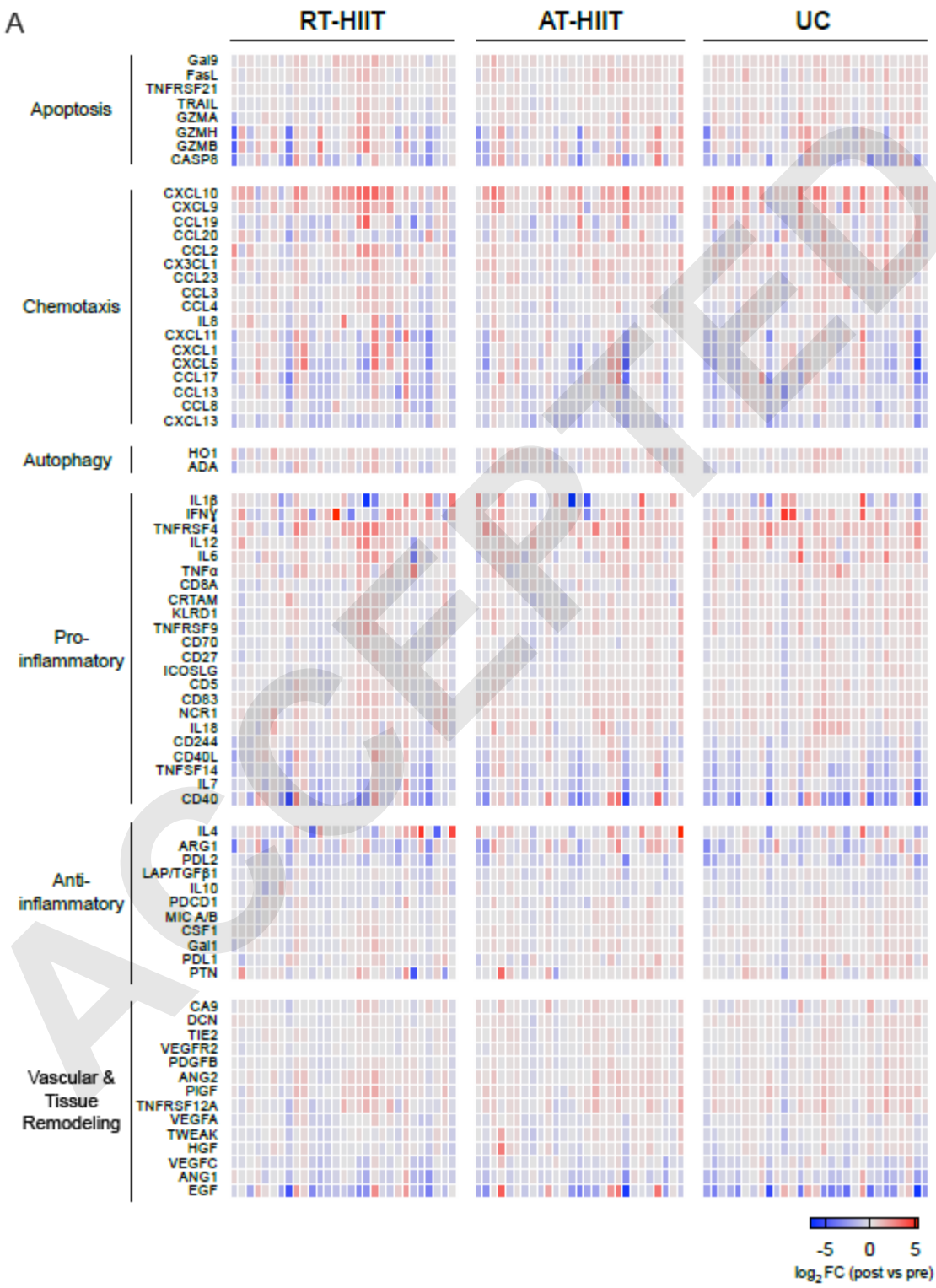


Figure 2B

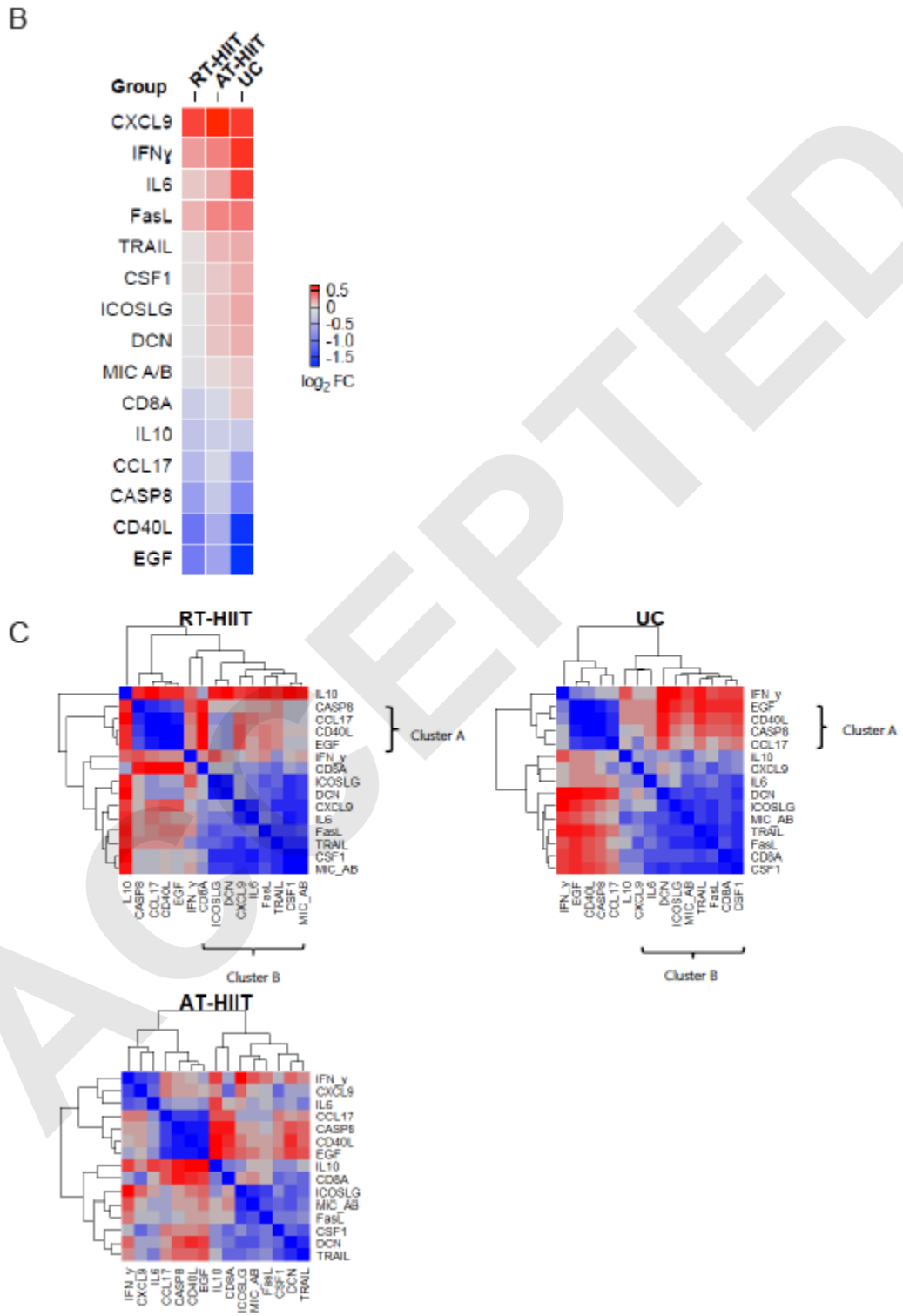


Table 1. Participant characteristics at baseline

	RT-HIIT *n* = 30	AT-HIIT *n* = 27	UC *n* = 29
Age in years	52.2 ± 10.1	53.9 ± 7.4	52.9 ± 10.1
BMI in kg/m^2	24.2 ± 3.6	24.2 ± 3.4	24.7 ± 4.4
Menopausal status			
Premenopausal	16 (53.3)	10 (37.0)	10 (34.5)
Postmenopausal	14 (46.7)	17 (63.0)	19 (65.5)
Comorbidities	6 (20.0)	4 (14.8)	10 (34.5)
Current smoker	1 (3.3)	0 (0.0)	1 (3.4)
Tumor profile			
Triple negative	1 (3.3)	5 (18.5)	6 (20.7)
HER2+, ER+, PR+	6 (20.0)	5 (18.5)	1 (3.4)
HER2+, ER-, PR-	0 (0.0)	2 (7.4)	4 (13.8)
HER2-, ER+, PR+	17 (56.7)	10 (37.0)	14 (48.3)
HER2-, ER+, PR-	3 (10.0)	4 (14.8)	2 (6.9)
HER2+, ER+, PR-	3 (10.0)	1 (3.7)	1 (3.4)
HER2-, ER-, PR+	0 (0.0)	0 (0.0)	1 (3.4)
Chemotherapy regimen			
Anthracycline	12 (40.0)	10 (37.0)	11 (37.9)
Taxane	2 (6.7)	2 (7.4)	0 (0.0)
Anthracycline + taxane	10 (33.3)	9 (33.3)	11 (37.9)
Anthracycline + taxane + Herceptin	6 (20.0)	6 (22.2)	7 (24.1)
MVPA (min/day)	92.6 ± 32.8	83.3 ± 28.6	68.0 ± 30.4
SED (min/day)	535.5 ± 104.4	555.0 ± 74.8	555.4 ± 93.1

Continuous variables are presented as mean ± SD, whereas dichotomous or categorical variables are presented as n (%).
Abbreviations: BMI Body Mass Index, *RT-HIIT* resistance and high-intensity interval training, *AT-HIIT* moderate-intensity aerobic and high-intensity interval training, *UC* usual care, *SD* standard deviation, *MVPA* objectively measured moderate-to-vigorous physical activity, SED objectively measured sedentary behavior.

Table 2. Exercise effects on inflammatory markers

Outcome	Group	Baseline mean (mean ± SD)	Post-intervention mean (mean ± SD)	Baseline to post-intervention	
				Within-group difference Mean [95% CI]	Between-group difference Mean [95% CI]
CD40-L	RT-HIIT	5.86 ± 1.87	4.90 ± 1.55	-1.06 [-1.79; -0.32]*	0.19 [-0.60; 0.99]
	AT-HIIT	6.07 ± 1.60	5.52 ± 1.18	-0.48 [-1.30; 0.35]	0.74 [-0.05; 1.53]
	UC	6.52 ± 1.33	4.82 ± 1.58	-1.73 [-2.47; -0.99]*	*ref.*
EGF	RT-HIIT	7.60 ± 1.54	6.65 ± 1.35	-1.06 [-1.76; -0.36]*	0.29 [-0.49; 1.07]
	AT-HIIT	7.76 ± 1.50	7.14 ± 1.34	-0.52 [-1.38; 0.34]	0.75 [-0.03; 1.52]
	UC	8.16 ± 1.46	6.39 ± 1.53	-1.77 [-2.55; -1.00]*	*ref.*
