

Physical exercise modulates the level of serum MMP-2 and MMP-9 in patients with breast cancer

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Abstract. Matrix metalloproteinases (MMPs) exhibit an important function in extracellular matrix degradation. MMPs modulate the activation of growth factors, cytokines and metastasis. At present, the effect of exercise on serum levels of MMP-2 and -9 remains unclear. The aim of the present study was to investigate the effect of various physical activities on the circulating levels of MMP-2 and -9 in breast cancer (BC) survivors and healthy subjects. A total of 66 female subjects were enrolled in the present study. The cohort included 46 BC survivors and 20 healthy subjects divided into 5 groups: Group A (17 BC survivors, participating in recreational dragon boat paddling), group B (14 BC survivors, participating in recreational physical activity), group C (15 sedentary BC survivors), group D (10 healthy subjects, participating in recreational physical activity) and group E (10 sedentary healthy subjects). ELISA assays revealed a significant increase in the level of circulating MMP-2 in group B compared with all other groups. Recreational physical activity increased the levels of MMP-9 in healthy subjects (group D vs. E), however, the differences were not statistically significant, while in the BC survivor groups the results were opposite, with exercise reducing MMP-9 levels (group B vs. C). Furthermore, a significant increase in MMP-2 was observed in group B lymph node metastasis-positive (N+) subjects compared with group A and C N+ subjects. Thus, the results of the present study indicate that various physical activities modulate the levels of circulating MMP-2 and -9 in BC survivors, and the same exercise program induces a different effect when undertaken by healthy subjects and BC survivors. These results

may have important implications with regard to the selection of appropriate physical activities for BC survivors, leading to improvements to their survival and prevention of recurrence, as well as amelioration of physical function, quality of life and fatigue.

Introduction

Breast cancer (BC) is the most common malignancy in women worldwide. Novel breast cancer cases and cancer-associated mortalities accounted for 29 and 15%, respectively, of all female cancers in 2015 (1). Lifestyle is considered an important risk factor for BC. All types of physical activity appear to reduce the risk of BC, although the evidence of this association is stronger for BC occurring in post-menopausal women than pre-menopausal women (2,3).

Studies have shown that the risk of BC is ~25% lower in the most active trained women compared with sedentary women (4,5). The BC risk is 17% higher in sedentary women compared with women that perform physical activity (6). In addition, the risk of BC decreases by 5% for every 2 h per week in women performing moderate or vigorous recreational activity (7). Physical activity has also been demonstrated to reduce the risk of BC recurrence in BC survivors (8,9). Therefore, The American Cancer Society recommends that cancer survivors partake in 150 min of physical activity per week (10,11).

The mechanism underlying the health benefits of regular exercise may involve the cumulative anti-inflammatory effects of repeated physical exercise (12,13). Regular exercise promotes the synthesis of myokines that modulate the inflammatory response (14). In addition, exercise may affect the levels of matrix metalloproteinases (MMPs) (15,16).

MMPs are a family of structurally and functionally related proteinases that are characterized by their ability to degrade the extracellular matrix (ECM), and their involvement in the normal functioning of various tissues during growth, development and aging (16,17). MMP expression is induced by a number of factors, including inflammatory cytokines, hormones, growth factors and oncogenes (18,19). In addition, MMPs are implicated in tumor invasion and metastasis (20,21).

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MMP-2 and -9 have been reported to exhibit an important ECM homeostatic function during muscle growth, development and repair processes (16). Previous studies have demonstrated that physical exercise affects the levels of MMPs following acute and chronic therapies in humans (22-24). A marginal increase in MMP-9, but not MMP-2, serum concentration has been demonstrated to occur following acute physical exercise (25). In particular, MMP-2 and -9 serum levels increase following exercise training that incorporates resistance-type exercise, as the mechanical stressors result in ECM remodeling (26). MMP-2 and -9 hydrolyze components of the basement membrane, and regulate tumor growth and metastasis (27). Activated MMP-2 is located in the protruding section of the cell-penetrating matrix. Its activation facilitates the passage of tumor cells through the ECM and the basal membrane of the blood vessel wall, thus promoting invasion of tumor cells (28). In addition, MMP-2 and -9 are gelatinases; thus, their role in the degradation of gelatin leads to the release of signaling molecules from the ECM that aid cell migration and angiogenesis (29). Previous studies have investigated the effects of acute short duration exercise on MMP concentrations in skeletal muscle and plasma (30,31). However, it remains unclear whether different exercise training programs promote different MMP responses (32). To the best of our knowledge, no previous studies have investigated the effect of exercise on the modulation of MMP-2 or -9 serum concentration in BC survivors.

