Potential Anticarcinogenic Effects From Plasma of Elderly After Exercise Training: A Pilot Study

Alessandra Peres

UFCSPA: Universidade Federal de Ciencias da Saude de Porto Alegre

Gilson Domeles (✉ gilsonpd@hotmail.com)

UFCSPA: Universidade Federal de Ciencias da Saude de Porto Alegre <https://orcid.org/0000-0001-6524-3204>

Gisele Branchini

UFCSPA: Universidade Federal de Ciencias da Saude de Porto Alegre

Fernanda Nunes

UFCSPA: Universidade Federal de Ciencias da Saude de Porto Alegre

Pedro Romão

UFCSPA: Universidade Federal de Ciencias da Saude de Porto Alegre

Luciele Guerra Minuzzi

UNESP: Universidade Estadual Paulista Julio de Mesquita Filho

Fabio Lira

UNESP: Universidade Estadual Paulista Julio de Mesquita Filho

Mateus Cavalcante

UFRGS: Universidade Federal do Rio Grande do Sul

Viviane Elsner

UFCSPA: Universidade Federal de Ciencias da Saude de Porto Alegre

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Short Report

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Abstract

This study aimed to evaluate the impact of exercise training plasma on *in vitro* prostate cancer cell viability and proliferation. PC3 prostate cancer cells were incubated with plasma obtained from young women with high and low physical fitness (PF) and with the plasma collected from institutionalized elderly before and after multimodal exercise training. Plasma from High PF women induced the lowest cell viability and proliferation after incubation time. PC3 cells presented lower cell viability and diminished rates of cell proliferation after the incubation with post-training plasma samples of elderly. The incubation of PC3 cells with post-training plasma of elderly decreased the mitochondrial membrane polarization and increased mitochondrial reactive oxygen species (ROS) production without changes in cytosolic ROS. Post-training plasma did not change apoptosis or necrosis rates in the PC-3 cell line. Multimodal exercise training increased the plasma levels of IL-2, IL-10, IFN- α , and FGF-1, and decreased TNF- α concentrations in institutionalized elderly. In conclusion, we showed that systemic adaptations in plasma mediators of institutionalized elderly may alter cell viability and proliferation by targeting mitochondrial ROS in a prostate cancer cell line.

Introduction

Biological aging impacts several aspects of the host immune system, characterized by a chronic inflammatory state associated with an accumulation of senescent exhausted myeloid and lymphoid cells (1). The chronic increases in inflammatory molecules are associated with the etiology and clinical course of most age-related diseases and mortality, including several cancer types (2). In this sense, prostate cancer becomes more prevalent with aging men, once that innate and adaptive inflammatory response enhancement contributes to prostate carcinogenesis. (3).

Epidemiological data from men with non-metastatic prostate cancer suggest that regular moderate-to-vigorous physical activity in a frequency > 3 hours per week significantly reduce the risk of prostate cancer-related death (4). Furthermore, emerging preclinical and clinical studies described several immunological and physiological adaptations that may prevent or treat prostate cancer progression, including enhanced T-cell and natural killer functional activity as well as increased myokine release by contracting muscle (5). Changes in systemic inflammation and in the composition of blood factors induced by exercise training adaptations may directly impact cancer cell viability. Past data highlighted the role of acute exercise-conditioned serum from young and aged humans to decrease cancer cell viability, indicating that changes in blood molecules (i.e., hormones, cytokines, and reactive oxygen species) during acute bouts contribute to the anticarcinogenic potential (6–8). However, few studies evaluated the potential effects of exercise training to induce anticarcinogenic effects in plasma of elderly individuals(6). Furthermore, it was previously reported that decreases in prostate cancer cell viability induced by pharmacological drugs is mediated by changes in mitochondrial function (9). Mitochondria are emerging players in the tumorigenic process by maintaining the energetic capabilities of cancer cells(10). In prostate cancer, it is now clearly that mitochondria are involved in the malignant process, cell proliferation aggression and metastasis formation(11). This study evaluated the role of plasma of the

elderly of elderly individuals submitted to multimodal exercise training on viability and proliferation of an immortalized prostate cancer androgen unresponsive PC3 cells.

