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Effects of short-term Pilates exercise on selected blood parameters

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Abstract. The aim of our prospective, interventional, pre-post, single arm study was to supplement the lack of knowledge of the effect of short-term Pilates intervention on selected blood parameters of healthy women. Female volunteers were recruited for 2-weeks Pilates intervention. Blood has been collected and anthropometric parameters were measured before and after exercise period (EP). Plasma insulin, cortisol, and dehydroepiandrosterone sulphate levels, erythrocyte antioxidant activity, glutathione levels, NK cytotoxicity and plasma cytokines were analysed. We found a decrease in erythrocyte antioxidant enzymes SOD and GPx activity; GSH levels; in the pro-inflammatory chemokine MCP-1 and trend to reduction in MIP-1β, PDGF and VEGF levels in plasma. NK cell cytotoxic activity increased after Pilates EP in the percentage of specific lysis at 25:1 effector: target (E:T) ratio and the same trend was observed at all E:T ratios as well as in the amount of lytic units *per* 107 cells. Our findings show that Pilates exercise may improve NK cell immune response and inflammatory milieu in plasma of healthy women.

Key words: Pilates — Inflammation — MCP-1 — NK cells

Introduction

Physical activity can positively influence and treat a variety of diseases associated with life style and those, that have a common inflammatory basis like type 2 diabetes, atherosclerosis, cancer, and others (Pradhan et al. 2002). Exercise reduces the risk of cardiovascular diseases (coronary artery disease, chronic heart failure and atherosclerosis) mortality by 35% (Nocon et al. 2008); reduces the risk of the overall incidence of diabetes and metabolic syndrome by 58% (Tuomilehto et al. 2001). It has preventive effects on weight control, the development of mental illnesses such as dementia (Rovio et al. 2005) and depression (Paffenbarger et al. 1994), and has a positive effect on muscle and bone strength, preventing osteoarthritis and osteoporosis.

Recent epidemiological studies have demonstrated an evident association between exercise and physical activity (PA) with markedly reduced cancer-related, and overall mortality, in various cancers, including breast, colon, and prostate (Holmes et al. 2005; Meyerhardt et al. 2006; Kenfield et al. 2011).

Evidence from observational studies has shown that physical activity reduces the risk of disease recurrence in colorectal, prostate, and breast cancer patients (Meyerhardt et al. 2006; Irwin et al. 2008; Friedenreich et al. 2016) and may be associated with a reduced risk of lung cancer (Brenner et al. 2016). Several mechanisms have been proposed, including reduced oxidative stress, the modification of metabolic hormone levels via a reduction in fat-produced estrogens, and a reduced contact with carcinogens through increased ventilation (Friedenreich and Orenstein 2002; Quadrilatero and Hoffman-Goetz 2003; Kruk and Aboul-Enein 2006).

The immune response to exercise is complex and specific to various components of the immune system, e.g. cells of the adaptive branch of the immune system (T lymphocytes and B lymphocytes); cells of the innate branch (monocytes, granulocytes, NK cells), and soluble factors such as immunoglobulins and cytokines/chemokines.

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Chemokine-binding proteins modulate immune responses through target cell surface receptors. This mechanism is similar to hormonal and may lead to new therapeutic approaches for treatment of inflammatory diseases.

Physical activity is of four main types: i/ aerobic (endurance), ii/ muscle-strengthening, iii/ bone-strengthening, and iv/ stretching. The intensity of the aerobic activities is divided into three groups: light, moderate or vigorous.