IL-6	RT-HIIT	2.51 ± 0.83	2.54 ± 0.74	-0.01 [-0.39; 0.37]	-0.47 [-0.87; -0.07]*
	AT-HIIT	2.85 ± 0.96	3.03 ± 0.75	0.21 [-0.09; 0.51]	-0.15 [-0.55; 0.25]
	UC	2.59 ± 1.01	3.11 ± 1.01	0.49 [0.12; 0.87]*	*ref.*
TRAIL	RT-HIIT	6.98 ± 0.29	7.02 ± 0.39	-0.004 [-0.19; 0.18]	-0.17 [-0.38;0.03]
	AT-HIIT	7.18 ± 0.55	7.33 ± 0.49	0.14 [-0.03; 0.30]	0.05 [-0.16; 0.26]
	UC	7.02 ± 0.51	7.21 ± 0.42	0.18 [0.03; 0.33]*	*ref.*
CD8A	RT-HIIT	8.19 ± 0.86	8.00 ± 0.72	-0.24 [-0.44; -0.03]*	-0.28 [-0.57; 0.004]
	AT-HIIT	8.07 ± 0.70	7.96 ± 0.74	-0.12 [-0.29; 0.06]	-0.20 [-0.48; 0.09]
	UC	7.99 ± 0.72	8.09 ± 0.80	0.10 [-0.10; 0.30]	*ref.*
DCN	RT-HIIT	3.55 ± 0.33	3.56 ± 0.39	-0.02 [-0.12; 0.09]	-0.14 [-0.31; 0.03]
	AT-HIIT	3.64 ± 0.50	3.75 ± 0.52	0.08 [-0.03; 0.20]	0.005 [-0.16; 0.17]
	UC	3.44 ± 0.54	3.62 ± 0.42	0.17 [0.03; 0.30]*	*ref.*
CCL17	RT-HIIT	7.23 ± 1.28	6.85 ± 1.23	-0.45 [-0.94; 0.03]	0.15 [-0.44; 0.74]
	AT-HIIT	7.28 ± 0.96	7.13 ± 1.08	-0.12 [-0.62; 0.38]	0.40 [-0.19; 0.99]
	UC	7.56 ± 1.07	6.84 ± 1.11	-0.73 [-1.22; -0.24]*	*ref.*
CASP-8	RT-HIIT	3.79 ± 1.25	3.18 ± 0.91	-0.75 [-1.34; -0.16]*	0.20 [-0.25; 0.66]
	AT-HIIT	3.70 ± 1.13	3.43 ± 0.74	-0.27 [-0.89; 0.35]	0.40 [-0.05; 0.86]
	UC	3.95 ± 1.01	3.03 ± 0.86	-0.96 [-1.39; -0.53]*	*ref.*
ICOSLG	RT-HIIT	3.77 ± 0.42	3.79 ± 0.48	-0.02 [-0.14; 0.11]	-0.14 [-0.32; 0.04]
	AT-HIIT	3.73 ± 0.55	3.84 ± 0.46	0.08 [-0.06; 0.23]	-0.05 [-0.23; 0.13]
	UC	3.65 ± 0.49	3.84 ± 0.43	0.18 [0.05; 0.31]*	*ref.*
CSF-1	RT-HIIT	6.73 ± 0.36	6.77 ± 0.41	-0.01 [-0.16; 0.13]	-0.13 [-0.30; 0.03]
	AT-HIIT	6.83 ± 0.51	6.92 ± 0.45	0.08 [-0.04; 0.20]	-0.03 [-0.19; 0.13]
	UC	6.68 ± 0.49	6.86 ± 0.45	0.17 [0.08; 0.27]*	*ref.*
IFN-y [a]	RT-HIIT	0.88 ± 1.59	1.01 ± 1.60	0.09 [-0.65; 0.82]	-0.51 [-1.20; 0.17]
	AT-HIIT	1.20 ± 0.98	1.46 ± 0.95	0.28 [-0.14; 0.69]	-0.14 [-0.82; 0.54]
	UC	0.88 ± 1.65	1.49 ± 1.25	0.57 [-0.05; 1.19]	*ref.*
IL-10[a]	RT-HIIT	0.92 ± 0.27	0.81 ± 0.26	-0.08 [-0.28; 0.13]	0.13 [-0.02; 0.29]
	AT-HIIT	0.95 ± 0.23	0.70 ± 0.18	-0.29 [-0.40; -0.19]*	-0.0004 [-0.14;0.14]
	UC	0.92 ± 0.26	0.71 ± 0.22	-0.27 [-0.41; -0.12]*	*ref.*
FasL	RT-HIIT	5.18 ± 0.49	5.38 ± 0.58	0.11 [-0.05; 0.27]	-0.15 [-0.39; 0.08]
	AT-HIIT	5.06 ± 0.64	5.38 ± 0.53	0.31 [0.13; 0.48]*	-0.02 [-0.25; 0.21]
	UC	5.06 ± 0.60	5.42 ± 0.63	0.33 [0.16; 0.51]*	*ref.*

