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Targeting the Tumor Microenvironment with Exercise:

The Role of Muscle Tissue-Derived Metabolites

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Hereby I declare:

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Table of contents

List of abbreviations

HRQoL– health-related quality-of-life

TNF- α – tumor necrosis factor-alpha

TME – tumor microenvironment

IL– interleukin

NK cells – natural killer cells

mTOR – mammalian Target of Rapamycin

ATP – adenosine triphosphate

VEGF – vascular endothelial growth factor

TAM - tumor-associated macrophage

Th – T helper

DAMPs – damage-associated molecular patterns

HMGB1 - High-Mobility-Group-Protein B1

Tregs – regulatory T cells

S1PR1 – sphingosine-1-phosphate receptor 1

S1PR2 – sphingosine-1-phosphate receptor 2

OSM – oncostatin M

SPARC – Secreted Protein Acidic and Rich in Cysteine

CXCL9 – chemokine C-X-C motif ligand 9

CCL 15 – chemokine C-C motif ligand 15

IL-6R – IL-6 Receptor

PD-1 – programmed cell death protein 1

PD-L1 – programmed cell death ligand 1

GH – growth hormone

IGF-1 – insulin-like growth factor 1

IL-6sR – soluble IL-6 receptor

VEGF-A – vascular endothelial growth factor-A

MDSC – myeloid-derived suppressor cells

CAF – carcinoma-associated fibroblast

EMT – epithelial-mesenchymal transition

ECM – extra cellular matrix

FGF – fibroblast growth factor

TGF-β – transforming growth factor-beta

MPP2 – matrix metalloproteinase

Gp130 – glycoprotein 130

OSMRβ chains – oncostatin M receptor β chains

OSMR – oncostatin M receptor

TGM2 – transglutaminase 2

CSC – cancer stem cell

STAT3 – signal transducer and activator of transcription 3

SMAD3 – mothers against decapentaplegic homolog 3

FNDC5 – fibronectin type III domain-containing protein 5

PGC-1α – peroxisome proliferator-activated receptor-gamma coactivator - 1α

HIIT – high-intensity interval training

HIF-1α – hypoxia-inducible factor-1-alpha

NF-κB – nuclear factor kappa-light-chain-enhancer of activated B cells

BDNF – brain derived neurotropic factor

TrkB T1– tropomyosin receptor kinase B T1

CXCR3 – chemokine Receptor CXCR3

CXCL10 – C-X-C motif chemokine ligand 10

CXCL11 – C-X-C motif chemokine ligand 11

TNBC – triple negative breast cancer

JAK/STAT – janus kinase/signal transducers and activators of transcription

PI3K/AKT– phosphatidylinositol 3-kinase/ protein kinase B

List of illustrations

1. Abstract

Cancer is one of the most widespread diseases globally, with nearly 20 million diagnoses in 2022 and 9.7 million deaths. The most common types include breast, lung, colorectal, and prostate cancer. The epidemiology of cancer varies significantly by region, influenced by factors such as genetics, lifestyle, environmental exposures, and healthcare access. Breast cancer is the most frequently diagnosed cancer worldwide after lung cancer, with approximately 2.3 million new cases each year.

Exercise has gained popularity as a supportive treatment, as it has been shown to alleviate treatment and disease-related side effects and improve the quality of life of survivors. Based on current guidelines from the American College of Sports Medicine®, it is recommended that cancer patients perform at least three 30-minute sessions of endurance exercise and two to three sessions of resistance exercise per week. These suggestions are based on epidemiological studies and not on physiological observations, so it is mandatory to further investigate the effects of exercise on tumors at the molecular level. This dissertation investigates the effects of myokines, a type of protein secreted by contracting muscles, on cancer.

Studies have shown that exercise entails multiple physiological changes that exert an anti-cancer effect. Animal studies have consistently shown that tumor size and progression are reduced in animals that exercise compared to controls. These observations are attributed to multiple effects.

This dissertation investigates the effects of myokines, a type of protein secreted by contracting muscles, on cancer. It includes two scientific publications: a narrative review and an intervention study. The narrative review describes the most commonly known myokines, their role in the tumor microenvironment, and which type of exercise induces them. The review shows that certain myokines, such as IL-6 and irisin, are significantly upregulated after exercise and demonstrate potent anti-tumor effects in vitro, including the inhibition of angiogenic signaling pathways and the induction of apoptotic pathways in tumor cells.

The human study investigated the effects of serum conditioned by an acute endurance intervention on breast cancer cells. The study showed that the conditioned serum significantly reduced the growth of breast cancer cells in vitro, particularly immediately after exercise. Cytokine analysis highlighted CXCL9 and CCL15 as potential mediators of the observed effects. Blocking the CXCL9 pathway with a CXCR3 antagonist further inhibited cell growth, supporting the hypothesis that exercise-induced cytokine changes contribute to anti-cancer effects.

Exercise is becoming an increasingly important element of cancer treatment as it offers a range of benefits for patients' well-being and potentially influences cancer progression. Studies suggest that physical activity can lower cancer-related mortality, particularly in breast and colorectal cancer survivors. The role of skeletal muscle as an endocrine organ is central to understanding how exercise exerts these effects. Muscle contractions stimulate the release of myokines, signaling proteins that influence local and systemic processes, including those within the tumor microenvironment. This dissertation shows that exercise-induced changes in cytokine concentration in serum collectively influence breast cancer cell growth through possible modulations of the tumor microenvironment. In particular, the chemokine CXCL9 emerged as a promising modulator, potentially influencing immune infiltration and angiogenesis in the tumor microenvironment.

Despite these advances, important questions remain. The impact of different types of exercise, including resistance training, on cancer biology has yet to be fully explored. Future research should also assess whether similar effects occur in different populations, for example divided by age, type of cancer, physical activity level and comorbidities. The findings of this dissertation suggest that acute exercise bouts may exert a more direct influence on cancer cells than previously appreciated.

Zusammenfassung der Promotionsarbeit

Krebs ist eine der weltweit am weitesten verbreiteten Krankheit, mit fast 20 Millionen Diagnosen im Jahr 2022 und 9,7 Millionen Todesfällen. Zu den häufigsten Krebsarten gehören Brust-, Lungen-, Darm- und Prostatakrebs. Die Epidemiologie von Krebs variiert erheblich je nach Region und wird durch Faktoren wie Genetik, Lebensstil, Umweltbelastungen und Zugang zur Gesundheitsversorgung beeinflusst. Brustkrebs ist mit etwa 2,3 Millionen neuen Fällen im Jahr nach Lungenkrebs am häufigsten diagnostizierte Krebserkrankung weltweit.

Sport hat sich als unterstützende Behandlung etabliert, da er nachweislich die Nebenwirkungen der Behandlung und der Krankheit lindert und die Lebensqualität der Überlebenden verbessert. Basierend auf den aktuellen Richtlinien des American College of Sports Medicine wird Krebspatienten empfohlen, mindestens drei 30-minütige Sitzungen Ausdauertraining und zwei bis drei Sitzungen Krafttraining pro Woche durchzuführen. Diese Empfehlungen basieren auf epidemiologischen Studien und nicht auf physiologischen Beobachtungen, daher ist es notwendig, die Auswirkungen von Sport auf Tumore auf molekularer Ebene weiter zu untersuchen.

Studien haben gezeigt, dass Sport zahlreiche physiologische Veränderungen bewirkt, die eine krebshemmende Wirkung haben. Tierstudien haben gezeigt, dass die Tumorgröße und das Fortschreiten der Krankheit bei Tieren, die Sport treiben, im Vergleich zu Kontrollgruppen konsistent reduziert sind. Diese Beobachtungen werden auf verschiedene Effekte zurückgeführt.

Diese Dissertation untersucht die Auswirkungen von Myokinen, einer Art von Proteinen, die von kontrahierenden Muskeln ausgeschüttet werden, auf Krebs. Sie umfasst zwei wissenschaftliche Publikationen: ein narratives Review und eine Interventionsstudie. Das narrative Review beschreibt die bekanntesten Myokine, ihre Rolle im Tumormikromilieu und welche Art von Sport sie induziert. Es wurde gezeigt, dass bestimmte Myokine, wie IL-6 und Irisin, nach dem Sport signifikant hochreguliert werden und starke antitumorale Effekte in

vitro zeigen, einschließlich der Hemmung angiogener Signalwege und der Induktion apoptotischer Signalwege in Tumorzellen.

Die Humanstudie untersuchte die Auswirkungen von Serum, das durch eine akute Ausdauerintervention konditioniert wurde, auf Brustkrebszellen. Die Studie zeigte, dass das konditionierte Serum das Wachstum von Brustkrebszellen in vitro signifikant reduzierte, insbesondere unmittelbar nach dem Sport. Die Analyse der Zytokine hob CXCL9 und CCL15 als potenzielle Mediatoren der beobachteten Effekte hervor. Die Blockierung des CXCL9-Wegs mit einem CXCR3-Antagonisten verstärkte das Zellwachstum weiter, was die Hypothese unterstützt, dass durch Sport induzierte Zytokinveränderungen zu antitumoralen Effekten beitragen.

Sport wird zunehmend als wichtiger Bestandteil der Krebsbehandlung anerkannt, da er eine Reihe von Vorteilen für das Wohlbefinden der Patienten bietet und möglicherweise das Fortschreiten der Krankheit beeinflusst. Studien zeigen, dass körperliche Aktivität die krebsbedingte Sterblichkeit senken könnte, insbesondere bei Brust- und Darmkrebspatienten. Die Rolle der Skelettmuskulatur als endokrines Organ ist zentral für das Verständnis, wie Sport diese Effekte ausübt. Muskelkontraktionen stimulieren die Freisetzung von Myokinen, die lokale und systemische Prozesse beeinflussen, einschließlich derjenigen im Tumormikromilieu. Diese Dissertation zeigt, dass durch Sport induzierte Veränderungen der Zytokinkonzentration im Serum das Wachstum von Brustkrebszellen durch mögliche Modulationen des Tumormikromilieus beeinflussen. Insbesondere das Chemokin CXCL9 erwies sich als vielversprechender Modulator, der möglicherweise die Immuninfiltration und Angiogenese im Tumormikromilieu beeinflusst.

Trotz dieser Fortschritte bleiben wichtige Fragen offen. Die Auswirkungen von verschiedenen Trainingsarten, unter anderem Krafttraining, auf die Krebsbiologie sind noch nicht vollständig erforscht. Künftige Forschungsarbeiten sollten auch untersuchen, ob ähnliche Effekte in verschiedenen Bevölkerungsgruppen auftreten, z. B. unterteilt nach Alter, Krebsart, körperlicher Aktivität und Begleiterkrankungen. Die Ergebnisse dieser Dissertation legen nahe, dass akute Sporteinheiten einen direkten Einfluss auf Krebszellen haben können.

2. Introduction and scientific background

Cancer is one of the most widespread diseases. Worldwide, almost 20 million people were diagnosed with cancer in 2022 while there were 9.7 million fatal cases (Bray et al., 2024). The most common types include breast, lung, colorectal, and prostate cancer. The epidemiology of cancer varies significantly by region, influenced by factors such as genetics, lifestyle, environmental exposures, and healthcare access (Bray et al., 2018). Among cancer malignancies, breast cancer is one of the most commonly represented, with approximately of 2.32 million new cases in 2022 (Bray et al., 2024). It is expected, that numbers will increase even further (Xu et al., 2023). Due to newly developed treatments, there are more and more cancer survivors at the same time. This is due to more specialized and individualized therapies (J. C. Brown et al., 2012).

Exercise has gained popularity as a supporting treatment, as it has been shown to alleviate treatment and disease-related side effects and improve quality of life of cancer survivors (Xinyan Zhang et al., 2019). As more research was conducted on the effects of exercise on cancer, it became apparent that exercise entails multiple physiological changes which exert an anti-cancer effect (Ruiz-Casado et al., 2017).

Based on the current guidelines from the American College of Sports Medicine it is recommend for cancer patients to perform at least three 30 min sessions of endurance exercise and two to three sessions of resistance exercise per week (K. L. Campbell et al., 2019). As these suggestions are based on epidemiological studies and not on physiological observations it is mandatory to further investigate the effects of exercise on tumors at the molecular level (Ruiz-Casado et al., 2017). While there is a variety of candidate pathways which mediate the effects of exercise on tumors, this thesis mainly investigates the effects of myokines given the current lack of clarity on several aspects. Myokines are a type of protein, which are secreted by contracting muscle (B. K. Pedersen, 2011). Their activity influences muscle growth and recovery following but beside these effects, myokines can also enter the periphery and exert their effects in multiple organs and tissues. Therefore, they are becoming of increasing interest in the context of diseases and conditions like obesity, dementia, diabetes and cancer, which were shown to be alleviated by exercise (B. K. Pedersen, 2011; B. K. Pedersen & Febbraio, 2012; Severinsen & Pedersen, 2020).

Understanding the underlying molecular mechanisms of myokines in the context of cancer can improve the design of more targeted exercise interventions that may support cancer therapies by enhancing their effectiveness or by limiting side effects (Liampas et al., 2022; Pagola et al., 2020; Spiliopoulou et al., 2021).

Accordingly, the aim of this thesis is to investigate the effects of exercise conditioned serum and mediators in the context of cancer and whether serum conditioned by an acute exercise intervention can influence breast cancer cell growth.

2.1 Physiological principles of exercise in cancer patients

Exercise plays a significant role in cancer care, providing numerous physiological benefits that improve both the overall health and health-related quality-of-life (HRQoL) of cancer patients. Additionally, exercise is linked to a reduced incidence of several cancer diseases (Lavín-Pérez et al., 2021; Machado et al., 2021; Xinyan Zhang et al., 2019). In studies that investigate the effects of exercise on patient reported outcomes, improvements were recognized (Hiensch et al., 2024). Furthermore, physical activity has been linked to a reduced risk of cancer recurrence, particularly in cases of breast and colorectal cancer (Idorn & Thor Straten, 2017).

As the integration of exercise into treatment plans is more discussed and investigated, it became more apparent, that exercise helps to mitigate treatment-related side effects like chemotherapy and radiation while promoting long-term recovery. The positive effects of exercise are grounded in several important physiological mechanisms. One of the most impactful benefits of exercise is its improvement of cardiovascular function. Aerobic exercises such as walking, cycling, or swimming enhance the heart and lung capacity by increasing the patients VO_{2max}. Improved cardiovascular function is essential for cancer patients, as treatments like chemotherapy often impair heart and lung health (Jones et al., 2012; Schmitz et al., 2010). A benefit of resistance training is the prevention of muscle wasting, or sarcopenia. Resistance exercise helps to preserve muscle mass, maintain physical function, and also reduce cancer-related fatigue (Schmitz et al., 2010; Winters-Stone et al., 2012).

The so-called hallmarks of cancer define which alterations cause cancer at the cellular level. These include immune system evasion, enhanced angiogenesis, increased proliferation, metastasis and cell invasion, altered cell metabolism and resisting apoptosis. All of these hallmarks can be effected by exercise. These are shown in Figure 1 (Ruiz-Casado et al., 2017). Patients with the same cancer disease can respond differently to the same exercise intervention based on the individual physiology and genetic predispositions (Bourke et al., 2016). Other factors that determine the patients response to exercise are the cancer stage and the type of treatment (Schmitz et al., 2010).

A key physiological principle behind the integration of exercise in cancer care is inflammation. Inflammation is a well-known contributor to cancer progression, and exercise can have anti-inflammatory effects. Regular exercise reduces levels of pro-inflammatory cytokines, such as tumor necrosis factor- α (TNF-α) and interleukin-6 (IL-6), which are often abnormal in cancer patients. By lowering systemic inflammation, exercise enhances the body’s immune response, potentially slowing tumor growth and supporting the effectiveness of treatments (Ballard-Barbash et al., 2012; Hojman et al., 2018). Alongside the reduction of the inflammatory response, immune function is also enhanced by exercise. As summarized in a meta-analysis, pro-inflammatory markers decrease in cancer populations following exercise. Following exercise, NK cell function has been shown to improve, as well as proliferation rates of lymphocytes (Khosravi et al., 2019). T cell function can be enhanced by exercise and is also a crucial component of ant-tumor immunity. In a melanoma mouse model, exercise improved T cell-mediated tumor control and sensitized tumors to anti-programmed cell death protein 1 (PD-1) therapy (Yan et al., 2023). The PD-1 receptor on T cells functions as an immune checkpoint that, after binding to its ligand programmed cell death ligand 1 (PD-L1), inhibits T cell activation and proliferation, allowing cancer cells to evade immune surveillance (Freeman et al., 2000).

Another system influenced by exercise is the endocrine system. Exercise helps regulate hormone levels, which can be disrupted by cancer and its treatments. This hormonal regulation is particularly beneficial in reducing the risk of cancer recurrence, especially in hormone-sensitive cancers such as breast and prostate cancer (Shang, 2007). Hormonal regulation in cancer plays an important role in the context of cancer cachexia (Tisdale, 2009). Elevated levels of catabolic hormones like cortisol and pro-inflammatory cytokines such as TNF-α and IL-6 contribute to muscle protein breakdown and systemic inflammation, which has mainly been demonstrated in animal models (H. J. Kim et al., 2012). At the same time, anabolic hormones including testosterone, growth hormone (GH), and insulin-like growth factor 1 (IGF-1) can be downregulated, which would reduce the body's capacity to maintain or rebuild muscle (Costelli et al., 2006; Trobec et al., 2011). As summarized in a recent systematic review regarding this topic, exercise can help rebalance this hormonal environment by lowering chronic cortisol and inflammatory cytokines while stimulating the release of anabolic hormones. These hormone-mediated effects of exercise may counteract muscle degradation and support better metabolic function in individuals with cancer cachexia (Libramento et al., 2025).

In conclusion, the physiological principles of exercise in cancer care are multifaceted, benefiting both physical and mental health. Exercise improves cardiovascular function, preserves muscle mass, reduces inflammation, regulates hormones and enhances immune response. These physiological mechanisms underscore the importance of incorporating exercise into cancer treatment plans, making it a critical supporting therapy in the management and recovery of cancer patients. Despite this knowledge, the underlying mechanisms are mostly unknown in humans and there is no consensus, which type of exercise is most beneficial (Spiliopoulou et al., 2021). We know from controlled animal studies, that exercise has a growth inhibitory effect on tumors. Tumor size and progression is consistently reduced in animals who exercise compared to their controls. These observations are attributed to multiple effects (C. de Lima et al., 2008). It is unclear if exercise can directly influence cancer cells, or if other mechanisms via the above described systems are more important. Another aspect of this question is which type of exercise may directly influence cancer cells and, if the effects are acute or chronic (Buss & Dachs, 2020; Hojman et al., 2018).

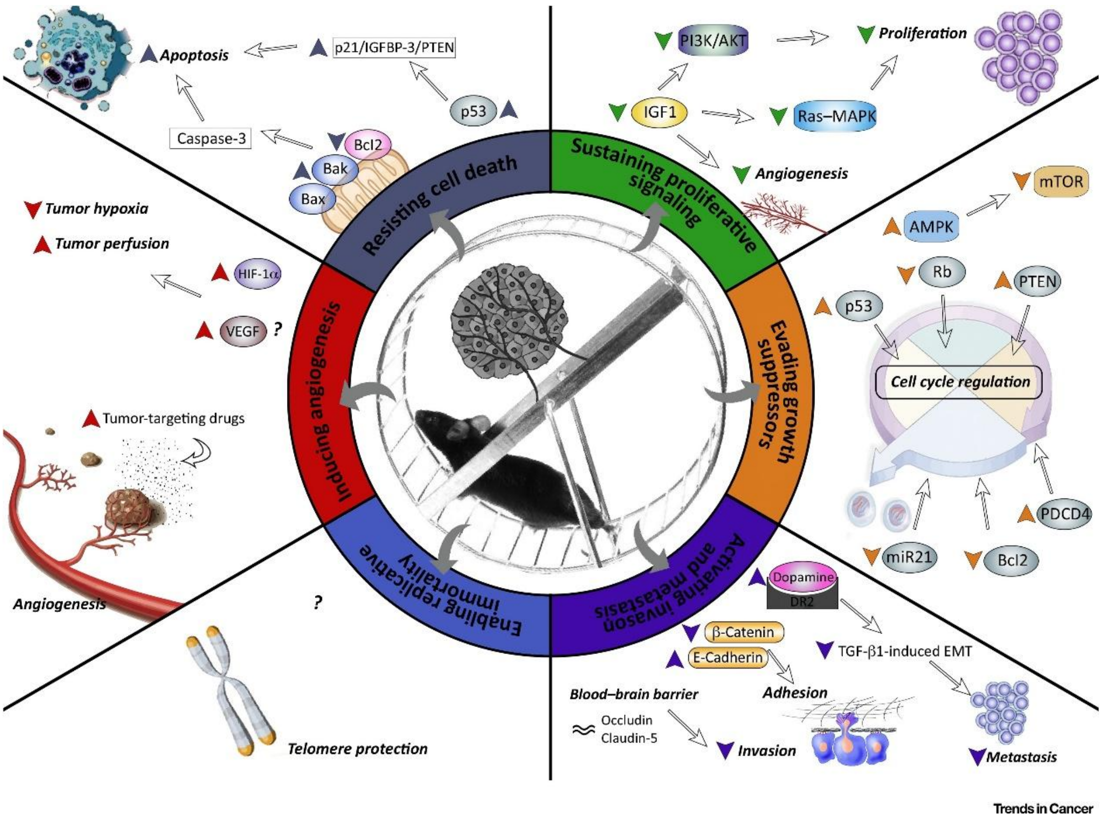

Figure 1. Hallmarks of cancer and the influence of exercise on them. This figure summarizes how exercise may modulate multiple cancer-related processes: it can reduce proliferative signaling by lowering circulating IGF-1, enhance tumor suppressor activity via protein 53 and adenosine monophosphate-activated protein kinase pathways, and inhibit invasion and metastasis through mechanisms such as stabilization of the blood–brain barrier and suppression of epithelial-to-mesenchymal transition. Exercise may also normalize tumor vasculature through upregulation of hypoxia-inducible factor-1-alpha (HIF-1α) and vascular endothelial growth factor (VEGF), promote apoptosis via proapoptotic proteins and modulate cell cycle regulators. However, its role in affecting telomerase activity and replicative immortality remains unclear. Reprinted from *Trends in Cancer*, Vol. 3, Issue 6, Ruiz-Casado, A., Martín-Ruiz, A., Pérez, L.M., Provencio, M., Fiuza-Luces, C., & Lucia, A., "Exercise and the Hallmarks of Cancer", pp. 423–441, Copyright (2017), with permission from Elsevier.

