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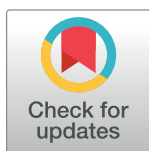
Physical exercise is associated with a reduction in plasma levels of fractalkine, TGF-β1, eotaxin-1 and IL-6 in younger adults with mobility disability

Parvin Kumar[1,2], Miranda Stiernborg[1,2], Anna Fogdell-Hahn[3], Kristoffer Månsson [4,5,6], Tomas Furmark [7], Daniel Berglind[8], Philippe A. Melas[2,4], Yvonne Forsell[8], Catharina Lavebratt [1,2]*

1 Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden, 2 Center for Molecular Medicine, L8:00, Karolinska University Hospital, Stockholm, Sweden, 3 Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden, 4 Centre for Psychiatry Research, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden, 5 Max Planck UCL Centre for Computational Psychiatry and Ageing Research, Berlin, Germany and London, United Kingdom, 6 Center for Lifespan Psychology, Max Planck Institute for Human Development, Berlin, Germany, 7 Department of Psychology, Uppsala University, Uppsala, Sweden, 8 Department of Public Health Sciences, Karolinska Institutet, Stockholm, Sweden

* catharina.lavebratt@ki.se

Abstract

Mobility disability (MD) refers to substantial limitations in life activities that arise because of movement impairments. Although MD is most prevalent in older individuals, it can also affect younger adults. Increasing evidence suggests that inflammation can drive the development of MD and may need to be targeted for MD prevention. Physical exercise has anti-inflammatory properties and has been associated with MD prevention. However, no studies to date have examined whether exercise interventions affect the peripheral inflammatory status in younger adults with MD. To this end, we used blood samples from young and middle-aged adults with MD (N = 38; median age = 34 years) who participated in a 12-week intervention that included aerobic and resistance exercise training. A pre-post assessment of inflammatory biomarkers was conducted in plasma from two timepoints, i.e., before the exercise trial and at follow-up (3–7 days after the last exercise session). We successfully measured 15 inflammatory biomarkers and found that exercise was associated with a significant reduction in levels of soluble fractalkine, transforming growth factor beta 1 (TGF-β1), eotaxin-1 and interleukin (IL) 6 (corrected α = 0.004). We also found significant male-specific effects of exercise on (i) increasing IL-16 and (ii) decreasing vascular endothelial growth factor-A (VEGF-A). In line with our results, previous studies have also found that exercise can reduce levels of TGF-β1, eotaxin-1 and IL-6. However, our finding that exercise reduces plasma levels of fractalkine in younger adults with MD, as well as the sex-dependent findings, have not been previously reported and warrant replication in larger cohorts. Given the suggested role of inflammation in promoting MD development, our study provides additional support for the use of physical exercise as a treatment modality for MD.

Introduction

Mobility disability (MD) represents substantial limitations in performance of activities and participation in life situations, which arise because of movement impairments [1,2]. In the United States, about 10% of the population aged 18 years or older has been estimated to live with MD [3] and, in Sweden, the corresponding proportion has been estimated at 8% for those 16 to 84 years of age [4]. MD is associated with significant health care and social needs in affected individuals, making its prevention a public health concern [5]. Specifically, MD has been linked to a number of negative conditions, including living alone, being impoverished and poorly educated, having low health-related quality of life, and suffering from depression, anxiety, obesity, diabetes or cardiometabolic disorders [6,7]. Although MD is most prevalent in older individuals and those with chronic conditions such as musculoskeletal diseases and neurological disorders [8], MD can also affect younger adults [9]. Although the treatment options for MD are still limited, there is evidence supporting the use of physical activity interventions for preventing MD and for improving physical functioning in those already affected [10,11]. Nonetheless, more than half of the adults (18–64 years old) with MD in the United States have been reported to be physically inactive, and those who visited a health care professional often did not receive physical activity recommendations [6].

Currently, the presence of systemic inflammation has emerged as a possible biological mechanism that can drive the development of MD [12–17]; inflammation may, therefore, need to be targeted for MD prevention. Regular moderate-intensity aerobic exercise is known to improve cardiorespiratory fitness (CRF) and cardiometabolic biomarkers [18], and has well-documented anti-inflammatory properties [19,20]. Regular resistance training is also known to protect against cardiovascular diseases, although there is less consensus in the literature about its anti-inflammatory effects [21–23]. The anti-inflammatory properties of exercise are, at least partly, mediated by its effect on reducing stress hormones, myokines (from muscle cells), and inflammatory cytokines (from adipose tissue) [20]. However, to our knowledge, no studies have examined how exercise interventions, which couple aerobic with resistance exercise, affect inflammatory biomarkers in younger adults with MD. Moreover, sex-specific effects of physical exercise on immune activity markers are not well explored. To this end, we utilized data and samples from a published randomized control trial that studied the effects of two exercise-motivation intervention arms (i.e., smartphone-app versus supervised exercise) on levels of physical activity and CRF in younger adults (aged 18 to 45 years) with mild-to-moderate MD [24].

Specifically, trial participants in both arms were encouraged to engage in moderate-to-vigorous physical activity, which included both aerobic and resistance training, for a minimum of 30 minutes per day. The 12-week trial included a total of 110 participants at baseline and showed that both exercise interventions were equally effective in providing motivation for physical activity, enhancing CRF and improving body composition [24]. In the present study, we hypothesized that the exercise intervention would also have anti-inflammatory effects in this group of MD individuals. To examine this hypothesis, we collected venous blood samples from the first 38 participants of the trial and measured a panel of 24 immune activity plasma analytes ("inflammatory biomarkers"). Blood sampling was performed both prior to the first exercise session (i.e., baseline) and post-intervention (i.e., at 12 weeks follow-up). Post-intervention samplings were performed 3–7 days after the last exercise session to avoid measuring the acute effects of exercise.

Conclusively, since no studies to date have examined whether exercise interventions affect the peripheral inflammatory status in younger adults with MD, the objective of the present study was to conduct a pre-post exercise assessment of inflammatory biomarkers in plasma

samples from young and middle-aged adults with MD. Here, we present the results from the successful pre-post measurements of 15 inflammatory biomarkers, some of which have not previously been well-studied in relation to physical exercise.

Results

Baseline characteristics of participants with mobility disability

The baseline characteristics of the study participants with MD (N = 38) are presented in Table 1. The participants reached moderate-to-vigorous physical activity levels corresponding to 43 (31–56), 48 (40–63) and 48 (35–61) min/day (median [25th-75th percentiles]) as measured on the 1st, 6th and 12th week of the intervention, respectively. As shown in Table 2, there was no change in metabolic markers in the study participants following the exercise intervention.

Bivariate correlations of inflammatory biomarkers and corrected threshold of significance

We successfully measured the following 15 inflammatory biomarkers in all study participants: (1) C-reactive protein (CRP), (2) eotaxin-1/CCL11, (3) soluble fractalkine/CX3CL1 (sFKN), (4) growth-regulated oncogene-alpha/CXCL1 (GRO-α), (5) soluble interleukin (IL)-2 receptor subunit alpha (sIL-2Rα), (6) IL-6, (7) IL-12/IL-23 subunit p40 (IL-12/IL-23p40), (8) IL-16, (9) IL-18, (10) serum amyloid A (SAA), (11) soluble intercellular adhesion molecule-1 (sICAM-1), (12) soluble vascular cell adhesion protein-1 (sVCAM-1), (13) transforming growth factor-

Table 1. Baseline characteristics of the study participants with mobility disability (N = 38).

Baseline characteristics	
Age (years); median (IQR)	34 (28–39)
Male sex; n (%)	11 (29.0%)
Daily smoking; n (%)	2 (5.26%)
Alcohol use[a]; median (IQR)	3 (3–3)
BMI (kg/m^2); median (IQR)	25.4 (22.3–30.9)
Fat mass (kg); median (IQR)	25.0 (17.1–34.5)
Fat-free mass (kg); median (IQR)	50.5 (45.3–59.5)
Ratio fat/fat-free mass; median (IQR)	0.472 (0.393–0.655)
VO$_2$max categories[b]	
Very low; n (%)	9 (23.7%)
Low; n (%)	6 (15.8%)
Somewhat low; n (%)	6 (15.8%)
Average; n (%)	6 (15.8%)
Somewhat high; n (%)	5 (13.2%)
High; n (%)	4 (10.5%)
Very high; n (%)	2 (5.3%)
VO$_2$max ([ml/min]/kg); median (IQR)	36.0 (28.0–40.0)

Abbreviations: BMI = Body Mass Index, VO$_2$max = submaximal VO$_2$max test, performed on a stationary bicycle, according to the Ekblom-Bak cycle ergometer test [25], and presented as ml/min per kg body weight.

[a]Alcohol use: 1 represents ≥ 4 times per week, 2 represents 2–3 times per week, 3 represents 2–4 times per month, 4 represents once per month, and 5 represents never.

[b]VO$_2$max categories are based on reference values from ~25,000 Swedish males and females in working age (https://www.gih.se/ekblombaktest; see also Methods).

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Table 2. Change (follow-up—Baseline, Δ) in metabolic markers during intervention.