CXCL9	RT-HIIT	6.21 ± 0.73	6.73 ± 0.84	0.48 [0.13; 0.83]*	0.04 [-0.37; 0.45]
	AT-HIIT	6.50 ± 1.03	7.13 ± 0.83	0.66 [0.32; 1.00]*	0.28 [-0.13; 0.69]
	UC	6.29 ± 0.95	6.82 ± 0.94	0.47 [0.10; 0.84]*	*ref.*
MIC A/B	RT-HIIT	3.11 ± 1.07	3.06 ± 1.04	-0.06 [-0.19; 0.06]	-0.14 [-0.30; 0.03]
	AT-HIIT	2.84 ± 1.16	2.88 ± 1.16	0.02 [-0.09; 0.14]	-0.06 [-0.22; 0.11]
	UC	2.97 ± 1.17	3.06 ± 1.29	0.09 [-0.03; 0.21]	*ref.*

Abbreviations: CASP-8 Caspase-8, *CCL17* C-C motif chemokine 17, *CD8A* T-cell surface glycoprotein CD8 alpha chain, *CD40-L* CD-40 ligand, *CSF-1* Macrophage colony stimulating factor-1, *CXCL9* C-X-C motif chemokine 9, *DCN* Decorin, *EGF* Pro-epidermal growth factor, *FasL* Fas antigen ligand, *ICOSLG* ICOS ligand, *IFN-y* Interferon-y, *IL-6* Interleukin-6, *IL-10* Interleukin-10, *MIC A/B* MHC class I polypeptide-related sequence A/B, *TRAIL* TNF-related apoptosis-inducing ligand, *SD* standard deviation, *CI* confidence interval, *RT-HIIT* resistance and high-intensity interval training, *AT-HIIT* moderate-intensity aerobic and high-intensity interval training, *UC* usual care.

Baseline values, within- and between-group differences were based on participants having baseline and post-intervention measurements (RT-HIIT=30, AT-HIIT=27, UC=29).

*$p<0.05$. [a]Log-transformed.

Table 3. Exercise effects on fatigue and physiological outcomes.

		Baseline	Baseline to post-intervention	
			Within-group differences	Between-group differences
		Mean (SD)	Mean [95% CI]	Mean [95% CI]
Fatigue				
Total fatigue	RT-HIIT	2.59 (3.46)	-0.02 [-1.45;1.42]	-1.25 [-2.58; 0.09]
	AT-HIIT	1.70 (2.31)	0.31 [-0.95;1.57]	-1.59 [-2.94; -0.24]*
	UC	2.27 (2.82)	1.57 [0.72;2.41]*	Reference
Physical fatigue	RT-HIIT	2.53 (3.35)	0.05 [-1.44;1.54]	-1.48 [-2.98; -0.02]*
	AT-HIIT	1.76 (2.56)	0.52 [-0.89;1.94]	-1.60 [-3.12; -0.09]*
	UC	2.62 (3.22)	1.64 [0.63;2.65]*	Reference
Physiological outcomes				
Cardiorespiratory fitness (L/min)	RT-HIIT	2.34 (0.49)	-0.09 [-0.23;0.05]	0.21 [-0.01;0.43]
	AT-HIIT	2.23 (0.48)	0.03 [-0.09;0.15]	0.31 [0.09;0.53]*
	UC	2.26 (0.49)	-0.29 [-0.44;-0.14]*	Reference
Lower-limb muscle strength (kg)	RT-HIIT	88.87 (28.94)	16.21 [8.72;23.81]*	21.65 [10.04;33.26]*
	AT-HIIT	81.27 (24.17	12.68 [7.11;18.25]*	17.53 [6.03;29.04]*
	UC	87.31 (26.02)	-6.00 [-13.49;1.49]	Reference
Handgrip strength (kg)	RT-HIIT	29.30 (5.82)	2.03 [0.95;3.11]*	3.07 [1.12;5.02]*
	AT-HIIT	29.65 (4.57)	0.19 [-1.13;1.50]	1.31 [-0.66;3.28]
	UC	29.64 (5.72)	-1.14 [-2.28;0.01]	Reference
BMI (kg/m^2)	RT-HIIT	24.22 (3.55)	0.19 [-0.15;0.52]	-0.68 [-1.32;-0.05]*
	AT-HIIT	24.22 (3.32)	0.10 [-0.24;0.44]	-0.67 [-1.31;-0.02]*
	UC	24.73 (4.35)	0.75 [0.26;1.24]*	Reference

Abbreviations: SD standard deviation, CI confidence interval, *RT-HIIT* resistance and high-intensity interval training, *AT-HIIT* moderate-intensity aerobic and high-intensity interval training, *UC* usual care, *BMI* Body Mass Index.

Baseline values, within- and between-group differences were based on participants having baseline and post-intervention measurements (RT-HIIT=30, AT-HIIT=27, UC=29). For exercise effects on fatigue and physiological outcomes in the whole OptiTrain study population, we refer to the original publications (18,20).

*p<0.05.

Table 4. The mediating effects of inflammatory markers on the relationship between exercise and fatigue.