Methods

Participants

Eight institutionalized older adults (aged 73.38 ± 11.28 y; body mass index 27.8 ± 4.9 kg/m²) living in a long-term facility in Porto Alegre City, south of Brazil. The inclusion and exclusion criteria were previously reported (12). Participants were not engaged in structured exercise training protocols in a period prior to 6 months before the trial. The Ethics Research Committee of Centro Universitario Metodista-IPA, Brazil approved the current study number 3.376.078. All participants signed written informed before enrollment and all procedures were in conformity with the Declaration of Helsinki. Additionally, we collected venous blood samples from young lean men with high physical fitness (PF) (High PF: age, 27.8 ± 5.6 y; body mass index, 24.3 ± 1.3 kg/m², VO_{2Peak} 49.7 ± 2.67 mL.kg.min) and low PF (Low PF: age, 29.8 ± 2.7 y; body mass index, 23.1 ± 2.1 kg/m², VO_{2Peak} 37.8 ± 3.1 mL.kg.min) as controls of the study. Physical fitness was evaluated by cardiopulmonary exercise test as previously described(13).

Study design and training protocol

Elderly participants participated in a multimodal exercise training (8 weeks, 2x/week, 60 minutes each session) in this noncontrolled trial. The multimodal protocol used in the current study was based on a previous study (14). Specifically, each session was divided into the four following moments: 1) warm-up (5 minutes), with stretching and active upper/lower limb exercises; 2) exercises focused on cardiovascular capacity, strength, balance/agility and flexibility (25 minutes), which included walking, stationary gait, resistance exercises for the main upper/lower muscle groups, unipodal support with open/closed arms over the chest, anterior/lateral inclination and static stretches; 3) exercises focused on perception and cognition (such as double-task), attention, memory and processing of requested actions (25 minutes), such as walking and naming fruit/colour names, completing previously established circuits, attending to requested verbal commands and memorizing motor/verbal signals; and 4) relaxation and breathing techniques (5 min).

Blood samples and Cytokine measurement

Fasting venous blood samples were collected from the antecubital vein into EDTA tubes (8 mL), centrifuged (1000 *g*, 10 minutes), aliquoted in microtubes and stored at -80°C. Blood collection was performed before the first exercise session and 48 h after the last exercise bout. The systemic levels of interleukin(IL)-1 α , IL-1 β , IL-2, IL-6, IL-10, IL-17, interferon (IFN)- α , tumour necrosis factor (TNF)- α (all from ThermoFisher, USA), fibroblast growth factor (FGF)-1, platelet-derived growth factor (PDGF) and transforming growth factor (TGF)- α (all from RayBiotech, USA) were determined by *Enzyme Linked Immunosorbent Assay* (ELISA) following manufacturer's instructions in a microplate reader (EzReader, Biochrom, USA). The coefficients of variation of all assays were always < 7.5%.

Cell Culture Experiments

The PC3 prostate cancer cell line from the American Type Culture Collection (ATCC® CRL-1435™) was used in this study. Cells were cultured in 75 cm² flasks using Roswell Park Memorial Institute medium-1640 (RPMI-1640) supplemented with 10% (v/v) fetal bovine serum (FBS), 0.1 mg/mL streptomycin, 100 U/mL penicillin. Cells were maintained in a humidified incubator at 37°C and at 5% CO₂, during a maximum of 15 passages. During experiments, 10% FBS was replaced with 10% of human plasma obtained before or after the training sessions.

After a 48-h treatment, the cell viability assay (MTT and LDH release) was performed by colorimetric reduction of MTT (3-(4,5-dimethylthiazole bromide-2-yl)-2,5-diphenyltetrazolium bromide) to formazan. Samples were read by a spectrophotometer at 492 nm (15). Appropriate controls with DMSO (extract solvent) and blank liposome were performed, to eliminate the membrane solvent hydrolysis effect in results interpretation. LDH activity was evaluated in the commercial kit (LDH Roche, Brazil). Released LDH in the culture media was coupled to an enzymatic assay yielding a red color, the intensity of which was measured at 490 nm by a microplate reader. All experiments were done in triplicate and results were shown as the mean of triplicates from three independent experiments.