2.1.1 Differences between resistance and endurance exercise in cancer patients

Exercise is defined as planned, structured, and repetitive physical activity with the goal of improving or maintaining components of physical fitness. Examples include walking on a treadmill, cycling, or resistance exercise (Riebe et al., 2018). Training on the other hand involves a systematic approach to exercise with the goal of achieving specific performance outcomes or health benefits. It typically includes progressive overload, periodization, and recovery phases that have to be tailored to the individual needs, especially in cancer patients (Courneya & Friedenreich, 2007). Difficulties when designing exercise prescriptions for cancer patients are the different risk factors like bone metastasis or cardio toxicity of treatment, cancer characteristics and treatment responses as cancer can present itself in many different forms and shapes (Siegel et al., 2021).

Exercise and training can be divided further into two main subcategories, which are resistance and endurance. Resistance training primarily focuses on improving muscle power, hypertrophy, and maximal strength. Exercises involve resistance movements such as weightlifting, which lead to increased muscle fiber recruitment and greater force production (Huiberts et al., 2024; Mikkonen et al., 2024). In contrast, endurance training emphasizes enhancing aerobic capacity and cardiovascular efficiency, often through prolonged, low-to-moderate intensity exercises like running, swimming, or cycling. This type of training improves the body's ability to sustain activity over extended periods by increasing oxygen utilization, measured as VO_{2max}, enhancing mitochondrial function, and improving muscle endurance (Huiberts et al., 2024; Jansson et al., 2022).

Both training modalities, resistance and endurance training, can benefit cancer patients. Multiple exercise guidelines for cancer survivors exist, while the consensus is that either 150 min moderate or 75 min intense supervised exercise, including both, endurance and resistance training, should be performed per week both during and after curative cancer treatment (K. L. Campbell et al., 2019; Ligibel et al., 2022). These guidelines specifically address the side-effects of cancer survivors (Wilson et al., 2023). Hiensch et al. (2024) showed in metastatic breast cancer patients, that this group also benefits from supervised mixed training interventions and demonstrated that health related quality of life improved. Women undergoing chemotherapy also benefit from exercise but there is so far no consensus for exercise recommendations of patients who are still undergoing treatment (Antunes et al., 2024; K. L. Campbell et al., 2019).

Although the beneficial effects of exercise on cancer- and treatment-related side effects during cancer treatment and rehabilitation as well as pre-habilitation are known, the exact biological pathways by which exercise affects cancer progression and recovery still need to be explored (Coderre et al., 2022; Stout et al., 2020; Watts et al., 2024). To further understand which exercise modality is best suited to support cancer treatment and prevention one has to explore the molecular adaptations caused by each exercise modality (Jones, 2020; Schumann et al., 2020). In the following, the known molecular adaptations to endurance and resistance exercise are presented. Given the ethical constraints of conducting certain human studies, studies on animal models, *in vitro* experiments, and comprehensive reviews of existing literature will be cited.

2.1.2 Molecular aspects of endurance exercise in relation to cancer

Adaptations to exercise and training can occur either acutely or chronically. If acute stimuli are repeated multiple times, they may result in chronic adaptions (Lambert, 2015). The effects of exercise on cancer cells follow the same principle (Schauer et al., 2022). Acute effects occur immediately or shortly after a single exercise session and may include temporary changes in cancer cell behavior, such as increased oxidative stress or alterations in hormone levels (Dethlefsen et al., 2016). In contrast, chronic effects result from repeated exercise over time and may the result of accumulated acute effects. Further distinctions can be made between direct or indirect effects on cancer cells. Indirect effects are changes in the TME, which result in growth inhibition or apoptosis. Direct effects include, for instance, the effects of myokines that can alter the cancer cell membrane (L. Pedersen & Hojman, 2012).

Acute effects of endurance exercise in relation to cancer

Acute endurance exercise has been found to exert several beneficial, immediate effects on cancer cell biology, primarily through immune cell mobilization and modulation of metabolic conditions that may help suppress tumor progression (Spiliopoulou et al., 2021). One of the primary responses involves the mobilization of immune cells, particularly cytotoxic T cells and natural killer (NK) cells, both critical in targeting and eliminating cancer cells. For example, endurance exercise can lead to an increase in circulating NK cells and T cells, which studies have shown can effectively target tumor cells. Research on a prostate cancer model found that NK cells were mobilized after exercise, but did not infiltrate tumor tissue. This suggests that while acute exercise prepares immune cells for their action, tissue-specific effects might require additional mechanisms to direct these cells into the tumor (Schenk et al., 2022; Sitlinger et al., 2020). Further research shows that endurance exercise can modify the inflammatory response by decreasing levels of pro-inflammatory cytokines such as TNF-α and IL-6 while increasing anti-inflammatory cytokines such as IL-10. This shift towards an anti-inflammatory profile may create a more favorable tumor microenvironment (TME), potentially inhibiting cancer progression (Hojman et al., 2018; Petersen & Pedersen, 2005).

In addition to immune responses, acute responses to endurance exercise also involve metabolic pathways, which may influence cancer cell viability. For example, studies on prostate cancer cells treated with post-exercise blood serum showed reduced cell proliferation and increased apoptosis. This is likely due to metabolic changes in the plasma that limit resources available for tumor growth. This anti-proliferative effect has been demonstrated across several cancer models, suggesting that metabolic shifts following exercise can produce a less hospitable environment for tumor cells (Rundqvist et al., 2013; Rundqvist et al., 2020). Contracting muscles also produce myokines, a subclass of cytokines that are likely to have an effect on tumors. Some are acutely increased while others are more reactant to repeated exercise bouts (Huang et al., 2022). These effects in particular will be discussed further in chapter 2.2.3.

Furthermore, Thompson et al. (2009) have described pathways that link exercise to the prevention of breast cancer, focusing in particular on the Mammalian Target of Rapamycin (mTOR) pathway. mTOR is a protein that regulates cellular metabolic activity in response to external stimuli, which can influence apoptosis, proliferation, growth, or survival (Hung et al., 2012). In breast cancer, mTOR dysregulation is associated with increased cell proliferation and vascularization. Exercise may suppress the mTOR pathway, contributing to its protective effects against breast cancer. However, Thompson et al. (2009) also note a dose-dependent relationship, suggesting that while moderate doses of exercise are beneficial, excessive exercise can induce cellular stress that may negate these effects. Additionally, exercise may limit cancer cell aggregation by restricting glucose availability. Due to their altered metabolism, cancer cells are dependent on glucose, which is only available to a limited extent during exercise, as glucose is primarily used for the aerobic metabolism of skeletal muscles (Ruiz-Casado et al., 2017). One of the hallmarks of cancer is the so called Warburg effect, which describes the altered metabolism. Cancer cells rely heavily on glucose because they predominantly use glycolysis—even in the presence of oxygen to generate energy and metabolic intermediates needed for cell growth quickly. This supports their high proliferation rate. This metabolic shift causes glucose to be converted into lactic acid instead of being fully oxidized, resulting in a lower adenosine triphosphate (ATP) yield per glucose molecule. Despite the reduced energy efficiency, this pathway allows cells to produce ATP more rapidly and supports cell proliferation. Moreover, cancer cells thereby gain the ability to bypass apoptosis, further supporting survival and proliferation. (Hanahan & Weinberg, 2000). By the accumulation of lactate within the TME, angiogenesis is increased via VEGF and immune escape is enhanced (Li et al., 2023).

A study found that a single 2-hour exercise session reduced breast cancer cell viability, while long-term training adaptations over six months had no significant impact. This finding challenges the traditional view that sustained training-induced reductions in baseline risk factors account for exercise's protective effects. Instead, the authors propose that cumulative acute exercise responses may drive cancer protection (Dethlefsen et al., 2016). These findings indicate that even a single session of moderate to high-intensity endurance exercise may prime the immune system and alter metabolic conditions, contributing to a less favorable environment for tumor development or direct inhibition of tumor cell growth. However, such acute effects are enhanced by regular, long-term exercise regimens that sustain immune and metabolic changes over time (Rundqvist et al., 2020; Sitlinger et al., 2020).

Chronic effects of endurance exercise in relation to cancer

Chronic endurance exercise affects cancer biology by engaging various physiological pathways that enhance immune function, reduce inflammation, and optimize metabolic health. Long-term endurance training promotes the production of muscle-derived proteins known as myokines, such as IL-6, IL-15, and IL-7. These myokines play an essential role in activating and sustaining immune cells, including T cells and NK cells, which are crucial for identifying and eliminating cancer cells (Jurdana, 2021; Valenzuela et al., 2022). While acute exercise mobilizes NK cells, chronic exercise appears to improve their cytotoxic function through receptor modulation, thereby enhancing their ability to target cancer cells (Pal et al., 2021). In aging individuals, the CD4+/CD8+ ratio can decrease, which corresponds to lower immune function. The ratio can be increased, and therefore normalized, by chronic endurance exercise (Despeghel et al., 2021).

Systemic inflammation is a major factor in the progression of cancer. By downregulating pro-inflammatory cytokines such as TNF-α and promoting anti-inflammatory cytokines such as IL-10, exercise helps to create an anti-inflammatory environment. Since chronic inflammation is closely linked to tumor growth, this anti-inflammatory environment not only contributes to a lower cancer risk but may also help to slow down tumor progression (Gustafson et al., 2021; Jurdana, 2021). Other inflammatory cytokines that are regulated by chronic endurance exercise are cytokines such as IL-6, IL-8, IL-10, and VEGF, which are often associated with chronic inflammation (Petersen & Pedersen, 2005). This shift in the cytokine profile creates a TME that may inhibit cancer progression (Hojman et al., 2018). In support of these findings, L. Pedersen et al. (2016) demonstrated in various murine tumor models that voluntary endurance exercise, such as wheel running, lowered tumor growth rates and recurrence. This effect was associated with increased NK cell infiltration into tumor cells and mobilization of IL-6-sensitive NK cells from the bone marrow, spleen and partly in peripheral blood mononuclear cells, showing that endurance exercise can activate specific immune mechanisms that are beneficial for cancer prevention and management. The expression of VEGF and other angiogenic factors is increased by regular exercise and will therefore lead to angiogenesis (Hoier & Hellsten, 2014). Regular endurance training increases insulin sensitivity and decreases insulin levels and related growth factors that can promote tumor growth. By maintaining lower insulin levels and enhancing the metabolism, exercise deprives cancer cells of an energy source, which helps to reduce the likelihood of tumor growth and recurrence over time (Gustafson et al., 2021; Jurdana, 2021).

Overall, chronic endurance exercise potentially influences cancer biology through multiple pathways, which strengthen the immune response, reduce inflammation and enhance the TME to inhibit cancer progression. This effect makes regular endurance exercise an important factor not only in reducing the risk of cancer, but also in supporting the body’s ability to fight cancer cells. Yet, these findings stem mostly from animal studies and a deeper understanding of molecular pathways and their interconnectedness in humans is required.

2.1.3 Molecular aspects of resistance exercise in relation to cancer

Cancer patients often face changes in body composition, either due to the treatment or the disease itself (Stene et al., 2013). In breast cancer patients, strength, body composition and other parameters improved following a resistance intervention (Schmitz et al., 2005). Higher lean body mass is associated with better outcomes in cancer patients (S. Tsai, 2012). It is also important to note that resistance exercise can have an effect on inflammatory markers and is therefore also highly relevant in regard to its influence on the TME and cancer cells (Hojman et al., 2018). Similar to endurance exercise, resistance exercise can have acute and chronic effects, which are presented below (Abernethy et al., 1994).

Acute effects of resistance exercise in relation to cancer

Acute resistance exercise triggers various molecular and cellular responses that could directly or indirectly influence tumor growth. These responses include changes in immune cell circulation, cytokine and myokine release, metabolic shifts, and oxidative stress, which together can alter the TME and create unfavorable conditions for cancer cell survival (Arazi et al., 2021; He et al., 2024).

One of the most significant acute effects of resistance exercise is, similar to endurance exercise, the mobilization and redistribution of immune cells, such as NK cells, cytotoxic T cells, and macrophages. This is partly driven by the release of catecholamines, like adrenaline, which mobilize these cells from lymphoid organs to the bloodstream (W. M. C. Brown et al., 2015; Idorn & Hojman, 2016). Studies suggest, that these immune cells are more likely to infiltrate the TME after exercise. NK cells, in particular, have been shown to target and destroy tumor cells in the TME, releasing cytotoxic granules that can induce apoptosis in cancer cells (Idorn & Hojman, 2016).

The secretion of exercise-induced cytokines and myokines, such as IL-6, IL-15, and irisin, further contributes to the anti-tumor effects. IL-6, which is acutely released from muscle tissue during resistance exercise, has dual roles in inflammation and immune modulation. Acute IL-6 spikes during exercise enhance immune surveillance by activating NK cells and promoting their migration to the TME, while IL-15 enhances immune cell proliferation (Nygaard et al., 2015; L. Pedersen & Hojman, 2012). Furthermore, irisin, has been shown in preliminary studies to induce apoptosis in cancer cells through metabolic stress pathways. However, this effect needs further investigation (Alizadeh Zarei et al., 2023).

Acute resistance exercise also influences metabolic factors in ways that might disadvantage cancer cells. During and immediately following intense resistance exercise, glucose uptake by muscle tissue is elevated, reducing glucose availability in the bloodstream. Given that cancer cells often rely on glycolysis for rapid ATP production, this temporary reduction in glucose could impose metabolic stress on tumor cells, particularly in highly glycolytic cancers (Hojman et al., 2018). Furthermore, resistance exercise leads to transient spikes in cortisol and adrenaline, which can increase oxidative stress within the TME. While chronic stress hormones may promote tumors, acute exercise-induced spikes can overwhelm cancer cells' antioxidant defenses, inducing DNA damage and disrupting survival pathways. These short-lived increases may favor cancer cell death without long-term tumor-promoting effects (Aboelella et al., 2021). Resistance exercise also has an effect on the vascular system in the TME. Exercise-induced myokines like SPARC influence endothelial cell function and tumor angiogenesis (Idorn & Hojman, 2016). A reduced or disrupted vascular network within the tumor can also make it more susceptible to immune cell infiltration and therapeutic agents, further supporting an anti-tumor environment (Liang et al., 2010).

The acute molecular and cellular responses to resistance exercise create a multifaceted anti-cancer effect through immune activation, metabolic stress, oxidative stress, and vascular modulation within the TME. These mechanisms highlight the potential for resistance exercise to serve as an adjunctive therapy in cancer treatment, although more research is necessary to clarify the optimal protocols and determine the specific molecular effects across different cancer types (He et al., 2024).

Chronic effects of resistance exercise in relation to cancer

Chronic resistance exercise modulates immune surveillance by increasing the activity and infiltration of immune cells in the TME. For instance, resistance exercise has been shown to enhance the cytotoxicity of NK cells (Idorn &

Hojman, 2016). This effect is likely mediated by long-term increases in circulating levels of immune-activating myokines like IL-15, Il-6 and irisin, which are regulated in response to sustained resistance exercise and enhance NK and T-cell proliferation and function, leading to sustained anti-inflammatory and metabolic effects within the TME (Pérez-López et al., 2018; Rautela & Huntington, 2017).

Additionally, chronic resistance exercise can also remodel the vasculature within tumors. As SPARC has been shown to influence angiogenesis and endothelial function in the TME, the resulting vascular remodeling can normalize tumor blood vessels and reduce hypoxia. A more normalized vasculature can enhance the delivery of immune cells and therapeutic agents to the tumor, amplifying the effectiveness of both natural immune responses and potential cancer therapies (Liang et al., 2010).

Lastly, resistance exercise could affect oxidative stress in cancer cells. An 8-week intervention altered oxidative stress markers in the plasma of head and neck cancer patients. These included reduced carbonyl content and 8-Hydroxydesoxyguanosin while anti-oxidative capacity was increased. These findings are important as oxidative stress can lead to inflammatory processes. A facilitator for oxidative stress in cancer patients is chemotherapy. The study found that chronic resistance exercise enhances antioxidant capacity in healthy cells while selectively increasing oxidative stress in cancer cells, which may benefit cancer patients undergoing chemotherapy (Yen et al., 2020).

2.2 The tumor microenvironment and its regulation by exercise

The TME is defined as the surrounding environment of a tumor and is composed of malignant as well as non-malignant cells (Balkwill et al., 2012). The main components of the TME are immune cells, the vascular system including endothelial cells, extracellular matrices as well as signaling molecules. The composition of these components, and therefore the TME, is highly individual and dependent on multiple factors like the type of cancer or its stage (Anderson & Simon, 2020). The composition of the TME can be a contributor to metastasis as well as therapy response and cancer cell survival, which makes it a highly interesting target for therapeutic approaches (Arneth, 2019). Exercise is one way to influence the TME, possibly towards a favorable outcome (Wiggins et al., 2018). The TME can be altered by exercise in at least four ways via myokines: the immune system, vascularization, cancer muscle cross talk and cancer cell metabolism (L. Pedersen et al., 2015; Spiliopoulou et al., 2021).These four ways will be presented in the following sections.

2.2.1 Immune system

The immune system has a complex dual role in cancer progression. It has the ability to either suppress or promote tumor growth depending on the interplay between immune rejection and immunosuppression. The TME is a critical determinant of disease outcomes, as it shapes the function and interactions of immune cells. Within this environment, immune cells can either enhance anti-tumor responses or promote tumor progression, highlighting the need to understand and potentially manipulate these dynamics (Goh et al., 2014).

Important components of the TME are macrophages, particularly tumor-associated macrophages (TAMs), which are found in breast cancer tissues and other tumors. It has been shown, that high TAM density correlates with poor prognosis, as these cells frequently adopt pro-tumor behaviors (M. J. Campbell et al., 2011). TAMs can be polarized into two phenotypes: M1 macrophages, which are activated by T-helper (Th)1 cytokines like IFN-γ and damage-associated molecular patterns (DAMPs) such as High-Mobility-Group-Protein B1 (HMGB1), display anti-tumor properties by stimulating natural killer (NK) cells and directly apoptosis (Goh et al., 2014; Kilinc et al., 2006). Conversely, M2 macrophages, which are activated by cytokines like IL-4, IL-10, and IL-13, support tumor growth through processes such as angiogenesis, degradation of the extracellular matrix and the recruitment of regulatory T cells (Tregs). Tregs have an immunosuppressive effect and inhibit effective anti-tumor immunity (M. J. Campbell et al., 2011; Kilinc et al., 2006). T cells also play a major role in cancer immunity, particularly CD8+ cytotoxic T cells, which are strongly associated with positive outcomes in breast cancer due to their ability to directly kill tumor cells (Kilinc et al., 2006). However, tumors often subvert this immune surveillance by recruiting Tregs, which suppress anti-tumor responses. A high infiltration of Tregs in the TME is correlated with poor prognosis in multiple cancers. This immunosuppressive mechanism presents a significant challenge to achieving sustained immune-mediated anti-tumor response. Exercise, however, has emerged as a potential modulator of these dynamics. Acute bouts of exercise increase the circulation of T cells, particularly CD8+ and CD4+, via redistribution of lymphocytes to peripheral tissues, including tumors. Chronic exercise further enhances these effects, improving immune surveillance and promoting anti-tumor responses. For instance, eight weeks of wheel running in mice with breast cancer resulted in a higher intratumoral CD8+/FoxP3+ ratio, reduction in tumor size, and extended survival compared to sedentary controls (Spiliopoulou et al., 2021).