Pilates is one type of physical activity systems developed in the early 1920s by Joseph Hubertus Pilates. It has developed in combination with Yoga, Zen and the regimens of ancient Greece and Rome (Latey 2001). Pilates improves balance (Marandi et al. 2013), flexibility (Phrompaet et al. 2011) and recently, according to a systematic review by Wells et al. (2012), it has been found that Pilates exercises improves center stability, strength, flexibility, muscle coordination, posture, breathing, etc. It also develops control and endurance in the entire body, including the *erector spinae* (Sekendiz et al. 2007), and it trains the mind and body organically through breathing exercises (Muscolino and Cipriani 2004). Pilates has been shown to improve muscle strength and balance (Granacher et al. 2013) and to increase bone mineral density (BMD) (Pilates Exercises for Osteoporosis - Osteoporosis Center - Everyday Health, available at: http://www.everydayhealth.com/osteoporosis/pilates-exercises-for-osteoporosis.aspx; Angın et al. 2015) and thus to prevent osteoporosis.

The aim of this prospective, interventional, pre-post, single arm study was, therefore, to determine the effects of short-term (two weeks) Pilates physical activity, of healthy women, on their plasma cytokines levels, NK cell cytotoxicity, and the erythrocyte activities of antioxidant enzymes and selected endocrine parameters.

Table 1. Clinical characteristics of the study subjects

Parameter	Before EP ($n = 10$)	After EP ($n = 10$)
Age (years)	46 ± 3	46 ± 3
Body weight (kg)	62.2 ± 3.0	62.8 ± 3.0
BMI (kg/m^2)	22.9 ± 1.2	23.1 ± 1.2
BF (%)	30 ± 2	32 ± 3
MET (h/day)	37 ± 6	40 ± 6
EE (kCal/24 h)	2707 ± 708	2986 ± 628
Morning fasting plasma cortisol (nmol/l)	234 ± 26	282 ± 30
Fasting plasma insulin (mIU/l)	2.92 ± 0.69	5.63 ± 2.11
DHEAS (mmol/l)	3.45 ± 0.54	3.55 ± 0.55

The data are expressed as mean ± SD. BF%, body fat percentage; EP, exercise period; BMI; body mass index; BF, body fat; MET, metabolic energy turnover; EE, energy expenditure estimated using activity score according to Lagerros et al. (2006); DHEAS, dehydroepiandrosterone sulphate.

Subjects and Methods

Study population

Initially, 13 volunteers recruited from the staff of the Cancer Research Institute of the Slovak Academy of Sciences, were included in the study; 10 underwent all examinations and interventions. Most of them did not take any medication; two probands were on substitutional therapy with L-thyroxine due to hypothyroidism. The inclusion criteria were volunteers without any cancer history, healthy adults (age between 20-65 years). Participants with a history of cardiovascular disease, hypertension, diabetes mellitus (type I and II), current smoking, hepatic or renal disease, acute or chronic infection were excluded from the study. The examinations were performed at the Biomedical Center, Institute of Clinical and Translational Research, Slovak Academy of Sciences. The basic clinical characteristics of all the study subjects are given in Table 1. All subjects gave informed written consent and the study was approved by the Ethics Committee of the Bratislava Self-Governing Region, Bratislava, Slovakia (106605/2014-ZDR), in accordance with the ethical guidelines of the Declaration of Helsinki, as revised in 2000.

Study design, sample collection and preparation

A prospective, interventional, pre-post, single arm research design was used. The examinations started in the morning at 08:00 AM. The subjects were asked to fast for 12 hours and restrain from incurring stress or doing any intensive physical activity 24 h before the study. Upon arrival in the outpatient's clinic, a baseline personal history was taken and body weight, height, body fat percentage (Omron 511BF, OMRON HEALTHCARE Co., Ltd. Kyoto, Japan) and waist circumference were measured. Thereafter, the cubital vein was cannulated (Terumo Europe N.V., Leuven, Belgium) and subjects rested for 30 min in a comfortable chair to relieve acute stress from the venipuncture. Their blood pressure (Dinamap Vital Sign Monitor, model 845 XT, Criticon X, Inc., Tampa, FL, USA) was measured on the arm during the rest. The blood of the probands was collected before the exercise period (ordinary physical activity period), and after 2 weeks of increased Pilates physical activity. Blood was collected into polyethylene tubes with EDTA as an anticoagulant and immediately cooled on ice. For immune assay analyses, the blood was centrifuged (1200 × *g*, 10 min, and 4°C) and all plasma aliquots were stored at –80°C until assayed. Plasma samples for cytokine analyses were prepared from peripheral blood (1 ml) by centrifugation at 1200 × *g* for 10 min, at room temperature. The supernatants were collected and filtered through sterile 0.22 μm filters, and aliquots were stored at –80°C until analysis. The erythrocytes for the determination of SOD and GPx activity were isolated from whole blood by