The proliferative response of PC3 was evaluated by the decay of CFSE fluorescence using the FACSCalibur (Becton Dickinson, San Jose, CA) flow cytometer equipped with a blue argon laser (488nm) and a 530/30nm bandpass filter. The CFSE fluorescence was analyzed in histograms of FL1 channel. The “M1 region” was defined as CFSE-stained cells derived from unstimulated cultures, which represented the peak of quiescent cells, and the M2 region was defined as proliferative cells according to the peaks of CFSE intensity. Apoptosis and necrosis of PC3 cells was measured using FITC Annexin V with propidium iodide Apoptosis/necrosis Detection Kit (556547, BD Biosciences) according to the manufacturer’s instructions using an FACSCalibur flow cytometer (BD Biosciences).

Mitochondrial membrane polarization, cytosolic and mitochondrial reactive oxygen species (ROS) analysis were performed after 12-h of *in vitro* PC3 incubation with pre- and post-exercise training plasma. The mitochondrial membrane potential ($\Delta\Psi$ m) was quantified according to a method previously described (16), using the fluorescent dye rhodamine 123 (Rh 123, Sigma-Aldrich, USA). Mitochondrial superoxide generation in live cells was assessed with MitoSOX Red (Invitrogen, ThermoFisher, USA). Cytosolic ROS production was evaluated using the reagent 20,70-dichlorofluorescein diacetate (DCF-DA), which becomes fluorescent when oxidated by ROS (Sigma Aldrich, USA). Analyses were performed by using CELLQuest Pro Software (BD Bioscience) on a FACSCalibur flow cytometer (BD Bioscience).

Statistical analysis

Data were analyzed in GraphPad Prism 8.0 (USA). Data were presented as mean $\pm$ standard deviation. Before-after exercise training comparisons were analyzed by Paired Student t-test. The effect of plasma obtained from High PF and Low PF as well as the plasma collected before and after exercise training from elderly individuals on cell viability evaluated by MTT assay, LDH release and PC3 cell proliferation

were compared by one-way ANOVA followed by Bonferroni's post-hoc. Pearson's correlations were applied to verify the relationship between systemic inflammatory mediators and *in vitro* PC3 measurements. p value ≤ 0.05 were considered statistically significant.

Results

Prostate cancer cell viability and proliferation are modulated by physical fitness status and exercise training practice

Firstly, we evaluated the effects of plasma obtained from young individuals with different PF status (low and high) and from elderly individuals who are engaged in a multimodal exercise training (Figure 1). Cell viability was lowest ($p=0.04$ vs. low PF; $p=0.001$ vs. pre-training plasma of elderly; $p=0.01$ vs. post-training plasma of elderly) and higher LDH released ($p=0.03$ vs. low PF; $p=0.001$ vs. pre-training plasma of elderly; $p=0.001$ vs. post-training plasma of elderly) were observed after incubation of PC3 cells with plasma collected from young individuals with high PF. The percentage of cells undergo proliferation was lower in the incubation of PC3 with plasma from High PF group compared to low PF ($p=0.03$), pre-training elderly ($p=0.001$), post-training elderly ($p=0.001$). The plasma of young individuals with low PF induced low PC3 cell viability ($p=0.02$ vs. pre-training elderly) and cell proliferation ($p=0.01$ vs. pre- and post-training elderly), and increased LDH release ($p=0.01$ vs. pre- and post-training elderly) compared to elderly group. Interestingly, the incubation of PC3 prostate cancer cell line with post-training plasma of elderly resulted in lower cell viability ($p=0.03$) and cell proliferation ($p=0.02$) and higher LDH release ($p=0.02$) compared to the pre-training plasma condition. Thus, higher PF status and exercise training practice have anticarcinogenic effects in a prostate cancer cell line.