Characteristics	Median (25th, 75th)
ΔVO_2max ([ml/min]/kg)	2.0 (0.0, 4.0)
ΔVO_2max categories	0.0 (0.0, 1.0)
ΔBMI (kg/m^2)	-0.30 (-0.84, 0.18)
ΔFat mass (kg)	-0.89 (-2.4, 0.43)
ΔFat free mass (kg)	-0.65 (-1.7, 0.33)
ΔRatio fat/fat free mass	-0.015 (-0.040, 0.022)

Abbreviations: Δ = Follow-up—Baseline, BMI = Body Mass Index, VO$_2$max = submaximal VO2max test, performed on a stationary bicycle, according to the Ekblom-Bak cycle, ergometer test [25], and presented as [ml/min]/kg.

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beta 1 (TGF-β1), (14) tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), and (15) vascular endothelial growth factor-A (VEGF-A). Bivariate correlation analyses of inflammatory analytes were conducted both at baseline and follow-up. At baseline, there were significant positive correlations (Spearman's r ≥ 0.50) between levels of (a) GRO-α, TGF-β1 and VEGF-A, (b) SAA and CRP, (c) IL-18 and IL-12/IL-23p40, and (d) sICAM-1 and sVCAM-1 (S1A Fig). Three correlations with r ≥ 0.50 remained significant at follow-up: (a) SAA and CRP, (b) IL-18 and IL-12/IL-23p40, and (c) sICAM-1 and sVCAM-1 (S1B Fig). Thus, there were 12 independent analyte groups and the corrected significance was set at α = 0.004 (0.05/12).

Exercise is associated with a significant reduction in sFKN, TGF-β1, eotaxin-1 and IL-6

Fig 1 shows the paired analyses of pre-post intervention changes in inflammatory biomarkers, which revealed significant exercise-associated reductions in levels of sFKN (p = 0.000054), TGF-β1 (p = 0.00053), eotaxin-1/CCL11 (p = 0.0031), and IL-6 (p = 0.0036). There was also a trend for a reduction in levels of sVCAM-1 (p = 0.0063). S1 Table shows the relationship between clinical baseline characteristics of the participants and (i) the baseline levels of these five inflammatory analytes, and (ii) the exercise-associated change (follow-up—baseline) in levels of these five inflammatory analytes. It should also be noted that although there were group-level exercise-associated reductions in levels of sFKN, TGF-β1, eotaxin-1, and IL-6, there was still variation in exercise response on the individual level (S2 Fig).

Differences between males and females in inflammatory biomarker changes following exercise

As shown in Fig 2, when we stratified for sex in our analyses, we found significant differences in pre-post change between sexes for IL-16 (p = 0.0053), sIL-2Rα (p = 0.013), TRAIL (p = 0.012), and VEGF-A (p = 0.0032). Post-hoc assessments showed that for males, but not for females, IL-16 levels increased (p = 0.00098) and VEGF-A levels decreased (p = 0.0029) following exercise. Conversely, females but not males, had a trend toward decreased levels of sIL-2Rα (p = 0.0065) following exercise.

Discussion

The anti-inflammatory properties of exercise [19,20,26,27], which are observed "chronically", i.e., following regular training [28], are of particular importance to MD because the presence of inflammation has emerged as a possible biological mechanism that can drive MD
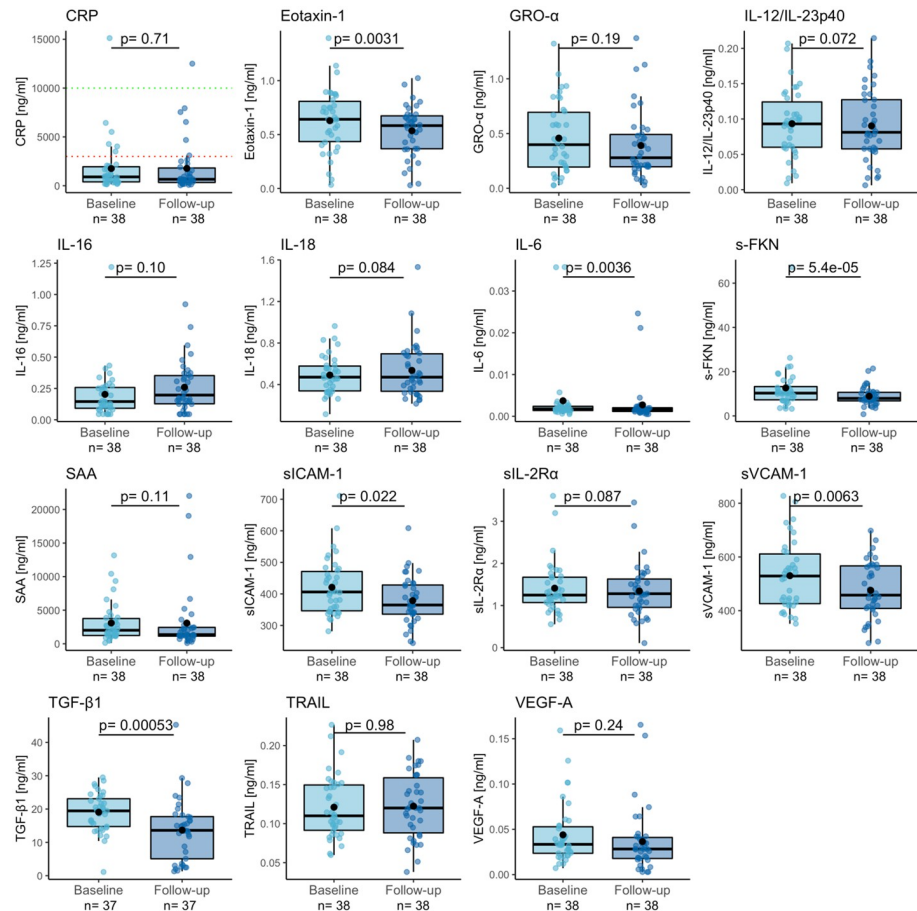
Fig 1. Exercise is associated with a reduction in sFKN, TGF-β1, eotaxin-1 and IL-6. The differences in plasma inflammatory biomarker levels between baseline and follow-up (i.e., before and after the 12-week physical exercise intervention program), were determined using Wilcoxon signed-rank tests. For CRP, the dotted red (3000 ng/ml) and the dotted green (10 000 ng/ml) lines represent the cut-offs for low-grade and mild-acute inflammation, respectively. Abbreviations: CRP = C-reactive protein, sFKN = soluble Fractalkine, GRO-α = Growth-regulated oncogene-alpha, IL-12/IL-23p40 = Interleukin (IL)-12/IL-23p40, IL-16 = Interleukin-16, IL-18 = Interleukin-18, sIL-2Rα = soluble Interleukin-2 receptor subunit alpha, IL-6 = Interleukin-6, SAA = serum amyloid A, sICAM-1 = soluble Intercellular adhesion molecule-1, sVCAM-1 = soluble Vascular cell adhesion molecule-1, TGF-β1 = Transforming growth factor beta 1, TRAIL = Tumor necrosis factor-related apoptosis-inducing ligand, VEGF-A = Vascular endothelial growth factor A.

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development [12–17]. In line with these "chronic" anti-inflammatory properties of regular exercise, our measurements (taken 3–7 days following the last exercise session of a 12-week intervention program) suggested that regular aerobic and resistance training led to an improvement in the peripheral inflammatory status of younger adults with mild-to-moderate MD. Specifically, following the exercise intervention, we found a significant reduction in plasma levels of four inflammatory biomarkers: (i) sFKN, (ii) TGF-β1, (iii) eotaxin-1 and (iv) IL-6. Moreover, (v) sVCAM-1 levels showed a trend for exercise-associated reduction, and sex-stratified analyses indicated different responses in males, compared to females, with post-exercise increases in IL-16 and decreases in VEGF-A levels in males only.