three-fold washing in a cold PBS solution and centrifugation at 800 × *g* for 10 min, at 4°C. Aliquots were stored at –80°C until analysis. Flow cytometry-based cytotoxicity assay was performed directly from fresh, non-frozen blood.

Determination of physical activity

Physical activity level (PAL) was assessed using a Slovak version of the Lagerro´s questionnaire (Lagerros et al. 2006). Physical activity during an average weekday was estimated using an instrument with nine physical activity steps, which graded physical activity according to its intensity. Each step was assigned a value expressed as a multiple of Metabolic Energy Turnover (MET) and exemplified by common activities. Participants were instructed to report the time they spent on each intensity level during an average day and night; hence, the total physical activity time should add up to 24 h and allow for an estimate of MET*hours *per* day (MET h/day) and a total physical activity score calculated as the sum of the individual level activities ($MET_1*t_1 + MET_2*t_2 + \ldots + MET_9*t_9$, where MET_i is assigned an MET value for the current level and t is the time spent executing the current activity).

Pilates exercise

All the participants were instructed to exercise 180 minutes *per* week, under the supervision of a certified STOTT Pilates instructor. STOTT PILATES (the contemporary approach to the original Pilates method) offers varying levels of movement – essential, intermediate and advanced – to meet the exercise needs of all clients, from post-rehab to elite athlete. Energy expenditure in units of metabolic equivalent of tasks (METs) was assigned to this activity using the Compendium of Physical Activities. The intensity of each 60 minutes exercise, expressed in METs, corresponded to 6 METs (Ainsworth et al. 2000).

Immunno assays

Plasma insulin, cortisol, and dehydroepiandrosterone sulphate levels were measured using radioimmunoassay kits (Immunotech a.s., a Beckman Coulter company, Praha, Czech Republic), according to the manufacturer's instructions.

Determination of superoxide dismutase activity

For the determination of superoxide dismutase activity (SOD; EC1.15.1.1), we used the RANSOD kit. The method employs xanthine and xanthine oxidase to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The activity was measured by the degree of reaction inhibition.

Determination of glutathione peroxidase activity

For the determination of glutathione peroxidase activity (GPx, EC1.11.1.9), according to the method of Paglia and Valentine (Paglia and Valentine 1967), we used cumene hydroperoxide as a substrate.

Glutathione assay

Intracellular total glutathione was measured by flow cytometry (CANTO II, Becton Dickinson) using Monochlorobimane (MCB) staining for glutathione (GSH). $1–2 \times 10^6$ cells were stained with 40 μM MCB at room temperature for 20 min. The cells were chilled by the addition of ice-cold PBS at 4°C to stop the enzyme-dependent staining reaction. Fluorescence of MCB-GSH conjugate was detected using a 405 nm excitation laser and a 450/50 emission bandpass filter. The cells were analysed by FCS Express 4.0 (de Novo) software. The relative fluorescence intensity (RFI) was expressed as a multiple of the fluorescence intensity of the control samples.