	Total fatigue	Proportion Mediated (%)	**Physical fatigue**	Proportion Mediated (%)
	Estimate [95% CI]		Estimate [95% CI]	
Effect of RT-HIIT on fatigue				
Total effect	-1.25 [-2.58; 0.09]		-1.48 [-2.98; -0.02]*	
Indirect effect through CD40-L	-0.01 [-0.34; 0.12]	0.8	-0.03 [-0.49; 0.11]	2.0
Indirect effect through IL-6	-0.40 [-1.11; -0.04]*	32.0	-0.41 [-1.01; -0.03]*	27.7
Indirect effect through EGF	-0.006 [-0.29; 0.14]	0.5	-0.03 [-0.41; 0.08]	2.0
Indirect effect through TRAIL	-0.13 [-0.69; 0.04]	10.4	-0.13 [-0.78; 0.06]	8.8
Indirect effect through CD8A	-0.39 [-1.11; -0.02]*	31.2	-0.39 [-1.20; -0.004]*	26.4
Indirect effect through DCN	-0.13 [-0.77; 0.05]	10.4	-0.12 [-0.69; 0.08]	8.1
Indirect effect through CCL17	-0.009 [-0.30; 0.10]	0.7	-0.02 [-0.43; 0.09]	1.4
Indirect effect through CASP-8	-0.05 [-0.52; 0.06]	4.0	-0.07 [-0.60; 0.06]	4.7
Indirect effect through ICOSLG	-0.17 [-0.81; 0.05]	13.6	-0.15 [-0.72; 0.05]	10.1
Indirect effect through CSF-1	-0.30 [-0.91; 0.03]	24.0	-0.29 [-0.93; 0.07]	19.6
Indirect effect through IFN-y	-0.22 [-0.86; 0.07]	17.6	-0.24 [-1.00; 0.08]	0.16
Indirect effect through IL-10	0.03 [-0.36; 0.56]	2.4	-0.06 [-0.78; 0.26]	4.1
Indirect effect through FasL	-0.11 [-0.61; 0.08]	8.8	-0.15 [-0.73; 0.12]	10.1
Indirect effect through CXCL9	0.03 [-0.25; 0.38]	2.4	0.05 [-0.27; 0.44]	3.4
Indirect effect through MIC A/B	-0.24 [-0.68; 0.02]	19.2	-0.23 [-0.73; 0.01]	15.5
Effect of AT-HIIT on fatigue				
Total effect	-1.59 [-2.94; -0.24]*		-1.60 [-3.12; -0.09]*	

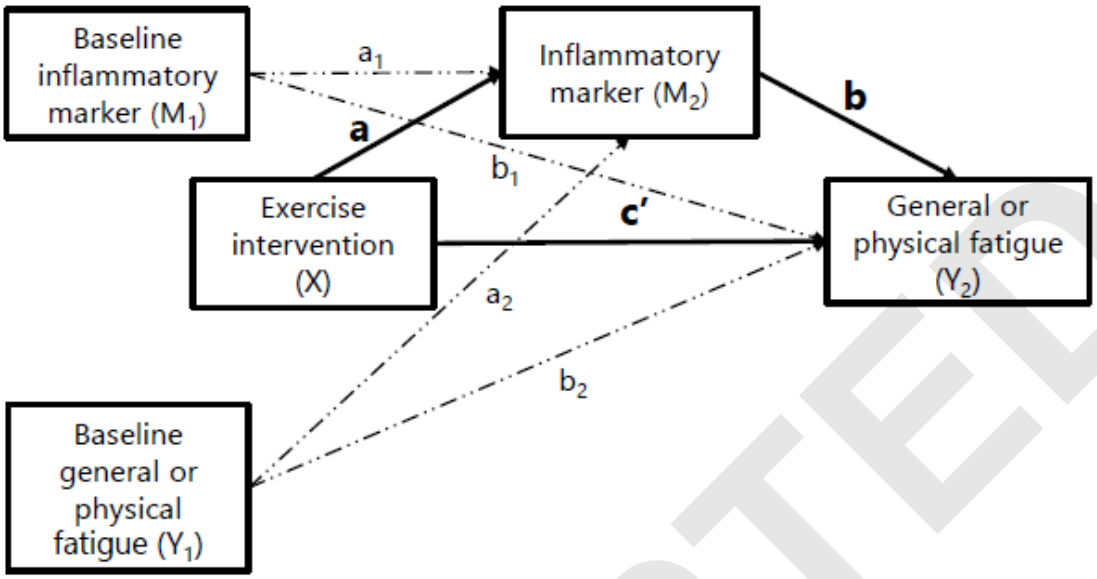
Indirect effect through CD40-L	-0.05 [-0.51; 0.21]	3.1	-0.11 [-0.64; 0.17]	6.9
Indirect effect through IL-6	-0.13 [-0.60; 0.18]	8.2	-0.12 [-0.64; 0.16]	7.5
Indirect effect through EGF	-0.02 [-0.45; 0.24]	0.9	-0.08 [-0.65; 0.17]	4.8
Indirect effect through TRAIL	0.04 [-0.09; 0.47]	2.5	0.05 [-0.11; 0.51]	3.1
Indirect effect through CD8A	-0.22 [-0.82; 0.03]	13.8	-0.22 [-0.95; 0.06]	13.8
Indirect effect through DCN	0.005 [-0.21; 0.27]	0.3	0.007 [-0.17; 0.37]	0.4
Indirect effect through CCL17	-0.02 [-0.46; 0.16]	1.3	-0.06 [-0.65; 0.12]	3.8
Indirect effect through CASP-8	-0.10 [-0.64; 0.10]	6.3	-0.14 [-0.74; 0.08]	8.8
Indirect effect through ICOSLG	-0.06 [-0.53; 0.11]	3.8	-0.06 [-0.49; 0.08]	3.8
Indirect effect through CSF-1	-0.07 [-0.50; 0.22]	4.4	-0.07 [-0.50; 0.27]	4.4
Indirect effect through IFN-y	-0.06 [-0.50; 0.16]	3.8	-0.06 [-0.53; 0.20]	3.8
Indirect effect through IL-10	-0.01 [-0.20; 0.22]	0.01	0.001 [-0.17; 0.24]	0.1
Indirect effect through FasL	-0.01 [-0.37; 0.18]	0.6	-0.006 [-0.40; 0.30]	0.4
Indirect effect through CXCL9	0.18 [-0.02; 0.64]	11.3	0.23 [-0.03; 0.72]	14.4
Indirect effect through MIC A/B	-0.10 [-0.57; 0.17]	6.3	-0.09 [-0.57; 0.11]	5.6

Abbreviations: CASP-8 Caspase-8, *CCL17* C-C motif chemokine 17, *CD8A* T-cell surface glycoprotein CD8 alpha chain, *CD40-L* CD-40 ligand, *CSF-1* Macrophage colony stimulating factor-1, *CXCL9* C-X-C motif chemokine 9, *DCN* Decorin, *EGF* Pro-epidermal growth factor, *FasL* Fas antigen ligand, *ICOSLG* ICOS ligand, *IFN-y* Interferon-y, *IL-6* Interleukin-6, *IL-10* Interleukin-10, *MIC A/B* MHC class I polypeptide-related sequence A/B, *TRAIL* TNF-related apoptosis-inducing ligand, *RT-HIIT* resistance and high-intensity interval training, *AT-HIIT* moderate-intensity aerobic and high-intensity interval training, *CI* confidence interval. *$p<0.05$.

Table 5. Pearson product-moment correlations between changes in physiological outcomes and changes in inflammatory markers.