(i). Fractalkine (FKN) is a chemokine, primarily expressed by vascular endothelial cells, which exists in a cell-surface-bound form and a soluble form (sFKN). FKN is made soluble by metalloproteinase-dependent cleavage of its membrane-bound form [29]; a
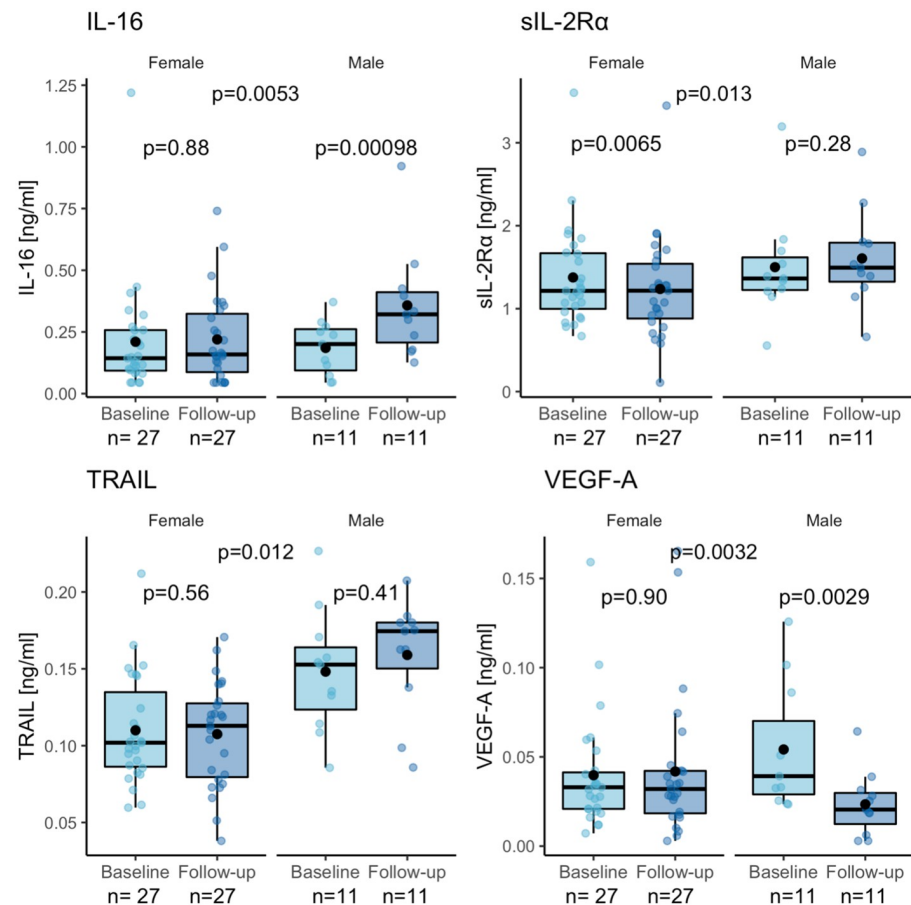
Fig 2. Sex-stratified analyses reveal differences in IL-16 and VEGF-A in males. Sex-stratified box plots of inflammatory biomarker levels before (baseline) and after (follow-up) the 12-week exercise intervention program. The differences in intervention response between sexes were evaluated with an ANCOVA model on analyte levels at follow-up adjusting for baseline analyte levels. The analytes with a difference in response between sexes at $\alpha = 0.05$ are shown: IL-16 ($p = 0.0053$; partial eta$^2 = 0.202$), sIL-2Rα ($p = 0.013$; partial eta$^2 = 0.164$), TRAIL ($p = 0.012$; partial eta$^2 = 0.166$), VEGF-A ($p = 0.0032$; partial eta$^2 = 0.223$). The post-hoc pre-post differences in analyte levels for each sex were assessed using Wilcoxon tests (significance set at $\alpha = 0.004$) and are presented under the corresponding sex. Abbreviations: IL-16 = Interleukin-16, sIL-2Rα = soluble Interleukin-2 receptor subunit alpha, TRAIL = Tumor necrosis factor-related apoptosis-inducing ligand, VEGF-A = Vascular endothelial growth factor A.

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process that is induced by proinflammatory signals [30]. The soluble form, sFKN, shows strong chemoattractant activity towards T cells and monocytes bearing the fractalkine receptor (CX3CR1), and promotes leukocyte integrin activation. The membrane-bound FKN facilitates adhesion of leukocytes to the vascular endothelium and their migration into tissue [31]. Elevated plasma levels of sFKN have been associated with the development of metabolic syndrome [32], cardiovascular outcomes [33] and atherosclerosis (16). Thus, the reduction in plasma levels of sFKN in MD participants, which was observed in our study, may indicate an exercise-induced reduction in vascular inflammation. While aerobic exercise is known to reduce vascular inflammatory activity [23,34], only few studies have previously investigated the effect of physical exercise on levels of sFKN. These previous studies, however, found that 12 months of combined aerobic and resistance training, or 12 weeks of balance exercise programs, were associated with slightly elevated circulating levels of serum sFKN in either patients with type 2

diabetes and coronary artery disease, or in those without a diagnosis or with Parkinson's disease [35,36]. Elevated levels of sFKN have also been found in serum of healthy individuals immediately following maximal exercise on a treadmill [37]. However, all of these previous studies measured sFKN levels in serum, which does not contain platelets, and FKN has a known role in mediating platelet adhesion [38]. Thus, since we measured sFKN levels using plasma, our findings of exercise-induced reductions in sFKN may indicate specificity to this liquid portion of blood, which warrants future investigation.

(ii). TGF-β1 is a multifunctional cytokine with an immune-modulatory role by inhibiting lymphocyte proliferation, stimulate IgA class switching of B cells and regulate tissue repair. Together with Il-6 it promotes the differentiation of pro-inflammatory CD4 + cells expressing IL-17. Higher plasma TGF-β1 levels have been associated with atherosclerosis, hypertension, hyperlipidemia and diabetes [39]. Moreover, there is evidence suggesting that increased TGF-β1 protein activity in skeletal muscle contributes to an impaired exercise response through inhibition of mitochondrial regulators and insulin signalling [40]. The expression of TGF-β1 is induced by mechanical loading in various tissues, including bone and cardiac tissue [41]. A previous study, using healthy individuals, found that TGF-β1 plasma levels increased transiently after two weeks of heavy resistance training, but that these chronic point-wise levels dropped below baseline following another two weeks of exercise [28]. These results are in agreement with our findings showing reduced TGF-β1 plasma levels after 12 weeks of regular exercise.

(iii). Eotaxin-1 is a chemokine expressed by the vascular smooth muscle cells located beneath the vascular endothelium, and also by, e.g., bronchial epithelial cells and dermal fibroblasts [42]. Eotaxin-1 expression is induced by proinflammatory cytokines (e.g., TNF-α) and it acts as a chemoattractant mainly for eosinophils, basophils, mast cells and Th2 lymphocytes. Higher plasma eotaxin-1 levels have been associated with asthma and allergy disorders but also neurodegeneration and accelerated aging [42]. In line with our findings of reduced eotaxin-1 levels following the 12-week exercise intervention, obese individuals who underwent eight-to-twelve week programs of aerobic and/or resistance exercise were also found to have reduced levels of eotaxin-1 [43–45].

(iv). IL-6 is a pleiotropic molecule with multiple immunologic and metabolic effects, which is both a pro-inflammatory cytokine and an anti-inflammatory myokine [46]. Higher levels of IL-6 have been found to predict, or to be associated with, physical impairment, disability and mortality in older persons [13,15,17,47,48]. Muscle-derived IL-6 is known to be released into the bloodstream during exercise, and is immediately followed by a transient increase in levels of anti-inflammatory IL-1 receptor antagonist (IL-1RA) and IL-10 [49–51]. Plasma levels of these three interleukins are back to pre-exercise levels in about 2 hours after a single exercise bout [52]. In line with our findings of reduced IL-6 levels following the 12-week exercise intervention, regular aerobic exercise has consistently been reported to decrease IL-6 levels in healthy adults [53,54]. The latter effect has also been proposed to be moderated by a genetic polymorphism in the *IL6* gene promoter, which regulates the beta-adrenergic activation of *IL6* transcription, in individuals with impaired glucose tolerance [55,56].

(v). One of the adhesion molecules measured, sVCAM-1, showed a trend for post-exercise reduction. sVCAM-1 levels correlate with levels of membrane-bound VCAM-1, which is expressed specifically by vascular endothelial and smooth muscle cells and is central to leukocyte–endothelial cell adhesion and leukocyte extravasation into the surrounding tissue [57,58]. Elevated levels of sVCAM-1 have been associated with vascular

inflammation, endothelial dysfunction and atherosclerosis, and—similar to our findings —sVCAM-1 has been found to be reduced by low-to-moderate aerobic exercise in a variety of study populations, but without any effect of resistance exercise [34].

Although the mechanistic connections between fractalkine, TGF-β1, eotaxin-1 and IL-6 were not explored in our study, some assumptions can be made based on previous literature and our correlation analyses. For instance, TGF-β1 has previously been found to stimulate fractalkine expression in human cancerous cell lines and rat microglia [59,60]. This could indicate that the exercise-induced reductions in levels of fractalkine found in our study, are mediated by a primary effect of exercise on lowering TGF-β1 levels. However, we found no significant correlations between plasma fractalkine and TGF-β1 at either baseline or follow-up (S1 Fig), or between fractalkine level change (follow-up—baseline) and change in TGF-β1 (S3A Fig). Moreover, other studies found that TGF-β1 actually reduced fractalkine mRNA and protein expression in glioma cells [61], which supports a complex interplay between the levels of these two molecules that may be dependent on the tissue or disease of interest. Similarly, studies using smooth muscle cells or fibroblast cultures, found that TGF-β1 (together with IL-13) can stimulate the expression of eotaxin-1 [62,63]. Indeed, we detected a correlation between change in TGF-β1 and change in eotaxin-1 levels (r = 0.33, p = 0.046, S3B Fig). Along the same lines, cell culture experiments have demonstrated a crosstalk between TGF-β1 and IL-6, with TGF-β1 controlling aspects of early innate inflammation through promotion of IL-6 secretion and/or induction of IL-6 protein and mRNA levels [64–66], but without any significant correlations between these two molecules found in the plasma samples of our study. Interestingly, however, studies have found that plasma fractalkine levels correlate positively with plasma levels of IL-6 [67], which is in line with our correlation data for these two molecules that were significant at baseline (Spearman's r = 0.42; S1 Fig), including the change in fractalkine that correlated with the change in IL-6 levels (r = 0.39, p = 0.015, S3C Fig). Indeed, previous studies using CD16+ monocytes suggested that membrane-bound fractalkine stimulates the production of IL-6 [68], providing a possible mechanistic link between the levels of these two molecules, which warrants additional investigation in physical exercise settings.