In our previous study, it was demonstrated that muscle-derived cytokines are released following a dragon boat training program and the resulting decreased oxidative stress conditions are responsible for the health benefits observed in patients with BC (14). In the present study, the effect of two different exercise training programs on MMP-2 and -9 serum concentration was investigated in BC survivors and healthy subjects. In addition, the association between MMP serum concentration and lymph node metastasis was investigated in BC survivors. The patients enrolled in the current study were active individuals involved in dragon boat paddling or other recreational physical activities.

Dragon boat paddling is a competitive sport that originated in China >2,000 years ago (33). Dragon boat paddling is a repetitive, vigorous physical exercise that increases flexibility, aerobic capacity and strength, and provides a novel approach for upper body rehabilitation following breast surgery and radiation treatment. This exercise has been reported to reduce the risk of developing lymphedema in women at the highest risk by 70% (34).

The present study may have important implications for BC survivors, with regard to their lifestyle choices and participation in physical activity.

Patients and methods

Patients. A total of 66 women were enrolled in the present study, between May and December 2014, in collaboration with Associazione Pagaie Rosa Dragon Boat Onlus (www.pagai-erosa.org). The cohort included 46 women who were BC survivors and 20 healthy subjects. Blood from patients was drawn at the Day Hospital of Don Gnocchi Foundation (Rome, Italy). BC survivors were divided into three different groups:

Group A, 17 women who participated in recreational dragon boat paddling (mean \pm standard deviation age, 53.4 ± 8.8 years); group B, 14 women who participated in other recreational physical activities (age, 53.9 ± 11.2 years); and group C, 15 sedentary women (age, 59.6 ± 9.9 years). The 20 healthy women were divided in two groups: Group D, 10 women who participated in other recreational physical activities (age, 49.4 ± 7.8 years); and group E, 10 sedentary women (age, 51.0 ± 8.1 years) (Table I). All the BC survivors were enrolled 10 \pm 5 years post-surgery. The exclusion criteria included cardiovascular disease, acute somatic symptoms, including fever or infection, uncontrolled hypertension, uncontrolled pain or any other condition that contraindicated exercise training in BC survivors or healthy subjects (35). It was not possible to enroll a control group of healthy subjects who participate in dragon boat racing as there is only a small number of individuals who play this sport in Rome. In addition, the effects of dragon boat mediated-release of MMPs in healthy subjects was not part of the scope of the present study.

Anthropometric parameters, including height, weight and body mass index (BMI), were determined for all participants at the beginning of the study (Table I). The TNM status was also determined for all the patients (Table II) (36). No clinical or radiographic evidence of distant metastases were present.

The present study was performed in accordance with the Declaration of Helsinki and written informed consent was provided by all subjects prior to enrolling in the study. The study was approved by the ethics committee of the Fondazione Policlinico Tor Vergata (Study Design DRAGON BOAT-REGISTRO SPERIMENTAZIONI protocol number 150/13).

Training program. The present study did not involve analysis of MMPs prior to training. Thus, the training program did not have a specific duration, and patients could be enrolled at any time during their training. All subjects from groups A, B and D were already performing identical physical activities to those described below prior to the start of the study. Sedentary subjects were only in groups C and E.

Group A. All subjects in group A participated in recreational dragon boat paddling once a week for 120 min, and were supervised by an exercise trainer.

