Universidade de Aveiro
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Estudo piloto para avaliar o efeito de um programa de exercício físico no perfil inflamatório de sobreviventes do cancro da mama

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Molecular e Celular, realizada sob a orientação científica do Doutora Luisa Alejandra Helguero, Professora auxiliar do Departamento de Ciências Médicas da Universidade de Aveiro e coorientação da Dra. Ana Joaquim, Médica Oncologista do Centro Hospitalar de Vila Nova de Gaia e Espinho.

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Este trabalho é dedicado à minha família, aos meus amigos e ao Carlos. Sem o vosso apoio, não seria possível. O meu sincero obrigado a vocês.

o júri

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palavras-chave

Cancro da mama, tratamentos oncológicos, sistema imunitário, metabolismo, inflamação, exercício físico

resumo

O cancro da mama é a patologia mais comum e a causa mais frequente de morte entre as mulheres. Os tratamentos oncológicos são agressivos, o que afeta negativamente a qualidade de vida não só dos pacientes, mas também dos sobreviventes do cancro. Os sobreviventes do cancro da mama vivem com sequelas deixadas pelos tratamentos. Entre essas sequelas, temos inflamação, fadiga, dores e disfunções no sistema imunitário. Uma forma de atenuar estes efeitos é a prática regular de exercício físico. O exercício físico traz inúmeros benefícios, desde ao bem-estar psicológico e físico, diminuição da inflamação até à estimulação do sistema imunitário. Posto isto, este projeto teve como objetivo investigar o efeito de um programa de exercício físico (MAMA_MOVE GAIA AFTER TREATMENT - MMGAT) com a utilização de treino combinado aeróbico e resistência com duração de 8 semanas no perfil inflamatório de sobreviventes do cancro da mama no período pós-menopausa. Foi realizada a quantificação de citocinas em amostras de soro recolhidas em 16 participantes em dois momentos: MC1 (antes da intervenção) e ME1 (8 semanas de intervenção). Células imunes e parâmetros bioquímicos também foram avaliadas. Uma análise de correlação foi feita de forma a averiguar correlações positivas entre as variáveis citocinas, células imunes e parâmetros bioquímicos. Não foram encontradas alterações significativas ao nível das citocinas, exceto IL-10, onde foi verificada uma diminuição desta. Porém, uma tendência para diminuir foi verificada em 50% dos participantes nas citocinas IL-2, IL-4, IL-6, IL-10 e TNF-α e em 12 participantes na citocina IFN-γ. Relativamente às células imunitárias, apenas se verificou uma diminuição significativa dos neutrófilos. Por último, os marcadores metabólicos e hormonais, estes se mantiveram dentro do normal após o exercício físico. Os resultados obtidos indicaram que o programa de exercício físico de treino combinado não alterou o perfil inflamatório dos sobreviventes do cancro da mama. Porém, o exercício físico mostrou uma tendência para diminuir as citocinas pró-inflamatórias, o que é benéfico para melhorar o perfil inflamatório das sobreviventes do cancro da mama. No futuro, estudos com uma maior amostragem e um maior prolongamento de programa de exercício físico poderá fornecer dados com maior significância estatística, face ao papel do exercício físico em sobreviventes do cancro da mama.

keywords Breast cancer, cancer treatments, immune system, metabolism, inflammation, physical exercise

abstract

Breast cancer is the most common cancer and the most frequent cause of death among women. Cancer treatments tends to be aggressive, which negatively affects the quality of life not only for patients, but also of cancer survivors. Breast cancer survivors suffer from sequelae left by treatments, such as inflammation, fatigue, pain and dysfunctions in the immune system and metabolism. Regular practice of physical exercise may mitigate those sequelae. Physical exercise brings benefits, from psychological and physical well-being, decreased inflammation to stimulation of the immune system. That said, this project aimed to investigate the effect of an 8-week physical program MAMA_MOVE GAIA AFTER TREATMENT (MMGAT) with the use of aerobic and resistance training in the inflammatory profile of breast cancer survivors. Cytokines were quantified in serum samples from 16 participants at two moments: MC1 (before exercise) and ME1 (8 weeks of intervention). Immune cells and biochemical parameters were also evaluated. A correlation analysis was performed between immune cells, cytokines and metabolic markers. No significant changes were found in the levels of cytokines, except IL-10, where a decreased occurred. However, a tendency to decrease was observed in 50% of the participants in the cytokines IL-2, IL-4, IL-6, TNF-α and in 12 participants in IFN-γ. For immune cells, there was only a significant decrease in neutrophils. Finally, the metabolic markers, these remained within normal. The results indicate that the combined physical training did not alter the inflammatory profile of breast cancer survivors. However, physical exercise showed a tendency to reduce pro-inflammatory cytokines, which is beneficial to improve the inflammatory profile of breast cancer survivors. Further studies with a large number of samples and with a physical program with more duration may provide data with statistical significance, regarding the role of physical exercise in breast cancer survivors.

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I. List of abbreviations

APCs – antigen-presenting cells
AD – axillary dissection
AT – Aerobic training
BC – breast cancer
CRP – C-reative protein
CT – chemotherapy
DC – ductal carcinoma in situ
DCs – dendritc cells
ER – estrogen receptor
ET – enlarged tumorectomy
HT - hormonotherapy
HR^+ - hormone receptor positive
$HER2^+$ – human epidermal growth factor receptor 2
IL-1β – Interleukin 1 beta
IL-2 – Interleukin 2
IL-4 – Interleukin 4
IL-6 – Interleukin 6
IL-10 – Interleukin 10
IFN-γ – Interferon gamma
LIC – lobular invase carcinoma
LMB – lean body mass
MT – mastectomy
MMGAT – MAMA_MOVE GAIA AFTER TREATMENT
NK – natural killer cells
PE – physical exercise
PR – progesterone receptor
PAMPs – pathogen associated molecular patterns
ROS – reactive oxygen species
RT – resistance training
NST –invasice carcinoma of no specific type

SN – sentinela nodes search

TNBC – triple negative breast cancer

TAMs – tumor-associated macrophages

TME – tumor microenviroment

TNF-α – Tumor Necrosis Factor alfa

VEGF – vascular endothelial growth factor

WAT – white adipose tissue

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CHAPTER I

INTRODUCTION

1. Introduction

Breast cancer (BC) is the most common cancer and the most frequent cause of cancer-related death among women worldwide. In 2020, 2 261 419 new cases of BC were diagnosed worldwide[1]. In Portugal, BC is the most common cancer and the second cause of cancer-related death. 7 041 new cases were reported, with a total of 27 051 cases recorded in 2020[1,2].

Cancer treatments tend to be aggressive. BC therapies include surgery (tumour excision and node dissection), radiation (external and internal beam) and systemic therapy (chemotherapy, hormonal therapy, and biologic therapy). Unfortunately, these treatments cause complications, which can be: pain, fatigue, injury in the muscle tissues and nerves, anaemia and inflammation[3]. The immune, uterine and musculoskeletal system can also be negatively affected by these treatments[4–7]. The immune system is fundamental, since this system is capable of protecting the organism from developing cancer, through immunosurveillance, a process when immune cells of innate and adaptive immunity participate together to eradicate cancer cells[8]. In fact, in order to cancer to survive, cancer cells take control of the immune system and cause immunosupression[9]. The tumor can create his own inflammatory environment, in order to promote his progression, through cytokines (glycoproteins that function as intercellular messengers) and immune cells that reduce immune response[10]. Therefore, it is important to counterbalance the detrimental effect of therapies in the immune system, so that immunity of BC survivors allows immunosurveillance to prevent or delay patient relapse.

Physical exercise (PE) is recommended for BC survivors, since improve the quality of life, reduce symptoms related to cancer treatments and to disease itself (fatigue), decreases the recurrence of cancer and stimulates the immune system[11–14]. Having said that, PE can be an important ally in the recovery of BC survivors. Therefore, after the end of the treatments, the practice of physical activity should be regular, to BC survivors achieve the beneficial effects of PE. It’s important to mention that, even during treatments, PE should be used as supportive treatment[15,16].

1.1. Breast cancer

BC is one of the diseases that most affects women, being the leading cause of death by cancer in females. In the last 5 years, about 7,8 million women have been diagnosed with this disease, with BC being the most common cancer in the world[17].

Lifestyle, family history, hormonal levels, reproductive factors, and age can contribute to the development of BC. Mutations in several genes (*BRCA1/2, p53, ATM* and *PIK3CA*) can likely to give rise to BC[18].

BC can be divided in several subtypes (Table 1). The distinction is made by histological and/or molecular criteria. Histological BC subtypes can be classified based on hormone and growth factor receptors present or absent in the tumor. These subtypes are hormone receptor – positive (HR^+) if the tumor expresses estrogen and/or progesterone receptors (ER and PR, respectively), receptor tyrosine kinase ($HER2^+$) or triple negative breast cancer (TNBC) if it lacks expression of these three proteins[19]. It is noteworthy that these proteins are the main therapeutic targets used in treatment, either with antiestrogens tamoxifen and fulvestrant or blocking antibodies such as Herceptin/Trastuzumab to inhibit HER-2 signalling. Additionally, aromatase inhibitors such as anastrozole and letrozole are also used to prevent the production of androgens, in order to reduce available estrogen to tumor cells [20].

Table 1 | Molecular classification of the subtypes of BC, their respective biomarkers and treatments. (Adapted from Nascimento et al. (2020)).

Classification	**Luminal A**	**Luminal B**	**HER2-enriched**	**TNBC**
Biomarkers	ER^+ and/or PR^+ $HER2^-$	ER^+ and/or PR^+ $HER2^{+/-}$ Ki67high	ER^- and PR^- $HER2^+$	ER^- and PR^- $HER2^-$
Prognosis	Good	Intermediate	Poor	Worst
Therapies	Endocrine	Endocrine Chemotherapy Target therapy	Chemotherapy Target therapy	Chemotherapy Immunotherapy

The molecular subtypes are classified according to gene expression signatures of the tumours and can be divided in at least 5 major subtypes: Luminal A (ER^+ and/or PR^+ or $HER2^-$); Luminal B (ER^+ and/or PR^+ or $^-/HER2^+$ and Ki67 high); HER2-enriched ($HER2^+$ and ER^- and PR^-), basal-like, claudin-low and metaplastic breast cancer (three subtypes that are histologically TNBC) that present the worst prognosis[21]. Luminal A BC is the most common (40-50% cases diagnosed), Luminal B follows (20-30%), with TNBC being the least frequent (10-20%)[22].

1.2. Immune system

The immune system has a fundamental task in the organism. It's responsible for the defense against strange agents, harmful substances and cellular alterations that may compromise the normal functioning of an organism. Having said that, in the face of all the menaces to which an organism is exposed, it's extremely important that all parts of the body and all cells be constantly monitored. In order for this task to be possible, the immune system consumes a lot of resources, with the production of a large number of cells. All these cells originate from the bone marrow. The activation of this system occurs when it recognizes foreign components in the organism, called antigens (e.g. bacterial proteins or altered proteins resulting from cancer cells)[23,24].

There are two types of immune response: innate and adaptive, with multiple cells with various functions participating in each line of defense. Innate response is very fast, however it's not specific and may not be efficient. In the event that innate immunity cannot cope with the danger present in the body, adaptive immunity comes into play. In adaptive immunity, the response is slower and can last for days. Nevertheless, it's more specialized and effective, and is also able to memorize different types of infection, in case they occur again[25].