Besides our primary pre-post analyses, we also conducted sex-stratified analyses that revealed significant increases in IL-16, and significant reductions in VEGF-A, in male individuals only. IL-16 is known to stimulate the expression of pro-inflammatory cytokines [69] and has been associated with inflammatory diseases, such as rheumatoid arthritis [70]. By contrast, there are also reports suggesting that IL-16 may possess protective effects against other inflammatory disorders, such as atherosclerosis [71,72]. However, to our knowledge, no previous studies have reported sex-specific effects of physical exercise on IL-16 levels. VEGF-A, on the other hand, is produced by many different cell types, including vascular endothelial cells, macrophages and cardiomyocytes, and has a key role in mediating angiogenesis and vasodilation. Although VEGF-A is essential for the cardiovascular system, aberrantly elevated levels have been implicated in inflammatory processes of cardiovascular disease and pathology following atherosclerosis [73], as well as in inflammatory diseases, such as inflammatory bowel disease [74] and psoriasis-like disease [75]. Similar to our findings, plasma VEGF-A levels have previously been found to decrease for at least 2 hours, following a single bout of exercise, in healthy men [76]. By contrast, in overweight postmenopausal women, there was no effect of regular moderate-to-vigorous aerobic exercise on plasma levels of VEGF-A [77].

Limitations

There are a number of limitations to this exploratory study that need to be acknowledged. (i) The low number of individuals included in our study (N = 38) render the significant

findings preliminary and exploratory in nature, and all results need to be interpreted with caution prior to replication in additional MD cohorts. Moreover, for those analytes that we could not detect a significant pre-post exercise change, we cannot exclude the presence of true small effect sizes. Finally, the number of participants was even lower in the sex-stratified analyses. Hence, only relatively large effect sizes could have been detected. (ii) The intervention study, from which the current data and samples derived from, included an additional N = 50 individuals with MD who donated blood but were not included in our study. Comparisons of all known baseline characteristics between the two groups showed only slight sex differences, i.e., with some male overrepresentation in our group (p = 0.046, S2 Table). (iii) The study lacks long-term post-intervention data on MD status, repeated sampling without active intervention in MD, as well as a control group without MD. (iv) The blood samples were collected in a time window of 3–7 days after the last exercise session. The transient inflammatory-resembling responses often observed "acutely", i.e., during or immediately after an exercise bout have been reported to last no longer than up to 24–36 h [37,52]. However, we cannot exclude the possibility of longer acute effects in some individuals. (v) As indicated by the levels of CRP in Fig 1, and in line with the hypothesis of inflammation in MD [12–17], a significant portion of the participants in our study had low-grade inflammation. However, CRP was not among the analytes that was significantly affected by exercise. Moreover, due to the lack of standardized reference levels for most of the remaining analytes, we cannot evaluate to which extent the participants' levels deviated from normal ones. (vi) Although only few of the study's MD participants reported a chronic disease, we cannot exclude the presence of undetected pathologies that could influence biomarker levels.

Conclusion

Our findings support a beneficial role of regular moderate aerobic exercise, combined with resistance training, on both CRF [24] and peripheral inflammatory status in young and middle-aged adults with MD. Importantly, the post-exercise reduction in plasma sFKN levels has to our knowledge not previously been reported, but is in agreement with reduced plasma levels of vascular adhesion molecules reported by others [34], including the trend for reduced sVCAM-1 levels observed in our study. The post-exercise reductions that we found in plasma levels of TGF-β1, eotaxin-1 and IL-6, are in agreement with findings from other study groups [28,43–45,53,54], although there is to our knowledge no report on these markers in younger adults with MD. The male-specific exercise effects that we detected in levels of IL-16 and VEGF-A are also novel for MD subjects, although sex-specific effects on VEGF-A are supported by previous reports in non-MD groups [76,77]. However, given the aforementioned limitations of the present pilot study, future studies are warranted to confirm these findings and translate them into outcomes of clinical significance. Moreover, future studies need to take into consideration putative confounding parameters such as disease comorbidity and medication. Nonetheless, given the proposed role of inflammation in promoting MD development [12–17], our study provides support for the continued evaluation of physical exercise as a preventive and treatment modality for MD.

Methods

Participants

Study participants were the first 38 subjects (of the total N = 110) that participated in a 12-week parallel-group randomized controlled trial that took place between May 2018 – December 2018, and which was designed to compare the effects of an app-based program with

a supervised exercise and health program on levels of physical activity and changes in CRF in individuals with mild-to-moderate MD [24]. As there was no difference in effects between the two motivation arms on amount of psychical activity or change in CRF or body composition, we did not account for intervention arm in this study. Participants were recruited from rehabilitation, primary care and occupational health care centres in Stockholm, Sweden. The eligibility criteria for study participation were as follows: male or female aged 18–45 years old with any mobility-related problems affecting everyday life, such as problems with transportation, work, dressing, and performing household or personal hygiene tasks, experienced during the three years prior to trial enrolment. Exclusion criteria included being bound to a wheelchair or having a medical condition preventing moderate-intensity walking (for a detailed description of the trial see also Berglind D *et al.* [78]). Only a few of the 38 participants in this study reported a chronic disease diagnosis. Of the 38, 19 were restricted in running, heavy lifts, and demanding sports, and 2 were restricted in household activities such as vacuum cleaning, walking in forest or gardening. Comparisons of the baseline characteristics of this study's participants (N = 38) with the baseline characteristics of those individuals who contributed blood in the trial but were not included in the study (N = 50), are presented in S2 Table and show an overrepresentation of males in the present study (p = 0.046). All participants gave written informed consent before entering the trial, which was approved by the Ethical Review Board in Stockholm (Dnr: 2017/1206-31/1) and was registered in the ISRCTN registry (registration number: ISRCTN22387524). The present study was also approved by the same ethical committee. Moreover, all methods were carried out in accordance with relevant guidelines and regulations, and all experimental protocols were approved by the Ethical Review Board in Stockholm.

Intervention, body composition and cardiorespiratory fitness (CRF)

The treatment arms were designed with an intrinsic motivation-to-change strategy in order to encourage participants to maintain sustained changes in moderate-to-vigorous physical activity and CRF [79]. In both treatment arms, participants were encouraged to engage in a minimum of 30 minutes of moderate-to-vigorous physical exercise every day, counting both aerobic and resistance exercise. The actual amount of physical activity was measured using Actigraph GT3X+ accelerometers, worn on the hip during all waking hours, for 7 consecutive days at each assessment. The assessments were at baseline, after 5 weeks and after 11 weeks of intervention. Management and analyses of the accelerometer data have been described elsewhere [24]. Briefly, valid measurements included $\geq$10 hours wear-time per day for $\geq$4 days. Wear-time and classification of bouts were computed using ActiLife v.6.13.3. Moderate-to-vigorous physical activity was defined as more than 3,208 counts per minute which in adults corresponds to a VO_2/resting $VO_2 \geq 3$ or walking at ~4.5 km per hour [80,81]. The exercise programs were designed in collaboration with personal trainers and individualized based on baseline levels of CRF (VO_2max), illness or physical symptoms. Body composition and CRF measurements in the study participants have been described in detail elsewhere [24]. In brief, fat mass (in kilograms; kg) and fat-free mass (kg) were measured through bioelectrical impedance [82] using an Omron body composition monitor (model HBF-511B-E/HBF-511T-E). Physical tests also included measurements such as height, weight and waist circumference. CRF measurements were performed using a submaximal VO_2max test on a stationary bicycle according to the Ekblom-Bak cycle ergometer test [25] and are presented in relative numbers (ml/min per kg body weight). VO_2max categories are based on age and sex-stratified reference values from ~25,000 Swedish males and females in working age (available at https://www.gih.se/ekblombaktest).

