Am J Transl Res 2014;6(5):422-438
www.ajtr.org /ISSN:1943-8141/AJTR0000794

Review Article

Exercise training and immune crosstalk in breast cancer microenvironment: exploring the paradigms of exercise-induced immune modulation and exercise-induced myokines

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Received May 14, 2014; Accepted June 16, 2014; Epub October 11, 2014; Published October 15, 2014

Abstract: Observational research suggests that exercise may reduce the risk of breast cancer and improve survival. One proposed mechanism for the protective effect of aerobic exercise related to cancer risk and outcomes, but has not been examined definitively, is the immune response to aerobic exercise. Two prevailing paradigms are proposed. The first considers the host immune response as modifiable by aerobic exercise training. This exercise-modulated immune-tumor crosstalk in the mammary microenvironment may alter the balance between tumor initiation and progression versus tumor suppression. The second paradigm considers the beneficial role of exercise-induced, skeletal muscle-derived cytokines, termed "myokines". These myokines exert endocrine-like effects on multiple organs, including the mammary glands. In this systematic review, we i) define the role of macrophages and T-cells in breast cancer initiation and progression; ii) address the two paradigms that support exercise-induced immunomodulation; iii) systematically assessed the literature for exercise intervention that assessed biomarkers relevant to both paradigms in human intervention trials of aerobic exercise training, in healthy women and women with breast cancer; iv) incorporated pre-clinical animal studies and non-RCTs for background discussion of putative mechanisms, through which aerobic exercise training modulates the immunological crosstalk, or the myokine-tumor interaction in the tumor microenvironment; and v) speculated on the potential biomarkers and mechanisms that define an exercise-induced, anti-tumor "signature", with a view toward developing relevant biomarkers for future aerobic exercise intervention trials.

Keywords: Exercise, breast cancer, immune, myokines, translational, immunotherapy

Introduction

Exercise training in women with early stage breast cancer, both during or following cancer treatment, enhances aerobic capacity, muscular strength and physical function, as well as improves quality of life, anxiety and depression [1]. There is now substantial observational evidence that regular participation in moderate-to-vigorous intensity physical activity reduces risk of developing postmenopausal breast cancer [2] and improves survival following a breast cancer diagnosis [3-5]. Several mechanisms for this protective effect have been proposed including alterations in sex hormones, metabolic hormones, DNA repair capacity and systemic low-grade inflammation [6, 7]. However, one proposed mechanism that has received less attention is host immune function.

The potential impact of exercise on the immune system has been examined related to both athletic performance [8] and risk of illness, such as increased risk of upper respiratory track infections in athletes with a heavy volume of training [9, 10]. More recently, there has been interest in the potential role of exercise in mitigating chronic disease outcomes that have an immune or inflammatory component, such as to prevent and reduce the risk of a breast cancer recurrence [11].

Although research to date support a modulatory role of exercise and physical activity on

immune function, the specific mechanisms relating exercise-induced immune responses to cancer outcomes in humans remains to be elucidated. Furthermore, the novel concept of skeletal muscle as an endocrine organ [12] has received substantial attention in muscle physiology research in the past decade. Bente Klarlund Pedersen and colleagues have conceptualized and demonstrated how contracting skeletal muscle is capable of secreting muscle-derived cytokines, which they termed "myokines". These myokines exerted endocrine-like functions in other tissues and organs, including the adipose tissue, colon liver, and brain. The extensive body of work by Pedersen's group as well as others [13-18], suggest that exercise may modulate the host immune response *via* skeletal muscle-organ crosstalk.

In this review, we will: i) define the prognostic role of macrophages and T-cells in breast cancer; ii) outline two proposed paradigms that support exercise-induced immunomodulation; iii) systematically review the literature for human exercise intervention that assessed biomarkers for breast cancer risk and survival; iv) incorporate pre-clinical animal studies and non-RCTs to discuss putative mechanisms through which exercise training modulates the immunological crosstalk, or the myokine-tumor interaction in the tumor microenvironment; and v) speculate on the potential biomarkers and mechanisms that define an exercise-induced, anti-tumor "signature", with a view toward developing relevant biomarkers for future exercise intervention trials.

The role of the immune system in breast cancer and potential immune biomarkers

The immune system has a Janus-like duality in breast cancer; on the one hand, various facets of the immune system become co-opted to support tumor growth. Conversely, the immune system remains capable of mounting an anti-tumor response against breast cancer cells. The balance between immunosuppression which allows tumor growth, versus immune rejection of neoplastic cells which limits tumor growth depends on the type of, and activation state of the leukocytes, as well as their crosstalk with other stromal cells and tumor cells in the tumor microenvironment [19]. This review will not cover all the immune cells involved in the cancer immunology literature, but rather focus on the role of macrophages and T cells in mediating tumor response and identified inflammatory biomarkers of breast cancer risk.

Macrophages

Tissue macrophages originate from circulating monocytes, and are found in large abundance in the breast tumor microenvironment; a trait associated with poor prognosis in cancer patients [20]. Specifically, a 77% increased risk of mortality was associated with increased double immunostaining of cluster of differentiation (CD)68^+ and proliferating cell nuclear antigen $(PCNA)^+$ macrophages in human breast adenocarcinomas [20]. These tumor-associated macrophages (TAMs) represent the largest proportion of leukocytes in the breast tumor microenvironment and are attractive targets for immunotherapy.

TAMs have been broadly classified into two different subsets, the M1-polarized macrophages, and the M2-polarized macrophages [21]. M1 macrophages have been termed classically activated macrophages, in that they are activated by T-helper (Th)1-type cytokines, such as interferon (IFN)-γ as well as molecules associated with pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) [22]. DAMPs, such as high mobility group box protein (HMGB)-1 which is secreted extracellularly, stimulates the production of IFN-γ from natural killer (NK) cells, with potential implications for tumor death [23].

Some notable characteristics of M1 macrophages include low expression of Th2-related cytokines, such as interleukin (IL)-4 and IL-10, and high expression of Th1-related cytokines such as IL-12, tumor necrosis factor (TNF)-α and IL-6 [19]. In addition, M1 macrophages are able to induce tumor destruction *in vitro*. C57BL/6 female mice bearing the Lewis Lung Carcinoma (3LLC) treated with microspheres containing IL-12 had significantly reduced primary tumor mass compared with placebo treated controls [24]. IL-12 can activate tumoricidal NK cells [25], as well as activate Th1 cells to mediate tumor killing [26]. IL-12 also induces the extracellular secretion of IL-15 from TAMs. Importantly, the IL-12-mediated cytotoxic activity of M1 macrophages appeared to be dependent on the presence of IL-15, where prior blockade with IL-15 antibody resulted in an

attenuated effect on shrinking primary tumor mass.