Plasma cytokines analyses

The plasma levels of 27 cytokines (IL-1b, IL-1 receptor antagonist (IL-1ra), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17A, Eotaxin, Basic FGF, G-CSF, GM-CSF, IFN-g, IP-10, MCP-1, MIP-1a, MIP-1b, PDGF-BB, RANTES, VEGF and TNF-α were analyzed using the Bio-Plex Suspension Array System (Bio-Rad Laboratories Inc., Hercules, CA, USA). The multiplex microbeads-based cytokine immunoassay was performed in 96-well filter microplates, according to the manufacturer's instructions. The cytokine standards and samples (50 μl were diluted in plasma dilution buffer and incubated with fluorescently-labelled microspheres coupled to specific monoclonal antibodies (50 μl) for 30 min on a plate shaker (300 rpm) in the dark). After one wash step, the beads were incubated with the biotinylated detection antibody cocktail (25 μl/well) for 30 min, followed by streptavidin-PE (50 μl/well) for 10 min. Finally, 125 μl of assay buffer was added to each well before reading the plate on a Bio-Plex system. The cytokine concentrations were calculated using Bio-Plex Manager Software.

Flow cytometry-based cytotoxicity assay

The preparation of effector peripheral blood mononuclear cells (PBMCs) and target (K562 cells) cells was performed as previously described (Cholujova et al. 2008).

The NK cytotoxic activity in PBMCs from healthy individuals included in this study was determined against target K-562 cells using the calcein acetoxymethyl ester (CAM) assay. The target K562 cells were loaded with CAM (stock

solution 1 mM in DMSO) complete culture medium (consisting of RPMI 1640 medium, 2 mM L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin and supplemented with 10% fetal calf serum) and further incubated for 15 min at 37°C in the dark. After washing with PBS, the target K-562 cells (2×10^4 *per* well) were seeded in 96-well V-bottomed microplates in 50 μl of CM.

Appropriate six serial two-fold dilutions of effector cells (PBMCs) were made, from which 100 μl aliquots were added to the K562 target cells to obtain the six effectors: target (E:T) ratios started at 50:1. Additionally, two sets of controls containing only effector cells or only target cells were prepared. The plates were centrifuged at 200 × *g* for 3 min and incubated for 3 h. The samples were then transferred into cytometric tubes and 2 μg/ml of 7-AAD (stock solution 1 mg/ml in PBS) was added to mark the dead cells. The samples were incubated on ice for 10 min, protected from light, and analyzed using a BD FACS CantoII flow cytometer. For the assessment of purified NK cell cytotoxicity, three 8:1, 4:1 and 2:1 effector (target ratios), an incubation time of 90 min, and 10^4 target K562 cells *per* well were used.

The cytotoxic activity toward the target K562 cells was determined based on the viable cells enumeration using flow cytometric data. The percentage of specific lysis (PSL) was calculated at each E:T ratio as follows: % specific lysis = (CT – TE / CT) × 100 (CT, mean the number of fluorescent target cells in control tubes; TE, the mean number of fluorescent cells in target + effector tubes). A lytic unit (LU) is defined as the number of effector cells required to lyse 20% of a predetermined standard number (TSTD = 2×10^4) of target cells. The results were reported as the number of LU contained in a specified number of effector cells (ESTD = 10^7). These calculations can be expressed by the formula: number of LU/10^7 effector cells = ESTD / (E:T20) × (TSTD), where e.g. E:T20 is the ratio at which 20% of the target cells are killed.

Flow cytometry – Phenotyping

Multi-parameter flow cytometry was performed using a lysed-whole-blood technique without isolation of cells on a density gradient, using a commercially available red cell lysing solution, Optilyse B (Beckman Coulter). The cell labelling and membrane fixation/permeabilization procedures were done by standard methods; the cell staining was performed as described in (Babusikova et al. 2008). We used monoclonal antibodies targeting membrane antigens: anti-CD45-ECD (Beckman Coulter), anti-CD16-FITC (Beckman Coulter), anti-CD11b-PE (Miltenyi Biotec), anti-CD3-FITC (Miltenyi Biotec), anti CD4-PE (Miltenyi Biotec), anti-CD8-APC (Miltenyi Biotec), anti-CD25-Pacific Blue (ExBio Praha), anti-CD19-FITC (Miltenyi Biotec) and anti-CD56-PE (Beckman Coulter), and FACS analysis was performed by FACS Canto II flow cytometer (BD Biosciences, San Jose, CA). The data were analysed using FCS Express 4.0 (De Novo Software). NK cells were identified as CD3-CD56 + cells. To determine the absolute number of NK cells, equal volumes (100 μl) of Flow-Count Fluorospheres (Beckman Coulter, CA, USA) and cells were mixed, and the NK cell concentration was calculated according to the formula: NK cells/μl = (count NK cells × concentration beads)/ (count beads).