Beyond T cells, exercise also influences NK cells, which are critical for their ability to kill tumor cells via perforin-mediated cytotoxicity. Acute exercise increases the number of circulating NK cells and their recruitment to tumors in mice, enhancing immune surveillance (Gustafson et al., 2021; Pardo et al., 2002). M1 macrophages further enhance NK cell activity by secreting cytokines like IL-12. However, the intensity of exercise plays a significant role, as moderate-intensity exercise is associated with improved NK cell function, whereas prolonged high-intensity exercise can suppress NK cell activity due to elevated stress hormone levels (B. K. Pedersen & Hoffman-Goetz, 2000; Xiaojie Zhang et al., 2019). Hypoxia within the TME, a common feature of tumors, inhibits macrophage and NK cell activity while impairing T-cell mobility. Exercise helps to restore these immune functions by increasing perfusion and reducing hypoxia (Aveseh et al., 2015; Xiaojie Zhang et al., 2019).

Another cell type of the immune system which contributes to cancer immunity are neutrophils, although their role is complex. Depending on their phenotype, N1 or N2, neutrophils can either suppress or promote tumor progression. High densities of neutrophils in tumors are associated with varying prognoses, and the specific phenotypes involved are rarely characterized in studies (Galdiero et al., 2018). Exercise-induced changes in cytokines, such as IL-6 and IL-10, may influence neutrophil behavior, but further research was needed to clarify these effects (Nieman et al., 1990). Recently, it was shown that neutrophils are released from the bone marrow in higher numbers if IL-6 levels increase; however this observation was made in a murine model (Florentin et al., 2021). Similarly, the role of B cells in exercise-mediated tumor suppression remains unclear, as studies have not yet reported significant changes in their number, cytotoxicity, or tumor infiltration after exercise interventions (Spiliopoulou et al., 2021). Exercise not only modulates immune cell activity but also alters the physical and biochemical properties of the TME.

In summary, physical activity exerts a multifaceted influence on the immune system and the TME in multiple cancer entities. By mobilizing T cells, enhancing NK cell cytotoxicity, and normalizing the TME, exercise promotes anti-tumor immunity and creates a more favorable environment for an immune-mediated tumor response (Gustafson et al., 2021; Pardo et al., 2002; Simpson et al., 2015; Spiliopoulou et al., 2021; Xiaojie Zhang et al., 2019). Moderate-intensity exercise, in particular, appears to maximize these benefits by balancing immune activation with the prevention of stress-related immune suppression (B. K. Pedersen & Hoffman-Goetz, 2000; Xiaojie Zhang et al., 2019).

2.2.2 Vascularization

Tumor vascularization in the TME is closely connected to immune cells and immune cell function in the TME, as increased vascularization facilitates better immune cell infiltration. Exercise-induced vascular normalization has been shown to enhance the expression of endothelial adhesion molecules, which are essential for the entry of leukocyte into tumor tissues. This normalization overcomes the diminished leukocyte-endothelial interactions caused by hypoxia and disorganized vasculature within tumors (Xiaojie Zhang et al., 2019). Studies on murine models demonstrated that voluntary running not only increased tumor vasculature but also significantly enhanced immune cell infiltration into the TME, leading to reduced tumor growth. These findings highlight the role of improved vascularization in supporting immune surveillance and anti-tumor immunity (Esteves et al., 2023).

Improved vascularization also addresses hypoxia, a hallmark of cancer cells which suppresses immune cell function. Hypoxic regions are formed from both structural and functional abnormalities in tumor vessels, such as incomplete smooth muscle coverage and reduced vasoconstriction as well as leaky vessels, which impair oxygen delivery (Ruiz-Casado et al., 2017; Wiggins et al., 2018). Exercise alleviates hypoxia by promoting a more homogeneous distribution of blood flow and sustaining oxygen delivery to the tumor (Wiggins et al., 2018). These changes reprogram the TME to favor immune cell transport and functionality, supporting the removal of waste products and oxygenation of tumor tissues. The effects of exercise extend to molecular pathways that stabilize vessel integrity and reduce leakage, thereby enhancing immune cell transport. Shear stress during physical activity activates endothelial cell surface receptors such as sphingosine-1-phosphate receptor 1 (S1PR1) and sphingosine-1-phosphate receptor 2 (S1PR2), which play key roles in regulating vascular leakage and permeability (Esteves et al., 2021). These molecular changes lead to a more robust and organized vascular structure, allowing for improved immune cell access to the TME (Faustino-Rocha et al., 2016). Additionally, exercise modulates angiogenic factors such as VEGF-A, which supports vascularization in tumors. While VEGF-B has been linked to leaky vasculature and metastasis, its interaction with VEGF-A is indirectly regulated by exercise, underscoring the complex interplay between these factors (Kivelä et al., 2007; X. Yang et al., 2015).

Evidence from preclinical models further highlights the multifaceted benefits of exercise via vascularization. Long-term exercise in animal models has been associated with improved vascularization, increased immune cell infiltration, and smaller tumor sizes (Esteves et al., 2023; Faustino-Rocha et al., 2016). For instance, in breast cancer models, exercise enhanced VEGF-A expression and improved histological tumor grading, demonstrating its capacity to remodel the TME into one more conducive to effective immune responses (Faustino-Rocha et al., 2016). In addition, studies in prostate cancer show that while exercise did not reduce tumor size, it significantly improved vascularization and reduced hypoxia, indicating that its benefits extend beyond direct tumor reduction (McCullough et al., 2013).

Emerging evidence suggests that voluntary, unconstrained exercise can be of great benefit to cancer patients, even during hospitalization and recovery. These findings will need to be verified in human studies (Esteves et al., 2023).

2.2.3 Cancer-Muscle Cross Talk

Exercise and higher levels of physical activity have been recognized as positive contributors to cancer patients' outcome. In people who exercise and who fulfill the criteria for a higher fitness level, the function of the immune cells is improved and the response to therapy is enhanced. Besides immune cells, there is another type of protein, which contributes to these effects. These are so called myokines, a type of cytokines derived from muscle cells. Myokines originate directly from muscle cells and their secretion is in relation to muscle contraction (Gustafson et al., 2021). The muscle itself has therefore been recognized as a secretory organ. The effects of myokines can therefore be either autocrine, paracrine or endocrine. While the functions of many remains to be explored, they are multifaceted. For instance, some myokines are involved in angiogenesis within the muscle and hypertrophy, while others are involved in metabolic processes or serve only as cross-talk mediators with other organs (B. K. Pedersen & Febbraio, 2012). Through this type of communication, a feedback loop between muscles and other systems can be created and whole-body effects can occur, which highlights the importance and relevance of myokines (Chen et al., 2021). It is therefore only plausible, that myokines are relevant in multiple diseases, via either direct or indirect interactions through the immune, endocrine or metabolic system (Severinsen & Pedersen, 2020).

To no surprise, exercise has consequently been regarded more and more as a type of treatment or supportive treatment in diseases, one of which is cancer (Huang et al., 2022). Conditions associated with obesity, like insulin-resistance, favor tumor growth and cancer development. This environment can be altered and reconstructed towards an anti-tumor environment by exercise-mediated myokines. In a more direct manner, myokines like IL-6, oncostatin M (OSM), IL-15, irisin and secreted protein acidic and rich in cysteine (SPARC) have been associated with reduced tumor growth, reduction in cancer cell proliferation rate and generally cytotoxic properties as well as immune cell infiltration (J.-S. Kim et al., 2021). In a murine experiment, which combined serum of exercised mice and a human breast cancer cell line, reduced cell proliferation and apoptosis was observed with serum taken directly after the intervention. Upon identifying relevant myokines, the authors found that several genes were upregulated directly after the exercise session, one of which controls OSM expression (Hojman et al., 2011). Similar growth inhibitory effects were seen with the combination of human serum after exercise and prostate cancer cells (Rundqvist et al., 2013).

Despite the positive effects of exercise on cancer patients and the recognition of growth inhibitory effects, the underlying mechanisms remain to be explored (Spiliopoulou et al., 2021). Myokines have the ability to exert paracrine effects and can therefore travel in the periphery after secretion to reach distant sites (Hoffmann & Weigert, 2017). When reaching the tumor site, myokines can directly influence the cancer cell itself or the TME (L. Pedersen & Hojman, 2012). This process may be alleviated by increased vascularization within the tumor through exercise (Natarajan et al., 2024).

While there is general agreement that exercise regulates the release of myokines, the specific type, intensity, and duration of exercise most beneficial for influencing the TME and cancer cells remains unclear (Papadopetraki et al., 2022). Studies suggest that both aerobic and resistance exercises can stimulate myokines secretion, but their distinct impacts on cancer biology require further investigation (Arazi et al., 2021; Dobashi et al., 2021; Kumari et al., 2016; Sponder et al., 2017).

As cancer-muscle cross talk mediated by myokines and the influence on the TME are the central aspect of this thesis, the topic is discussed in more depth in chapter 2.3 and 2.4.

2.2.4 Cancer cell metabolism

Another hallmark of cancer is the altered cell metabolism in cancer cells. This phenomenon is called the Warburg effect. For the sake of faster cell reproduction, mitochondrial activity is downregulated through an increase of glycolysis. This entails a higher production and accumulation of lactate (Hanahan & Weinberg, 2000; Ruiz-Casado et al., 2017). Lactate is the final product of aerobic or anaerobic glycolysis and its accumulation creates an acidic TME. Lactate is mostly consumed by aerobic tumor cells, which ensures survival of hypoxic tumor cells as they can consume only glucose (Xiaojie Zhang et al., 2019). As tumors are not isolated entities though, metabolic shifts caused by exercise can influence the metabolism of tumors as well (Hojman et al., 2018). It was shown in a murine breast cancer model, that moderate endurance exercise reduced the tumors mitochondrial capacity. Genetic expression was also altered, specifically those of genes related to glycolic metabolism. The effects were only present if mice exercised for the whole study period but nor if the mice had a rest period in between exercising regimens. It was therefore concluded, that only continuous training might have an efficient favorable effect on cancer cell metabolism (Vulczak et al., 2020). Through these mechanisms, exercise can make the TME less conducive to the survival and growth of cancer cells (Koelwyn et al., 2017).

Lactate is another metabolism dependent factor, which can influence the TME. Under normal conditions, muscle cells can share certain metabolic characteristics with cancer cells during high-intensity exercise. Lactate buildup during exercise influences metabolic gene expression as well, but with occasional bouts of exercise and consistent training, these lactate exposures are temporary and promote beneficial metabolic adjustments through negative feedback mechanisms. In cancer cells on the other hand, these do not apply. Lactate in the TME is a major contributor to all cancer forming processes like angiogenesis, migration and metastasis and the immune escape (San-Millán & Brooks, 2017). High-intensity exercise enhances mitochondrial oxidative metabolism, reducing the reliance on glycolysis and thereby limiting lactate accumulation in tumors. This acute shift can counteract the acidification and immune suppression caused by lactate, fostering a less favorable environment for tumor growth. Additionally, exercise-induced vascular improvements increase oxygen supply, mitigating hypoxia - a driver of lactate production in cancer cells (La Cruz-López et al., 2019; Xiaojie Zhang et al., 2019). Chronic endurance training in mice reduced lactate concentrations in tumors and shifted their metabolism to a less aggressive form by increasing lactate dehydrogenase isoenzyme 1. This was accompanied by a reduction of other tumor growth promoting proteins (Aveseh et al., 2015). Combining exercise with therapeutic strategies targeting lactate metabolism, such as lactate dehydrogenase inhibitors, shows promise for enhancing cancer treatment outcomes (Li et al., 2023).

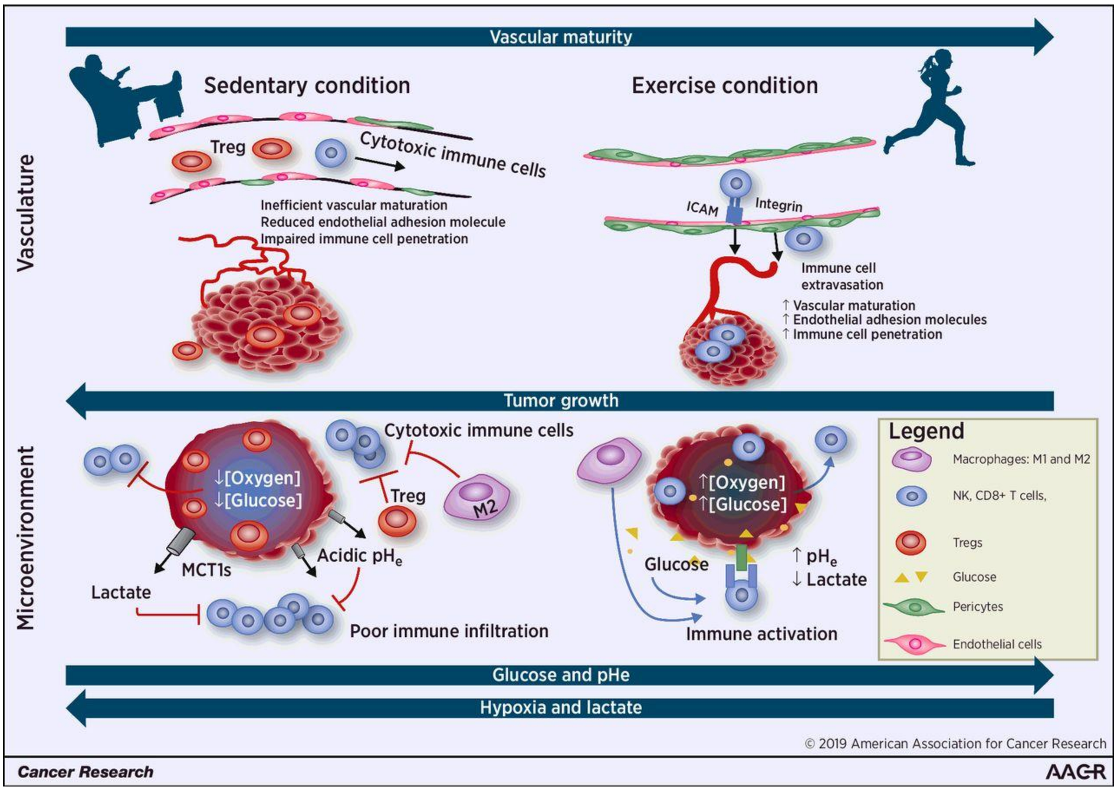

Figure 2. The connectedness of exercise induced effects. Exercise promotes a more aerobic, metabolically favorable, and immunologically active tumor microenvironment. The figure illustrates macrovascular (upper half) and microenvironmental (lower half) changes on in tumors from individuals who exercise compared to sedentary individuals, which were described in the previous chapters. It highlights how exercise can promote vascular normalization, increasing endothelial adhesion molecule expression and facilitating the extravasation and infiltration of cytotoxic immune cells into the tumor tissue. In contrast, tumors in sedentary individuals are typically hypoxic and exhibit high glycolytic activity. The lower half shows possible cross-sections of tumors from sedentary and exercised individuals. In sedentary tumors, elevated lactate levels, low extracellular pH, and limited glucose availability create an immunosuppressive environment that favors Tregs and impairs the function of cytotoxic immune cells. Exercise, by improving perfusion and oxygenation, enhances glucose availability and reduces lactate accumulation, collectively fostering a favorable TME. The result would be enhanced immune activation. It is important to note, that the interconnectedness depicted is a hypothesis and has not been demonstrated as such. Reprinted from *Cancer Research*, 2019, Vol. 79, Issue 10, pp. 2447–2456, Ashcraft, K. A. et al., *"Can Exercise-Induced Modulation of the Tumor Microenvironment Inhibit Tumor Growth?"*, with permission from the American Association for Cancer Research (AACR).

2.3 Myokines and their importance in cancer disease and the tumor microenvironment

While the main function of myokines appears to be the preservation of the muscle and increasing its performance, myokines have gained popularity in cancer research over the past years (Hoffmann & Weigert, 2017). More and more myokines are being discovered and their functions explored. Currently, there are about 3000 molecules, that are defined as myokines (Whitham & Febbraio, 2016). In connection to cancer disease, myokines are associated with decreased proliferation and cell-migration, reduced risk of metastasis and other antitumor effects (Huang et al., 2022). In colon cancer for example, increased apoptosis and reduced proliferation following exercise transmitted by SPARC, were shown. Furthermore, interactions with adipokines have been shown in prostate cancer. These positive effects have been reported in animal models (Aoi et al., 2013). IL-6 probably exerts its anti-cancer effect by enhancing the efficiency of NK-cells (Lucia & Ramírez, 2016). Myokines may also support cancer treatment. In breast cancer for example, it has been shown that irisin can enhance treatment efficiency while simultaneously reducing cancer aggressiveness (Roy et al., 2018). Aside from anti-tumor effect, cancer promoting effects of myokines have also been observed. It is therefore relevant, to gain further insights into their function (Huang et al., 2022). It appears, that the effect of myokines within the TME are more prominent directly after an exercise session, as cell viability in *in vitro* experiments decreases when treated with serum directly after a session but not with serum after multiple months of training (Dethlefsen et al., 2016).

In terms of cancer treatment efficacy, myokines play a supportive role by sensitizing the TME to chemotherapeutic agents and immune checkpoint inhibitors. Enhanced immune cell infiltration, facilitated by cytokines such as IL-15 and IL-6, makes tumors more responsive to these therapies. By altering the TME and supporting immune-mediated and direct cytotoxic mechanisms, myokines are an example of how exercise-induced physiological changes can contribute to the suppression of cancer progression and the enhancement of treatment outcomes (Huang et al., 2022).

Myokines that are most likely to influence the tumor and the TME according to current knowledge are IL-6, irisin, OSM, SPARC, IL-6 and BDNF (Huang et al., 2022). Their function is described in the following chapters. Another factor, which has been discovered during experiments is the chemokine C-X-C motif ligand 9 (CXCL9), which is therefore also described in the following chapter. All myokines except CXCL9 and their connection with the TME have been described in detail in publication 1. The following descriptions will highlight the most relevant aspects and incorporate findings that have been made since publication.

2.4 Myokines discussed in this thesis

This chapter describes the myokines and chemokines, which are also discussed in the publications, in detail. Here, more information is added compared to the publications as the most recent literature, which was not published at the publication time of Publication 1, has been taken into account. Figure 3 depicts the main effects of each myokines discussed.

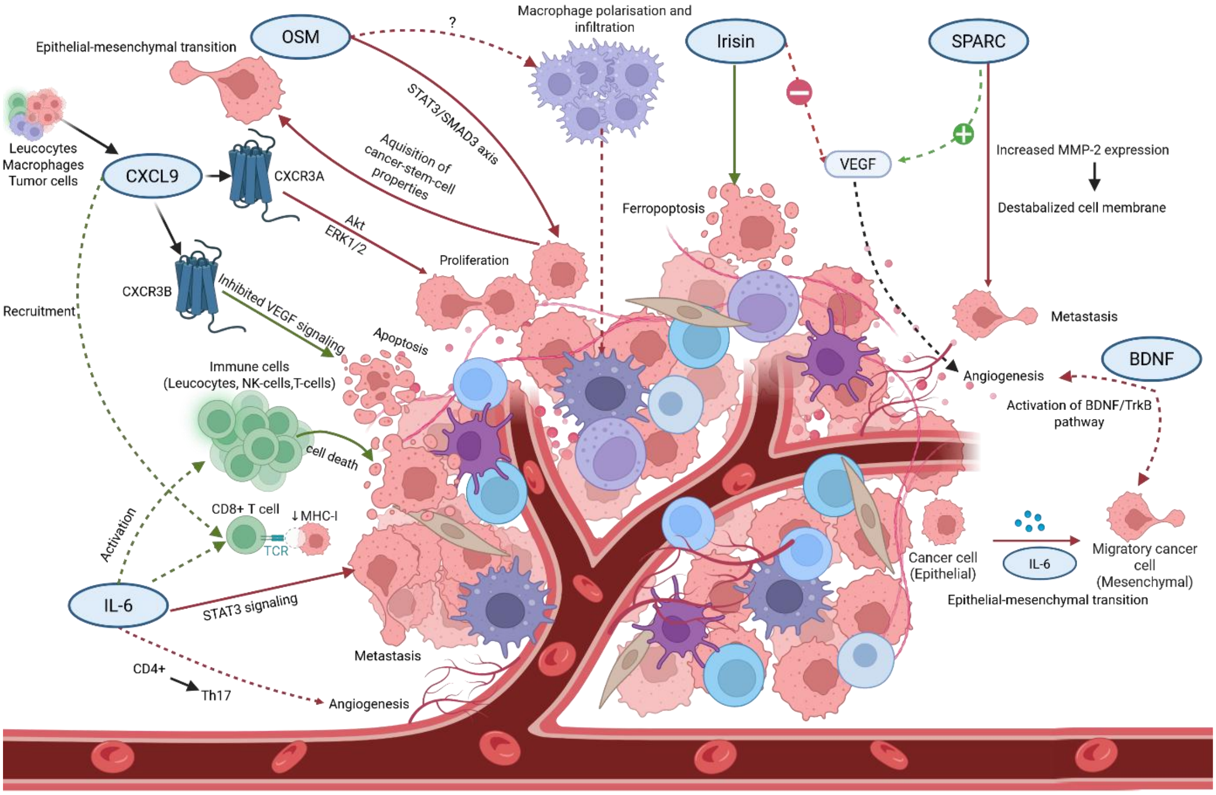

Figure 3. Adapted representation of exercise-induced changes in myokine expression and their impact on the tumor microenvironment. The myokines discussed in this thesis and their roles in the TME are pictured. Dotted arrows indicate an indirect effect, solid arrows a direct effect. Red arrows indicate a negative influence and green arrows a positive one. Black arrows indicate a dependence of the effect on the effector, it can be either positive or negative. CXCL9 was added to demonstrate its impact on the TME. It has to be taken into account, that CXCR3A and CXCR3B are membrane receptors, which is not pictured here as their influence is the main focus. Adapted from Sports Medicine International Open, Gunasekara, N., Clauss, D., Bloch, W., "Effects of Exercise-Induced Changes in Myokine Expression on the Tumor Microenvironment," 8.continuous publication, Copyright (2024), with permission from the author.