Innate immunity is the first line of defense to act, meaning that the cells of this immunity do not require a pre-exposure to an antigen. Once innate immunity is activated, recruitment of immune cells occurs to the necessary site (infection or inflammation)[26]. The cells that participate in this type of immune response are macrophages and dendritic cells (DCs), and their main features are listed in

Table 2. An important function of of macrophages and DCs is their ability to present antigens to T cells, therefore they are called antigen-presenting cells (APCs). In case of infection, these are the first cells to arrive to the location, making contact with the antigens. After this interaction, they move, through the lymphatic vessels, to the secondary lymphatic organs (spleen, tonsils, appendix). The goal is to trigger the adaptive response by presenting the antigens to T lymphocytes. In addition, DCs can also contribute to immune memory, since they can remain in the lymphoid organ[27].

Table 2 | Immune cells from innate immunity and their features and functions[28,29]. (Adapted from Warrington et al. (2011)).

Type of immune cell	Distinguishing features	Function	Lifetime	Image
Neutrophils	Multilobed nucleus, granulated cytoplasm	Phagocytosis, release cytotoxic chemicals from granules	7 hours - Few days	
Eosinophils	Bilobed nucleus, granulated cytoplasm	Antibacteria action, induce inflamation	Few hours	
Basophils	Lobed nucleus, large number of granules	Release of granules, antiparasitic action, induce inflamation	8 - 12 days	
Macrophage	Large cytoplasm, central round nucleus	Phagocytosis, antigen presentation to T cells	Months - to years	
Dendritic cells	Long dendrites	Antigen presentation to T cells	3 days	
Natural Killer cells	Large and round nucleus	Kills tumor cells and virus-infected cells	Less than 10 days	
Mast cells	Oval or irregularly shaped cells, single central nucleus	Realise of histamina and heparine, wound healing, defense against pathogens	Few months	
Monocytes	Nucleus round with kidney shape	Transforms into macrophages or dendritic cells	Hours-few days	

Neutrophils represent 50-70% of the leucocytes circulating in the bloodstream. Phagocytosis is mostly responsible by these cells and are the first to be recruited to the site of inflammation. Eosinophils are also phagocytic, however their circulating percentage in the blood is lower (1-3%). These cells seem to be an important ally in the defense against parasitic organisms. Unlike these cells, Basophils are not phagocytic cells but participate in allergic responses. Their percentage in blood is less than 1%. Also responsible for phagocytosis, we have Monocytes, which are in the blood with a 1-6% percentage. These cells, after leave the bone marrow and enter the blood, circulate for 8h and then migrate to tissues, where they will differentiate into macrophages[31].

In adaptive immunity, multiple specialized cells are found (Figure 1). Cytotoxic T cells (also known as $CD8^+$) possess the function of triggering apoptosis of infected cells, through release of cytotoxic granules and perforin. Helper T cells (also known as $CD4^+$) don't have cytotoxic or phagocytic activity. These cells can stimulate humoral immunity (TH_2 cells) or may stimulate $CD8^+$ cells (TH_1 cells). Regulatory T cells are important as they limit or supress the activity of $CD8^+$ and TH cells[23,30]. It's important to note that, after activation of the adaptive response, innate immunity works in conjunction with adaptive immunity. Specialized T cells attract cells such as macrophages and neutrophils to site of infection for better combat[24].

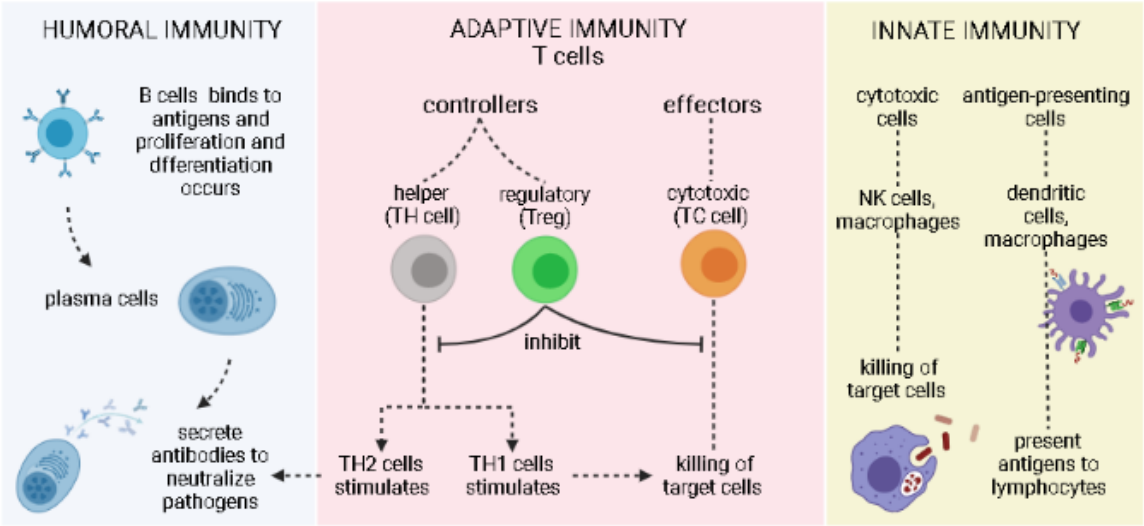


Figure 1 | Types of immune responses and their respective cells. (Adapted from Weinberg, 2014).

Within adaptive immunity, the humoral immune response is a crucial defense strategy, where antibodies are produced by B cells. Once B cells are activated by antigens, these cells are going to differentiate into memory B cells or antibody-secreting plasma cells[32]. Antibodies are very specific and have the ability to recognize and bind to antigens. Once bound to infected cells, virus or bacterias, the target is destroyed by macrophages or T cytotoxic cells. Cytotoxic T cells are not able, by themselves, to identify the antigen, so the antibodies play a important role in the bridge between innate and adaptive response. The antibodies bind to the antigens and recruit immune cells[23].

1.3. Immunosurveillance

In 1909, the scientist Paul Erlich formulated the theory that the immune system could prevent the formation of cancer. However, at that time, the theory was not proven. Later, Lewis Thomas and Frank Burnet reformulated that hypothesis in the 50s and presented the concept of “immunological surveillance mechanism”. These biologists suggested that the immune system can detect malignant cells through specific tumor antigens[33].

Immunological surveillance is a rudimentary component of an organism, as it guarantees only the proliferation of healthy and normal cells. This constant surveillance allows the identification of cancer cells and/or pre-carcinogenic cells and their elimination before imbalances occur in the body. The immune system can act in many ways to prevent the development of cancer such as: extinction of viral infection; inhibiting the inflammatory environment that is known to promote carcinogenesis, and constant surveillance of cells, allowing the recognition and elimination of malignant cells. Cancer cells have tumor specific antigens, which makes it possible to identify many of these cells[34]. Multiple immune cells participate in the immune surveillance process and help to prevent the development and growth of cancer (Table 3).

Table 3 | Immune cells and their action against cancer**.**

Type of cell	Mode of action
$CD4^+$ T cells (TH_1)	Help $CD8^+$ T cells in tissue destruction and tumor rejection
$CD8^+$ T cells	Tumor destruction
Natural Killer (NK) cells	Lyse MHC class I-deficient tumor cells
M1 macrophages	Production of nitric oxide Antigen presenting cell (to activate cytotoxic $CD8^+$ T)

1.4. Immunoediting and carcinogenesis

The immune system is not always fool proof in detecting and destroying cancer cells. Some cancer cells can escape immune surveillance. This process is known as immunoediting, which can be divided into three stages: elimination, equilibrium, and escape (Figure 2). This concept of immunoediting was created by Gavin Dunn and Robert Schreiber in 2002[33] and since then research to understand its mechanisms has been intensified.

The first stage elimination corresponds to immunological surveillance, where the immune system is always on alert, so that it's possible to recognize malignant cells and destroy them. Although, if these cells are not all extinct, the second stage of immunoediting is carried out[34].

In the second stage, equilibrium, residual malignant cells persist in the body. However, the immune system exercises control over these cells so that they don't develop and there's no incident of metastases. This stage can have two resolutions: either the immune system can control cancer cells and stop cancer progression, or the cancer will continue to evolve. Faced with the situation of not being able to control the ungoverned division of malignant cells, despite this continuous effort, the immune system fails at this sage and the most resistant cancer cells will escape[34].

In the last stage, cancer cells will increase in number. These cells will continue to progress and at this stage it's possible to detect cancer[34]. The scape stage requires the cancer cells to edit their phenotype to supress the immune surveillance – elimination response as well as to recruit immune cells that will stimulate tumour growth.

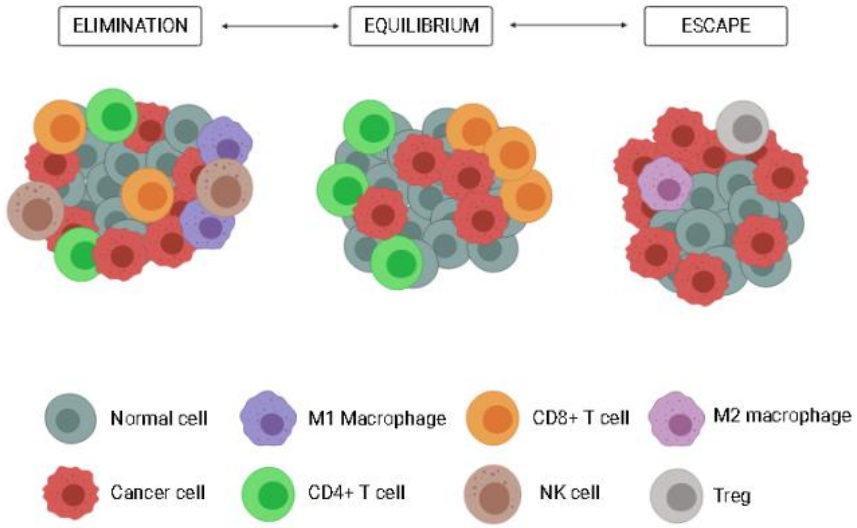


Figure 2 | Stages of immunoediting. In the elimination stage, immune cells are recruite to destroy cancer cells. M1-like macrophage is an innate immune cell with pro-inflammatory functions. These cells promote the inflammatory response, by recruiting more immune cells to the site such as NK cells. Also, is responsible to activate and recruite immune cells of adaptative response ($CD4^+$ and $CD8^+$ T cells and T_{reg}). In the equilibrium phase, the immune cells can control the cancer cells, however if that control it's not enough, the more resistant cancer cells will escape. In the last stage, immune cells are recruited by cancer cells, to promote their growth. M2-like macrophages, (also known as Tumor-associated macrophages, TAMs), can promote angiogenesis and metastasis, since those cells have pro-tumor functions. Also, T_{reg} can block the activity of $CD8^+$ T cells, therefore inhibiting tumor suppression.

1.5. Inflammation and carcinogenesis

Inflammation is a physiological response, which can be activated by various stimuli (e.g., infection, tissue damage or oxidate stress). Inflammation is responsible for the defense against pathogens, but also damages in the tissues. After activation of the inflammatory response, there's the recruitment of immune cells to the indicated site. In the case of acute inflammation, this process has a limited period of action and is sufficient to restore homeostasis. However, if the inflammation-causing agent is not eliminated and inflammation becomes chronic[35]. Chronic inflammation is persistent and can cause serious damage to the body such as compromise of the immune system, development of infections and tumor and metabolic syndrome. The origins of chronic inflammation can also be associated chronic infections, lack of physical activity and lifestyle[36].

Worldwide, 25% of cancer cases are due to chronic infection and inflammation. The persistence of an inflammatory environment, which contains cytokines, reactive oxygen species (ROS) and growth factors can lead to the initiation and progression of cancer[37].