Statistical analysis

The General Linear Model repeated measures (GLM-RM) procedure was used to determine the main effects on measured parameter responses to exercise. A statistical evaluation was performed using the SPSS 11.5 program (SPSS Inc., Chicago, IL, USA). Measured parameters were compared by paired and unpaired *t*-tests or Mann-Whitney tests and Wilcoxon tests, according to the data distribution. The results were expressed as the mean ± SD, unless otherwise specified. Differences were considered statistically significant at $p < 0.05$.

Table 2. Activities of antioxidant enzymes SOD, GPx and levels of GSH

	Before EP	After EP
GPx (U/ml)	0.0298 ± 0.000371	0.0271 ± 0.000434**
SOD (U/ml)	0.914 ± 0.0289	0.767 ± 0.0362*
GSH (RFI)	30.195 ± 8.265	5.453 ± 0.933**

The data are expressed as mean ± SD; * $p \leq 0.05$; ** $p \leq 0.002$. EP, exercise period; GPx, glutathione peroxidase; SOD, superoxide dismutase; GSH, glutathione; U/ml, units *per* ml; RFI, relative fluorescence intensity.

Results

Physiological effects, antioxidant changes and stress hormones

The general and anthropometric characteristics of the study population are shown in Table 1.

After 2 weeks of Pilates exercise, all probands remained weight stable; neither did the percentage of their body fat change. Physical activity measured as the mean MET h/day and 24 h energy expenditure was higher during the intervention. The mean fasting glucose and insulin, cortisol, and dehydroepiandrosterone sulphate (DHEAS) levels were comparable before and after the intervention (Table 1).

We observed a statistically significant decrease in the activities of antioxidant enzymes SOD and GPx, as well as a statistically significant decrease in GSH levels (Table 2) before and after Pilates intervention.

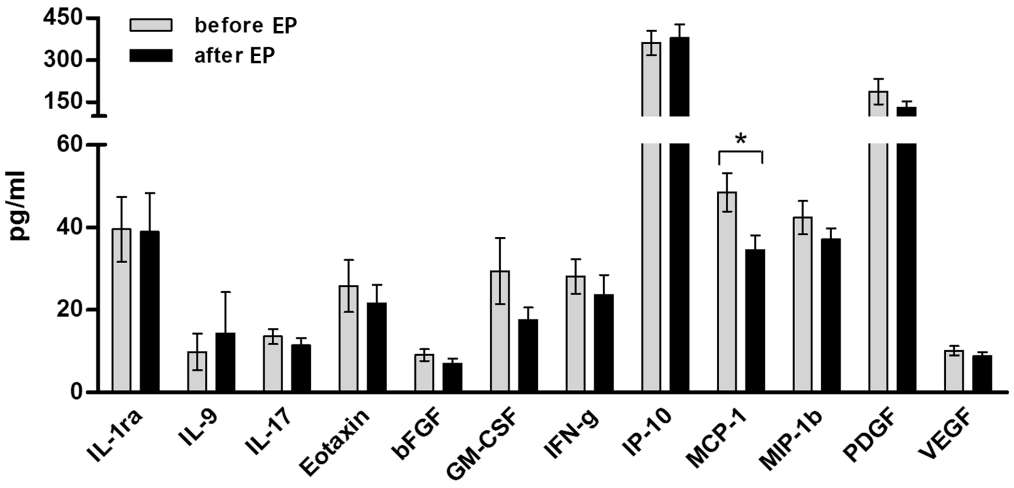

Figure 1. Cytokines and chemokines changes. Plasmatic levels of cytokines and chemokines measured before and after Pilates exercise period (EP). Data are means ± SD. * $p = 0.005$. IL, interleukin; bFGF, basic fibroblasts growth factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN, interferon; MCP-1, monocyte chemoattractant protein-1; IP-10, proinflammatory chemokine; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor.

