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Effect of exercise in Breast Cancer and its association with tumor characteristics, risk factors for recurrence and lifestyle

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ABSTRACT

Triple-negative breast cancer (TNBC) does not express estrogen receptors, progesterone receptors, or human epidermal growth factor receptor 2, and it is characterized by its aggressive nature, lack of targeted therapies and early peak of recurrence. Recent observational research suggests that inactivity is associated with an increased risk of breast cancer (BC) including the Triple Negative subtype. Little is known about the potential driving molecular events for each subtype of BC and the mechanisms through which exercise yields protective effects against disease progression and recurrence risk. The present thesis focuses on the protective effects of exercise against TNBC through the induction of biochemical metabolic system modifications able to reduce the cell proliferation of BC.

Organization of the thesis and experimental approaches

The **first chapter** provides possible explanations of causative mechanisms for cancer control examining recent findings regarding the basic biological effects of exercise on modulation of the mTOR pathway in TNBC and the benefits induced by various exercise and training protocols.

The **second chapter** of the thesis examines how circulating serum factors released after exercise (High-Intensity Endurance Cycling (HIEC) Test), before (pre) and after (post) a training protocol, could reduce TNBC cell line proliferation, *ex vivo*.

The **third chapter** evaluates the effect of structured and supervised physical activity (PA) intervention on breast cancer survivors (BCS) - “Lifestyle program”.

We showed that PA counseling and supervised exercise training improve functional exercise capacity and could motivate subjects suffering from BC to adopt a healthier lifestyle based on the maintenance of physical activity levels (PAL) recommended in this population. Women enrolled in the “Lifestyle program” took part in the Dragon Boat Race Day event, representing the first team of women in pink from the Marche Region.

CONTENT

ORIGINAL PAPERS

This Thesis is based on the following original research articles, which will be referred to by their Roman numerals.

I. Barbieri E, Falcieri E, De Santi M, Natalucci V, Vallorani L, Agostini D, Annibalini G, Stefani L, Szychlinska MA and Musumeci G. 2018. *Editorial* The "Journal of Functional Morphology and Kinesiology" Journal Club Series: Highlights on Recent Papers in Physical Activity and Sedentary Behavior. ISSN 2411-5142. doi: 10.3390/jfmk3020023

II. Agostini D^, Natalucci D^, Baldelli G^, De Santi M, Donati Zeppa S, Vallorani L, Annibalini G, Lucertini F, Federici A, Izzo R, Stocchi V and Barbieri E. New Insights into the Role of Exercise in Inhibiting mTOR Signaling in Triple-Negative Breast Cancer – Oxidative Medicine and Cellular Longevity, 2018 Sep 30; 2018:5896786. doi: 10.1155/2018/5896786.
^*Authors contributed equally to this work.*

III. Natalucci V. et al. Effects of exercise on triple-negative breast cancer cell proliferation.
In preparation.

IV. Natalucci V. et al. Promotion and prescription of exercise in breast cancer survivors to improve quality of life and health outcomes.
In preparation.

INTRODUCTION

Breast Cancer

Today breast cancer (BC) is the most common invasive tumor in women worldwide and is the most common cancer in all age groups, albeit at different rates (41% in young women vs. 22% in older women). In Italy, there were an estimated 50,500 new cases in 2017 and the average 5-year survival rate (87%) in our country is higher than the European average (82%) [1]. Studies have shown that BC is the result of an intricate interaction among genetic, epigenetic and environmental factors. Among the most significant factors influencing the risk of BC onset and relapse are aging, gender, family history, inherited factors (mutations in the BRCA1 and BRCA2 genes), menstrual and reproductive history, dense breasts, race and ethnicity, radiation exposure, birth control pills, combined post-menopausal hormone therapy (PHT), and diethylstilbestrol exposure (DES).

In recent years a growing body of evidence points to the important effect that healthy lifestyle choices (modifiable factors) such as not smoking, adopting a healthy diet and doing regular PA could have on the onset and clinical course of BC.

Growing amounts of data indicate a relationship between PA, biological makers of BC aggressiveness (*e.g*., levels of insulin, estrogen and IGF-1, and inflammatory status) and lower recurrence risk.

BC is a heterogeneous disease and differs greatly among patients (inter- intra-tumor heterogeneity) [2]. Although in Western countries the incidence of BC is increasing, mortality is steadily decreasing mainly due to prevention strategies, including regular mammography screenings for early detection of BC and primary prevention based on healthy lifestyle choices (not smoking, healthy diet and regular PA).

Several epidemiological studies suggest that physically active women have a significantly lower risk (a reduction of about 25%) of developing BC than sedentary women [3].

Exercise could also reduce the side effects of cancer treatments such as chemotherapy, radiotherapy and hormone therapy and have a positive effect on psychological wellbeing since woman undergoing cancer therapy are more at risk of developing depressive and cognitive disturbances [4-6]. Additional positive effects of PA include a reduction in body fat, improvement in immune system regulators, alterations in free-radical generation and an improvement in the ability of cancer patients and survivors to deal with fatigue, lymphedema and bone metastasis [7]. Another important consideration is that, after

diagnosis, most BC patients and survivors reduce PA level (by 11%) [8], and sedentary behaviors are strongly associated with overweight and obesity, both recognized risk factors for BC. Indeed, adipose tissue contributes to the establishment of a systemic low-grade inflammatory state characterized by activation and infiltration of pro-inflammatory immune cells and a deregulated production of pro-inflammatory cytokines [9,10,11].

Molecular and Systemic Effects Linking Exercise to Cancer Prevention and Cancer Control*

It has been 20 years since skeletal muscle was recognized as an endocrine organ, and skeletal muscle contraction and myokine release have received attention as exercise-induced health benefits. Physical activity (PA) has been shown to reduce cancer incidence, inhibit tumor growth, and improve clinical outcomes following a diagnosis of a primary disease [12], yet daily energy expenditure from PA is decreasing at an alarming rate in Western society [13]. In particular, inactivity is associated with an increased risk of breast cancer (BC). Pernille Hojman and collaborators from Bente K. Pedersen's research team describe the latest molecular understanding of the systemic effect of exercise on cancer progression control. They defined a direct effect on tumor-environment factors linked to whole-body exercise remodeling, mitigation of cancer-related adverse events, and amelioration of anti-cancer treatment efficacy. Several preclinical studies on the effect of exercise on cancer outcomes point to a reduction in the rate of tumor growth [14,15]. However, an important finding of these studies is that exercise, per se, is not capable of directly eliminating already established tumors. For example, in response to exercise-conditioned serum treatment, BC cell growth is inhibited by 10–15% compared to tumor growth observed in the control setting, but there is not a complete eradication of cancer cells [16,17]. Recent investigations have stressed the role of circulating growth factors, prohormones, and sexual hormones, which are able to stimulate cancer cell proliferation through the activation of their respective receptors [18,19]. In particular, the phosphoinositide 3-kinase-AKT-mammalian target of rapamycin (PI3K-AKT-mTOR) pathway is a frequently hyperactivated pathway in cancer and is important for tumor cell growth and survival. Many studies have shown a down-regulation of PI3K-AKT-mTOR signaling after exercise; however, to date, none have established the exercise-dependent mechanisms underlying this association. Exercise

inhibits mTOR functions in both AMPK-independent and -dependent manners. AMPK is also induced in tumors during exercise [20,21], probably as a transient condition. It is known that intra-tumoral metabolism is regulated, but how this affects tumor growth and metastatic rate during exercise is not currently understood. Chronic exercise induces physiological adaptation across homeostatic control circuits, such as oxidative metabolism, mitochondrial biogenesis in several tissues, angiogenesis, immune regulation, and metabolism. Moreover, it diminishes BMI and blood concentrations of estrogens, and insulin, decreases insulin resistance, and strengthens anti-inflammatory pathways against tumor cells. Hence, exercise has an increasingly protective tumorigenic effect [22,23]. These findings have wide-ranging implications for society and may lead to improve the way cancer is managed.

Physical Activity Counseling in Breast Cancer Survivors*

The overall prevalence of women living with a diagnosis of breast cancer (BC) is increasing in industrialized countries. Survivors who maintain a healthy weight and stay physically active (PA) have a better response to treatment and better survival outcomes. Thus, it is necessary to identify an appropriate promotion and prescription of regular PA for BC survivors to improve their prognosis, response to therapy, and quality of life. In this issue, Fong AJ and colleagues [24] examine factors affecting exercise counseling by clinicians and discuss future strategies to address oncology clinicians' perceptions of PA counseling and barriers to such counseling in BC survivors. Focus group discussions were transcribed verbatim and analyzed using inductive thematic analysis. In order to facilitate PA counseling, healthcare facilities, and clinicians need to better support and promote patient-managed PA, making it available on multiple platforms and providing better referral pathways to exercise professionals and physiologists.

Protocol for Exercise Prescription among Breast Cancer Survivors*

Recent systematic reviews and meta-analyses tend to aggregate exercise programs into general categories and rarely investigate the specific features of these programs, which may make them more or less effective. What physical activity (PA) should be prescribed for breast cancer (BC) survivors? Not all types of exercise carry the same benefits.

Specificity is a core principle of exercise training to promote the desired adaptations, and the specificity of adaptation is underpinned by the acronym FITT, which stands for frequency, intensity, time and type. The FITT principle outlines the key components of an effective exercise program Overall recommendations for cancer survivors are consistent with the American Cancer Society, who recommends that cancer survivors engage in a minimum of 150 weekly minutes of moderate-intensity exercise, and it is important to note that the American College of Sports Medicine [25] has established FITT guidelines that are easy to follow for exercise prescription that everyone should know. Frequency refers to how often exercise is performed, intensity refers to the intensity of the exercise undertaken, time refers to the amount of time spent exercising, and type refers to the kind of exercise that is performed. However, adherence to any exercise program is the most difficult thing to achieve, and currently there is a growing number of initiatives designed to increase the effectiveness of long-term PA in BC patients in the most comfortable environment: at home. The aim of the study carried out by Mascherini G. and collaborators was to verify the long-term effectiveness of a home-based program for active lifestyle change in overweight BC survivors. They enrolled premenopausal women, whose PA levels, baseline aerobic capacity, flexibility, strength, and anthropometric measurements were assessed. These parameters were assessed six times during one year of unsupervised exercise. After being prescribed an individual exercise program, a significant reduction in BMI and skinfold sum was observed, as well as maintenance of muscle cell mass. Assessments also showed an increase in lower limb muscle fitness and a reduction in diastolic blood pressure after a six-minute walking test. Exercise is highly recommended for cancer patients, and the prescription of a home-based model of unsupervised exercise described by Mascherini G. et al. seems to ensure ideal compliance, favoring long-term therapeutic efficacy [26]. This intervention strategy could target all patients affected by BC and might motivate these subjects to adopt a healthier lifestyle based on regular PA, as well as proper nutrition.

High Intensity Interval Training (HIIT): new evidence in BCS

In recent years, it has been suggested that a reassessment of the exercise prescription guidelines for BCS is needed. Generally, the beneficial effects induced by exercise are commonly attributed to two types of adaptation, long-term (months/years), such as

improvement in cardiorespiratory fitness, strength and body composition [27,28,29] and short-term effects (hours/days) due to stimulation of muscle contraction. In particular, the marked systemic response to a single bout of exercise reduces the vitality of BC [30]. The available exercise guidelines for BCS emphasize the importance of participating in moderate aerobic and resistance exercise and recommend flexibility building exercises or combined exercises (aerobic and resistance exercise) [31]. However, research on the effects of different types of exercise on physical fitness and various biomarkers in BCS is ongoing, and in the past year, a great deal of evidence has emerged regarding a novel strategy of training for BCS: High-Intensity Interval Training (HIIT). HIIT is a cardio session consisting of short sets of intense exertion, and it represents a valid alternative to traditional endurance-based exercise training for preventing cardiovascular disease (CVD) in BCS [32]. In particular, the principle of HIIT is based upon high intensity aerobic exercise training bouts that are separated by periods of lower intensity that allow for recovery. The target intensity during HIIT is between 85-95% of peak heart rate (HR_{peak}) for four minutes, while during the two-to-three-minute lower intensity recovery periods, the target intensity is between 60-70%.

Only a few studies have been conducted on HIIT training in BCS where it was shown that HIIT can be performed by female cancer survivors without adverse health effects, and it may be an effective way to improve certain aspects of health, including protection against chronic diseases, especially CDV, in this population.

The beneficial effects of HIIT consist of improved quality of life, reduced cancer-related fatigue [33] and reduces the adverse cardiotoxic effects due to treatments in different stage of disease. An ongoing pilot study is examining the HIIT effects on endothelial function and maximal oxygen consumption (VO2max) [34].

In the HIIT protocol, the exercise training intensity is relative to the individual's peak oxygen uptake (VO2peak) and HR_{peak} and should be measured for each individual before starting a HIIT program to control the exercise training intensity properly. Furthermore, it has been shown that VO2max and other cardiovascular efficiency measures, such as endothelial function, improve both with HIIT exercise and with higher volumes of moderate continuous intensity exercise [35]. Both types of exercise enhance the patient's quality of life (QoL), reduce fatigue, and improve energy expenditure and body composition. However, one of the added benefits of HIIT workouts is the reduced time

this protocol requires compared to other exercise programs and the fact that there are currently no contraindications for its use by BCS.

**A part of the introduction was taken by the Editorial The "Journal of Functional Morphology and Kinesiology" Journal Club Series: Highlights on Recent Papers in Physical Activity and Sedentary Behavior. Barbieri E, Falcieri E, De Santi M, Natalucci V, Vallorani L, Agostini D, Annibalini G, Stefani L, Szychlinska MA and Musumeci G. 2018.*

AIMS OF THE THESIS

The first aim of this thesis was to provide new evidence of the effects of exercise on breast cancer prevention, control and healthy benefits.

The primary outcome of this thesis was to examine if systemic responses to acute exercise and training could modulate breast cancer cell proliferation and/or intracellular pathways, *ex vivo*. Particular attention will be focused on the anti-proliferative effect of exercise serum on Triple-Negative Breast Cancer cells.

The secondary outcome was an ameliorating effect induced from the introduction of targeted exercise counseling and a structured physical activity intervention on functional exercise capacities and lifestyle in breast cancer survivors.

CHAPTER 1

Review Article

New Insights into the Role of Exercise in Inhibiting mTOR Signaling in Triple-Negative Breast Cancer

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New Insights into the Role of Exercise in Inhibiting mTOR Signaling in Triple-Negative Breast Cancer

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Abstract

Triple-negative breast cancer (TNBC) does not express estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2, and is characterized by its aggressive nature, lack of targets for targeted therapies and early peak of recurrence. Due to these specific characteristics, chemotherapy does not usually yield substantial improvements and new target therapies and alternative strategies are needed. The beneficial responses of TNBC survivors to regular exercise, including a reduction in the rate of tumour growth, are becoming increasingly apparent. Physiological adaptations to exercise occur in skeletal muscle but have an impact on the entire body through systemic control of energy homeostasis and metabolism, which in turn influence the TNBC tumour microenvironment. Gaining insights into the causal mechanisms of the therapeutic cancer control properties of regular exercise is important to improve the prescription and implementation of exercise and training in TNBC survivors. Here we provide new evidence of the effects of exercise on TNBC prevention, control and outcomes, based on the inhibition of the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (PKB also

known as Akt)/mammalian target of rapamycin (mTOR) (PI3K-Akt-mTOR) signaling. These findings have wide-ranging clinical implications for cancer treatment, including recurrence and case management.

Keywords

Exercise, Physical Activity, Cancer control, mTOR, Triple Negative Breast Cancer

1. Introduction

Breast cancer (BC) is one of the most common carcinomas and one of the main causes of cancer-related death worldwide [1]. Among the various subtypes, triple-negative BC (TNBC) accounts for approximately 20% of BC cases. The absence of estrogen and progesterone receptors and human epidermal receptor 2 (HER2) in malignant cells reduces treatment options and increases the risk of recurrence and death, especially in the first 3–5 years of follow-up after surgery [2]. Thus, TNBC exhibits a more aggressive clinical course than non-TNBC. Most TNBC cases are diagnosed in women under the age of 60, and in 20% of diagnosed cases, there is a mutation of the germinal BC (BRCA) gene [3–7]. In patients with metastatic TNBC, there are currently no available targeted therapies and chemotherapy is the only possible treatment option. In addition to the biological-molecular aspects associated with prognosis and BC development, a growing body of evidence highlights the impact of lifestyle on disease-related outcomes. Unhealthy lifestyles with low levels of physical activity (PA) result in overweight and obesity, which appear to have a negative impact on BC [8], increasing the risk of recurrence and death in all subtypes, including TNBC [9]. Conversely, proper diet, weight loss, and increased PA lead to more favourable outcomes in the short and long term [10, 11]. The mechanisms underlying the effects of exercise on breast carcinogenesis are not clear, but experimental evidence suggests that PA induces phosphatidylinositol-3-kinase (PI3K)/protein kinase B (PKB also known as Akt)/mammalian target of rapamycin (mTOR) (PI3K-Akt-mTOR) signaling inhibition and slows TNBC tumor cell growth [12–14]. Physiological adaptations to exercise occur primarily in skeletal muscle, but the effects of exercise and training also impact other tissues through systemic control of energy homeostasis and metabolism, thus influencing the TNBC tumor microenvironment and mTOR inhibition [15].

Given the scope of this review, we summarise recent discoveries related to the underlying biology of exercise-induced modulation of the mTOR pathway in TNBC, examining the benefits induced by different exercise and training protocols.

We also consider how exercise affects the level of microRNAs (miRNAs) linked to the mTOR pathway involved in TNBC initiation and progression [16, 17], and how nutrients can influence mTOR signaling.

Finally, we discuss how exercise induces beneficial adaptations and why it should be prescribed as a coadjuvant "medicine," which has the potential to improve TNBC outcomes.

2. mTOR Signaling

2.1. mTOR Pathway and mTOR Activation in BC

mTOR is a serine-threonine kinase that interacts with several proteins to form two distinct complexes, mTORC1 and mTORC2, which show different sensitivities to rapamycin [18]. mTORC1 is acutely sensitive to rapamycin and responds to growth factors, stress, amino acids, and energy, promoting protein translation and synthesis, cell growth, mass, division, and survival. mTORC1 comprises mTOR, the regulatory associated protein of mTOR (Raptor), the G-protein β-subunit-like protein (GβL), also known as mLST8, DEP domain-containing mTOR-interacting protein (Deptor), proline-rich Akt substrate of 40 kDa (PRAS40), and Tti1/Tel2 complex. mTORC2 is insensitive to acute rapamycin treatment and contains mTOR, the rapamycin-insensitive companion of mTOR (Rictor), the mammalian stress-activated map kinase-interacting protein 1 (mSIN1), GβL, Deptor, protein observed with Rictor-1/2 (Protor 1/2), and Tti1/Tel2. Raptor and PRAS40 are unique to mTORC1, while Rictor, mSIN1, and Protor 1/2 are unique to mTORC2 [18].

The various components of mTORC1, which is the most widely studied complex, have several regulatory effects: Raptor, Tti1, and Tel2 are positive regulators, whereas PRAS40 and Deptor are negative regulators [19]. Several factors regulating mTORC1 activation converge in the tubular sclerosis complex (TSC), consisting of hamartin (TSC1), tuberin (TSC2), and TBC1 domain family member 7 (TBC1D7) [20]; the complex works via the Ras homolog enriched in brain (Rheb) GTPase, negatively regulating mTORC1 [21].

An upstream regulator of TSC is the PI3K/Akt pathway activated by growth factors such as insulin-like growth factor 1 (IGF-1) and insulin. PI3K phosphorylates phosphatidylinositol (3,4)-bis-phosphate (PIP2) lipid to phosphatidylinositol (3,4,5)-tris-phosphate (PIP3), which recruits phosphoinositide-dependent kinase-1 (PDK1) and Akt. Akt phosphorylates TSC2 and PRAS40 inactivating them and inducing, in turn,

mTORC1 activation [22]. TSC2 can also be phosphorylated and inactivated by the activated Ras/extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) signaling pathway [19].

Another critical regulator of mTORC1 is the adenosine monophosphate-activated protein kinase (AMPK), which is activated when cellular energy level is low. AMP linking to AMPK allows its phosphorylation (while ATP availability prevents it) triggering repression of energy-consuming processes, also inhibiting mTOR, and enhancing energy-producing processes. AMPK phosphorylates TSC2 in different sites than Akt, activating rather than inactivating TSC2, and phosphorylates Raptor, thus achieving mTORC1 repression [23].

mTORC1 activation requires sufficient amino acid levels, though it is not clear how these levels are sensed. Amino acid regulation requires the formation of a Rag GTPase complex, which binds Raptor, in order to translocate mTORC1 to the lysosome allowing its association with Rheb, and thus its activation [24].

The activation of mTORC1 leads to several downstream effects, including protein synthesis promotion. Raptor binds to the eukaryotic translation initiation factor 4E-(eIF4E-) binding protein 1 (4E-BP1) and the ribosomal protein S6 kinase beta-1 (S6K1), recruiting them to the mTORC1 complex and allowing their phosphorylation [25, 26]. Hyperphosphorylation of 4E-BP1 by mTOR prevents the association of 4E-BP1 and eIF4E, allowing eIF4E to bind eIF4G to begin translation. Phosphorylation of S6Ks, including several S6K1 isoforms and S6K2, by mTOR promotes their activation and thus the phosphorylation of their targets involved in mRNA translation. S6K1 is also involved in negative feedback on mTORC1 and mTORC2 [27].

The mTORC1 complex and AMPK also regulate the autophagic process, a cellular mechanism through which cells eliminate damaged components associated with a wide range of diseases, including cancer. After glucose deprivation, AMPK associates with, and directly phosphorylates, the serine/threonine Unc-51-like autophagy activating kinase (ULK1), an upstream component of the autophagy mechanism. By contrast, when nutrients are plentiful, mTORC1 phosphorylates ULK1, preventing its association with and activation by AMPK, inhibiting autophagy [28].

Aberrant activation of the PI3K/Akt/mTOR pathway is often found in human cancers and promotes cell proliferation [29]. Activation has been shown in the lung, head, and neck and breast, gynaecologic, colorectal, and prostate cancers and glioblastoma multiforme

[30] and also in B-lineage acute lymphoblastic leukemia [31]. PI3Ks are pivotal molecules in this pathway and possess eight isoforms grouped into class I, class II, and class III. Class I PI3Ks (PI3Kα, β, γ, and δ), stimulated by Tyr kinases, G protein-coupled receptors, and Ras, are currently the focus of research in drug development. Mutation of the PIK3CA gene, which encodes the catalytic subunit α (p110α), one of the class I PI3K isoforms, is found in several cancers [32]. The signaling and biological roles of class II and III PI3Ks are not clear, and they have not been implicated in oncogenesis [32].

In TNBC, the activation of the PI3K/Akt/mTOR pathway is induced by an overexpression of upstream regulators (i.e., growth hormone receptors), mutations of the PIK3CA gene, and by decreased activity of the phosphatase and tensin homolog (PTEN) and of the proline-rich inositol polyphosphatase, which are downregulators of PI3K [33–35]. By contrast, activation of downstream effectors of PI3K (e.g., Akt and mTOR) and activation of downstream effectors of parallel pathways (MAPK and Ras) are rare events in TNBC [36]. Furthermore, other oncogenic pathways (i.e., FGFR, cMET, and RAF) regulated by P53 inactivation converge to activate the PI3K pathway [37].

Due to the frequent activation of the PI3K/Akt/mTOR pathway in human cancers, more than 50 inhibiting drugs are in development, and several clinical trials are ongoing [38]. The first established therapeutic anticancer agents targeting this pathway are everolimus and temsirolimus, which abrogate mTOR signaling, and have been approved by the U.S. Food and Drug Administration. Based on the results obtained with everolimus in pancreatic neuroendocrine tumors [39], and temsirolimus for advanced renal cell cancer [40], these agents are now approved for treatment of these diseases.

Therapies targeting other pathway members have been described. Monotherapy using pan-class I PI3K, which inhibits all class I PI3K isoforms, has effects at dose-limiting toxicity, leading to prolonged disease stabilization in some patients with advanced solid tumors (especially the lung) during phase I clinical trials [41]. Isoform-specific PI3K inhibitors have also been tested and have shown an antitumor activity in tumors such as p110δ- (isoform δ-) driven hematologic malignancies [42] or PIK3CA-mutant HR-positive BC [43]. Akt inhibitors and mTORC1/2 inhibitors aimed to suppress not only mTORC1, but also the feedback activation of Akt by mTORC2 [44], are currently being investigated in clinical studies [45]. The use of PI3K/Akt/mTOR pathway inhibitors is often associated with MAPK inhibitors, growth factor receptor inhibitors, and endocrine

therapy. Furthermore, they might sensitize tumors to chemotherapy synergistically inducing apoptosis, as showed in sarcomas [46].

These promising strategies are now under investigation for the treatment of several tumors, including nonsmall cell lung cancer [47], colorectal cancer [48], nonmedullary thyroid carcinoma [49], and B-lineage acute lymphoblastic leukemia [31]. Although these strategies have been shown to be effective, there is great variability in the duration and quality of their benefits and the long-term side effects for patients. Thus, the identification of protein and/or genetic biomarkers to recognize subjects that will benefit the most from these therapeutic strategies is essential [50]. In TNBC, the development of PI3K/Akt/mTOR-targeted therapies, taking into account the inhibitors of this pathway alone or in combination with other strategies, will provide new tools to control disease progression and improve outcomes [51]. In a recent phase 2 clinical trial, the efficacy of ipatasertib (an Akt inhibitor) in association with paclitaxel (an antineoplastic agent used in TNBC treatment) was shown [52].