Cancer cells have the ability to induce and prolong an inflammatory (chronic) state because they can recruit immune cells (neutrophils, DCs, macrophages, eosinophils and lymphocytes) into the tumor microenvironment (TME) through production of cytokines. Subsequently, these immune cells can block the cytotoxic response of T cells and stimulate angiogenesis, so that cancer progression occurs, blocking anti-tumor immunity[38]. Also, the production of ROS by macrophages and neutrophils in the TME can lead to mutations in the parenchymal cells which increases the DNA damage and mutation load of cancer cells and thus contributes to genomic instability and cancer progression[23,39].

1.6. Metabolism, inflammation and immune system

All organisms need energy to maintain all physiological and cellular process by a process called metabolism. Metabolism is a group of chemical reactions where food is converted in energy. Besides his role of storing energy, the white adipose tissue (WAT) is an endocrine organ and has an important task in the homeostasis. WAT can produce cytokines, hormones, acute phase proteins and growth factors[40]. Immune cells also depend on metabolic process for activation, differentiation, proliferation and to perform their functions[41,42]. Obesity can interfere with the normal function of the immune system[43].

Obesity (excess of WAT) is a chronic disease that can compromise the normal functioning of the organism, including the metabolism and immune system. This disease cause complications referred as metabolic syndrome such as hypertension, insulin resistance, diabetes and hyperglycemia and high levels of triglycerides. This condition is responsible for promoting an inflammatory state in the organism, through increase of cytokines such as Tumor necrosis factor alfa (TNF-α) and Interleukin 6 (IL-6)[44–47]. Furthermore, obesity is a risk factor for development and recurrence of BC. This condition is associated with resistance to endocrine therapy[48].

1.7. Cytokines

Cytokines are proteins or glycoproteins, with a molecular weight less than 30 kDa (<200 amino acids). They're produced by various types of cells, mainly by T_h

cells, DCs and macrophages. The name cytokine is general and there are 200 cytokines known to date which are grouped into different groups or families (i.e. interferons, tumour necrosis factors, among others)[49,50].

These proteins can be pro- and anti-inflammatory. Pro-inflammatory cytokines promote the inflammatory and immune response[51], whereas anti-inflammatory cytokines are responsible for the control of the immune and inflammation response, in a negative way, meaning that these can inhibit other cytokines and immune cells. It's important to mention that cytokines, taking into account their properties, are very important targets in the treatments of diseases[52].

Cytokines are pleiotropic, that is, they can cause multiple effects on the same target and may stimulate or inhibit the production/activity of another cytokine. Also, they have redundant activity since many cytokines can perform similar functions. These glycoproteins may act in multiple ways: on the cells that secrete them (autocrine action), on nearby cells (paracrine action) and on distant cells (endocrine action)[53]. Therefore, cytokines function as intercellular messenger to exert a certain effect on a cell and influence biological activities. This effect is achieved by binding to receptors present in the membrane of target cells. Once the connection is made, intracellular signals will interfere with the expression of proteins, which results in a specific biological response[54].

Induction of an inflammatory response, regulation of haematopoiesis, wound healing, control of cell proliferation and onset of immune and humoral response are some examples of the effects caused by cytokines[28].

Cytokines play here an important role in the regulation of the immune system by allowing immune cells to communicate between them and to initiate the defence response. Therefore, cytokines play a pivotal role in preventing the formation and progression of cancer. In the following sections, the main characteristics related to the cytokines studied in this work will be described.

1.7.1. Interleukin 1β (IL-1β)

The IL-1 cytokine family consists of 11 members, including pro-inflammatory cytokine IL-1β[55]. This cytokine can be produced by macrophages and monocytes

in inactive form that subsequently requires cleavage to be activated and secreted[56].

IL-1β production and release can be triggered by various factors. The presence of antigens can elevate the levels of this cytokine to act as pyrogen[51], therefore stimulating inflammation and immune response. Also, the presence of PAMPs (Pathogen associated molecular patterns) stimulates the production of these cytokine. The levels of IL-1β may differ according to type cell that produces it[57].

This cytokine is important in the defense of the organism, since it stimulates inflammation[58]. Also, is responsible for the activation of phagocytic cells, which are required in the defense against microbes and bacteria[59].

1.7.2. Interleukin 2 (IL-2)

IL-2 is a pro-inflammatory cytokine. The source of this cytokine is in the secondary lymphoid organs, being produced mainly by $CD4^+$ T helper cells. However, it can also be produced by other cells (DCs and mast cells) but at low concentrations [60].

$CD8^+$ T cells through autocrine action, release IL-2 and stimulate their own expansion and cytotoxicity. In the thymus, this cytokine is responsible for the initiation of naive T cell proliferation and maturation of T_{reg}. IL-2 is fundamental to maintain the homeostasis of T_{reg}, since deregulation of these cells can lead to autoimmunity. NK cells are also dependent of IL-2 for their growth and activation. These cells can produce IL-2, to support autocrine signalling or can be activated by IL-2 produced by T cells or DCs [61].

Antigens can trigger the production and secretion of IL-2, to be possible to initiate and promote an immune and inflammatory response. As well when memory T cells meet again one antigen, IL-2 production is triggered to promote the expansion of these cells, to rapidly erratic the foreign agent. During chronic infections, the levels of this cytokine are also elevated[62].

1.7.3. Interleukin 4 (IL-4)

Consisting of 129 amino acids and with molecular weight 18 kDa, IL-4 is an anti-inflammatory cytokine and is produced by $CD4^+$ T cells (TH_2), basophils, mast cells and eosinophils[50,63].

Activation and differentiation of naive T cells is possible through IL-4, influencing these cells to produce IL-4 (autocrine stimulation). This cytokine also has a strong influence under the specificity of immunoglobulins. In B cells, IL-4 is responsible for the transformation into IgE and IgG4[63]. Not only does this cytokine influence lymphocytes (inhibition of TH_1 cells) but also influence the proliferation, apoptosis and gene expression in macrophages, fibroblasts, endothelial and epithelial cells. This cytokine can also stimulate the polarization of macrophages to M2 (anti-inflammatory phenotype). So, in situations where a immune response starts being prejudicial, high levels of IL-4 occurs in order to attenuate the immune and inflammation response[64].

1.7.4. Interleukin 6 (IL-6)

IL-6 is formed by a polypeptide chain of 184 amino acids and has broad functions. This cytokine was first discovered as B-cell stimulatory factor. This cytokine is produced by a various number of cells, causing multiple effects. In acute inflammation, immune cells (monocytes and macrophages) produce IL-6, with the aim of recruiting neutrophils to the site. This cytokine can prolong the lifetime of neutrophils. Also, IL-6 is responsible for transforming B cells into plasma cells (antibody producers)[65].

In the immune cell production chain, IL-6 plays an important role, since it can stimulate haematopoiesis. In the defense of the organism, IL-6 ensures the production of proteins of the acute phase (C-reactive protein (CRP), fibrinogen, amyloid A serum AA, among others). Also this cytokine may act as a pyrogen, so can cause fever[66].

IL-6 production can be initiated by various stimulus: foreign pathogens, infections, tissue lesions and pro-apoptotic cells. It’s natural that, in these conditions, IL-6 levels rapidly elevate. However, if IL-6 levels persists for a long period, negative effects can occurs, which can lead to diseases[67].

1.7.5. Interleukin 10 (IL-10)

With strong anti-inflammatory properties, IL-10 is produced by macrophages, DCs, B cells and $CD4^+$ and $CD8^+$ T cells. It's a fundamental cytokine in regulating innate and adaptive immunity. This cytokine functions as the key regulator of the duration and intensity of immune and inflammatory response [68].

The function of IL-10 is the suppression of the immune response. Therefore, IL-10 can have multiple immune cells under control. In DCs, through the autocrine action, the production of chemokines is inhibited, so that the dislocation of DCs to lymph nodes does not occur. Thus, there's no activation or differentiation of naive T cells. In $CD4^+$ (specifically TH_1 cells) and $CD8^+$, IL-10 can mitigate the effects of these cells, as they can cause excessive effects during an infection. In $CD4^+$ T cells, IL-10 may also have direct action on these cells so that they don't produce cytokines (IL-2, IL-4, IL-5, IFN-γ and TNF-α). Since IL-10 is important to mitigate and prevent that inflammatory response provokes damage in the tissues and pathologies, in these conditions, IL-10 levels are elevated[68].

1.7.6. Interferon gamma (IFN-γ)

This molecule belongs to a group of molecules that "interfere" with pathogens and with a viral infection and are called interferons. The production of IFN-γ is triggered by mitogens and cytokines (IL-2, IL-5 and IL-18), so levels of this glycoprotein is expected to be elevated in conditions of infections and inflammation. With molecular weight 50 kDa, this cytokine is mainly produced by TH_1 cells, cytotoxic $CD8^+$ T cells and NK cells. NKT cells, B cells and APCs can also produce this glycoprotein[69].

IFN-γ, being a pro-inflammatory cytokine, stimulate inflammatory responses, therefore can activate multiple immune cells. In adaptive immunity, TH_1 cells produce large amounts of IFN-γ, so that there's a continuous population of cells of this type. Thus, IFN-γ inhibits the production of other cells such as TH_2 and directly inhibits the production of IL-4. IFN-γ also inhibited T_{reg} cell differentiation. The proliferation of cytotoxic T cell precursors is induced by IFN-γ and subsequently activates cytotoxic $CD8^+$ T cells [69]. In innate immunity, IFN-

γ activates macrophages, so that they acquire a pro-inflammatory phenotype, in order to cause an increase in their phagocytosis capacity. NK cells are also activated by IFN-γ[70].

1.7.7. Tumor necrosis factor alfa (TNF-α)

TNF-α was identified in 1975, when it was discovered that this cytokine can induce necrosis of tumors through the action of endotoxins[71]. This cytokine is made up of 157 aminoacids (17 kDa) and it's a potent pro-inflammatory cytokine. TNF-α plays an important role in the inflammation process, since through the production of other pro-inflammatory cytokines, it activates the inflammatory response. In addition, TNF-α can activate and recruit cells (e.g., macrophages) to the site of inflammation. This cytokine can be produced by various immune cells, mainly macrophages[72,73].

TNF-α plays a role in initiation fever, production of prostaglandins and acute phase proteins. Also, it's responsible for increase the bloodstream in the site of inflammation, through activation of endothelial cells[74].

1.8. Breast cancer and cytokines

Cytokines can play an important role in cancer, as these can stimulate the growth of cancer cell populations and, more dangerously, metastases. BC cells can synthesize and secrete a variety of cytokines to promote autocrine and paracrine signalling. The effect of most commonly studied cytokines in the progression of breast cancer is shown in Table 4.

BC treatments can negatively affect the organism of the patients, including altering the normal levels of cytokines. This dysregulation of cytokines levels depends on multiple factors: type, dose and duration of treatment. For example, radiotherapy can increase the levels of cytokines, therefore these glycoproteins can be used as biomarkers of the development of the disease[75].

Table 4 | Cytokine and their roles in BC.