Cytokines and chemokines changes

In the study, we measured the plasmatic levels (pg/ml) of 27 cytokines/chemokines of healthy volunteers obtained before and after two weeks of instructed Pilates exercise. We observed changes in 13 of them (Figure 1), as values of the rest were extrapolated beyond the standard range and were thus excluded from the evaluation. A statistically significant decrease ($p = 0.005$) in the pro-inflammatory chemokine MCP-1 (Figure 1) was observed, together with a reduction in MIP-1β, PDGF and VEGF levels, almost approaching statistical significance ($p = 0.071$).

Nk cell cytotoxic activity

NK cell cytotoxic activity had trend to increase after Pilates intervention ($p = 0.152$) as shown by amount of lytic units *per* 10^7 cells (Fig. 2). In addition, percentage of specific lysis showed tendency to increase at all E: T ratios, with statistically significant increase ($p = 0.02$) at 25:1 ratio (Figure 3). Interestingly, neither percentage nor absolute counts of NK cells did change in our study (Figure 4). There were no significant changes in other PBMC subsets (data not shown).

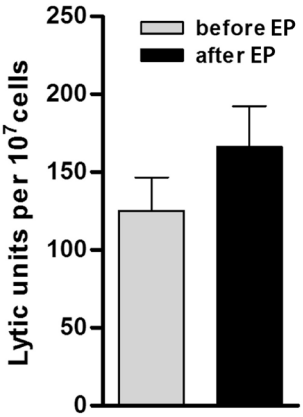

Figure 2. NK cell cytotoxic activity – amount of lytic units before and after exercise period (EP). A lytic unit is defined as the number of effector cells (PBMCs) required to lyse a predetermined number of target cells (K562 cells) = % specific lysis *per* 10^7cells. Data are means ± SD.

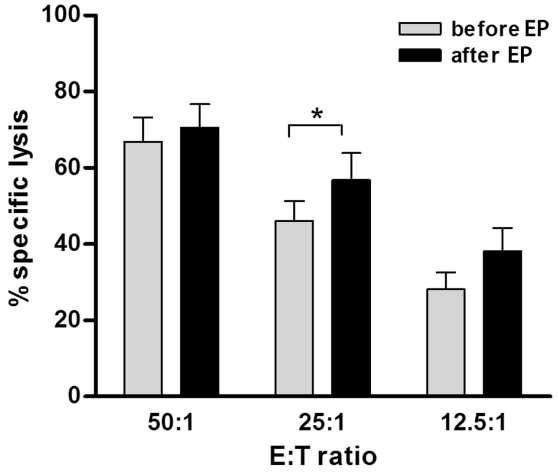

Figure 3. NK cytotoxic activity (E : T ratio) in effector cells (PBMCs) against target Nk cell cytotoxic activity – % of specific NK cell lysis cells (K-562 cells). Statistically significant ratio E : T = 25 : 1. Data are means ± SD. * $p = 0.02$; where 25% of the target cells are killed.

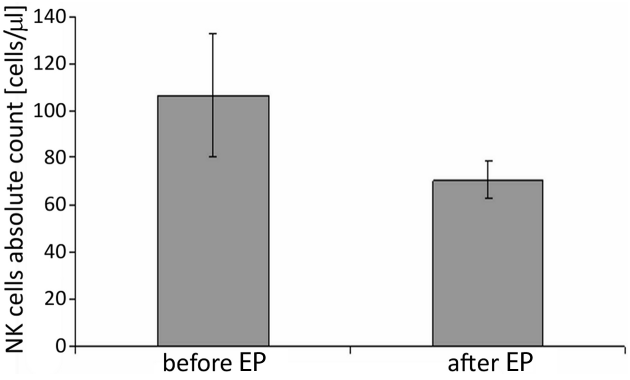

Figure 4. Absolute number of NK cells. Mean of absolute NK cells count [cells/µl] before and after EP. Data are means ± SEM.