The 120-min dragon boat paddling session included a 30 min dry land warm up as activation exercise for the whole body, followed by 90 min of dragon boat paddling training. The dragon boat paddling training consisted of: A 20-min rowing warm up on the right and left sides of the boat (changing side every 10 min for symmetric training); 50 min rowing with stroke-rate regulated by sound feedback (tambourine) and rowing technical skill exercises (resting in relation to the intensity of the exercise); and 10 min training on competition distances (200 or 500 meters repeated at last twice). Finally, the subjects performed a 10 min cool-down and 10 min of stretching exercises, focusing on the upper limbs.

Groups B and D. All subjects in groups B and D participated in other recreational physical activities (the same for the two groups) 3 times a week for 60 min, and were supervised by an exercise trainer.

The 60-min recreational physical activities included a 10-min warm-up, 30 min endurance training (treadmill 6.5-8.0 km/h, cyclette 55-65 rpm or aerobic dance) and 20 min

Table I. Anthropometric parameters of enrolled subjects.

Group	Subject status	Type of physical activity	Enrolled subjects, n	Age, years ^a	Height, cm ^a	Weight, kg ^a	BMI ^a
A	BC	Dragon boat paddling	17	53.4±8.8	161.9±7.5	60.2±9.4	22.9±2.4
B	BC	Recreational physical activity	14	53.9±11.2	164.2±7.9	64.3±10.2	23.9±3.5
C	BC	Sedentary	15	59.6±9.9	164.1±5.8	67.4±9.1	25.0±3.3
D	H	Recreational physical activity	10	49.4±7.8	164.3±2.5	61.6±5.4	22.9±2.4
E	H	Sedentary	10	51.1±8.1	163.3±4.1	56.3±6.0	21.2±3.0

^aMean ± standard deviation. BMI, body mass index; BC, breast cancer; H, healthy.

strength training (2-4 kg). The warm up involved large muscle groups with low-intensity activities (slow run and execution of joint mobility exercises), gradually preparing the body to pass from the rest condition to the state of activation, with progressive increase of intensity. Aerobic dance, performed to music, was physical exercise that combined rhythmic aerobic exercise with strength training routines to improve flexibility, muscular strength and cardiovascular fitness. Finally, the subjects performed 10 min of stretching exercises. Each subject enrolled in groups B and D performed treadmill, cyclelette or aerobic dance alternately during the week.

Measurement of MMP-2 and -9 levels. Serum samples were obtained during the training program. Blood was drawn without anticoagulant a total of 48 h after training (and between two training sessions) from all subjects between 9:00 and 10:00 a.m., following an overnight fast, and was allowed to coagulate for 20-30 min. Blood samples were not obtained before the training program began as the aim of the present study was to compare the effects of various physical activities on the circulating levels of MMP-2 and MMP-9 in well-trained subjects. Serum was separated by centrifugation (800 x g for 10 min at 4°C), and all specimens were aliquoted, frozen and stored at -80°C within 2 h of collection. Commercially available human ELISA kits (high-sensitivity Quantikine; catalog nos., MMP200 and DMP900; R&D Systems, Inc., Minneapolis, MN, USA) were used to determine the MMP-2 and -9 levels, according to the manufacturer's instructions. Optical density was measured using a microplate reader at a wavelength of 450 nm (Model 550; Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Statistical analysis. All data were initially entered into a Microsoft Excel for Office 2007 database (Microsoft Corporation, Redmond, WA, USA) and statistical analysis was performed using SPSS version 19.0 (IBM SPSS, Armonk, NY, USA). Data are presented as the mean ± standard deviation for parameters with (normal) Gaussian distributions (following confirmation with histograms and the Kolgomorov-Smirnov test) or as frequencies (%) for occurrences.

All data obtained were compared by using one-way analysis of variance. Significant results were examined by Bonferroni's test for one or more factors, respectively. $P < 0.05$ was considered to indicate a statistically significant difference.

Table II. Tumor stage and lymph node metastasis of breast cancer survivors (n=46).

Parameter	Patients, n
Tumor stage	
I	24
II	14
III	8
Lymph node metastasis	
Present	25
Absent	21
Histological type	
Ductal	42
Lobular	4

To determine the number of samples necessary in each group, power analysis was performed based on an α of 5% and variation of 40% of the variable outcome MMP-2. The t-test showed that an amplitude of $n=8$ for both groups reached a power ($1-\beta$) of 94%. This was only calculated for MMP-2 as it was the only significantly modified parameter. The variation of MMP-9 concentration was not significant.