	BMI (kg/m^2]	**Cardiorespiratory fitness (L/min)**	**Lower-limb muscle strength (kg)**	**Handgrip strength (kg)**
CASP-8	0.04	0.05	0.19	0.13
CCL17CD8a	-0.05	0.10	0.13	0.22**
CD40L	0.11			-0.18*
CSF-1	-0.07	-0.06	-0.23*	0.14
CXCL9	0.08	0.07	0.11	-0.16
	0.14	-0.02	-0.16	-0.03
	0.004	-0.01	-0.04	-0.03
DCN	-0.05	0.21*	-0.13	0.19*
EGF	0.003	0.03	0.11	-0.12
ICOSLG	-0.04	0.22*	-0.14	0.04
IFN-y	0.03	-0.33**	-0.06	-0.01
IL-6	0.02	-0.10	-0.14	-0.01
IL-10	0.10	0.13	-0.03	-0.16
MIC A/B	0.14	-0.02	-0.14	-0.16
TRAIL		0.10	-0.13	

Abbreviations: BMI Body Mass Index, *CASP-8* Caspase-8, *CCL17* C-C motif chemokine 17, *CD8A* T-cell surface glycoprotein CD8 alpha chain, *CD40-L* CD-40 ligand, *CSF-1* Macrophage colony stimulating factor-1, *CXCL9* C-X-C motif chemokine 9, *DCN* Decorin, *EGF* Pro-epidermal growth factor, *FasL* Fas antigen ligand, *ICOSLG* ICOS ligand, *IFN-y* Interferon-y, *IL-6* Interleukin-6, *IL-10* Interleukin-10, *MIC A/B* MHC class I polypeptide-related sequence A/B, *TRAIL* TNF-related apoptosis-inducing ligand, *p<0.10, **p<0.05



Supplemental Digital Content 1. Schematic representation of the mediation model.

Supplemental Digital Content 2. Exercise effects on 92 different cytokines.

	Baseline mean ± SD			Baseline to post-intervention						
				Within-group change [95% CI]			Unadjusted between-group difference *(p-value)*		Adjusted between-group difference *(p-value)*	
	RT-HIIT (*n*=30)	**AT-HIIT** (*n*=27)	**UC** (*n*=29)	**RT-HIIT**	**AT-HIIT**	**UC**	**RT-HIIT vs UC**	**AT-HIIT vs UC**	**RT-HIIT vs UC**	**AT-HIIT vs UC**
Interleukins										
IL-1 like cytokines are produced upon inflammation, injury and infection										
IL-1b[a]	-1.65 ± 1.41	-1.79 ± 1.08	-1.42 ± 2.15	-0.28 [-1.05;0.49]	-0.27 [-1.29;0.75]	-0.11 [-0.75;0.53]	-0.24 (0.69)	-0.09 (0.89)	-0.18 (0.77)	-0.04 (0.95)
IL-18	8.26 ± 0.74	8.26 ± 0.68	8.23 ± 0.69	0.03 [-0.19;0.26]	0.22 [-0.06;0.49]	0.15 [-0.09;0.39]	-0.09 (0.56)	0.07 (0.67)	-0.07 (0.68)	0.08 (0.64)
Common γ-chain cytokines invoke lymphocyte activation and differentiation										
IL-4[a]	1.45 ± 1.93	1.45 ± 1.20	1.98 ± 1.01	0.21 [-0.63;1.04]	0.53 [-0.09;1.14]	-0.07 [-0.64;0.49]	-0.10 (0.81)	0.23 (0.58)	-0.08 (0.86)	0.25 (0.56)
IL-7	4.66 ± 0.97	4.72 ± 0.79	4.79 ± 0.78	-0.67 [-1.05;-0.29]*	-0.59 [-0.90;-0.28]*	-0.67 [-1.06;-0.29]*	-0.07 (0.76)	0.03 (0.88)	-0.05 (0.83)	0.04 (0.87)
IL-6 like cytokines are mediators in various immune processes, including hematopoiesis and the APR										
IL-6	2.51 ± 0.83	2.85 ± 0.96	2.59 ± 1.01	0.10 [-0.28;0.47]	0.18 [-0.10;0.46]	0.52 [0.14;0.90]*	**-0.52 (0.01)**	**-0.16 (0.43)**	**-0.47 (0.02)**	**-0.15 (0.47)**
IL-12	4.56 ± 0.92	4.63 ± 0.95	4.41 ± 1.05	0.40 [0.04;0.76]*	0.34 [0.11;0.57]*	0.55 [0.23;0.88]*	-0.08 (0.68)	-0.07 (0.69)	-0.07 (0.70)	-0.08 (0.69)
CSF-1	6.73 ± 0.36	6.83 ± 0.51	6.68 ± 0.49	0.02 [-0.12;0.15]	0.09 [-0.03;0.21]	0.18 [0.07;0.29]*	**-0.15 (0.06)**	**-0.04 (0.65)**	**-0.13 (0.11)**	**-0.03 (0.70)**
IL-10 like cytokines play a major role in suppressing inflammatory responses										
IL-10[a]	0.92 ± 0.27	0.95 ± 0.23	0.92 ± 0.26	-0.10 [-0.30;0.09]	-0.27 [-0.38;-0.16]*	-0.26 [-0.40;-0.13]*	**0.12 (0.14)**	**0.006 (0.94)**	**0.13 (0.09)**	**-0.0004 (1.00)**
Interferons play a role in pathogen resistance										
IFN-γ[a]	0.88 ± 1.59	1.20 ± 0.98	0.88 ± 1.65	0.19 [-0.50;0.88]	0.28 [-0.14;0.69]	0.56 [-0.04;1.15]	**-0.48 (0.17)**	**-0.11 (0.75)**	**-0.51 (0.14)**	**-0.14 (0.69)**
Tumor necrosis factors are involved in cell death										
CD27	6.77 ± 0.51	6.65 ± 0.62	6.55 ± 0.52	-0.15 [-0.30;0.002]	-0.01 [-0.20;0.18]	0.04 [-0.13;0.21]	-0.10 (0.40)	-0.001 (1.00)	-0.07 (0.57)	0.007 (0.95)