2.2. MicroRNAs and mTOR Signaling in BC

Several studies highlight the role of circulating microRNAs (miRNAs), in different tumors, including BC and the TNBC subtype [16]. In particular, recent evidence has shown that miR10a is downregulated in triple-negative BC cells [53]. Furthermore, overexpression of miR-10a decreases the proliferation and migration of TNBC cell lines via PI3K/Akt/mTOR signaling and through the mitochondrial apoptotic pathway [53]. Recently, Phua et al. [54] demonstrated that miR-184 is also downregulated in TNBC patients and that miR-184 overexpression in TNBC cells leads to a reduced expression of mTOR. The decreased cancer cell proliferation, due to mTOR reduction, has been confirmed in vivo: mice injected with mir-184-transfected MDA-MB-231 cells showed a delayed primary tumor formation and reduced metastatic burden. Emerging evidence points to epigenetic silencing by hypermethylation as a possible mechanism through which these tumor suppressor/growth inhibitor miRNAs are downregulated in TNBC [55]. In metastatic breast tumors, miR-184 has been found to be hypermethylated compared to the methylation status of miR-184 in normal breast tissue, suggesting a selective pressure in silencing this miRNA during the metastatic process [54].

Upregulation of miR-21 was detected in TNBC tissues and in MDA-MB-468 cells by Fang et al. [56]. Inhibition of this miRNA resulted in decreased proliferation, viability,

and invasiveness of TNBC cells and enhanced apoptosis. Experiments to identify miR-21 targets have shown that PTEN is downregulated, suggesting an activation of mTOR and the oncogenic properties of miR-21 in TNBC, with increased proliferation and invasion by TNBC cells. Another miRNA that has been found to be upregulated in TNBC tissues in comparison to non-TNBC or adjacent tissues is miR-146a. Indeed, it has been reported to be significantly related to tumor size and histological stage: patients with elevated miR-146a expression have lower survival rates and worse prognoses than low-expression individuals [57]. In addition, miR-146a has been shown to bind the 3-UTR region of BRCA1, inhibiting its expression; the BRCA1 protein is absent or present at very low levels in about one-third of sporadic BCs [58]. Evidence suggests that downregulation of BRCA1 expression leads to Akt/mTOR oncogenic pathway activation [59]. Hence, strategies that could modify the deregulated status of these miRNAs in TNBC could have a pivotal role in inhibiting the Akt/mTOR pathway and could affect TNBC initiation and progression. It is not yet known how these miRNAs might be modulated by exercise and whether they can be associated positively or negatively with TNBC progression, for which there are no reliable prognostic factors.

2.3. Autophagy and mTOR Signaling

Autophagy is the cellular mechanism responsible for the degradation of cytoplasmic components. It is through this mechanism that cells maintain cellular homeostasis by eliminating damaged proteins and organelles and by providing substrates for energy generation and biosynthesis under stress conditions. The mTOR complex is a major negative regulator of autophagy. It suppresses autophagy in response to nutrients, growth factors, and hormone availability, promoting protein synthesis, cell division, and metabolism. The mTOR signaling pathway is frequently activated in tumor cells, resulting in the activation of its growth-promoting functions and the inhibition of autophagy [60]. In cancer, the cytoprotective role of autophagy could prevent tumorigenic transformation by inhibiting chronic tissue damage. By contrast, once cancer occurs, cancerous cells could utilize autophagy to enhance fitness and survive in the hostile tumor microenvironment, providing energy via substrate degradation. Autophagy could therefore be tumor suppressive (for example, via elimination of damaged cellular components), as well as tumor promoting in established cancers [61]. In addition, autophagy has recently been shown to play a role in necroptosis, and, together with

apoptosis, autophagy also regulates other death pathways, including immunogenic cell death, entosis, and pyroptosis [62]. It has been demonstrated that suppression of autophagy in epidermal growth factor receptor- (EGFR-) driven nonsmall cell lung adenocarcinoma xenografts promotes cell proliferation, tumor growth, and dedifferentiation, as well as resistance to EGFR tyrosine kinase inhibitor therapy [63]. Moreover, autophagy suppresses early oncogenesis in lung adenocarcinoma through effects on regulatory T cells [64], and autophagy genes are often required for the cytotoxic effects of chemotherapy [65]. In view of the complex- and context-dependent role of autophagy in cancer progression and response to therapy, it could be hypothesized that the inhibition of the mTOR pathway and the consequent induction of autophagy may be useful in certain cancers through autophagy-dependent antitumor immunity, autophagy-dependent cytotoxic effects, or other tumor-suppressor effects [66]. In addition to its effects on skeletal muscle, exercise has also been found to induce autophagy in the liver, pancreas, adipose tissue, and cerebral cortex in transgenic mouse models [67, 68]. Whether exercise-induced stress activates autophagy in healthy cells (or cells primed for malignant transformation), or cancer cells themselves, and whether such effects inhibit or potentiate tumorigenesis, is not known and needs further investigation [15].

3. Evidence of mTOR Modulation by Exercise in TNBC

3.1. mTOR and Exercise

PA reduces mortality for all diseases, including tumors [69], reducing the incidence of primary development and ameliorating the prognosis [15]. Hence, it should be prescribed like a medication indicating the correct typology, dose, and timing, i.e., the type, intensity, duration, and frequency of exercise as described in Exercise Prescription in BC Survivors. Physiological adaptations to exercise occur not only in skeletal muscle but also systemically in other metabolically active tissues involved in the exercise response (such as the bone, heart, adipose, endothelium tissue, and brain) profoundly altering the systemic milieu, in turn influencing the tumor microenvironment and cancer hallmarks [15]. In order to understand the effect of PA on mTOR and BC, muscular, systemic, and microenvironment effects should be considered.

3.1.1. Aerobic Exercise and Muscular Effects

In skeletal muscle, aerobic exercise activates several adaptive pathways, including protein kinases, transcription, and coregulatory factors that, by gene expression modification, increase mitochondrial biogenesis and stimulate metabolic reprogramming [70]. Exercise induces a depletion of nutrients, energetic substrates, and nicotinamide adenine dinucleotide (NAD)H that elevate the ratios of AMP : ATP and NAD+ : NADH, directly activating AMPK and other metabolic sensors, including NAD-dependent protein deacetylase sirtuin 1 (SIRT1) and kinases, such as ERK1/2, p38 MAPK, and Jun N-terminal kinase (JNK) [71]. These energy sensors trigger the transcriptional regulator peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α), which regulates the expression of mitochondrial biogenesis, increase the expression of mitochondrial transcription factor A (TFAM), which, once transferred to the mitochondria, controls transcription of mitochondrial DNA [71]. Moreover, aerobic exercise, through PGC1α phosphorylation, influences other transcription factors, including peroxisome proliferator-activated receptor-γ (PPARγ), an important regulator of fatty acid oxidation and estrogen-related receptor-α (ERRα) and ERRγ, which directly regulate mitochondrial energy metabolism by oxidative phosphorylation, fatty acid oxidation, and the tricarboxylic acid (TCA) cycle [72,73]. In this regard, the reactive oxygen species (ROS) and reactive nitrogen species produced by exercise also directly or indirectly regulate contraction-induced mitochondrial biogenesis [74] and skeletal muscle metabolic reprogramming via AMPK and PGC-1α [75]. AMPK-mediated cell survival requires inhibition of mTOR. Therefore, AMPK and mTOR play antagonistic roles in cells and inhibition of mTOR is essential for AMPK-mediated metabolic homeostasis [76].

3.1.2. Resistance Exercise and Muscular Effects

In skeletal muscle, resistance exercise causes an increase in muscle size and strength via mTOR activation. In canonical growth factor signaling, mTOR is activated by PI3K/Akt, through IGF-1 and insulin signaling, but a considerable body of evidence suggests that mTORC1 is also likely activated by a growth factor-independent movement of proteins to and from the lysosome, via resistance exercise-induced phosphorylation of TSC2 [77]. Cellular trafficking of mTOR and its association with positive regulators that occur in

human skeletal muscle leading to protein synthesis after resistance exercise, in fed condition, were recently confirmed by Song and colleagues [78].

3.1.3. Systemic and Microenvironment Effects of Exercise

Exercise stimulates the release of molecular signals such as muscle-derived regulatory RNAs, metabolites, and myokines with autocrine, paracrine effect on energetic substrate oxidation, hypertrophy, angiogenesis, inflammation, and regulation of the extracellular matrix. To better evaluate the systemic response to PA, a distinction must be drawn between long term (training) and acute exercise. Training induces a reduction of basal concentration of circulatory sex hormones and lowers adiposity, both recognized risk factors [79], while acute exercise causes a sharp increase in circulating hormones, cytokines, and immune cells [80–82]. Both the systemic adaptations to training and the strong response to acute exercise support plausible mechanisms that inhibit carcinogenesis by suppressing the activation of mTOR signaling network. Hence, exercise may improve BC outcomes [14] (Figure 1). Moreover, both long-term training and a single bout of exercise control energy availability and induce a hormetic response that accounts for the physiological cellular stress adaptation [83,84].

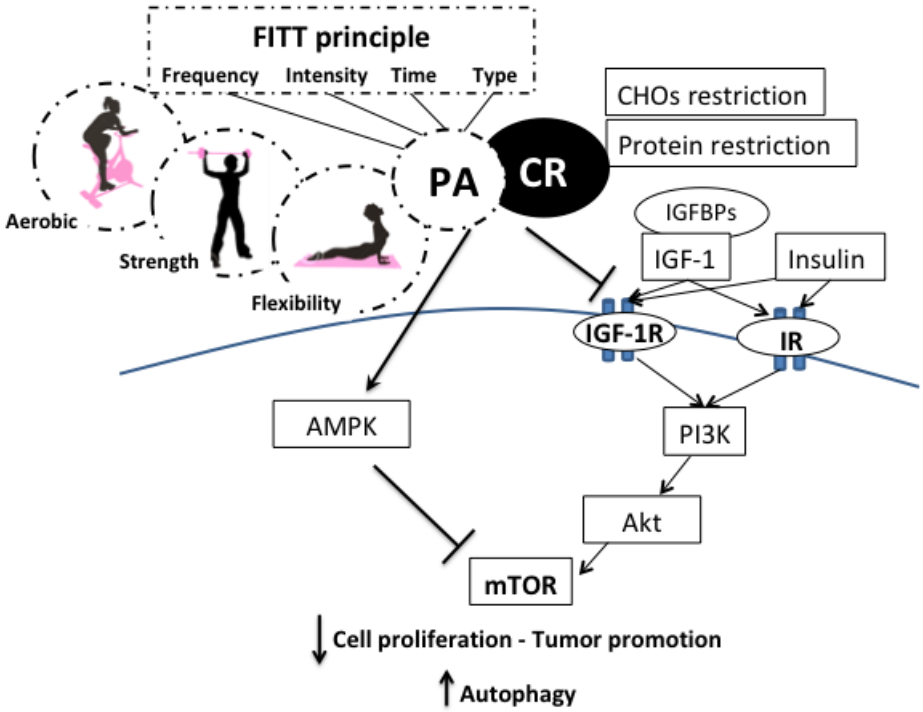

Figure 1: In this figure, we consider potential mechanisms regulated by physical activity and caloric restriction in inhibiting the mTOR pathway. Both refer to energy availability inhibiting carcinogenesis by suppressing the activation of the mTOR signaling network in this subtype of mammary carcinoma. The mTOR inhibition is mediated through the effects of vigorous PA or long-term exercise on systemic response such as concentrations of the circulating growth factors and hormones (i.e., IGF-1 and insulin) that regulate the mTOR network. The network is controlled through the PI3K/Akt signaling pathway, the glycaemia and glutamine levels, inducing apoptosis and reversing malignancy-associated metabolic programming. Moreover, the control of energy availability by both exercise and CR induces a mitohormetic response that accounts for a physiological cellular stress adaptation through AMPK activation inducing mTOR inhibition. In this context, exercise should be considered in terms of its four components: frequency, intensity, time, and type; however, dose-dependent effects of each component on cancer protection via mTOR inhibition have not yet been clarified. Most data indicate that vigorous PA, either long-term or in adulthood, may reduce a woman's risk of mammarian cancer, especially TNBC relapse. The inhibition of the mTOR complex and its cell growth-promoting functions leads to a reduction of cell proliferation, control of cancer progression, and consequent autophagy induction probably involved in tumorigenesis prevention. Thus, we hypothesized that the exercise-induced inhibition of the mTOR pathway may be useful in the control of cancer progression, including TNBC. PA: physical activity; CR: caloric restriction; CHOs: carbohydrates; mTOR: mammalian

target of rapamycin; IGF-1: insulin-like growth factor 1; IGF-1R: insulin-like growth factor receptor 1; IR: insulin receptor; IGFBPs: insulin-like growth factor binding proteins; PI3K: phosphatidylinositol-3-kinase; AMPK: adenosine monophosphate-activated protein kinase; TNBC: triple-negative breast cancer. FITT-VP principle, which reflects the frequency (F), intensity (I), time (T), and type (T) of exercise, and its volume (V) and progression (P) over time, in an individualized exercise training program.

Hormesis is a process whereby exposure to a low dose of a potential stress favours adaptive changes in the cell that enables it to better tolerate subsequent stress [85,86]. This type of stress is often related to reactive oxygen species (ROS) originating from the mitochondrial respiratory chain [87]. The accumulation of transient low doses of ROS through exercise influences signaling from the mitochondrial compartment to the cell [88]. Remarkably, this coordinated response to mild mitochondrial stress appears to induce mitochondrial metabolism, increase stress resistance, stimulate various long-lasting cytoprotective pathways, and favour the establishment of an oxidant-resistant phenotype, hence preventing oxidative damage and chronic diseases. Accordingly, low levels of ROS elicit positive effects on physiological cellular and systemic responses and ultimately increase lifespan [83,88–93]. The hormetic nature of exercise, which produces low levels of ROS, emerges as a key feature for cancer control. Indeed, in the tumor microenvironment, the activation of exercise-induced hormesis of the AMPK-p38-PGC1-α axis supports oxidative metabolism maintaining the cellular ATP pool and conserving cellular energy and viability during the metabolic stress condition: AMPK regulates metabolism and energy homeostasis [94,95]. Exercise-induced mitochondrial biogenesis improves mitochondrial function in addition to the upregulation of antioxidant defenses that function as back regulators of intracellular ROS levels, and leads to improved redox homeostasis [96,97] as well as significantly improved insulin sensitivity. By contrast, high levels of ROS cause functional oxidative damage to proteins, lipids, nucleic acids, and cell components, induce a significant increase in intracellular Ca^{2+}, and promote signaling cascades for apoptosis or autophagy via NF-κB or forkhead box sub group O (FoxO) pathways. High ROS levels are therefore reputed to act as etiological, or at least exacerbating factors in chronic/aging-related diseases.

The typical hormetic response modulated by exercise involves kinases, deacetylases, and transcription factors; many of which have also been shown to be involved in the

carcinogenic process [86]. The most studied are sirtuins (SIRT), which are histone deacetylases, and the FoxO family of transcription factors. The pathways in which NF-kappaB and the Nrf-2/ARE are components are also involved in hormetic responses and implicated in carcinogenesis and are modulated by exercise [86].

FoxO transcription factors play a critical role in cell cycle control and cellular stress responses. FoxOs are known to be regulated by the insulin signaling pathway; however, recently, the research group of Burnet demonstrated that AMPK phosphorylates 6 specific residues on FoxO and opposes the phosphorylation of other FoxO sites by Akt [98]. Phosphorylation of FoxO by AMPK affects the conformation of the protein in such a way that sirtuin-mediated deacetylation is also modified [99]. The dependence of sirtuins on nicotinamide adenine dinucleotide (NAD(+)) links their activity to cellular metabolic status. Emerging evidence indicates that deacetylation of FoxO by SIRT1 favours expression of cell survival/stress resistance and the downregulation of proapoptotic genes [85,100,101]. Sirtuins therefore protect against cancer development as they regulate the cellular stress responses and ensure that damaged DNA is not propagated and that mutations do not accumulate [99]. However, how FoxO activation is influenced by exercise remains unclear. In addition, cytokines such as those that we and others have found to be regulated by exercise and training [14,102–104] have been reported to have direct and indirect effects on cellular stress responses modulated by acetylation/deacetylation reactions, and these effects can be further modified by cortical steroids, which exercise dramatically induces [105].

Similarly, various chemical mimetics of PA and caloric restriction (CR) such as AICAR, PPARδ agonist, resveratrol, and metformin can trigger a beneficial response by activation of key regulators of stress tolerance at the level of transcription, posttranscriptional modifications, and regulation of energy metabolism [92,106]. Cross talk between major CR hormesis-induced pathways, especially AMPK/PPAR and antioxidant systems, IGF-1, and homeostatic energy balance, reveals the correlation between CR and exercise mimetics [107].

Likewise, depending on the exercise, the level/persistence could induce an adaptive response that might turn the same process from “physiologic” into “pathologic,” as in the case of inflammation. Careful titration of ROS levels within specific tumor microenvironments may lie at the crossroads between the prevention, protection, and/or initiation and progression of disease, in particular, as regards the induction of

mitochondrial functionality, cellular homeostasis, and more generally, cellular metabolic health.

Considering the type of exercise, both aerobic and resistance training increase glucose uptake in skeletal muscle via insulin-independent mechanisms, with a subsequent decrease in circulating levels of insulin, IGF-1, and glucose [108]. In a model of mammary carcinogenesis, PA caused a delay in carcinogenesis with a concomitant activation of AMPK and reduction in Akt and mTOR activation and reduction in insulin and IGF-1 in circulation [12]. Reduction of insulin levels is an important aspect given that hyperinsulinemia and insulin resistance are commonly observed in obesity with adipokine alterations, conditions associated with increased risk of BC and poor prognosis [8]. Insulin resistance is a condition in which the target tissues of insulin such as skeletal muscle, adipose tissue, and liver show a reduction in their response to physiological concentrations of the insulin hormone. As a consequence, the pancreatic β-cells produce more of the hormone to compensate for the defective response of target tissues, thus leading to hyperinsulinemia. BC cells express high levels of the insulin receptor (IR), and increased circulating insulin is associated with BC recurrence and death [109]. In contrast, PA has a fundamental role in reducing muscle insulin resistance and normalizing circulating insulin levels. Regular exercise in both healthy and oncological conditions ameliorates glycemic control including glycated hemoglobin (HbA1c) and insulin sensitivity in a "dose"-dependent manner according to duration and intensity [110,111]. Skeletal muscle in virtue of its mass and high rate of insulin and exercise-stimulated glucose transport, represents the most important tissue in glucose uptake. Exercise per se increases trafficking of glucose transporter 4 (GLUT4) to the plasma membrane through insulin-independent mechanisms [112]. Under normal physiological conditions, in skeletal muscle, insulin actions are mediated by the IR-catalyzed phosphorylation of the IR substrates 1 and 2 (IRS1 and IRS2). The tyrosine-phosphorylated IRS proteins then interact with and activate PI3K, a critical player in insulin signaling, particularly with regard to glucose homeostasis. Activation of PI3K generates PIP3 that induces membrane translocation of the serine/threonine kinase Akt. PIP3 activation of PDK1 and the Rictor/mTOR complex 2 leads to phosphorylation and subsequent activation of Akt [113]. Akt phosphorylates TBC1D4 (also known as Akt substrate of 160 kDa, AS160) and TBC1D1 promoting the translocation of GLUT4 vesicles from intracellular compartments to membrane for glucose uptake [114].

Although recent findings help to better understand the effect of exercise on glycemic control, the specific exercise-induced signaling mechanisms leading to the acute and long-term adaptations favouring enhanced glycemic control are less clear [112,115].

Endurance and, to a lesser extent, resistance exercise represent a significant metabolic stress, activating AMPK and thus inhibiting mTOR also in nonmuscular tissue such as liver, fat, and tumor tissues. In order to better evaluate the impact of exercise on mTOR in the BC microenvironment, not only AMPK, but also other circulating factors, should be considered. IGF-1, as well as insulin, activates the MAPK pathway and the PI3K pathway, which are both involved in cancer development and progression. The importance of IGF-1 axis in the development and progression of BC has been clearly shown [116]. The overexpression of IGF-1R in BC has been reported and related to poorer survival rates [117].

The IGF signaling system is composed by IGF-1 and IGF-2, insulin-like growth factor binding proteins (IGFBPs), a family of binding proteins regulating IGF half-lives and available in circulation and extracellular fluids, IGF receptors, and insulin receptors. Furthermore, we recently evaluated the complexity of the IGF-1 gene [118] and the biological activity of IGF-1 isoforms in BC cell lines [119] showing that the IGF-1 isoforms induced cell proliferation via IGF1R phosphorylation. Some studies have reported conflicting results regarding the regulation of IGF-1. Such studies report an increase, no difference or a decrease in circulating IGF-1 levels associated with PA [120–123]. These results are not surprising because the IGF-1 levels are influenced by several clinical factors such as gender, age, body mass index (BMI), sex steroid concentrations, nutrition, stress, level of PA, and intervening illness. Thus, exercise prescription should take into consideration most of these variables.

Another process through which exercise might regulate tumor metabolism is the autophagic machinery [15], as described in Autophagy and mTOR Signaling.

It is clear that exercise can ameliorate the BC microenvironment and can be very important in reducing BC risk and tumor burden when canonical radiochemotherapies or chemical mTOR inhibitors are not working, as in TNBC. Exercise workouts for these subjects will be explained in Exercise Prescription in BC Survivors. Ex vivo experimental data, using TNBC cell lines stimulated with sera collected before and after a single aerobic exercise bout (pre- or postexercise serum/a), are described in Experimental Evidence of mTOR Inhibition.

3.2. Experimental Evidence of mTOR Inhibition

As regards the mechanisms involved in the exercise-induced reduction of TNBC risk and tumorigenesis, few data are available. Ex vivo experiments, working with TNBC cells stimulated with sera collected before and after a single aerobic exercise bout (pre- or postexercise serum/a), are a good starting point to understand how exercise could affect the progression and recrudescence of TNBC. The research group of Dethlefsen has demonstrated that incubation of MCF-7 estrogen-responsive BC cells and MDA-MB-231 TNBC cells treated with postexercise serum, from both healthy volunteers [124] and operated cancer patients [14,124], resulted in a reduction of BC cell viability in comparison with BC cells incubated with preexercise sera. In particular, it has been demonstrated that MCF-7 and MDA-MB-231 stimulation with sera leads to a viability reduction of 11% in MCF-7 cells and 9% in MDA-MB-231 cells in the case of supplementation with postexercise serum from operated cancer patients receiving adjuvant chemotherapy compared to preexercise serum [124]. Furthermore, the viability of both BC cell lines supplemented with sera from healthy women was also significantly reduced by the exercise-conditioned sera, resulting in a 10% and 19% reduction in MCF-7 viability and a 14% and 13% reduction in MDA-MB-231 viability by 1 h and 2 h postexercise sera, respectively. The reduced viability of MDA-MB-231 supplemented with 5% of healthy women 2-hour postexercise serum has also been confirmed by a pilot study that we performed working with culture medium with a physiological concentration of glucose (80mg/dl), resulting in a statistically significant reduction in cell proliferation of about 10% compared to cells supplemented with preexercise human serum [103]. Promising data on the tumorigenic potential of cancer cells in mice are also available. As reported by Dethlefsen et al. in 2017 [124], different outcomes in incidence and growth of tumors were detected inoculating NMRI-Foxn1nu mice with MCF-7 or MDA-MB-231 BC cells preincubated for 48 hours with pre or postexercise sera from healthy volunteers. In particular, only 45% of the mice inoculated with MCF-7 supplemented with postexercise human serum formed tumors compared with 90% of mice inoculated with MCF-7 preincubated with at rest sera, and the volume of tumors was reduced by 76%. Moreover, tumor incidence in mice inoculated with MDA-MB-231 cells preincubated with postexercise sera tended to be lower than it was in mice inoculated with MDA-MB-231 cells preincubated with rest sera, but no difference in tumor volume was observed between the two groups. These results show that exercise-stimulated changes suppress

BC cell proliferation and reduce the tumorigenic potential of BC cells, also in the case of TNBC cells. Another important aspect to be considered is the fact that PA has been reported to lead to an increased level of the catecholamines epinephrine (EPI) and norepinephrine (NE) [82]; this result has also been confirmed in BC survivors two hours after a single exercise session [124]. Moreover, by blocking the β-adrenergic signaling pathway in BC cells, the effects of postexercise sera in BC cell viability is completely blunted, indicating the crucial role of catecholamines in inhibiting BC cells viability and tumor growth [124]. Their role in exercise-induced effects on BC cell viability has also been confirmed by MCF-7 and MDA-MB-231 treatment with different doses of EPI and NE, resulting in a dose-dependent growth inhibitory effect in both BC cell lines. Catecholamines have been shown to induce a dose-dependent phosphorylation of yes-associated protein (YAP) in MDA-MB-231 cells [125]; YAP is the main downstream target of the mammalian Hippo pathway and, when phosphorylated, it is retained in the cytoplasm. Hippo pathway is a tumor suppressor signaling cascade that regulates cell growth, and it has been shown to be a dysregulated pathway in several types of cancers, including BC, in which there is an activation of YAP oncoproteins and transcriptional coactivators with the PDZ-binding motif (TAZ) associated with tumor formation, growth and progression, metastasis, and drug resistance [126]. Dethlefsen et al. showed that the Hippo pathway is regulated by exercise-conditioned sera: incubation of BC cells with postexercise sera led to a time-dependent phosphorylation of YAP in MCF-7 BC cells and to a decreased expression of YAP target genes, due to phosphorylated-YAP cytoplasmic retention, in both MCF-7 BC cells and MDA-MB-231 TNBC cells [124]. Studies performed by Tumaneng et al. demonstrated that Hippo pathway is related to the mTOR signaling cascade: YAP mediates the effects of the Hippo pathway regulating target genes, including the miR-29; this miRNA family has been proven to inhibit PTEN, an upstream activator of mTOR [127]. In summary, the Hippo pathway can be activated by exercise through the production of the catecholamines EPI and NE and can inhibit BC cell growth through the action of YAP and miR-29, inactivating the mTOR pathway.

As mentioned above, several miRNAs have been found to be deregulated in TNBC cells and patients; evidence suggests that different types of exercise can regulate these miRNAs in different ways. One of these miRNAs is miR-21, which has been found to be upregulated in TNBC patients; it has an oncogene activity and plays a crucial role in tumor cell proliferation and invasion, repressing PTEN [128].