Results

No significant differences in age or height were observed between the groups. Conversely, the weight (group C vs. group E, $P=0.024$) and BMI (group C vs. group E, $P=0.035$) were significantly higher in sedentary BC survivors in comparison with healthy sedentary women (Table I). Table II shows tumor stage and lymph node metastasis of breast cancer survivors enrolled in the study.

To determine the effect of physical exercise on MMP-2 and -9 serum concentration in BC survivors and healthy subjects, two different exercises training programs were compared. The results revealed a difference in MMP serum concentration between BC survivors and healthy subjects participating in different exercise programs.

Specifically, the MMP-2 serum concentration was significantly increased in the sera of BC survivors participating in recreational physical activity (group B) compared with those participating in dragon boat paddling (group A; B vs.

Table III. Serum matrix metalloproteinase-9 concentration in BC dragon boat group (A), BC physical activity group (B), BC sedentary group (C), H physical activity group (D) and H sedentary group (E).

Dragon boat/physical activity group (mean \pm sd)	Sedentary group (mean \pm sd)	P-value
A (99.9 \pm 54.5)	C (160.9 \pm 131.2)	0.679
B (114.3 \pm 87.8)	C (160.9 \pm 131.2)	0.999
D (93.6 \pm 58.3)	E (67.8 \pm 22.5)	0.999

BC, breast cancer; H, healthy; sd, standard deviation.

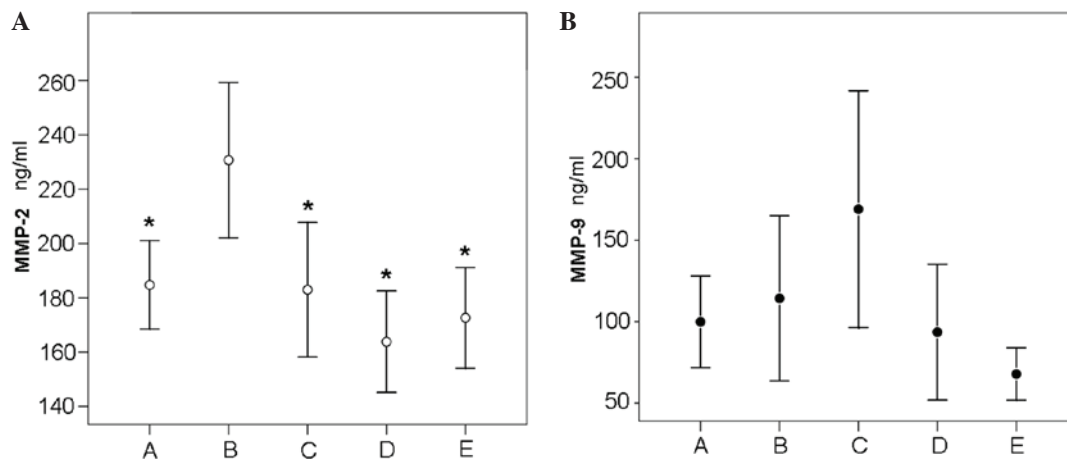


Figure 1. MMP-2 and MMP-9 serum concentration in breast cancer survivors and healthy subjects. (A) Significant differences in MMP-2 levels were identified in group B when compared with groups A ($P=0.028$), C ($P=0.008$), D ($P=0.001$) and E ($P=0.02$). * $P<0.05$ vs. B. (B) No significant differences in MMP-9 levels were identified between groups A-E. MMP, matrix metalloproteinase.

A, $P=0.028$), or with sedentary BC survivors (group C; B vs. C, $P=0.008$; Fig. 1A). Statistically significant differences were observed between group B (230.7 \pm 49.5) and group A (184.7 \pm 31.7; $P=0.028$) and between group B and group C (182.9 \pm 44.8; $P=0.008$).