Cytokine	Type of cytokine	Effect	Cells type mediating the effect	References
IL-1β	Pro-inflammatory	BC bone metastasis	Cancer cells	[76]
IL-2	Pro-inflammatory	Inhibit anti tumor immunity	Cancer cells	[77]
IL-4	Anti-inflammatory	Survival of cancer cells (induction of expression of Bcl-2)	Cancer cells	[78]
		Expasion of MDSCs	MDSCs	[79]
		Estimulate the tumor-promoting activity of macrophages	TH_2	[80]
IL-6	Pro-inflammatory	Increases VEGF in tumor cells (angiogenesis)	Tumor-infiltrating lymphocytes	[81]
		Promote tumor metastasis	TAMs	[82]
		Polarization to M2 macrophages	Cancer cells/TAMs	[82]
IL-10	Anti-inflammatory	Apoptosis of $CD8^+$ T cells	TAMs	[80]
		Suppression of adaptive immune responses	Cancer cells/TAMs	[82]

IFN-γ	Pro-inflammatory	Tumor-specific antigen loss, induction of a more malignant phenotype in cancer cells	NK cells, TH_1	[83]
		$CD8^+$ T cells apoptosis	Cancer cells	[84]
TNF-α	Pro-inflammatory	Increases VEGF in tumor cells (angiogenesis)	Tumor-infiltrating lymphocytes	[81]
		Increase inflammation in TME	M1 macrophages	[85]

Legend: TAMs – tumor-associated macrophages; **NK cells** – natural killer cells; **VEGF** – vascular endothelial growth factor.

1.9. Physical exercise and health

PE can be defined as any movement performed by skeletal muscles where energy is spent. However, exercise training has a different definition: it's a subcategory of PE, because it's planned, structured and repetitive[86].

Regular exercise comes with many benefits. PE reduces the risk of chronic diseases such as diabetes, cardiovascular diseases, cancer and hypertension. Therefore, increases the quality of life, contributes to the feeling of well-being and increases muscle strength[87]. In 2016, more than 25% of the global population did not exercise enough[88]. Having said that, physical inactivity is the fourth risk factor of mortality, worlwide[89].

1.10. Impact of physical exercise on breast cancer survivors

As mentioned above, oncologic treatments cause undesired alterations in the organism of patients, even after the end of treatments (Table 4). BC survivors suffer from sequeale, which can last for years. In this present study, the most used treatments were surgery, hormonotherapy, chemotherapy, radiotherapy and

biological therapy (monoclonal antibodies – trastuzumab and pertuzumab), all of which have side effects.

Table 5 | Consequences of BC treatments[90–92].

Cancer treatment	Sife effects during treatment	Side effects after treatment
Surgery	Lymphedema, pain, tissue injuries	Reduced arm and shoulder mobility, pain, lymphedema
Hormonotherapy (or endocrine therapy)	Weight gain, menopause symptoms, vaginal dryness	Osteoporosis, blood clots
Chemotherapy	Alopecia, weight gain, fatigue, nausea, infections, reduced number of white blood cells, cardiotoxicity	Cardiotoxicity, ischemia, arrhythmias, amenorrhea or menopause, problems in short-term memory, ovarian failure
Radiotherapy	Irritation, fatigue	Reduced arm and shoulder mobility, lymphedema, damage in the heart
Biological therapy (monoclonal antibodies)	Similar to influenza, cardiotoxicity	Cardiotoxicity

However, weight loss can also occur. Cachexia is a consequence of cancer and cancer treatments where loss of adipose tissue and lean body mass (LMB) occurs[93]. Cancer treatments also significantly alter the metabolism. The levels of triglycerides, total cholesterol, LDL cholesterol can suffer an increase and HDL a decrease. CRP, glucose and insulin levels can also increase[94–97].These consequences and with the addition of lower or inactivity of physical activity can negatively affect the quality of life.

Cancer rehabilitation is a process that helps BC survivors recover important aspects of daily life. That is, the goal is to improve the quality of life of survivors of BC[98]. PE can be an important factor in the recovery, since it can improve fatigue, chronic inflammation, depression, immune function, bone loss and reduce the risk of BC recurrence[99,100]. Also, PE can improve lipid profile, through a reducing of triglycerides and LDL cholesterol[101].

There are two types of exercise: aerobic and resistance. Aerobic training (AT) may have a low or high intensity, which requires aerobic metabolism. This type of exercise improves the cardiovascular and musculoskeletal system and lipid profile. AT also has a positive influence on mental health. Squats, abdominal leg raise, dance, walks and cycling are some examples of AT activities[102,103]. Resistance training (RT) can increase muscle mass and bone mineral density and improve the cardiovascular system. Also, reduce body fat and the risk of development type 2 diabetes[104].

It's fundamental to study the beneficial effects of PE in BC survivors, to know what the correct type, duration and frequency of PE that BC survivors must address to achieve better quality of life. However, the role of PE in the inflammatory and immunity profile of BC survivors are still controversial. Previous reviews showed that combined exercise (AT + RT) is the one that more positive outcome have on BC survivors, such as decrease of pro-inflammatory cytokine profile, reduced inflammation, increased musculoskeletal strength and bone mineral density. However, no significant alterations were observed in immune cells[105,106]. Nevertheless, only one exercise type had different results. Studies with AT showed no significant alterations on pro-inflammatory cytokines[107], where RT intervention showed significant reduction of pro-inflammatory cytokine (TNF-α) produced by immune cells and improve muscle strength[108,109].

CHAPTER II
AIMS AND OBJECTIVES

2. Aims and objectives

BC survivors, due to cancer treatments, suffer from sequelae, which can negatively affect quality of life. These survivors have a higher risk of developing cardiovascular diseases and those treatments can interfere with the metabolism. Since metabolism is important to produced energy to be used by other cells (including immune cells), this process can be dysregulated. As a consequence of their treatment, BC survivors suffer from inflammation and immune system dysfunctions. Exercise can be an ally in the recovery of BC survivors, since it can improve many aspects of BC survivors' daily life. Having said that, the objectives of this work were to analyse the serum samples from BC survivors undergoing an adapted PE program in order to evaluate how PE can influence the cytokine levels and immune cell numbers in serum and infer a possible effect on systemic immunity. For this purpose, the samples obtained from the MAMA MOVE GAIA AFTER TREATMENT clinical trial (NCT04024280) were used to:

1) To quantify the changes induced by 8-week PE in IL-1β, IL-2, IL-4, IL-6, IL-10, IFN-γ and TNF-α.
2) To analyse changes in immune cell number in BC survivors after 8-week PE.
3) To correlate the cytokine and immune cell changes induced by PE.
4) To characterize the effect of PE on glycemia, lipidemia and serum estrogen.
5) To correlate changes induced by PE metabolic markers with changes in cytokine and immune cells.

CHAPTER III
MATERIAL AND METHODS

3. Material and Methods

3.1. Study design

This protocol describes a run-in control period followed by intervention clinical trial on breast cancer survivors, designed to test the effects of a supervised and adapted physical exercise program that combines muscle strength and aerobic training (RT and AT, respectively) with progressive intensity, with a duration of 16 weeks and frequency of 3 training sessions per week. In this study, the impact of PE on the level of cytokines, leukocytes and clinical pathology will be investigated on breast cancer survivors after 8 weeks of PE. The study design is described in Figure 3.

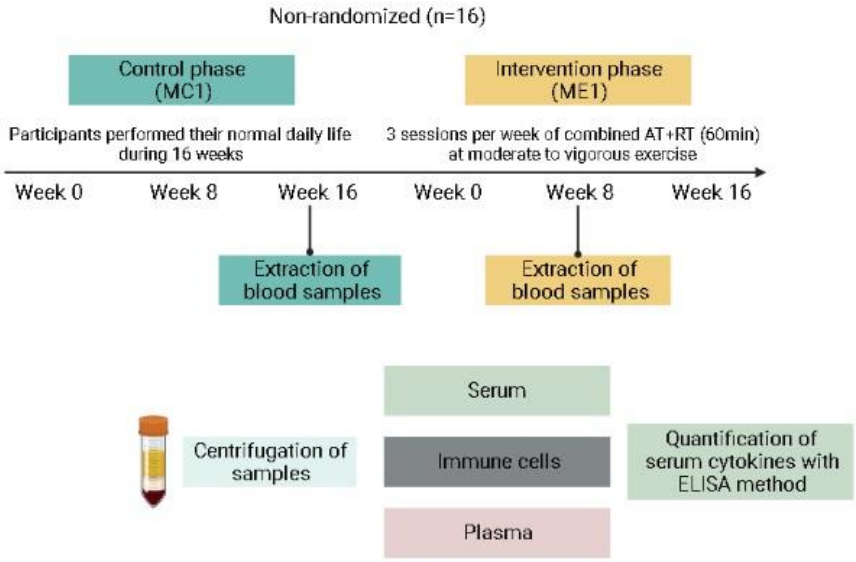


Figure 3 | Study design of the program MMGAT, were quantification of cytokines and analysis of immune cells and metabolic parameters occurred.

This study was approved approved by the Ethics Committee of the Centro Hospitalar de Vila Nova de Gaia/Espinho (CHVNGE; Vila Nova de Gaia, Portugal) (reference number: 145/2018-1). This study is registered in Clinical Trials (NCT04024280). Each change in the protocol was submitted for approval by the CHVG/E ethics committee.

3.2. Characteristics of participants

Eighty breast cancer survivors were recruited for the clinical trial, 16 of which were included in this present study. The admission of participants to the study followed the criteria presented in Table 6. The characteristics of the participant cohort are presented in Table 7. LDL cholesterol was calculated for each participant according to[110].

Table 6 | Summary of inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Histological diagnosis of breast carcinoma	Severe anaemia (Hb ≤ 8 g/dL)
Stages 0 to IIIC	Symptomatic moderate anaemia (Hb >8 and ≤ 10 g/dL); considered symptoms are: sustained tachycardia, exertion dyspnoea, thoracic pain or syncope
Having undergone primary treatment with curative intent, defined as surgery that can have been complemented with neoadjuvant, or adjuvant, chemotherapy and/or radiotherapy	Uncontrolled hypertension
Conclusion of the last of the following treatments at least one month before: surgery, chemotherapy or radiotherapy	Uncontrolled diabetes
At least one consultation in the Medical Oncology Department of the Centro Hospitalar de Vila Nova de Gaia/Espinho	Cardiac failure grade >1 in the New York Heart Association evaluation
Assistant medical oncologist consent for the physical exercise practice	History of osteoporosis with Tscore <-2.5 in the lumbar spine and/or femur in the menopause
Not meeting the physical activity guidelines of the American College of Sports Medicine (moderate activity ≥ 150 minutes/week or vigorous activity ≥ 75 minutes/week and ≥ 2 resistance training/week).	Contraindication given by the assistant surgeon

Table 7 | Characteristics of the 16 study subjects.

ID	Age (years)	BMI (kg/m^2)	Disease stage at diagnosis	Histology	Therapy	HT	Type of surgery
26	59	28,8	III	LIC	RT+QT	Letrozole	MT+AD
28	68	33,5	II	NST	RT+QT	Letrozole	ET+SN
29	56	28,6	0	DC	RT	Tamoxifen	ET+SN
35	64	37,3	II	NST	RT+QT	Anastrozole	MT+AD
39	55	25,3	II	NST	RT+QT	Letrozole	MT+AD
41	58	38,1	0	DC	RT	Tamoxifen	ET+SN
42	55	31,7	IV	NST	RT+QT	Tamoxifen	MT+AD
43	50	36,3	I	NST	RT	Tamoxifen	ET+SN
47	56	24,5	I	NST	-	Tamoxifen	MT+AD
52	72	31,9	I	NST	RT	Letrozole	ET+SN
55	48	32,9	III	NST	RT+QT	Tamoxifen	ET+SN
57	69	24,9	I	NST	RT	Letrozole	ET+SN
58	63	34,2	I	NST	RT+QT	Anastrozole	ET+SN
60	73	28,3	II	NST	RT+QT	Letrozole	ET+SN
64	73	22,6	I	NST	-	Letrozole	MT+AD
66	44	26,9	II	NST	RT+QT	Tamoxifen	MT+AD
Mean ± SD (n=16)	60,18 ± 8,79	30,26 ± 4,67	I (37,5%) II (31,2%) III (18,7%) 0 (12,5%) IV (6,2%)	LIC (6,25%) DC (12,5%) NST (81,2%)	RT+QT (56,2%) RT (31,2%)	Letrozole (43,7%) Tamoxifen (43,7%) Anastrozole (12,5%)	MT+AD (43,7%) ET+SN (56,2%)

Legend: LIC (Lobular invasive carcinoma); NST (invasive carcinoma of no specific type); DC (Ductal carcinoma in situ); HT (hormonotherapy), RT (radiotherapy), CT (chemotherapy), MT (mastectomy), AD (axillary dissection), ET (enlarged tumorectomy), SN (sentinel nodes search).