Interleukin – 6

The effects of IL-6 in response to exercise have first been described by B. K. Pedersen et al. (2004). The group found that muscle contractions increase IL-6 gene expression and consequently found elevated IL-6 levels in plasma.

Here, IL-6 was first connected to the anti-inflammatory effect of exercise. IL-6 is nowadays known as a multifunctional cytokine involved in immune regulation, inflammation, metabolism, and cell survival. It can act in a pro-inflammatory or anti-inflammatory manner depending on the signaling pathway engaged. Signaling can happen either via the IL-6 receptor (IL-6R) or the protein gp30 (Scheller et al., 2011). The IL-6R is usually membrane bound but can also be found soluble in inflammatory environments (IL-6sR) (Fisher et al., 2014). By binding to soluble factors, IL-6 can shift an acute inflammatory response towards a less favorable chronic inflammatory response (Scheller et al., 2011). Under these circumstances IL-6 promotes inflammation while inflammation in acute responses is reduced by IL-6 (Gabay, 2006).

In the context of cancer, IL-6 is a key mediator in the TME, promoting tumor growth, immune evasion, and angiogenesis (Abdulrauf et al., 1998; Ruoslahti, 2002). The tumor vasculature is typically unstable and disorganized, with angiogenesis influenced by factors like vascular endothelial growth factor-A (VEGF-A), which tumor cells produce themselves. IL-6 enhances VEGF expression through the STAT3 pathway, fostering tumor-friendly conditions (Abdulrauf et al. 1998; Ruoslahti 2002; Schaaf et al. 2018; Wei et al. 2003). However, IL-6 can also positively impact the immune response by modulating T-cell activity (Kumari et al., 2016). Elevated serum IL-6 levels by exercise can influence cancer progression. For instance, acute exercise increased serum IL-6, reducing colon cancer cell proliferation and suggesting improved DNA repair with regular exercise (Orange et al., 2022). Yet, sustained high serum IL-6 levels might promote tumorigenesis (Kistner et al., 2022; Orange et al., 2022; Procházka et al., 2014). IL-6 affects the immune system in cancer, with both positive and negative effects. It supports T-cell survival, proliferation, and antitumor activity, as seen in exercise-induced IL-6 increases that slowed tumor growth by boosting CD8+ T-cell metabolism in mice (Ene et al., 2022; Rundqvist et al., 2020). Conversely, IL-6 can suppress immunity by increasing myeloid-derived suppressor cells (MDSCs), as seen in hepatocellular carcinoma, or promoting pro-tumor Th17 cell differentiation in colorectal cancer (Gulubova et al., 2022; Hsieh et al., 2019). Acute IL-6 spikes from exercise may inhibit tumor growth by activating NK cells, suggesting a dual role depending on its duration and context (L. Pedersen et al., 2016).

Besides entering the tumor from the periphery, sources of IL-6 in the TME include tumor cells, CD4+ T cells, stromal cells, and macrophages (Fisher et al., 2014). In the TME, IL-6 activates STAT3 signaling, altering cancer cell gene expression and promoting an inflammatory, pro-tumor environment. Carcinoma-associated fibroblasts (CAFs) in the TME are a major IL-6 source, inducing epithelial-mesenchymal transition (EMT), which enhances migration, metastasis, and drug resistance. For example, IL-6 reduces E-cadherin and tumor protein 53 expression, facilitating invasiveness and chemotherapy resistance in prostate cancer cells (Lin et al., 2020; Sehgal, 2022; L. Wang et al., 2018; Y. Wang & Zhou, 2011).

Due to its characteristics, IL-6 pathways have become targets for cancer treatment. Promising results, like reduction in tumor growth and altering the immune response have already been observed in pre-clinical trials (Soler et al., 2023).

SPARC

SPARC, a myokine commonly found in the ECM and TME, is a family of proteins with diverse functions in both embryonic and adult tissues (M. K. Kim et al., 2020; Porter et al., 1995). It influences various biological processes, including cell cycle regulation, vascularization, matrix mineralization, and cell adhesion, though its role varies depending on the tumor type and tissue context (Arnold & Brekken, 2009; Clark & Sage, 2008). SPARC modulates cell–cell interactions and ECM communication, impacting cancer cell adhesion and migration, which can enhance metastasis. In a murine melanoma model, reduced SPARC levels lowered invasiveness and migration, yet in bladder carcinoma, SPARC appears to inhibit tumor progression, potentially by limiting inflammatory responses (Ledda et al., 1997; Nagaraju & Sharma, 2011; Said et al., 2013).

SPARC's direct effects on cancer cells remain unclear due to the lack of known receptors, though studies show its preferential binding to the extra cellular matrix (ECM) and interactions with factors such as VEGF, fibroblast growth factor (FGF), and transforming growth factor-beta (TGF-β), particularly in malignant tissues (Arnold & Brekken, 2009; Porter et al., 1995). Indirectly, SPARC expression correlates with VEGF in colon cancer, linking low expression to poorer outcomes. In renal carcinoma, SPARC acts downstream of TGF-β, promoting invasion via increased matrix metalloproteinase-2 (MPP2) expression, while in ovarian cancer, SPARC normalized the TME by downregulating VEGF (Bao et al., 2021; Liang et al., 2010; Said et al., 2007).

Exercise elevates SPARC levels acutely and longitudinally, with plasma levels returning to baseline within six hours in both humans and mice. A 12-week exercise intervention in prostate cancer patients showed trends of increased SPARC levels, though significance was not reached (Catoire & Kersten, 2015; J.-S. Kim et al., 2022; Y.-P. Liu & Hsiao, 2013). In a murine colorectal cancer model, SPARC increased after an acute exercise session and chronic, low intensity exercise has been connected to apoptotic effects mediated by SPARC (Aoi et al., 2013).

The dual role of SPARC - either inhibitory or promoting - seems dependent on the cell type and tissue of origin, warranting further investigation into these factors (Arnold & Brekken, 2009; Liang et al., 2010). Overall, SPARC is clearly influenced by exercise and holds a complex role in cancer progression, though its functions within the TME require further exploration (J.-S. Kim et al., 2022; Liang et al., 2010).

Oncostatin-M

OSM, a cytokine of the IL-6 family, binds to glycoprotein 130 (gp130) complexes and oncostatin M receptor β chains (OSMRβ chains), which are expressed on a variety of cells (Queen et al., 2005; Richards & Botelho, 2019). Produced by immune cells such as macrophages and dendritic cells, as well as by skeletal muscle, OSM qualifies as a myokine (Queen et al., 2005; Won Seok Hyung et al., 2019). It plays a role in the development of the liver, the production of blood cell, inflammation, and neural protection, making it a potential therapeutic target for several diseases. Although initially viewed as inhibitory in cancer, OSM is now recognized for its role in promoting tumor progression (Komori & Morikawa, 2022; Masjedi et al., 2021; Won Seok Hyung et al., 2019).

In the TME, OSM contributes to cancer advancement by recruiting M2 macrophages and altering CAFs. Elevated OSM levels in serum and the TME correlate with disease progression across cancer types (Caligiuri et al., 2022). Overexpression of the oncostatin M receptor (OSMR) in cancer cells enhances OSM signaling, activating STAT3 and driving angiogenesis, invasiveness, and migration through upregulation of VEGF-A and transglutaminase 2 (TGM2) (Caffarel & Coleman, 2014). Studies suggest that neutrophils in the TME increase OSM production, further promoting metastasis and tumor growth, particularly in triple-negative breast cancer (Queen et al., 2005). Further studies showed that aerobic exercise increases OSM levels in muscle and serum. This effect was also shown in mouse models of breast cancer, where tumor-associated OSM also increased after exercise (Karimi & Behboudi Tabrizi, 2018; Komori & Morikawa, 2022).

OSM directly affects the TME by facilitating EMT and cancer stem cell (CSC) characteristics through OSM/ signal transducer and activator of transcription 3 (STAT3) signaling. This pathway drives the nuclear accumulation of mothers against decapentaplegic homolog 3 (SMAD3), a transcription factor influenced by TGF-β, enhancing gene expression that promotes EMT, invasiveness, and drug resistance (Fuxe et al., 2010; Junk et al., 2017). In skin cancer models, OSM absence reduced tumor size and M2 macrophage polarization, implicating OSM as both a direct and indirect driver of cancer progression (Simonneau et al., 2018).

Indirectly, OSM promotes tumor growth by mediating stromal crosstalk among cancer cells, immune cells, and fibroblasts, creating a feedback loop that reinforces its effects (Araujo et al., 2022). A 12-week exercise study in prostate cancer patients found significant increases in serum OSM levels after aerobic and resistance training, correlating with lean body mass. However, while conditioned serum slowed cell growth in culture, a direct link was not confirmed (J.-S. Kim et al., 2022). In another recent model of oral cancer, a clear connection between slowed cancer cell growth and rising OSM levels in serum after exercise could be drawn (Yoshimura et al., 2024). Although the role of OSM in the TME remains complex, its involvement in OSM/STAT3 signaling presents a promising target for reducing drug resistance (Junk et al., 2017).

Irisin

Irisin, a myokine cleaved from the transmembrane protein fibronectin type III domain-containing protein 5 (FNDC5), is released into the bloodstream during and after exercise in both humans and mice (Boström et al., 2012; Cebulski et al., 2022). Irisin is located in various tissues, including skeletal muscle, where its expression is upregulated by the exercise-induced coactivator peroxisome proliferator-activated receptor-gamma coactivator - 1α (PGC-1α). Irisin levels rise acutely after exercise, particularly resistance or high-intensity interval training (HIIT), with higher responses observed in trained individuals, while chronic exercise appears to decrease circulating irisin levels. Notably, irisin levels decline with age and have been linked to muscle hypertrophy in mice through injection studies (Maalouf & El Khoury, 2019; Qiu et al., 2015; C.-L. Tsai et al., 2021; Tsuchiya et al., 2015).

In cancer, irisin’s role has gained interest due to its potential metabolic influence in TME. For example, reduced irisin levels in breast cancer correlate with advanced tumor progression and poorer survival outcomes, while elevated levels in renal cancer patients have been observed in some studies (Altay et al., 2018; Cebulski et al., 2022). Despite these findings, most in vitro studies suggest that irisin inhibits cancer progression (Nowinska et al., 2019).

Irisin directly affects cancer cells by influencing their metabolism and survival. In ovarian cancer cell lines, it suppressed proliferation, migration, and colonogenic potential while enhancing sensitivity to chemotherapy in a dose- and time-dependent manner. Additionally, irisin reduced HIF-1α and VEGF expression, induced apoptosis, and increased the activity of apoptosis-related proteases like caspase-3/7 in breast cancer cells (Alizadeh Zarei et al., 2023; Gannon et al., 2015). It also reduced nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activity, which regulates inflammation and cancer progression (Dolcet et al., 2005). In pancreatic cancer cells, irisin enhanced ferroptosis, a form of iron-dependent apoptosis, suggesting its potential as a therapeutic target (B. C. Yang & Leung, 2020).

Irisin levels fluctuate with exercise, increasing after acute interventions but remaining stable during chronic exercise. Its inhibitory effects on VEGF expression and varying influence on metalloproteases in aerobic metabolism highlight its complex role in cancer biology (Alizadeh Zarei et al., 2023; Löffler et al., 2015). In a recently published study on irisin and its role in metastasis formation in breast cancer, researchers found that an increase in irisin decreased cell migration and the expression of multiple factors associated with metastasis. There are for example MMP-2 and MMP-9. As an exercise intervention, the researchers chose a steady state endurance exercise, where they found only increases in irisin levels but not in other myokines (Lee et al., 2024). These results further highlight the importance of investigating which exercise type is most favorable in the context of cancer.

BDNF

Brain-derived neurotrophic factor (BDNF), a myokine and neurotrophin, plays a role in both mental health and metabolism. BDNF influences insulin secretion by binding to tropomyosin receptor kinase B T1 (TrkB T1) in pancreatic cells, demonstrating its regulation by both hippocampal and muscle activity, with peripheral metabolic effects. For instance, BDNF serum levels increased in obese patients following an eight-week moderate- or high-intensity training intervention (Fulgenzi et al., 2020; N. S. de Lima et al., 2022, 2022; Shimizu et al., 2003). However, other studies have found elevated BDNF levels within muscle rather than in the bloodstream, suggesting that BDNF primarily acts in an autocrine and paracrine manner, limiting its peripheral release and potential influence on the TME (Brandt & Pedersen, 2010; Matthews et al., 2009).

Despite uncertainties about its systemic effects, BDNF is implicated in cancer progression. It enhances properties that promote metastasis, angiogenesis, and resistance to chemotherapy. The discrepancies in findings regarding BDNF's systemic presence—whether from central nervous system or muscle activity—and its role in serum levels leave its potential impact on the TME unresolved (Malekan et al., 2023).

CXCL9

CXCL9 is a chemokine involved in multiple processes but particularly in immune regulation and inflammation. It interacts with the G-protein-coupled chemokine receptor CXCR3, a receptor it shares with C-X-C motif chemokine ligand 10 (CXCL10) and C-X-C motif chemokine ligand 11 (CXCL11). Depending on its isoform, CXCR3 mediates distinct cellular responses: CXCR3A promotes tumor cell proliferation and survival via pathways like ERK1/2 and Akt, while CXCR3B induces apoptosis and inhibits angiogenesis by counteracting VEGF signaling. A range of cells, including leukocytes, macrophages, dendritic cells, fibroblasts, and tumor cells secretes CXCL9. Its effects within the TME can be both autocrine and paracrine in nature (Pan et al., 2023).

In cancer in general, CXCL9 displays a dual role, reflecting its ability to modulate immune cell activity and influence tumor behavior. Elevated serum levels of CXCL9 have been detected in various cancers and are associated with immune cell recruitment to the TME, particularly CD8+ T cells, which enhance anti-tumor activity. However, its effect varies by cancer type. For instance, in breast cancer, high CXCL9 expression correlates with increased tumor cell proliferation, as evidenced by elevated Ki67 levels, and generally indicates a poor prognosis. Conversely, in triple-negative breast cancer (TNBC), high CXCL9 levels are associated with better outcomes, likely due to their role in enhancing immune responses (Razis et al., 2020). CXCL9 is predominantly expressed by macrophages in TNBC, and its expression decreases when macrophages polarize to an M2 phenotype. This suggests that CXCL9 could serve as a marker for effective immunotherapy in TNBC, with its effects mediated through pathways such as Janus kinase/signal transducers and activators of transcription (JAK/STAT) and phosphatidylinositol 3-kinase/ protein kinase B (PI3K/AKT), which influence macrophage polarization and immune cell activity (Wu et al., 2023).

In the TME, CXCL9 regulates immune infiltration, angiogenesis, and metastasis. In prostate cancer models, CXCL9 signaling has been linked to reduced cytokine activity in T cells, resulting in cancer progression (Tan et al., 2018). Additionally, CXCR3B-mediated signaling on vascular endothelial cells limits tumor angiogenesis, further highlighting its anti-tumor potential under certain conditions (Pan et al., 2023). On the other hand, higher circulating CXCL9 levels in older men have been associated with increased mortality, suggesting a complex and context-dependent role (Da Seo et al., 2024).

Exercise has been shown to influence CXCL9 expression and its downstream effects, which could have implications for cancer therapy. For instance, regular physical activity can enhance CXCL9 expression and its receptor CXCR3, potentially normalizing tumor vasculature and reprogramming the immune TME to improve CD8+ T cell-mediated antitumor activity. Murine models of breast cancer demonstrated that these exercise-induced changes enhanced the efficacy of immunotherapy (Gomes-Santos et al., 2021). Interestingly, while exercise benefits are often linked to CXCL9, not all studies report significant changes in its expression following physical activity. For example, a 12-week home-based training protocol did not alter CXCL9 levels in cancer patients (Filgueira et al., 2022). Nevertheless, these findings suggest a promising avenue for integrating exercise into cancer treatment strategies, particularly for patients undergoing immunotherapy (J. Liu et al., 2024).

3. Description of the Scientific Publications

Results of one narrative review and one human study conducted as part of the present doctoral thesis were each published in a peer-reviewed scientific journal:

Gunasekara, N, Clauss, D., & Bloch, W. (2024). Effects of Exercise-Induced Changes in Myokine Expression on the Tumor Microenvironment. Sports Medicine International Open, 8, a22831663. https://doi.org/10.1055/a-2283-1663

Gunasekara, N., Clauss, D., Voss, A., Schurz, K., Fleck, K., Neu-Gil, P., & Bloch, W. (2025). The Influence of an Acute Endurance Intervention on Breast Cancer Cell Growth—A Pilot Study. International Journal of Molecular Sciences, 26(9), 3976. https://doi.org/10.3390/ijms26093976

The literature research for the narrative review (Gunasekara et al., 2024) was conducted between January and July 2023 and handed in in October 2023. The aim of this review was to describe the most commonly known myokines, their role in the TME and which type of exercise, if known, induces them. Specifically, it was proposed that myokines released during physical activity would contribute to modulating the TME by enhancing anti-inflammatory responses, promoting immune cell infiltration, and reducing tumor-supportive processes such as angiogenesis and tissue hypoxia.

Given that prior research had largely focused on the systemic benefits of exercise for cancer prevention and recovery, the study aimed to address a significant gap by examining localized effects of myokine expression on the cellular and molecular composition of the TME. As study density on this topic is low, in vitro and in vivo models were taken into account.

Key outcomes of the studies included assessments of changes in inflammatory cytokines, immune cell recruitment markers, and tumor cell apoptosis rates within the TME. Furthermore, oxygenation levels, angiogenesis markers, and extracellular matrix remodeling were monitored in some studies to understand the broader implications of myokine activity in altering the TME. The results show that certain myokines, IL-6 and irisin, were significantly upregulated after exercise. They further demonstrated potent anti-tumor effects in vitro, including the inhibition of angiogenic signaling pathways and the induction of apoptotic pathways in tumor cells (Gunasekara et al., 2024).

In conclusion, the review showed that exercise-induced myokine expression plays a critical role in modulating the TME, supporting the notion that physical activity is not only beneficial for overall health but may also serve as an adjunctive strategy in cancer treatment. In the context of this doctoral thesis, these findings lay the foundation for further investigation of the molecular pathways underlying myokine-TME interactions, with potential implications for the development of pharmacological agents that mimic exercise.

The second publication is based on a pilot study that aimed to investigate the effects of a serum conditioned by an acute endurance intervention on breast cancer cells (Gunasekara et al., 2025). The study had a cross over design to investigate whether the changes observed can be clearly related to the exercise intervention or if other factor, e.g. the circadian rhythm, influence cancer cell growth. Triple negative breast cancer cells (MDA-MB-231) were chosen, because breast cancer is the most diagnosed type of cancer in women and by choosing hormone insensitive cells, these effects could be ruled out. The main hypothesis of the pilot study was that serum conditioned by an acute endurance exercise would inhibit the growth of MDA-MB-231 breast cancer cells in vitro. It was presumed that changes in cytokine concentrations induced by exercise would directly impact tumor cell proliferation and viability, possibly through alterations in the TME. To investigate this, serum samples were collected from 11 sedentary female participants at three time points: before (T0), immediately after (T1), and two hours post-exercise (T2). The participants performed a one-hour cycling session at moderate intensity, and a control condition with rest instead of exercise was included. The serum samples were then used to culture MDA-MB-231 cells. Cellular proliferation and viability were assessed using MTT assays and Ki-67 immunohistochemistry. A cytokine array identified significant exercise-induced changes in specific cytokines, and further experiments utilized CXCR3 inhibitors to explore mechanistic pathways.