The utility of IL-12 and IL-15 as anti-tumor therapeutics has been documented in other preclinical models. For instance, intraperitoneal injection of IL-12 into AB6F1 mice bearing Sa1 ascites was successful in clearing tumors in 40% of the mice [27]. As well, the IL-12 treatment resulted in massive macrophage infiltration in the Sa1 ascites, comprising up to 75% of the total cell population, compared with 9%, in untreated conditions. Comes *et al.* [28] reported a synergistic anti-tumor effect of combining IL-12 and IL-15. In this study, N592 small cell lung cancer cells were transfected with plasmid vectors carrying IL-12, IL-15 or both, and subsequently implanted in female athymic CD1 mice. The investigators found that N592 cells that produced only either IL-12 or IL-15 had either modest or significant effects on attenuating tumor growth *in vivo*, whereas mice implanted with N592 cells expressing both cytokines had complete abrogation of tumor growth. As well, immunohistochemical analyses revealed that mice implanted with the combined treatment had higher expression of inducible nitric oxide (iNOS) synthase, TNF-α and IFN-γ in resected tumor tissue. These molecular characteristics suggest that the macrophage subsets in these IL-12/IL-15-treated mice belong to the M1 phenotype.

M2 macrophages are myeloid cells belonging to the opposite extreme of polarized TAMs. They are activated by typical Th2-type cytokines such as IL-4, IL-10 and IL-13 and participate in tumor angiogenesis [29], extra-cellular matrix (ECM) degradation and invasion into stromal parenchyma, metastasis, and recruitment of immunosuppressive T cells [30]. Furthermore, an increased M1 compared to M2 population in the tumor microenvironment may reflect better prognosis, at least in non-small cell lung cancer [31, 32]. Tumors resected from patients showed an association between increased median survival times (92.7 months versus 7.7 months) and high percentages of M1 macrophages in the tumor islets (>75% versus <5%) [32].

T-cells

T-cells are generally divided into $CD4^+$ T helper (Th), $CD8^+$ cytotoxic T-cells and regulatory T cells (Tregs). The increased presence of infiltrating T lymphocytes, especially $CD8^+$ T-cells, is associated with a favorable prognosis. For instance, BALB/c mice were injected with lung alveolar carcinoma cell line-1 and then treated with IL-12 and a NO inhibitor (N-nitro-L-arginine methyl ester; L-NAME) [33]. Treatment of these mice resulted in enhanced viability and proliferation of $CD8^+$ T-cells, compared with treating with IL-12 alone. In another study [34], adoptive transfer of $CD8^+$ T-cells was effective in mediating tumor regression in mice implanted with B16 melanoma cells.

Clinical studies in humans have also shown positive correlations between intratumoral T-cell infiltration and favorable prognosis in breast cancer treatment [35-37]. A pathologic complete response to neoadjuvant treatment in breast cancer patients was associated with greater immunohistochemical staining of $CD3^+$, $CD8^+$ and forkhead box (FOX) P3 T-cells in resected tumor samples obtained at diagnosis [35]. In another study [36], increased concentration of $CD3^+CD4^+$ or $CD8^+CD28^+$ cells in peripheral blood was associated with improved survivorship for patients with metastatic breast cancer, whereas patients with increased blood concentration of $CD8^+CD28^-$ cells was associated with shorter survival time. Interestingly, patients with greater number of $CD8^+CD28^-$ cells in peripheral blood also presented with decreased IFN-γ. This suggests that IFN-γ-mediated tumor destruction depends on the co-stimulatory signals of CD28 for $CD8^+$ T-cell activation.

Immune biomarkers of breast cancer prognosis in peripheral blood and tumor microenvironment

The oncology community routinely samples peripheral blood to obtain a snapshot of general immune function of the cancer patient in order to monitor if the patient can maintain the planned chemotherapy schedule. However, the circulating leukocytes at the time of treatment may also provide an idea of activation state of the immune cells that are recruited to eliminate the tumor and in turn serve as blood-borne biomarker of tumor progression. Breast cancer patients have been shown to demonstrate a higher concentration of peripheral blood $CD14^+$ $CD16^+$ monocytes compared with healthy controls, with the number of the pro-inflammatory

$CD16^+$ monocytes correlating negatively with tumor size and early stage tumor growth [38]. The intriguing findings suggest that this specific subset of monocytes represent an early recruitment of pro-inflammatory monocytes into the tumor microenvironment. Furthermore, this monocyte subset becomes less frequent as the tumor progresses, implying its potential utility in early diagnosis. $CD14^+CD16^+$ monocytes are also found in the tumor tissue, which raises the question if these are the same population of monocytes that will become polarized into M1 and M2 macrophages? While the answer is presently unknown, this represents a relevant paradigm to explore in future pre-clinical and clinical studies.

In addition to monocytes subsets as potential biomarkers in breast cancer, T-cell subsets such as $CD3^+CD8^+CD28^+$ cells may be useful prognosticators of outcomes, such as responsiveness to therapy and likelihood of residual disease and recurrence. In addition, the intracellular and extracellular gene and protein expression of cytokines associated with T-cell subsets as well as monocytes/macrophages can be further quantitated to give an indication of the molecular signatures associated with disease progress. These cytokines and chemokines include the Th-1/Th-2 or M1/M2 related cytokines such as IFN-γ, TNF-α, transforming growth factor (TGF)-β, IL-4, IL-6, IL-10, IL-12, IL-13, and IL-15.

Analyzing peripheral blood leukocytes for subset differences, as well as gene and protein expression may account for their functional status, but may miss a complete picture of how these leukocytes interact with the tumors within the microenvironment. Combining both the blood-borne and intratumoral panel from tissue collected through needle biopsy, to examine plausible biomarkers will be more useful than either alone, in determining immune response during cancer treatment.

Exercise modulates immune function and inflammation

Currently, there are two intriguing concepts in the areas of exercise and the immune response that have potential implications in cancer biology. One concept deals with the host immune response to exercise, specifically, changes in functional status of cells of the immune system, such as monocytes and T-cells. The second concept is that cytokine production is not restricted to immune cells alone; that other organs, such as skeletal muscle, can be induced to secrete "myokines" during exercise. The two concepts remain disparate areas of inquiry, despite potential overlap. Thus, there is a current knowledge gap which needs to be addressed: do the "myokines" crosstalk with the immune system, and do they contribute to the protective effects of exercise that is consistently reported in epidemiological studies?

Cancer has been described as an inflammatory disease [39], in that the immune cells found within the tumor microenvironment promote a feedback loop of pro-inflammatory signaling *via* the up-regulation and production of specific cytokines and chemokines. These inflammatory signals further recruit other leukocytes that can be immunosuppressive. The protective effect of exercise may be attributed to its ability to modulate the host immune response, with direct effects on the immune-tumor cross-talk, or *via* the mobilization of myokines from contracting skeletal muscle, which indirectly influences cancer *via* anti-inflammatory and metabolic pathways.