3.3. Exercise intervention

Participants performed a supervised physical exercise program specifically developed for BC patients, based on the guidelines of the American College of Sports Medicine[111]. The program consisted of 3 sessions per week of physical exercise. In the first and second week, aerobic training lasted 10 minutes and, every two weeks, two more minutes were added. In aerobic sessions, walking and stepping were performed. The intensity of aerobic training was 12-17 in the Borg scale. In the first week, resistance sessions lasted 30 minutes and without load then, 15 submaximal repetitions. Using free weights, the lower body exercises were squat, leg extension, leg curl and calf raise; the upper body

exercises were frontal and lateral arm raise, chest press, seated row and bicep curl.

The participants were serially evaluated. Each participant passed through 2 evaluation moments: at baseline (M0), at 16 weeks before beginning the study (MC1), at 8 weeks of physical exercise (ME1) and 16 weeks of physical exercise at the end of the program (MC2).

3.4. Blood samples

Blood samples were collected from the participants at 16 weeks before the beginning the study (MC1) and at 8 weeks of physical exercise (ME1) at CHVGE. The immune cells, plasma and serum were separated by centrifugation. Both serum and plasma samples were stored at -80ºC.

3.5. Immunoassays

Serum cytokines were quantified using the sandwich Enzyme-Linked Immunosorbent Assay (ELISA; Peprotech Laboratories Inc., Rocky Will, New Jersey, USA). The following cytokines were selected based on their pro- and anti-inflammatory effects and quantified individually: IL-1β, IL-2, IL-4, IL-6, IL-10, IFN-γ e TNF-α (Table 8 and 9).

The ELISA assays were carried out according to the manufacture's protocol. Briefly, antibodies and standards were reconstituted with sterile water. The ELISA set up was done in a 96 well-plate (Greiner 96 Flat Bottom Transparent Polystyrene Cat. No.: 655101/655161/655192), then the capture antibody (50µL/well) was incubated in a plate sealed with aluminium foil, overnight at room temperature. The following day, the liquid was removed, and the plate washed 4x with 50µL/well wash buffer solution (0,05% Tween-20 in PBS). After washing, blocking buffer (1% BSA in PBS) was added (50µL/well), the plate was sealed and incubated for 1 hour. Next, the plate was washed 4x with wash buffer, followed by addition of the standard curve samples (50µL/well) and serum samples (50 µL/well). The plate was covered and incubated for 2-4 hours depending on the cytokine. Then, the liquid was removed, and plates washed 4x. The detection antibody was added (50µL/well) and was incubated for 2 hours in the sealed plate at room temperature. Then, the liquid was removed, and wells washed 4x. The avidin-HRP conjugate or streptavidin-HRP conjugate was added

(50µL/well) and incubated for 30 minutes. The plate was washed 4x and the TMB liquid substrate was added (50µL/well). Stop solution was added to prevent over-development and colour saturation. The plate was read on the Tecan i-control Infinite 200. All sample were analysed in technical triplicates in the same plate.

Table 8 | Reagents included in each of ELISA kits.

Analyte	Reagent	Description	Catalogue#	Lot#
IL-1β	Capture	Mouse anti-human IL-1β	900-TM95	0615T095-M
	Standard	Recombinant human IL-1β		
	Detection	Biotinylated goat anti-human IL-1β		
IL-6	Capture	Rabbit anti-human IL-6	900-M16	0618016-M
	Standard	Recombinant human IL-6		
	Detection	Biotinylated goat anti-human IL-6		
IL-2	Capture	Goat anti-human IL-2	900-M12	0613012-M
	Standard	Recombinant Human IL-2		
	Detection	Biotinylated goat Anti-human IL-2		
IL-4	Capture	Rabbit anti-human IL-4	900-M14	1019014-M
	Standard	Recombinant anti-human IL-4		
	Detection	Biotinylated rabbit anti-human IL-4		
IL-10	Capture	Rabbit anti-human IL-10	900-M21	1215021-M
	Standard	Recombinant human IL-10		
	Detection	Biotinylated rabbit anti-human IL-10		
IFN-γ	Capture	Rabbit anti-human IFN-γ	900-M27	1116027-M
	Standard	Recombinant anti-human IFN-γ		
	Detection	Biotinylated rabbit anti-human IFN-γ		
TNF-α	Capture	Rabbit anti-human TNF-α	900-M25	1017025-M
	Standard	Recombinant human TNF-α		
	Detection	Biotinylated rabbit anti-human TNF-α		

Table 9 | Concentration of antibodies and the respective range of each ELISA kit.

Cytokine	Capture antibody concentration (µg/ml)	Detection antibody concentration (µg/ml)	Standard curve range (pg/mL)
IL-1β	0,25	0,25	6-750
IL-2	2,0	0,25	63-4000

IL-4	0,50	0,50	16-1000
IL-6	0,50	1,0	16-2000
IL-10	1,0	0,50	23-3000
IFN-γ	1,0	0,5	8-3000
TNF-α	1,0	0,50	23-3000

3.6. Data analysis

The quantification of the cytokines was performed using a standard curve and values were analysed using the GraphPad Prism 5.01 software. To evaluate the effect of the intervention on the levels of cytokines in each individual and per group, Wilcoxon *t-tests for paired samples was* used and $p<0.05$ was considered significant. Spearman correlation analysis between variables was performed using the online tool MetaboAnalyst 5.0 setting a minimum correlation coefficient $r=0.5$ and $p<0.05$.

CHAPTER IV
RESULTS

4. Results

The impact of the MMGAT PE intervention program on the levels of cytokines was evaluated using the ELISA method. Data corresponding to immune cell frequencies and clinical pathology was obtained from Germano de Sousa Labs. IL-1β was not possible to quantify, since all samples were below the level of detection. Due to small sample size of ID47, only one cytokine was quantified.11

The data was analysed using unvaried methods (quantitative analysis). However, due to the reduced number of samples, a qualitative description was also performed [112].

4.1. Analysis of serum cytokines

4.1.1. Interleukin 2

The mean IL-2 baseline values for the participants of this study (MC1) were (70.5 ± 47.7 pg/mL), which is within the normal IL-2 range in healthy pos-menopausal women 66.7-128.7 pg/mL[113]. The levels remained in the normal range after the intervention (ME1) and no significant changes were observed in IL-2 levels after exercise training (Table 10).

Table 10 | Effect of 8 weeks MMGAT program on serum IL-2 levels.

	MC1	**ME1**
n	15	15
Median	67.9	49.6
25% Percentile	31.7	26.2
Mean ± SD	70.5 ± 47.7	103.6 ± 168.8
SD error	12.3	44.6
Lower 95% CI	44.1	10.1
75% Percentile	95.18	124.5
One-tailed Wilcoxon test	0.2444	

The median values at ME1 (49.6 pg/mL) were lower than at MC1 (67.9 pg/mL) which is indicative that at least half of the participants showed a reduction of IL-2.

Qualitatively, the differences between median values were confirmed since 11 participants showed lower IL-2 after the intervention (7 participants showing significant changes at the individual level; Table 11).

Normal baseline IL-2 levels according to Cioffi et al. (2002) were only verified in 8 participants with 5 of them (IDs: 26, 28, 35, 42, 43) maintaining normal levels after exercise, and 3 (IDs: 55, 57, 66) experienced a decreased to levels below normal. None of the participants starting at baseline with abnormal levels normalized after the intervention. Therefore, the intervention did not improve IL-2 levels in the participants that showed abnormal values at MC1.

Table 11 | IL-2 levels (mean ± SD) in serum of breast cancer survivors before (MC1) and after (ME1) 8 weeks of MMGAT program.

ID	MC1		ME1		
	Mean	**SD**	**Mean**	**SD**	***p. value***
26	**69.75**	**21.50**	**94.55**	**10.50**	**<0.0001**
28	**95.18**	**9.61**	**84.11**	**3.60**	**<0.0001**
29	25.14	14.26	15.03	18.16	0.1561
35	109.6	18.86	132.6	21.24	0.2000
39	**18.86**	**4.11**	**10.26**	**2.39**	**<0.0001**
41	**41.96**	**5.05**	**30.60**	**14.77**	**<0.0001**
42	67.86	20.16	130.7	13.75	0.1000
43	**132.7**	**14.25**	**124.5**	**0.51**	**<0.0001**
52	**31.73**	**2.92**	**22.55**	**17.47**	**<0.0001**
55	62.39	9.42	49.58	10.28	0.2000
57	**90.31**	**5.82**	**56.92**	**0.29**	**<0.0001**
58	**186.3**	**88.95**	**38.47**	**23.30**	**<0.0001**
60	**5.67**	**2.11**	**694.2**	**247.9**	**0.0296**
64	43.68	21.89	26.16	2.75	0.4000
66	76.73	13.08	43.47	1.45	0.1000

Note: bold – statistically significant.

4.1.2. Interleukin 4

The IL-4 range in healthy pos-menopausal women is 11.3-22.5 pg/mL[113] and the mean values for all participants at baseline were above this range (91.8 ± 219.8). No significant changes were observed in IL-4 levels after exercise training (Table 12). The large standard deviation (SD) was noted for MC1, which is due mainly to the levels of ID43, still, even if this participant was excluded from the analysis, the differences in IL-4 at MC1 and ME1 were not significant (Table 13).

At the individual level, only 5 participants showed IL-4 levels at MC1 within the normal values. Out of the 10 participants with abnormally high IL-4 at MC1, 5 had a reduction to normal levels or levels close to normal (ID29, ID35, ID42, ID52 and ID66). Therefore, while the intervention did not significantly improve IL-4 values, qualitatively it could be associated to the return to normal of 5 participants (Table 14).

Table 12 | Effect of 8 weeks MMGAT program on serum IL-4 levels.

	MC1	**ME1**
n	15	15
Median	31	27.1
25% Percentile	20.8	17.1
Mean ± SD	91.8 ± 219.8	52.1 ± 52.7
SD error	56.8	13.6
Lower 95% CI	-30	23
75% Percentile	46.77	71.37
One-tailed Wilcoxon test	0.2487	

Table 13 | Effect of 8 weeks MMGAT program on serum IL-4 levels. (ID43 not present).