Key findings included a significant reduction in cell proliferation and viability immediately post-exercise (T1), but not after two hours (T2). Cytokine analysis highlighted CXCL9 and CCL15 as potential mediators of the observed effects. Blocking the CXCL9 pathway with a CXCR3 antagonist (AMG487) further inhibited cell growth, supporting the hypothesis that exercise-induced cytokine changes contribute to anti-cancer effects. In conclusion, the study showed that acute endurance exercise alters serum composition in a way that directly inhibits breast cancer cell growth in vitro, with CXCL9 implicated as a key regulatory factor. These findings underline the importance of acute exercise in modulating the TME and provide a basis for exploring exercise as an adjunctive strategy in cancer management.

Above-described scientific papers are enclosed as published in their respective journal.

4. Summarizing discussion

Exercise is becoming an increasingly important part of cancer treatment as it offers a range of benefits for patients' well-being and potentially influence cancer progression. It has been consistently shown to alleviate treatment-related side effects, such as fatigue and reduced physical function, which are common among cancer patients, improving their overall quality of life (Idorn & Thor Straten, 2017; Schmitz et al., 2010). Observational studies also suggest that physical activity can lower cancer-related mortality, particularly in breast and colorectal cancer survivors, reinforcing its value as part of comprehensive care (Ballard-Barbash et al., 2012; Hojman et al., 2018). Evidence from animal studies further supports these findings, demonstrating that regular exercise reduces tumor size, potentially through mechanisms that include enhanced immune surveillance and altered tumor metabolism (Hojman et al., 2018; L. Pedersen et al., 2016).

The role of skeletal muscle as an endocrine organ is central to understanding how exercise exerts these effects. Muscle contractions stimulate the release of myokines, signaling proteins that influence local and systemic processes, including those within the TME (B. K. Pedersen & Febbraio, 2012). Myokines may play an important role in regulating immune cell recruitment, vascular function, and cell metabolism, which are critical components of the TME. Prior research has predominantly focused on singular myokines, such as IL-6 or irisin, highlighting their ability to reduce tumor proliferation and induce apoptosis in vitro in human studies and in vivo in animal models (Dethlefsen et al., 2016; Rundqvist et al., 2020). However, these studies often overlook the interconnected nature of myokine activity, which can result in synergistic or antagonistic effects as well as direct effects on cancer cells themselves (L. Pedersen & Hojman, 2012).

This thesis proposes that changes of cytokine concentration in serum through exercise collectively influence breast cancer cell growth through possible modulations of the TME. Using cytokine arrays to analyze serum-conditioned media from an acute moderate endurance exercise intervention, this thesis demonstrates that exercise induces significant changes in cytokine concentrations (Gunasekara et al., 2025). These findings align with the seminal work of L. Pedersen and Hojman (2012), who identified the regulatory role of muscle-derived cytokines in mediating immune responses and metabolic shifts during exercise. Further, the study of Rundqvist et al. (2013) showed that changes in prostate cancer cell growth were caused by conditioned serum. These results were connected to changes in CD8+ cell activation later on (Rundqvist et al., 2020). This thesis underlines that exercise can have a direct impact on cancer cells, with acute endurance exercise eliciting immediate changes in TME-related pathways, as evidenced by reduced proliferation of breast cancer cells in vitro (Dethlefsen et al., 2016; Gunasekara et al., 2025). In particular, the chemokine CXCL9 emerged to be a promising modulator, potentially influencing immune infiltration and angiogenesis in the TME (Gunasekara et al., 2025). Although the role of CXCL9 requires further investigation, these findings underscore its potential relevance in exercise-mediated cancer modulation.

Despite these advances, important questions remain. The impact of resistance training on cancer biology has yet to be fully explored. Endurance and resistance exercise lead to changes in the concentration of different myokines, each of which can trigger individual biological responses. As a result, the physiological effects and potential benefits of different types of exercise in cancer patients can vary. These differences highlight the importance of tailoring training approaches towards the individual needs (Gunasekara et al., 2024). Given that resistance exercise promotes muscle hypertrophy and may increase the amount of myokines secretion, it is possible that resistance exercise has greater benefits than currently known (L. Pedersen & Hojman, 2012). Additionally, the study performed in the context of this thesis was conducted with sedentary participants; future research should assess whether similar effects occur in physically active or athletic populations, where baseline myokine levels and responses to exercise may differ (Hojman et al., 2018; B.K. Pedersen & Febbraio, 2012). Current evidence suggests that the anti-cancer benefits of exercise may be cumulative, driven by repeated immune and metabolic adaptations over time, yet acute exercise bouts also appear to hold significant therapeutic potential, particularly when integrated with immunotherapy (Dethlefsen et al., 2016; L. Pedersen et al., 2016).

4.1. Myokines and their relatedness to cancer

Myokines play complex roles in cancer biology, influencing both tumor progression and suppression depending on the type of cancer, tumor stage, and exercise modality. These dual effects are due to their involvement in processes such as the regulation of inflammatory reactions, angiogenesis, and metabolism. While some myokines such as IL-6, SPARC, and irisin demonstrate anti-tumor effects by enhancing immune cell infiltration or inducing apoptosis in cancer cells, others may promote tumor growth under chronic inflammatory conditions or when their signaling pathways are dysregulated (Hojman et al., 2018; B. K. Pedersen & Febbraio, 2012). This variability underscores the necessity to understand myokine activity in the context of the TME, which is highly individualized and dependent on factors such as cancer type and exercise characteristics (Brandt & Pedersen, 2010; J.-S. Kim et al., 2021; L. Pedersen & Hojman, 2012; Severinsen & Pedersen, 2020).

Exercise can influence cancer progression through both direct and indirect mechanisms, with each pathway playing a distinct but complementary role. Direct effects refer to the immediate changes that occur within the TME due to factors released during and shortly after physical activity. Indirect effects, on the other hand, involve systemic adaptations that emerge over time, such as enhanced immune surveillance and metabolic health, which collectively contribute to a less favorable environment for tumor growth and metastasis (Gunasekara et al., 2024).

Direct effects of exercise are primarily mediated by the acute release of myokines and other metabolites into the bloodstream. These molecules can act directly on cancer cells and the TME, modulating cellular processes such as proliferation, apoptosis, angiogenesis, and immune cell recruitment. For instance, acute endurance exercise has been shown to increase levels of myokines like IL-6 and irisin, which can suppress cancer cell growth through pathways involving mitochondrial stress and caspase-mediated apoptosis. IL-6, for example, acts in an immune-modulating capacity by enhancing the activity of NK cells and CD8+ T cells, which are crucial for the immune surveillance of tumor cells (Orange et al., 2022; L. Pedersen & Hojman, 2012; Petersen & Pedersen, 2005). Furthermore, irisin has been demonstrated to reduce cancer cell proliferation and migration in breast and ovarian cancer cells, possibly by influencing energy metabolism and reducing hypoxia-induced resistance (Alizadeh Zarei et al., 2023). These direct effects suggest that exercise induces rapid, localized changes in the TME that may directly inhibit cancer cell survival and growth, which is supported by the finding that conditioned serum after an acute exercise session significantly reduced breast cancer cell growth (Gunasekara et al., 2025).

In addition to these direct impacts on tumor biology, exercise also elicits a series of indirect effects that influence the TME, which can support longer-term cancer control. These effects include improvements in immune function, reductions in systemic inflammation, and enhanced metabolic health. Chronic exercise, particularly moderate-intensity endurance training, has been shown to promote the production of myokines such as IL-15 and SPARC, which activate and sustain immune cells, such as NK cells and T cells enable the body to better recognize and eliminate cancer cells (J.-S. Kim et al., 2021; J.-S. Kim et al., 2022). Additionally, exercise reduces levels of pro-inflammatory cytokines like TNF-α and IL-6, which are often elevated in cancer patients and contribute to tumor progression by promoting immune suppression and angiogenesis. By shifting the cytokine profile towards a more anti-inflammatory state, chronic exercise creates a more favorable environment for immune-mediated tumor suppression (Ene et al., 2022).

Furthermore, exercise influences metabolic factors, such as insulin sensitivity and glucose metabolism, which play a significant role in cancer cell survival. The exercise-induced improvement in insulin sensitivity and glucose uptake by muscle cells lower circulating glucose levels, potentially starving tumor cells that rely heavily on glycolysis for energy (Richter & Hargreaves, 2013; Shang, 2007). Regular exercise also enhances fat oxidation and reduces the accumulation of fatty acids, which may disrupt the metabolic environment that tumors depend on for rapid growth (Matthews et al., 2009).

The findings in this thesis support the notion that exercise exerts both direct and indirect effects on cancer progression, which may work in tandem to reduce tumor growth and improve treatment outcomes. The acute effects of exercise, such as the release of myokines and other metabolites, have direct impacts on cancer cells by inducing apoptosis and modulating the TME, while chronic exercise promotes systemic changes that improve immune function, reduce inflammation, and optimize metabolic health, all of which help to create a less favorable environment for cancer progression. This dual mechanism suggests that exercise should be considered not only as an adjunct therapy to enhance immune function but also as a direct means to influence tumor biology, particularly when integrated with immunotherapy and other treatment modalities (Gunasekara et al., 2024; Gustafson et al., 2021; Sanft et al., 2023; Wiggins et al., 2018).

The findings of this thesis, alongside existing research also suggest that moderate and regular exercise elicits the most favorable myokine-mediated responses in cancer care. Moderate-intensity endurance exercise optimizes the release of myokines that promote anti-inflammatory effects, improve vascularization, and recruit cytotoxic immune cells to the TME (Gunasekara et al., 2024). For example IL-6 exhibits both pro-inflammatory and anti-inflammatory properties depending on the signaling pathway engaged. Exercise-induced acute spikes in IL-6 activate anti-inflammatory effects via the IL-6R and STAT3 signaling, improving immune surveillance and promoting CD8+ T-cell activity in the TME. Conversely, chronic, low-grade elevations in IL-6, often observed in sedentary individuals, are associated with tumor-promoting inflammation and immune suppression (Dethlefsen et al., 2016; L. Pedersen & Hojman, 2012). SPARC, another key myokine, is known for its role in extracellular matrix remodeling and angiogenesis. It exhibits tumor-suppressive effects in certain cancers, such as colon cancer, by normalizing the vasculature within the TME and inhibiting metastasis. In contrast, SPARC can enhance tumor invasiveness in cancers like melanoma, depending on its interaction with MMPs and growth factors such as VEGF (Aoi et al., 2013; Liang et al., 2010). Similarly, irisin, derived from the cleavage of FNDC5 during exercise, has been shown to reduce cancer cell proliferation and migration by modulating mitochondrial function, VEGF expression, and apoptosis-related pathways, making it a promising target for further investigation (Alizadeh Zarei et al., 2023).

This thesis extends previous research by highlighting the interconnectedness of myokine activity, suggesting that myokines influence one another and interact synergistically to modulate the TME. Acute moderate exercise significantly alters the composition of circulating cytokines, as demonstrated by the cytokine array analysis in this study, which revealed a notable upregulation of CXCL9. This chemokine is implicated in the recruitment of immune cells, particularly CD8+ T cells, to the TME. Through interactions with the CXCR3 receptor, CXCL9 enhances anti-tumor immunity while reducing angiogenesis by competing with VEGF signaling (Pan et al., 2023; Tan et al., 2018).

The results also suggest that acute exercise bouts may exert a more direct influence on tumor cells than previously appreciated. For instance, myokines such as IL-6 and irisin can directly inhibit cancer cell proliferation through mechanisms like mitochondrial stress and activation of caspase-mediated apoptosis. Furthermore, their ability to transiently alter the TME, reducing factors like hypoxia and lactate accumulation, creates a less hospitable environment for cancer cell survival. This direct effect highlights the therapeutic potential of each exercise session, particularly in conjunction with immunotherapy, where enhanced immune infiltration and TME normalization could amplify treatment efficacy (Dethlefsen et al., 2016; L. Pedersen et al., 2016). Together, these direct and indirect effects highlight the multifaceted role of exercise in cancer therapy, emphasizing the need for further research to better understand the underlying molecular mechanisms and the optimal exercise protocols to maximize therapeutic benefit. A potential modulator of breast cancer cell growth, influenced by acute endurance exercise, will be discussed in the following chapter.

4.2 CXCL9 as a potential modulator of the tumor microenvironment of breast cancer cells

CXCL9, a chemokine belonging to the CXC family, has emerged as a promising modulator of the TME. This chemokine plays a pivotal role in immune cell recruitment, particularly T cells, to the TME, where it can influence both tumor progression and immune responses. CXCL9 functions through its receptor, CXCR3, which is expressed on a variety of immune cells, including CD8+, NK cells and macrophages. Its expression is often associated with favorable outcomes in cancer, as it enhances immune infiltration into tumors, potentially improving the body's ability to control cancer growth (Pan et al., 2023; Razis et al., 2020).

The research conducted in this thesis underscores the role of CXCL9 as a key player in the exercise-induced modulation of the TME, particularly in the context of breast cancer. Acute endurance exercise was shown to downregulate CXCL9 concentration in serum samples, indicating that physical activity may increase its expression and thus enhance immune surveillance within the TME. CXCL9 has the ability to recruit and activate immune cells, especially CD8+ T cells, which are crucial for targeting and eliminating cancer cells. This process is central to the potential therapeutic effects of exercise in combination with immunotherapy, as CXCL9 could enhance the recruitment of cytotoxic T cells to the tumor site, where they can exert their anti-tumor activity (Gunasekara et al., 2025; Pan et al., 2023).

Additionally, CXCL9 appears to exert its anti-tumor effects by modulating angiogenesis and reducing the supply of nutrients to the tumor. It has been shown that CXCL9, which is signaled via CXCR3B on endothelial cells, inhibits angiogenesis and thus restricts the vascular supply to tumors. This is particularly important because tumor blood vessels are often disorganized and leaky, leading to hypoxia and an environment that promotes tumor growth. By modulating angiogenesis, CXCL9 can normalize the blood vessels in the TME, thereby improving oxygen and nutrient delivery to tissues and potentially making the tumor more susceptible to immune cell infiltration (Pan et al., 2023).

Despite these promising findings, the role of CXCL9 in cancer progression is complex and may be context-dependent. In some cancers, elevated CXCL9 levels have been associated with poorer prognosis, particularly when the chemokine’s activity promotes the recruitment of immune suppressive cells, such as Tregs which can dampen anti-tumor responses (Razis et al., 2020). This duality in CXCL9’s role suggests that its effects may vary depending on factors such as the stage of cancer, the immune cell populations present, and the specific characteristics of the TME (Da Seo et al., 2024; Pan et al., 2023; Tan et al., 2018).

In breast cancer, particularly TNBC, CXCL9 has been associated with more favorable outcomes, as it recruits immune cells that are critical for anti-tumor immunity. The exercise-induced upregulation of CXCL9 observed in this study supports the idea that physical activity may enhance the immune system's ability to target and eliminate tumor cells, particularly in immune-responsive cancers like TNBC. Moreover, CXCL9's ability to inhibit angiogenesis and normalize tumor vasculature could further strengthen the TME's anti-tumor properties, making it a potential target for therapies aimed at enhancing tumor blood flow or promoting immune cell activity within the TME (Razis et al., 2020; Wu et al., 2023).

While the results of this thesis suggest that CXCL9 plays a promising role in modulating the TME, further research is necessary to fully elucidate its molecular mechanisms in different cancer types and stages. The effects of CXCL9 may also depend on the type and intensity of exercise, as well as the presence of other myokines and inflammatory cytokines that could influence its expression and activity. For example, understanding how CXCL9 interacts with other immune modulators like IL-6, SPARC, and irisin will be critical for developing more targeted exercise interventions that maximize its therapeutic potential (L. Pedersen & Hojman, 2012; Razis et al., 2020; Tan et al., 2018; Wu et al., 2023).

In summary, CXCL9 may be a main modulator of the TME, with the potential to improve immune cell infiltration and angiogenesis in breast cancer. Exercise appears to increase CXCL9 expression, making it a promising target for therapeutic strategies that integrate physical activity with cancer treatments, such as immunotherapy. These findings underscore the importance of continued investigation into the role of CXCL9 and other myokines in cancer biology, as they may provide new pathways for enhancing cancer treatment and improving patient outcomes. Further detailed investigations are needed to investigate the specific effects of different exercise modalities and their frequency on physiological and molecular adaptations (Gunasekara et al., 2025).

It should be taken into account, that only a small number of female participants was tested in the pilot study described. To gain further insight it is necessary to increase this number and include male participants. Furthermore, it is possible that other cancer types react differently to conditioned serum, which highlights the importance to repeat the described experiments on different cancer cell lines. These interventions would make the findings more robust. Aside from these limitations, the mechanisms that were observed apply to healthy women. In the future, these investigations should be performed on a homogenous group of cancer patients to find out if exercise can have the same effects in people who are already in treatment for the disease as this would be more closely related to the conditions exercise is applied as treatment support.

4.3 Summary and outlook

In summary, moderate and regular exercise has the most substantial impact on cancer biology, both through direct effects on tumor cells and indirect systemic adaptations that enhance immune function and tissue homeostasis. Acute exercise bouts appear particularly relevant for their immediate influence on the TME, supporting the notion that every exercise session contributes meaningfully to cancer care. This finding is especially significant in the context of immunotherapy, where myokine-mediated changes in immune infiltration and tumor metabolism may synergize with treatment to improve clinical outcomes (Gunasekara et al., 2024; B. K. Pedersen & Febbraio, 2012). Moderate and regular exercise also appears to have the most substantial impact on the TME, balancing direct effects on cancer cells with broader systemic changes that enhance immune function and tissue oxygenation. Consistent with current exercise recommendations of 150 mins per week, this thesis supports that every exercise session matters, especially in the context of direct anti-cancer effects (Wilson et al., 2023). These findings not only support the integration of exercise into cancer care but also pave the way for more precise, myokine-focused interventions that could further optimize patient outcomes.

The chemokine CXCL9 is already discussed as an important component of TME and immune modulation (Da Seo et al., 2024). The findings of this thesis support the fact, that CXCL9 might an important mediator of anti-cancer effects in triple negative breast cancer cells. It highlights its role in exercise mediated alterations of cancer-cell growth. Furthermore, this thesis found that conditioned serum drawn immediately after an intervention but no after a 2 hours rest period had a direct growth inhibitory effect on breast cancer cells. These are important new findings that requires further investigations. The results discussed in this thesis were achieved with an acute moderate endurance exercise. To complete the picture of CXCL9s role in exercise mediated changes, it is necessary to investigate the impact of acute resistance training as well as chronic effects of exercise on the chemokine. The same goes for the growth inhibitory effects of conditioned serum from the above mentioned conditions.

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5. Appendix

Appendix A - Publication 1

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Review

Thieme

Effects of Exercise-Induced Changes in Myokine Expression on the Tumor Microenvironment

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ABSTRACT

In this narrative review, we summarize the direct and indirect effects that myokines have on the tumor microenvironment. We took studies of various cancer types and species into account. Systematic reviews and meta-analyses that matched the search terms were also considered. We searched databases for six months. As a narrative approach was chosen, no data was analyzed or reanalyzed. The goal of this narrative review is to create an overview on the topic to identify research gaps and answer the questions as to whether myokine expression may be relevant in cancer research in regard to the tumor microenvironment. Six commonly known myokines were chosen. We found strong links between the influence exercise has on interleukin-6, oncostatin M, secreted protein acidic and rich in cysteine, and irisin in the context of tumor progression and inhibition via interactions with the tumor microenvironment. It became clear that the effects of myokines on the tumor microenvironment can vary and contribute to disease progression or regression. Interactions among myokines and immune cells must also be considered and require further investigation. To date, no study has shown a clear connection, while multiple studies suggest further investigation of the topic, similar to the effects of exercise on myokine expression.

Introduction

The positive influence of exercise on cancer has been shown in multiple contexts. For instance, exercise may prevent the onset of colon cancer and improve overall quality of life in patients with various cancer types while also reducing side effects caused by medication, such as fatigue [1–5]. Additionally, in vivo studies suggest that exercise might inhibit tumor growth itself [6]. However, the underlying mechanisms of these effects as well as the cause of slowed tumor growth through exercise remain to be elucidated [7]. One explanatory approach toward this topic is changes within the surroundings of the tumor itself, the so-called tumor microenvironment (TME) [8].