PC-3 Prostate cancer cell line were incubated with plasma of young high physical fitness (PF), young low PF, and with the plasma of institutionalized elderly individuals obtained before training and 48 h after 8 weeks of exercise training. Cell viability was evaluated by MTT (A) and LDH activity (B), and proliferation by the drop of CFSE fluorescence in PC-3 cells (C and D) after 48 h of cell culture.

Plasma acquired after multimodal exercise training changes the mitochondria membrane potential and increases mitochondrial reactive oxygen species production

Next, we evaluated the role of mitochondria-induced reactive oxygen species generation in the anticarcinogenic effects of plasma of trained elderly individuals. After 12-h of PC-3 cell culture, post-training plasma decreased mitochondrial membrane polarization compared to pre-training plasma incubation condition ($p<0.01$). The mitochondria membrane depolarization was accompanied by increased mitochondrial ROS production in the PC3 cell line incubated with post-training serum ($p=0.04$), without changes in cytosolic ROS generation ($p>0.05$). Finally, PC-3 cell line incubation with post-training plasma did not change apoptosis or necrosis cell events ($p>0.05$).

PC-3 Prostate cancer cell line were incubated with plasma of institutionalized elderly individuals obtained before training and 48 h after 8 weeks of exercise training. Histogram of rhodamine-123 (A) evaluated the

mitochondrial membrane potential (B), histogram of Mitosox (C) quantified mitochondrial superoxide (D) generation, and histogram of DCF (E) evaluated cytosolic reactive oxygen species (F) production after 12-h of PC-3 cell line incubation with plasma obtained before and after exercise training. Apoptosis (Annexin-V+ cells) or necrosis (Propidium iodide+ cells) did not differ before – after comparison (G).

Statistical analysis conducted through Paired student t-test ($p < 0.05$).

Multimodal exercise training alters systemic cytokine levels of institutionalized elderly individuals

Increases in the plasma levels of Interleukin (IL)-2 ($p = 0.01$), Interferon-alpha (IFN- α) ($p = 0.01$), and Fibroblast growth factor (FGF)-1 ($p = 0.01$) occurred after the training period. On the other hand, tumor necrosis factor-alpha (TNF- α) concentrations decreased ($p = 0.03$) after training. In addition, IL-10 levels tended to be higher after training compared to the baseline values ($p = 0.06$). No statistical differences were observed in IL-1ra, IL-1 β , IL-6, platelet-derived growth factor (PDGF), IL-17a, or transforming growth factor-alpha (TGF- α) ($p > 0.05$).

Statistical analysis performed by Paired t-test ($p < 0.05$).

Post training cytokine plasma levels were correlated with *in vitro* experiments MTT, LDH, CFSE cell proliferation, cytosolic ROS, mitochondrial membrane polarization and mitochondrial ROS in PC3 prostate cancer cell line, and heat map correlation is presented in Figure 3. TNF- α levels correlated with mitochondrial ROS ($r = 0.82$; $p = 0.04$). IL-6 levels correlated with supernatant LDH content ($r = 0.94$; $p = 0.005$) and CFSE cell proliferation ($r = 0.82$; $p = 0.04$). IL-1ra concentrations significantly correlated with mitochondrial membrane polarization ($r = 0.88$; $p = 0.01$). IL-17a levels correlated with mitochondrial ROS ($r = 0.74$; $p = 0.04$). Finally, TGF- α inversely correlated with LDH content ($r = -0.83$; $p = 0.01$).

Discussion

The main results of this study were: 1) the incubation of PC3 prostate cancer cell line with plasma acquired from young men with high PF and with post-exercise training plasma of elderly individuals leads to lower cell viability and proliferation rates after 48 h of treatment; 2) conditioned post-training plasma-induced mitochondrial membrane depolarization and higher mitochondrial ROS, but not cytosolic ROS, in PC3 prostate cancer cells without changes in apoptosis/necrosis rate; 3) 8 weeks of multimodal exercise training increases the systemic levels of IL-2, IFN- α , and FGF-1 and decreases the TNF- α concentrations in aged individuals. Taken together, we showed for the first time that multimodal exercise training induces systemic inflammatory adaptations in institutionalized elderly individuals in parallel to the enhanced anticarcinogenic potential of blood mediators against prostate cancer through changes in mitochondrial membrane polarization and mitochondrial ROS generation.