Nielsen et al. [129] showed how miR-21 level significantly decreased 3–5 days after endurance training (60 min of cycle ergometer exercise at 65% of P_{max}, 5 times a week for 12 weeks), at rest. However, levels of miR-21 were also found to be upregulated immediately after a single exhausting cycling exercise at a low heart rate, just as it was after a training period of 90 days [130]. Discrepancies between data obtained by these two studies could be explained by the different types of exercise considered, as confirmed by Wardle et al. [131].

The microRNA precursor miR-146a has also been found to be an upregulated miRNA in TNBC tissues, and its level is related to tumor size and survival rate. Nielsen et al. [129] showed that miR-146a levels significantly decreased immediately after a single session of pedaling exercise performed at 65% of the maximal power output. In this case, depending on the different exercise considered, miR-146 levels can be dysregulated: after a single exhausting cycling exercise at a low heart rate, it has been found to be upregulated [130]. Variations in miR-146 levels when comparing strength or endurance exercise groups to controls were observed; levels increase in the endurance group, while they decrease in the strength group [131]. The downregulation of miR-146a after strength exercise was also confirmed by a study that involved a single strength exercise session performed at 70% of one-repetition maximum [132] in which the miR-146a level was found to have decreased 3 days after exercise.

In short, a subset of circulating miRNAs, including miR-21 and miR-146a, are associated with the whole-body adaptive response to differential forms of exercise and training. These miRNAs have been found to be upregulated in TNBC patients and related to the repression of PTEN or BRCA1 with consequent mTOR pathway activation. Hence, their downregulation with specific types of exercises could be a very promising approach to control TNBC initiation and progression.

4. Energy Intake in TNBC and mTOR Modulation

mTORC1 is a key regulator of cell growth and proliferation, and at the same time, it is also at the centre of nutrient regulation and utilization. In this regard, a large number of studies have demonstrated the role of excessive energy intake on cancer development, and by contrast, the protective effects of CR [133]. While the antitumorigenic effects of CR are well established, the mechanism behind this relationship is not completely clear, though it is believed that the tumor suppressive effects are mediated, as they are for exercise, by enhanced apoptosis, modulation of systemic signals such as IGF-1, insulin, metabolic, and inflammatory pathways, as well as by reduced angiogenesis [134]. Specifically, a large quantity of data points to the role of mTOR activation in cancer development through protein-induced IGF-1 signaling and to the beneficial effects of caloric and protein restriction not only on aging-associated diseases such as cancer but also on life span [135,136] (Figure 1).

CR increases the level of the circulating adiponectin, which can exert anticancer effects through mechanisms that include an increase in insulin sensitivity, a decrease in insulin/IGF-1 and mTOR signaling via AMPK activation as well as a reduction in the proinflammatory cytokine expression via inhibition of the nuclear factor κ-light-chain-enhancer of activated B-cells (NF-κB) [136,137].

AMPK, as mentioned above, is an important mediator in the maintenance of cellular energy homeostasis, and recently, it has gained attention for its possible role as a metabolic tumor suppressor and in cancer prevention and control. Since AMPK phosphorylation is regulated by energy availability (AMP : ATP ratio), AMPK activators, such as metformin, CR, and aerobic exercise, reduce the incidence of cancer.

Leptin is a peptide hormone produced by white adipose tissue. It affects several tissues and acts on the hypothalamus to regulate appetite and energy expenditure. It also impacts carcinogenesis, angiogenesis, immune responses, cytokine production, and other biological processes [138,139].

Intermittent CR is associated with the suppression of murine mammary tumor incidence and a decrease in the leptin-to-adiponectin ratio [139]. This ratio, when elevated, is related to metabolic syndrome and some cancers [140,141]. In TNBC metastases, CR decreases proliferation, increases apoptosis, and downregulates the IGF1-1R pathway, coadiuvating canonical therapies [142]. Taken together, these findings show that dietary

interventions can ameliorate the systemic milieu and tumor microenvironment. Chronic CR is not suitable for cancer patients at risk for weight loss, cachexia, and immunosuppression, but it can be substituted with intermittent CR, fasting-mimicking diets, low carbohydrate/ketogenic diets, or CR mimetic drugs.

Fasting and low carbohydrate diets have been shown to reduce side effects and to enhance the effectiveness of chemotherapy and radiation therapy in animal models, and there is a great deal of interest in the potential clinical value of these interventions.

Protein consumption has different effects on cancer mortality, which vary according to age, with an increased risk in middle age and a reduction in the elderly [143].

Protein restriction (PC) for the middle-aged followed by moderate protein intake in elderly subjects may increase longevity and health span since protein restriction is sufficient to reduce growth hormone receptor (GHR)-IGF1 activity and can reduce cancer incidence in model organisms regardless of energy intake [144].

Moreover, L-type amino acid transporter 1 (LAT1), which transports large quantities of neutral amino acids, was found highly expressed in human BC tissues. The upregulation of LAT1 plays an important role in BC progression because more amino acids are required for protein synthesis and cellular proliferation [145].

The activation of the mTOR/S6K1 signaling pathway depends on the availability of amino acids (AA), particularly branched chain AA, such as leucine, and also glucose [106]. Growth factor signals, which usually activate mTORC1 signaling, have little or no impact in the absence of AA.

Leucine deprivation causes an upregulation of insulin-like growth factor binding protein 1 through transcriptional activation and mRNA stabilization, probably decreasing the effects of IGF1 and thus lowering cell proliferation [146].

However, in most BC cell lines with constitutively activated Akt/mTOR signaling, leucine restriction is not efficient in inhibiting mTOR signaling since it is associated with activation of survival molecule Akt, making leucine deprivation an undesirable approach for BC therapy [146].

Glutamine is another AA involved in the regulation of the mTOR pathway inducing the uptake of leucine [147]. Tumor cells are more sensitive to amino acid deprivation than normal cells; thus, glutamine restriction and/or transporter inhibition decrease mTOR activity [147].

A novel therapeutic approach based on whey protein concentrate (WPC) supplementation for BC treatment has been suggested by Cheng et al. [148]. WPC is rich in bioavailable cysteine, which can be used for glutathione synthesis, and contains all nine essential AAs. WPC promotes muscle protein synthesis [149] and can be used as a nutritional supplement during chemotherapy [150]. WPC has also been shown to enhance rapamycin sensitivity in MDA-MB-231 TNBC cells, a cell line resistant to rapamycin and other mTOR inhibitors [148].

The combination of conventional therapies and *n*-3 polyunsaturated fatty acid (PUFA) supplementation (nutritional interventions) increases the sensitivity of tumor cells to conventional therapies, possibly improving their efficacy especially against cancers resistant to treatment, as suggested by D'Eliseo and Velotti [151]. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have anticancer effects on different cancer types by inducing apoptotic cell death in human cancer cells either alone or in combination with canonical therapies. EPA and/or DHA also have proapoptotic effects in both triple-negative [152] and ER+ BC subtypes [153], although when compared at the same dose, DHA appears to be more effective. This might be due to the structural differences between DHA and EPA. The proapoptotic effects occur with increases in plasma membrane incorporation and decreases in cell viability [152–154], PI3K/Akt pathway [155], and pEGFR activation [152].

In agreement, CR and other nutritional interventions could play an important role in support of conventional therapies to improve TNBC outcomes.

5. Exercise Prescription in BC Survivors

In general, reviews and meta-analyses tend to group PA and exercise interventions into general categories and rarely examine the specific exercise protocols employed in the studies. Therefore, which characteristics make an exercise protocol safe and effective for BC survivors and, particularly, for TNBC patients?

Since the 2009 roundtable consensus statement on exercise guidelines for cancer survivors [156], which outlined the situations in which deviations from the 2008 US Physical Activity Guidelines for Americans (PAGA) were appropriate and included relevant implementation strategies [157], exercise recommendations from several

internationally recognized institutions, such as the American Cancer Society [158] and the National Comprehensive Cancer Network [159], have been published for BC survivors. Fortunately, all of the abovementioned publications have recently been reviewed within the framework for exercise prescription of the American College of Sports Medicine (ACSM) [160], along with others providing practical guidance for exercise prescription in these patients [161,162]. ACSM's framework for exercise prescription employs the so-called FITT-VP principle [160], which considers the frequency (*F*), intensity (*I*), time (*T*), and type (*T*) of exercise and its volume (*V*) and progression (*P*) over time in an individualized exercise training program.

A detailed description of the FITT-VP principle for each type of exercise—i.e., aerobic, resistance, and flexibility—adapted to BCS needs is provided in Tables 1, 2, and 3. Note that the following guidelines should not be regarded as specific for BC patients because no studies, to date [163], have adopted (and/or reported) the proper application of the principles of specificity, progression, overload, initial values, and adherence, within their exercise interventions. Therefore, although specific exercise guidelines for cancer survivors still need to be outlined, particularly for TNBC survivors, the following information represents the most up-to-date adaptations of the PAGA to BCS, including TBNC patients. Improving the reporting of exercise prescriptions will also allow for more specific recommendations regarding types and doses of exercise for BCS (and, hopefully, for the TNBC subgroup), in order to identify effective exercise interventions to be delivered to this growing community.

Table 1: Aerobic (cardiorespiratory endurance) exercise recommendations.

Intensity (I)	Frequency (F)	Time (T) (duration)	Type (T) (mode) (examples)	Volume (V) (quantity)	Progression (P) (rate of)	Specific notes
Light: 30–39% VO_2R/HRR; 57–63% HR_{max}; 9–11 RPE.	At least 5 d wk^{-1}.	30 to 60 min each session (i.e., at least 150 min wk^{-1}).	Continuous and rhythmic exercises that involve major muscle groups (walking, cycling, slow dancing, jogging, running, rowing, stepping, fast dancing, etc.).	≥500–1,000 MET min wk^{-1}.	Increase gradually any of the FITT components as tolerated by the patient (gradual progression is required to minimize the risks of muscular soreness, injury, undue fatigue, and the long-term risk of overtraining). Initiate increasing exercise duration (as tolerated): an example for healthy people is adding 5–10 min every 1–2 wk over the first 4–6 wk and adjusting upward over the next 4–8 months to meet the recommended FITT components, but slower progression may be needed for BCS.	If tolerated without adverse effects of symptoms or side effects, moderate to vigorous intensity and 3–5 d wk^{-1} frequency are recommended, but lower (light) intensities and frequencies are still beneficial when the current physical activity level is low. Avoid prescribing and monitoring intensity using %HRR (using $\%HR_{max}$ or RPE is recommended in BCS). Be aware of fracture risk, because bone is a common site of metastases in breast cancer: BCS with metastatic disease to the bone will require modification of their exercise program (e.g., reduced impact, intensity, and volume) given the increased risk of bone fragility and fractures.
Moderate: 40–59% VO_2R/HRR; 64–75% HR_{max}; 12-13 RPE.	At least 5 d wk^{-1}.	30 to 60 min each session (i.e., at least 150 min wk^{-1}).				
Vigorous: 60–89% VO_2R/HRR; 76–95% HR_{max}; 14–17 RPE.	At least 3 d wk^{-1}.	20 to 60 min each session (i.e., at least 75 min wk^{-1}).				

Modified from [160]. VO2R: oxygen uptake reserve, calculated as the difference between maximal oxygen uptake and resting oxygen uptake; HRR: heart rate reserve, calculated as the difference between maximal heart rate and resting heart rate; HR_{max}: maximal heart rate; RPE: rate of perceived exertion on the 6–20 scale; MET-min: metabolic equivalents (MET) of energy expenditure for a physical activity performed for a given number of minutes (min), calculated as MET × min; FITT: frequency, intensity, time, and type of exercise.

Table 2: Resistance (strength) exercise recommendations.

Intensity (I)	Frequency (F)	Time (T) (duration)	Type (T) (mode) (examples)	Volume (V) (quantity)	Progression (P) (rate of)	Specific notes
Light: 30–49% 1-RM.	2-3 d wk^{-1}.	Depends on exercise volume (number of sets, repetitions for each set, and rest intervals in-between) and is not associated with effectiveness.	Any form of movement designed to improve muscular fitness by exercising a muscle or a muscle group against external resistance: exercise and breathing techniques are of paramount importance and symptom-limited ROMs should be adopted according to BCS responses to exercise (free weights, resistance machines, weight-bearing functional tasks, etc.).	2–4 sets of 8–15 repetitions (at least 1 set of 8–12 repetitions can be effective in BCS) with 2-3 min rest between sets.	BCS should start with a supervised program of at least 16 sessions and very low resistance (<30% 1-RM), and progress with smallest increment possible (e.g., 2–10% 1-RM, depending on muscular size and involvement, is recommended for healthy adults). If a break is taken, lower the level of resistance by 2 wk worth for every week of no exercise.	No upper limit on the account of weight to which BCS can progress. Individuals with lymphedema should wear a compression sleeve during resistance training activity. Watch for arm/shoulder symptoms including lymphedema and reduce resistance or stop specific exercises according to symptom response. Be aware of risk of fracture (see aerobic exercise for details).
Moderate: 50–69% 1-RM.	2-3 d wk^{-1}.					
Vigorous: 70–84% 1-RM.	2-3 d wk^{-1}.					

Modified from [160]. 1-RM: one-repetition maximum, i.e., the load that can be lifted one time only; ROM: range of motion; BCS: breast cancer survivors.

Table 3: Flexibility (stretching) exercise recommendations.

Intensity (I)	Frequency (F)	Time (T) (duration)	Type (T) (mode) (examples)	Volume (V) (quantity)	Progression (P) (rate of)	Specific notes
Stretch to the point of feeling tightness or slight discomfort.	≥2-3 d wk^{-1} (stretching on a daily basis is most effective).	Hold a static stretch for at least 10–30 s (30–60 s may confer greater benefit). Accumulate a total of 60 s of stretching for each flexibility exercise by adjusting time/duration and repetitions (see volume) according to individual needs.	Stretching exercise that increases the ability to move a joint through its complete ROM (provided individual specific conditions are accounted for) (static active flexibility, static passive flexibility, dynamic flexibility, ballistic flexibility, proprioceptive neuromuscular facilitation, etc.).	Repeat each exercise 2–4 times in order to attain the goal of 60 s stretch time (e.g., two 30 s stretches or four 15 s stretches). A stretching routine can be completed approximately in ≤10 min.	Optimal progression is still unknown.	BCS should focus on joints in which a loss of ROM occurred because of surgery, corticosteroid use, and/or radiation therapy. Flexibility exercises are most effective when the muscles are warm.

Modified from [160]. ROM: range of motion; BCS: breast cancer survivors.

6. Benefits of Exercise Pre- and Postdiagnoses

Humans have not been “designed” for a sedentary lifestyle. The absence of an adequate level of PA puts us at increased risk of developing cancer. This has been highlighted by the European Breast Cancer Conference [164], issued an important statement: regular PA reduces the risk of BC for woman of any age and body weight by 12%.

PA as a nonpharmacological treatment to combat the collateral effects associated with BC is under considerable scientific attention [160,165,166]. To allow physicians to prescribe PA to patients before and after treatment, scientific clarity and evidence supporting the thesis that PA programs reduce the damaging effects of cancer and its treatment are needed. Very little is known about the effect of exercise on TNBC outcomes, but data suggest that pre- and postdiagnosis PA may be one of the factors, which, if appropriately prescribed, could bring benefits to patients.

Generally, TNBC has poor treatment outcomes because of a lack of receptor targets for conventional drugs to act upon. However, there is irrefutable evidence of the effectiveness of regular PA in primary and secondary prevention of premature death from any cause, including BC. Thus, different types of exercise can influence the prevention and progression of disease through several common mechanisms such as reduction of insulin resistance and improvement in immunity and cardiovascular function. Research in humans shows that exercise can regulate inflammation [13,167], oxidation [168,169], and gene expression [170].

Together with the potential mechanisms underlying the effects of exercise on breast carcinogenesis, Thompson [12] proposed three interesting hypotheses: (i) the hormesis hypothesis: oncological response to exercise is antithetical to a physiological cellular stress response; (ii) the metabolic reprogramming hypothesis: exercise reduces the glucose and glutamine available to mammary carcinomas, inducing apoptosis and reversing tumor-associated metabolic program; and (iii) the mTOR network hypothesis: exercise inhibits carcinogenesis by suppressing the activation of the mTOR signaling network in mammary carcinomas.

Recent investigations have revealed that the most active women had, on average, a 25–30% lower BC risk than women in the lowest category of recreational PA [171]. Data from the California Teachers Study (CTS) suggest that PA has a protective role in prediagnoses and may reduce a woman’s risk of BC, especially the TNBC subtype. An

analysis of the risk index (HR) associated with variations in the amount of PA hours among TNBC women yielded significant results. The HR results show significant associations when moderate-intense activity is considered as the only variable. When they are considered as separate variables, there are no statistically significant associations between moderate activity and TNBC, whereas intense activity is inversely associated with TNBC [172]. The reduction risk associated with baseline strenuous recreational PA was statistically significant among overweight or obese pre- or postmenopausal women, but not among their leaner counterparts.

In patients with BC postdiagnosis, acute and chronic symptoms, such as muscle mass loss, fatigue, weight gain, hormone alterations, bone loss, cachexia, and adverse psychological effects, may all be favourably influenced by regular exercise. A prospective cohort study analysed modifiable lifestyle factors, including exercise, associated with total mortality and recurrence/disease-specific mortality in patients with TNBC [173]. The association between TNBC prognoses and exercise postdiagnosis yielded important results: women who engaged in exercise regularly during the first 6 months postdiagnosis had a lower risk of total mortality and recurrence/disease-specific mortality, with adjusted HRs of 0.58 and 0.54, respectively. In addition, those who engaged in PA for a long time (2.5 h/wk) or women who exercised ≥7.6 metabolic equivalent hours/wk had a reduced risk of all causes and recurrence/disease-specific mortality compared with nonexercisers. Survivors who maintain a healthy weight and stay physically active have a better response to treatment and better survival outcomes. Thus, it is necessary to identify an appropriate promotion and prescription of regular PA for BC survivors in order to improve their prognosis, response to therapy, and quality of life. As previously described, the mTOR signaling pathway is differentially regulated by different exercise modalities, and it represents one of the main key regulators of the protective effects of exercise.

7. Conclusions

In this review, we presented new insights into the downregulation of mTOR signaling in TNBC by exercise and CR. It has been shown that mTOR network inhibition is mostly mediated through the effects of CR and vigorous PA as well as long-term exercise, which decrease the level of circulating growth factors and hormones.

During exercise, the body is exposed to different types of stressors, including temperature, metabolism, hypoxic, oxidative, and mechanical stress. These stressors initiate biochemical targets, which in turn actuate different signaling pathways that regulate gene expression and adaptive responses. Beneficial adaptation likely depends on the basal state of oxidative stress and inflammation at the beginning of exercise training. In turn, this basal state may depend on the periodization of training and recovery, together with age, health status, and diet.

Exercise, as a hormetic agent, has the potential for beneficial energy upregulation. The dose response effects are complex and reflect activation of major defensive pathways in both systemic and local environments. A mitohormetic stimulus that occurs through a physiological cellular stress adaptation and AMPK activation across hormetic control circuits, such as increase of oxidative metabolism, mitochondrial biogenesis, angiogenesis, immune regulation and a decrease in BMI, and insulin secretion, are induced by exercise. Moreover, PA increases glucagon, catecholamines, and other hormones and influences miRNAs involved in cancer. Exercise as well as CR limit glycaemia and glutamine availability to mammary carcinomas, inducing apoptosis and reversing malignancy-associated metabolic programming. It is also known that intratumoral metabolism is regulated by exercise, but how this affects tumor growth and metastatic rate is not clearly understood. Although the signal for these hormonal and autonomic changes has been partially described in ex vivo experiments, such changes are difficult to transfer in vivo. Currently, there is an agreement in the literature that there is a role for exercise as a coadiuvant “medicine” in canonical therapies and that it has an increasingly protective tumorigenic effect. In this context, PA needs to be broken down into its main components: frequency, intensity, time, and type; however, the dose-dependent effects of each of these components on cancer protection via mTOR inhibition are still unclear. Most data suggest that both vigorous and long-term PA in adulthood may reduce a woman’s risk of mammalian cancer, especially the TNBC subtype.

Finally, we can assert that there is a sufficient evidence showing that sedentary behaviour and nutritional risk factors for TNBC are modifiable. Hence, the suggestions regarding the modification of such risk factors highlighted in this review could have wide-ranging implications for society and may improve public healthcare cancer management. Accordingly, we would like to emphasize the importance of promoting physically active lifestyles to reduce the risk of relapse in TNBC. Fostering active lifestyles can provide important support during conventional cancer treatment, preventing the potential negative impacts on patients' physical condition, as well as their emotional and social well-being.

References

1. A. Jemal, F. Bray, M. M. Center, J. Ferlay, E. Ward, and D. Forman, "Global cancer statistics," CA: A Cancer Journal for Clinicians, vol. 61, no. 2, pp. 69–90, 2011.
2. G. Bianchini, J. M. Balko, I. A. Mayer, M. E. Sanders, and L. Gianni, "Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease," Nature Reviews Clinical Oncology, vol. 13, no. 11, pp. 674–690, 2016.
3. P. Boyle, "Triple-negative breast cancer: epidemiological considerations and recommendations," Annals of Oncology, vol. 23, Supplement 6, pp. vi7–vi12, 2012.
4. R. Dent, M. Trudeau, K. I. Pritchard et al., "Triple-negative breast cancer: clinical features and patterns of recurrence," Clinical Cancer Research, vol. 13, no. 15, pp. 4429–4434, 2007.
5. W. D. Foulkes, I. E. Smith, and J. S. Reis-Filho, "Triple-negative breast cancer," New England Journal of Medicine, vol. 363, no. 20, pp. 1938–1948, 2010.
6. B. G. Haffty, Q. Yang, M. Reiss et al., "Locoregional relapse and distant metastasis in conservatively managed triple negative early-stage breast cancer," Journal of Clinical Oncology, vol. 24, no. 36, pp. 5652–5657, 2006.
7. C. Oakman, G. Viale, and A. Di Leo, "Management of triple negative breast cancer," The Breast, vol. 19, no. 5, pp. 312–321, 2010.
8. C. Duggan, M. L. Irwin, L. Xiao et al., "Associations of insulin resistance and adiponectin with mortality in women with breast cancer," Journal of Clinical Oncology, vol. 29, no. 1, pp. 32–39, 2011.
9. F. O. Ademuyiwa, A. Groman, T. O'Connor, C. Ambrosone, N. Watroba, and S. B. Edge, "Impact of body mass index on clinical outcomes in triple-negative breast cancer," Cancer, vol. 117, no. 18, pp. 4132–4140, 2011.
10. E. M. Ibrahim and A. Al-Homaidh, "Physical activity and survival after breast cancer diagnosis: meta-analysis of published studies," Medical Oncology, vol. 28, no. 3, pp. 753–765, 2011.
11. D. R. Brenner, N. T. Brockton, J. Kotsopoulos et al., "Breast cancer survival among young women: a review of the role of modifiable lifestyle factors," Cancer Causes & Control, vol. 27, no. 4, pp. 459–472, 2016.
12. H. J. Thompson, W. Jiang, and Z. Zhu, "Candidate mechanisms accounting for effects of physical activity on breast carcinogenesis," IUBMB Life, vol. 61, no. 9, pp. 895–901, 2009.
13. A. M. W. Petersen and B. K. Pedersen, "The anti-inflammatory effect of exercise," Journal of Applied Physiology, vol. 98, no. 4, pp. 1154–1162, 2005.
14. C. Dethlefsen, C. Lillelund, J. Midtgaard et al., "Exercise regulates breast cancer cell viability: systemic training adaptations versus acute exercise responses," Breast Cancer Research and Treatment, vol. 159, no. 3, pp. 469–479, 2016.
15. G. J. Koelwyn, D. F. Quail, X. Zhang, R. M. White, and L. W. Jones, "Exercise-dependent regulation of the tumour microenvironment," Nature Reviews Cancer, vol. 17, no. 10, pp. 620–632, 2017.
16. L. Cascione, P. Gasparini, F. Lovat et al., "Integrated microRNA and mRNA signatures associated with survival in triple negative breast cancer," PLoS One, vol. 8, no. 2, article e55910, 2013.

17. M. Polakovičová, P. Musil, E. Laczo, D. Hamar, and J. Kyselovič, "Circulating microRNAs as potential biomarkers of exercise response," International Journal of Molecular Sciences, vol. 17, no. 10, p. 1553, 2016.
18. M. Laplante and D. M. Sabatini, "mTOR signaling in growth control and disease," Cell, vol. 149, no. 2, pp. 274–293, 2012.
19. S. H. Hare and A. J. Harvey, "mTOR function and therapeutic targeting in breast cancer," American Journal of Cancer Research, vol. 7, no. 3, pp. 383–404, 2017.
20. C. C. Dibble, W. Elis, S. Menon et al., "TBC1D7 is a third subunit of the TSC1-TSC2 complex upstream of mTORC1," Molecular Cell, vol. 47, no. 4, pp. 535–546, 2012.
21. A. R. Tee, B. D. Manning, P. P. Roux, L. C. Cantley, and J. Blenis, "Tuberous sclerosis complex gene products, tuberin and hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb," Current Biology, vol. 13, no. 15, pp. 1259–1268, 2003.
22. B. A. Hemmings and D. F. Restuccia, "PI3K-PKB/Akt pathway," Cold Spring Harbor Perspectives in Biology, vol. 4, no. 9, article a011189, 2012.
23. D. M. Gwinn, D. B. Shackelford, D. F. Egan et al., "AMPK phosphorylation of raptor mediates a metabolic checkpoint," Molecular Cell, vol. 30, no. 2, pp. 214–226, 2008.
24. Y. Sancak, T. R. Peterson, Y. D. Shaul et al., "The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1," Science, vol. 320, no. 5882, pp. 1496–1501, 2008.
25. S. S. Schalm, D. C. Fingar, D. M. Sabatini, and J. Blenis, "TOS motif-mediated raptor binding regulates 4E-BP1 multisite phosphorylation and function," Current Biology, vol. 13, no. 10, pp. 797–806, 2003.
26. H. Nojima, C. Tokunaga, S. Eguchi et al., "The mammalian target of rapamycin (mTOR) partner, raptor, binds the mTOR substrates p70 S6 kinase and 4E-BP1 through their TOR signaling (TOS) motif," Journal of Biological Chemistry, vol. 278, no. 18, pp. 15461–15464, 2003.
27. B. Magnuson, B. Ekim, and D. C. Fingar, "Regulation and function of ribosomal protein S6 kinase (S6K) within mTOR signalling networks," Biochemical Journal, vol. 441, no. 1, pp. 1–21, 2012.
28. D. Egan, J. Kim, R. J. Shaw, and K. L. Guan, "The autophagy initiating kinase ULK1 is regulated via opposing phosphorylation by AMPK and mTOR," Autophagy, vol. 7, no. 6, pp. 643-644, 2011.
29. S. Z. Millis, S. Ikeda, S. Reddy, Z. Gatalica, and R. Kurzrock, "Landscape of phosphatidylinositol-3-kinase pathway alterations across 19 784 diverse solid tumors," JAMA Oncology, vol. 2, no. 12, pp. 1565–1573, 2016.
30. P. M. LoRusso, "Inhibition of the PI3K/AKT/mTOR pathway in solid tumors," Journal of Clinical Oncology, vol. 34, no. 31, pp. 3803–3815, 2016.
31. C. Simioni, A. M. Martelli, G. Zauli et al., "Targeting the phosphatidylinositol 3-kinase/Akt/mechanistic target of rapamycin signaling pathway in B-lineage acute lymphoblastic leukemia: an update," Journal of Cellular Physiology, vol. 233, no. 10, pp. 6440–6454, 2018.
32. B. Vanhaesebroeck, J. Guillermet-Guibert, M. Graupera, and B. Bilanges, "The emerging mechanisms of isoform-specific PI3K signalling," Nature Reviews Molecular Cell Biology, vol. 11, no. 5, pp. 329–341, 2010.