The TME surrounds the tumor and consists of nonmalignant as well as malignant cells. The interactions between these various cell types create the TME [9]. While the cells that make up the TME vary between individuals as well as cancer types, the main components are the extracellular matrix, immune cells, the vascular system, and stromal cells [10]. The TME is an important contributor to metastasis, tumor formation, and therapy response. It plays a role in each step of tumorigenesis, from ensuring cancer cell survival in the early stages to cell evasion in the later stages [11]. The tumor influences the TME to favor vascularization and evade the body's immune response, and depending on the type of immune cells within the TME, they can either inhibit tumor growth or promote inflammation and thereby favor it [7, 11]. Furthermore, the efficiency of therapies depends on the type of immune cells in the TME [12]. As the composition of the TME may determine the outcome and alter the prognosis, it is favorable to find ways in which it can be influenced [13]. In this review, the components of the TME are not described in detail as a review on this topic has been previously published [11].

Changes in the TME are an important factor in tumor regulation and can be caused by exercise [14]. As there are strong indications that exercise can influence tumor growth, changes in the TME may be one important aspect [15]. Four known entities can alter the TME through exercise. These are vascularization, the immune system, cancer cell metabolism, and myokines [7, 16]. While recent reviews on the topic have focused on the changes in the TME as a whole, to our knowledge there are no reviews focusing on cancer-muscle crosstalk concerning the TME. In recent years, there have been a number of studies on cancer-muscle crosstalk, which evokes the necessity to summarize these findings to further concentrate on the direct influence of muscle activity on changes in the TME and therefore possibly tumor growth [8].

Myokines are proteins that are released by contracting skeletal muscles and function in a similar way to hormones. Some are also classified as cytokines. They play a role in the prevention of multiple chronic diseases, e. g., breast cancer, type 2 diabetes and cardiovascular diseases. While these are fundamentally different diseases, there appear to be similar underlying mechanisms that can be influenced by myokines [17]. To date, only a few myokines have been identified and connected with a specific function that can be executed in an endocrine, paracrine or autocrine manner [18]. Myokines are involved in various communication pathways, including muscle-organ crosstalk and metabolism as well as vascularization [7]. Involvement in other pathways is very likely. First hints toward the effects that muscle activity has on cancer were presented in a study that showed that patients with higher muscle strength had a lower risk of developing cancer compared to patients with lower muscle strength [19]. Aerobic exercise has been suggested to increase vascularization in previously oxygen-low areas of the tumor, which can enhance the immune and drug response [8]. Myokines influence the transcription factors responsible for vascularization; therefore, exercise can normalize vascularization and metabolism within the TME [7].

While the number of known myokines is within thousands, the most prominent ones that are likely to influence the TME will be discussed in this review [18]. The effects of myokines can be local or systemic, thereby affecting cells directly and indirectly [20]. Most myokines show effects within the muscle tissue, but the ones that enter the bloodstream can have direct as well as indirect effects, depending on the target cell via the bloodstream. Myokines can influence immune cells and have an indirect effect on tumor cells via various immune cells, or they can have a direct effect if they come in contact with the tumor cells [16,21,22]. Therefore, one can assume that there are direct and indirect effects on the TME [18]. This distinction will be used as a structure to describe the effects of myokines on the TME.

Materials and Methods

A literature search of PubMed and Google Scholar was conducted between January and July 2023. As literature density regarding the topic of this review is low, a narrative approach was chosen to give an overview of the topic [23]. First, the search terms in ▶ **Table 1** were defined. We used a primary search term, the name of the myokine, and added the listed variations to our primary search terms. We included systematic reviews, meta-analyses and original studies on all species. Articles were excluded if they were not in English, were grey literature or if the full text version was unavailable to us. Supplementary references were recognized from the articles we found during our first search.

▶ **Table 1** Search terms.

Primary search term	***Variations added to the primary search term***						
Interleukin-6	AND myokine	AND cancer	AND tumor microenvironment	AND exercise	AND cancer AND tumor microenvironment	AND exercise AND cancer	AND exercise AND tumor microenvironment
IL-6	AND myokine	AND cancer	AND tumor microenvironment	AND exercise	AND cancer AND tumor microenvironment	AND exercise AND cancer	AND exercise AND tumor microenvironment
Oncostatin M	AND myokine	AND cancer	AND tumor microenvironment	AND exercise	AND cancer AND tumor microenvironment	AND exercise AND cancer	AND exercise AND tumor microenvironment
SPARC	AND myokine	AND cancer	AND tumor microenvironment	AND exercise	AND cancer AND tumor microenvironment	AND exercise AND cancer	AND exercise AND tumor microenvironment
Osteonectin	AND myokine	AND cancer	AND tumor microenvironment	AND exercise	AND cancer AND tumor microenvironment	AND exercise AND cancer	AND exercise AND tumor microenvironment
Irisin	AND myokine	AND cancer	AND tumor microenvironment	AND exercise	AND cancer AND tumor microenvironment	AND exercise AND cancer	AND exercise AND tumor microenvironment
BDNF	AND myokine	AND cancer	AND tumor microenvironment	AND exercise	AND cancer AND tumor microenvironment	AND exercise AND cancer	AND exercise AND tumor microenvironment

Results

Myokines and their response to exercise

Different types of exercise can have different influences on myokine expression [24]. Effects by exercise can be distinguished in acute changes in myokine expression that are present directly after exercise or chronic changes that are only present after a prolonged intervention period [25].

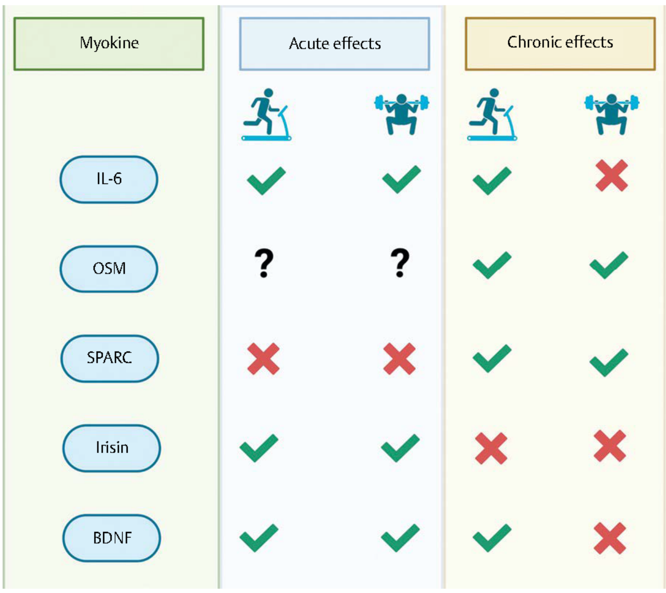
Interleukin-6 (IL-6) levels in men and women decreased after an endurance intervention over the time period of eight months, which was observed in combination with a lowered inflammatory status [26]. Contrary to these findings, IL-6 levels remained similar in men who performed either strength, concurrent, or endurance training for 16 weeks [27]. In a study on middle-aged women who performed resistance training for 16 weeks, a decrease in IL-6 levels was observed 48 h after a training session [28]. In marathon runners, an increase in IL-6 plasma concentration was observed directly after the race, while values correlated with the intensity of the run [29]. Changes in oncostatin M (OSM) levels were observed after a 12-week resistance training intervention in prostate cancer patients and also after a six-month intervention which included endurance and resistance training [30, 31]. The levels of secreted protein acidic and rich in cysteine (SPARC) are not changed through sprints or directly after resistance training in young men [32, 33]. On the other hand, in prostate cancer patients serum and relative SPARC levels increased after a six-month intervention phase consisting of endurance and resistance training [31]. Irisin levels in plasma were elevated directly after endurance and resistance training in men and women [34]. It was also recently observed that the increase in irisin levels in endurance exercise is related to its intensity. High intensity interval training therefore resulted in higher levels compared to high volume training [35]. After a 26-week intervention of combined exercise, no changes in irisin levels were found [36]. Brain-derived neurotropic factor (BDNF) levels in older men were elevated acutely after resistance as well as endurance exercise [37]. After a 26-week endurance intervention in younger men, BDNF levels were decreased overall [38]. In older women, a 22-week resistance training intervention resulted in no changes in BDNF levels [39].

▶ **Fig. 1** summarizes the acute and chronic changes of myokine concentrations discussed in this review in serum or plasma, divided by resistance and endurance exercise.

Myokines and their influence on the TME

Interleukin-6

The most popular and first discovered myokine is IL-6, which is involved in pathways that regulate muscle hypertrophy as well as cellular oxygen uptake and fat metabolism [18]. In addition to its



▶ **Fig. 1** The myokines discussed in this review are listed on the left. The middle column shows whether or not acute effects caused either by resistance or endurance training are reported for each myokine. On the right it is shown if chronic effects have been reported. These can either be elevations or reductions of myokine concentration in serum or plasma. Abbreviations: IL-6 = Interleukin-6, OSM = Oncostatin M, SPARC = secreted protein acidic and cysteine rich, BDNF = brain derived neurotropic factor. Created with BioRender.com [rerif] metabolic function, IL-6 is also a key player in chronic and acute inflammation. In acute inflammatory processes, IL-6 stimulates the production of most proteins whose increase marks the beginning of an acute inflammatory response [40]. Overall, the effect of IL-6 in acute inflammation is preservative, as the amount of anti-inflammatory cytokines remains intact while pro-inflammatory cytokines are repressed. Normally, IL-6 binds to the membrane-bound non-signaling IL-6 receptor α (IL-6Rα). The resulting complex can then bind to the signal-transducing subunit glycoprotein 130 (gp130), which is expressed on most cell types. This process actively limits the IL-6 pathway, as only two cell types, hepatocytes and leukocytes, express IL-6Rα. Apart from the usual pathway, IL-6 can bind to soluble interleukin-6 receptor α (sIL-6Rα) [41]. This receptor is usually membrane-bound but can be found in fluids after being shed from neutrophil membranes in highly inflammatory environments. The resulting IL-6/IL-6Rα complex favors chronic inflammation by promoting the shift of neutrophils into monocytes [40]. The soluble complex also binds to gp130, and as the local restriction is forfeited, IL-6 signaling can take place in every cell [41]. In the context of inflammation, IL-6 is therefore a two-sided sword, as it mediates the transition from acute to chronic inflammation by interacting with the sIL-6Rα receptor. While it exhibits anti-inflammatory properties in the acute response, it promotes inflammation in chronic events [42]. In chronic inflammatory diseases, IL-6 is therefore already used as a target for treatment [43].

In cancer, IL-6 generally has a negative impact, as its signaling is connected to disease progression in humans and mouse models. Among these negative impacts are the avoidance of apoptosis, favoring migration and metastasis as well as angiogenesis. The vasculature of a tumor corresponds to its malignancy, and the process of angiogenesis in tumors diverges from normal angiogenic processes [44, 45]. The vasculature of a tumor is generally more unstable and unorganized than healthy vasculature [45]. Vascular endothelial growth factor-A (VEGF-A), a promotor of early-stage angiogenesis, is highly available in the TME, as it is produced directly by tumor cells [46]. IL-6 has been shown to favor angiogenic processes by upregulating vascular endothelial growth factor (VEGF) via the signal transducer and activator of transcription 3 (STAT3) pathway [47]. In the TME itself, IL-6 favors a tumor-friendly environment, but similar to inflammation, IL-6 can also have a positive effect in cancer [48]. In an indirect manner, IL-6 can influence the response of T cells toward an active immune response. Janus kinases (JAKs) within the TME are activated as part of the IL-6 pathway, which results in STAT3 signaling, which will be discussed in more depth later on. Downstream of this pathway, multiple transcription factors can be activated that navigate the pro-tumorigenic IL-6 response [41].

According to current knowledge, the origin of IL-6 in the TME is tumor cells themselves, $CD4^+$ T cells, stromal cells, and macrophages [41]. IL-6 levels can also be increased in the serum. The main source here is contracting muscles [49]. For example, an acute endurance exercise intervention led to an increased IL-6 concentration in the serum of men who have lifestyle risks for colon cancer. Colon cancer cells were treated with this serum, and decreased proliferation was observed. The authors suggest that cancer cells show enhanced DNA repair when they are exposed to exercise regularly. A sign or this suggestion is the fact that the effects of IL-6 on colon cancer cells were dependent on the dose with which they were treated [50]. Whether IL-6 that is released into the serum has an effect on the TME remains to be explored but appears plausible, as it is likely that sIL-6Rα is present as a binding factor [51]. Therefore, chronically increased IL-6 levels in serum may increase tumorigenesis [52].

Direct effects

IL-6, when located in the TME, can have multiple effects, such as STAT3 signaling activation and other metastasis-promoting effects [53]. STAT3 signaling by IL-6 can influence gene expression in cancer cells. Soluble IL-6 forms a complex with its receptors, which can induce STAT3 signaling via activation of JAK. Activated STAT3 can change the gene expression of the cell, which will lead to anti-inflammatory gene transcription via membrane-bound activation but pro-inflammatory transcription if the activating IL-6 complex is soluble as it is in the TME. Soluble IL-6 in the TME will therefore directly change gene expression in cancer cells to favor an inflammatory environment, which contributes to cancer progression [54]. As previously mentioned, IL-6 in the TME can have multiple sources. In addition to the cell types that were mentioned, carcinoma-associated fibroblasts (CAFs) can be a main source. CAFs that produce IL-6 are suspected to be the main cause of epithelial-mesenchymal transition (EMT) [55]. EMT is a process that is necessary for embryogenesis, wound healing, stem cells, and cancer progression and is characterized by cell differentiation [56]. During this differentiation, epithelial cells that are immobile and interact with other cell basement membranes transition into mesenchymal cells that can move freely. This new phenotype enhances the ability of cells to migrate [57]. In cancer, this process favors metastasis and drug resistance [58]. During EMT, cell–cell adhesions are loosened through genes whose transcription factors are induced by different processes [57]. Factors that induce EMT include cytokines and other soluble factors [59]. In breast cancer, an EMT phenotype can be induced, and IL-6 has been identified as a direct inducer of this phenotype in MCF-7 cells. In this context, the MCF-7 cells produced IL-6 themselves, leading to a feedback loop. Additionally, proliferation was increased. E-cadherin, a protein in the cell membrane, is responsible for cell–cell adhesion, and its absence can cause elevated invasiveness in cancer cells. In the presence of autocrine IL-6 in MCF-7 cells, a complete halt of E-cadherin expression was observed [60]. The expression of gene tumor protein 3 (TP53), which encodes tumor suppressor protein p53, is also influenced by IL-6 via the IL-6/JAK/STAT3 pathway. Similar to E-cadherin, p53 expression is attenuated by IL-6 originating from CAFs via ubiquitination. This results in chemotherapy resistance against the drug doxorubicin in prostate cancer cells (LNCaP) and possibly against other chemotherapies by resisting cell death [61].

Indirect effects

The indirect effects of IL-6 on the TME mostly revolve around its influence on the immune system [62]. Similar to the IL-6-driven inflammatory response, the influence of IL-6 on the immune system in a cancer context can be just as equivocal [41]. Recently, the positive effects of IL-6 were highlighted. As a part of this response, the effect of IL-6 occurs in the lymph nodes and modulates the immune system [63]. The activated immune cells then travel to the TME and influence it locally. IL-6 can modulate the T-cell response by enhancing the survival and proliferation of leukocytes. Additionally, IL-6 favors the transport of antitumor T cells toward the TME [41]. While the positive properties of IL-6 in a tumor response are still to be discovered, there is recent progress in understanding. It was shown in a mouse model that animals with access to aerobic training exhibited slower tumor growth, which was linked to increased $CD8^+$ T-cell metabolism induced by muscle activity. One can therefore assume that exercise can shift the IL-6 response in tumors toward a positive response [64]. In general, infiltration of the TME with T cells favors a good prognosis. $CD8^+$ cells can differentiate into interleukin-21 (IL-21)-producing $CD8^+$ cells via IL-6-induced STAT3 signaling, which supports B cells in viral responses [65]. In the context of chronic inflammation, forkhead box protein P3 (Foxp3+) CD8 + cells develop in the presence of IL-6 and suppress autoimmune responses [66].

T-cell immunity can also be reduced via IL-6 signaling. IL-6, as a soluble factor, increases the number of myeloid-derived suppressor cells (MDSCs) in vitro. These cells are an immature form of myeloid cells and can inhibit innate and adaptive immune responses. In hepatocellular carcinoma (HCC), the number of MDCs increased through IL-6 signaling and resulted in a reduction in T-cell immunity. This mechanism causes cancer progression. It is important to note that the authors mention a strong hint toward this mechanism but that further experiments are needed to show a direct link [67]. Another example of the negative effects of STAT3 signaling via IL-6 was found in colorectal cancer. STAT3 phosphorylation by IL-6 in the presence of transforming growth factor β (TGF-β) in colorectal cancer caused the differentiation of $CD4^+$ cells into Th17 cells, which can cause disease progression by onco- and angiogenesis [54]. In a study with colorectal cancer patients, a positive correlation was found between $STAT3^+$ cells in the TME and patient survival. The authors also showed that $IL\text{-}6^+$ immune cells were found significantly more often in early-stage tumors than in later-stage tumors [68]. Multiple mouse model studies showed that tumor growth is either suppressed or slowed when mice exercised prior to tumor injections. The authors found that the slowed tumor growth rate correlated with natural killer cell (NK cell) infiltration within the tumor. In further studies, they found that this effect is caused by acute IL-6 increases, as NK cells are IL-6 sensitive, and elevated IL-6 levels were shown in serum after acute intervention. Therefore, the authors suggest that the acute rise in IL-6 that causes an acute inflammatory process may inhibit tumor growth, while repeated exercise bouts before the disease can slow or prevent tumor progression by immune system activation [69].

Oncostatin M

OSM belongs to the family of IL-6 cytokines, as it can also bind to gp130 complexes [70]. In addition to gp130 complexes, OSM can bind to OSMRβ chains, which are expressed on a variety of cells [71]. While OSM is produced by multiple cells of the immune system, such as macrophages and dendritic cells, it is also secreted by skeletal muscle, which classifies it as a myokine [70, 72]. It is involved in multiple processes, such as liver development and blood cell production, and has been suggested as a target for treatment in common diseases, as OSM is also involved in the inflammatory response and can prevent neural cell damage [72, 73]. In cancer, OSM can promote cancer progression but was originally regarded as inhibitory [74].

OSM in the TME contributes to cancer progression by recruiting M2 macrophages into the tumor environment and by altering the phenotype of CAFs. In general, elevated OSM levels in serum as well as in the TME have been associated with disease progression in different cancer types [75]. While aerobic exercise increases OSM concentration in muscle tissue, it has been shown that OSM concentration also increases in serum after aerobic exercise in mice that were previously injected with breast cancer cells [73, 76]. In cancer cells, the oncostatin M receptor (OSMR) can be overexpressed. This overexpression leads to increased OSM signaling, which will cause angiogenesis, invasiveness, and cell migration. OSMR overexpression will therefore favor disease progression. As OSM binds to OSMR, STAT3 signaling is activated, and gene transcription of VEGF-A and transglutaminase 2 (TGM2) is induced. VEGF-A induces angiogenesis, while TGM2 causes cell migration [77]. An in vitro experiment with triple negative breast cancer cells that were cocultured with neutrophils suggests that neutrophils in the TME will increase OSM production, which will then promote metastasis and tumor progression [70]. Similar to IL-6, OSM concentration appears to increase in serum and tumor tissue after exercise in mice [76].

Direct effects

In the TME, OSM has been brought into the context of EMT and was identified as the largest contributor toward the attainment of cancer stem cell characteristics (CSCs). Similar to IL-6, OSM can bind to STAT3. OSM/STAT3 signaling will then lead to an accumulation of mothers against decapentaplegic homolog 3 (SMAD3) in the nucleus [78]. SMADS are intracellular proteins that function as transcription factors that are activated by TGF-β and control the transcription of TGF-β target genes in a cofactor-dependent manner [79]. The altered transcription by OSM/STAT3 signaling favors gene transcription that will enhance EMT as well as CSCs. This increases the invasiveness and drug resistance of the tumor [78].

Indirect effects

Overexpression and promotion of tumor growth by OSM in vitro was observed in a study by Simonneau et al. [80] in skin cancer. In the same study, the authors showed that tumor size in vitro and the polarization of M2 macrophages are reduced if OSM is absent, which suggests that OSM is an indirect promotor of cancer progression. The authors of a 12-week intervention study on prostate cancer patients analyzed serum myokine levels before and after the intervention and found a significant rise in OSM serum concentration, which correlated with lean body mass. The intervention consisted of aerobic and resistance training. In cell culture, the growth rate of cells with the conditioned serum slowed. The authors mention, however, that a direct connection between the rise of myokines and slowed cell growth could not be shown [30]. OSM was identified as a promoter of breast cancer and metastasis by directing stromal intracellular crosstalk between cancer cells, immune cells, and cancer cell-associated fibroblasts [81]. In the context of this study, the authors took OSM produced by myeloid cells into account and found a feedback loop between these cells and cancer cells with OSM receptors. In summary, the authors state that the role of OSM within the TME remains unclear, while it is also suggested that OSM/STAT3 signaling is a promising target to reduce drug resistance [78].