Acute aerobic exercise influences monocyte characteristics in humans

The effects of exercise on the biological functions of human monocytes have been investigated. In general, the type, intensity and duration of exercise appear to play a role in modulating the behavior of human monocytes. Acute aerobic exercise resulted in increased: i) monocyte mobilization [40-43], ii) insulin binding by monocytes [8], and iii) density of beta-adrenergic receptors on monocyte surfaces [44]. However, acute prolonged cycling (4 h at 70% of anaerobic threshold) did not result in changes in either oxidative burst or phagocytosis of monocytes [45]. Monocyte subsets also appeared to be altered differentially by the duration of acute aerobic exercise, with a bout of exhaustive exercise of short duration (approx. 87 min) increasing $CD14^+$ bright and $CD14^+$ low cell counts, whereas a bout of exhaustive exercise of long duration (100 km, 4 h) resulted in an increase in CD14 $bright^+$ and a decrease in $CD14^+$ low cells [46]. A seminal study by Steppich *et al*. [47] showed that $CD14^+CD16^+$ monocytes were mobilized after a short bout of

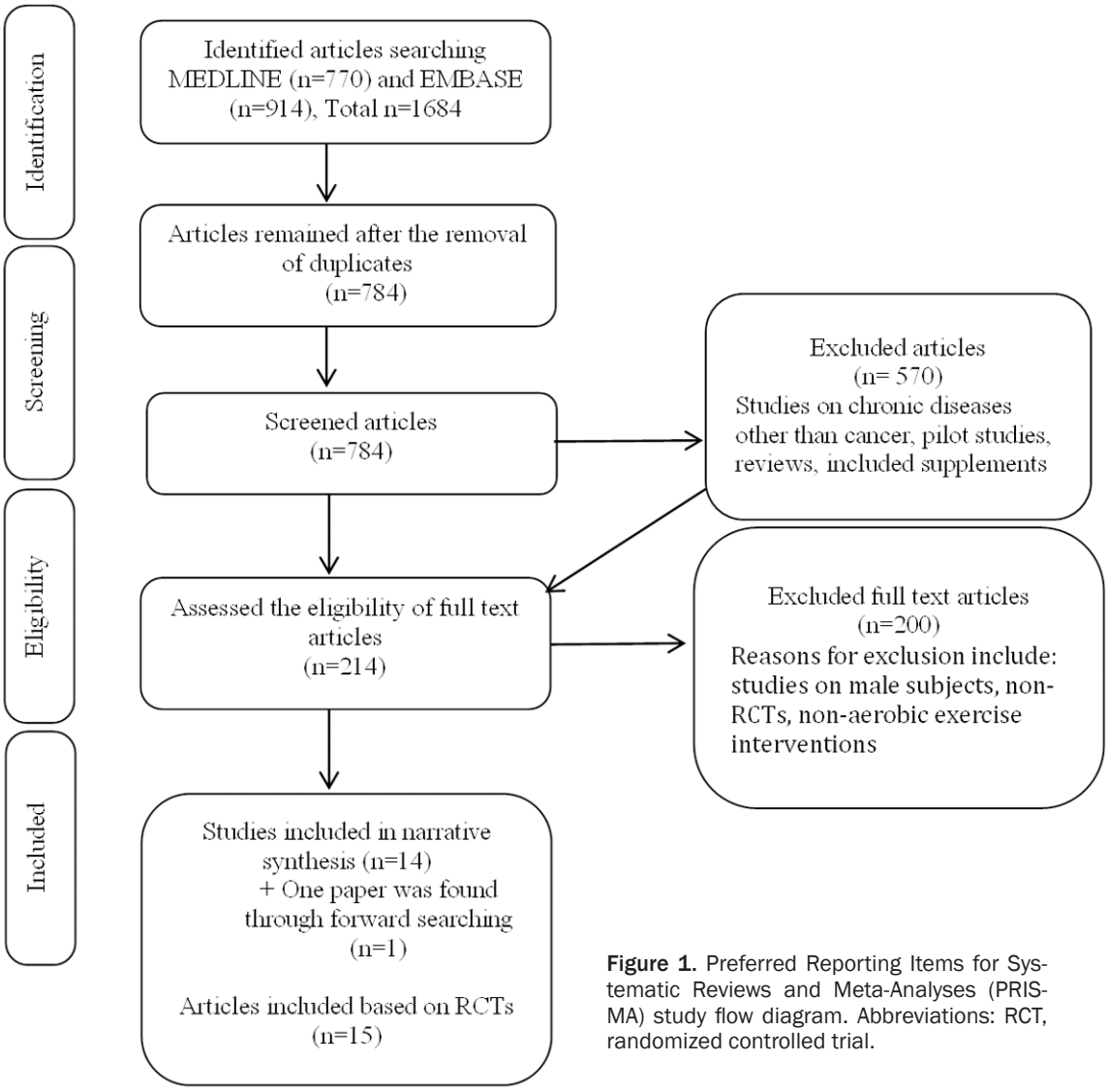

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) study flow diagram. Abbreviations: RCT, randomized controlled trial.

cycling (2 min at 400 W) above anaerobic threshold, increasing by almost 2-fold compared with pre-exercise values.

The production of different cytokines and chemokines by monocytes is instrumental in recruiting other immune cells, as well as contributing to antigen presentation and stimulation of cellular cytotoxicity against malignant cells. In addition, the cytokine profile may reflect the polarization state of macrophage and T cells. In this regard, a brief 30-minute bout of cycling was able to induce the up-regulation of TNF-α, IL-4 and IL-6 in Th cells, IFN-γ, and IL-4 in monocytes, as determined by flow cytometry [48]. The results of this study suggested that a short acute bout of aerobic exercise (30 min at 80% VO_{2max}) does not necessarily skew the immune profile towards either a Th1/M1 or Th2/M2 state, given that both polarized states are extremes in the continuum. Instead, it seems that acute aerobic exercise may up-regulate both pro-and anti-inflammatory immune mediators, which can alert and activate the leukocytes to cellular stress and danger.

Exercise training status is related to monocytes subset and cytokine profile in humans

Training status may influence the functionality of monocytes. CD64 expression is relevant to IgG binding and phagocytosis, and its surface expression on monocytes was reported to be

Table 1. Results of systematic review of literature of aerobic exercise on outcomes of interest by group