33. T. Liu, R. Yacoub, L. D. Taliaferro-Smith et al., "Combinatorial effects of lapatinib and rapamycin in triple-negative breast cancer cells," Molecular Cancer Therapeutics, vol. 10, no. 8, pp. 1460–1469, 2011.
34. P. Cossu-Rocca, S. Orru, M. R. Muroni et al., "Analysis of PIK3CA mutations and activation pathways in triple negative breast cancer," PLoS One, vol. 10, no. 11, article e0141763, 2015.
35. L. M. Ooms, L. C. Binge, E. M. Davies et al., "The inositol polyphosphate 5-phosphatase PIPP regulates AKT1-dependent breast cancer growth and metastasis," Cancer Cell, vol. 28, no. 2, pp. 155–169, 2015.
36. Cancer Genome Atlas Network, "Comprehensive molecular portraits of human breast tumours," Nature, vol. 490, no. 7418, pp. 61–70, 2012.
37. H. Liu, C. J. Murphy, F. A. Karreth et al., "Identifying and targeting sporadic oncogenic genetic aberrations in mouse models of triple-negative breast cancer," Cancer Discovery, vol. 8, no. 3, pp. 354–369, 2018.
38. J. Rodon, R. Dienstmann, V. Serra, and J. Tabernero, "Development of PI3K inhibitors: lessons learned from early clinical trials," Nature Reviews Clinical Oncology, vol. 10, no. 3, pp. 143–153, 2013.
39. J. C. Yao, M. H. Shah, T. Ito et al., "and for the Rad001 in Advanced Neuroendocrine Tumors-Third Trial (RADIANT-3) Study Group, Everolimus for advanced pancreatic neuroendocrine tumors," New England Journal of Medicine, vol. 364, no. 6, pp. 514–523, 2011.
40. G. Hudes, M. Carducci, P. Tomczak et al., "Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma," New England Journal of Medicine, vol. 356, no. 22, pp. 2271–2281, 2007.
41. G. I. Shapiro, J. Rodon, C. Bedell et al., "Phase I safety, pharmacokinetic, and pharmacodynamic study of SAR245408 (XL147), an oral pan-class I PI3K inhibitor, in patients with advanced solid tumors," Clinical Cancer Research, vol. 20, no. 1, pp. 233–245, 2014.
42. R. R. Furman, J. P. Sharman, S. E. Coutre et al., "Idelalisib and rituximab in relapsed chronic lymphocytic leukemia," New England Journal of Medicine, vol. 370, no. 11, pp. 997–1007, 2014.
43. A. M. Gonzalez-Angulo, D. Juric, G. Argilés et al., "Safety, pharmacokinetics, and preliminary activity of the α-specific PI3K inhibitor BYL719: results from the first-in-human study," Journal of Clinical Oncology, vol. 31, 15_Supplement, pp. 2531–2531, 2013.
44. R. J. O. Dowling, I. Topisirovic, B. D. Fonseca, and N. Sonenberg, "Dissecting the role of mTOR: lessons from mTOR inhibitors," Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics, vol. 1804, no. 3, pp. 433–439, 2010.
45. R. Dienstmann, J. Rodon, V. Serra, and J. Tabernero, "Picking the point of inhibition: a comparative review of PI3K/AKT/mTOR pathway inhibitors," Molecular Cancer Therapeutics, vol. 13, no. 5, pp. 1021–1031, 2014.
46. M. Kilic-Eren, T. Boylu, and V. Tabor, "Targeting PI3K/Akt represses hypoxia inducible factor-1αactivation and sensitizes rhabdomyosarcoma and Ewing's sarcoma cells for apoptosis," Cancer Cell International, vol. 13, no. 1, p. 36, 2013.
47. C. Fumarola, M. A. Bonelli, P. G. Petronini, and R. R. Alfieri, "Targeting PI3K/AKT/mTOR pathway in non small cell lung cancer," Biochemical Pharmacology, vol. 90, no. 3, pp. 197–207, 2014.

48. A. K. Pandurangan, "Potential targets for prevention of colorectal cancer: a focus on PI3K/Akt/mTOR and Wnt pathways," Asian Pacific Journal of Cancer Prevention, vol. 14, no. 4, pp. 2201–2205, 2013.
49. M. S. Petrulea, T. S. Plantinga, J. W. Smit, C. E. Georgescu, and R. T. Netea-Maier, "PI3K/Akt/mTOR: a promising therapeutic target for non-medullary thyroid carcinoma," Cancer Treatment Reviews, vol. 41, no. 8, pp. 707–713, 2015.
50. T. K. Owonikoko and F. R. Khuri, "Targeting the PI3K/AKT/mTOR pathway: biomarkers of success and tribulation," American Society of Clinical Oncology Educational Book, vol. 33, pp. e395–e401, 2013.
51. R. L. B. Costa, H. S. Han, and W. J. Gradishar, "Targeting the PI3K/AKT/mTOR pathway in triple-negative breast cancer: a review," Breast Cancer Research and Treatment, vol. 169, no. 3, pp. 397–406, 2018.
52. S.-B. Kim, R. Dent, S.-A. Im et al., "Ipatasertib plus paclitaxel versus placebo plus paclitaxel as first-line therapy for metastatic triple-negative breast cancer (LOTUS): a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial," The Lancet Oncology, vol. 18, no. 10, pp. 1360–1372, 2017.
53. K. Ke and T. Lou, "MicroRNA-10a suppresses breast cancer progression via PI3K/Akt/mTOR pathway," Oncology Letters, vol. 14, no. 5, pp. 5994–6000, 2017.
54. Y. W. Phua, A. Nguyen, D. L. Roden et al., "MicroRNA profiling of the pubertal mouse mammary gland identifies miR-184 as a candidate breast tumour suppressor gene," Breast Cancer Research, vol. 17, no. 1, p. 83, 2015.
55. M. Esteller, "Epigenetic gene silencing in cancer: the DNA hypermethylome," Human Molecular Genetics, vol. 16, no. R1, pp. R50–R59, 2007.
56. H. Fang, J. Xie, M. Zhang, Z. Zhao, Y. Wan, and Y. Yao, "miRNA-21 promotes proliferation and invasion of triple-negative breast cancer cells through targeting PTEN," American Journal of Translational Research, vol. 9, no. 3, pp. 953–961, 2017.
57. Q. Wang, C. Wang, J. Guo, J. Fan, and Z. Zhang, "Expression of miR-146a in triple negative and its clinical significance," International Journal of Clinical and Experimental Pathology, vol. 9, no. 11, pp. 11832–11837, 2016.
58. A. I. Garcia, M. Buisson, P. Bertrand et al., "Down-regulation of BRCA1 expression by miR-146a and miR-146b-5p in triple negative sporadic breast cancers," EMBO Molecular Medicine, vol. 3, no. 5, pp. 279–290, 2011.
59. T. Xiang, Y. Jia, D. Sherris et al., "Targeting the Akt/mTOR pathway in Brca1-deficient cancers," Oncogene, vol. 30, no. 21, pp. 2443–2450, 2011.
60. H. Y. Chen and E. White, "Role of autophagy in cancer prevention," Cancer Prevention Research, vol. 4, no. 7, pp. 973–983, 2011.
61. E. White, "Deconvoluting the context-dependent role for autophagy in cancer," Nature Reviews Cancer, vol. 12, no. 6, pp. 401–410, 2012.
62. Y. Liu and B. Levine, "Autosis and autophagic cell death: the dark side of autophagy," Cell Death & Differentiation, vol. 22, no. 3, pp. 367–376, 2015.

63. *Y. Wei, Z. Zou, N. Becker et al., "EGFR-mediated Beclin 1 phosphorylation in autophagy suppression, tumor progression, and tumor chemoresistance," Cell, vol. 154, no. 6, pp. 1269–1284, 2013.*
64. *S. Rao, L. Tortola, T. Perlot et al., "A dual role for autophagy in a murine model of lung cancer," Nature Communications, vol. 5, no. 1, p. 3056, 2014.*
65. *R. L. Macintosh and K. M. Ryan, "Autophagy in tumour cell death," Seminars in Cancer Biology, vol. 23, no. 5, pp. 344–351, 2013.*
66. *B. Levine, M. Packer, and P. Codogno, "Development of autophagy inducers in clinical medicine," Journal of Clinical Investigation, vol. 125, no. 1, pp. 14–24, 2015.*
67. *C. He, M. C. Bassik, V. Moresi et al., "Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis," Nature, vol. 481, no. 7382, pp. 511–515, 2012.*
68. *C. He, R. Sumpter Jr, and B. Levine, "Exercise induces autophagy in peripheral tissues and in the brain," Autophagy, vol. 8, no. 10, pp. 1548–1551, 2012.*
69. *P. D. Neufer, M. M. Bamman, D. M. Muoio et al., "Understanding the cellular and molecular mechanisms of physical activity-induced health benefits," Cell Metabolism, vol. 22, no. 1, pp. 4–11, 2015.*
70. *B. Egan and D. P. D'Agostino, "Fueling performance: ketones enter the mix," Cell Metabolism, vol. 24, no. 3, pp. 373–375, 2016.*
71. *J. A. Hawley, M. Hargreaves, M. J. Joyner, and J. R. Zierath, "Integrative biology of exercise," Cell, vol. 159, no. 4, pp. 738–749, 2014.*
72. *W. Fan and R. Evans, "PPARs and ERRs: molecular mediators of mitochondrial metabolism," Current Opinion in Cell Biology, vol. 33, pp. 49–54, 2015.*
73. *W. Fan, W. Waizenegger, C. S. Lin et al., "PPARδ promotes running endurance by preserving glucose," Cell Metabolism, vol. 25, no. 5, pp. 1186–1193.e4, 2017.*
74. *L. R. Silveira, H. Pilegaard, K. Kusuhara, R. Curi, and Y. Hellsten, "The contraction induced increase in gene expression of peroxisome proliferator-activated receptor (PPAR)-γ coactivator 1α (PGC-1α), mitochondrial uncoupling protein 3 (UCP3) and hexokinase II (HKII) in primary rat skeletal muscle cells is dependent on reactive oxygen species," Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, vol. 1763, no. 9, pp. 969–976, 2006.*
75. *I. Irrcher, V. Ljubicic, and D. A. Hood, "Interactions between ROS and AMP kinase activity in the regulation of PGC-1alpha transcription in skeletal muscle cells," American Journal of Physiology-Cell Physiology, vol. 296, no. 1, pp. C116–C123, 2009.*
76. *B. Chaube and M. K. Bhat, "AMPK, a key regulator of metabolic/energy homeostasis and mitochondrial biogenesis in cancer cells," Cell Death and Disease, vol. 7, no. 1, article e2044, 2016.*
77. *K. Watson and K. Baar, "mTOR and the health benefits of exercise," Seminars in Cell & Developmental Biology, vol. 36, pp. 130–139, 2014.*
78. *Z. Song, D. R. Moore, N. Hodson et al., "Resistance exercise initiates mechanistic target of rapamycin (mTOR) translocation and protein complex co-localisation in human skeletal muscle," Scientific Reports, vol. 7, no. 1, p. 5028, 2017.*

79. A. McTiernan, "Mechanisms linking physical activity with cancer," Nature Reviews Cancer, vol. 8, no. 3, pp. 205–211, 2008.
80. B. K. Pedersen and L. Hoffman-Goetz, "Exercise and the immune system: regulation, integration, and adaptation," Physiological Reviews, vol. 80, no. 3, pp. 1055–1081, 2000.
81. B. K. Pedersen, A. Steensberg, C. Fischer, C. Keller, K. Ostrowski, and P. Schjerling, "Exercise and cytokines with particular focus on muscle-derived IL-6," Exercise Immunology Review, vol. 7, pp. 18–31, 2001.
82. H. Zouhal, C. Jacob, P. Delamarche, and A. Gratas-Delamarche, "Catecholamines and the effects of exercise, training and gender," Sports Medicine, vol. 38, no. 5, pp. 401–423, 2008.
83. E. Barbieri, D. Agostini, E. Polidori et al., "The pleiotropic effect of physical exercise on mitochondrial dynamics in aging skeletal muscle," Oxidative Medicine and Cellular Longevity, vol. 2015, Article ID 917085, 15 pages, 2015.
84. E. Barbieri, P. Sestili, L. Vallorani et al., "Mitohormesis in muscle cells: a morphological, molecular, and proteomic approach," Muscle, Ligaments and Tendons Journal, vol. 3, no. 4, pp. 254–266, 2013.
85. V. Calabrese, C. Cornelius, A. T. Dinkova-Kostova, E. J. Calabrese, and M. P. Mattson, "Cellular stress responses, the hormesis paradigm, and vitagenes: novel targets for therapeutic intervention in neurodegenerative disorders," Antioxidants & Redox Signaling, vol. 13, no. 11, pp. 1763–1811, 2010.
86. M. P. Mattson, "Hormesis defined," Ageing Research Reviews, vol. 7, no. 1, pp. 1–7, 2008.
87. M. Ristow and K. Schmeisser, "Mitohormesis: promoting health and lifespan by increased levels of reactive oxygen species (ROS)," Dose-Response, vol. 12, no. 2, pp. 288–341, 2014.
88. T. L. Merry and M. Ristow, "Mitohormesis in exercise training," Free Radical Biology & Medicine, vol. 98, pp. 123–130, 2016.
89. E. Barbieri, M. Battistelli, L. Casadei et al., "Morphofunctional and biochemical approaches for studying mitochondrial changes during myoblasts differentiation," Journal of Aging Research, vol. 2011, Article ID 845379, 16 pages, 2011.
90. E. Barbieri and P. Sestili, "Reactive oxygen species in skeletal muscle signaling," Journal of Signal Transduction, vol. 2012, Article ID 982794, 17 pages, 2012.
91. M. V. Blagosklonny, "Hormesis does not make sense except in the light of TOR-driven aging," Aging, vol. 3, no. 11, pp. 1051–1062, 2011.
92. J. S. Sonneborn, "Mimetics of hormetic agents: stress-resistance triggers," Dose-Response, vol. 8, no. 1, pp. 97–121, 2010.
93. J. Yun and T. Finkel, "Mitohormesis," Cell Metabolism, vol. 19, no. 5, pp. 757–766, 2014.
94. D. G. Hardie, "AMP-activated protein kinase—an energy sensor that regulates all aspects of cell function," Genes & Development, vol. 25, no. 18, pp. 1895–1908, 2011.
95. D. G. Hardie, "The LKB1-AMPK pathway—friend or foe in cancer?" Cancer Cell, vol. 23, no. 2, pp. 131-132, 2013.
96. J. A. Hawley and S. J. Lessard, "Exercise training-induced improvements in insulin action," Acta Physiologica, vol. 192, no. 1, pp. 127–135, 2008.

97. M. Ristow, K. Zarse, A. Oberbach et al., "Antioxidants prevent health-promoting effects of physical exercise in humans," Proceedings of the National Academy of Sciences, vol. 106, no. 21, pp. 8665–8670, 2009.
98. E. L. Greer, D. Dowlatshahi, M. R. Banko et al., "An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in C. elegans," Current Biology, vol. 17, no. 19, pp. 1646–1656, 2007.
99. L. R. Saunders and E. Verdin, "Sirtuins: critical regulators at the crossroads between cancer and aging," Oncogene, vol. 26, no. 37, pp. 5489–5504, 2007.
100. A. Brunet, L. B. Sweeney, J. F. Sturgill et al., "Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase," Science, vol. 303, no. 5666, pp. 2011–2015, 2004.
101. H. Vaziri, S. K. Dessain, E. N. Eaton et al., "hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase," Cell, vol. 107, no. 2, pp. 149–159, 2001.
102. G. Annibalini, F. Lucertini, D. Agostini et al., "Concurrent aerobic and resistance training has anti-inflammatory effects and increases both plasma and leukocyte levels of IGF-1 in late middle-aged type 2 diabetic patients," Oxidative Medicine and Cellular Longevity, vol. 2017, Article ID 3937842, 10 pages, 2017.
103. V. Natalucci, G. Baldelli, M. De Santi et al., "Effects of exercise on breast cancer triple-negative cell proliferation in vitro," in SISMES IX National Congress, vol. 13 of Sport Science for Health, Supplement 1, Brescia, Italy, September 29–October 1 2017.
104. L. Pedersen, J. F. Christensen, and P. Hojman, "Effects of exercise on tumor physiology and metabolism," The Cancer Journal, vol. 21, no. 2, pp. 111–116, 2015.
105. A. M. Bodles and S. W. Barger, "Cytokines and the aging brain - what we don't know might help us," Trends in Neurosciences, vol. 27, no. 10, pp. 621–626, 2004.
106. W. De Haes, L. Frooninckx, R. Van Assche et al., "Metformin promotes lifespan through mitohormesis via the peroxiredoxin PRDX-2," Proceedings of the National Academy of Sciences, vol. 111, no. 24, pp. E2501–E2509, 2014.
107. V. A. Narkar, M. Downes, R. T. Yu et al., "AMPK and PPARdelta agonists are exercise mimetics," Cell, vol. 134, no. 3, pp. 405–415, 2008.
108. K. I. Stanford and L. J. Goodyear, "Exercise and type 2 diabetes: molecular mechanisms regulating glucose uptake in skeletal muscle," Advances in Physiology Education, vol. 38, no. 4, pp. 308–314, 2014.
109. P. Pasanisi, F. Berrino, M. De Petris, E. Venturelli, A. Mastroianni, and S. Panico, "Metabolic syndrome as a prognostic factor for breast cancer recurrences," International Journal of Cancer, vol. 119, no. 1, pp. 236–238, 2006.
110. Y. Liubaoerjijin, T. Terada, K. Fletcher, and N. G. Boule, "Effect of aerobic exercise intensity on glycemic control in type 2 diabetes: a meta-analysis of head-to-head randomized trials," Acta Diabetologica, vol. 53, no. 5, pp. 769–781, 2016.
111. C. Jelleyman, T. Yates, G. O'Donovan et al., "The effects of high-intensity interval training on glucose regulation and insulin resistance: a meta-analysis," Obesity Reviews, vol. 16, no. 11, pp. 942–961, 2015.

112. *E. A. Richter and M. Hargreaves, "Exercise, GLUT4, and skeletal muscle glucose uptake," Physiological Reviews, vol. 93, no. 3, pp. 993–1017, 2013.*

113. *D. D. Sarbassov, D. A. Guertin, S. M. Ali, and D. M. Sabatini, "Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex," Science, vol. 307, no. 5712, pp. 1098–1101, 2005.*

114. *G. D. Cartee, "Roles of TBC1D1 and TBC1D4 in insulin- and exercise-stimulated glucose transport of skeletal muscle," Diabetologia, vol. 58, no. 1, pp. 19–30, 2015.*

115. *C. Frosig and E. A. Richter, "Improved insulin sensitivity after exercise: focus on insulin signaling," Obesity, vol. 17, no. n3s, pp. S15–S20, 2009.*

116. *D. LeRoith and C. T. Roberts Jr., "The insulin-like growth factor system and cancer," Cancer Letters, vol. 195, no. 2, pp. 127–137, 2003.*

117. *S. E. Hankinson, W. C. Willett, G. A. Colditz et al., "Circulating concentrations of insulin-like growth factor-I and risk of breast cancer," The Lancet, vol. 351, no. 9113, pp. 1393–1396, 1998.*

118. *G. Annibalini, P. Bielli, M. De Santi et al., "MIR retroposon exonization promotes evolutionary variability and generates species-specific expression of IGF-1 splice variants," Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms, vol. 1859, no. 5, pp. 757–768, 2016.*

119. *M. De Santi, G. Annibalini, E. Barbieri et al., "Human IGF1 pro-forms induce breast cancer cell proliferation via the IGF1 receptor," Cellular Oncology, vol. 39, no. 2, pp. 149–159, 2016.*

120. *J. F. Meneses-Echavez, E. G. Jimenez, J. S. Rio-Valle, J. E. Correa-Bautista, M. Izquierdo, and R. Ramirez-Velez, "The insulin-like growth factor system is modulated by exercise in breast cancer survivors: a systematic review and meta-analysis," BMC Cancer, vol. 16, no. 1, p. 682, 2016.*

121. *C. M. Dieli-Conwright, K. S. Courneya, W. Demark-Wahnefried et al., "Effects of aerobic and resistance exercise on metabolic syndrome, sarcopenic obesity, and circulating biomarkers in overweight or obese survivors of breast cancer: a randomized controlled trial," Journal of Clinical Oncology, vol. 36, no. 9, pp. 875–883, 2018.*

122. *D. W. Kang, J. Lee, S. H. Suh, J. Ligibel, K. S. Courneya, and J. Y. Jeon, "Effects of exercise on insulin, IGF axis, adipocytokines, and inflammatory markers in breast cancer survivors: a systematic review and meta-analysis," Cancer Epidemiology Biomarkers & Prevention, vol. 26, no. 3, pp. 355–365, 2017.*

123. *K. M. Winters-Stone, L. J. Wood, S. Stoyles, and N. F. Dieckmann, "The effects of resistance exercise on biomarkers of breast cancer prognosis: a pooled analysis of three randomized trials," Cancer Epidemiology Biomarkers & Prevention, vol. 27, no. 2, pp. 146–153, 2018.*

124. *C. Dethlefsen, L. S. Hansen, C. Lillelund et al., "Exercise-induced catecholamines activate the hippo tumor suppressor pathway to reduce risks of breast cancer development," Cancer Research, vol. 77, no. 18, pp. 4894–4904, 2017.*

125. *F.-X. Yu, B. Zhao, N. Panupinthu et al., "Regulation of the Hippo-YAP pathway by G-protein coupled receptor signaling," Cell, vol. 150, no. 4, pp. 780–791, 2012.*

126. *F. Zanconato, M. Cordenonsi, and S. Piccolo, "YAP/TAZ at the roots of cancer," Cancer Cell, vol. 29, no. 6, pp. 783–803, 2016.*

127. K. Tumaneng, K. Schlegelmilch, R. C. Russell et al., "YAP mediates crosstalk between the Hippo and PI(3)K-TOR pathways by suppressing PTEN via miR-29," Nature Cell Biology, vol. 14, no. 12, pp. 1322–1329, 2012.

128. L. Z. Liu, C. Li, Q. Chen et al., "MiR-21 induced angiogenesis through AKT and ERK activation and HIF-1α expression," PLoS One, vol. 6, no. 4, article e19139, 2011.

129. S. Nielsen, T. Akerstrom, A. Rinnov et al., "The miRNA plasma signature in response to acute aerobic exercise and endurance training," PLoS One, vol. 9, no. 2, article e87308, 2014.

130. A. L. Baggish, A. Hale, R. B. Weiner et al., "Dynamic regulation of circulating microRNA during acute exhaustive exercise and sustained aerobic exercise training," The Journal of Physiology, vol. 589, no. 16, pp. 3983–3994, 2011.

131. S. L. Wardle, M. E. S. Bailey, A. Kilikevicius et al., "Plasma microRNA levels differ between endurance and strength athletes," PLoS One, vol. 10, no. 4, article e0122107, 2015.

132. S. Sawada, M. Kon, S. Wada, T. Ushida, K. Suzuki, and T. Akimoto, "Profiling of circulating microRNAs after a bout of acute resistance exercise in humans," PLoS One, vol. 8, no. 7, article e70823, 2013.

133. V. D. Longo and L. Fontana, "Calorie restriction and cancer prevention: metabolic and molecular mechanisms," Trends in Pharmacological Sciences, vol. 31, no. 2, pp. 89–98, 2010.

134. C. H. O'Flanagan, L. A. Smith, S. B. McDonell, and S. D. Hursting, "When less may be more: calorie restriction and response to cancer therapy," BMC Medicine, vol. 15, no. 1, p. 106, 2017.

135. S. D. Hursting, S. M. Dunlap, N. A. Ford, M. J. Hursting, and L. M. Lashinger, "Calorie restriction and cancer prevention: a mechanistic perspective," Cancer & Metabolism, vol. 1, no. 1, p. 10, 2013.

136. S. C. Johnson, P. S. Rabinovitch, and M. Kaeberlein, "mTOR is a key modulator of ageing and age-related disease," Nature, vol. 493, no. 7432, pp. 338–345, 2013.