CD40_L	5.86 ± 1.87	6.07 ± 1.60	6.52 ± 1.33	-1.05 [-1.73;-0.38]*	-0.55 [-1.33;0.24]	-1.70 [-2.46;-0.94]*	**0.18 (0.64)**	**0.74 (0.07)**	**0.19 (0.63)**	**0.74 (0.07)**
CD40	10.49 ± 0.80	10.71 ± 0.68	10.79 ± 0.70	-0.22 [-0.57;0.13]	-0.18 [-0.50;0.13]	-0.37 [-0.68;-0.06]*	-0.08 (0.66)	0.12 (0.47)	-0.04 (0.80)	0.13 (0.45)
CD70	2.69 ± 0.36	2.86 ± 0.54	2.66 ± 0.52	-0.16 [-0.31;-0.01]*	-0.08 [-0.24;0.08]	-0.0001 [-0.16;0.16]	-0.13 (0.24)	-0.006 (0.96)	-0.10 (0.34)	0.003 (0.98) (1.00)
FasL	5.18 ± 0.49	5.06 ± 0.64	5.06 ± 0.60	0.17 [0.01;0.33]*	0.32 [0.13;0.50]*	0.36 [0.17;0.55]*	**-0.17 (0.15)**	**-0.02 (0.87)**	**-0.15 (0.19)**	**-0.02 (0.89)**
TNF-a[a]	2.42 ± 0.53	2.43 ± 0.47	2.31 ± 1.00	0.34 [0.10;0.57]*	0.26 [0.09;0.44]*	0.33 [0.17;0.50]*	-0.07 (0.57)	-0.08 (0.52)	-0.04 (0.75)	-0.07 (0.58)
TNFRSF-4	2.45 ± 0.53	2.35 ± 0.29	2.21 ± 0.43	0.74 [0.35;1.13]*	0.79 [0.53;1.04]*	0.98 [0.74;1.21]*	-0.16 (0.46)	-0.11 (0.62)	-0.19 (0.36)	-0.12 (0.56)
TNFRSF9	4.19 ± 0.55	4.08 ± 0.67	4.08 ± 0.59	0.02 [-0.19;0.23]	0.12 [-0.08;0.31]	0.20 [-0.01;0.41]	-0.13 (0.33)	-0.07 (0.63)	-0.09 (0.51)	-0.06 (0.67)
TNFRSF-12	5.04 ± 0.62	4.89 ± 0.62	4.71 ± 0.66	0.02 [-0.24;0.29]	0.22 [-0.007;0.44]	0.32 [0.08;0.57]*	-0.11 (0.51)	-0.02 (0.92)	-0.07 (0.69)	-0.004 (0.98)
TNFRSF14	2.91 ± 0.92	2.94 ± 0.84	3.28 ± 0.79	-0.64 [-0.93;-0.36]*	-0.41 [-0.79;-0.03]*	-0.74 [-1.07;-0.40]*	-0.18 (0.29)	0.06 (0.74)	-0.13 (0.42)	0.07 (0.69)
TNFRSF-21	6.74 ± 0.36	6.66 ± 0.45	6.58 ± 0.38	0.04 [-0.07;0.16]	0.17 [0.04;0.29]*	0.18 [0.08;0.28]*	-0.06 (0.43)	0.03 (0.71)	-0.05 (0.53)	0.03 (0.68)
TRAIL	6.98 ± 0.29	7.18 ± 0.55	7.02 ± 0.51	0.02 [-0.16;0.20]	0.15 [-0.002;0.30]	0.19 [0.02;0.36]*	**-0.18 (0.09)**	**0.05 (0.60)**	**-0.17 (0.10)**	**0.05 (0.62)**
TWEAK	7.95 ± 0.47	7.87 ± 0.58	8.02 ± 0.52	-0.20 [-0.36;-0.04]*	-0.02 [-0.24;0.20]	-0.12 [-0.29;0.05]	-0.10 (0.40)	0.04 (0.73)	-0.09 (0.45)	0.05 (0.72)
Transforming growth factor B family is involved in development, immune regulation, immune tolerance, carcinogenesis										
LAP TGF-beta-1	0.90 ± 0.42	0.98 ± 0.40	1.02 ± 0.37	-0.24 [-0.41;-0.07]*	-0.21 [-0.42;-0.01]*	-0.28 [-0.46;-0.10]*	-0.05 (0.63)	0.03 (0.78)	-0.03 (0.80)	0.03 (0.74)
CC Chemokines target monocytes, T cells, dendritic cells, eosinophils and NK cells										
CCL2	8.89 ± 0.67	8.95 ± 0.64	9.05 ± 0.87	0.44 [0.07;0.81]*	0.42 [0.23;0.60]*	0.38 [0.12;0.65]*	-0.06 (0.74)	-0.02 (0.93)	-0.07 (0.67)	-0.02 (0.90)
CCL3	4.10 ± 0.58	4.23 ± 0.53	4.20 ± 0.55	0.17 [-0.04;0.39]	0.16 [0.0001;0.31]*	0.26 [0.06;0.45]*	-0.11 (0.38)	-0.09 (0.51)	-0.06 (0.62)	-0.07 (0.56)

CCL4	6.18 ± 0.63	6.36 ± 0.57	6.44 ± 0.49	-0.03 [-0.25;0.19]	-0.06 [-0.27;0.16]	-0.14 [-0.35;0.07]	0.04 (0.81)	0.05 (0.72)	0.08 (0.61)	0.06 (0.67)
CCL8	6.43 ± 0.74	6.72 ± 0.74	6.50 ± 1.02	-0.30 [-0.60;-0.004]*	-0.30 [-0.52;-0.08]*	-0.35 [-0.63;-0.08]	0.01 (0.95)	0.13 (0.50)	0.05 (0.77)	0.14 (0.46)
CCL13	7.35 ± 0.79	7.38 ± 0.59	7.70 ± 0.90	-0.52 [-0.92;-0.12]*	-0.33 [-0.61;-0.05]*	-0.64 [-0.97;-0.32]*	-0.07 (0.76)	0.11 (0.61)	-0.05 (0.81)	0.12 (0.60)
CCL17	7.23 ± 1.28	7.28 ± 0.96	7.56 ± 1.07	-0.43 [-0.88;0.03]	-0.15 [-0.63;0.34]	-0.72 [-1.20;-0.24]*	**0.11 (0.72)**	**0.39 (0.19)**	**0.15 (0.62)**	**0.40 (0.18)**
CCL19	8.25 ± 1.02	8.43 ± 1.25	8.17 ± 1.01	-0.23 [-0.69;0.23]	-0.20 [-0.57;0.17]	0.06 [-0.26;0.38]	-0.24 (0.22)	-0.06 (0.76)	-0.20 (0.30)	-0.05 (0.78)
CCL20	5.19 ± 0.96	5.30 ± 0.81	5.32 ± 0.98	-0.26 [-0.63;0.11]	-0.05 [-0.36;0.26]	-0.12 [-0.48;0.24]	-0.16 (0.46)	0.09 (0.70)	-0.17 (0.46)	0.08 (0.71)
CCL23	9.47 ± 0.62	9.44 ± 0.81	9.58 ± 0.63	-0.02 [-0.25;0.21]	0.10 [-0.14;0.33]	0.03 [-0.20;0.27]	-0.10 (0.51)	-0.01 (0.95)	-0.05 (0.73)	0.0003 (1.00)
CXC chemokines mediate neutrophil chemotaxis										
CXCL1	8.97 ± 1.04	9.23 ± 0.86	9.19 ± 0.73	-0.16 [-0.59;0.28]	-0.39 [-0.80;0.01]	-0.44 [-0.85;-0.03]*	0.11 (0.63)	0.09 (0.71)	0.14 (0.56)	0.09 (0.70)
CXCL5	10.94 ± 1.60	11.37 ± 1.16	11.17 ± 1.23	-0.33 [-0.89;0.23]	-0.56 [-1.10;-0.02]*	-0.65 [-1.18;-0.13]*	0.16 (0.61)	0.22 (0.48)	0.20 (0.51)	0.23 (0.46)
CXCL9	6.21 ± 0.73	6.50 ± 1.03	6.29 ± 0.95	0.51 [0.18;0.84]*	0.63 [0.31;0.94]*	0.54 [0.11;0.96]*	**-0.06 (0.78)**	**0.24 (0.27)**	**0.04 (0.83)**	**0.28 (0.18)**
CXCL8 (IL-8)	4.74 ± 0.71	4.98 ± 0.54	4.95 ±0.69	0.09 [-0.28;0.45]	-0.17 [-0.35;0.02]	-0.19 [-0.41;0.04]	0.19 (0.31)	0.02 (0.92)	0.18 (0.34)	0.02 (0.93)
CXCL10	6.89 ± 1.03	7.20 ± 1.12	7.25 ± 1.38	1.17 [0.68;1.65]*	1.11 [0.74;1.49]*	1.24 [0.80;1.68]*	-0.27 (0.33)	-0.13 (0.65)	-0.23 (0.40)	-0.12 (0.66)
CXCL11	6.76 ± 1.18	6.97 ± 0.99	6.90 ± 0.97	-0.30 [-0.83;0.23]	-0.19 [-0.53;0.14]	-0.32 [-0.77;0.13]	-0.03 (0.91)	0.20 (0.47)	0.01 (0.97)	0.21 (0.45)
CXCL13	8.29 ± 0.93	8.43 ± 0.71	8.41 ± 0.85	-0.71 [-0.96;-0.45]*	-0.83 [-1.07;-0.59]*	-0.67 [-0.99;-0.36]*	-0.05 (0.77)	-0.16 (0.36)	-0.06 (0.74)	-0.16 (0.36)
CX3C chemokines attract T cells and monocytes										
CX3CL1	5.21 ± 0.48	5.17 ± 0.52	5.01 ± 0.53	0.33 [0.10;0.56]*	0.41 [0.20;0.62]*	0.50 [0.34;0.67]*	-0.05 (0.71)	-0.008 (0.95)	-0.05 (0.70)	-0.01 (0.94)