	MC1	**ME1**
n	14	14
Median	30.4	26.5
25% Percentile	20.8	17.1
Mean ± SD	35.28 ± 22.41	59.79 ± 54.37
SD error	5.9	14.5
Lower 95% CI	22.34	19.40
75% Percentile	45.96	60.73
One-tailed Wilcoxon test	0.4908	

Table 14 | IL-4 levels (mean ± SD) in serum of breast cancer survivors before (MC1) and after (ME1) 8 weeks of MMGAT program*.*

ID	MC1		ME1		
	Mean	**SD**	**Mean**	**SD**	***p. value***
26	**22.12**	**4.64**	**11.00**	**2.01**	**<0.0001**
28	45.69	16.84	119.2	66.33	0.1340
29	41.01	3.37	11.23	5.91	0.0126
35	27.80	18.42	17.12	18.64	0.2858
39	**20.77**	**2.63**	**53.15**	**32.94**	**<0.0001**
41	**14.84**	**5.54**	**12.07**	**4.32**	**<0.0001**
42	**99.26**	**30.49**	**26.00**	**2.31**	**0.0386**
43	882.5	438.4	71.37	8.72	0.0602
52	**34.18**	**20.23**	**25.79**	**2.02**	**<0.0001**
55	30.99	4.17	27.06	3.49	0.2000
57	46.77	12.60	208.1	75.68	0.1000
58	13.68	6.52	76.48	6.48	0.1000
60	53.30	41.99	55.46	65.15	0.5000
64	13.77	0.76	43.61	26.54	0.1254
66	**29.74**	**3.92**	**24.76**	**0.45**	**<0.0001**

Note: bold – statistically significant.

4.1.3. Interleukin 6

It has previously been shown that the IL-6 range in healthy pos-menopausal women is 6,8-15 pg/mL[113,114] but in obese women with mean age of 47 ± 10 years the values reported were in average 45.16 ± 83.88pg/mL[115]. The mean baseline values for the participants in this study were (39.2 ± 24 pg/mL) which is around the expected since they are all overweight or obese. Only 2 participants had MC1 IL-2 levels in the normal range for healthy post-menopausal women (ID60 and ID64) and no significant changes were observed in IL-6 levels after exercise training (Table 15).

The median values at MC1 were 38.1 pg/mL and were reduced to 29 mg/mL, indicating that in at least 50% of the participants IL-6 serum levels improved. This was confirmed when looking at the individual level changes, significant reductions at ME1 were observed in 6 participants (ID28, ID39, ID24, ID43, ID51 andID58; Table 16).

Table 15 | Effect of 8 weeks MMGAT program on serum IL-6 levels.

	MC1	ME1
n	14	14
Median	38.10	29
25% Percentile	24.98	12.58
Mean ± SD	39.2 ± 24	35.4 ± 26.6
SD error	6.41	7.10
Lower 95% CI	25.28	20.00
75% Percentile	45.92	53.80
One-tailed Wilcoxon test	0.5830	

Table 16 | IL-6 levels (mean ± SD) in serum of breast cancer survivors before (MC1) and after (ME1) 8 weeks of MMGAT program.

ID	MC1		ME1		
	Mean	**SD**	**Mean**	**SD**	***p. value***
26	41.47	10.73	53.43	3.68	0.2000
28	**46.91**	**15.15**	**33.73**	**2.45**	**<0.0001**
29	27.17	4.42	12.99	3.55	0.1000
35	**44.29**	**5.05**	**54.92**	**2.20**	**<0.0001**
39	**105.6**	**9.40**	**4.03**	**1.13**	**<0.0001**
41	59.98	12.43	67.35	10.76	0.3500
42	**1131**	**871.5**	**55.92**	**6.01**	**0.0506**
43	**45.59**	**1.96**	**42.25**	**1.08**	**<0.0001**
52	**25.21**	**5.51**	**11.35**	**0.43**	**<0.0001**
55	33.33	1.49	24.25	5.65	0.1000
57	34.72	15.30	47.28	12.82	0.2000
58	**42.55**	**13.16**	**15.15**	**6.15**	**0.0500**
60	**11.24**	**2.10**	**23.27**	**4.27**	**0.0500**
64	5.78	0.59	10.66	3.65	0.1018
66	24.27	2.32	98.59	14.78	0.1000

Note: bold – statistically significant.

4.1.4. Interleukin 10

It has previously been shown that the IL-10 range in healthy pos-menopausal women is 9.4-22.6 pg/mL[113,116]. However, other studies in healthy males+female group with age above 65 years showed variations of 0.01-41.7 pg/mL[114] and in an obese cohort of 40.5 ± 13 years-old the levels were 1.43 ± 0.86 pg/mL[117]). The mean baseline value for this study was much higher (473.5 ± 1262 pg/mL), but this was due to only two participants (ID35 and ID43), the remaining ones

were all within the reported values for aged and obese populations (Table 17 and 18).

Table 17 | Effect of 8 weeks MMGAT program on serum IL-10 levels.

	MC	**ME**
n	15	15
Median	2.02	1.89
25% Percentile	1.50	1.01
Mean ± SD	473.5 ± 1262	561.9 ± 1385
SD error	337.3	357.6
Lower 95% CI	-255.2	-250.1
75% Percentile	9.63	5.67
One-tailed Wilcoxon test	0.2852	

When ID35 and ID43 participants were excluded from the analysis and now the mean values were near / below the normal range (Table 19). Therefore, the intervention had a significant effect in the participant cohort reducing IL-10 levels.

Table 18 | IL-10 levels (mean ± SD) in serum of breast cancer survivors before (MC1) and after (ME1) 8 weeks of MMGAT program.

ID	**MC1**		**ME1**		
	Mean	**SD**	**Mean**	**SD**	***p. value***
26	3.16	0.95	3.52	1.90	0.3500
28	**2.13**	**1.26**	**0.87**	**0.28**	**0.0500**
29	**1.20**	**0.61**	**1.04**	**0.09**	**<0.0001**
35	**2245**	**980**	**555.3**	**65.62**	**<0.0001**
39	1.57	0.30	0.41	0.39	0.1000
41	**25.75**	**5.08**	**1.01**	**0.48**	**0.0500**
42	4.26	0.02	2.22	0.65	0.1000
43	4336.33	*	4409.33	*	ND
52	**1.92**	**0.28**	**2.53**	**0.06**	**<0.0001**
57	1.71	1.16	1.10	0.48	0.2760
58	1.89	0.60	1.89	0.39	0.5000
60	2.18	0.82	1.60	0.61	0.3500
64	1.30	0.33	5.67	6.87	-
66	0.79	0.29	0.70	0.16	0.3500

"*" represents the values that were above the maximum detectable level. (ND – not detectable).
Note: bold – statistically significant.

Table 19 | Effect of 8 weeks MMGAT program on serum IL-10 levels. (ID35 and ID43 not present).

	MC	**ME**
n	12	12
Median	1.9	1.1
25% Percentile	1.4	0.8
Mean ± SD	4.0 ± 6.9	1.5 ± 0.9
SD error	1.9	0.3
Lower 95% CI	-0.4038	0.8984
One-tailed Wilcoxon test	**0.0171**	

4.1.5. Interferon gamma

In healthy pos-menopausal women, the IFN-γ range was reported to be 0.01-0.08 pg/mL[113]. However, other studies in healthy males+female group with age above 65 years showed variations of 1-117.7 pg/mL[114] and in an obese cohort of 40.5 ± 13 years-old the levels were 4.79 ± 0.62 pg/mL[117]. The mean baseline value in the present study was clearly much higher (384.2 ± 254.9 pg/mL). Nevertheless, in this cytokine, a tendency to decrease after exercise was observed (p=0.0636), although the mean levels at ME1 were still higher than the previously reported[113] (Table 20).

Table 20 | Effect of 8 weeks MMGAT program on serum IFN-γ levels.

	MC	**ME**
n	16	16
Median	343.4	222.7
25% Percentile	223.9	181.5
Mean ± SD	384.2 ± 254.9	377.4 ± 531.7
SD error	63.72	132.9
Lower 95% CI	248.4	94.04
75% Percentile	437.1	323.7
One-tailed Wilcoxon test	*0.0636*	

Qualitatively, in 11 participants, exercise reduced IFN-γ levels, with 5 of them showing significant decrease (ID26, ID42, ID55, ID58 and ID60) but still the

levels were higher than levels reported in other studies [113,114,117] (Table 21). Therefore, the intervention program did not significantly improve IFN-γ levels.

Table 21 | IFN-γ levels (mean ± SD) in serum of breast cancer survivors before (MC1) and after (ME1) 8 weeks of MMGAT program.

ID	MC1		ME1		
	Mean	**SD**	**Mean**	**SD**	***p. value***
26	**315.4**	**71.36**	**179.3**	**45.26**	**<0.0001**
28	450.0	23.86	250.9	48.51	0.1000
29	313.0	40.51	343.0	15.90	0.2161
35	272.9	0.00	144.6	48.14	-
39	378.3	*	217.8	89.64	ND
41	1269	132.0	385.2	113.6	0.1000
42	**398.3**	**42.52**	**188.4**	**22.40**	**<0.0001**
43	**462.7**	**56.76**	**2316**	**338.2**	**0.0083**
47	466.0	16.53	627.1	99.00	0.0756
52	199.8	62.68	187.9	0.00	-
55	**224.3**	**48.40**	**97.64**	**28.19**	**0.0427**
57	195.0	12.26	265.8	65.97	0.1370
58	**223.8**	**67.64**	**204.4**	**52.72**	**<0.0001**
60	**371.3**	**64.35**	**143.4**	**22.55**	**0.0210**
64	397.9	78.92	258.6	89.78	0.1205
66	209.5	89.75	227.6	69.33	0.2578

"*" representes the values that were above the maximum detectable level. (ND – not detectable).
Note: bold – statistically significant.

4.1.6. Tumor necrosis factor alfa

The mean TNF-α baseline values were (91.9 ± 28.1 pg/mL), which is above the normal TNF-α range in healthy pos-menopausal women (2.2-6.4 pg/mL). However, other studies in healthy males+female group with age above 65 years showed variations of 0.86-20.8 pg/mL[114] and in an obese cohort of 47 ± 10 years-old the levels were 209.06 ± 301.49 pg/mL[117]. No significant changes were observed in TNF-α levels after exercise training (Table 22).

Qualitatively, 6 participants showed a tendency to decrease the levels of TNF-α after intervention, with 3 of them showing significant alterations (ID35, ID39 and ID52) (Table 23). Therefore, the intervention did not have a significant effect in TNF-α levels.

Table 22 | Effect of 8 weeks MMGAT program on serum TNF-α levels.

t test	MC	ME
n	15	15
Median	82.02	92.76
25% Percentile	74.14	69.55
Mean ± SD	91.9 ± 28.1	90.44 ± 28.5
SD error	7.24	7.34
Lower 95% CI	76.32	75.7
75% Percentile	122.7	114.0
One-tailed Wilcoxon test	0.4452	

Table 23 | TNF-α levels (mean ± SD) in serum of breast cancer survivors before (MC1) and after (ME1) 8 weeks of MMGAT program.

ID	MC1		ME1		
	Mean	**SD**	**Mean**	**SD**	***p. value***
26	82.02	14.66	76.21	9.82	0.5000
28	**49.93**	**2.02**	**75.86**	**11.29**	**<0.0001**
29	64.37	18.19	69.55	9.52	0.5000
35	**122.7**	**26.67**	**92.76**	**4.37**	**<0.0001**
39	**129.1**	**26.38**	**60.49**	**11.16**	**0.0386**
41	79.70	5.07	94.41	14.45	0.2000
42	133.8	69.87	125.8	17.15	0.4444
43	143,00	40,11	155,05	*	ND
52	**75.65**	**1.39**	**52.43**	**1.98**	**<0.0001**
55	**102.0**	**8.28**	**115.1**	**2.74**	**<0.0001**
57	90.50	12.51	95.30	24.77	0.5000
58	78.49	45.93	114.0	19.62	0.2000
60	86.65	6.43	52.33	3.09	0.1000
64	65.91	6.66	83.85	0.98	0.1000
66	74.14	24.01	93.43	0.00	-

"*" representes the values that were above the maximum detectable level. (ND – not detectable).
Note: bold – statistically significant.