Author, Year	Sample size	Participant characteristics Age: (yrs) BMI (kg/m^2) Mean (SD)	Intervention	Outcome measure	Pre-intervention Mean (SD) or (95% confidence interval)	Post-intervention Mean (SD) or (95% confidence interval)	Pre-from post-intervention (Mean change)	Source of blood, Assay
Breast Cancer Survivors								
Fairey, 2005	n=53	Age: Ex: 59 (5) Con: 58 (6) BMI: Ex: 29.4 (7.4) Con: 29. (6.1)	15 wks, 3 x/wk (supervised), 70-75% VO_{2max}, 15 min for wk 1-3, increased by 5 min every 3 wk and 35 min for wk 13-15	NK cell cytotoxic activity	Ex: 55.5 (12.1) Con: 58.0 (12.9)	Ex: 61.4 (9.8) Con: 56.4 (10.5)	Ex: 5.9** Con: -1.6	Peripheral blood mononuclear cells, ELISA
				Spontaneous ([3H]-thymidine dpm × 10^6 cells)	Ex: 863 (425) Con: 776 (417)	Ex: 1.042 (290) Con: 811 (247)	Ex: 179** Con: 35	
				CRP (mg/L)	Ex: 5.19 (3.56) Con: 4.28 (3.05)	Ex: 3.79 (2.30) Con: 4.39 (3.87)	Ex: -1.4 Con: 0.11	
Payne, 2008	n=20	Age: All: 64.7 (6.3) BMI: NR	14 wks, 4 x/wk, moderate walking activity 20 min/session	IL-6 Range: 2-29 (ng/ml)				Serum IL-6, NR
Nieman, 1994	n=12	Age: Ex: 60.8 (4.0) Con: 51.2 (4.7) Weight: (Kg) Ex: 67.6 (3.7) Con: 75.5 (9.8)	8 wks, 3 x /wk (supervised) 75% HR_{max}, 60 min/session	NK cells- (10^9/1)	Ex: 0.3 (0.1) Con: 0.2 (0.1)	Ex: 0.3 (0.1) Con: 0.2 (0.1)	Ex: 0 Con: 0	Whole blood, chromium release assay for all measures
				Lymphocyte (10^9/1)	Ex: 1.4 (0.2) Con: 1.4 (0.2)	Ex: 1.1 (0.2) Con: 1.6 (0.3)	Ex: -0.3 Con: 0.2	
				Neutrophil (10^9/1)	Ex: 3.7 (0.3) Con: 3.8 (0.7)	Ex: 3.0 (0.4) Con: 3.9 (0.8)	Ex: -0.7 Con: 0.1	
				Total leukocytes (10^9/1)	Ex: 5.7 (0.3) Con: 5.9 (0.9)	Ex: 4.9 (0.4) Con: 6.1 (0.9)	Ex: -0.8 Con: 0.2	
Sprod, 2012	n=21	Age: TCC: 54.3 (3.6) SST: 52.7 (2.1) BMI: Ex: 24.9 (1.9) Con: 25.0 (1.4)	12 wks, 3 times/wk (supervised), Intensity>NA, 60 min/session	IL-6 (pg/mL)	Ex: 2.63 (1.32) Con: 2.44 (0.56)	Ex: 4.63 (2.32) Con: 2.42 (0.55)	Ex: 2 Con: -0.01	Serum, ELISA
				IL-8 (pg/mL)	Ex: 9.37 (1.80) Con: 11.06 (2.57)	Ex: 9.69 (2.05) Con: 7.24 (1.93)	Ex: 0.32 Con: -3.82	
Jones, 2012	n=115	Age: Ex: 60.5 (7.0) Con: 60.9 (6.8) BMI: Ex: 30.2 (4.0) Con: 30.4 (3.8)	24 wks, 5 days/wk, 3 days/wk (supervised), 2 days/wk (home-based), 60-75% HR max, at least 45 min by eight wk of trial.	IL-6 (pg/mL) CRP (mg/L)	Ex: 3.55 (6.29) Con: 1.91 (1.01)	Ex: 3.59 (6.03) Con: 1.91 (1.19)	Ex: 0.04 Con: 0	Serum, ELISA
					Ex: 2.47 (2.35) Con: 2.43 (2.55)	Ex: 2.39 (2.26) Con: 2.23 (2.60)	Ex: -0.08 Con: -0.2	
				TNF-α (pg/mL)	Ex: 1.15 (0.52) Con: 1.28 (0.60)	Ex: 1.17 (0.40) Con: 1.35 (0.63)	Ex: 0.02 Con: 0.08	
Ergun, 2013	n=40	Age: Ex: 49.7 (8.3) Con: 50.3 (10.4) BMI: Ex: 26.6 (4.4) Con: 28.6 (5.1)	12 wks, 3 days/wk, 45 min/day and brisk walking for 30 min/day	IL-6	Ex: 3.30 (2.18) Con: 2.23 (1.84)	Ex: 2.89 (1.89) Con: 2.18 (1.92)	Ex: 0.7 Con: -0.05	Serum and whole blood, ELISA
				IL-8	Ex: 10.37 (3.60) Con: 9.99 (3.70)	Ex: 7.76 (3.10) Con: 8.68 (3.33)	Ex: -2.61* Con: -1.31	
				TNF-α	Ex: 11.12 (3.25) Con: 11.99 (5.74)	Ex: 11.85 (4.13) Con: 12.98 (5.11)	Ex: 0.73 Con: 0.99	
				RANTES	Ex: 169.30 (27.14) Con: 167.36 (25.89)	Ex: 161.47 (30.77) Con: 169.05 (25.64)	Ex: -7.89 Con: 1.69	
				MCP-1	Ex: 20.05 (6.05) Con: 19.60 (6.79)	Ex: 20.64 (8.75) Con: 22.98 (10.15)	Ex: 0.59 Con: 3.38*	

Premenopausal women								
Arikawa, 2011	n=319	Age: Ex: 25.2 (3.4) Con: 25.2 (3.5) BMI: <25: 101 (66%) 25-30: 30 (19.6%) >30: 22 (14.4%)	16 wks, 5 days/wk (supervised), 65%-70>85% HR_{max}, 45 min/session	CRP ($mg.L^{-1}$)	Ex (162): 5.02 (4.17-6.03) Con (149): 3.94 (3.25-4.78)	Ex: 4.32 (3.60-5.19) Con: 3.90 (3.22-4.73)	Ex: -0.7** Con: -0.04	Plasma, Multiplex Bead based Assay
				SAA ($mg.L^{-1}$)	Ex: 4.60 (3.83-5.53) Con: 3.56 (2.94-4.32)	Ex: 4.04 (3.42-4.78) Con: 3.72 (3.12-4.44)	Ex: -0.56 Con: 0.16	
Horne, 1997	n=9	Age: All: 22.3 (3.3) Weight: 73.4 (11.6) Kg	12 wks, 3 times/wk (supervised), 90% of VO_{2max}, 30-42 min/session	TNFα (pg/ml)	Ex: 5.6 (3.7) Con: NR	Ex: 17.6 (6.4) Con: 11.2 (15.9)	Ex: 12** Con: NA	Whole blood, ELISA
Jimenez, 2007	n=8	Age: All: 22.3 (3.3) Weight: 73.4 (11.6) Kg	One session of acute exercise, 65% VO_{2max}, 2 hours	Leukocyte ($10^3.mL^{-1}$)	Ex: 0 Con: 4.4 (0.4) SEM	Ex: -1.6 (1.1) Con: 5.1 (0.5)	Ex: -1.6** Con: 0.7	Whole blood, Assay (leukocytes) and culture supernatant (cytokines)
				Lymphocyte ($10^3.mL^{-1}$)	Ex: 1.8 (0.2) SEM Con: 1.5 (0.2)	Ex: 1.7 (0.1) Con: 1.7 (0.1)	Ex: 0.1** Con: 0.2	
				LPS-stimulated ΔTNF-α (%)	Ex: 0 Con: 0	Ex: -38 (6)% SEM Con: 10	Ex: -38** Con: 10	
				Monocyte ($10^3.mL^{-1}$)	Ex: 0.39 (0.04) SEM Con: 0.36 (0.03) SEM	Ex: 0.58 (0.05) Con: 0.35 (0.03)	Ex: 0.19** Con: 0.01	
				LPS-stimulated ΔIL-10 (%)	Ex: 0 Con: 0	Ex: 79 (19) SEM Con: 0	Ex: 79 Con: 0	
Nehlsen-Cannarella, 1990	n=36	Age: Ex: 36 (1.6) Con: 32.8 (1.4) BMI: Ex: 28.3 (0.7) Con: 27.8 (0.8)	15 wk, 5 days/wk (supervised), 60% HR reserve, 45 min/session	Total Leukocyte ($10^9.l^{-1}$)	Ex: 6.03 (0.23) (SE) Con: 5.96 (0.39)	Ex: 6.38 (0.31) Con: 6.79 (0.33)	Ex: 0.35 Con: 0.83	Whole blood, lymphocyte subsets
				Lymphocyte ($10^9.l^{-1}$)	Ex: 2.40 (0.17) (SE) Con: 2.17 (0.12)	Ex: 2.24 (0.16) Con: 2.35 (0.13)	Ex: -0.16** Con: 0.18	
				T cells (CD5) ($10^9.l^{-1}$)	Ex: 1.87 (0.14) (SE) Con: 1.73 (0.1)	Ex: 1.71 (0.12) Con: 1.84 (0.1)	Ex: -0.16** Con: 0.11	
				B cells (CD20) ($10^9.l^{-1}$)	Ex: 0.274 (0.03) (SE) Con: 0.157 (0.02)	Ex: 0.286 (0.041) Con: 0.306 (0.025)	Ex: 0.012** Con: 0.149	
				T helper cells (CD4) ($10^9.l^{-1}$)	Ex: 1.22 (0.1) (SE) Con: 1.09 (0.08)	Ex: 1.10 (0.07) Con: 1.13 (0.06)	Ex: -0.12 Con: 0.4	
				T cytotoxic (CD8) ($10^9.l^{-1}$)	Ex: 0.684 (0.053) (SE) Con: 0.698 (0.050)	Ex: 0.614 (0.047) Con: 0.712 (0.067)	Ex: 0.561 Con: 0.067	
Postmenopausal women								
Campbell, K.L. 2008	n=100	Age: Ex: 54.4 (7.1) Con: 53.7 (5.6) BMI: Ex: 28.9 (5.5) Con: 28.5 (4.8)	12 months, 6 d/wk, at least 3 days/wk (supervised) and home-based sessions 60-85% of HR_{max}, 60 min/day	CRP (mg/L)	Ex: 2.08 (1.48-2.91) Con: 2.16 (1.57-2.97)	Ex: 1.87 (1.35-2.59) Con: 2.16 (1.57-2.98)	Ex: -0.21 Con: 0	Plasma, Latex-enhanced nephelometry by high-sensitivity assays