137. D. Barb, C. J. Williams, A. K. Neuwirth, and C. S. Mantzoros, "Adiponectin in relation to malignancies: a review of existing basic research and clinical evidence," The American Journal of Clinical Nutrition, vol. 86, no. 3, pp. 858S–866S, 2007.

138. E. C. Villanueva and M. G. Myers, "Leptin receptor signaling and the regulation of mammalian physiology," International Journal of Obesity, vol. 32, Supplement 7, pp. S8–12, 2008.

139. O. P. Rogozina, M. J. L. Bonorden, C. N. Seppanen, J. P. Grande, and M. P. Cleary, "Effect of chronic and intermittent calorie restriction on serum adiponectin and leptin and mammary tumorigenesis," Cancer Prevention Research, vol. 4, no. 4, pp. 568–581, 2011.

140. C. H. Jung, E. J. Rhee, J. H. Choi et al., "The relationship of adiponectin/leptin ratio with homeostasis model assessment insulin resistance index and metabolic syndrome in apparently healthy Korean male adults," Korean Diabetes Journal, vol. 34, no. 4, pp. 237–243, 2010.

141. D.-C. Chen, Y.-F. Chung, Y.-T. Yeh et al., "Serum adiponectin and leptin levels in Taiwanese breast cancer patients," Cancer Letters, vol. 237, no. 1, pp. 109–114, 2006.

142. B. A. Simone, T. Dan, A. Palagani et al., "Caloric restriction coupled with radiation decreases metastatic burden in triple negative breast cancer," Cell Cycle, vol. 15, no. 17, pp. 2265–2274, 2016.

143. M. E. Levine, J. A. Suarez, S. Brandhorst et al., "Low protein intake is associated with a major reduction in IGF-1, cancer, and overall mortality in the 65 and younger but not older population," Cell Metabolism, vol. 19, no. 3, pp. 407–417, 2014.

144. L. Fontana, R. M. Adelaiye, A. L. Rastelli et al., "Dietary protein restriction inhibits tumor growth in human xenograft models," Oncotarget, vol. 4, no. 12, pp. 2451–2461, 2013.

145. Y. Zhao, L. Wang, and J. Pan, "The role of L-type amino acid transporter 1 in human tumors," Intractable & Rare Diseases Research, vol. 4, no. 4, pp. 165–169, 2015.

146. G. Singh, A. Akcakanat, C. Sharma, D. Luyimbazi, K. Naff, and F. Meric-Bernstam, "The effect of leucine restriction on Akt/mTOR signaling in breast cancer cell lines in vitro and in vivo," Nutrition and Cancer, vol. 63, no. 2, pp. 264–271, 2011.

147. P. Nicklin, P. Bergman, B. Zhang et al., "Bidirectional transport of amino acids regulates mTOR and autophagy," Cell, vol. 136, no. 3, pp. 521–534, 2009.

148. S. H. Cheng, Y. M. Tseng, S. H. Wu, S. M. Tsai, and L. Y. Tsai, "Whey protein concentrate renders MDA-MB-231 cells sensitive to rapamycin by altering cellular redox state and activating GSK3β/mTOR signaling," Scientific Reports, vol. 7, no. 1, article 15976, 2017.

149. C. Mitchell, R. McGregor, R. D'Souza et al., "Consumption of milk protein or whey protein results in a similar increase in muscle protein synthesis in middle aged men," Nutrients, vol. 7, no. 10, pp. 8685–8699, 2015.

150. K. Marshall, "Therapeutic applications of whey protein," Alternative Medicine Review, vol. 9, no. 2, pp. 136–156, 2004.

151. D. D'Eliseo and F. Velotti, "Omega-3 fatty acids and cancer cell cytotoxicity: implications for multi-targeted cancer therapy," Journal of Clinical Medicine, vol. 5, no. 2, 2016.

152. P. A. Corsetto, G. Montorfano, S. Zava et al., "Effects of n-3 PUFAs on breast cancer cells through their incorporation in plasma membrane," Lipids in Health and Disease, vol. 10, no. 1, p. 73, 2011.

153. H. Chamras, A. Ardashian, D. Heber, and J. A. Glaspy, "Fatty acid modulation of MCF-7 human breast cancer cell proliferation, apoptosis and differentiation," Journal of Nutritional Biochemistry, vol. 13, no. 12, pp. 711–716, 2002.

154. A. Barascu, P. Besson, O. Lefloch, P. Bougnoux, and M. Jourdan, "CDK1-cyclin B1 mediates the inhibition of proliferation induced by omega-3 fatty acids in MDA-MB-231 breast cancer cells," The International Journal of Biochemistry & Cell Biology, vol. 38, no. 2, pp. 196–208, 2006.

155. W. Q. Cao, Z. F. Ma, M. M. Rasenick, S. Y. Yeh, and J. Z. Yu, "N-3 poly-unsaturated fatty acids shift estrogen signaling to inhibit human breast cancer cell growth," PLoS One, vol. 7, no. 12, article e52838, 2012.

156. K. H. Schmitz, K. S. Courneya, C. Matthews et al., "and American College of Sports, M., American College of Sports Medicine roundtable on exercise guidelines for cancer survivors," Medicine & Science in Sports & Exercise, vol. 42, no. 7, pp. 1409–1426, 2010.

157. K. Y. Wolin, A. L. Schwartz, C. E. Matthews, K. S. Courneya, and K. H. Schmitz, "Implementing the exercise guidelines for cancer survivors," The Journal of Supportive Oncology, vol. 10, no. 5, pp. 171–177, 2012.

158. C. L. Rock, C. Doyle, W. Demark-Wahnefried et al., "Nutrition and physical activity guidelines for cancer survivors," CA: A Cancer Journal for Clinicians, vol. 62, no. 4, pp. 243–274, 2012.

159. C. S. Denlinger, J. A. Ligibel, M. Are et al., "Survivorship: healthy lifestyles, version 2.2014," Journal of the National Comprehensive Cancer Network, vol. 12, no. 9, pp. 1222–1237, 2014.

160. American College of Sports Medicine, D. Riebe, J. K. Ehrman, G. Liguori, and M. Magal, ACSM's Guidelines for Exercise Testing and Prescription, Lippincott Williams & Wilkins, Philadelphia, 10th edition, 2017.

161. A. A. Kirkham, K. L. Campbell, and D. C. McKenzie, "Comparison of aerobic exercise intensity prescription methods in breast cancer," Medicine & Science in Sports & Exercise, vol. 45, no. 8, pp. 1443–1450, 2013.

162. F. Scharhag-Rosenberger, R. Kuehl, O. Klassen et al., "Exercise training intensity prescription in breast cancer survivors: validity of current practice and specific recommendations," Journal of Cancer Survivorship, vol. 9, no. 4, pp. 612–619, 2015.

163. S. E. Neil-Sztramko, K. M. Winters-Stone, K. A. Bland, and K. L. Campbell, "Updated systematic review of exercise studies in breast cancer survivors: attention to the principles of exercise training," British Journal of Sports Medicine, 2017.

164. P. Autier, C. Pizot, M. Boniol et al., "Physical activity, hormone replacement therapy and breast cancer risk: a meta-analysis of prospective cohort studies," in European Breast Cancer Conference, p. 102, Glasgow, Scotland, 2014.

165. E. Barbieri, E. Falcieri, M. De Santi et al., "The "Journal of Functional Morphology and Kinesiology" Journal Club Series: highlights on recent papers in physical activity and sedentary behavior," Journal of Functional Morphology and Kinesiology, vol. 3, no. 2, 2018.

166. M. De Santi, "Physical activity promotion for cancer prevention," Journal of Cancer Research Forecast, vol. 1, no. 1, p. 1008, 2018.

167. K. Ogawa, K. Sanada, S. Machida, M. Okutsu, and K. Suzuki, "Resistance exercise training-induced muscle hypertrophy was associated with reduction of inflammatory markers in elderly women," Mediators of Inflammation, vol. 2010, Article ID 171023, 7 pages, 2010.

168. D. Gonzalez, R. Marquina, N. Rondon, A. J. Rodriguez-Malaver, and R. Reyes, "Effects of aerobic exercise on uric acid, total antioxidant activity, oxidative stress, and nitric oxide in human saliva," Research in Sports Medicine, vol. 16, no. 2, pp. 128–137, 2008.

169. F. Seifi-Skishahr, M. Siahkohian, and B. Nakhostin-Roohi, "Influence of aerobic exercise at high and moderate intensities on lipid peroxidation in untrained men," Journal of Sports Medicine and Physical Fitness, vol. 48, no. 4, pp. 515–521, 2008.

170. D. Ornish, M. J. M. Magbanua, G. Weidner et al., "Changes in prostate gene expression in men undergoing an intensive nutrition and lifestyle intervention," Proceedings of the National Academy of Sciences, vol. 105, no. 24, pp. 8369–8374, 2008.

171. C. M. Friedenreich and A. E. Cust, "Physical activity and breast cancer risk: impact of timing, type and dose of activity and population subgroup effects," British Journal of Sports Medicine, vol. 42, no. 8, pp. 636–647, 2008.

172. *H. Ma, X. Xu, J. Clague et al., "Recreational physical activity and risk of triple negative breast cancer in the California Teachers Study," Breast Cancer Research, vol. 18, no. 1, p. 62, 2016.*

173. *P. P. Bao, G. M. Zhao, X. O. Shu et al., "Modifiable lifestyle factors and triple-negative breast cancer survival: a population-based prospective study," Epidemiology, vol. 26, no. 6, pp. 909–916, 2015.*

CHAPTER 2

Original Article

Effects of Exercise on Triple-Negative Breast Cancer Cell Proliferation

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Effects of exercise on triple-negative breast cancer cell proliferation

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Abstract

Purpose: The purpose of this study was to examine if systemic responses to acute endurance exercise before and after 9 weeks of training in sedentary healthy women could modulate TNBC cell proliferation in vitro.

Methods: Twelve healthy sedentary women (age: 21 ± 0.8 years, body height: 160 ± 4.7 m, body mass: 53 ± 5.2 kg, BMI: 21 ± 1.2 kg/m^2) performed 36 indoor cycling training sessions over a 9-week period. Before (pre) and after (post) this training protocol, subjects were asked to perform a High-Intensity Endurance Cycling (HIEC) test. The HIEC Test consists of a 20 minutes warm-up composed of four 5 minutes steps at 50%, 60%, 65% and 70% of individual maximal power (Pmax), previously determined by an incremental test. After the warm-up, subjects performed ten 90 seconds. Sprints ("SPR" steps) at 90% Pmax separated by 3 minutes recovery intervals ("REC" steps) at 55% Pmax. At the end of the last REC step, subjects performed an eleventh SPR step to exhaustion. Blood samples were collected before, immediately after, and 4 h and 24 h post-HIEC Test and creatine kinase (CK) was determined to evaluate muscle adaptation response to exercise. The sera obtained were used to supplement a culture medium for TNBC MDA-MB-231 cells to evaluate cell proliferation. TNBC cells were seeded at 20.000 cells/well in 24-well plates. After overnight incubation, the culture medium was replaced with DMEM red-phenol free with a physiological concentration of glucose (0.8 mg/mL) and 5% conditioned human serum collected pre- or post- exercise. After 48 hours, the cells were counted by trypan blue exclusion assay using a hemocytometer.

Results: Sera collected after acute endurance exercise performed pre- and post-training induced the same effect on TNBC cell proliferation. In particular, 4 h post-exercise serum, both pre- and post-training, led to a statistically significant reduction in proliferation of about 24-25% compared to cells supplemented with pre-exercise serum (one-way ANOVA followed by Dunnett's Multiple Comparison Test; $p<0.001$). The

reduction in proliferation found in 4 h post-exercise serum was also maintained in 24 h post-exercise serum. Furthermore, we observed an increase of CK level in blood after HIEC exercise session in both pre- and post-training. However, trained women showed a lower level of CK 24 h post-exercise and a faster recovery of muscle response to physical activity.

Conclusions: The systemic responses induced by a high-intensity endurance cycling session, pre- and post-training, might reduce cancer cell proliferation.

Keywords

Exercise, high-intensity endurance cycling; triple-negative breast cancer

1. Introduction

About 10-20% of breast cancers (BC) are found to be triple-negative (TNBC). TNBC is characterized by the absence of estrogen receptors (ER) and progesterone receptors (PR), and does not show an overexpression of human epidermal growth factor receptor 2 (HER-2) [1]. Since the tumor cells lack the necessary receptors, common treatments such as hormone therapy and drugs that target estrogen, progesterone, and HER-2 are ineffective and chemotherapy is the main treatment option [2].

TNBC exhibits similar characteristics to basal-like BC with distinct pathological and clinical features that make this BC subtype an aggressive phenotype: the 5-year survival rate for TNBC is 70% lower than other BC subtypes which have 80% survival rates [2] Owing to its lack of expression of hormonal receptors and intrinsic properties, TNBC shows high metastatic capability and poor prognosis [3-6]. A large body of epidemiological evidence suggests that regular exercise protects against BC development [7-17].

Among the cancer preventive effects of physical activity (PA) are the reduction of adiposity, changes in endogenous hormones, in particular, the reduction of basal concentrations of circulatory sex hormones, metabolic hormones as well as inflammatory factors, all of which are known to be risk factors for BC [18].

In last few years it has been shown that contracting skeletal muscle releases myokines that exert their role in an autocrine, paracrine and endocrine manner [19]. In addition to their acknowledged role as mediators in the cross talk between muscle and fat, it has been suggested that myokines may also play a role in cancer protection [20, 21, 22].

In this regard, observational data show that physically active breast cancer survivors (BCS) have a 30–50 % reduced risk of disease recurrence and mortality [23, 24]. Hence, PA would seem to have a significant controlling effect on BC progression.

PA might reduce systemic inflammation alone or in combination with a reduction in body weight or leading to a reduction in inflammatory cytokines in adipose tissue. Although PA may improve BC outcomes, no studies have provided mechanistic data to differentiate between different types of exercise and relative systemic responses, and very little is known about pathways and mechanisms linking PA and TNBC.

The purpose of this study was to examine if systemic responses to acute endurance exercise before and after training in sedentary healthy women could modulate TNBC cell

proliferation, *in vitro*. Specifically, we stimulated BC MDA-MB 231 cell lines with serum obtained in healthy sedentary women pre- and post- High Intensity Endurance Cycling (HIEC) Test.

2. Materials and Methods

The subjects in the study were healthy sedentary women from the University of Urbino. The study was performed at the Department of Biomolecular Sciences (DISB) of the University of Urbino between September and December 2017 according to the guidelines of the Helsinki Declaration for research with human volunteers (1975) and after the approval by the Ethics Committee of the University of Urbino Carlo Bo (date of approval July 10, 2017). Following a medical health-screening, all participants provided written informed consent to participate in the study.

Participants

Twelve healthy sedentary women (age: 21 ± 0.8 years, body height: 160 ± 4.7 m, body mass: 53 ± 5.2 kg, BMI: 21 ± 1.2 kg/m^2) were recruited for this study.

The exclusion criteria included major cardiovascular disease risks, musculoskeletal injuries, upper respiratory infection, smoking or having taken any medication in the previous 3 months.

None of the participants had performed any physical activity for at least six months prior to the start of the study. The participants were advised to maintain their dietary routine and were also instructed to refrain from all training activities except those sessions included in the experimental design.

Outcome measures

The outcome measures evaluated in this study were the effects on cell proliferation and viability of MDA-MB-231 BC cell lines. Cells were cultured with the sera of women obtained pre-exercise, immediately after exercise sessions and 4 h and 24 h post-exercise together with a physiological concentration of glucose. Cell proliferation was evaluated by cell count using a hemocytometer and trypan blue exclusion assay.

Preliminary testing

The subjects were informed of the research activities and were asked to sign an informed consent form to undergo a preliminary medical examination to ascertain their suitability for participation in the study. During the first week in the laboratory the subjects underwent an anthropometric examination which included the measurement of body height (cm), body mass (kg), BMI (kg/m^2), waist circumference (cm) of the major muscular groups (waist, thighs, legs, arms) and Bio-Electrical Impedance Analysis (BIA). In the second week the subjects performed a maximal incremental test to exhaustion on an SRM cycle ergometer (SRM Italia, Lucca, Italy), during which maximum oxygen consumption was measured by the Cosmed K4b2 metabolimeter (Cosmed Italia Srl).

Experimental Design

Incremental Test for VO2max, Pmax, and Lactate Thresholds

Each subject performed an incremental test to assess maximal oxygen consumption (VO_{2max}), lactate thresholds (to define the individual training intensity zones) and maximal power (Pmax), to determine the individual workloads for the experimental trial.

The test was performed on an SRM ergometer (SRM Italia, Lucca, Italy). Subjects began cycling at 50 W, and power output was thereafter increased by 20 W every 3 min until volitional exhaustion or a drop in the cadence below 60 rpm. Oxygen consumption was monitored for the duration of the trial (breath-by-breath) using the Cosmed K4b2 metabolimeter (COSMED, Rome, Italy), heart rate (HR) was recorded with the Polar RS-800 heart rate monitor (POLAR, Kempele, Finland), and blood lactate was measured (before starting the test and within 30 s before the end of each stage) using the Lactate Pro portable blood lactate meter (Arkray, Kyoto, Japan) in micro blood samples drawn from the tip of the index finger according to the manufacturer's instructions. VO_{2max} was identified as the maximum value derived from the 15-breath moving average of oxygen consumption of the entire test, as suggested by Robergs et al. [25]. Blood lactate measurements were used to identify the training intensity zones, based on the following concentration values: zone 1, $\leq$ 2.0 mM, zone 2, >2.0 and < 4.0mM, zone 3, $\geq$ 4.0mM. HR was monitored to determine values corresponding to LT1 and LT2, as well as maximal heart rate (HRmax) [26]. Percentages of the Pmax were used to determine the

workloads to be undertaken during the experimental trials (*e.g.,* power output (W) at a given % Pmax).

The incremental test was repeated three days after the end of the training period to assess the training effects on VO_{2max}, lactate thresholds and Pmax (useful to determine the new workloads for the experimental trial).

High Intensity Endurance Cycling (HIEC) Test

On the day of the experiment subjects arrived at the laboratory at 06.00 AM in a fasted state 2 h before the test. All the subjects then had a standardized breakfast (200 ml of fruit-juice and two slices of tart with marmalade (96g) (480 kCal, 88g CHO, 5g protein; 10g fat), and a blood sample was collected just before starting the trial.

HIEC tests were performed on a "Technogym Group Cycle™ Connect" equipped with a power meter (Technogym S.p.A., Cesena, Italy). Subjects completed an initial 2 min warm-up period allowing them to build up to the starting workload, followed by four 5 min continuous progressive increments of cycling completed at a workload corresponding to 50%, 55%, 60% and 70% Pmax. This was followed by ten 90 s sprints (SPR) at a workload corresponding to 90% Pmax, separated by 180 s recovery (REC) at a workload corresponding to 55% Pmax. If a subject completed all 10 sprints, after a 3 min interval at 55% Pmax, a time to exhaustion step was undertaken at 90% Pmax. Exhaustion during the 10 sprints at 90% Pmax or the time to exhaustion at 90 % Pmax was defined as an inability to maintain power output within 5 W of the expected power and an inability to restore this power output within 15 s despite verbal encouragement. No feedback on elapsed time was provided, as this would have introduced potential bias with subjects targeting previous times. The power output during the experimental trials was controlled by the power meter display, with the subjects using a predefined cadence [27]. Blood samples were collected immediately after, and 2 h, 4 h and 24 h post-HIEC test (Figure 1). The HIEC Test was repeated three days after the final incremental test.

Training Protocol

36 indoor cycling training sessions were performed over a 9-week period. Training sessions were structured as follows (Figure 1):

(A) 3 weeks of 3 sessions/week of 53.1 ± 1.3 min,

(B) 3 weeks of 4 sessions/week of 59.1 ± 1.2 min,

(C) 3 weeks of 5 sessions/week of 68.2 ± 1.4 min.

The 12 subjects were trained by an expert instructor whose aim was to have the subjects follow the same training program. The class was choreographed based on conventional principles (*i.e.*, warm-up, systematic interval exercise, and cool-down) widely used in the indoor cycling community [28]. The training program for each session was designed following the same intensity distribution based on a polarized model, with 69 ± 3% of the session time spent in zone 1 (< LT1), 11 ± 2% spent in zone 2 (> LT1; < LT2), 20 ± 2% spent in zone 3 (> LT2) as described by Seiler et al. [26].

During the training sessions the HR of each subject (instructor included) was monitored using a Polar Team Pack 2 (POLAR, Kempele, Finland) and projected onto the wall. The subjects were asked to maintain the same HR intensity zone as the instructor.

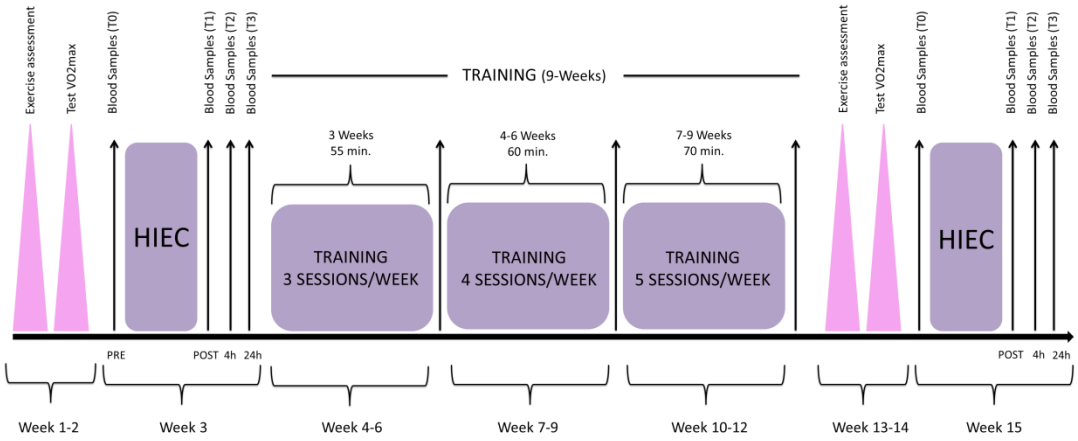

Figure 1. *Experimental Design.*

Diet monitoring

For the duration of the study subjects were asked to keep a daily food diary and their nutrient intake was monitored using the appropriate software (Metadieta). In general, subjects were not asked to change their eating habits, except in cases where an excessive or reduced protein intake was found, in which case the subject was asked to make changes. On trial days, breakfast was provided two hours before the acute session.

Blood profile

Blood samples were collected pre-, immediately after and 4 h and 24 h post-exercise. The sera obtained were used to supplement culture medium for TNBC MDA-MB-231 cells to evaluate cell proliferation.

Cell culture experiments

The MDA-MB-231 human breast adenocarcinoma cell lines (ER-, PR-, HER2-) were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 2 mmol/L L-glutamine, 1 × MEM non-essential amino acid solution, 0.1 mg/ml streptomycin and 0.1 U/L penicillin (growth media) and 1 mM sodium pyruvate. Cells were maintained in a humidified incubator (5% CO2) at 37 °C during a maximum of fifteen cellular passages . For the experiments, the MDA-MB-231 cells were seeded at 20.000 cells/well in 24-well plates. After overnight incubation, the culture medium was replaced with DMEM red-phenol free with a physiological concentration of glucose (0.8 mg/mL) and with 5% of conditioned human sera collected pre/post-HIEC Test. After 48 hours, cells were counted by trypan blue exclusion assay using a hemocytometer. All cell culture materials were purchased from Sigma-Aldrich (St. Louis, MO, USA).
Blood samples were collected in BD Vacutainer® Plus plastic serum tubes, inverted 6 times and allowed to clot for 30 min at room temperature. Serum was obtained by a centrifugation step at 1,000 x g for 15 min at 4° C and was then aliquoted and stored at -80° C until the experiments.

Statistical analyses

One-way ANOVA followed by Dunnett's Multiple Comparison Test ($p<0.001$) were used to compare pre- and post-exercise cell proliferation at different times. Twelve subjects were recruited in the acute exercise study, and all the subjects with available blood samples (n=12 healthy women) were used in the training study.

3. Results and Discussion

In this study we used the serum obtained from healthy sedentary women before and after a single session of HIEC (exercise session lasting about 75/80 minutes with alternated stages of high, 90% Pmax, and low intensity, 55% Pmax, ending with a time to exhaustion, 90% Pmax), to treat TNBC cells.

The HIEC session was performed before and after 9 weeks of training, and the serum was added to a culture medium of MDA-MB 231 cells. This *ex vivo* approach allowed us to analyze the effect of systemic changes induced by exercise on the cellular viability of this type of aggressive tumour.

The serum taken after the single HIEC exercise session significantly reduced the proliferation of MDA-MB 231 cells. This effect was particularly evident after the acute exercise, while the serum taken after 9 weeks of training, at basal level (before the single exercise session) showed only a slight insignificant reduction in cell proliferation (Figure 3) compared to the serum taken at the pre-training basal level, when subjects were still in a sedentary state. Thus, cell proliferation of MDA-MB 231 cell lines after the single exercise session was significantly reduced and the experiments were reproducible (Figure 2). In particular, the reduction in cell proliferation was observable immediately after exercise: the rate of cell proliferation decreased significantly by about 10-15% in the cells grown with the sera collected immediately after exercise. Moreover, 4 h after exercise, the proliferation rate decreased by about 24-25% compared to the cells grown with pre-exercise sera, in both experimental conditions, *i.e.*, pre- and post- training (unidirectional ANOVA followed by the multiple comparison test of Dunnett, $p < 0.001$). The reduction in proliferation observed using the sera obtained 4 h after exercise was also maintained by the sera taken 24 h after exercise.

The results of our study show that a single exercise session has a significant braking effect on the proliferation of cancer cells, in agreement with Dethlefsen C. et al., 2016 [29]. The acute exercise protocol examined in a part of the 2016 study by Dethlefsen C. et al. consisted of a concurrent protocol lasting 2 h: 30 min of warm-up, 60 min of resistance exercises (3 sets of 8-10 repetitions at 70-90% of 1-RM) followed by 30 min of high intensity pedaling on a cycle ergometer.