4.2. Analysis of immune cells number

The number of immune cells, after the PE intervention, were analyzed using the data obtained from Germano de Sousa Labs. Only one significant decrease between groups was observed in Neutrophils, which were in lower levels than the normal range at baseline and were significantly reduced (p=0.0103) (Table 24; Figure 4).

Table 24 | Immune cells response (mean ± SD) in breast cancer survivors before (MC1) and after (ME1) 8 weeks of MMGAT program.

Type of immune cell	MC1		ME1			Normal range (10^3/µL)
	Mean	SD	Mean	SD	*p. value*	
Leukocytes	5220	1822	5093	1043	0.3818	4000-10000
Neutrophils	**3423**	**1017**	**2920**	**701.7**	**0.0103**	**40000-80000**
Eosinophils	122.0	87.85	125.3	89.35	0.3654	1000-6000
Basophils	21.33	13.02	20.67	11.63	0.3872	0.000-2000
Lymphocytes	1660	554.1	2300	1934	0.1200	20000-40000
Monocytes	278.0	61.44	266.0	79.62	0.2582	2000-10000

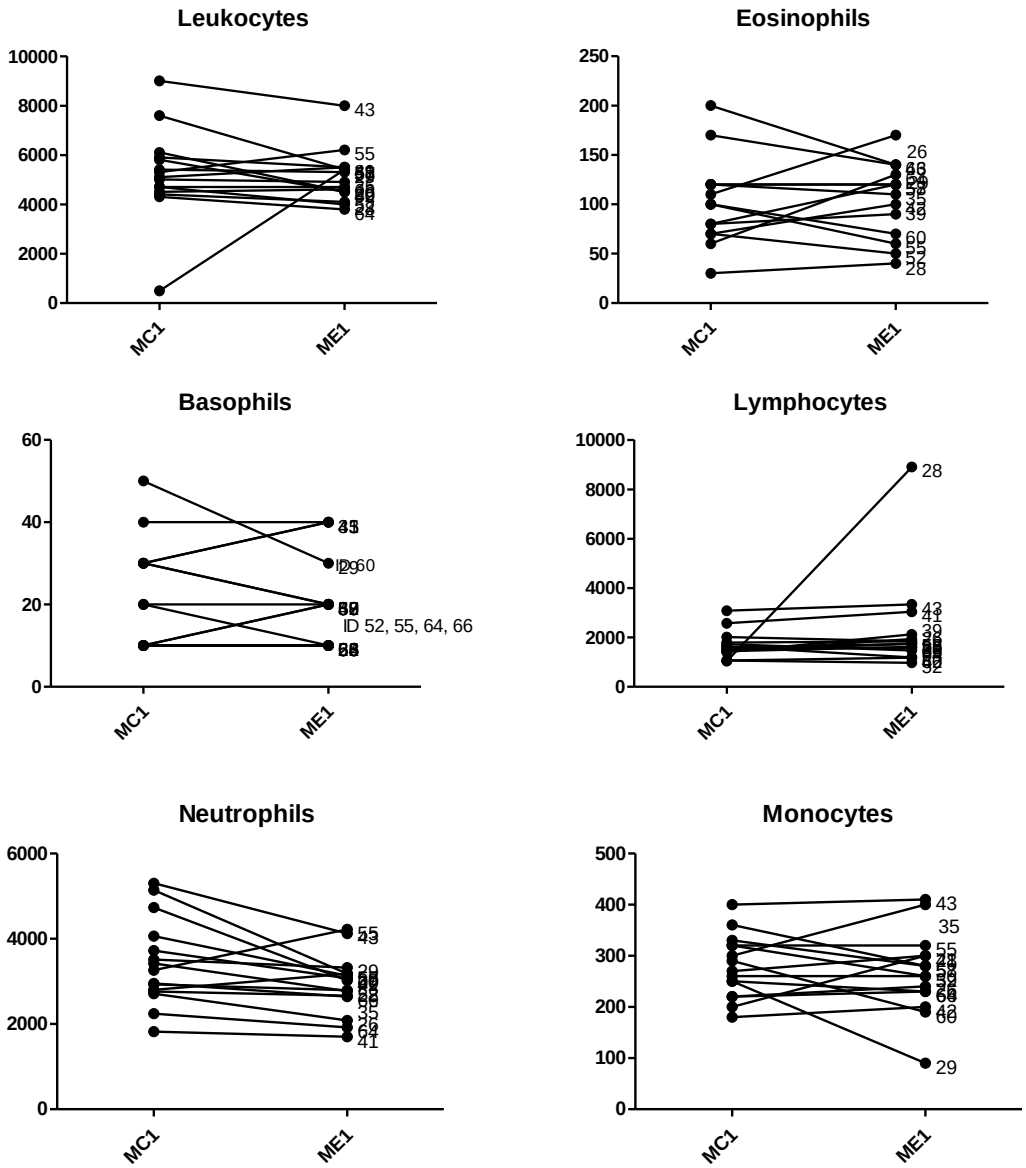


Figure 4 | Effect of 8 weeks MMGAT program on immune cells.

At baseline level, leucocytes and basophils levels were normal in all 15 participants. After intervention, the levels of these immune cells remained within

the normal range. These results show that intervention did not alter the levels of these immune cells.

Neutrophils, eosinophils, lymphocytes, and monocytes levels were below baseline level in all 15 participants. A tendency to increase was observed in the levels of lymphocytes after intervention, however, did not restore the normal levels. These results suggests that exercise did not improve the levels of these immune cells.

4.3. Metabolic profile

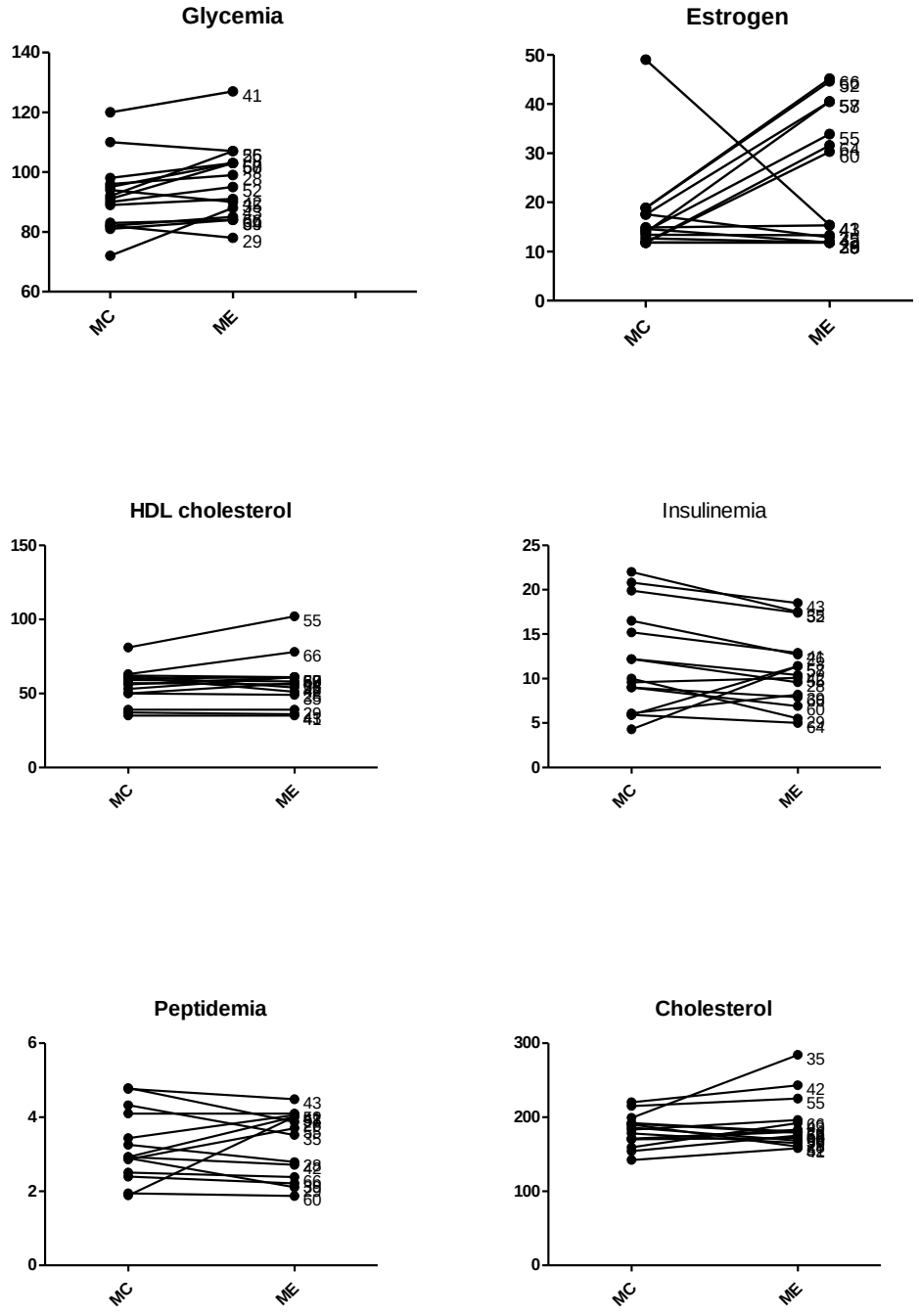
The data from the clinical pathology of the participants was also evaluated. A significant increase was observed between groups in glycemia (p=0.0063) and estrogen (p=0.0455), although in both cases the values remained within the normal range (Table 25; Figure 5).

Table 25 | Changes in metabolic profile (mean ± SD) in breast cancer survivors before (MC1) and after (ME1) 8 weeks of MMGAT program.

ID	Before		After			Normal range
	Mean	SD	Mean	SD	*p. value*	
Glycemia	**91.67**	**11.91**	**96.27**	**12.58**	**0.0063**	**70-100 mg/dL**
Insulinemia	11.91	5.773	11.03	4.225	0.1666	3.0-25.0 µUI/ml
Peptidemia	3.212	0.9565	3.273	0.8877	0.3949	1.10-4.40 ng/ml
Cholesterol	181.3	21.41	190.7	34.70	0.1034	<190 mg/dL
HDL cholesterol	55.00	11.81	57.27	16.67	0.1456	45-65 mg/dL
Triglycerides	152.4	78.25	145.7	80.97	0.3333	<150 mg/dL
C-reactive protein	0.2807	0.2046	0.3551	0.3505	0.1374	0.050-1.000 mg/dL
Estrogen	**16.84**	**9.254**	**24.71**	**13.60**	**0.0455**	**0-37.0 pg/ml**
Basal IGF1	86.09	27.32	95.57	29.31	0.1034	51.0-187.0 ng/ml
LDL cholesterol	95.85	18.20	105.4	29.01	0.2214	<100 mg/dL

The levels of insulinemia, C-reactive protein, LDL cholesterol and basal IGF1 were normal in all 15 participants before exercise and after intervention. However, one participant showed an increased in C-reactive protein to abnormal levels (ID28) after intervention. Similarly, LDL levels increased to abnormal levels in one participant (ID 35).