However, no significant differences in MMP-2 serum concentration were identified between BC patients participating in dragon boat paddling (group A) and sedentary BC survivors (group C; $P=0.999$). Notably, the mean MMP-2 serum concentration was significantly higher in BC survivors participating in recreational physical activity (group B, 230.7 \pm 49.5) compared with healthy subjects participating in recreational physical activity (group D; 163.8 \pm 26.1; $P=0.001$) and between group B and sedentary healthy subjects in group E (172.6 \pm 25.9; $P=0.021$). By contrast, no significant differences in mean MMP-2 levels were identified between the sedentary BC survivors (group C; 182.9 \pm 44.8) and sedentary healthy subjects (group E; 172.6 \pm 25.9; $P=0.999$).

The effect of exercise on MMP-9 serum concentration revealed a different trend between BC survivors and healthy subjects. Notably, physical exercise decreased the MMP-9 levels in BC survivors in group A and group B compared to group C, while it increased the MMP-9 serum levels in healthy subjects in group D compared with group E (Fig. 1B).

Although variations in mean MMP-9 levels were identified between dragon boat paddling BC survivors and sedentary BC survivors (group A, 99.9 \pm 54.5; group C, 169.0 \pm 131.2; $P=0.679$),

recreational physical activity BC survivors and sedentary BC survivors (group B, 114.3 \pm 87.8; group C, 169.0 \pm 131.2; $P=0.999$), and healthy recreational physical activity and healthy sedentary individuals (group D, 93.6 \pm 58.3; group E, 67.8 \pm 22.5; $P=0.999$), these differences were not statistically significant (Table III).

The association between MMP-2 and -9 serum levels, and the presence of lymph node metastasis were also investigated in groups A-C. No statistical differences in mean MMP-2 serum levels were identified between lymph node metastasis positive (N+) BC survivors of the dragon boat paddling group (group A; 186.5 \pm 24.4) and N+ sedentary BC survivors (group C; 188.4 \pm 45.3) or between the lymph node metastasis negative (N-) BC survivors of the dragon boat paddling group (group A; 181.5 \pm 44.7) and the N- sedentary BC survivors (group C; 176.7 \pm 46.8) (Fig. 2).

However, significant differences in mean MMP-2 serum levels were identified between N+ BC survivors that participated in recreational physical activity (group B; 260.7 \pm 58.2) and N+ sedentary BC survivors (group C; 188.4 \pm 45.3; $P=0.013$). No significant differences were identified between N- BC survivors participating in recreational physical activity (group B; 208.2 \pm 28.1) and N- sedentary BC survivors (group C; 176.7 \pm 46.8; $P=0.913$). MMP-2 serum levels were significantly higher in N+ BC survivors of the recreational physical activity group (group B; 260.7 \pm 58.2) compared with N+ BC survivors of the dragon boat paddling group (group A;