Discussion

Many beneficial effects of Pilates exercise have gradually been revealed, such as an improvement in strength, flexibility, muscle coordination, posture, breathing, etc. (Phrompaet et al. 2011; Marandi et al. 2013). Recent studies have shown that long term Pilates exercise can improve osteoporosis, which is a problem in elderly people, and can improve the quality of life for patients with osteoporosis (Granacher et al. 2013; Küçükçakır et al. 2013).

In our study, we addressed some regulatory mechanisms which are activated by short term Pilates exercise and might be perceived as potentially prophylactic against cancer, since its mechanisms of action in cancer patients have not yet been described. It has only been observed that Pilates exercise decreased pain, improved mood (Keays et al. 2008), cardiopulmonary outcome (Eyigor et al. 2010) and patients were more interested in daily living activities, felt less irritated, less fatigue (Eyigor et al. 2010) and were more confident in communicating with family members and relatives without hesitation. Our observations showed reduction in MCP-1 plasma levels as well as tendency to reduce angiogenetic chemokines and increase of NK cell cytotoxicity, what has not yet been studied in Pilates interventions. To the best of our knowledge, our study is the first one dealing with short term Pilates and the only one among all (short term as well as long term Pilates interventions) describing its effects on immune parameters in human blood.

First, we observed, that 2 weeks Pilates intervention caused a significant decrease in pro-inflammatory chemokine Monocyte chemotactic protein-1 (MCP-1) plasma levels. This chemoattractant protein-1 (MCP-1/CCL2) is one of the key chemokines that regulate the migration and infiltration of monocytes/macrophages. Both MCP-1 and its receptor CCR2 have been demonstrated to be induced and involved in various diseases. As such, it has been shown to be a potential intervention point for the treatment of various diseases, including multiple sclerosis (Sørensen et al. 2004), rheumatoid arthritis (Hayashida et al. 2001), atherosclerosis (Kusano et al. 2004), and insulin-resistant diabetes (Sartipy and Loskutoff 2003). MCP-1 was also found to be associated with pancreatic cancer cachexia (Talbert et al. 2018). MCP-1 expression in tumour cells is significantly correlated with the extent of tumour-associated-macrophage (TAM) infiltration (Sato et al. 1995), and both MCP-1 and VEGF expressions have been positively correlated with TAM infiltration, angiogenesis, and poor survival in breast cancer (Valković et al. 2002; Deshmane et al. 2009). Thus, a decrease in these chemokines might be beneficial for breast cancer patients and survivors.

One of the mechanisms by which exercise and PA exert their beneficial effects is through peripheral immune system adaptations. The effects of voluntary wheel running on the neuroimmune reactions of mice were found to be modulated by MCP-1 (Spielman et al. 2017). Our finding that there was already a reduction in MCP-1 after 2 weeks of Pilates exercise complements the findings of others who reported that, in long term physical activity regimes, total levels of biomarkers of inflammation improved (Lunde et al. 2017). Similarly, endurance training (ET) reduced inflammatory markers (TNF-α, IL-6), macrophage recruitment (MCP-1 and F4/80) and increased the IL-10/TNF-α ratio in plasma (Rocha-Rodrigues et al. 2017). MCP-1 and TNF-α also decreased in the group of healthy men who performed aerobic and resistance training on alternating days (AD) 4–6 d/wk (Ihalainen et al. 2018). MCP-1 has also been recognized as an angiogenic chemokine. Previous microarray analysis identified MCP-1 as a TGF- beta target gene in endothelial cells (Ma et al. 2006). In addition, other studies have revealed that MCP-1 participates in VEGF-mediated angiogenesis, increases vascular permeability and up-regulates VEGF expression (Hong et al. 2005).