The levels of peptidemia, cholesterol total, triglycerides, cholesterol HDL e estrogen levels were abnormal at baseline in some participants. Two participants (ID26 and ID42) restored the normal levels of peptidemia after intervention. Cholesterol levels decreased to normal levels in 3 participants (IDs: 52, 57, 64). Also, triglycerides levels restored the normal levels in 2 participants (ID 57 and ID 66). Estrogen in ID43 restored the normal levels after intervention. Cholesterol HDL remain within abnormal levels at MC1 and ME1.



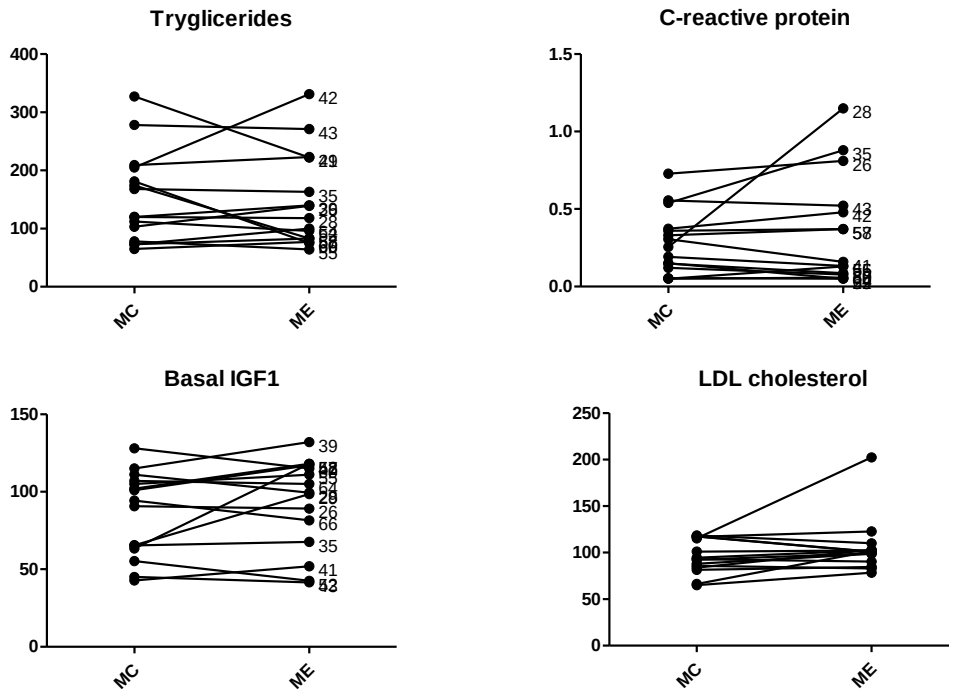


Figure 5 | Effect of 8 weeks MMGAT program on the metabolic parameters.

4.4. Correlation analysis between immune cells, cytokines and biochemical parameters

To investigate the existence of a relationship between cytokines, immune cells and biochemical parameters, a correlation analysis was performed. A positive correlation between TNF-α and leucocytes (p=0.001; r=0.55) was found. C-reactive protein was correlated with IL-2 (p=0.0008; correlation=0.57) and IL-6 (p=0.001; correlation=0.54). A correlation between IL-10 and peptidemia was also found (p=0.0006; correlation=0.58).

A correlation between cytokines was also carried out. It was found only one positive correlation, between TNF-α and IL-6 (p=0.006; correlation=0.48).

CHAPTER V

DISCUSSION

5. Discussion

In this study, the effect of PE on the profile of cytokines, immune cells and metabolic and hormonal in BC survivors was studied. The main finding was a decrease of IL-10 and neutrophils levels.

IL-10, being a immunosuppressive cytokine, plays an important role in the homeostasis of the organism. It functions as an preventive agent of inflammatory and autoimmune diseases. This cytokine is mainly produced by macrophages and neutrophils[118]. During PE, occurs the production of pro-inflammatory cytokines (IL-6 and TNF-α) in the muscle. The presence of these cytokines stimulates the production of IL-10, in order to the body returns to homeostasis, with PE here having an anti-inflammatory effect[119]. In this study, a significant decrease in IL-10 levels were found between MC1 group (baseline) and ME1 group (after 8 week of intervention). Since BC survivors suffer from inflammation, it would be expected to find an increase in IL-10 levels. However, this result was not obtained. A previous study showed that AT (150 minutes per week) and RT (2 times per week) in BC survivors didin't significantly change IL-10 levels[120]. However, another study with duration of 12 weeks (3 sessions per week, 20-30 minutes each session) showed that moderate to high intensity exercise on a bicycle ergometer in overweight/obese BC survivors significantly increased IL-10 levels[121]. In our study, of the participants with obese BMI (ID28, ID35, ID4, ID42, ID43, ID52, ID55 and ID58) none showed significant increase in IL-10 and 2 showed a significant decrease. Despite being a significant result, decrease in IL-10 by 8 weeks of PE did not correlate with changes in any of the pro-inflammatory cytokines or in the immune cell numbers, which suggests that this was not sufficient to alter the inflammatory profile of BC survivors in this present study.

Lymphedema is one of the sequels after BC treatment with radiation or chemotherapy. All the participants in the study received radiotherapy, while 56% also received chemotherapy. Local inflammation is promoted by adipose tissue expansion and fibrosis of lymphatic tissue and is critical in the pathophysiology of lymphedema. In turn, lymphedema leads to a chronic inflammatory response. It has been shown that blocking the differentiation of TH2 cells or blocking IL-4

action prevented the development of lymphedema, that inflammation is caused by IL-6 produced by adipose tissue macrophages and that congestion keeps levels of TNF-α and IFN-γ increased[122]. In agreement with this, the participants of this trial had higher levels of TNF-α and IFN-γ than those reported for healthy post-menopausal women.

No significant alterations were found in IL-2, IL-4, IL-6, IFN-γ e TNF-α. These results are compatible with previous studies. Gómez et al. (2011) studied the effect of an 8-week exercise program (AT + RT) on BC survivors but no significant changes in cytokines levels were reported. However, in the present study, IL-2, IL-4, IL-6 and TNF-α tended to decrease for about 50% of the participants, while INF-γ tended to be reduced for 11 out of 15 participants. Therefore, although these results are not significant and should taken with caution, they are promising regarding the effect of the intervention on pro-inflammatory cytokines and IL-4, involved in the pathophysiology of lymphedema. Moreover, the reduction of pro-inflammatory cytokines and no increase in anti-inflammatory one suggests a tendency of PE to improve the inflammatory profile of BC survivors. The tendencies in IL-6 reduction observed in this study are in agreement with one previous study that showed a significant reduction of IL-6 levels in BC survivors who performed at least 150 minutes per week of AT[124], where 86% of the participants performed the recommended weekly training.

PE can have an impact on the immune system and, depending on its intensity, its effects may be beneficial or harmful. During muscle contraction, calcium (Ca^{2+}) is released, and pro-inflammatory cytokines are produced. Thus, neutrophils are recruited to the site of inflammation[125]. In the present study, a significant reduction in serum neutrophils was observed after the intervention. This result may suggest that PE induces local inflammation which consequently, causes the recruitment of neutrophils to the tissues. This is consistent with previous studies showing that PE stimulates the activation and recruitment of neutrophils to sites of inflammation[126]. As a consequence, the serum neutrophile levels would decrease, as observed by us. A recent study that investigated the effect of a 16-week exercise program (AT + RT) in samples of BC survivors showed PE could activate and improve neutrophils[127]. Although a significant

reduction was observed, the levels of neutrophils did remain below normal after PE. Therefore, a reduction of neutrophils observed by 8 weeks of PE in our study needs to be further investigated to verify the activation state of the circulating neutrophils to better understand if it improved the immune system.

It is known that TNF-α is a cytokine that participates in the process of inflammation. This cytokine can cause fever, edema and vasodilation. The displacement of leucocytes to the site of inflammation is facilitated by this cytokine[128]. Previous studies have shown a relationship between TNF-α and leucocytes[129]. In the present study, the results showed a medium positive correlation between these two variables. Although there were no significant changes in TNF-α and leukocytes, PE induces similar changes in these two variables.

The effect of PE on the metabolic markers of BC survivors was also evaluated. The practice of PE must be regular, in order for the organism to be able to achieve its benefits. Improvement of lipid profile, weight loss and decreased of blood pressure are some of the positive effects of PE[130]. A previous study showed that an 8-week AT program in obese subjects showed significant reducing of triglycerides and LDL cholesterol. Also, a significant increase in HDL cholesterol was observed[131]. In the present study, no significant alterations in the participant weight were observed. The metabolic profile was already within normal levels for most participants and was not improved by 8 weeks of PE. A slight tendency to increase LDL levels was observed after PE, however that may be due to only one participant who showed abnormal levels after PE.

Associated with inflammation, we also have CRP and IL-6. CRP is an acute phase protein, which actively participates in the inflammatory process and infections. IL-6 is a trigger of CRP production in the liver[132]. In this study, no significant changes were observed in IL-6 and CRP, however there was a medium positive correlation. The presence of this correlation may be a potential indicator of the anti-inflammatory role of PE since PE can reduce inflammation and CRP levels[133,134]. A medium positive correlation was also found between IL-2 and CRP. In the literature, there's limiting data regarding to the relationship between these two variables, however a recent study showed that the levels of

IL-2 are significantly correlated with CRP in obese subjects[135]. A medium positive correlation was also found between IL-10 and peptidemia. There's lack of literature between these two variables, as far we know.

Linked with IL-6, we have TNF-α, where a medium positive correlation was found. As stated earlier, both cytokines are pro-inflammatory and participate in the inflammatory process. The presence of this correlation indicates that the effect of PE on IL-6 or TNF-α was similar. It's important to highlight that the participants of these study, except 3 were either overweight (BMI ≥25 kg/m^2) or obese (BMI≥30 kg/m^2) and IL-6 and TNF-α in obese subjects are in high concentrations, according to the literature[136,137]. Also, this may explain the high levels of TNF-α and IFN-γ[117] obtained in this study.

The present study has some limitations. Although 80 BC survivors were recruited to the MMGAT clinical trial, enough samples were collected only for 16 participants. Therefore, the analysis was done in a small number of samples, lower than the needed to observe significant effects. Also, the duration of the study may have been a limiting factor. The PE intervention was designed for 16-weeks, but we could only collect samples after 8 weeks of initiating the PE program, which may not be enough time to cause significant changes in the metabolic or immune profile of BC survivors.

CHAPTER VI

CONCLUDING REMAKS AND FUTURE PERSPECTIVES

6. Concluding remarks and future perspectives

The aim of this work was to investigate if the adapted exercise program MMGAT had a beneficial effect on the inflammatory and metabolic profiles of BC survivors.

The exercise program did significantly reduce the levels of IL-10 and neutrophils. Even though overall no statistically significant results were found for most of the variables tested, this pilot study showed that 8 weeks of combined training (AT + RT) tended to reduce pro-inflammatory cytokines. This is important to emphasize, since these can be preliminary results indicating that PE can reduce inflammation, which is beneficial for BC survivors, since they suffer with inflammation and lymphedema due to cancer treatments. In addition, a significant decrease of IL-10 could be due to the tendency to reduce pro-inflammatory cytokines observed. This could be evidence of the anti-inflammatory effect of PE. Additionally, no significant alterations occurred in inflammatory makers, these parameters remained within normal after PE.

In the future, studies aimed at evaluating the role of PE in BC survivors should be conducted with a larger number of samples and also a program that could last the entire 16 weeks, in order to clearly identify the significant effects induced by the MMGAT PE program.

CHAPTER VII

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