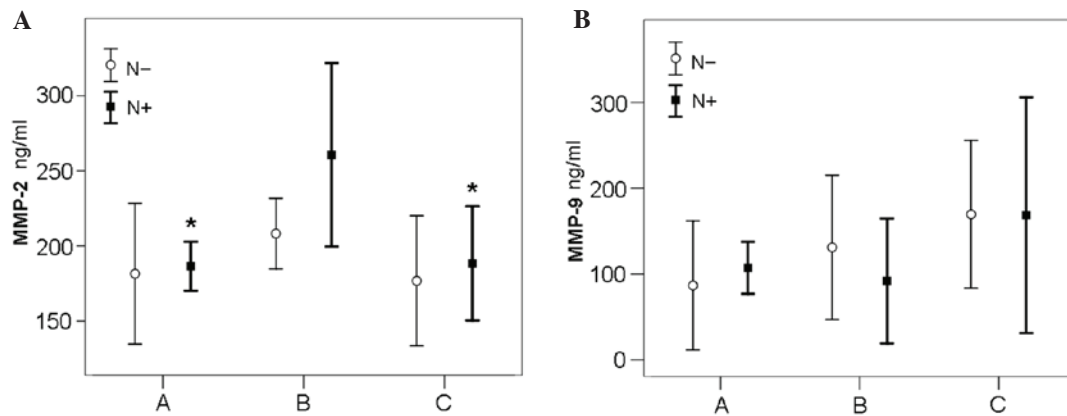


Figure 2. MMP-2 and MMP-9 serum levels in N+ and N- breast cancer survivors. (A) Significant differences in MMP-2 levels were identified in the BN+ group when compared with the CN+ ($P=0.013$) and AN+ ($P=0.007$) groups. * $P<0.05$ vs. N- group. (B) No significant differences in MMP-9 levels were identified between groups A-C. Number of survivors: Group A, N- 6; N+ 11; group B, N- 8; N+ 6; group C, N- 7; N+ 8. MMP, matrix metalloproteinase; N+, lymph node metastasis positive; N-, lymph node metastasis negative.

186.5 \pm 24.4; $P=0.007$) (Fig. 2). No significant differences were observed in MMP-9 serum levels between the N- and N+ subgroups among groups A-C.

Discussion

Regular physical exercise improves physical function, quality of life and reduces fatigue in BC survivors (37,38). It also decreases the risk of BC recurrence and reduces BC-specific mortality in BC survivors (39,40).

Physical exercise is considered an essential factor for improving cancer survival. Previous studies have indicated that exercise interventions are efficacious in men with prostate cancer (41,42) and in multiple myeloma (43,44). In endometrial, ovarian and colorectal cancer survivors, lifestyle interventions that include regular participation in physical activity are associated with positive health outcomes (45,46). Furthermore, it has been demonstrated that individuals who are physically active after being diagnosed with breast or colon cancer exhibit a higher rate of survival compared with those that are physically inactive (47).

Circulating MMPs are involved in the breakdown of the ECM in carcinogenesis and cardiovascular diseases (48). MMPs modulate the activation of growth factors, cytokines and angiogenesis, facilitating physiological adaptations to exercise (18,49).

However, high levels of mechanical stress, such as that induced by high physical impact exercise, may activate the local production of MMPs in skeletal muscle. Rullman *et al* (50) reported that a single session of exercise induces MMP-9 activation, causing a marked increase in serum MMP-9 concentration. Kadoglou *et al* (24) demonstrated that exercise reduces MMP-9 serum levels, but not MMP-2 levels, in patients with type 2 diabetes. Additional studies have investigated the function of circulating MMP-2 and MMP-9 in BC patients (51,52); the results revealed increased MMP-9 expression and activity in malignant tumors, and enhanced MMP-2 activity in malignant tumors exhibiting high estrogen receptor expression. These studies indicate that serum levels of MMP-2 or -9 may present useful markers for the staging and prognosis

of BC and other types of cancer (53), such as prostate adenocarcinoma, in association with prostate-specific antigen levels, gastric cancer and lung carcinoma (54-56).

At present, the effect of different exercise training programs on MMP-9 and -2 serum levels in human subjects remains unclear (57). It was previously demonstrated that endurance training decreases MMP-9 concentration (58) and aerobic training reduces MMP-2 plasma levels in individuals with pathological conditions (25). Urso *et al* (26) observed that the variation in MMP-2 and -9 serum levels is dependent on different exercise training regimens. Thus, understanding which type of exercise and intensity may reduce the risk of recurrence in BC patients is important.

The aim of the present study was to determine the effect of two different exercise training programs on the serum MMP-2 and -9 levels of healthy subjects and BC survivors. The results did not identify any significant differences in MMP-2 serum levels in healthy subjects that participated in recreational physical activity; there were no significant differences in MMP-2 serum levels in group D compared with group E, and although MMP-9 levels increased in group D compared with group E, these differences were not statistically significant. Among the BC survivors, no significant differences in circulating MMP-2 levels were identified between the dragon boat paddling (group A) and sedentary (group C) groups. However, a significant increase in serum MMP-2 was identified in the recreational physical activity BC group (group B) compared with the sedentary BC group (group C). This difference in circulating MMP-2 levels following exercise appears to be dependent on the different exercise training programs undertaken by the BC survivors; however, the same exercise program did not induce significant increases in the MMP-2 levels of healthy subjects.