Friedenreich, 2012	n=320	Age: Ex: 61.2 (5.4) Con: 60.6 (5.7) BMI: Ex: 61.2 (5.4) Con: 60.6 (5.7)	12 months, 3 days/wk (supervised), 2 days/wk (home-based), 70%-80% heart rate reserve, 45 min/session	CRP (mg/L)	Ex: 1.4 (1.2-1.6) Con: 1.3 (1.1-1.5)	Ex: 1.1 (1.0-1.3) Con: 1.3 (1.1-1.5)	Ex: -0.3** Con: 0	Serum, ELISA (IL-6), Solid-phase chemiluminescent immunometric assay (TNF-α)
				IL-6 (pg/mL)	Ex: 1.5 (1.4-1.6) Con: 1.4 (1.4-1.5)	Ex: 1.4 (1.3-1.5) Con: 1.4 (1.3-1.4)	Ex: -0.1 Con: 0	
				TNFα (pg/mL)	Ex: 1.5 (1.4-1.6) Con: 1.4 (1.4-1.5)	Ex: 1.4 (1.3-1.5) Con: 1.4 (1.3-1.4)	Ex: -0.1 Con: 0	
Lee, 2012	n=22	Age: Con: 38.3 (4.9) LI: 41.6 (4.5) HI: 41.7 (4.3) BMI: Con: 27.3 (2.7) LI: 27.4 (2.7) HI: 25.4 (2.7)	14 wks, LI and HI: 3 x/wk (wk 1-4), 4 x/wk (5-9 wk), 5 x/wk (10-14) (supervised) 50-70% max O_2 consumption, Duration of each exercise at the predetermined exercise intensity was calculated based on body weight	IL-6 ($pg{\cdot}mL^{-1}$) CRP ($mg{\cdot}dL^{-1}$)	Con: 0.73 (0.81) LI: 0.70 (0.49) HI: 0.86 (1.03) Con: 0.10 (0.06) LI: 0.12 (0.15) HI: 0.16 (0.14)	Con: 0.95 (0.60) LI: 0.72 (0.62) HI: 0.71 (0.69) Con: 0.06 (0.03) LI: 0.10 (0.10) HI: 0.06 (0.03)	Con: 22 LI: 0.2 HI: -0.15 Con: -0.04 LI: -0.02 HI: -0.1	Serum, ELISA
				TNFα ($pg{\cdot}mL^{-1}$)	Con: 1.32 (0.20) LI: 1.32 (0.32) HI: 1.30 (0.35)	Con: 2.05 (0.90) LI: 1.79 (0.58) HI: 2.04 (0.85)	Con: 0.73 LI: 0.47 HI: 0.74	
Campbell, P.T, 2008	n=115	Age: Ex: 60.5 (7.0) Con: 60.9 (6.8) BMI: Ex: 30.2 (4.0) Con: 30.4 (3.8)	48 wks, 5 days/wk, 3 days/wk (supervised), 2 days/wk (home-based), 60-75% HR_{max}, at least 45 min by eight wk of trial.	CRP ($mg{\cdot}L^{-1}$)	Ex: 2.39 (1.85-3.10) Con: 2.36 (1.88-2.97)	Ex: 2.15 (1.66-2.78) Con: 2.65 (2.09-3.36)	Ex: -0.24** Con: 0.2	Serum, ELISA
				SAA ($mg{\cdot}L^{-1}$)	Ex: 4.87 (4.04-5.87) Con: 5.11 (4.43-5.90)	Ex: 4.57 (3.81-5.48) Con: 5.29 (4.55-6.16)	Ex: -0.3 Con: 0.18	
				IL-6 ($mg{\cdot}L^{-1}$)	Ex: 2.36 (1.82-3.05) Con: 2.26 (1.80-2.83)	Ex: 2.66 (2.02-3.52) Con: 2.36 (1.88-2.96)	Ex: 0.3 Con: 0.1	
Imayama, 2012	n=204^	Age: Ex: 58.1 (5.0) Con: 57.4 (4.4) BMI: Ex: 30.7 (3.7) Con: 30.7 (3.9)	48 wks, 5 days/wk, 3 days/wk (supervised), 2 days/wk (home-based), 70-85% HR_{max}, at least 45 min by seventh wk of trial.	Hs-CRP (mg/L)	Ex: 2.48 (2.00-3.06) Con: 1.90 (1.50-2.40)	Ex: 2.46 (2.23-2.72) Con: 2.06 (1.84-2.30)	Ex: -0.02 Con: 0.16	Serum, ELISA
				SAA, (mg/L)	Ex: 5.17 (4.47-5.99) Con: 5.20 (4.42-6.11)	Ex: 5.86 (5.43-6.31) Con: 5.21 (4.79-5.66)	Ex: 0.69 Con: 0.01	
				IL-6, (pg/mL)	Ex: 1.47 (1.30-1.66) Con: 1.43 (1.25-1.64)	Ex: 1.57 (1.48-1.65) Con: 1.60 (1.50-1.69)	Ex: 0.1 Con: 0.17	
				Leukocytes ($\times 10^9$/L)	Ex: 5.54 (5.26-5.83) Con: 5.39 (5.10-5.71)	Ex: 5.47 (5.34-5.60) Con: 5.36 (5.12-5.50)	Ex: -0.07 Con: -0.03	

Legend: ^, a 4-arm RCT (n=438) but we have not reported the results of the dietary weight loss arm (n=118) or the combined dietary weight loss and exercise arm (n=116). *Statistically significant in the specified group compared to its baseline. **Statistically significant in the exercise group compared to control. Abbreviations: Ex, Exercise intervention arm; Con, Control arm; RANTES, Regulated upon Activation Normal T-cell Expressed and presumably Secreted; MCP, Monocyte chemotactic protein; HI, High intensity; LI, Low intensity; ELISA, enzyme-linked immunosorbent assay; NR, Not reported.

higher in endurance athletes than sedentary controls [49]. In a combined endurance and resistance training intervention in elderly men and women, a significant decrease in the inflammatory $CD14^+CD16^+$ monocyte subsets was observed, as well as lower endotoxin-stimulated TNF-α production *in vitro* [50]. This finding supports the anti-inflammatory effects mediated by regular exercise, given that the $CD14^+CD16^+$ monocyte subset is related to a pro-inflammatory profile as well as an early cancer stage [38].