Measurement of inflammatory biomarkers

Venous blood was collected from participants at baseline (prior to the first exercise session) and at the 12-week follow-up (3–7 days after the last exercise session) using lithium heparin tubes. Participants rested 40 minutes immediately prior to sampling. Refrigerated centrifugation was performed at 1,700 g for 20 minutes and plasma aliquots were stored at −80˚C for 0.5–1.5 years until analysis. A total of 24 inflammatory/immune activity analytes were measured using the multiplex Meso Scale Discovery (MSD) Platform (Meso Scale Diagnostics; Maryland, USA) according to the manufacturer's protocol at the MSD core facility, Center for Molecular Medicine, Stockholm. The analytes were the following: C-Reactive Protein (CRP), eotaxin-1/CCL11, soluble fractalkine/CX3CL1 (sFKN), growth-regulated oncogene-alpha (GRO-α), Interferon-γ (IFN-γ), interleukin (IL)-10, IL-12 subunit p40 (IL-12p40), IL-17A, IL-18, IL-1β, IL-2, IL-16, soluble interleukin-2 receptor subunit alpha (sIL2Rα), IL-6, monocyte chemoattractant protein 1 (MCP1), serum amyloid A (SAA), transforming growth factor beta 1, 2, and 3 (TGF-β1–3), tumor necrosis factor alpha (TNFα), TNF-related apoptosis-inducing ligand (TRAIL), soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular cell adhesion protein 1 (sVCAM-1), and vascular endothelial growth factor A (VEGF-A). The V-plex vascular injury panel 2 (MSD, Cat. no. K15198G) was used to measure CRP, SAA, ICAM-1 and VCAM-1, and the U-plex panels (MSD, Cat. nos. K15067L and K15241K) were used to measure the remaining analytes in multiplexes of 3, 7 and 10 analytes at experimental conditions designed by the manufacturer to give high sensitivity and avoid interference between analytes. Analytes with more than five samples under the lowest level of detection (LLOD) were excluded from further analyses, i.e., IL-17A, IL-1β, MCP1, TNFα, IFN-γ, IL-10, IL-2, TGF-β2, TGF-β3 (S3 Table). The LLOD for the remaining analytes were the following: On V-plex [ng/ml]; CRP = 3.34, SAA = 14.2, sICAM-1 = 1.86, sVCAM-1 = 9.27, and on U-plex [pg/ml]; eotaxin-1 = 9.12, sFKN = 103, GRO-α = 0.991, IL-6 = 0.502, IL-12/IL-23p40 = 1.37, IL-16 = 44.7, IL-18 = 0.438, sIL-2Rα = 81.2, TGF-β1 = 10.0, TRAIL = 2.84, VEGF-A = 2.91. All plasma samples were freeze-thawed twice. The manufacturer-delivered standard curve calibrators were measured in duplicates, and the patient samples were run in singlets based on empirically low coefficients of variation (CV) from MSD measurements in our lab. All samples were run on the same plate for each analyte and all standard curves had a robust correlation ($r^2 > 0.997$). Within plate CVs were determined from the standard curve of each analyte and presented as median (IQR): CRP = 7.39% (6.10%–8.58%), SAA = 11.64% (10.56%–14.51%), sICAM-1 = 10.1% (7.31%–11.29%), sVCAM-1 = 9.49% (6.35%–10.94%), eotaxin-1 = 6.95% (4.18%–9.4%), sFKN = 2.46% (1.34%–3.63%), GRO-α = 5.31% (4.08%–5.84%), IL-6 = 6.3% (4.51%–8.28%), IL-12/IL-23p40 = 2.91% (1.6%–4.4%), IL-16 = 5.94% (4.52%–10.07%), IL-18 = 2.34% (1.39%–4.41%), sIL2Rα = 4.58% (3.97%–11.86%), TGF-β1 = 6.28% (3.47%–9.1%), TRAIL = 2.57% (1.49%–5.1%), VEGF-A = 2.74% (1.2%–4.75%). Data are presented as calculated values of undiluted plasma.

Statistical analyses

To detect an effect of regular exercise on inflammatory biomarkers with the effect sizes previously reported [28,43,53,76,83], at an α = 0.05 and power = 0.80 for paired differences, a sample size of N = 10–35 individuals was estimated to be needed. Specifically, as several of the analytes measured by us were not previously included in studies of regular aerobic exercise, the sample size calculation was based only on eotaxin-1, IL-6, sICAM-1, sVCAM-1, TGF-β1 and VEGF-A. Group differences between those with and those without blood samples were tested using Mann-Whitney U tests (for continuous variables) and chi-square tests (for categorical variables). The bivariate correlations between clinical characteristics and the analyte

levels were estimated with a non-parametric Spearman's rank correlation test. Differences between baseline and follow-up were tested using non-parametric Wilcoxon tests and (as described in the main text) the corrected significance was set at $\alpha = 0.004$ (0.05/12) since there were 12 independent analyte groups. Sex differences in pre-post analyte level response were tested using an ANCOVA model on the analyte levels at follow-up, adjusting for baseline levels. For analytes with a sex difference at $\alpha = 0.05$, the post-hoc pre-post analyte level difference in each sex was assessed using Wilcoxon tests and with a significance set at $\alpha = 0.004$. No possible outlier was excluded from the analyses. The detected pre-post differences were confirmed not to be biased by different time-of-the-day sampling at baseline compared to follow-up by removing from the analysis the nine samples with different pre-post time-of-the-day samplings (data not shown).

Supporting information

S1 Fig. Bivariate correlation analyses of inflammatory biomarkers. Correlation analyses between inflammatory biomarker levels at **(A)** baseline and **(B)** follow-up. Numbers represent Spearman's rank correlation coefficients (r) and blue circles indicate a positive r different from zero at $\alpha = 0.01$. Abbreviations: CRP = C-reactive protein, sFKN = soluble Fractalkine, GRO-α = Growth-regulated oncogene-alpha, IL-12/IL-23p40 = Interleukin (IL)-12/IL-23p40, IL-16 = Interleukin-16, IL-18 = Interleukin-18, sIL-2Rα = soluble Interleukin-2 receptor subunit alpha, IL-6 = Interleukin-6, SAA = serum amyloid A, sICAM-1 = soluble Intercellular adhesion molecule-1, sVCAM-1 = soluble Vascular cell adhesion molecule-1, TGF-β1 = Transforming growth factor beta 1, TRAIL = Tumor necrosis factor-related apoptosis-inducing ligand, VEGF-A = Vascular endothelial growth factor A.
(DOCX)

S2 Fig. Individual presentation of differences in inflammatory biomarkers. The differences in inflammatory biomarker levels before (baseline) and after (follow-up) the 12-week exercise intervention program were determined using Wilcoxon signed-rank tests and using lines to indicate the intervention responses on the individual level. Abbreviations: CRP = C-reactive protein, sFKN = soluble Fractalkine, GRO-α = Growth-regulated oncogene-alpha, IL-12/IL-23p40 = Interleukin (IL)-12/IL-23p40, IL-16 = Interleukin-16, IL-18 = Interleukin-18, sIL-2Rα = soluble Interleukin-2 receptor subunit alpha, IL-6 = Interleukin-6, SAA = serum amyloid A, sICAM-1 = soluble Intercellular adhesion molecule-1, sVCAM-1 = soluble Vascular cell adhesion molecule-1, TGF-β1 = Transforming growth factor beta 1, TRAIL = Tumor necrosis factor-related apoptosis-inducing ligand, VEGF-A = Vascular endothelial growth factor A.
(DOCX)

S3 Fig. Correlation between biomarkers, analysis on plasma level changes from baseline (bl) to follow up (fu). Spearman's correlation analyses between **(A)** TGF-β1 and sFKN, **(B)** TGF-β1 and Eotaxin-1, and **(C)** sFKN and IL-6. Change is defined as level at follow up (fu) minus level at baseline (bl). Numbers represent Spearman's rank correlation coefficients (r) and p-values. Abbreviations: sFKN = soluble Fractalkine, IL-6 = Interleukin-6, TGF-β1 = Transforming growth factor beta 1.
(DOCX)

S1 Table. The relationship between levels of inflammatory biomarkers at baseline and clinical baseline characteristics, as well as the relationship between the change (follow-up—Baseline) in levels of inflammatory biomarkers and clinical baseline characteristics.
(DOCX)

S2 Table. Baseline characteristics of the study participants with mobility disability (N = 38) and comparisons with those MD individuals who contributed blood but were not included in this study (N = 50).
(DOCX)

S3 Table. Lower Level Of Detection (LLOD) values for all measured analytes.
(DOCX)

Author Contributions

Conceptualization: Parvin Kumar, Yvonne Forsell, Catharina Lavebratt.

Data curation: Parvin Kumar, Miranda Stiernborg, Daniel Berglind.

Formal analysis: Parvin Kumar, Miranda Stiernborg.

Funding acquisition: Daniel Berglind, Catharina Lavebratt.

Investigation: Parvin Kumar, Daniel Berglind, Philippe A. Melas, Yvonne Forsell, Catharina Lavebratt.

Methodology: Parvin Kumar, Philippe A. Melas, Catharina Lavebratt.

Project administration: Yvonne Forsell.

Resources: Daniel Berglind, Yvonne Forsell.

Supervision: Daniel Berglind, Yvonne Forsell, Catharina Lavebratt.

Validation: Catharina Lavebratt.

Visualization: Miranda Stiernborg, Catharina Lavebratt.

Writing – original draft: Philippe A. Melas.

Writing – review & editing: Parvin Kumar, Miranda Stiernborg, Anna Fogdell-Hahn, Kristoffer Månsson, Tomas Furmark, Daniel Berglind, Yvonne Forsell, Catharina Lavebratt.

References

1. WHO. World report on disability: World Health Organization; 2011 [cited 2021]. https://www.who.int/disabilities/world_report/2011/report.pdf.

2. Clarke P, Ailshire JA, Bader M, Morenoff JD, House JS. Mobility disability and the urban built environment. Am J Epidemiol. 2008; 168(5):506–13. Epub 2008/08/01. https://doi.org/10.1093/aje/kwn185 PMID: 18667526.

3. Centers for Disease C, Prevention. Prevalence and most common causes of disability among adults—United States, 2005. MMWR Morb Mortal Wkly Rep. 2009; 58(16):421–6. Epub 2009/05/02. PMID: 19407734.

4. Boström G. Hälsa på lika villkor?: hälsa och livsvillkor bland personer med funktionsnedsättning. Östersund: Statens folkhälsoinstitut; 2008.

5. Welfare TNBoHa. Swedish disability policy—service and care for people with functional impairments 2009. https://www.mindbank.info/item/1201.