Angiopoietins play a role in angiogenesis										
TIE2	6.41 ± 0.46	6.36 ± 0.51	6.32 ± 0.47	0.02 [-0.09;0.13]	0.11 [-0.03;0.25]	0.14 [0.01; 0.27]*	-0.09 (0.30)	-0.02 (0.87)	-0.09 (0.33)	-0.01 (0.88)
ANG-1	7.92 ± 1.00	8.11 ± 0.72	8.18 ± 0.77	-0.63 [-0.96;-0.29]*	-0.66 [-0.94;-0.38]*	-0.76 [-1.11;-0.41]*	-0.03 (0.88)	0.06 (0.77)	0.002 (0.99)	0.07 (0.74)
ANG-2	4.07 ± 0.54	4.00 ± 0.51	3.98 ± 0.45	0.19 [-0.003;0.38]	0.26 [0.10;0.42]*	0.21 [0.07;0.36]*	0.0002 (1.00)	0.06 (0.61)	0.0001 (1.00)	0.06 (0.61)
Matrix metalloproteinases are involved in chemokine/cytokine inactivation and the release of apoptotic ligands										
MMP-7	8.89 ± 0.70	8.88 ± 0.50	8.89 ± 0.60	0.21 [-0.08;0.50]	0.26 [0.09;0.42]*	0.21 [-0.03;0.46]*	0.004 (0.98)	0.03 (0.87)	0.05 (0.75)	0.03 (0.83)
MMP-12	5.96 ± 0.70	6.17 ± 0.67	5.97 ± 0.88	-0.17 [-0.41;0.07]	-0.02 [-0.23;0.19]	-0.03 [-0.23;0.17]	-0.13 (0.36)	0.09 (0.52)	-0.14 (0.33)	0.09 (0.54)
CD244	5.42 ± 0.61	5.57 ± 0.54	5.45 ± 0.60	-0.20 [-0.40;-0.002]*	-0.20 [-0.43;0.03]	-0.20 [-0.44; 0.03]	0.01 (0.95)	0.08 (0.54)	0.03 (0.81)	0.09 (0.52)
EGF	7.60 ± 1.54	7.76 ± 1.50	8.16 ± 1.46	-1.00 [-1.65;-0.35]*	-0.63 [-1.45;0.20]	-1.77 [-2.58;-0.96]*	**0.28 (0.47)**	**0.74 (0.06)**	**0.29 (0.46)**	**0.75 (0.06)**
PlGF	7.38 ± 0.40	7.36 ± 0.52	7.33 ± 0.48	0.23 [0.005;0.46]*	0.32 [0.14;0.50]*	0.38 [0.21;0.56]*	-0.15 (0.27)	-0.04 (0.77)	-0.14 (0.31)	-0.04 (0.78)
CRTAM	3.32 ± 0.61	3.34 ± 0.79	3.06 ± 0.48	-0.01 [-0.22;0.20]	0.01 [-0.19;0.21]	0.19 [0.01;0.37]*	-0.11 (0.41)	-0.09 (0.53)	-0.06 (0.64)	-0.08 (0.57)
CD4	-0.28 ± 0.33	-0.15 ± 0.45	-0.21 ± 0.45	-0.009 [-0.14;0.12]	0.002 [-0.12;0.13]	0.14 [0.002;0.28]*	**-0.17 (0.06)**	**-0.11 (0.23)**	**-0.15 (0.11)**	**-0.10 (0.26)**
CD8A	8.19 ± 0.86	8.07 ± 0.70	7.99 ± 0.72	-0.24 [-0.43;-0.04]*	-0.12 [-0.29;0.05]	0.10 [-0.09;0.29]	**-0.31 (0.02)**	**-0.17 (0.17)**	**-0.30 (0.02)**	**-0.17 (0.16)**
CA9	2.82 ± 0.48	2.81 ± 0.55	2.83 ± 0.63	-0.03 [-0.19;0.13]	0.20 [0.03;0.37]*	0.06 [-0.16;0.27]	-0.06 (0.58)	0.14 (0.22)	-0.06 (0.63)	0.15 (0.22)
Gal-9	6.72 ± 0.33	6.80 ± 0.48	6.70 ± 0.38	0.31 [0.15;0.47]*	0.31 [0.20;0.42]*	0.33 [0.23;0.43]*	-0.03 (0.72)	0.01 (0.89)	-0.01 (0.91)	0.02 (0.82)
VEGFR-2	6.16 ± 0.35	6.15 ± 0.49	6.03 ± 0.45	-0.11 [-0.22;-0.01]*	0.03 [-0.11;0.16]	0.04 [-0.08;0.15]	-0.09 (0.22)	0.04 (0.62)	-0.07 (0.33)	0.04 (0.58)
PDGF	9.64 ± 0.64	9.72 ± 0.63	9.78 ± 0.62	-0.78	-0.67	-0.81	-0.03 (0.91)	0.11 (0.62)	0.02 (0.93)	0.12 (0.59)