MMP-9 serum levels were higher in sedentary BC survivors (group C) than in healthy subjects (group E). Furthermore, MMP-9 serum levels were decreased in both groups of recreationally active BC survivors (groups A and B) compared with sedentary BC survivors (group C). This result may be associated with tumor necrosis factor- α (TNF- α) expression, as TNF- α is a stimulator of MMP-9 production and is decreased following exercise (59,60).

However, exercise increased MMP-9 serum levels in the healthy subjects (group D vs. E). This difference reflects the impact of exercise training and may be associated with an inflammatory response (61). Rullman *et al* (62) reported that endurance exercise enhances MMP-9 but not MMP-2 levels in human skeletal muscle in healthy subjects.

Our previous study regarding exercise in BC patients contributed to the understanding of the modulatory effects of exercise on MMP-2 and MMP-9 regulation. It was demonstrated that muscle-derived cytokines are released following a training program, while decreasing the oxidative stress (14). Changes in MMP serum levels may reflect the impact of exercise on the production of inflammatory molecules. It is hypothesized that exercise causes a reduction in free radicals and an increase in antioxidant defenses, which may influence the inflammatory conditions and subsequently alter MMP serum concentrations. The different effects of exercise observed on MMP-2 and -9 serum levels may be associated with different dose-dependent responses to physical activity (57).

The results of the present study indicate that the type of physical exercise (intensity, volume, frequency and length) may effect MMP-2 and MMP-9 plasma levels. The effect of the same exercise program was different in healthy subjects and BC survivors. To the best of our knowledge, it remains to be elucidated why this differential response occurs. To understand these findings, it is helpful to analyze the weekly frequency of exercise, as well as the period of rest and the muscle strength applied. In the present study, exercise was performed once a week for 120 min with a rest time of 6 days in the dragon boat paddling group (group A). The muscle strength applied during paddling is proportionate to an anti-gravity exercise in accordance with the variables of hydrodynamics. The other recreational physical activity groups (group B and D) trained 3 times a week for 60 min, and thus the rest time was shorter than that of the dragon boat paddling group. For groups B and D, the muscle strength applied was proportionate to the extra load used during the exercise session without the benefit of hypogravity. All these variables have been shown to affect MMP-2 serum levels in BC survivors.

Coagulation enzymes are involved in MMP-2 activation for example, urokinase plasminogen converts plasminogen to plasmin, which promotes tumor growth and angiogenesis, degrading the ECM and basement membrane, and activating pro-MMPs (63). MMPs and urokinase plasminogen activators are involved in tumor invasion and metastasis. The results of the present study demonstrated that BC survivors exhibit increased MMP-2 levels when compared with healthy subjects. These results may be due to an increase in urokinase plasminogen activator expression, as it has been demonstrated that exercise increases plasma fibrinolytic activity. Furthermore, exhaustive exercise is associated with increased expression of plasminogen activator in plasma (64), and exercise-stimulated thrombin and plasmin generation were previously identified in healthy subjects that exercised for a significantly longer duration (65). Prolonged physical exercise is associated with numerous changes in blood hemostasis and higher levels of aerobic fitness are associated with increased fibrinolytic activity (66,67), while MMP-2 activation is inhibited by plasmin inhibitors (68).

Previous studies have reported that serum levels of MMP-2 and MMP-9 are significantly higher in BC patients

than in control subjects (69,70). Furthermore, patients with N+ cancer exhibit significantly higher MMP-2 and MMP-9 activity than those with N- cancer (53). The results obtained in the present study revealed a significant increase in MMP-2 but not MMP-9 concentration in N+ BC survivors compared with N- BC survivors. Furthermore, different exercise regimes exhibited different effects on circulating MMP-2 and MMP-9 levels.

In conclusion, the contradictory results obtained in the present study may be due to the fact that exercise of various intensities were performed in previous studies. Therefore, further studies are required to determine the biological significance of MMP levels in the adaptation of skeletal muscle to physical activity in cancer patients and to explore how different types of physical exercise affect MMP production. Overall, the results of the present study indicate that various physical activities modulate the levels of circulating MMP-2 and -9 in BC survivors, and an identical exercise program induces a contrasting effect when undertaken by healthy subjects and BC survivors.

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