An *ex vivo* study described by Rundqvist H. et al., 2013 [30] showed that the incubation of prostate cancer cells (LNCaP) with serum obtained after 65 min of aerobic exercise on

the cycle ergometer, performed by young and healthy subjects with increasing intensity (20 min to 50% VO2max and the following 40 min to 65% Vo2max), resulted in a 31% inhibition of prostate cancer cell growth ($p < 0.05$) compared to incubation with a pre-exercise serum pool.

The exercise protocol described by Rundqvist H. et al., 2013 was recently applied in a preliminary study in sedentary premenopausal women; the exercise serum (taken immediately after and 2 h post-exercise) led to a reduction in cell proliferation. In particular, the serum taken 2 h after exercise inhibited the proliferation of MDA-MB 231 cell lines by about 10% compared to the pre-exercise serum (31).

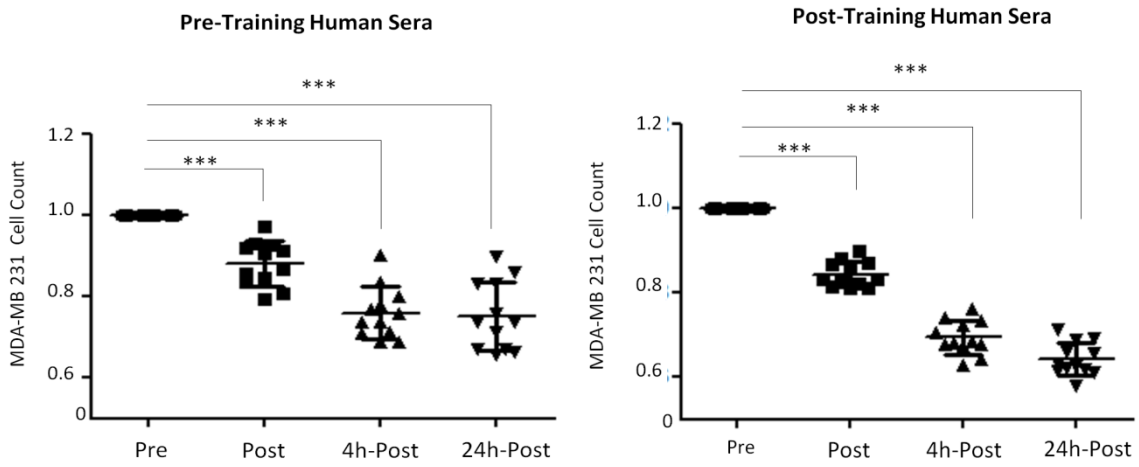

Figure 2. MDA-MB-231 cell proliferation analysis. Sera were collected before, immediately after, and 4 h and 24 h after (post) the HIEC Test (Pre- and Post-training). Cells were stimulated with 5% of conditioned human sera collected pre/post-HIEC Test before (pre) and after (post) 9 weeks of indoor cycling training. Data are expressed as cell count relative to cells stimulated with pre-HIEC Test serum (set as one-fold). Statistical significance was tested by one-way ANOVA followed by Dunnett's Multiple Comparison Test; *** $p<0.001$.

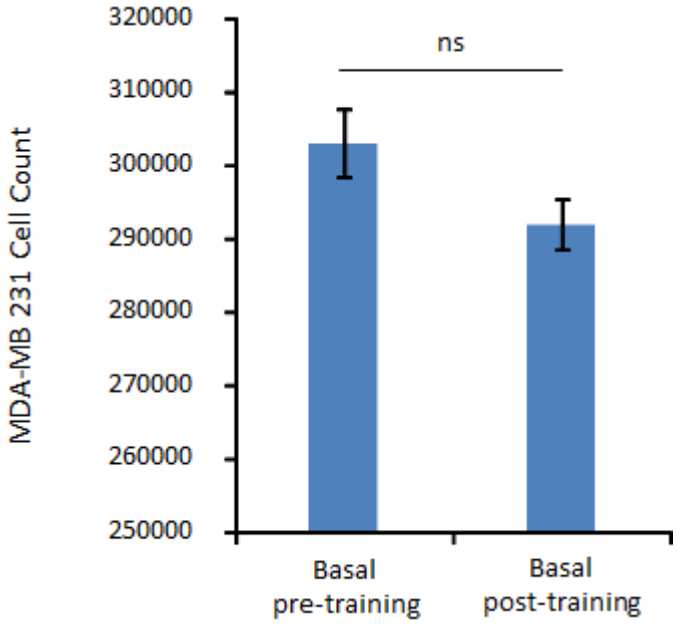

Figure 3. Basal effect of human sera on MDA-MB-231 cell proliferation. Cells were stimulated with 5% conditioned human sera collected at rest before (pre) and after (post) 9 weeks of indoor cycling training. Data are expressed as total cell count. Statistical significance was tested by two-tailed paired t-test ($p<0.05$).

Furthermore, data from our study show that the HIEC exercise session resulted in a release of creatine kinase (CK) in the blood immediately after exercise (Figure 4).

Our results related to the CK muscle damage marker show a significant increase between pre- and post-exercise (13.33 ± 5.8 vs 24.91 ± 8.6; p = 0.003) in the samples taken during the acute session before the training period; the CK values return to baseline values after 4 h and increase again after 24 h, *i.e.*, 24 h after the HIEC Test (13.9 ± 6.3 vs. 21.4 ± 9.7 p = 0.00) (Figure 4). In the samples taken during the acute exercise session carried out after the training period, we also found a significant increase in CK between pre- and post-exercise (22.2 ± 7.5 vs 32.2 ± 7.0 p = 0.00) and then a return to baseline values after 4 h and a further reduction after 24 h (20.8 ± 5.0 vs 13.8 ± 5.4 p = 0.01) (Figure 4).

The presence of CK in the blood is generally used as an indirect marker of muscle damage. In fact, individuals who regularly participate in intense exercise at high volume tend to have significantly increased CK base levels compared to sedentary or moderately active individuals [32]. Elevated serum CK levels were also found after regular exercise

in pre-menopausal women compared to sedentary individuals of the same age [33]. This suggests that the release of CK into serum is a normal reaction to regular exercise; however, the molecular mechanisms that trigger its release after high intensity exercise are not yet clear and further investigation of these mechanisms could provide important insights into TNBC. Interestingly, in a large study by Pan H. et al., 2013 [34], an association between serum CK levels and BC was observed. The authors found that serum CK levels in BC patients were significantly lower than those with benign breast disease. Therefore, it is possible to deduce that CK levels represent an important part of the immune response that influences the development and progression of BC and low CK levels would seem to favor pathways to tumorigenesis [35]. Hence, an increase in serum CK levels due to mechanisms induced by muscle contraction may exert protective action against TNBC.

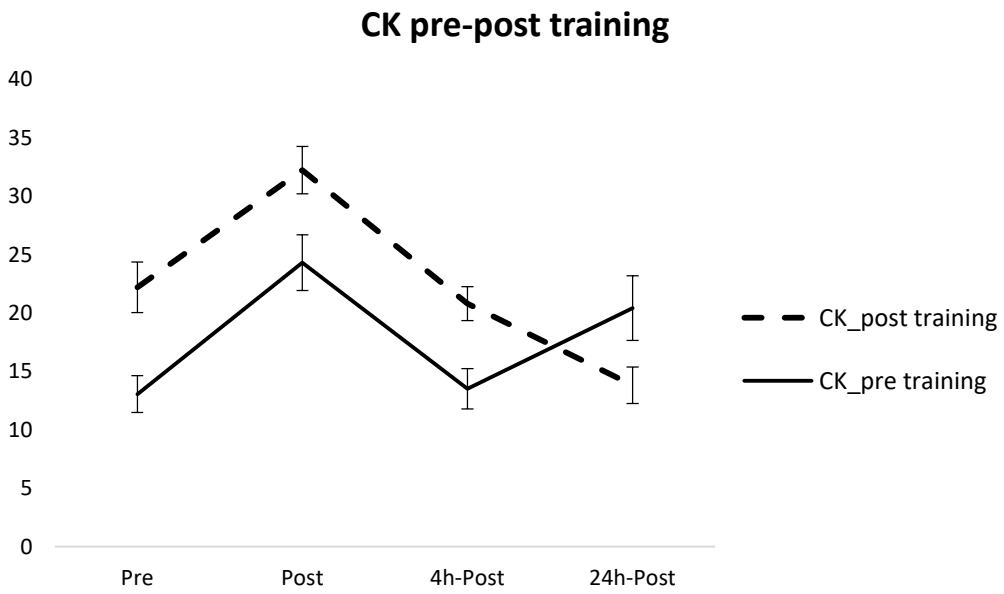

Figure 4. CK dosage. Sera were collected before, immediately after, 4 h and 24 h after the HIEC Test (Pre- and Post-training).

4. Conclusions

This study provides strong evidence of the protective effects of exercise against a particularly aggressive tumor, TNBC, which is considered less sensitive to therapies. There are few investigations that have dealt with this area of research rigorously, particularly in TNBC cell lines. Our investigation showed that systemic changes, occurring during and performance of a 75/80 min HIEC Test, had inhibitory effects on BC viability in the tested TNBC cell lines, and this effect was maintained for at least for 24 h. Hence, we propose that high-intensity exercise of 75/80 min could have a positive effect on TNBC outcomes.

This study provides new data on factors released through exercise, which may have inhibitory effects on cancer cell proliferation. Such factors could have therapeutic value for TNBC survivors. In the future, research should focus on a detailed characterization of different types of exercise, aiming to identify potential exercise-induced anti-oncogenic factors and establishing how these are regulated by different modes of exercise.

References

1. *Boyle, P., Triple-negative breast cancer: epidemiological considerations and recommendations. Ann Oncol, 2012. 23 Suppl 6: p. vi7-12.*
2. *Carey, L.A., et al., The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res, 2007. 13(8): p. 2329-34.*
3. *Irvin, W.J., Jr. and L.A. Carey, What is triple-negative breast cancer? Eur J Cancer, 2008. 44(18): p. 2799-805.*
4. *Tseng, L.M., et al., Distant metastasis in triple-negative breast cancer. Neoplasma, 2013. 60(3): p. 290-4.*
5. *Bauer, K.R., et al., Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. Cancer, 2007. 109(9): p. 1721-8.*
6. *Kassam, F., et al., Survival outcomes for patients with metastatic triple-negative breast cancer: implications for clinical practice and trial design. Clin Breast Cancer, 2009. 9(1): p. 29-33.*
7. *Dirx, M.J., et al., Baseline recreational physical activity, history of sports participation, and postmenopausal breast carcinoma risk in the Netherlands Cohort Study. Cancer, 2001. 92(6): p. 1638-49.*
8. *Fraser, G.E. and D. Shavlik, Risk factors, lifetime risk, and age at onset of breast cancer. Ann Epidemiol, 1997. 7(6): p. 375-82.*
9. *Lahmann, P.H., et al., Physical activity and breast cancer risk: the European Prospective Investigation into Cancer and Nutrition. Cancer Epidemiol Biomarkers Prev, 2007. 16(1): p. 36-42.*
10. *McTiernan, A., et al., Recreational physical activity and the risk of breast cancer in postmenopausal women: the Women's Health Initiative Cohort Study. JAMA, 2003. 290(10): p. 1331-6.*
11. *Patel, A.V., et al., Recreational physical activity and risk of postmenopausal breast cancer in a large cohort of US women. Cancer Causes Control, 2003. 14(6): p. 519-29.*
12. *Rockhill, B., et al., A prospective study of recreational physical activity and breast cancer risk. Arch Intern Med, 1999. 159(19): p. 2290-6.*
13. *Tehard, B., et al., Effect of physical activity on women at increased risk of breast cancer: results from the E3N cohort study. Cancer Epidemiol Biomarkers Prev, 2006. 15(1): p. 57-64.*
14. *Thune, I., et al., Physical activity and the risk of breast cancer. N Engl J Med, 1997. 336(18): p. 1269-75.*
15. *Ballard-Barbash, R., et al., Physical activity, biomarkers, and disease outcomes in cancer survivors: a systematic review. J Natl Cancer Inst, 2012. 104(11): p. 815-40.*
16. *Brown, J.C., et al., Cancer, physical activity, and exercise. Compr Physiol, 2012. 2(4): p. 2775-809.*
17. *Moore, S.C., et al., Association of Leisure-Time Physical Activity With Risk of 26 Types of Cancer in 1.44 Million Adults. JAMA Intern Med, 2016. 176(6): p. 816-25.*
18. *Thivat, E., et al., Weight change during chemotherapy changes the prognosis in non metastatic breast cancer for the worse. BMC Cancer, 2010. 10: p. 648.*

19. *Hojman, P., et al., Molecular Mechanisms Linking Exercise to Cancer Prevention and Treatment. Cell Metab, 2018. 27(1): p. 10-21.*
20. *Hojman, P., et al., Exercise-induced muscle-derived cytokines inhibit mammary cancer cell growth. Am J Physiol Endocrinol Metab, 2011. 301(3): p. E504-10.*
21. *Barnard, R.J., et al., Effects of a low-fat, high-fiber diet and exercise program on breast cancer risk factors in vivo and tumor cell growth and apoptosis in vitro. Nutr Cancer, 2006. 55(1): p. 28-34.*
22. *Davis AR, Schilling E, Wellberg B, Macias W, Porter S, Riechman SE, Fluckey JD. Chemotherapeutic properties of myokines released from "E-stim" skeletal muscle. 14th International Conference Biochemistry of Exercise (Abstract no. 30), 2009.*
23. *Yager, J.D. and N.E. Davidson, Estrogen carcinogenesis in breast cancer. N Engl J Med, 2006. 354(3): p. 270-82.*
24. *Beasley J.M., Kwan M.L., Chen W.Y. et al., Meeting the physical activity guidelines and survival after breast cancer: findings from the after breast cancer pooling project. Breast Cancer Res, 2012. Treat 131:637–643.*
25. *Robergs, R.A., D. Dwyer, and T. Astorino, Recommendations for improved data processing from expired gas analysis indirect calorimetry. Sports Med, 2010. 40(2): p. 95-111.*
26. *Seiler, K.S. and G.O. Kjerland, Quantifying training intensity distribution in elite endurance athletes: is there evidence for an "optimal" distribution? Scand J Med Sci Sports, 2006. 16(1): p. 49-56.*
27. *O'Hara, J.P., et al., Reliability of a high-intensity endurance cycling test. Int J Sports Med, 2012. 33(1): p. 18-25.*
28. *Battista, R.A., et al., Physiologic responses during indoor cycling. J Strength Cond Res, 2008. 22(4): p. 1236-41.*
29. *Dethlefsen, C., et al., Exercise regulates breast cancer cell viability: systemic training adaptations versus acute exercise responses. Breast Cancer Res Treat, 2016. 159(3): p. 469-79.*
30. *Rundqvist, H., et al., Effect of acute exercise on prostate cancer cell growth. PLoS One, 2013. 8(7): p. e67579.*
31. *Natalucci V., et al., Effects of exercise on breast cancer triple-negative cell proliferation in vitro. IX National Congress SISMES, vol. 13 of Sport Science for Health, Brescia, Italy, September 29–October 1 2017, Supplement 1, (Abstract no. 4GS).*
32. *Chevion, S., et al., Plasma antioxidant status and cell injury after severe physical exercise. Proc Natl Acad Sci U S A, 2003. 100(9): p. 5119-23.*
33. *Thompson, H.S., S.P. Scordilis, and M.J. De Souza, Serum creatine kinase activity varies with ovulatory status in regularly exercising, premenopausal women. Horm Res, 2006. 65(3): p. 151-8.*
34. *Pan, H., et al., Low serum creatine kinase levels in breast cancer patients: a case-control study. PLoS One, 2013. 8(4): p. e62112.*
35. *Patra, S., et al., Progressive decrease of phosphocreatine, creatine and creatine kinase in skeletal muscle upon transformation to sarcoma. FEBS J, 2008. 275(12): p. 3236-47.*

CHAPTER 3

Original Article

Promotion and prescription of exercise in breast cancer survivors to improve quality of life and health outcomes

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In preparation, 2018.

Promotion and prescription of exercise in breast cancer survivors to improve quality of life and health outcomes

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Abstract

Background: Healthy lifestyle habits have been associated with improved quality of life for breast cancer survivors (BCS). Physical activity (PA) during and following Breast Cancer (BC) treatments has been shown to improve both physical and emotional health, and overall quality of life. It can help to improve body weight and muscle strength and attenuate physical side effects of BC treatments, such as fatigue, pain and lowered bone density. The aim of this pilot study was to evaluate the effect of a structured PA intervention on measures of physical performance and quality of life among BCS attending the Breast Unit, University Hospital of Ancona.

Material and methods: Twenty sedentary women with BC (age range: 56.05 ± 11.56 years) who had undergone primary surgery were recruited consecutively at the Breast Cancer Unit facilities of the University Hospital of Ancona, between February and March 2018 for a controlled pilot study. Patients, that were not engaged in physical training for six months previously, belonged-to either a control (n=10) or an intervention arm (n=10). Patients in both arms were assigned to sixteen weeks of "Lifestyle Program", consisting of lifestyle counseling, periodical functional tests and follow-up. The intervention arm (IA) underwent eight weeks of supervised exercise program and eight weeks of follow-up. The supervised exercise program includes two sessions/week (~60 min) of aerobic, resistance and flexibility exercise in the same session (circuit training program) and one session *per* week devoted to aerobic unsupervised training. The control arm (CA) received only lifestyle counseling and the periodical tests. Participants were evaluated at

the beginning of the study to establish a baseline and reevaluated after four, eight and sixteen weeks.

PA level (evaluated by the International Physical Activity Questionnaire, IPAQ), quality of life (tested by QoL - 36-Item Short Form Health Survey questionnaire, SF-36), and functional exercise capacities (tested by 6-minute Walk Test, 6MWT; Hand Grip test; Sit and Reach test) were assessed along sixteen weeks for both arms: at baseline, after four, eight and sixteen weeks.

Results: At baseline, the two groups showed comparable outcome measures results. After the first four-week of follow-up, the quality of life measured through SF-36 improved in both groups, but the "General Health" domain (D8) significantly more in the IA rather in the CA (F 8.0; p=.01) and the amount of daily physical activity quantified in MET increased significantly in both groups (IA: Z = -53.0; p <.0001; CA: Z = -2.2; p = .03); however, in the IA subjects the MET value increased two-fold compared to the CA subjects (F = 21.0; p = .0002). 6MWT highlighted that subjects of the IA improved significantly more than CA (F=7.6; p=.01) increasing on average by 49 meters [range: 190: -4] while the CA subjects worsened on average by about 15 meters [range: 42: -95] compared to baseline. Also, right hand squeeze force improved significantly in the IA (Z = -2.66; p = .008), while no differences were found within and between groups in the Sit and Reach test. All subjects reported that they appreciated the training or counseling.

Conclusions: The "Lifestyle Program", including exercise counseling and supervised exercise program, improved functional exercise capacity and motivated BCS to adopt a healthier lifestyle based on regular PA.

Keywords

Breast cancer; Breast cancer survivors; PA counseling; exercise; healthy lifestyle

1. Background

Breast cancer (BC) is the most common cancer among women worldwide [1]. Breast cancer survivors (BCS) therefore face unique challenges to their health and well-being. A large body of evidence shows that physical activity (PA) is able to improve the psycho-physical well-being of BCS [2-9]. The beneficial effects induced by exercise are commonly attributed to long-term adaptations (i.e., months/years) and related to improvements in cardiorespiratory fitness, strength, body composition and resistance to fatigue [10-12]. In addition to these training related improvements, exercise also yields short-term effects (i.e., hours/days) mainly due to muscles releasing molecular factors induced by contraction. Indeed, during exercise transient but marked increases in a wide range of circulating hormones, cytokines, and immune cells occur [13].

It has been reported that these systemic responses are able to reduce BC viability [14]. Although the role of PA in improving BC outcomes is well documented, it is not yet known which anti-oncogenic mechanisms are triggered by acute exercise or long term training.

In general, reviews and meta-analyses tend to group PA and exercise interventions into general categories, and infrequently examine the specific exercise protocols employed in the studies. The American Cancer Society (ACS) recommends that cancer survivors engage in a minimum of 150 weekly minutes of moderate-intensity exercise [15, 16]. However, PA levels are generally low among BCS, and many women decrease their PA following diagnosis [17, 18].

Thus, it is of primary importance to identify an appropriate promotion and prescription of regular PA for BCS to improve their prognoses, response to therapy, and quality of life (QoL).

For this purpose hospitals need to have an integrated team of professionals, including a physiologist and a qualified and specialized trainers of exercise and sport science, who can approach the disease not only from a pharmacological and surgical point of view. It is important that PA becomes an increasingly personalized tool, like drugs use, and it should be included in a personalized lifestyle program. To achieve this goal, PA counseling, which could promote both physical and mental well-being, must be integrated into primary care. Moreover, PA counseling is important for most patients and its benefits can translate into considerable benefits for the health of the community in terms of both

improved individual health and reduction in the economic and social costs of physical inactivity.

The “Lifestyle program” aims to promote positive lifestyle and behavior changes that are the basis of an individual’s motivational readiness and will to integrate PA into daily routines.

The primary aim of this pilot study was to evaluate the effects of a structured PA intervention, in particular, a circuit training program, on measures of physical performance and quality of life among BCS attending the Breast Unit, University Hospital of Ancona.

The secondary aim was the introduction of targeted PA counseling for BCS in order to optimize recruitment and adherence to a healthy lifestyle, as well as to improve health outcomes in BCS. Furthermore, the present study proposed a PA diary as a reliable and simple tool to assess the level of daily PA and the effect of PA counseling.

2. The Study Design

This pilot study was a controlled trial with two arms, the control arm (CA) and intervention arm (IA). After the oncological or physiatrist visit, BCS were assigned to sixteen weeks of “Lifestyle Program”, consisting of lifestyle counseling for both groups and supervised exercise programs for the IA participants. Those patients who did not give the consensus to participate in the supervised exercise programs organized at the Ancona University Hospital were enrolled in the CA, those who ensured adhesion to the proposed training sessions were enrolled in the IA. All reasons of denied consensus were recorded by the assessor clinicians.

PA level (International Physical Activity Questionnaire Short Form, IPAQ-SF) and functional exercise capacities (6-Minute Walk Test, 6MWT; Hand Grip test; Sit and Reach test) were assessed along sixteen weeks for both arms: at baseline, after four, eight and sixteen weeks. Supervised exercise was administrated to the IA for the first eight weeks of the “Lifestyle program” after one week of familiarization sessions. The PA intervention was planned individually in terms of the Frequency (F), Intensity (I), Time (T), Type (T), Volume (V), and Progression (P), known as the FITT-VP principle of the American College of Sports Medicine [19] and included a circuit training program. The

intensity of aerobic exercise on a treadmill was prescribed as % of the average speed of the 6MWT [20]. Resistance and flexibility exercises were included in the circuit training program as well.

2.1. Methods

2.1.1 Participants and Setting

Twenty sedentary women with BC (age 56.05 ± 11.56 years; body mass 67.9 ± 13.23 kg; body height 163.1 ± 6.04 m; waist circumference 93.55 ± 10.26 cm; BMI 25.49 ± 4.46 kg/m^2) (Table 1) were recruited from the Department of Medical Oncology, University Hospital of Ancona between February and March 2018 and randomized to CA (n=10 women) or IA (n=10 women) for a controlled pilot study. Inclusion criteria included: histologically confirmed diagnosis of BC; woman between 30 and 70 yrs of age; mastectomy and/or quadrantectomy +/- lymphadenectomy surgery; both with positive or negative lymph nodes; ≤5 year post-surgery status; time since completion of chemotherapy ≥6 months; sedentary ≥6 months.

Major exclusion criteria were contraindications to exercise training; bone and brain metastases, or symptomatic cardiac disease.

The Institute's Ethics Committee approved the study. All participants were informed about the objectives of the study and provided written consent was obtained.

Table 1. Clinical characteristics of the pilot study population

Sample characteristics (n=20)

Age (years)	56.05 ± 11.56
Body mass (kg)	67.9 ± 13.23
Body height (m)	163.1 ± 6.04
Waist circumference (cm)	93.55 ± 10.26
BMI (kg/m^2)	25.49 ± 4.46

Note: Data are reported as means ± SD. BMI: body mass index.

Measures

The assessment included: (1) health and lifestyle questionnaires, (2) test of functional exercise capacity and PA levels, (3) training in the use of treadmill. The measures are briefly summarized hereafter.

Health and lifestyle questionnaires

Participants completed three questionnaires: (1) (Baseline) Health Questionnaire which included demographic characteristics, menopausal status, menstrual and reproductive history, personal health history, family history of cancer, nutrition information, and smoking and alcohol use histories; (2) IPAQ, used for monitoring health-related PA and sedentary behavior [21, 22]; (3) SF-36, used to measure quality of life (QoL) and general health [23].

Health-related fitness assessments

The functional exercise capacities and PA levels were measured objectively using standardized testing protocols. The assessments were typically completed in the following order: resting blood pressure and heart rate (HR); anthropometric data (age, in years; body mass, in kilograms; body height, in meters; waist circumference, in centimeters; BMI, in kg/m^2); flexibility (Sit and Reach Test); grip strength (Hand Grip Test); cardiovascular fitness (6MWT).

Muscle fitness and flexibility

Flexibility and muscle strength evaluations were performed with easily executable and reproducible tests, namely the Sit and Reach for flexibility [24] and the Hand Grip test [25], which uses a hand dynamometer to estimate the overall static strength of the upper limbs (Jamar® Hydraulic Dynamometer). Handgrip strength test (HGS) is easy to perform, non-invasive, low cost and it is also used as screening tool to track patients' deterioration of health [26] and as predictor of post-operative morbidity [27].

Cardiovascular fitness

The 6MWT is a practical and simple test that measures the distance a patient can walk rapidly on a hard flat surface in a period of 6 min. Currently, guidelines for the 6MWT established by the American Thoracic Society (ATS) recommend the use of an indoor or outdoor corridor with a flat surface 30 m in length [28]. Patients were instructed to walk along the corridor at their own pace, trying to cover as much distance as possible in 6 min. This test reflects the subject's functional exercise level for daily physical activities [29] because most everyday activities are performed at sub-maximal levels of exertion.