Although other studied cytokines and chemokines (MIP-1β, PDGF and VEGF) showed only a trend in reduction approaching statistical significance after Pilates exercise, our findings reflect the complex regulatory mechanisms involved in inflammation and angiogenesis that seem to be positively influenced by Pilates exercise. Observed systemic decrease of these cytokines/chemokines could mediate beneficial effects also in cancer (Valković et al. 2002; Deshmane et al. 2009) and its frequent comorbidity diabetes (Sartipy and Loskutoff 2003).

Although, we observed a decrease in MCP-1 in the absence of weight/fat loss in healthy women, it is interesting that similar exercise settings in obese adults did not cause any changes in circulating chemokines, but only effected the chemokine receptors CCR2, CCR5, and CXCR2 expression on leukocytes (Barry et al. 2017). In our study, we did not observe any changes in peripheral blood subpopulations (data not shown); however, this was expected as Gustafson

et al. (2017) showed a 122% increase of leukocytes immediately after exercise, peaking at 140% at the three-hour time point, for an endurance test, and returning back to base level after 24 h. There is growing evidence that acute exercise can increase reactive oxygen species (ROS) production. In our system, we applied 2 weeks Pilates exercise corresponding to 18 METs/week. We observed a decrease in SOD and GPx activities, as well as a decrease in GSH in erythrocytes, which suggests that our intensity levels approached vigorous intensity. There are similar observations in various other studies that have appeared recently. A treadmill maximal cardiopulmonary exercise test resulted in increased levels of lipid peroxidation and diminished superoxide dismutase activity (de Medeiros Lima et al. 2016). The 30-min. aerobic cycle ergometer test at an intensity of approximately 80% of HR_{max}, followed by recovery at RT, decreased erythrocytes' GPx activity in 18-year-old footballers (Sutkowy et al. 2017). Short-term monitoring of ultramarathon mountain race-induced oxidative stress showed a significant increase in static oxidation-reduction potential marker levels and a significant decrease in the capacity oxidation-reduction potential and GSH levels post-race compared with pre-race (Spanidis et al. 2017).

Our data also showed increased NK cell activity, although the number of NK cells did not change. We have already discussed the unchanged number of PB cells higher, and Evans et al. made a similar observation, exclusively for NK cells (Evans et al. 2015). In their study, they showed that exercise similarly affects the magnitude of the NK cell response in breast cancer survivors and physically similar women without a history of cancer. With this evidence, we can envisage similar NK cell response to Pilates both in healthy women and cancer survivors.

Moreover, we agree with others, who also observed exercise- dependent activation of NK cells cytotoxicity, specifically in breast cancer (Nieman et al. 1995; Fairey et al. 2005) and stomach cancer (Na et al. 2000) survivors and believe that this can provide a mechanistic explanation for the protective effects of exercise on cancer patients (Idorn and Hojman 2016). Idorn and Hojman (2016) propose that exercise represents a potential strategy, as an adjuvant therapy, in cancer treatment, by improving NK cell functions. Since we do not assume different mechanisms leading to NK cell response in Pilates in comparison with other types of exercise, we deduce that STOTT Pilates might be one of the exercise types suitable for cancer patients as it can be easily adjusted to the degree of their physical performance.

To summarize, we found that MCP-1 proinflammatory and angiogenic chemokine plasma levels were decreased after a short period of Pilates exercise. The observed increase in NK cell cytotoxic activity may be the underlying mechanism by which Pilates might be beneficial for healthy people, improving their NK immune functions, and as such, it might be also useful in cancer patients and survivors, who often have impaired effectors of innate immunity. However, this presumption needs to be confirmed by specific cancer-oriented interventional studies.

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