SPARC

Another myokine that was observed in an intervention study is SPARC [82]. SPARC is a common protein within the extracellular matrix (ECM) that can be found in the TME. SPARC was described as a family of closely related proteins that have multiple functions in adult as well as embryonic tissue [83]. As the authors reported, SPARC can influence the cell cycle in late phases, vascularization, matrix mineralization, and cell adhesion. Its role is not clearly understood, but according to the current literature, the role of SPARC within the tumor is dependent on the cell type and the tissue in which the tumor lies [84, 85].

As a protein of the cellular matrix, SPARC regulates the interaction among cells and the communication between cells and the extracellular matrix (ECM). In cancer, SPARC influences cell–cell adhesions and can therefore increase the migratory properties of cancer cells, which may lead to metastasis [86]. Low SPARC levels in a murine melanoma model in vitro and in vivo appear to reduce invasiveness and cell migration, supporting the previous statement [87]. Contrary to this finding, SPARC is suspected to inhibit tumor progression and metastasis in bladder carcinoma, partly by limiting the inflammatory response [88].

Direct effects

There are no known receptors of SPARC in humans, but there are a few suspicions on how SPARC might directly influence cancer cells [89].

An antibody study with different human tissues was conducted to determine the amount of SPARC within those tissues. The most prominent findings were that SPARC appeared to be binding on the ECM rather than being incorporated in it. Additionally, it was shown that the SPARC concentration is higher in malignant tissues [83]. SPARC can directly bind to collagen and interacts with factors such as VEGF, fibroblast growth factor (FGF), and TGF-β [85].

Indirect effects

In biopsies of colon cancer patients, SPARC expression correlated positively with VEGF, and low SPARC expression was associated with a poor outcome [90]. In renal cell carcinoma, SPARC is a downstream effector of TGF-β, and its expression is increased by TGF-β concentration. In this context, matrix metalloproteinase-2 (MPP2) expression was increased in vitro, which promotes invasion and therefore metastasis [91]. On the other hand, SPARC normalized the TME of ovarian cancer cells in vitro and in vivo via downregulation of VEGF [92]. SPARC is released through acute as well as longitudinal training interventions. An increase in SPARC in the plasma was shown in mice as well as humans, while gene expression after acute and longitudinal training was also elevated [24]. Plasma levels of SPARC appear to return to pre-exercise levels within 6 hours in mice and humans [3]. In prostate cancer patients specifically, no elevation in serum SPARC levels was observed, but a trend could be seen after a 12-week exercise training intervention [30]. The inhibitory and promoting properties of SPARC may be dependent on the origin or the cell type, which can be malignant or stromal. Therefore, it may be beneficial to involve these factors in further studies [90].

In summary, the role of SPARC within the TME remains to be elucidated and may be altered by multiple factors, while it is clear that SPARC expression is influenced by exercise and plays a role in cancer progression [82, 85, 90].

Irisin

Irisin was first described as a hormone that is secreted after exercise in mouse models as well as humans through fibronectin type III domain-containing protein 5 (FNDC5) cleavage [93]. FNDC5 is a transmembrane protein that is located in multiple tissues, one of which is skeletal muscle. Upon physical exercise, the extracellular part of the protein, which is irisin, is cleaved from FNCD5 and will enter the bloodstream, but it is unclear what causes this cleavage [94, 95]. Additionally, FNDC5 expression is upregulated by peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), which is an exercise-induced coactivator. In humans and mice, irisin levels in serum increase after exercise, while the increase is higher in trained humans, while irisin levels decrease with age. Additionally, irisin injections may induce muscle hypertrophy in mice [96, 97]. In an acute setting, resistance exercise provoked the strongest irisin response compared to endurance or combined exercise [98]. In a recent study in which adults performed an acute high-intensity interval training (HIIT) intervention, irisin levels in serum increased compared to moderate exercise and control [97]. Interestingly, a meta-analysis demonstrated that chronic exercise decreases the circulating concentration of irisin [99].

Myokine was first shown to promote brown fat development in vivo via mitochondrial uncoupling protein 1 (UCP1) expression [93]. Soon after, irisin became linked not only to obesity but also to multiple diseases [100]. The link between cancer and irisin has been drawn, as obesity favors an inflammatory environment that increases cancer cell survival and proliferation [96]. Recently, the role of irisin in breast cancer was examined [94]. The authors found that tumor progression relates to decreased irisin levels and that high levels respond to an increased survival time. Irisin levels also appear to play a role in renal cancer. FNDC5/irisin levels were tested in the serum of patients and compared to a healthy control group with an enzyme-linked immunosorbent assay (ELISA). The study revealed elevated FNDC5/irisin levels in the patient group compared to the control group [101]. In contrast to this study, most in vitro experiments have shown that irisin has an inhibitory effect on cancer progression [102].

Direct effects

Irisin was recently brought into context with exercise and the TME. The underlying idea is that irisin has a metabolic effect that may be transferable to cancer cells, as one hallmark of cancer is altered glucose metabolism. This was tested in vitro with multiple ovarian cancer cell lines. In a time- and dose-dependent manner, irisin suppressed cell proliferation and migration as well as the clonogenic potential of ovarian cells, in addition to a heightened sensitivity toward chemotherapy treatment [103].

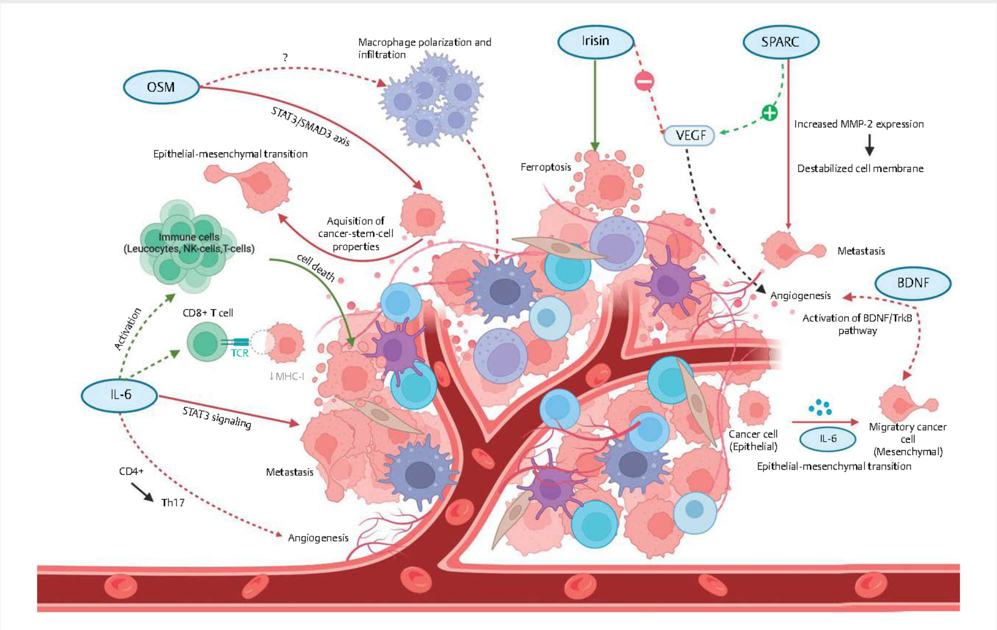
An in vitro experiment with breast cancer cells showed that the activity of caspase-3/7 is increased, while activity of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) is suppressed after irisin treatment. This leads to a lower count of breast cancer cells and decreased cell migration [104]. Caspase-3/7 are both proteases that can directly induce apoptosis and are therefore important markers, e. g., in cancer drug efficiency [105]. NF-kB summarizes a group of transcription factors that regulate inflammation as well as cell migration and other mechanisms that are important in cancer development [106]. Irisin treatment of OC cells decreased hypoxia-inducible factor-1-alpha (HIF-1α) and VEGF expression, possibly favoring tumorigenesis. Aside from these observations, an induction of apoptosis was also observed [103]. In another in vitro study on pancreatic cancer, ferroptosis, an iron-dependent type of apoptosis in which reactive oxygen species accumulate, was enhanced when cells were treated with irisin. These findings suggest that irisin may have a direct effect on cell death and is therefore an interesting therapeutic target [107].

Indirect effects

Serum irisin levels decrease in humans with age, while they are increased after acute exercise interventions but remain unaffected by chronic exercise [108]. In regard to aerobic metabolism genes, irisin had an inhibitory effect on VEGF expression, while the expression of other observed genes varied among cell lines. The effects on metalloproteases are still inconclusive and will need further studies that involve the effect of different exercise interventions on myokines and cancer cells [103].

BDNF

BDNF is a myokine as well as a neurotrophin that is known to influence multiple mental disorders [109]. Another important aspect of BDNF is its metabolic effects. BDNF binds to tropomyosin receptor kinase B T1 (TrkB. T1) in pancreatic cells and thereby increases insulin secretion in a murine model. These findings support the notion that BDNF is regulated not only by hippocampal activity but also by muscle activity and has a peripheral effect [110]. These findings are supported by the discovery of increased BDNF serum levels in obese patients after an eight-week moderate- or high-intensity training intervention [111]. In contrast, in other studies, increasing levels of BDNF were detected within the muscle but not the periphery, leading to the conclusion that BDNF exhibits its function in an autocrine and paracrine manner, while it may have an effect on peripheral metabolic activity. While the amount and mRNA expression of BDNF is increased in muscle through exercise, the effects appear to be local without release into the bloodstream, which makes an influence on the TME unlikely [25, 112]. Despite this, there is evidence that BDNF contributes to cancer progression by increasing metastasis-promoting cell properties, angiogenesis, and chemotherapy resistance [113]. As there appears to be no consensus on the effects of BDNF and it is unclear whether central nervous system or muscle activity causes increased serum BDNF levels, it remains elusive whether BDNF can have an effect on the TME.



▶ **Fig. 2** The effects of myokines on the tumor microenvironment.

Discussion

Exercise is an important factor in cancer prevention, treatment, and rehabilitation due to its multiple positive effects on patients. The question that remains unanswered is which molecular mechanisms contribute to these findings. A summary of the currently known effects of the myokines presented above is presented in ▶ **Fig. 2**. The current literature shows that myokines are a promising aspect for answering these questions. All myokines that we described above may contribute to cancer development in different manners, but all of them need further exploration. Further studies also need to be conducted to understand which exercise has the greatest impact on the different myokines to determine which exercise mode is most helpful as supportive cancer treatment and rehabilitation. As pictured in ▶ **Fig. 1**, the serum and plasma levels of myokines are influenced by different types of exercise. While the majority appears to be more affected by acute exercise, it is unknown if the alterations in serum or plasma concentration by acute exercise remain persistent in regularly trained individuals or if adaptions can be observed. This would mean that repeated training sessions are required to have a direct effect on the TME, while long-term adaptations may lead to enhanced perfusion through angiogenesis and therefore a better response towards treatment [8]. This is supposedly caused by chronic changes in myokine concentration in serum, which can be either elevated or depleted [17, 21, 26, 108]. Overall, the intensity of exercise may be related to the levels found in serum, therefore one could assume that overall high intensities in exercise are favorable in the context of cancer prevention and rehabilitation [29]. Currently, the acute effects of myokines appear to be of higher importance.

An important question that remains to be answered is whether myokines from the periphery have a direct influence on signaling pathways within the TME. As a part of cancer research, the discovery of new approaches in cancer treatment is imminent. This includes research directed toward drug resistance as well as the discovery of new target pathways. One approach toward this goal is to understand the molecular aspects of the TME and how modulations influence tumor growth. Tumor growth has been shown to be slowed or inhibited by different modes of exercise in animal studies and in vitro. As there is no consensus about the exact molecular mechanisms of exercise on the TME, we propose the approach of investigating the role of myokines in the TME [74]. In this review, we presented the most commonly known myokines and their influences on the TME and consequently tumor growth and progression. It was shown that myokines can have tumor progression as well as inhibitory effects. These appear to depend on multiple aspects, e. g., dose-dependent effects. Additionally, the interactions between myokines themselves and between myokines and cytokines may contribute to the effects on the TME.

In summary, the findings of this review show tumor progression as well as inhibitory properties for all myokines discussed. These effects may be dose-dependent, and exercise can therefore have negative as well as positive effects on tumors. Another important aspect is the differentiation between acute and chronic myokine effects in addition to interactions between myokines and between myokines and other cytokines, as this can also alter the effects on the TME. Exercise is a promising contributor to altering the TME, and the inhibitory effects of exercise on cancer have been demonstrated by in vivo and in vitro studies. Myokines likely contribute to these effects, which makes them an interesting target to further elucidate the effect of exercise on cancer disease.

Conclusion

The objective that this narrative review aimed to answer is the question as to whether the influence of myokines on the TME may be one of the reasons why positive effects of exercise in cancer are observed as the underlying molecular mechanisms are still unknown. In doing so, six common myokines were first described and then brought into the context of TME based on a literature review. While it cannot be clearly stated which type of exercise enhanced the expression of which myokines, there are clearly parts within the TME that respond to myokine regulation. Similar to inflammatory processes, it appears that all myokines discussed exhibit progressive as well as inhibitory properties within the TME and in cancer disease in general. To gain further understanding, future studies may focus on the currently known myokines and which type of exercise promotes their expression. There are no studies with similar cohorts investigating the changes of myokine expression in different types of exercise. It therefore remains questionable if one can reliably state which myokine is influenced by which exercise. In further human studies this should be compared to cancer patients, including in regard to their treatment. Furthermore, cell culture studies with conditioned serum may show if myokines that can be found in the serum have direct interactions with the TME. These findings can enhance existing exercise recommendations for cancer patients as they add to an in-depth understanding of the positive effects that different training modalities have on cancer patients.

One limitation of this review is that it is narrative. Therefore, there is no quantitative support for our observations. Moreover, existing literature was used to create a more concise picture of a topic within sports medicine that may be worth exploring. This review therefore lacks the clarity a systematic review may offer. Due to a lack of literature concerning the direct and indirect effects of myokines on the TME, a systemic review was not possible but should be reconsidered in the future. There may also be more possible interactions of myokines discussed within the TME that may not have been included if they did not match the search terms.

Despite these limitations there are clearly molecular mechanisms that were pointed out in this review and that may explain the positive influence of exercise on cancer and are therefore worth further investigations.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Appendix B - Publication 2

International Journal of Molecular Sciences

MDPI

Article

The Influence of an Acute Endurance Intervention on Breast Cancer Cell Growth—A Pilot Study

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Abstract: Exercise potentially inhibits tumor growth. It remains unclear which processes mediate these effects. Alterations of cytokine concentration in serum can influence cancer cell growth and may cause cell growth inhibition. This pilot study examines whether exercise-induced conditioning in serum can directly affect tumor cells. It focuses on serum collected before and after acute endurance exercise and its impact in vitro. Participants underwent a 1 h endurance training on a cycle ergometer. Samples were collected before, after, and two hours post-exercise. MDA-MB-231 cells were incubated with serum, and cell vitality and proliferation were assessed. Cytokine arrays identified relevant cytokine concentration changes. After identifying CXCL9 as a possible contributor to inhibitory effects, we inhibited the CXCR3 pathway and reassessed vitality. Exercise-conditioned serum significantly reduced cell vitality and proliferation post-intervention and after resting. Cytokine arrays revealed changes in multiple concentrations, and the inhibition of CXCL9 resulted in growth inhibitory effects. Our findings suggest that serum conditioned by an endurance intervention causes changes in cancer cell growth. Based on our observations, the alterations in serum cause growth-inhibitory effects, possibly mediated through the CXCR3 axis. This study provides preliminary evidence supporting the role of exercise in modulating the cancer cell growth directly by changes in serum.

Keywords: exercise oncology; myokines; sports medicine

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1. Introduction

In women, breast cancer is the most common cancer type, with approximately two million new cases diagnosed each year [1]. Exercise is regarded as a component of cancer prevention as it contributes to lower incidences [2]. Exercise is an important component during cancer rehabilitation and is recommended as part of breast cancer treatment [3,4]. Apart from reducing disease- and treatment-related side effects in humans, numerous animal studies report a decrease in tumor weight and tumor growth, an increased immune response, and a reduced pathological score following exercise interventions [5–7]. An indirect effect on cancer cell growth through exercise via the immune system, mediated by cytotoxic T-cells, has been shown in animal studies [8]. In human studies, it is essential to identify the direct impact of exercise and the cytokines released on cancer cell growth. Additionally, the underlying mechanisms driven by myokines need to be investigated [9]. Regular physical activity promotes the mobilization and function of immune cells, such as cytotoxic T-cells and natural killer (NK) cells that infiltrate tumors and help fight cancer. Exercise also improves the body's cytokine profile, enhancing the immune response in the tumor microenvironment (TME) while reducing the presence of immunosuppressive cells like myeloid-derived suppressor cells (MDSCs) [10,11]. The most commonly described factors contributing to changes in the TME are increased vascularization, enhanced immune surveillance, and metabolic changes in cancer-muscle cross-talk [12,13]. The communication between the tumor itself, cancer cells, and muscles is mediated by myokines, which are classified as a subcategory of cytokines that are released by contracting muscle tissue and, thus, released during exercise. Myokines are known to act as communicators between the muscle and other organs or within the muscle itself to promote hypertrophy. When released into the periphery, myokines have effects on multiple tissues. This applies to both healthy and cancerous tissues [14]. While these aspects may each be explored individually, there is likely an interaction among them [15]. The extent to which these mechanisms slow breast cancer growth remains unclear, and it is uncertain which type of exercise has the greatest impact. While there is evidence that exercise benefits the immune system and TME, further research is required to determine the optimal exercise approach [16].

The direct impact of metabolites and cytokines released during exercise on the TME may partly explain the observed effects of exercise on cancer. These molecules, such as interleukins and myokines, can modulate immune activity, enhance anti-tumor responses, and influence the TME by promoting immune cell infiltration and altering the inflammatory status. Moreover, a direct regulation of cancer cell growth by myokines should be taken into account. This interaction may be one of the mechanisms by which exercise indirectly supports tumor suppression in various cancer types. However, further research is needed to understand the extent of these effects [13].

The main components of the TME are the extracellular matrix, immune cells, stromal cells, and cells of the vascular system, with malignant and non-malignant cells surrounding the tumor and forming the TME through interactions with various surrounding cells, though its exact composition varies depending on the tumor type [17]. All of these components may be influenced by myokines [18]. The most well-characterized myokine is interleukin-6 (IL-6), which plays a central role in promoting muscle hypertrophy [14]. In the context of cancer, IL-6 has been recognized as directly affecting metastatic processes by altering the TME through multiple pathways that promote metastasis. Other direct effects of myokines on cancer cells include the induction of an epithelial–mesenchymal transition and the modulation of STAT3 signaling, thereby increasing proliferation. On the other hand, myokines can decrease cell migratory properties by increasing caspase-3/7 activity [16,19]. The increased expression of caspase-3/7 activity by altered myokine concentrations following resistance exercise has already been demonstrated in breast cancer cells that were treated with conditioned serum of breast cancer patients [19]. While the influence of muscle metabolites, such as lactate, has already been demonstrated, the influence of myokines via the immune or endocrine system is also likely, as myokines affect multiple tissues [15,20,21]. While myokines can have direct effects on cancer cells and the TME, as described above, indirect effects should also be taken into account [14]. Recently, Rundqvist et al. [8] demonstrated in a breast cancer animal model that muscles produce molecules, which caused an enhanced efficiency of $CD8^+$ T-cells and resulted in a decrease in tumor growth. In a different study investigating a murine breast cancer model, mice that performed continuous endurance exercise exhibited lower tumor volume and decreased levels of IL-6 and vascular endothelial growth factor (VEGF) compared to a control group [22,23]. In cancer survivors, changes in the concentration of several myokines have been observed following acute high-intensity endurance interventions [24]. As there is a cross-talk between the muscle and the tumor, it is reasonable to assume that myokines play a role in TME regulation and, consequently, tumor growth [25]. In this context, the alteration of cancer cell extracellular matrix by myokine-regulated expression of matrix metalloprotease (MMP), which can enhance migratory properties, should be considered [26]. Rundqvist et al. demonstrated that incubation of prostate cancer cells with post-exercise pooled serum inhibited cancer cell growth in vitro, a finding confirmed in a murine model [27]. Beyond myokines and cytokines, metabolic changes from exercise-induced lactate fluctuations should also be considered when investigating the effects of conditioned serum on cancer cells [28]. Higher than normal lactate concentrations within the TME may favor the immune system escape while also providing an energy source for cancer cells [29]. In contrast to these findings, higher lactate concentrations in the tumor environment also have the potential to support $CD8^+$ cells in developing stem-cell-like characteristics and thereby increasing anti-tumor immunity [30].