In a more clinically relevant study [51], breast cancer survivors responded to 5 weeks of cycle ergometry training (5 days per week, for 30-40 min per session at 60% of heart rate reserve) by exhibiting a decrease in circulating concentrations of monocytes at rest. Functionally, exercise training showed a divergent effect on phagocytosis, depending on whether the stimulus used was enzyme treated sheep red blood cells (RBCs) or Anti-D loaded human RBCs [51]. In a different study, breast cancer survivors were randomized into a control or a combined aerobic and strength training program (3 days per week, 90 min per session) for 2 months [52]. Peripheral blood concentrations of the immune-related cytokines such as TNF-α, IFN-γ, IL-1α, IL4, IL-6, IL-10, IL-12, IL-13, IL15, as well as chemokines such as monocyte chemotactic protein (MCP)-1, and stromal derived factor (SDF)-1α were unchanged after the exercise training program.

Exercise affects tissue macrophage behavior in pre-clinical mouse studies

To date, no exercise studies in cancer survivors have reported on both human tissues and blood markers collected before and after an intervention. Therefore, pre-clinical studies, predominantly in animal models, that are relevant to exercise-induced immunomodulation in cancer provide some insight. Similar to studies performed on human monocytes, acute exercise, such as a single bout of exhaustive treadmill running, inhibited sarcoma cell growth *in vitro* by peritoneal macrophages of NMRI mice [53]. As well, short-term (several days) exercise training studies in mice of various genetic backgrounds demonstrated an increased *in vitro* cytotoxic effect of peritoneal macrophages against adenocarcinoma cells [54, 55].

Exercise training for cancer prevention may involve a shift in macrophage polarization in the tumor microenvironment [56]. The cytokines induced by exercise are frequently associated with M1 or Th1 cells. A previous study [57] showed that MHC II^{high} tumor-associated macrophages appeared in the tumor microenvironment during the early stages of the disease. These cells also expressed genes that are synonymous with the M1 macrophage phenotype (IL-6, IL-12, iNOS). In contrast, MHC II^{low} tumor-associated macrophages were found during the later stages of tumor progression, and expressed genes that are associated with the M2 macrophage phenotype (Ym1, fizz1, Arg1, IL-10, TGF-beta).

T-cells

A bout of acute treadmill running (45min at 80% VO_{2max}) increased relative changes in $CD8^+$ cells in young, healthy and endurance-trained human volunteers, resulting in a decline in the $CD4^+/CD8^+$ cell ratio in peripheral blood [58]. Comparatively, 3 months of aerobic training with cycle ergometry did not result in differences in phytohemagglutin (PHA)- or pokeweed mitogen (PMA)-stimulated T-cell proliferation in healthy young men [59].

Well-trained athletes provide a useful benchmark with which to compare exercise-induced changes in immune function from the normal, sedentary population. It was reported recently that compared with sedentary individuals, peripheral blood obtained under resting conditions from endurance-trained athletes presented with higher concentrations of IL-10, as well as a greater population of $CD4^+CD25^+CD127^{low}$ Tregs [60]. It is possible that prolonged periods of intensive endurance training up-regulates an anti-inflammatory profile in order to suppress pro-inflammatory signalling due to muscle damage.

Exercise-induced, skeletal muscle-derived cytokines possess endocrine-like properties

Role of exercise-induced, skeletal muscle-derived cytokines or "myokines"

An exciting breakthrough in exercise immunology research is the novel paradigm conceptualized by Bente Pedersen and research group in Copenhagen. Pedersen *et al.* have proposed

that the contracting skeletal muscle is an endocrine organ; secreting specific cytokines, known as myokines [12, 62]. To date, at least 12 myokines have been reported.

Myokines with reported paracrine functions on the original tissue or organ of secretion have been reported and include leukemia inhibitory factor (LIF) [15] and IL-15 [17]. Other myokines purported to exhibit endocrine functions include the well-studied IL-6 [63], Irisin [14], calprotectin [16], myonectin [18], Oncostatin M (OSM) [64] and secreted protein acidic and rich in cysteine (SPARC) [13]. The mechanistic concept is that skeletal muscle contraction results in the transient rise of myokine concentration, such as IL-6 from contracting muscles, and subsequent release of other anti-inflammatory cytokines, such as IL-1 receptor agonist (IL-1ra) and IL-10 [65]. This in turn reduces the number of pro-inflammatory cytokines, with a subsequent inhibition of migratory mononuclear cells such as macrophages and T-cells towards the inflamed microenvironment, such as adipose tissue [66].

Therefore, this line of research suggests that exercise training, and the accumulation of repeated bouts of skeletal muscle contraction may mediate an anti-inflammatory milieu, which may be beneficial for the primary prevention of cancer. In other words, muscle contraction during exercise induces the secretion of cytokines such as IL-6, which further recruits other anti-inflammatory cytokines such as IL-1ra and IL-10. Changes in the circulating concentrations of the pro- and anti-inflammatory cytokines may also shift the circulating monocyte or T-cell population to that of a M2 or Th2 profile. A M2 or Th2 profile may be beneficial given that during carcinogenesis, there is increased pro-inflammatory signaling due to increased oxidative stress and cellular/tissue damage. The possibility is that regular aerobic exercise training may prevent cancer initiation by reducing pro-inflammatory signaling. Thus, from a primary prevention perspective, aerobic exercise training induces an anti-inflammatory profile, at least in whole blood. However, aerobic exercise training may induce a subtle shift in the subsets of T-cells and macrophages to be may play a role in reducing risk of cancer (e.g. Th1 and M1 polarized), consistent with animal models of breast cancer.

Some myokines are putative candidates for exercise-induced chemoprotection, including SPARC, OSM and IL-15. SPARC is a glycoprotein involved in multiple cellular functions, such as proliferation, apoptosis, as well as mitigating the interactions between cells and the extracellular matrix [67]. Further, a C allele polymorphism was found to be more frequent in patients with hepatocellular carcinoma (HCC) than in healthy controls [68]. Aoi *et al.* [13] found an increase in circulating SPARC protein concentrations after an acute aerobic exercise (30 min of treadmill run at 30 m/min) in wild type mice and healthy human volunteers. Wild-type mice given 4 weeks of progressive aerobic exercise training (20 min at 18 m/min to 60 min at 30 m/min) and subsequent injections of azoxymethane (AOM; which induces colon tumorigenesis) presented with decreased aberrant crypts compared with sedentary mice. Conversely, SPARC-null mice had greater aberrant crypts in their colons after they were given the same treatments. Increased SPARC protein expression was also detected in both C2C12 myocytes that were stretched *in vitro* and in the culture media, compared with non-stretched myocytes. The results from this innovative study suggest that SPARC may serve as a myokine, and can potentially alter the interaction of the tumor cells with the extra-cellular matrix.