6. Carroll DD, Courtney-Long EA, Stevens AC, Sloan ML, Lullo C, Visser SN, et al. Vital signs: disability and physical activity—United States, 2009–2012. MMWR Morb Mortal Wkly Rep. 2014; 63(18):407–13. Epub 2014/05/09. PMID: 24807240.

7. Holmgren M, Lindgren A, de Munter J, Rasmussen F, Ahlstrom G. Impacts of mobility disability and high and increasing body mass index on health-related quality of life and participation in society: a population-based cohort study from Sweden. BMC Public Health. 2014; 14:381. Epub 2014/04/20. https://doi.org/10.1186/1471-2458-14-381 PMID: 24742257.

8. Picavet HS, van den Bos GA. The contribution of six chronic conditions to the total burden of mobility disability in the Dutch population. Am J Public Health. 1997; 87(10):1680–2. Epub 1997/11/14. https://doi.org/10.2105/ajph.87.10.1680 PMID: 9357354.

9. Iezzoni LI, McCarthy EP, Davis RB, Siebens H. Mobility difficulties are not only a problem of old age. J Gen Intern Med. 2001; 16(4):235–43. Epub 2001/04/25. https://doi.org/10.1046/j.1525-1497.2001.016004235.x PMID: 11318924.

10. Pahor M, Guralnik JM, Ambrosius WT, Blair S, Bonds DE, Church TS, et al. Effect of structured physical activity on prevention of major mobility disability in older adults: the LIFE study randomized clinical trial. JAMA. 2014; 311(23):2387–96. Epub 2014/05/29. https://doi.org/10.1001/jama.2014.5616 PMID: 24866862.

11. de Vries NM, van Ravensberg CD, Hobbelen JS, Olde Rikkert MG, Staal JB, Nijhuis-van der Sanden MW. Effects of physical exercise therapy on mobility, physical functioning, physical activity and quality of life in community-dwelling older adults with impaired mobility, physical disability and/or multi-morbidity: a meta-analysis. Ageing Res Rev. 2012; 11(1):136–49. Epub 2011/11/22. https://doi.org/10.1016/j.arr.2011.11.002 PMID: 22101330.

12. Osawa Y, Semba RD, Fantoni G, Candia J, Biancotto A, Tanaka T, et al. Plasma proteomic signature of the risk of developing mobility disability: A 9-year follow-up. Aging Cell. 2020; 19(4):e13132. Epub 2020/03/12. https://doi.org/10.1111/acel.13132 PMID: 32157804.

13. Cesari M, Kritchevsky SB, Nicklas B, Kanaya AM, Patrignani P, Tacconelli S, et al. Oxidative damage, platelet activation, and inflammation to predict mobility disability and mortality in older persons: results from the health aging and body composition study. J Gerontol A Biol Sci Med Sci. 2012; 67(6):671–6. Epub 2012/03/06. https://doi.org/10.1093/gerona/glr246 PMID: 22389462.

14. Verghese J, Holtzer R, Lipton RB, Wang C. High-sensitivity C-reactive protein and mobility disability in older adults. Age Ageing. 2012; 41(4):541–5. Epub 2012/03/16. https://doi.org/10.1093/ageing/afs038 PMID: 22417984.

15. Ferrucci L, Harris TB, Guralnik JM, Tracy RP, Corti MC, Cohen HJ, et al. Serum IL-6 level and the development of disability in older persons. J Am Geriatr Soc. 1999; 47(6):639–46. Epub 1999/06/12. https://doi.org/10.1111/j.1532-5415.1999.tb01583.x PMID: 10366160.

16. Vasunilashorn S, Ferrucci L, Crimmins EM, Bandinelli S, Guralnik JM, Patel KV. Association of inflammation with loss of ability to walk 400 meters: longitudinal findings from the Invecchiare in Chianti Study. J Am Geriatr Soc. 2013; 61(10):1743–9. Epub 2013/10/03. https://doi.org/10.1111/jgs.12446 PMID: 24083386.

17. Wannamethee SG, Whincup PH, Lennon L, Papacosta O, Lowe GD. Associations between fibrin D-dimer, markers of inflammation, incident self-reported mobility limitation, and all-cause mortality in older men. J Am Geriatr Soc. 2014; 62(12):2357–62. Epub 2014/12/18. https://doi.org/10.1111/jgs.13133 PMID: 25516032.

18. Lin X, Zhang X, Guo J, Roberts CK, McKenzie S, Wu WC, et al. Effects of Exercise Training on Cardiorespiratory Fitness and Biomarkers of Cardiometabolic Health: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. J Am Heart Assoc. 2015; 4(7). Epub 2015/06/28. https://doi.org/10.1161/JAHA.115.002014 PMID: 26116691.

19. Beavers KM, Brinkley TE, Nicklas BJ. Effect of exercise training on chronic inflammation. Clin Chim Acta. 2010; 411(11–12):785–93. Epub 2010/03/02. https://doi.org/10.1016/j.cca.2010.02.069 PMID: 20188719.

20. Scheffer DDL, Latini A. Exercise-induced immune system response: Anti-inflammatory status on peripheral and central organs. Biochim Biophys Acta Mol Basis Dis. 2020; 1866(10):165823. Epub 2020/05/04. https://doi.org/10.1016/j.bbadis.2020.165823 PMID: 32360589.

21. Valenzuela PL, Castillo-Garcia A, Morales JS, de la Villa P, Hampel H, Emanuele E, et al. Exercise benefits on Alzheimer's disease: State-of-the-science. Ageing Res Rev. 2020; 62:101108. Epub 2020/06/21. https://doi.org/10.1016/j.arr.2020.101108 PMID: 32561386.

22. Fiuza-Luces C, Santos-Lozano A, Joyner M, Carrera-Bastos P, Picazo O, Zugaza JL, et al. Exercise benefits in cardiovascular disease: beyond attenuation of traditional risk factors. Nat Rev Cardiol. 2018; 15(12):731–43. Epub 2018/08/18. https://doi.org/10.1038/s41569-018-0065-1 PMID: 30115967.

23. Boeno FP, Ramis TR, Munhoz SV, Farinha JB, Moritz CEJ, Leal-Menezes R, et al. Effect of aerobic and resistance exercise training on inflammation, endothelial function and ambulatory blood pressure in middle-aged hypertensive patients. J Hypertens. 2020; 38(12):2501–9. Epub 2020/07/23. https://doi.org/10.1097/HJH.0000000000002581 PMID: 32694343.

24. Berglind D, Yacaman-Mendez D, Lavebratt C, Forsell Y. The Effect of Smartphone Apps Versus Supervised Exercise on Physical Activity, Cardiorespiratory Fitness, and Body Composition Among Individuals With Mild-to-Moderate Mobility Disability: Randomized Controlled Trial. JMIR Mhealth Uhealth. 2020; 8(2):e14615. Epub 2020/02/06. https://doi.org/10.2196/14615 PMID: 32014846.

25. Bjorkman F, Ekblom-Bak E, Ekblom O, Ekblom B. Validity of the revised Ekblom Bak cycle ergometer test in adults. Eur J Appl Physiol. 2016; 116(9):1627–38. Epub 2016/06/18. https://doi.org/10.1007/s00421-016-3412-0 PMID: 27311582.

26. Heesch KC, Brown WJ. Do walking and leisure-time physical activity protect against arthritis in older women? J Epidemiol Community Health. 2008; 62(12):1086–91. Epub 2008/11/15. https://doi.org/10.1136/jech.2007.072215 PMID: 19008369.

27. Thompson PD, Buchner D, Pina IL, Balady GJ, Williams MA, Marcus BH, et al. Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease: a statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity). Circulation. 2003; 107(24):3109–16. Epub 2003/06/25. https://doi.org/10.1161/01.CIR.0000075572.40158.77 PMID: 12821592.

28. Hering S, Jost C, Schulz H, Hellmich B, Schatz H, Pfeiffer H. Circulating transforming growth factor beta1 (TGFbeta1) is elevated by extensive exercise. Eur J Appl Physiol. 2002; 86(5):406–10. Epub 2002/03/08. https://doi.org/10.1007/s00421-001-0537-5 PMID: 11882926.

29. Garton KJ, Gough PJ, Blobel CP, Murphy G, Greaves DR, Dempsey PJ, et al. Tumor necrosis factor-alpha-converting enzyme (ADAM17) mediates the cleavage and shedding of fractalkine (CX3CL1). J Biol Chem. 2001; 276(41):37993–8001. Epub 2001/08/10. https://doi.org/10.1074/jbc.M106434200 PMID: 11495925.

30. Fraticelli P, Sironi M, Bianchi G, D'Ambrosio D, Albanesi C, Stoppacciaro A, et al. Fractalkine (CX3CL1) as an amplification circuit of polarized Th1 responses. J Clin Invest. 2001; 107(9):1173–81. Epub 2001/05/09. https://doi.org/10.1172/JCI11517 PMID: 11342581.

31. Bazan JF, Bacon KB, Hardiman G, Wang W, Soo K, Rossi D, et al. A new class of membrane-bound chemokine with a CX3C motif. Nature. 1997; 385(6617):640–4. Epub 1997/02/13. https://doi.org/10.1038/385640a0 PMID: 9024663.