The following parameters were recorded during the 6MWT: distance covered (6MWD), peak heart rate (HR_{peak}), which was measured using a heart rate monitor, systolic and diastolic blood pressure at rest and at the end of the test, and self-perception of effort (Borg Scale) [30].

Assessment timing

The assessment was provided at the baseline, at the start of the "Lifestyle Program"; after the first four weeks of follow-up (4-weeks) at the end of the PA program, *i.e.* after eight weeks of follow-up (8-weeks) and at the end of the "Lifestyle Program" (16-weeks), *i.e.* at the sixteenth week follow-up.

Familiarization sessions

The subjects enrolled in IA familiarized themselves with treadmill walking three times a week for one week. Walking duration was increased during the familiarization period as tolerated, up to a maximum of 30 min, with a maximum of three rest periods. In the first session, walked at a self-selected pace on a treadmill, and in the subsequent two sessions, individuals were encouraged to train at an intensity of 50-60% of the average speed of the 6MWT.

In the same sessions, participants familiarized themselves with resistance and stretching exercises, and each patient was instructed on how to correctly perform both types of exercise.

2.1.2 Intervention

The "Lifestyle Program" coordinated by the Oncologists and Physiatrists of the Breast Cancer Unit, University Hospital of Ancona, was implemented with exercise counseling and a supervised exercise program to improve functional exercise capacity [31]. A diary of PA was also proposed to monitor the patient's PA level and help BCS to exercise regularly.

Physical Activity Diary

The Diary contains information about the benefits of exercise, the amount of exercise that is recommended for patients, types of exercises and practical tips to encourage BCS to exercise regularly. The booklet cover (Figure 1a) contains the diary name, "Active Women". The cover image was selected by research team and represents a group of BCS. The first page of the Diary (Figure 1b) provides a summary of recommendations for BCS, while the second page (Figure 1c) contains instructions on PA programming and

describes the “Talk test”. These sections aim to increase awareness of the many benefits of exercise for BCS. In the light of the scientific evidence on the benefits of PA, the PA diary is presented and women are invited to complete the diary for four consecutive weeks (Figure 1d). The following session includes instructions (Figure 1e) for completing “My Physical Activity Diary”. Finally, instructor, before delivering the PA diary, completes the table with the clinical characteristics of the woman (body height, body mass, waist circumference, BMI) and invites the woman to complete “My Physical Activity Diary”, indicating 'Starting time', 'Type of PA', 'Duration' and 'Comments or notes' for four consecutive weeks (Figure 1f).

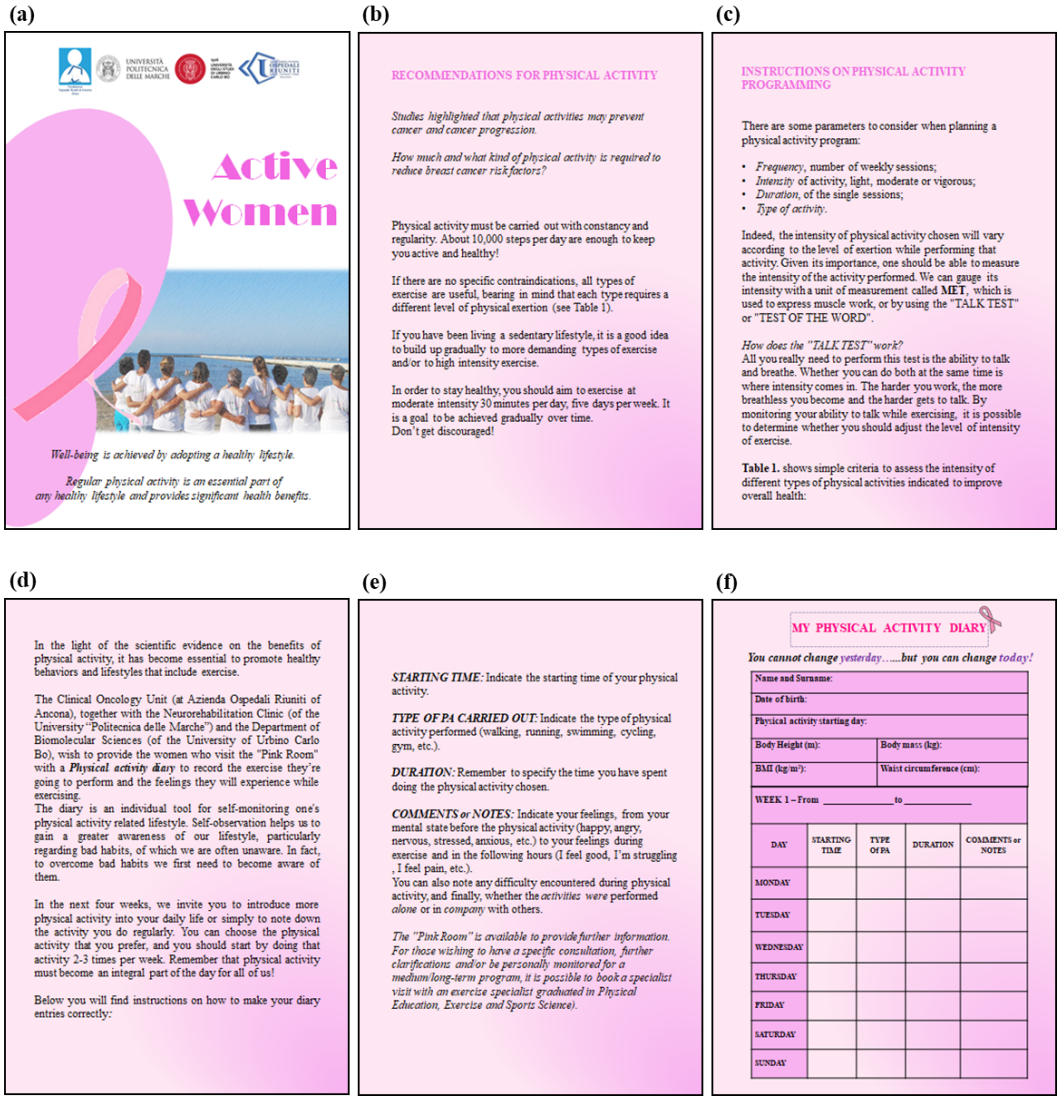

Figure 1. *Physical Activity Diary*: (a) cover; (b) recommendations; (c) instructions on PA programming; (d) (e) and (f) instructions for completing the diary “My Physical Activity Diary”.

Physical Activity Interventions

Cancer survivors were enrolled in a CA and an IA. For both groups an exercise counseling activity was offered consisting of an individual meeting (30 min), based on principles of narrative and cognitive theory [32]. The counseling was held by a qualified and specialized exercise trainer, who provided the patient with the appropriate information and support for practicing regular PA. At the end of the counseling session, the PA diary was given to each patient.

Subjects in the IA then started on a program of PA and exercise, training three times a week on Monday, Wednesday (supervised sessions) and Friday (unsupervised session, monitored by PA diary) for eight weeks. The training protocol was developed based on the recommendations of the ACSM [19] and its general aim was to have subjects trained regularly for at least 3 days a week (in accordance with the main guidelines - at least 150 min/week of moderate intensity exercise or 75 min/week of vigorous intensity exercise). Subjects in the CA maintained their routine and were not involved in any supervised training.

The supervised training sessions were held at the gym of the Neurorehabilitation Clinic - University Hospital of Ancona and were structured as follows: each session lasting about 60 min and exercise intervention consisted in circuit training that included both aerobic and resistance exercises. Each training session was held in groups and on non-consecutive days, for eight weeks. The women of IA completed a total of sixteen supervised training sessions.

Procedures

The supervised exercise sessions were structured as follows: a 5 min warm-up phase during which women performed dynamic warm-up exercises for the joints and for the main muscle groups (both upper and lower body) (Table 2); the main part consisted of 45 min of circuit training (Table 4); a final 5 min cool-down phase where women performed static stretching exercises mainly for the pectoral muscle, hamstrings and lumbar region (Table 3). All the exercises were explained and previously demonstrated by a qualified specialized exercise trainer and the women were invited to practice the exercises for a few minutes before starting the first training session.

Table 2. *Dynamic warm-up exercise*

1. Side-to-side neck tilt. Tilt neck towards one shoulder and then towards the other.
2. Neck turn. Turn head to one side, keeping chin at the same level.
3. Shoulder rolls. From a position of proper alignment, roll shoulders upwards, then back, then downwards in a fluid motion.
4. Raise body on toes and swing alternating arms.
5. March on the spot. Keep knees up and move the opposite arm with the opposite knee.

Table 3. *Static stretching exercises*

1. Head chest stretch. Seated or standing, interlock your fingers, bend your elbows and raise your arms above your head. Gently squeeze your shoulder blades together and move your elbows and hands backward. Vary the height of your hands to involve shoulders and/or chest (hands behind head, hands on top of head, hands a few inches above head).
2. The simple hamstring stretch: sit on the floor with both legs out straight. Extend your arms and reach forward by bending at the waist as far as possible while keeping your knees straight. Relax back into the starting position and repeat.
3. Cat-camel back stretch. On hands and knees, slowly alternate between arching and rounding your back so that all three sections of your spine-lumbar (lower), thoracic (middle) and cervical (upper)-extend together and then flex together.
4. Supine cross-leg spinal twist. Lying on your back with knees bent and feet on the floor, stretch arms out to sides, palms facing down. Cross right knee over left knee. Shift hips to the right and drop knees to the left.
5. Twisting glute stretch. Sitting on the floor, with your legs stretched out in front of you, bend one knee, keeping the other extended. Drape the opposite arm across the bent knee and twist toward it until you feel your glute muscles stretching.

Circuit training protocol

The circuit training protocol included eight stations (seven stations dedicated to resistance exercise and one station for aerobic exercise), repeated twice in each training session (Table 4). The exercise routine was designed to involve the whole body and to alternate opposing muscle groups work and rest periods. The goal was to make the lower body work while the upper body was not called into play, and vice versa. In order to obtain aerobic and metabolic benefits, each station consisted of very few breaks between the exercises and the women were given three levels of difficulty at each resistance station (Table 4) so that the intensity of the exercise was approximately customized for each patient.

Table 4. *Circuit training session*

Phase/Exercises	Exercise Time	Series	Intensive progression (level 1/2/3)*	Materials
Warm-up	5 min			
Main part	45 min			
Circuit training stations				
a. Push-ups	30s; 10-20s Rest	2	Wall push-ups/ Push-ups from knees/Push-ups	Met
b. Squats	30s; 10-20s Rest	2	Wall-squats/Bodyweight squats/Counterbalance squats	Medicine ball (MB) - 2kg
c. Triceps extension	30s; 10-20s Rest	2	Low/ medium/ high resistance	Elastic band (Therabands)
d. Crunches	30s; 10-20s Rest	2	Arms stretched forward/ chest/ backwar	Mat
e. Bridging	30s; 10-20s Rest	2	Basic bridge (Two legs)/Single Leg bridge/ Basic bridge with band	Mat and Elastic band (Therabands)
f. Calf-Raise	30s; 10-20s Rest	2	Wall Calf-raise, doubles legs/ Calf-raise single leg/ Calf-raise single leg, knee straight	Step
g. Rowing	30s; 10-20s Rest	2	Low/ medium/ high resistance	Elastic band (Therabands)
Aerobic exercise				
Treadmill walking	15-20 min	2	80%–110% of the average 6MWT speed	Treadmill
Cool-down	5 min			
Static stretching				

* see text for details about intensity progression mode.

Each resistance exercise was performed for 30s and was followed by 10 to 20s of rest between one exercise and the other (gradually reduced during training). During the 30s of exercise, women were invited to complete the highest possible number of repetitions in a controlled manner up to the maximum allowed speed. All participants began the intervention program exercising at the first level of difficulty. When the participant was able to reach the maximum exercise speed for the given time she was instructed to adopt the next intensity level in the following exercise session. Aerobic exercise was performed on a treadmill (Runner EE-0720 MTR, Italy) and walking intensity was based on the average speed of the 6MWT [20]. The duration of the walk varied from a minimum 15 to maximum 20 min per session and the walk was repeated twice during the circuit protocol. The walking speed was increased by 10% during the eight weeks of training considering the average speed reached during the 6MWT at baseline and after four weeks of training. During the first four weeks of training, the walking speed was set considering the average speed during the 6MWT at baseline and was increased as follows: first week, 80% of the average speed of the 6MWT; second week, 90% of the average speed of the 6MWT; third week, 100% of the average speed of the 6MWT; fourth week, 110% of the average speed of the 6MWT. In the following four weeks, the intensity was increased as in the first four-weeks, but the walking speed was set considering the new average speed reached during 6MWT performed after the first four-week intervention. During aerobic exercise the Borg scale was shown (values from 6 to 20), to allow subjects to describe the intensity of their exertion and the operator to identify a given percentage of the maximum heart rate (HRmax) [30]. Moreover, during the aerobic exercise the “Talk test” was also proposed: this allowed the subject to focus on the frequency of their breathing and thus to become aware of its intensity (this test was also prescribed in the PA diary as a useful means to set the intensity of autonomous exercise) [33].

Statistical Analysis

Statistical analysis was performed using StatView SAS software (version 5.0.1.). Descriptive analysis of clinical characteristics (mean, standard deviation) was performed and variables were found to be normal for age, body mass, body height, waist circumference, BMI. The analysis of the intra-group comparison was performed using the Wilcoxon signed-rank test, applied to non-parametric variables. The Friedman Test was

used to analyze the differences between the times in the intervention arm. Analysis of variance (ANOVA) for repeated measure was performed to obtain intergroup comparison (ANOVA for repeated measure, followed by Dunnett's Multiple Comparison Test; $p < 0.05$).

Specifically, the results are presented after 4 weeks for both groups. The control group is still being completed and we will publish our results of the complete study, once finished.

3. Results

Baseline Assessment Results

At baseline no differences were find as for demographic and clinical data between the two groups. Subjects of the CA chose to not participate to the PA at the Hospital because of yet engaged in a autonomous PA (n=2) or because of logistic reasons, they live too far from the Hospital to reach it every weeks (n=8).

36-Item Short Form Health Survey questionnaire (SF-36)

The quality of life assessed by the SF-36 scale showed that at baseline the two groups were comparable in the different scales of the perception of well-being. After four weeks both groups had improved in terms of 'Role limitation due to emotional problems' (D3), Energy fatigue (D4), Emotional well-being (D5), while only the IA subjects improved in terms of 'Physical functioning' (D1), 'Role limitation due to physical health' (D2), 'Social functioning' (D6), 'Pain' (D7) and 'General health' (D8). In the D8, a significant positive effect was found for the IA subjects with respect to CA (F 8.0; p=.01) (Figure 2, A).

International Physical Activity Questionnaire (IPAQ)

At baseline, the amount of daily physical activity quantified in MET was similar between the two groups. After four weeks, in both groups, the MET were significantly increased (IA: $Z = -53.0$; $p < .0001$; CA: $Z = -2.2$; $p = .03$); however, in the IA subjects the MET value increased two-fold compared to the CA subjects ($F = 21.0$; $p = .0002$) (Figure 2, B). After eight weeks of intervention the patients in the IA maintained the level of activity

which then tended to decrease two months after the end of the training; however, it remained higher than the baseline (Chi2 = 26.3; p <.0001).

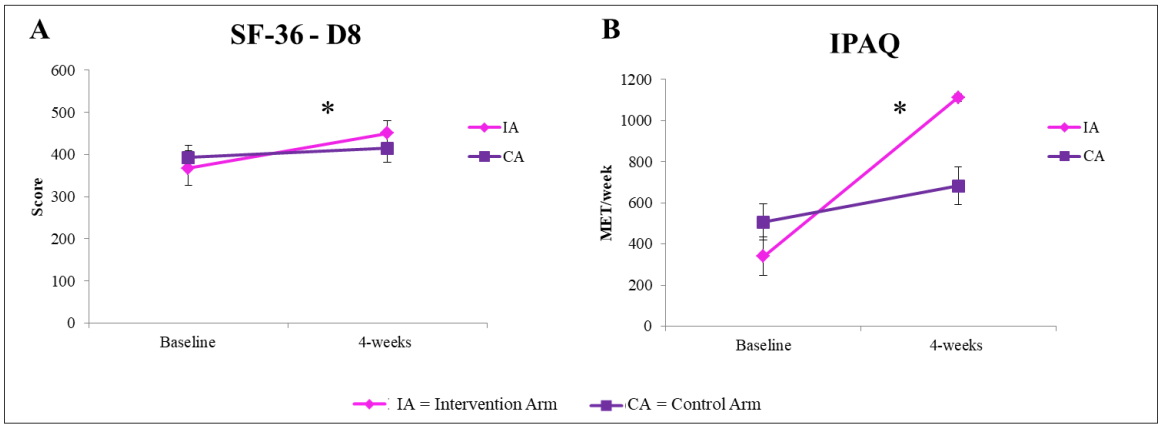

Figure 2. *Health and lifestyle questionnaire results.* SF-36 – D8 = 'General health', show a significant positive effect was found for the IA subjects after 4-weeks of intervention (**A**). IPAQ, International Physical Activity Questionnaire: the IA subjects the MET value increased two-fold compared to the CA subjects (F = 21.0; p = .0002) after 4-weeks of intervention (**B**).

Physical Activity Diary

The PA diary monitored daily PA from the first day after PA counseling. The results were translated into MET/week. The formal analysis of the data using comparative statistics did not show significant differences, but St. Err and Std. Dev were very high in the CA subjects. The amount of daily physical activity quantified in MET increased significantly in both groups (IA: Z = -53.0; p <.0001; CA: Z = -2.2; p = .03); however, in the IA subjects the MET value increased two-fold compared to the CA subjects (F = 21.0; p = .0002). The descriptive analysis of the data shows that three subjects the IA increased the MET from the first to the fourth week by about 1500 MET, while the others remained stable. In the CA subjects, after the first week there is great variability in behavior: three subjects did not perform any physical activity (MET ~ 0), two performed very intense activity (MET ~ 10000), while the others performed moderate activity (3000-4000 MET). At the fourth week the two patients who had carried out intense activity were no longer active (MET ~ 0), two increased from the baseline activity by about 1500 MET, six subjects did not change their activity level, including three patients who were completely sedentary (Figure 3).

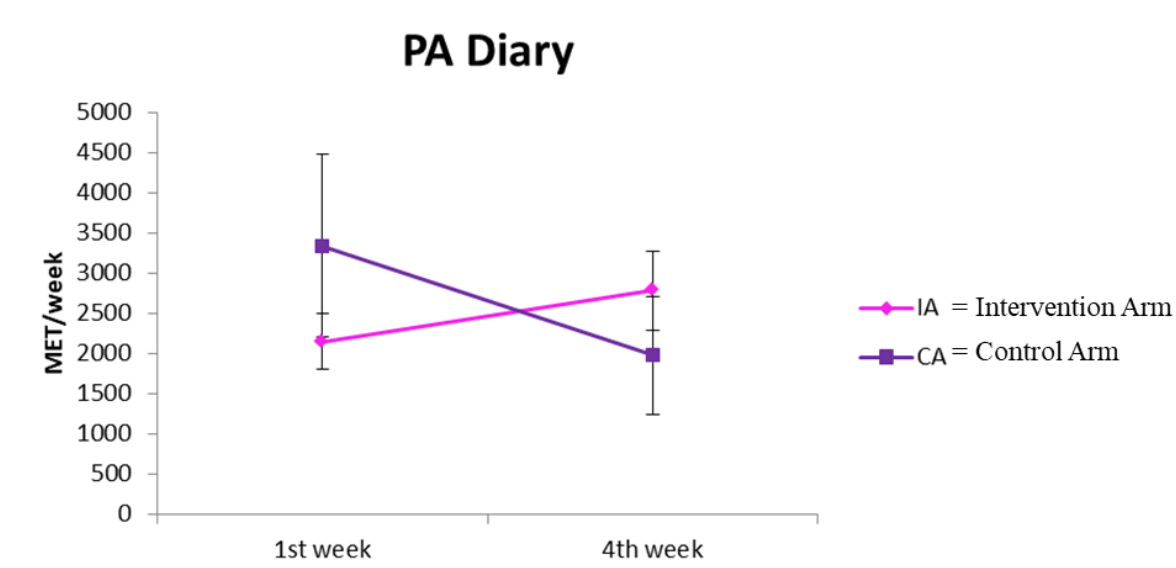

Figure 3. *Data obtained from "My Physical Activity Diary".* The PA diary monitored daily PA from the first day after PA counseling. The results were translated into MET/week for the 1a and the 4a-week in the IA and CA. The amount of daily physical activity quantified in MET increased significantly in both groups (IA: Z = -53.0; p <.0001; CA: Z = -2.2; p = .03); in the IA subjects the MET value increased two-fold compared to the CA subjects (F = 21.0; p = .0002).

Six-Minute Walk Test (6MWT)

Analysis of the results obtained for the 6MWT showed that five subjects in the IA improved over 30 meters, after four weeks of intervention (MCID according to Bohannon RW et al., 2017)[34] and no subject's performance worsened compared to the baseline. Overall, subjects in the IA improved on average 49 meters [range: 190: -4], while subjects in the CA worsened on average by about 15 meters [range: 42: -95]. In the CA, only two subjects improved by more than 20 meters, while four subjects showed a significant drop in performance (less than 20 meters compared to baseline); the difference in walked meters between the two groups after four weeks of intervention was statistically significant (Z = -2.6; p = .009, Figure 4, A). In agreement with this data, subjects in the IA showed a positive improvement trend over time. This statistically significant result is evident after four weeks of intervention compared to baseline (Z = -2.1; p = .02) (Figure 4, A) and is maintained through eight weeks of intervention (Chi2 =

12.0; p = .006). The intra-group comparison in the CA showed no statistically significant difference between the first four weeks and baseline evaluations (Figure 4, A).

Sit-And-Reach Test (SRT)

Results for the Sit and Reach Test showed no significant amelioration after four weeks in both groups, although a positive trend of improvement over time in the IA subjects is observable throughout the period of supervised intervention, although it notable a decreasing trend during the unsupervised period (Figure 4, B).

Hand Grip Strength (HGS)

The results of the Hand Grip Test (HGS) clearly showed that the right hand force improved significantly in subjects in the IA (Z = -2.66; p = .008) (Figure 4, C-D). Overall, the right hand of subjects in the IA showed a positive trend of improvement over time from baseline to eight weeks of intervention: Chi2 = 18.1; p = .0004 (Friedman test). The hand force however tends to decrease in both hands two months after the end of training. The left hand of IA subjects showed a positive trend from baseline to eight weeks of intervention: Chi2 = 10.1; p = .01, which tends to decrease in the two months following the training period. The CA subjects did not show statistically significant changes in force in either hand (Figure 4, C-D). In both groups, no statistically significant differences emerged between the force of the operated limb and the contralateral.

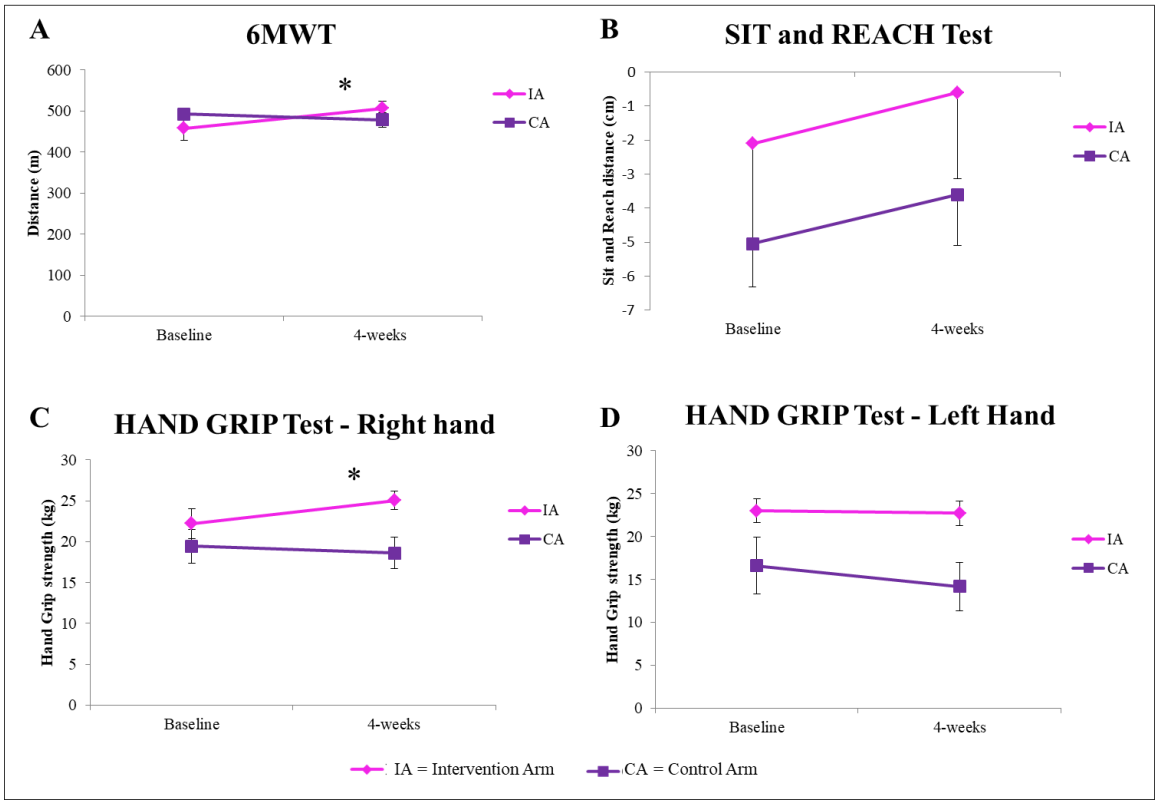

Figure 4. *Functional exercise capacity tests results.* 6MWT (6-minute Walk Test), show a statistically significant (Z = -2.6; p = .009) in the difference in walked meters between the two groups after 4-weeks of intervention (**A**). Sit and Reach Test (SRT) show no significant amelioration after 4-weeks in both groups (**B**). Hand Grip strength (HGS) (Kg) show a statistically significant in the right hand of subjects in the IA (Z = -2.66; p = .008) after 4-weeks of intervention (**C**); the left hand of IA subjects showed a positive trend from baseline to 4-weeks of intervention (**D**). In both groups, no statistically significant differences emerged between the force of the operated limb and the contralateral.