Here, the plasma collected from highly conditioned men, but not from low PF individuals, decreased the PC3 cell viability and lowered cell proliferation. These results are in line with the previously reported by a

series of systematic reviews and meta-analysis recently published who demonstrated the anticarcinogenic potential of peripheral blood factors of exercised individuals (6, 7, 16). Interestingly, the incubation of conditioned plasma obtained from institutionalized elderly engaged in multimodal exercise training decreased both cell viability and proliferation in PC3 prostate cancer cell line. It was hypothesized that exercise-induced biochemical mediators release, mainly myokines and immunomodulatory cytokines, have a critical role in decreasing cancer cell viability and growth (5). Interestingly, apoptosis and necrosis rate were unchanged after the incubation of PC3 prostate cell line with conditioned exercised-plasma, confirming previously data who demonstrate that exercised mediators reduce cell viability without changes in cell death pathways (17, 18). Notably, several studies were conducted using acute exercise session models and the tumor-suppressive effects of chronic longitudinal exercise training is poorly studied.

Here, we describe for the first time the mitochondrial dysfunction in PC3 prostate cell line incubated with post-exercise training plasma of elderly. Targeting cancer cell mitochondria has been long suggested as a therapeutic approach to control cell proliferation and growth. In this sense, several pharmacological therapies alter mitochondria function to induce cell death and lower tumor progression (17). In the present study we show that depolarization of the mitochondrial membrane potential associated with increasing superoxide production (mitochondrial ROS) after 12h of PC3 incubation with conditioned plasma of elderly. Furthermore, mitochondria membrane depolarization leads to translocation of apoptosis-induced factor (AIF) to the nuclei and activation of caspase-12 associated with endoplasmic reticulum to induce cell death(18). Moreover, mitochondrial membrane depolarization directly affects complex II and its function in electric chain transport, leading to ROS generation and the activation of apoptotic cascade(19). However, the lack of changes in apoptosis rate after conditioned plasma incubation may indicate the need of repeated or prolonged incubation time to induce cancer cell death.

Here, exercise training was able to decrease proinflammatory TNF- α levels, suggesting a role to induce anti-inflammatory profile in institutionalized elderly. However, other classic proinflammatory mediators, such as IL-6, IL-17a and IL-1 β , did not change after 8 weeks of multimodal exercise. These results may suggest that the potential anti-inflammatory adaptations observed in previous observational and longitudinal studies (20) may need a longer intervention time than 8 weeks to be achieved. On the other hand, we are the first study to observe increased FGF-1 increased after exercise training period. FGF-1, also called acidic FGF, plays an important role in the regulation of cell survival, cell division, angiogenesis, cell differentiation and migration (21). Interestingly, experimental studies shows that mice treated with FGF-1 restore blood glucose levels and endothelial function, highlighting the role of this growth factor in the vascular health and metabolic control(22). Furthermore, mutated *fgf1* gene is linked to accelerated neurological senescence profile in mice(23). Thus, FGF-1 emerges as an important biological mediator to the control of aging through exercise training.

We found an increases in IL-2 and IFN- α levels in the peripheral blood of elderly after exercise training period. Both IL-2 and IFN- α have strong anti-tumorigenic directly effects against cancer cell, and *in vitro* cytokine treatment of prostate tumor cell lines can effectively alter a number of prostate carcinoma

properties closely associated with tumor invasion and metastatic phenotype(24). In addition, the correlation between post-training cytokine levels and PC3 cell viability, proliferation and mitochondrial function revealed some associations between changes in systemic inflammatory mediators and cancer cell phenotype.

In conclusion, this longitudinal study described for the first time the potential of conditioned plasma to decrease cell viability and proliferation in PC3 prostate tumor cell line. We also demonstrated a new mechanistic pathway by which exercise may alters prostate cell function through mitochondrial function, mainly by mitochondrial membrane depolarization and superoxide formation. These changes were accompanied by alterations in several systemic inflammatory mediators after multimodal exercise training. Collectively, changes in blood factors composition by exercise training contribute to the control of prostate tumorigenesis, suggesting the role of exercise as an adjuvant therapeutic in cancer treatment and prevention.