In a similar study by Hojman *et al.* [64], an increase in serum concentrations of OSM was observed after an acute, 1-hour bout of swim session in mice. This increase was most evident immediately post-exercise, and returned to baseline values after 2 hours. Importantly, serum taken from exercising animals increased caspase activity in MCF-7 tumor cells *in vitro*. As well, incubating the tumor cells with human recombinant OSM resulted in *in vitro* growth inhibition after 5 days. To ascertain that skeletal muscle was a source of OSM secretion, electrical stimulation of murine C2C12 myocytes was performed and the media from stimulated and non-stimulated cells were then added to MCF-7 cells. MCF-7 cells treated with serum from electrically stimulated myocytes showed a 100% increase in caspase activity, which was attenuated in half when anti-OSM antibodies were added to the culture.

Other putative cytokines/myokines such as IL-15 may also be relevant in exercise-induced

cancer protection. However, unlike IL-6 which has been demonstrated to be increased by up to 100-fold after an acute bout of prolonged aerobic exercise, the plasma concentrations of IL-15 were reported to be unchanged [69] or increased by up to 1-fold in young healthy men [70]. These conflicting differences may be attributed to training status in subjects from the two studies, with untrained subjects apparently demonstrating an increase in circulating IL-15 after exercise, compared with trained subjects. A more plausible explanation is that in the Ostrowski *et al.* study, data for IL-15 was only available for 2 subjects, limiting the ability to interpret the data.

In summary, the majority of the studies that investigated the biological functions of myokines have not considered their interactions with the immune system, and specifically, how these myokines can cross-talk with immune cells to potentially impact the process of tumorigenesis. With the exception of SPARC protein [13] and OSM [64], the majority of myokines has not been studied in the context of immune-tumor crosstalk after exercise training, and are attractive targets for future work.

Systematic review of the effect of aerobic exercise on immune function: human intervention trials in women without breast cancer and following a breast cancer diagnosis

In an effort to examine the currently available literature on the role of aerobic exercise on immune function, a systematic literature review was performed related to cancer prevention (i.e., in women who had not had a breast cancer diagnosis) and related to cancer outcomes in "patients" (i.e., women currently undergoing adjuvant cancer treatment; namely chemotherapy and/or radiation) and "survivors" (i.e., women who have completed adjuvant cancer treatment, but hormone therapy may be ongoing). The search was limited to: full text English-language; randomized controlled trials (RCT) of aerobic exercise in women; and published in peer-reviewed journals. Search terms related to breast cancer (e.g., breast neoplasm, breast cancer, breast tumor) and exercise (e.g., aerobic exercise, running) were used and modified for the individual databases (MEDLINE and EMBASE) (See Supplemental Table 1). Studies were excluded if multiple cancer sites were included and separate information for women with breast cancer was not available. In order to address the two key paradigms, the search included immunological markers, specifically monocytes, T cells, lymphocytes and leukocytes, and inflammation markers proposed to be affected by exercise and relevant to cancer prevention and survival, namely, interleukins (IL-1, 6, 18, 10, 12, 15), chemokine (CCLs) and (CXCLs), tumor necrosis factor (TNF-α), Serum Amyloid A (SAA), and C-reactive protein (CRP).

The search yielded 1684 potentially relevant articles, which were then screened for inclusion by title and abstract (**Figure 1**). All eligible papers underwent a full text review and for the eligible papers, the reference list was hand-searched to identify any additional relevant studies, which yielded one additional study [71]. Duplicate papers from a single study were handled by including individual studies rather than individual papers. Two papers [72, 73] from a single study were identified and therefore counted as one single study. The search strategy identified 15 studies that met the inclusion criteria and relevant data was extracted on study design and study outcomes (**Table 1**).

Four out of 15 studies were in premenopausal women [74-77], five in postmenopausal women [71, 78-81] and six in breast cancer survivors [11, 72, 82-85]. Sample sizes ranged from 8 to 320, and the mean number of participants was 100. The aerobic exercise interventions were a mean of 23 weeks in length (range, 8-48 weeks). In 10 out of 15 studies, the inflammatory and immunological markers were specified as the primary outcome [11, 71-78, 82, 83] and as a secondary outcome in the remaining five studies [79-81, 84, 85].

The quality and risk of bias for all studies were assessed using criteria from the Cochrane Collaboration risk of bias assessment protocol, risk of bias was low in all included studies (data not shown) [86].

Aerobic exercise training interventions

The length of the exercise interventions ranged from 8 to 48 weeks, while one study reported the result of a single acute exercise bout [76]. The frequency of exercise interventions ranged from three times per week to six times per week. The intensity of the prescribed exercise moderate to vigorous (i.e., 60-85% of maximum

heart rate), however, exercise training intensity was not reported in three studies [82, 84, 85]. The duration of each exercise session ranged from 15 to 60 minutes or was calculated with consideration of subjects' body weight in one study [81]. The duration of the acute exercise study was a two-hour exercise bout [76]. For supervised interventions, 11 RCTs reported attendance as the mean adherence rate of 84%; ranging from 49% to 98% [11, 71-74, 76, 78-80, 82, 83, 85]. Three studies did not report the adherence rate [75, 77, 81].

Impact of aerobic exercise training on biomarkers of interest in healthy female populations

Five studies reported significant changes in immunological and inflammation markers due to aerobic exercise among pre- and post-menopausal healthy women with no prior diagnosis of cancer [71, 74-76, 79]. In premenopausal women, significant reduction in the pro-inflammatory cytokine TNF-α was reported in a 12-week aerobic exercise intervention [75], and in one study of an acute 2-hour exercise bout [76]. In addition to changes in concentrations of TNF-α, a significant reduction of exercise-induced production of IL-10 after 120 min of exercise, and a significant mobilization of blood leukocytes and monocytes were also noted in the recovery period of the acute exercise study [76]. A 16-week aerobic exercise intervention in premenopausal women significantly reduced the plasma CRP concentrations in the exercise group compared to control; however, no significant reduction of SAA was observed [74]. In a 15-week aerobic exercise intervention among obese premenopausal women, the percentage and number of lymphocytes, numbers of T cells ($CD5^+$), and B cells ($CD20^+$) significantly decreased compared to controls [77]. However, no significant changes in total leukocytes, T helper cell ($CD4^+$), and T cytotoxic cell ($CD8^+$) numbers were found.

In postmenopausal women, similar reductions in CRP were noted with aerobic exercise interventions of varying lengths in some [71, 79], but not all studies [78, 80, 81]. No changes were observed in serum SAA [71, 80], IL-6 [79-81], TNF-α [71, 79, 81] and total leukocytes [80] among postmenopausal women in different aerobic exercise interventions. Seven studies investigated the factors that may mediate the effect of aerobic exercise and adjusted the model for baseline BMI, adherence rate, body fat and physical fitness [71, 74, 78-80, 81, 83]. Three studies found that exercise-induced significant reduction of CRP concentrations were independent of weight loss [74, 78, 81].