32. Xueyao Y, Saifei Z, Dan Y, Qianqian P, Xuehong D, Jiaqiang Z, et al. Circulating fractalkine levels predict the development of the metabolic syndrome. Int J Endocrinol. 2014; 2014:715148. Epub 2014/06/03. https://doi.org/10.1155/2014/715148 PMID: 24883062.

33. Shah R, Matthews GJ, Shah RY, McLaughlin C, Chen J, Wolman M, et al. Serum Fractalkine (CX3CL1) and Cardiovascular Outcomes and Diabetes: Findings From the Chronic Renal Insufficiency Cohort (CRIC) Study. Am J Kidney Dis. 2015; 66(2):266–73. Epub 2015/03/22. https://doi.org/10.1053/j.ajkd.2015.01.021 PMID: 25795074.

34. Koh Y, Park J. Cell adhesion molecules and exercise. J Inflamm Res. 2018; 11:297–306. Epub 2018/08/14. https://doi.org/10.2147/JIR.S170262 PMID: 30100749.

35. Njerve IU, Byrkjeland R, Arnesen H, Akra S, Solheim S, Seljeflot I. Effects of long-term exercise training on adipose tissue expression of fractalkine and MCP-1 in patients with type 2 diabetes and stable coronary artery disease: a substudy of a randomized controlled trial. Diabetes Metab Syndr Obes. 2016; 9:55–62. Epub 2016/04/05. https://doi.org/10.2147/DMSO.S96299 PMID: 27042130.

36. Szymura J, Kubica J, Wiecek M, Pera J. The Immunomodulary Effects of Systematic Exercise in Older Adults and People with Parkinson's Disease. J Clin Med. 2020; 9(1). Epub 2020/01/16. https://doi.org/10.3390/jcm9010184 PMID: 31936624.

37. Crabb EB, Franco RL, Caslin HL, Blanks AM, Bowen MK, Acevedo EO. The effect of acute physical and mental stress on soluble cellular adhesion molecule concentration. Life Sci. 2016; 157:91–6. Epub 2016/06/05. https://doi.org/10.1016/j.lfs.2016.05.042 PMID: 27259811.

38. Meyer dos Santos S, Klinkhardt U, Scholich K, Nelson K, Monsefi N, Deckmyn H, et al. The CX3C chemokine fractalkine mediates platelet adhesion via the von Willebrand receptor glycoprotein Ib. Blood. 2011; 117(18):4999–5008. Epub 2011/03/15. https://doi.org/10.1182/blood-2011-02-335471 PMID: 21398580.

39. Toma I, McCaffrey TA. Transforming growth factor-beta and atherosclerosis: interwoven atherogenic and atheroprotective aspects. Cell Tissue Res. 2012; 347(1):155–75. Epub 2011/06/01. https://doi.org/10.1007/s00441-011-1189-3 PMID: 21626289.

40. Bohm A, Hoffmann C, Irmler M, Schneeweiss P, Schnauder G, Sailer C, et al. TGF-beta Contributes to Impaired Exercise Response by Suppression of Mitochondrial Key Regulators in Skeletal Muscle. Diabetes. 2016; 65(10):2849–61. Epub 2016/07/01. https://doi.org/10.2337/db15-1723 PMID: 27358493.

41. Heinemeier K, Langberg H, Kjaer M. Exercise-induced changes in circulating levels of transforming growth factor-beta-1 in humans: methodological considerations. Eur J Appl Physiol. 2003; 90(1–2):171–7. Epub 2003/09/25. https://doi.org/10.1007/s00421-003-0881-8 PMID: 14504950.

42. Teixeira AL, Gama CS, Rocha NP, Teixeira MM. Revisiting the Role of Eotaxin-1/CCL11 in Psychiatric Disorders. Front Psychiatry. 2018; 9:241. Epub 2018/07/03. https://doi.org/10.3389/fpsyt.2018.00241 PMID: 29962972.

43. Cho SY, Roh HT. Effects of aerobic exercise training on peripheral brain-derived neurotrophic factor and eotaxin-1 levels in obese young men. J Phys Ther Sci. 2016; 28(4):1355–8. Epub 2016/05/18. https://doi.org/10.1589/jpts.28.1355 PMID: 27190482.

44. Roh HT, Cho SY, So WY. A Cross-Sectional Study Evaluating the Effects of Resistance Exercise on Inflammation and Neurotrophic Factors in Elderly Women with Obesity. J Clin Med. 2020; 9(3). Epub 2020/04/05. https://doi.org/10.3390/jcm9030842 PMID: 32244926.

45. Choi KM, Kim JH, Cho GJ, Baik SH, Park HS, Kim SM. Effect of exercise training on plasma visfatin and eotaxin levels. Eur J Endocrinol. 2007; 157(4):437–42. Epub 2007/09/26. https://doi.org/10.1530/EJE-07-0127 PMID: 17893257.

46. Shephard RJ. Cytokine responses to physical activity, with particular reference to IL-6: sources, actions, and clinical implications. Crit Rev Immunol. 2002; 22(3):165–82. Epub 2002/12/25. PMID: 12498381.

47. Cesari M, Penninx BW, Pahor M, Lauretani F, Corsi AM, Rhys Williams G, et al. Inflammatory markers and physical performance in older persons: the InCHIANTI study. J Gerontol A Biol Sci Med Sci. 2004; 59(3):242–8. Epub 2004/03/20. https://doi.org/10.1093/gerona/59.3.m242 PMID: 15031308.

48. Taaffe DR, Harris TB, Ferrucci L, Rowe J, Seeman TE. Cross-sectional and prospective relationships of interleukin-6 and C-reactive protein with physical performance in elderly persons: MacArthur studies of successful aging. J Gerontol A Biol Sci Med Sci. 2000; 55(12):M709–15. Epub 2000/12/29. https://doi.org/10.1093/gerona/55.12.m709 PMID: 11129392.

49. Kandola A, Ashdown-Franks G, Hendrikse J, Sabiston CM, Stubbs B. Physical activity and depression: Towards understanding the antidepressant mechanisms of physical activity. Neurosci Biobehav Rev. 2019; 107:525–39. Epub 2019/10/06. https://doi.org/10.1016/j.neubiorev.2019.09.040 PMID: 31586447.

50. Steensberg A, Fischer CP, Keller C, Moller K, Pedersen BK. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. Am J Physiol Endocrinol Metab. 2003; 285(2):E433–7. Epub 2003/07/15. https://doi.org/10.1152/ajpendo.00074.2003 PMID: 12857678.

51. Cabral-Santos C, de Lima EA Junior, Fernandes I, Pinto RZ, Rosa-Neto JC, Bishop NC, et al. Interleukin-10 responses from acute exercise in healthy subjects: A systematic review. J Cell Physiol. 2019; 234(7):9956–65. Epub 2018/12/12. https://doi.org/10.1002/jcp.27920 PMID: 30536945.

52. Pereira Nunes Pinto AC, Natour J, de Moura Castro CH, Eloi M, Lombardi I Junior. Acute effect of a resistance exercise session on markers of cartilage breakdown and inflammation in women with rheumatoid arthritis. Int J Rheum Dis. 2017; 20(11):1704–13. Epub 2017/11/07. https://doi.org/10.1111/1756-185X.13204 PMID: 29105312.

53. Monteiro-Junior RS, de Tarso Maciel-Pinheiro P, da Matta Mello Portugal E, da Silva Figueiredo LF, Terra R, Carneiro LSF, et al. Effect of Exercise on Inflammatory Profile of Older Persons: Systematic Review and Meta-Analyses. J Phys Act Health. 2018; 15(1):64–71. Epub 2017/08/05. https://doi.org/10.1123/jpah.2016-0735 PMID: 28771081.

54. Jankord R, Jemiolo B. Influence of physical activity on serum IL-6 and IL-10 levels in healthy older men. Med Sci Sports Exerc. 2004; 36(6):960–4. Epub 2004/06/05. https://doi.org/10.1249/01.mss.0000128186.09416.18 PMID: 15179165.

55. Oberbach A, Lehmann S, Kirsch K, Krist J, Sonnabend M, Linke A, et al. Long-term exercise training decreases interleukin-6 (IL-6) serum levels in subjects with impaired glucose tolerance: effect of the -174G/C variant in IL-6 gene. Eur J Endocrinol. 2008; 159(2):129–36. Epub 2008/05/13. https://doi.org/10.1530/EJE-08-0220 PMID: 18469018.

56. Cole SW, Arevalo JM, Takahashi R, Sloan EK, Lutgendorf SK, Sood AK, et al. Computational identification of gene-social environment interaction at the human IL6 locus. Proc Natl Acad Sci U S A. 2010; 107(12):5681–6. Epub 2010/02/24. https://doi.org/10.1073/pnas.0911515107 PMID: 20176930.

57. Blankenberg S, Barbaux S, Tiret L. Adhesion molecules and atherosclerosis. Atherosclerosis. 2003; 170(2):191–203. Epub 2003/11/13. https://doi.org/10.1016/s0021-9150(03)00097-2 PMID: 14612198.

58. Muller N. The Role of Intercellular Adhesion Molecule-1 in the Pathogenesis of Psychiatric Disorders. Front Pharmacol. 2019; 10:1251. Epub 2019/12/12. https://doi.org/10.3389/fphar.2019.01251 PMID: 31824303.