subunit B				[-1.03;-0.52]*	[-0.99;-0.34]*	[-1.17;-0.46]*				
PDCD1	2.00 ± 0.59	2.00 ± 0.62	1.92 ± 0.49	-0.05 [-0.24;0.14]	0.02 [-0.19;0.23]	0.11 [-0.13;0.35]	-0.11 (0.43)	-0.02 (0.89)	-0.08 (0.56)	-0.01 (0.91)
Gal-1	5.64 ± 0.27	5.61 ± 0.43	5.62 ± 0.47	0.04 [-0.13;0.21]	0.16 [0.03;0.29]*	0.11 [-0.01;0.24]	-0.07 (0.49)	0.05 (0.64)	-0.05 (0.65)	0.05 (0.60)
PD_L1	3.90 ± 0.80	3.80 ± 0.59	3.74 ± 0.44	-0.16 [-0.36;0.03]	-0.03 [-0.23;0.18]	0.03 [-0.15;0.20]	-0.10 (0.41)	-0.02 (0.86)	-0.07 (0.59)	-0.01 (0.91)
HGF	6.82 ± 0.53	6.81 ± 0.66	6.87 ± 0.61	-0.25 [-0.41;-0.08]*	0.05 [-0.24;0.35]	-0.17 [-0.37;0.04]	-0.11 (0.46)	0.18 (0.22)	-0.09 (0.53)	0.18 (0.22)
GZMA	4.49 ± 0.70	4.37 ± 0.60	4.28 ± 0.54	0.005 [-0.24;0.25]	0.18 [-0.01;0.36]	0.31 [0.07;0.55]*	-0.19 (0.17)	-0.05 (0.72)	-0.14 (0.28)	-0.04 (0.77)
HO-1	11.58 ± 0.50	11.58 ± 0.59	11.57 ± 0.45	0.12 [-0.09;0.33]	0.27 [0.05;0.50]*	0.16 [-0.02;0.35]	-0.04 (0.76)	0.11 (0.40)	-0.03 (0.79)	0.11 (0.40)
CD5	3.50 ± 0.50	3.47 ± 0.41	3.46 ± 0.37	0.007 [-0.16;0.17]	0.09 [-0.04;0.21]	0.13 [-0.03;0.29]	-0.11 (0.30)	-0.03 (0.78)	-0.07 (0.48)	-0.02 (0.83)
NCR1	2.91 ± 0.55	2.87 ± 0.41	2.91 ± 0.38	0.27 [0.09;0.45]*	0.37 [0.22;0.51]*	0.40 [0.25;0.54]*	-0.13 (0.22)	-0.04 (0.73)	-0.11 (0.31)	-0.03 (0.77)
DCN	3.55 ± 0.33	3.64 ± 0.50	3.44 ± 0.54	-0.02 [-0.12;0.09]	0.11 [-0.01;0.23]	0.17 [0.02;0.33]*	**-0.15 (0.09)**	**0.003 (0.97)**	**-0.14 (0.10)**	**0.005 (0.95)**
MIC A/B	3.11 ± 1.07	2.84 ± 1.16	2.97 ± 1.17	-0.04 [-0.15;0.07]	0.03 [-0.08;0.14]	0.09 [-0.02;0.20]	**-0.14 (0.08)**	**-0.06 (0.49)**	**-0.14 (0.10)**	**-0.06 (0.50)**
LAMP-3	2.83 ± 0.70	3.01 ± 0.78	2.85 ± 0.68	0.95 [0.57;1.33]*	0.82 [0.56;1.08]*	0.95 [0.67;1.23]*	-0.01 (0.95)	-0.07 (0.75)	0.02 (0.94)	-0.06 (0.79)
CASP-8	3.79 ± 1.25	3.70 ± 1.13	3.95 ± 1.01	-0.68 [-1.21;-0.15]*	-0.28 [-0.87;0.31]	-0.91 [-1.38;-0.45]*	**0.16 (0.48)**	**0.39 (0.09)**	**0.20 (0.37)**	**0.40 (0.08)**
ICOSLG	3.77 ± 0.42	3.73 ± 0.55	3.65 ± 0.49	-0.005 [-0.13;0.12]	0.11 [-0.03;0.25]	0.20 [0.06;0.33]*	**-0.15 (0.10)**	**-0.05 (0.55)**	**-0.14 (0.13)**	**-0.05 (0.56)**
PD-L2	1.48 ± 0.45	1.42 ± 0.44	1.37 ± 0.47	-0.06 [-0.18;0.06]	0.04 [-0.09;0.17]	0.08 [-0.06;0.22]	-0.11 (0.22)	-0.01 (0.88)	-0.10 (0.29)	-0.01 (0.91)
VEGF-A	7.46 ± 0.49	7.53 ± 0.52	7.50 ± 0.58	-0.21 [-0.42;-0.01]*	-0.12 [-0.28;0.05]	-0.13 [-0.29;0.03]	-0.11 (0.34)	0.02 (0.84)	-0.08 (0.50)	0.03 (0.79)
KLRD1	4.52 ± 0.66	4.51 ± 0.72	4.42 ± 0.65	0.09	0.23	0.28	-0.18 (0.15)	-0.01 (0.92)	-0.14 (0.26)	-0.002 (0.99)

				[-0.09;0.26]	[0.05;0.42]*	[0.10;0.46]*				
CD83	1.37 ± 0.44	1.33 ± 0.46	1.30 ± 0.38	0.15 [-0.02;0.32]	0.28 [0.14;0.42]*	0.30 [0.16;0.43]*	-0.14 (0.18)	0.004 (0.97)	-0.13 (0.23)	0.007 (0.94)
GZMB	3.22 ± 1.49	3.24 ± 1.19	3.11 ± 1.04	-0.05 [-0.68;0.58]	-0.03 [-0.48;0.42]	0.02 [-0.32;0.36]	0.06 (0.78)	0.08 (0.74)	0.10 (0.69)	0.08 (0.72)
PTN	1.31 ± 1.44	1.54 ± 1.25	1.11 ± 0.87	-0.11 [-0.60;0.37]	0.17 [-0.17;0.52]	0.17 [-0.07;0.41]	-0.20 (0.41)	0.17 (0.49)	-0.21 (0.41)	0.17 (0.50)
ARG-1	2.74 ± 1.05	2.39 ± 0.92	2.66 ± 0.78	-0.47 [-0.98;0.05]	0.04 [-0.45;0.54]	-0.45 [-0.88;-0.02]*	0.10 (0.70)	0.23 (0.38)	0.08 (0.77)	0.22 (0.40)
VEGF-C	0.84 ± 0.66	0.65 ± 0.55	0.88 ± 0.80	-0.47 [-0.68;-0.26]*	-0.33 [-0.56;-0.10]*	-0.45 [-0.70;-0.20]*	-0.05 (0.66)	-0.03 (0.78)	-0.02 (0.83)	-0.03 (0.83)
GZMH	3.77 ± 1.57	3.74 ± 1.34	3.61 ± 1.15	-0.15 [-0.73;0.43]	0.03 [-0.44;0.50]	0.03 [-0.38;0.44]	-0.06 (0.82)	0.12 (0.67)	0.02 (0.94)	0.13 (0.62)
ADA	2.59 ± 0.64	2.42 ± 0.50	2.60 ± 0.54	-0.04 [-0.33;0.26]	0.06 [-0.16;0.28]	-0.10 [-0.29;0.10]	0.05 (0.68)	0.02 (0.89)	0.05 (0.68)	0.02 (0.89)

CI confidence interval, *SD* standard deviation

[a]Log-transformed

* Significant at level p<0.05

** Significant at level p<0.20. It will be assessed whether the cytokine mediates the effect of exercise on cancer-related fatigue.