4. Discussion

Lifestyle can play an important role in BC prevention, and one of the modifiable factors that can have a strong impact on the disease is PA.

An increasing number of studies worldwide highlight the importance of PA in counteracting BC, and there is a large body of scientific evidence showing that PA plays a fundamental role in the promotion of health and wellbeing, prevention, treatment and the reduction of the risk of recurrence.

Based on these emerging data, physicians and other health professionals have a moral, ethical and professional obligation to inform patients of the risk of being physically inactive and to provide them with the right exercise prescription. In fact, although the healthcare community often provides recommendations on PA for patients with chronic-degenerative diseases, it has not yet developed a health prevention plan for cancer patients after surgery including a precise exercise and PA prescription. To date, PA is recommended based on general guidelines provided by the Health Ministry or the American College of Sports Medicine for a specific population and healthcare professionals assume that patients fully adhere to the exercise protocols provided to them. However, this does not happen in practice; a systematic review of the literature shows that self-reported measurement of adherence to exercise is not a valid and reliable measure to support patients undertaking a PA program that provides optimal doses of exercise [35]. Moreover, recording self-reported PA using a simple diary is not a valid lifestyle change tool. In fact, as our study shows, after patients have received PA counseling and a diary, they perceive the importance of an active lifestyle; nevertheless, they fail to maintain the right levels of PA recommended showing sudden increases and/or drastic reductions in PA. An inconsistent irregular exercise program, especially with spikes in MET and uncontrolled HR, could pose cardiovascular risks and potentially damage patient health rather than providing a powerful means of prevention as demonstrated in the literature.

To be successful in the promotion of exercise it is essential that, alongside the patient's clinical program and initial counseling session on PA, a structured educational program is needed. Our study demonstrates the importance and effectiveness of an exercise program provided in the hospital, prescribed and supervised by a qualified and specialized exercise trainer, who starts working with the patient at the end of her therapeutic regime and then

continues to provide support to help the patient move gradually towards an active lifestyle. Numerous studies have highlighted the importance of PA at different stages of the disease [4] and how different types of exercise can improve the prognosis and reduce the risk of both metastasis and recurrence. Patients with BC receive various forms of medical therapy, such as surgery, chemotherapy, hormone therapy, antibodies and/or radiotherapy, and have to deal with various side effects such as fatigue [36]. In addition, after diagnosis and treatment, patients begin to decrease their PA level, remaining physically inactive [37]. Movement enhances the subject's sense of well-being acting on a mental level and in terms of controlling the progression of the disease. The promotion of exercise during the therapeutic treatment, with suitable spaces within the clinical facilities, where patients can move and train safely, should become an essential part of cancer care.

Limitations of the study

On the basis of the results obtained, we can state that the proposed exercise protocol was effective in achieving the pre-established objectives. However, although the training program led to improvements in the outcomes foreseen by the study, the exercise protocol was designed on the basis of sub-maximal and non-maximal tests, and thus had limitations related to the personalization of the exercise intensity adopted during the workout. Regarding improvements in muscle tone, we could only offer exercises with bodyweight or with small pieces of equipment. The intensity of the load, during counter-resistance exercises, was gradually increased by using small overloads or resistances (a medicine ball or elastic bands). Undoubtedly, carrying out a maximum oxygen consumption test would have ensured better personalization of the aerobic intensity prescribe for treadmill exercise. Furthermore, the use of isotonic machines would have allowed us to calculate the maximum specific and personalized load to use for each patient and therefore we could have adapted and increased the training stimulus more effectively with muscle strengthening exercises. By doing so, we could have obtained better results in the different outcomes related to the physiological parameters under consideration.

5. Conclusions

In conclusion, the results of the present pilot study showed that the supervised exercise program was able to make significant changes in terms of aerobic capacity, muscle strength, perceived fatigue and quality of life. We were able to ascertain that many of the consequences of the disease, such as muscle weakness, fatigue and depression, were largely due to de-conditioning. The positive effects obtained in terms of physical and mental conditioning were sufficient to avoid cases of drop-out during the entire period of intervention. This pilot study with a training program of this type and duration, is a possible useful model for an applicable everyday exercise protocol and can also be propose in hospitals that offer limited-equipped gym.

Future studies on BCS should consider proposing the exercise protocol for a longer period both for BCS and for patients undergoing treatment. Furthermore, based on the availability of hospital resources, future exercise protocols could be based on maximal testing.

The approach to cancer patients must include, in addition to specific medical therapies, particular attention to the communicative and relational dimensions. For the cancer patient, exercise can help patients to cope better with the post-surgery phase and foster recovery, both physically and psychologically, after the debilitating treatment phase.

For the BC patients, the "post-surgery" phase is a long and difficult period from a psychological point of view, and it includes frequent checkups and follow-ups: the inclusion of care programs can help to create a physio-psychological support network during this delicate/difficult phase. An exercise promotion activity involving PA counseling and a supervised exercise program also provides opportunities to socialize in groups, a fundamental element from a psychological standpoint. In fact, BC patients often tend to isolate themselves following diagnosis: having a personalized health plan that also helps the patient to avoid isolation and to face and share with the other patients their difficulties can be an important resource for the patient in facing daily challenges.

References

1. *Jemal, A., et al., Global cancer statistics. CA Cancer J Clin, 2011. 61(2): p. 69-90.*
2. *Holmes, M.D., et al., Physical activity and survival after breast cancer diagnosis. JAMA, 2005. 293(20): p. 2479-86.*
3. *Abrahamson, P.E., et al., Recreational physical activity and survival among young women with breast cancer. Cancer, 2006. 107(8): p. 1777-85.*
4. *Irwin, M.L., et al., Influence of pre- and postdiagnosis physical activity on mortality in breast cancer survivors: the health, eating, activity, and lifestyle study. J Clin Oncol, 2008. 26(24): p. 3958-64.*
5. *Kellen, E., et al., Lifestyle changes and breast cancer prognosis: a review. Breast Cancer Res Treat, 2009. 114(1): p. 13-22.*
6. *Kim, C.J., D.H. Kang, and J.W. Park, A meta-analysis of aerobic exercise interventions for women with breast cancer. West J Nurs Res, 2009. 31(4): p. 437-61.*
7. *Spence, R.R., K.C. Heesch, and W.J. Brown, Exercise and cancer rehabilitation: a systematic review. Cancer Treat Rev, 2010. 36(2): p. 185-94.*
8. *Ibrahim, E.M. and A. Al-Homaidh, Physical activity and survival after breast cancer diagnosis: meta-analysis of published studies. Med Oncol, 2011. 28(3): p. 753-65.*
9. *Stebbing, J., et al., A metabolic phenotyping approach to understanding relationships between metabolic syndrome and breast tumour responses to chemotherapy. Ann Oncol, 2012. 23(4): p. 860-6.*
10. *Zeng, Y., et al., Meta-analysis of the effects of exercise intervention on quality of life in breast cancer survivors. Breast Cancer, 2014. 21(3): p. 262-74.*
11. *Giallauria, F., et al., Exercise training improves heart rate recovery in women with breast cancer. Springerplus, 2015. 4: p. 388.*
12. *Giallauria, F., et al., Exercise training improves cardiopulmonary and endothelial function in women with breast cancer: findings from the Diana-5 dietary intervention study. Intern Emerg Med, 2016. 11(2): p. 183-9.*
13. *Pedersen, B.K. and L. Hoffman-Goetz, Exercise and the immune system: regulation, integration, and adaptation. Physiol Rev, 2000. 80(3): p. 1055-81.*
14. *Dethlefsen, C., et al., Exercise regulates breast cancer cell viability: systemic training adaptations versus acute exercise responses. Breast Cancer Res Treat, 2016. 159(3): p. 469-79.*
15. *Rock, C.L., et al., Nutrition and physical activity guidelines for cancer survivors. CA Cancer J Clin, 2012. 62(4): p. 243-74.*
16. *Pate, R.R., et al., Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. JAMA, 1995. 273(5): p. 402-7.*
17. *Lahart, I.M., et al., Physical activity, risk of death and recurrence in breast cancer survivors: A systematic review and meta-analysis of epidemiological studies. Acta Oncol, 2015. 54(5): p. 635-54.*

18. *Irwin, M.L., et al., Physical activity levels among breast cancer survivors. Med Sci Sports Exerc, 2004. 36(9): p. 1484-91.*
19. *American College of Sports Medicine, D.Riebe, J.K.Ehrman, G. Liguori, and M. Magal, ACSM's Guidelines for Exercise Testing and Prescription, Lippincott Williams & Wilkins, Philadelphia, 10th edition, 2017.*
20. *Enright, P.L. and D.L. Sherrill, Reference equations for the six-minute walk in healthy adults. Am J Respir Crit Care Med, 1998. 158(5 Pt 1): p. 1384-7.*
21. *Stringuetta-Belik, F., et al., [Greater level of physical activity associated with better cognitive function in hemodialysis in end stage renal disease]. J Bras Nefrol, 2012. 34(4): p. 378-86.*
22. *Lee, P.H., et al., Validity of the International Physical Activity Questionnaire Short Form (IPAQ-SF): a systematic review. Int J Behav Nutr Phys Act, 2011. 8: p. 115.*
23. *Ware, J.E., Jr. and C.D. Sherbourne, The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care, 1992. 30(6): p. 473-83.*
24. *Mayorga-Vega, D., R. Merino-Marban, and J. Viciana, Criterion-Related Validity of Sit-and-Reach Tests for Estimating Hamstring and Lumbar Extensibility: a Meta-Analysis. J Sports Sci Med, 2014. 13(1): p. 1-14.*
25. *Gomes, P.R., et al., Short-term changes in handgrip strength, body composition, and lymphedema induced by breast cancer surgery. Rev Bras Ginecol Obstet, 2014. 36(6): p. 244-50.*
26. *Rantanen, T., et al., Handgrip strength and cause-specific and total mortality in older disabled women: exploring the mechanism. J Am Geriatr Soc, 2003. 51(5): p. 636-41.*
27. *Webb, A.R., et al., Hand grip dynamometry as a predictor of postoperative complications reappraisal using age standardized grip strengths. JPEN J Parenter Enteral Nutr, 1989. 13(1): p. 30-3.*
28. *ATS statement: guidelines for the six-minute walk test. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. Am J Respir Crit Care Med 2002;166 (1): 111-117.*
29. *Solway, S., et al., A qualitative systematic overview of the measurement properties of functional walk tests used in the cardiorespiratory domain. Chest, 2001. 119(1): p. 256-70.*
30. *Borg G. Borg's perceived exertion and pain scales. In: Champaign IL, Hum Kinetics 1998.*
31. *Battelli N et al (2015) Annals of Oncology, 26(suppl 6):vi132¿vi151.*
32. *Midtgaard, J., Theoretical and practical outline of the Copenhagen PACT narrative-based exercise counselling manual to promote physical activity in post-therapy cancer survivors. Acta Oncol, 2013. 52(2): p. 303-9.*
33. *Persinger, R., et al., Consistency of the talk test for exercise prescription. Med Sci Sports Exerc, 2004. 36(9): p. 1632-6.*
34. *Bohannon, R.W. and R. Crouch, Minimal clinically important difference for change in 6-minute walk test distance of adults with pathology: a systematic review. J Eval Clin Pract, 2017. 23(2): p. 377-381.*
35. *Bollen, J.C., et al., A systematic review of measures of self-reported adherence to unsupervised home-based rehabilitation exercise programmes, and their psychometric properties. BMJ Open, 2014. 4(6): p. e005044.*

36. *Schmitz, K.H., et al., American College of Sports Medicine roundtable on exercise guidelines for cancer survivors. Med Sci Sports Exerc, 2010. 42(7): p. 1409-26.*
37. *Lucas AR, Levine BJ, Avis NE: Post-treatment trajectories of physical activity in breast cancer survivors. Cancer 2017; 123:2773-2780.*

CONCLUSIONS

***"Dragonette"* – An example of a project for women who have undergone breast cancer surgery, which shows a high level of adherence to the exercise program**

Today we can no longer think that women who have undergone surgery following a diagnosis of breast cancer will not return to a full and active life. There is growing scientific interest in the types of exercise that can best play a role both in terms of primary and secondary prevention of breast cancer, and women who have undergone surgery following diagnosis of breast cancer are becoming more and more aware of the benefits of an active lifestyle. Many of these "active" women are now part of an important project called "Abreast in a Boat", an initiative started in 1996 in Canada, at the behest of Dr. Don McKenzie.

This project is for women who have undergone breast cancer surgery with the aim of debunking the myth that, for those who have undergone partial or total breast removal due to cancer, it is not possible to engage in physical activity given the very high risk of lymphedema. The project, therefore, started with a group of volunteers who engaged in an activity that essentially involved using the arms and the bust. The project has since been adopted in many countries in the Americas and Europe. In Italy, the first team of women in pink was founded in Rome in 2003 and a group of breast cancer survivors in Turin have been involved in dragon boating for years.

The women who participate in this project are called Dragonettes because of the type of boat that is used. In fact, the 12.66-meter long dragon boat is an ancient boat of Chinese origin with the head and the tail in the shape of a dragon; the paddles are similar to those of the Canadian canoe and weigh about 1 kg. There are 20 athletes in the boat, 10 on the right and 10 on the left, with the helmsman and a drummer. At the bow, facing the crew, a drummer beats time on a large drum, while at the stern is the helmsman, right on the tail of the dragon, struggling with 3-meter long oars.

Today, Dragonette crews are able to participate in events and non-competitive dragon boat races, and they are also supported by women who have not undergone surgery who train with the "otherwise lucky" (as the Dragonettes call themselves) and this has made it possible to form solid teams. Indeed, the Dragonettes have already been recognized by the Italian Dragon Boat Federation, as the women in pink section.

Rowing to fight breast cancer is a challenge that the women of the Marche region have also met. On 22 July 2018 the first dragon boat of the Marche, promoted by the

Department of Medical Oncology, University Hospital of Ancona and the Fondazione Ospedali Riuniti of Ancona, in collaboration with other local companies, took part in a joyous sporting event dedicated to women with breast cancer. The aim of the event was to raise awareness of breast cancer prevention, emphasizing the importance of respecting the calendar of checkups/screening and pledging support for all women affected by the disease.

Twenty breast cancer survivors took part in the event and ten of them had taken part in the "Lifestyle program" coordinated by the Oncologists of the Breast Cancer Unit, University Hospital of Ancona. This crew of Dragonettes, has also ventured with its own boat on a journey of about 6 km in the Adriatic Sea.

Sport and medicine are coming together more and more: the specific breast cancer guidelines of the ACSM show that this activity, although not empirically tested, is a valid safe form of exercise given the large number of women who take part in Dragon Boating. Paddling is very useful for the full recovery of the upper limbs. But even more important are the psychological benefits: sharing, teaming up, discovering, getting back into the game, even at a difficult time, setting new goals to be stronger than before. Side by side in a boat, to win in life.

Dragonettes – "Side by side in a boat, to win in life". First Dragon Boat of the Marche region, Italy, 22 July 2018.

Nothing makes a woman more fragile and vulnerable than the onset of a serious illness. When the diagnosis is "breast cancer", the negative connotations associated with this pathology generate the impression of being invaded and overwhelmed by something malignant. It was in this particular scenario, that the role of exercise in primary and secondary cancer prevention was examined. In addition, the possible mechanisms activated and/or regulated by exercise were identified and investigated by examining recent evidence regarding the effects of muscle contraction on the inhibition of the mTOR pathway in a particular subtype of BC, TNBC. In this regard, a group of young healthy women were recruited to perform acute endurance exercise (high-intensity endurance cycling (HIEC)) and we investigated how the circulating serum factors released after exercise can reduce TNBC cell proliferation, in vitro. We showed that a single exercise session exerted a significant protective effect against tumor proliferation in triple-negative cell lines. In addition, we revealed how the proposed training led to systemic changes, including the exertion of inhibitory effects on the viability of the analyzed BC cell line.

Given the growing body of evidence regarding the importance of lifestyle changes for women diagnosed with BC (before, during and after the long therapeutic journey), we investigated the effect of structured and supervised PA in BCS in collaboration with the Breast Cancer Unit, University Hospital of Ancona, within the "Lifestyle Program", which promotes exercise in the therapeutic treatment of BC.

In this context, we stressed that PA counseling can motivate women with BC to adopt a healthier lifestyle, helping them to maintain PA levels that are recommended by the guidelines for this population. However, changing one’s lifestyle is a complex process: providing information is not enough, and it does not ensure the implementation of a PA program. In fact, a social support network is needed, allowing these patients to embark on a personalized journey and providing them with support at every stage of the disease. Indeed, as the data of our pilot study show, an approach involving the promotion, prescription and administration of a structured and supervised training program, represents an important step in changing patients’ lifestyles and improving their quality of life.

Hence, PA can only be promoted effectively by creating a support system, involving various professionals, including oncologists; physiatrists; physiotherapists; graduates in physical education, exercise and sports science; nutritionist and psychologists. The

multidisciplinary interventions of these professionals provide a coordinated program of care for the patient, creating the proper conditions for the promotion and practice of PA, which is vital for the prevention and treatment of female tumors.

REFERENCES

Introduction

1. *Associazione Italiana Registro Tumori (AIRTUM), I numeri del cancro 2017. (http://www.registri-tumori.it/PDF/AIOM2017/2017_numeri_del_cancro.pdf).*
2. *Turashvili G and Brogi E. Tumor Heterogeneity in Breast Cancer. Front Med (Lausanne), 2017. 4: p. 227.*
3. *Friedenreich CM. The role of physical activity in breast cancer etiology. Semin Onco., 2010. 37:297–302.*
4. *Mijwel S et al. Exercise training during chemotherapy preserves skeletal muscle fiber area, capillarization, and mitochondrial content in patients with breast cancer. FASEB J, 2018. 32(10): p. 5495-5505.*
5. *Ammitzboll G, et al. Physical activity and survival in breast cancer. Eur J Cancer, 2016. 66: p. 67-74.*
6. *Mijwel S, et al. Highly favorable physiological responses to concurrent resistance and high-intensity interval training during chemotherapy: the OptiTrain breast cancer trial. Breast Cancer Res Treat, 2018. 169(1): p. 93-103.*
7. *Courneya KS, Mackey JR, Bell GJ, Jones LW, Field CJ, Fairey AS. Randomized controlled trial of exercise training in postmenopausal breast cancer survivors: cardiopulmonary and quality of life outcomes. J Clin Oncol 2003; 21:1660-8.*
8. *Irwin ML, Crumley D, McTiernan A, Bernstein L, Baumgartner R, Gilliland FD, Kriska A, Ballard Barbash R. Physical activity levels before and after a diagnosis of breast carcinoma. Cancer 2003; 97: 1746-57.*
9. *Apostolopoulos V, de Courten MP, Stojanovska L, Blatch GL, Tangalakis K, de Courten B. The complex immunological and inflammatory network of adipose tissue in obesity. Mol Nutr Food Res. 2016; 60:43-57.*
10. *Huh JY, Park YJ, Ham M, Kim JB. Crosstalk between adipocytes and immune cells in adipose tissue inflammation and metabolic dysregulation in obesity. Mol Cells. 2014; 37:365-371.*
11. *Catalan V, Gomez-Ambrosi J, Rodriguez A, Fruhbeck G. Adipose tissue immunity and cancer. Front Physiol. 2013; 4:275.*
12. *Moore SC, Lee IM, Weiderpass, E, Campbell PT, Sampson JN, Kitahara CM, Keadle SK, Arem H, Berrington de Gonzalez A, Hartge P, et al. Association of Leisure-Time Physical Activity With Risk of 26 Types of Cancer in 1.44 Million Adults. JAMA Intern Med. 2016, 176, 816–825.*
13. *Hallal PC, Andersen LB, Bull FC, Guthold R, Haskell W, Ekelund U. Lancet Physical Activity Series Working Group. Global physical activity levels: Surveillance progress, pitfalls, and prospects. Lancet 2012, 380, 247–257.*
14. *Ashcraft KA, Peace RM, Betof AS, Dewhirst MW, Jones LW. Efficacy and mechanisms of aerobic exercise on cancer initiation, progression, and metastasis: A critical systematic review of in vivo preclinical data. Cancer Res. 2016, 76, 4032–4050.*
15. *Pedersen L, Christensen JF, Hojman P. Effects of exercise on tumor physiology and metabolism. Cancer 2015, 21, 111–116.*
16. *Dethlefsen C, Lillelund C, Midtgaard J, Andersen C, Pedersen BK, Christensen JF, Hojman P. Exercise regulates breast cancer cell viability: Systemic training adaptations versus acute exercise responses. Breast Cancer Res. Treat. 2016, 159, 469–479.*
17. *Hojman P, Dethlefsen C, Brandt C, Hansen J, Pedersen L, Pedersen BK. Exercise-induced muscle-derived cytokines inhibit mammary cancer cell growth. Am. J.Physiol. Endocrinol. Metab. 2011, 301, E504–E510.*

18. *De Santi M, Annibalini G, Barbieri E, Villarini A, Vallorani L, Contarelli S, Berrino F, Stocchi V, Brandi G. Human IGF1 pro-forms induce breast cancer cell proliferation via the IGF1 receptor. CellOncol. 2016, 39, 149–159.*
19. *Annibalini G, Bielli P, De Santi M, Agostini D, Guescini M, Sisti D, Contarelli S, Brandi G, Villarini A, Stocchi V, et al. MIR retroposon exonization promotes evolutionary variability and generates species-specific expression of IGF-1 splice variants. Biochim. Biophys. Acta 2016, 1859, 757–768.*
20. *Piguet AC, Saran U, Simillion C, Keller I, Terracciano L, Reeves HL, Dufour JF. Regular exercise decreases liver tumors development in hepatocyte-specific PTEN-deficient mice independently of steatosis. J. Hepatol. 2015, 62, 1296–1303.*
21. *Theriau CF, Shpilberg Y, Riddell MC, Connor MK. Voluntary physical activity abolishes the proliferative tumor growth microenvironment created by adipose tissue in animals fed a high fat diet. J. Appl. Physiol. 2016, 121, 139–153.*
22. *Barbieri E, Agostini D, Polidori E, Potenza L, Guescini M, Lucertini F, Annibalini G, Stocchi L, De Santi M, Stocchi V. The pleiotropic effect of physical exercise on mitochondrial dynamics in aging skeletal muscle. Oxid. Med. Cell Longev. 2015, 2015, 917085.*
23. *Thompson HJ, Jiang W, Zhu Z. Candidate mechanisms accounting for effects of physical activity on breast carcinogenesis. IUBMB Life 2009, 61, 895–901.*
24. *Fong AJ, et al., A qualitative analysis of oncology clinicians' perceptions and barriers for physical activity counseling in breast cancer survivors. Support Care Cancer, 2018. **26**(9): p. 3117-3126.*
25. *American College of Sports Medicine ACSM. ACSM's Guidelines for Exercise Testing and Prescription; Lippincott, Williams & Wilkins: Philadelphia, PA, USA, 2013; pp. 264–273.*
26. *Mascherini G, Tosi B, Giannelli C, Grifoni E, Degl'innocenti S, Galanti G. Breast cancer: Effectiveness of a one-year unsupervised exercise program. J. Sports Med. Phys. Fitness 2018.*
27. *Zeng Y, et al., Meta-analysis of the effects of exercise intervention on quality of life in breast cancer survivors. Breast Cancer, 2014. 21(3): p. 262-74.*
28. *Giallauria F, et al. Exercise training improves heart rate recovery in women with breast cancer. Springerplus, 2015. **4**: p. 388.*
29. *Giallauria F, et al. Exercise training improves cardiopulmonary and endothelial function in women with breast cancer: findings from the Diana-5 dietary intervention study. Intern Emerg Med, 2016. **11**(2): p. 183-9.*
30. *Dethlefsen C, et al. Exercise regulates breast cancer cell viability: systemic training adaptations versus acute exercise responses. Breast Cancer Res Treat, 2016. **159**(3): p. 469-79.*
31. *Cheema BS, Gaul CA. Full-body exercise training improves fitness and quality of life in survivors of breast cancer. J Strength Cond Res 2006; 20:14-21.*
32. *Boerman LM, Berendsen AJ, van der Meer P, et al. Long-term follow-up for cardiovascular disease after chemotherapy and/or radiotherapy for breast cancer in an unselected population. Support Care Cancer 2014; 22:1949–58.*
33. *Schmitt J, et al. A 3-week multimodal intervention involving high-intensity interval training in female cancer survivors: a randomized controlled trial. Physiol Rep, 2016. **4**(3).*

34. *Lee K, et al. Effects of high-intensity interval training on vascular function in breast cancer survivors undergoing anthracycline chemotherapy: design of a pilot study. BMJ Open, 2018.* ***8****(6): p. e022622.*
35. *Tsukamoto H, Suga T, Takenaka S, et al. Greater impact of acute high-intensity interval exercise on post-exercise executive function compared to moderate-intensity continuous exercise. Physiol Behav 2016; 155:224–30.*

Chapter 1

Deborah Agostini, Valentina Natalucci, Giulia Baldelli, Mauro De Santi, Sabrina Donati Zeppa, Luciana Vallorani, Giosuè Annibalini, Francesco Lucertini, Ario Federici, Riccardo Izzo, Vilberto Stocchi, and Elena Barbieri. New Insights into the Role of Exercise in Inhibiting mTOR Signaling in Triple-Negative Breast Cancer. *Oxidative Medicine and Cellular Longevity*, 2018 Sep 30; 2018:5896786. doi: 10.1155/2018/5896786.

Chapter 2

Natalucci V. et al. Effects of exercise on triple-negative breast cancer cell proliferation – *In preparation.*

Chapter 3

Natalucci V. et al. Promotion and prescription of exercise in breast cancer survivors to improve quality of life and health outcomes – *In preparation.*