Declarations

Compliance with Ethical Standards

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Conflict of interest

The author(s) declare no conflict of interests.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Research Committee of Centro Universitario Metodista-IPA, Brazil (protocol number: 3.376.078) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

1. Bauer ME, De la Fuente M (2013) Oxidative Stress, Inflammaging, and Immunosenescence. In: Inflammation, Advancing Age and Nutrition: Research and Clinical Interventions.
2. F B-T GF, CRR S, RM M ME B. The interplay between immunosenescence and age-related diseases. Semin Immunopathol [Internet]. 2020 Oct 1 [cited 2021 Aug 2];42(5):545–57. Available from: <https://pubmed.ncbi.nlm.nih.gov/32747977/>

3. G T, M S, G G, R H, P G, S S, et al (2014) Senescent remodeling of the innate and adaptive immune system in the elderly men with prostate cancer. *Curr Gerontol Geriatr Res* [Internet]. [cited 2021 Aug 2];2014. Available from: <https://pubmed.ncbi.nlm.nih.gov/24772169/>
4. Aicr WCRF, Diet Nutrition, Physical Activity and Cancer: a Global Perspective A summary of the Third Expert Report. [cited 2021 Aug 2]; Available from: <http://gco.iarc.fr/today>
5. JS K, DA G, RU N, DR EG (2021) T. Exercise-induced myokines and their effect on prostate cancer. *Nat Rev Urol* [Internet]. Jun 22 [cited 2021 Aug 3]; Available from: <https://pubmed.ncbi.nlm.nih.gov/34158658/>
6. CM S, AM T, FM HS, MC S R, M F, Effect of exercise-conditioned human serum on the viability of cancer cell cultures: A systematic review and meta-analysis. *Exerc Immunol Rev* [Internet]. 2021 [cited 2021 Aug 2];27:24–41. Available from: <https://pubmed.ncbi.nlm.nih.gov/33965899/>
7. ST O, AR J JM S. The serological responses to acute exercise in humans reduce cancer cell growth in vitro: A systematic review and meta-analysis. *Physiol Rep* [Internet]. 2020 Nov 1 [cited 2021 Aug 2];8(22). Available from: <https://pubmed.ncbi.nlm.nih.gov/33207085/>
8. JH H, GM JM, PA M, JM DGLR H, et al (2020) Mobilizing serum factors and immune cells through exercise to counteract age-related changes in cancer risk. *Exerc Immunol Rev* [Internet]. [cited 2021 Aug 2];26:80–99. Available from: <https://pubmed.ncbi.nlm.nih.gov/32139350/>
9. Seo JH, Agarwal E, Chae YC, Lee YG, Garlick DS, Storaci AM et al Mitochondrial fission factor is a novel Myc-dependent regulator of mitochondrial permeability in cancer. *EBioMedicine* [Internet]. 2019 [cited 2021 Nov 22];48:353–63. Available from: <https://doi.org/10.1016/j.ebiom.2019.09.017>
10. Boland ML, Chourasia AH, Macleod KF (2013) Mitochondrial dysfunction in cancer. *Front Oncol* [Internet]. [cited 2021 Nov 30];3. Available from: <https://pubmed.ncbi.nlm.nih.gov/24350057/>
11. Zichri S, Ben, Kolusheva S, Shames AI, Schneiderman EA, Poggio JL, Stein DE et al Mitochondria membrane transformations in colon and prostate cancer and their biological implications. *Biochim Biophys acta Biomembr* [Internet]. 2021 Jan 1 [cited 2021 Nov 30];1863(1). Available from: <https://pubmed.ncbi.nlm.nih.gov/32931774/>
12. WB IFCW G, L I, M W, G M, Effects of a multimodal exercise protocol on functional outcomes, epigenetic modulation and brain-derived neurotrophic factor levels in institutionalized older adults: a quasi-experimental pilot study. *Neural Regen Res* [Internet]. 2021 Dec 1 [cited 2021 Aug 3];16(12):2479–85. Available from: <https://pubmed.ncbi.nlm.nih.gov/33907037/>
13. Dorneles GP, da Silva I, Boeira MC, Valentini D, Fonseca SG, Dal Lago P et al (2019) Cardiorespiratory fitness modulates the proportions of monocytes and T helper subsets in lean and obese men. *Scand J Med Sci Sport.* ;(May):1–11
14. C P, A C-F HR J M. Effects of a 10-week multimodal exercise program on physical and cognitive function of nursing home residents: a psychomotor intervention pilot study. *Aging Clin Exp Res* [Internet]. 2018 Aug 3 [cited 2021 Oct 14];30(5):471–9. Available from: <https://pubmed.ncbi.nlm.nih.gov/28776280/>

15. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods*.
16. Ferlini C, Scambia G (2007) Assay for apoptosis using the mitochondrial probes, Rhodamine123 and 10-N-nonyl acridine orange. *Nat Protoc*.
17. Wen S, Zhu D, Huang P Targeting cancer cell mitochondria as a therapeutic approach. *Future Med Chem* [Internet]. 2013 Jan [cited 2021 Aug 10];5(1):53. Available from: [/pmc/articles/PMC3587793/](#)
18. Wang C, Youle RJ (2009) The Role of Mitochondria in Apoptosis. *Annu Rev Genet* [Internet]. Dec 1 [cited 2021 Aug 10];43:95. Available from: [/pmc/articles/PMC4762029/](#)
19. Zorov DB, Juhaszova M, Sollott SJ Mitochondrial Reactive Oxygen Species (ROS) and ROS-Induced ROS Release. *Physiol Rev* [Internet]. 2014 Jul 1 [cited 2021 Aug 10];94(3):909. Available from: [/pmc/articles/PMC4101632/](#)
20. M S, NL B, B A, MA E. The Impact of Acute and Chronic Exercise on Immunoglobulins and Cytokines in Elderly: Insights From a Critical Review of the Literature. *Front Immunol* [Internet]. Apr 14 [cited 2021 Aug 10];12. Available from: <https://pubmed.ncbi.nlm.nih.gov/33936044/>
21. Li X FGFs in Injury Repair and Regeneration. *Fibroblast Growth Factors*. 2018 Jan1;17–144
22. WY TKAK K, V D, I B, J C, et al (2019) Resistance to visceral obesity is associated with increased locomotion in mice expressing an endothelial cell-specific fibroblast growth factor 1 transgene. *Physiol Rep* [Internet]. Apr 1 [cited 2021 Aug 10];7(7). Available from: <https://pubmed.ncbi.nlm.nih.gov/30972920/>
23. TA C, JA G, S Y, S F, R H, A S, et al (2005) Mechanisms of aging in senescence-accelerated mice. *Genome Biol* [Internet]. [cited 2021 Aug 10];6(6). Available from: <https://pubmed.ncbi.nlm.nih.gov/15960800/>
24. Westdorp H, Sköld AE, Snijer BA, Franik S, Mulder SF, Major PP et al (2014) Immunotherapy for Prostate Cancer: Lessons from Responses to Tumor-Associated Antigens. *Front Immunol* 0(MAY):191

Figures

Figure 1

The effects of plasma from young high physical fitness (PF), young low PF, and elderly individuals submitted to multimodal exercise training on viability and proliferation of PC3 prostate cancer cell line.

Figure 2

Anticarcinogenic effects of plasma from trained elderly individuals in PC3 prostate cancer cell line.

Figure 3

The impact of 8 weeks of multimodal exercise training on systemic cytokine levels of institutionalized elderly individuals.

Statistical analysis performed by Paired t-test ($p < 0.05$).

Figure 4

Heat map correlation between plasma cytokine levels and *in vitro* analysis of cell viability (MTT and LDH), cell proliferation (CFSE), reactive oxygen species production (cytosolic ROS and mitochondrial ROS) and mitochondria membrane polarization in PC3 prostate cancer cell line.