59. Chen S, Luo D, Streit WJ, Harrison JK. TGF-beta1 upregulates CX3CR1 expression and inhibits fractalkine-stimulated signaling in rat microglia. J Neuroimmunol. 2002; 133(1–2):46–55. Epub 2002/11/26. https://doi.org/10.1016/s0165-5728(02)00354-5 PMID: 12446007.

60. Son SH, Park J, Jung MJ, Lee SK, Kim H, Kim KR, et al. Transforming growth factor-beta-regulated fractalkine as a marker of erosive bone invasion in oral squamous cell carcinoma. Eur J Oral Sci. 2021; 129(1):e12750. Epub 2021/01/28. https://doi.org/10.1111/eos.12750 PMID: 33503283.

61. Sciume G, Soriani A, Piccoli M, Frati L, Santoni A, Bernardini G. CX3CR1/CX3CL1 axis negatively controls glioma cell invasion and is modulated by transforming growth factor-beta1. Neuro Oncol. 2010; 12(7):701–10. Epub 2010/06/01. https://doi.org/10.1093/neuonc/nop076 PMID: 20511186.

62. Zhou X, Hu H, Balzar S, Trudeau JB, Wenzel SE. MAPK regulation of IL-4/IL-13 receptors contributes to the synergistic increase in CCL11/eotaxin-1 in response to TGF-beta1 and IL-13 in human airway fibroblasts. J Immunol. 2012; 188(12):6046–54. Epub 2012/05/11. https://doi.org/10.4049/jimmunol.1102760 PMID: 22573806.

63. Matsukura S, Odaka M, Kurokawa M, Kuga H, Homma T, Takeuchi H, et al. Transforming growth factor-beta stimulates the expression of eotaxin/CC chemokine ligand 11 and its promoter activity through binding site for nuclear factor-kappabeta in airway smooth muscle cells. Clin Exp Allergy. 2010; 40 (5):763–71. Epub 2010/03/11. https://doi.org/10.1111/j.1365-2222.2010.03474.x PMID: 20214667.

64. Saito A, Osuga Y, Yoshino O, Takamura M, Hirata T, Hirota Y, et al. TGF-beta1 induces proteinase-activated receptor 2 (PAR2) expression in endometriotic stromal cells and stimulates PAR2 activation-induced secretion of IL-6. Hum Reprod. 2011; 26(7):1892–8. Epub 2011/05/07. https://doi.org/10.1093/humrep/der125 PMID: 21546388.

65. Ganeshan K, Johnston LK, Bryce PJ. TGF-beta1 limits the onset of innate lung inflammation by promoting mast cell-derived IL-6. J Immunol. 2013; 190(11):5731–8. Epub 2013/05/01. https://doi.org/10.4049/jimmunol.1203362 PMID: 23630359.

66. Liton PB, Li G, Luna C, Gonzalez P, Epstein DL. Cross-talk between TGF-beta1 and IL-6 in human trabecular meshwork cells. Mol Vis. 2009; 15:326–34. Epub 2009/02/12. PMID: 19209241.

67. Franco L, Williams FM, Trofimov S, Surdulescu G, Spector T, Livshits G. Elevated plasma fractalkine levels are associated with higher levels of IL-6, Apo-B, LDL-C and insulin, but not with body composition in a large female twin sample. Metabolism. 2013; 62(8):1081–7. Epub 2013/03/13. https://doi.org/10.1016/j.metabol.2013.02.001 PMID: 23477808.

68. Ancuta P, Wang J, Gabuzda D. CD16+ monocytes produce IL-6, CCL2, and matrix metalloproteinase-9 upon interaction with CX3CL1-expressing endothelial cells. J Leukoc Biol. 2006; 80(5):1156–64. Epub 2006/10/24. https://doi.org/10.1189/jlb.0206125 PMID: 17056766.

69. Mathy NL, Scheuer W, Lanzendorfer M, Honold K, Ambrosius D, Norley S, et al. Interleukin-16 stimulates the expression and production of pro-inflammatory cytokines by human monocytes. Immunology. 2000; 100(1):63–9. Epub 2000/05/16. https://doi.org/10.1046/j.1365-2567.2000.00997.x PMID: 10809960.

70. Kaufmann J, Franke S, Kientsch-Engel R, Oelzner P, Hein G, Stein G. Correlation of circulating interleukin 16 with proinflammatory cytokines in patients with rheumatoid arthritis. Rheumatology (Oxford). 2001; 40(4):474–5. Epub 2001/04/20. https://doi.org/10.1093/rheumatology/40.4.474 PMID: 11312391.

71. Gronberg C, Asciutto G, Persson A, Fredrikson GN, Nilsson J, Goncalves I, et al. Endarterectomy patients with elevated levels of circulating IL-16 have fewer cardiovascular events during follow-up. Cytokine. 2016; 85:137–9. Epub 2016/06/28. https://doi.org/10.1016/j.cyto.2016.06.009 PMID: 27344024.

72. Gronberg C, Bengtsson E, Fredrikson GN, Nitulescu M, Asciutto G, Persson A, et al. Human Carotid Plaques With High Levels of Interleukin-16 Are Associated With Reduced Risk for Cardiovascular Events. Stroke. 2015; 46(10):2748–54. Epub 2015/09/04. https://doi.org/10.1161/STROKEAHA.115.009910 PMID: 26330445.

73. Braile M, Marcella S, Cristinziano L, Galdiero MR, Modestino L, Ferrara AL, et al. VEGF-A in Cardiomyocytes and Heart Diseases. Int J Mol Sci. 2020; 21(15). Epub 2020/07/30. https://doi.org/10.3390/ijms21155294 PMID: 32722551.

74. Scaldaferri F, Vetrano S, Sans M, Arena V, Straface G, Stigliano E, et al. VEGF-A links angiogenesis and inflammation in inflammatory bowel disease pathogenesis. Gastroenterology. 2009; 136(2):585–95 e5. Epub 2008/11/18. https://doi.org/10.1053/j.gastro.2008.09.064 PMID: 19013462.

75. Benhadou F, Glitzner E, Brisebarre A, Swedlund B, Song Y, Dubois C, et al. Epidermal autonomous VEGFA/Flt1/Nrp1 functions mediate psoriasis-like disease. Sci Adv. 2020; 6(2):eaax5849. Epub 2020/01/15. https://doi.org/10.1126/sciadv.aax5849 PMID: 31934626.

76. Rullman E, Rundqvist H, Wagsater D, Fischer H, Eriksson P, Sundberg CJ, et al. A single bout of exercise activates matrix metalloproteinase in human skeletal muscle. J Appl Physiol (1985). 2007; 102 (6):2346–51. Epub 2007/01/27. https://doi.org/10.1152/japplphysiol.00822.2006 PMID: 17255365.

77. Duggan C, Tapsoba Jde D, Wang CY, McTiernan A. Dietary Weight Loss and Exercise Effects on Serum Biomarkers of Angiogenesis in Overweight Postmenopausal Women: A Randomized Controlled Trial. Cancer Res. 2016; 76(14):4226–35. Epub 2016/07/16. https://doi.org/10.1158/0008-5472.CAN-16-0399 PMID: 27417562.

78. Berglind D, Nyberg G, Willmer M, Persson M, Wells M, Forsell Y. An eHealth program versus a standard care supervised health program and associated health outcomes in individuals with mobility disability: study protocol for a randomized controlled trial. Trials. 2018; 19(1):258. Epub 2018/04/29. https://doi.org/10.1186/s13063-018-2646-z PMID: 29703242.

79. Foster C, Richards J, Thorogood M, Hillsdon M. Remote and web 2.0 interventions for promoting physical activity. Cochrane Database Syst Rev. 2013; 9:CD010395. Epub 2013/10/03. https://doi.org/10.1002/14651858.CD010395.pub2 PMID: 24085594.

80. Haskell WL, Lee IM, Pate RR, Powell KE, Blair SN, Franklin BA, et al. Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. Med Sci Sports Exerc. 2007; 39(8):1423–34. Epub 2007/09/01. https://doi.org/10.1249/mss.0b013e3180616b27 PMID: 17762377.

81. Santos-Lozano A, Santin-Medeiros F, Cardon G, Torres-Luque G, Bailon R, Bergmeir C, et al. Actigraph GT3X: validation and determination of physical activity intensity cut points. Int J Sports Med. 2013; 34(11):975–82. Epub 2013/05/24. https://doi.org/10.1055/s-0033-1337945 PMID: 23700330.

82. Kyle UG, Genton L, Karsegard L, Slosman DO, Pichard C. Single prediction equation for bioelectrical impedance analysis in adults aged 20–94 years. Nutrition. 2001; 17(3):248–53. Epub 2001/04/20. https://doi.org/10.1016/s0899-9007(00)00553-0 PMID: 11312069.

83. Adamopoulos S, Parissis J, Kroupis C, Georgiadis M, Karatzas D, Karavolias G, et al. Physical training reduces peripheral markers of inflammation in patients with chronic heart failure. Eur Heart J. 2001; 22 (9):791–7. Epub 2001/05/15. https://doi.org/10.1053/euhj.2000.2285 PMID: 11350112.