Impact of aerobic exercise training on biomarkers of interest patients or breast cancer survivors

No studies examined the effect of aerobic exercise on inflammation and immunological markers in breast cancer patients (i.e., during chemotherapy or radiation treatment). Six studies examined the influence of aerobic exercise intervention on inflammation and immunological markers among breast cancer survivors [11, 72, 73, 82-85]. Two studies examined CRP [73, 83] and while a slight reduction was reported in one study [73], it was not statistically significant and no change was reported in another study [83]. Two studies examined IL-8 [82, 85], and a reduction was noted in one study [82], but not in the other [85]. No significant changes were observed in serum IL-6 [82-85], and TNF-α concentrations [82, 83]. One study examined the impact of an aerobic exercise intervention on Regulated upon Activation Normal T-cell Expressed and secreted (RANTES), and while a greater reduction was noted in the exercise group [82], no statistically significant changes were reported in RANTES or monocyte chemotactic protein-1 (MCP-1) concentrations compared to the education group. In addition, comparing the post-treatment to baseline concentrations, interleukin-8 was significantly decreased in the home exercise group while concentrations of MCP-1 were significantly increased in the education group. Two studies examined natural killer cell cytotoxic activity [11, 72]. The first study observed an increased natural killer cell cytotoxic activity, total lytic unit and spontaneous lymphocyte proliferation in postmenopausal breast cancer survivors with a 15-week exercise intervention [72]. However, in the second study, natural killer cell activity (NKCA), lymphocyte and total leukocytes concentrations were not altered significantly by the aerobic exercise intervention, although NKCA tended to be higher in the exercise group [11].

Summary of results

Our systematic review of the literature revealed that although biomarkers of immune function

and inflammation have been examined in healthy women or breast cancer survivors, the results have been mixed and very few RCTs included biomarkers relevant to immunomodulation, as opposed to a more generic inflammatory profile. Furthermore, only some investigators performed flow cytometry [11, 72] to determine changes in immune subsets or changes in intracellular concentrations of cytokines after acute exercise or exercise training.

Protective molecular phenotypes related to exercise-induced immunomodulation

Determination of an exercise-induced molecular "signature" could provide a rationale to examine the effect of exercise combined with chemotherapy or immunotherapy in mediating disease prognosis. The putative mechanisms are likely to involve direct modulation of monocyte/macrophage and T-cell functions, as well as indirect modulation of myokine (OSM, SPARC) crosstalk with stromal and tumor cells from the tumor microenvironment. In fact the integration of these two paradigms begets the compelling questions: do myokines communicate with innate and adaptive immune cells, and what is the direction of the cross-talk? Gaps in the exercise immunology literature represent new opportunities for cross-disciplinary collaborations. In addition, long-term profiling of chronic aerobic exercise training on leukocyte and myokine expression in individuals without a diagnosis of cancer could help to elucidation a more complete understanding of the immune response to long-term aerobic exercise training, specifically how aerobic exercise modulates gene and protein expression of molecular mediators involved in the immune surveillance and recognition of potential malignancies.

Conclusion

We envision a future where aerobic exercise training may play a larger role in an integrated approach to enhance breast cancer prevention and treatment outcomes. The immune system plays an instrumental role in surveying the host microenvironment for potential malignancies, and its efficacy can be modulated with exercise training. In order for the research and clinical community to improve breast cancer prevention and treatment outcomes, it is necessary to elucidate the potential mechanisms involved. Proposed mechanisms include immune-relevant pathways, as well as myokine signaling. We suggest that these two areas represent a significant opportunity to further understand how exercise can positively influence immune cross-talk relevant to breast cancer prevention and treatment outcomes.

Disclosure of conflict of interest

None.

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Supplemental Table 1. Risk of Bias (aka Quality Ratings)

	Selection Bias			Performance Bias			(Measurment Bias)	(Attrition Bias)		
Author, publication year	Randomization	Allocation concealed (investigators)	Groups similar at baseline	Blinding of patients	Blinding of treating providers	Completion of study	Blinding of outcomes assessors	Dropout	Intention to treat	Funding source
Fairey et al., 2005	Y	Y	Y	NR	Y	52/53 (98%) Exercise: 24 Control: 28	Y	Exercise: (1) 4.2%	Y	National Cancer Institute of Canada (NCIC), Canadian Cancer Society (CCS), CCS/NCIC Sociobehavioral Cancer Research Network.
Campbell P.T. et al., 2008	Y	Y	Y	NR	Y	108/112 (96%) Exercise: 50 Control: 58	Y	0	Y	National Institutes of Health
Arikawa et al., 2011	Y	N	Y	NR	N	319/391 (82%) Exercise: 166 Control: 153	N	Exercise: (46) 21.7% Control: (26) 14.5%	N	National Institutes of Health/National Cancer Institute
Horne et al., 1997	Y	N	Y	NR	N	Exercise: 4 Control: 5	N	0	NR	NR
Jimenez et al., 2007	Y	Y	Y	NR	Y	8/8 (100%)	Y	0	NR	NR
Nehlsen-Cannarella et al., 1991	Y	N	Y	NR	N	36/36 (100%)	N	0	NR	Steele Foundation grant
Campbell K.L. et al., 2008	Y	Y	Y	NR	Y	95/100 Control: 49 Exercise: 46	Y	Overall: 3% Exercise: (1) 2.08% Control: (2) 3.92%	N	National Institutes of Health
Friedenreich et al., 2012	Y	Y	Y	NR	Y	310/320 (97%) Exercise: 154 Control: 156	Y	Overall: 2.8% Exercise: (5) 3.1% Control: (4) 2.5%	Y	Canadian Breast Cancer Research Alliance, the Alberta Cancer Foundation, Alberta Heritage for Medical Research Health, Canadian Institutes of Health Research.
Lee et al., 2012	Y	Y	Y	NR	Y	22/27 (81%) Control: 7 LI: 8 HI: 7	Y	(5/27) 20%	N	Kyung Hee University
Imayama et al., 2012	Y	Y	Y	NR	Y	186/204 (91%) Exercise: 106/117 Control: 80/87	Y	Overall: 39.9%	Y	National Cancer Institute
Sprod et al., 2012	Y	N	Y	NR	N	21/35 (60%) Exercise: 9 Control: 10	N	Overall: 52% Exercise: (6) 66% Control: (4) 40%	N	Sally Schindel Cone Foundation (KMM), NCI

Nieman et al., 1995	Y	N	Y	NR	N	16/20 (80%) Exercise: 8 Control: 8	N	Overall: 40% Exercise: (2) (10%) Control: (2) (10%)	N	National Institute of Aging
Payne et al., 2008	Y	N	Y	NR	N	18/20 (90%) Exercise: 9 Control: 9	N	Overall: 10% Exercise: (1) (10%) Control: (1) (10%)	N	National Institute of Nursing Research
Ergun et al., 2013	Y	Y	Y	NR	Y	38/40 (95%) Exercise: 18 Control: 20	Y	2/40 (5%) Exercise: 2	NR	Ege University Medical Faculty BAP project
Jones et al., 2013	Y	Y	Y	NR	Y	67/68 (99%) Exercise: 36 Control: 31	Y	1/68 (1.5%) Control: 1	Y	American Cancer Society

Abbreviations: Y, yes; N, no; NR, not reported.