As breast cancer is the most common cancer among women, and positive effects of exercise on cancer- and treatment-related side effects have been shown in humans, it is reasonable to explore whether exercise-conditioned serum and associated cytokines directly influence the growth of breast cancer cells [31]. Therefore, the purpose of this pilot study is to identify whether conditioned serum has an effect on breast cancer cell growth in vitro. Secondly, we tested whether changes in protein or cytokine concentrations within the serum can be detected. We further explored whether the concentration changes we observed could be directly linked to growth inhibition. This pilot study serves as a basis for future research to build upon.

2. Results

2.1. Cell Proliferation

We compared cell proliferation in cells that were treated with pre-exercise ($T0_{intervention}$), post-exercise ($T1_{intervention}$), and rest serum ($T2_{intervention}$). An immunohistochemistry with Ki-67 was performed. The ratio of Ki-67-negative to Ki-67-positive cells as well as the relative change of Ki-67-negative vs. Ki-67-positive cells from $T0_{intervention}$ to $T1_{intervention}$, $T1_{intervention}$ to $T2_{intervention}$, and $T0_{intervention}$ to $T2_{intervention}$ was calculated. For this purpose, two independent researchers counted 100 cells per participant at each time point. The relation of negative cells to cells counted in total was assessed for each participant, and means were calculated for each condition. We found statistically significant differences between the proliferation rates of each time point (Chi^2 (2) = 20.182, p < 0.001, n = 11). We found that the proliferation rates decreased from T0 to T1 (p = 0.032) as well as from T0 to T2 (p < 0.001), but not from T1 to T2 (p = 0.165). The effect sizes of T0–T1 (r = 0.315) and T0–T2 (r = 0.417) showed medium efficiency according to Cohen's classification, while T1–T2 (r = 0.273) showed low efficiency [32].

2.2. Analysis of the Cytotoxic Effects

Cell viability was determined via the optical density (OD) measured following an MTT assay. Cell viability was highest in the pre-exercise condition (T0) (M = 1.05, SD = 0.194), followed by the rest condition (T2) two hours after the endurance exercise (M = 0.924, SD = 0.164). The lowest cell viability was measured immediately after the intervention at $T1_{intervention}$ (M = 0.887, SD = 0.218), as shown in Figure 1. Significant differences in cell viability were found between the time points $T0_{intervention}$ and $T1_{intervention}$, as determined by an rmANOVA (p = 0.046), indicating that cell viability is significantly reduced in cells treated with conditioned serum from blood which was drawn directly after the intervention.

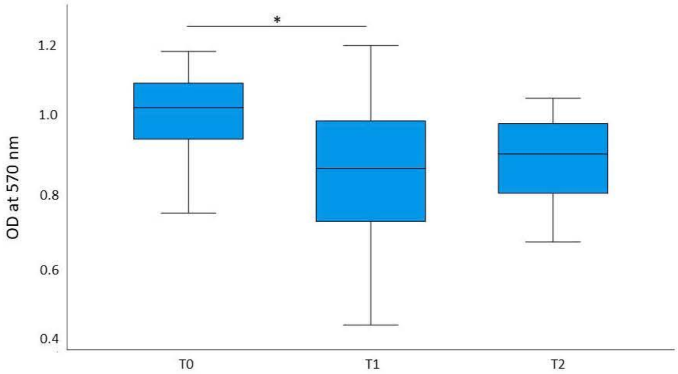

Figure 1. Results of the MTT assay. MDA-MB-231 cells were treated with individual sera of all 11 participants and an MTT assay was conducted to assess cell viability. The blood samples were drawn before the endurance intervention (T0), directly after the endurance intervention (T1), and after 2 h of rest (T2). The optical density (OD) was measured at 570 nm. Median values of the optical density measured during MTT assay for T0, T1, and T2 with serum taken on the day of the intervention are displayed. The mean values of each time point were tested for statistical differences. Statistically significant differences of mean values following an rmANOVA are marked in the figure (* = $p < 0.05$). Cell viability was significantly decreased in cells treated with post-exercise conditioned serum (T1) compared to pre-exercise (T0) and rest serum conditions (T2). Therefore, serum conditioned by an acute moderate intensity endurance exercise decreased cell viability in MDA-MB-231 cells.

2.3. Cytokine Array

After processing the protein arrays according to the manufacturer's instructions with pooled serum for each condition, a 2D densitometry analysis was conducted on cytokine array membranes using ImageJ. Signal intensities of individual cytokine spots were quantified from scanned images. Values were normalized to internal controls for comparative analysis. The following cytokines were selected for densitometry analysis: Leptin, PDGF-BB, CCL5, MCP1, IL-15, Angiogenin, BDNF, TNF- α, CXCL9, IL-3, CCL 15, IGFBP-1, NAP-2, EGF, IGFBP-2, Eotaxin-1, ICAM-1, TIMP-1, EGFR, TIMP-2, CXCL5, IGFBP-6, CCL4, TRAILR3, Adiponectin, MIP-3-beta, MSP, uPAR, ANGPT2, OPG CXCL1, gp130, HCC-4, and IL-6 R. Full cytokine names can be viewed in the list of abbreviations.

Densitometry was analyzed for intervention and control conditions to determine if differences were due to the intervention or factors like circadian rhythm. A Friedman Test assessed whether changes between T0–T1, T1–T2, and T0–T2 were exercise-related or influenced by other factors. We compared absolute densitometry values of each condition and time point (Chi^2 (5) = 28.353, $p < 0.001$, $n = 34$) and a post hoc analysis, which showed significant differences between values for all cytokines combined at $T1_{rest}$ and $T1_{intervention}$ ($p < 0.001$) and $T2_{rest}$ and $T1_{intervention}$ ($p < 0.001$) but not between $T0_{rest}$ and $T0_{intervention}$ ($p = 0.846$) and $T2_{rest}$ and $T2_{intervention}$ ($p = 0.92$). Changes in concentrations are presented in Table A2.

Cytokine concentrations were similar between intervention and control conditions at the first serum collection time point. The exercise intervention altered cytokine levels compared to the control condition, where participants rested for 1 h instead of cycling. Figure 2 presents an excerpt of the array with CXCL9 marked in the pre-exercise condition, post-exercise condition, and after resting.

Repeating the analysis of change rates confirmed significant differences between $T0_{rest}$-$T1_{rest}$ and $T0_{intervention}$-$T1_{intervention}$, while other changes were not statistically significant.

Thus, densitometry changes are likely due to the exercise intervention and independent of other factors like the circadian rhythm or nutritional status.

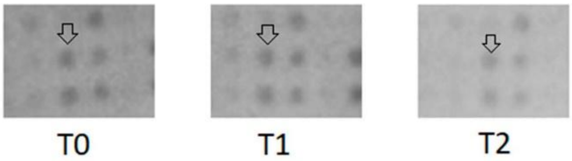

Figure 2. Cytokine array results for CXCL9. Arrays were incubated with pooled serum from 11 participants at three time points: pre-exercise (T0), post-exercise (T1), and after rest (T2). A control was included in the experiment but is not shown in this figure. The excerpt presented highlights the CXCL9 spot, indicated by the arrows. A gradual decrease in CXCL9 concentration across time points is visible and was quantified using densitometric analysis in ImageJ (V. 1.51j8). All arrays were processed and imaged under identical conditions.

2.4. *Blocking CXCR3 with AMG 487*

Previous experiments showed decreased concentrations of multiple cytokines when comparing pre- vs. post-exercise serum. MTT assays and immunohistochemistry revealed significant differences in cell proliferation and vitality from $T0_{intervention}$ to $T1_{intervention}$ and between intervention and control conditions. Given CXCL9′s role in the TME, we investigated whether reduced cell activity was linked to CXCL9 concentration [33]. To test this, we used AMG 487, a CXCR3 antagonist, to block the CXCL9 pathway and repeated cytotoxicity and proliferation assays with pre-exercise (T0) serum and T0 serum plus an inhibitor (T0 + IH).

2.4.1. Analysis of the Cytotoxic Effects

The inhibition of the CXCR3 axis resulted in reduced cell viability. OD was determined at 570 nm for cells incubated in the T0 condition (T0) and the T0 condition with an inhibitor (IH). Cell viability at T0 ($M = 1.53$, $SD = 0.07$) was higher than T0 + IH ($M = 1.406$, $SD = 0.14$). The analysis yielded a t-value of $t\ (10) = 3.064$ and a p-value of $p = 0.012$. Figure 3 shows that the cell viability was significantly reduced in cells that were treated with the T0 serum and the inhibitor in individual participants. Two participants had opposing effects, which did not affect the overall results. This might be due to a measurement error or individual responses of said participants.

2.4.2. Proliferation

Inhibition of the CXCR3 pathway reduced cell proliferation. MDA-MB-231 cells were treated with conditioned serum of T0 and T0 serum with the inhibition of the CXCR3 pathway by AMG 487 (T0 + IH). A Ki-67 immunohistochemistry was performed for both conditions. Consequently, we compared the conditions T0 and T0 + IH and found that the cell proliferation rate was reduced in the cells that were treated with the T0 + IH serum ($t\ (10) = -11.734$, $p < 0.01$). Figure 4 shows an example of microscopic imagery, while Figure 5 displays the statistical results. When inhibiting the CXCR3 pathway in pre-exercise serum, cell proliferation was significantly reduced. This was similarly observed in the post-exercise condition.

In summary, treating MDA-MB-231 cells with serum from healthy, minimally active women after acute endurance exercise reduced cell vitality and proliferation. Follow-up experiments using a baseline serum with a CXCL9 pathway inhibitor also reduced cell viability and growth, highlighting the relevance of CXCL9 in the context of exercise effects on cancer cells.

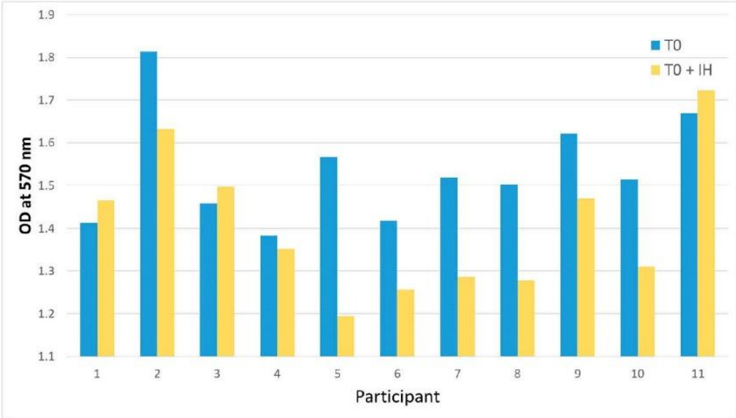

Figure 3. Results of the MTT assay after the inhibitor experiment. An MTT assay was performed on MDA-MB-231 cells, which were treated with each participant's pre-exercise (T0) serum and each participant T0 serum plus the CXCR3 pathway inhibitor AMG 497 (T0 + IH). Optical density was measured at 570 nm. Except for the serum of two participants, cell viability decreased in the T0 + IH condition, which was confirmed by a two-sided paired *t*-test.

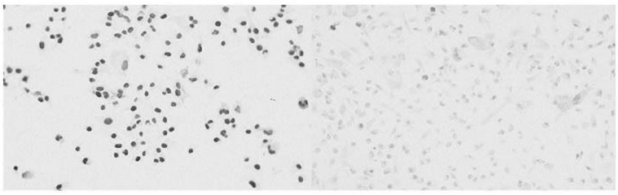

Figure 4. Immunohistochemistry results before and after inhibition of CXCR3. On the left, MDA-MB-231 cells were treated with the conditioned pre-exercise serum (T0) and Ki-67. The darker appearance of the cells indicates the presence of Ki-67, an indicator of proliferative cells. On the right, cells were treated with the conditioned serum of the pre-exercise condition (T0), Ki-67, and CXCR3 inhibitor AMG 486. These samples stem from one participant, but experiments were performed and evaluated for all conditions and all 11 participants. It became evident that cells treated with the CXCR3 inhibitor express less Ki-67 and therefore lack proliferative properties. This was confirmed by a two-sided paired *t*-test. Images were taken at 20× magnification.

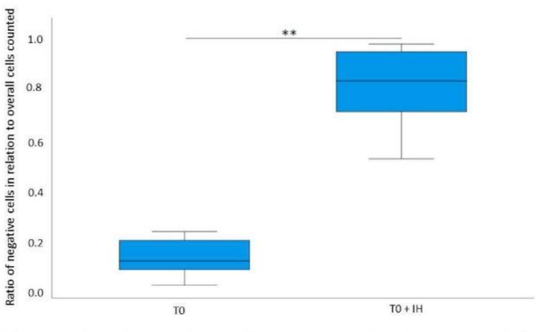

Figure 5. Statistical analysis of immunohistochemistry results before and after inhibition of CXCR3. This boxplot shows the results of the immunohistochemistry, where MDA-MB-231 cells were treated

with pre-intervention (T0) conditioned serum and T0 conditioned serum with CXCR3 partway inhibitor AMG 487 (T0 + IH). All participants were included. Images of the slides were analyzed with ImageJ (V. 1.51j8), and cells were counted by two independent observers. The median and distribution of the ratio of non-proliferative to overall counted cells in the conditions T0 and T0 + IH are shown. Two outliers at T0 were excluded for graphical reasons but were included in the statistical analysis. The mean values of the two groups were compared by a paired two-sided test. The T0 + IH condition shows a significantly higher count of non-proliferative cells. Therefore, inhibition of CXCR3 inhibited cell proliferation. Statistically significant differences are marked (** = $p < 0.01$).

3. Discussion

We could confirm that breast cancer cell growth decreased after being treated with the conditioned serum of healthy women drawn immediately after a 1 h moderate intensity endurance exercise session. This demonstrates the feasibility of the growth inhibitory effects of conditioned serum after exercise, which was the primary aim of this pilot study. Additionally, we examined the concentration and changes in multiple cytokines in the serum at different time points. We observed concentration changes in multiple cytokines across all time points, accompanied by decreased proliferation and vitality. The densitometry results indicated that these concentrations also fluctuate in the control condition, albeit to a lesser extent.

Acute and chronic endurance exercise interventions are recognized as important components in influencing tumor growth. In cancer survivors of multiple entities, it was demonstrated that concentration changes in myokines occurred acutely after a high-intensity session but not chronically [24]. Exercise is generally associated with beneficial outcomes in various cancer types, and emerging guidelines suggest that exercise can be effectively integrated into cancer treatment [34]. Breast cancer patients undergoing treatment are advised to engage in 150 min of moderate-intensity endurance exercise weekly, based on its benefits for HRQoL. Combined data from multiple studies suggest that endurance exercise may also affect survival rates [35]. Despite these recommendations, it remains uncertain which type of exercise or dosage is most beneficial in directly affecting the tumor, as the studies investigating this topic are heterogeneous [36–38]. The promising effects of resistance exercise on myokine concentration and, consequently, growth inhibition in triple-negative breast cancer cells have already been demonstrated [19]. In our pilot study, we showed that acute endurance intervention at moderate intensity had an inhibitory effect on triple-negative breast cancer cell growth in vitro [27]. Known effects of endurance exercise that can reduce tumor growth include a reduction in C-reactive protein (CRP) levels and the release of inflammatory markers such as IL-6, sex hormones, insulin response, and vascularization [21,39,40]. Besides these factors, immune cells play a vital role not only in peripheral tissues but also within the TME, where they can either promote or inhibit tumor progression [41].

Although these effects impact the tumor, they may not be directly responsible for the reduction in cancer cell growth observed with exercise. In a study comparing the effects of an acute exercise intervention to a six-month exercise intervention in breast cancer patients, reduced cancer cell growth in vitro was only present in cells treated with serum after the acute exercise intervention. Although cytokine changes in both sera were detected, cell viability in MDA-MB-231 cells remained the same when treated with the chronic condition serum. While the effects of chronic exercise favor a positive outcome and generally lower inflammatory environments, the authors concluded that the accumulated effects of acute exercise can directly inhibit breast cancer cell growth [42]. Our results support these findings. The release of myokines from the muscle into the periphery during and after exercise and their function in the TME may be one factor contributing to cancer cell growth inhibition. While research on myokines is still limited, they likely influence oncogenic pathways as ligands or activate suppressor pathways directly [40]. The relevance of myokines was additionally highlighted by a pilot study, which found that myokine concentrations are altered by exercise in breast cancer patients [43]. Supporting these findings, this pilot study found that serum from sedentary women, conditioned by acute endurance exercise, inhibited breast cancer cell growth immediately after the intervention but not after two hours of rest. Cytokines, which could mediate this exercise-dependent effect on triple-negative breast cancer cell growth, were identified, and we focused on the CXCR3 axis in further experiments. Additionally, we took the lactate concentration within the serum into account. Higher lactate concentrations within the TME are associated with tumor growth and metastasis formation, when the lactate is produced by cancer cells as an energy source [44]. When higher lactate concentrations from external sources surround cancer cells, cell proliferation is inhibited [45]. We saw inhibitory effects in cells that were treated with higher lactate concentrations after the intervention, but those effects were irrelevant for the inhibition of the CXCR3 pathway, as cells were treated with pre-exercise serum and added inhibitor. Therefore, our observations are likely independent of the serum lactate concentration.

We found that the concentration of CXCL9 was lower after exercise. Therefore, we chose to inhibit the CXCL9 pathway via its receptor CXCR3, as this cytokine has been described as influencing tumor cell growth [33]. This approach was previously demonstrated by Liu et al. (2011) [46]. In this condition, we also observed growth inhibitory effects. CXCR3 is a cell membrane receptor that can be activated by CXCL9, C-X-C motif chemokine 10 (CXCL10), and C-X-C motif chemokine 11 (CXCL11). The two major mechanisms in which CXCR3 and its ligands are involved are angiogenesis and the recruitment of different immune cells at inflammatory sites [47]. There are two common isoforms of the CXCR3 receptor, CXCR3 A and CXCR3 B. Overall, CXCR3 is associated with cancer progression by altering the TME, but also with anti-tumor effects and growth reduction in vivo [48,49]. In a recent study, the CXCR3 A isoform was associated with modulation of cancer stem-cell-like properties, leading to cancer progression and treatment resistance. Isoform B is also associated with inhibiting effects, while this isoform can also have tumor-promoting effects, but only when it is overexpressed [48]. Breast cancer cells, including MDA-MB-231 cells, typically express CXCR3 A receptors [50].

The interaction of ligands with CXCR3 can be described as ambiguous. The ligands CXCL9, CXCL10, and CXCL11 all bind to CXCR3 and are primarily secreted by immune cells such as leukocytes and macrophages, as well as by dendritic cells, fibroblasts, and tumor cells. These cytokines are normally expressed at low levels but are upregulated in response to inflammation [33]. CXCL9, CXCL10, and CXCL11 interact with CXCR3 on tumor cells, immune cells, and vascular endothelial cells, influencing tumor growth, immune cell activity, and blood vessel formation. Tumor cells can secrete these cytokines and bind them to their receptors in an autocrine manner, promoting tumor growth and metastasis. Additionally, this signaling axis regulates the immune response, enhancing the activity of immune cells and inhibiting angiogenesis. Another important component of the acute effects of exercise is the activation of the p53 protein, which causes apoptosis [51]. The p53 and CXCR3 pathways may intersect, as p53 activation can regulate immune responses by modulating cytokine signaling, potentially enhancing CXCR3-driven T-cell recruitment. Furthermore, CXCR3 expression correlates with better cancer prognosis in some cases, suggesting that exercise-induced p53 activation and immune modulation could synergize to suppress tumor growth [52,53].

The effects of the CXCL9/CXCL10/CXCL11-CXCR3 axis can vary, even within the same organ, depending on the distribution of CXCR3 subtypes in tumor cells. For instance, in breast cancer patients, elevated serum levels of CXCL9 and CXCL10 are observed compared to healthy controls, and high levels of CXCL9 transcripts have been linked to better outcomes. While it is apparent that the CXCR3/CXCL9 axis is important in cancer, it may depend on the target cell if the interaction has a progressive or inhibitory effect on the cancer cell [54]. In a murine breast cancer model, different ligands of the CXCR3 receptor were elevated in varying breast cancer types after exercise. In this case, multiple cell types expressed CXCR3, CXCL9, and other ligands, but there was no predominant cell type [55]. When interacting with CXCR3, CXCL9 promotes tumor growth and metastasis in certain contexts, but it also recruits immune cells like cytotoxic T-cells to the tumor, supporting anti-tumor immunity. CXCL9, therefore, can either promote or inhibit tumor progression by influencing the tumor microenvironment. This complex behavior currently makes CXCL9 a controversial target in cancer therapy [56]. It is suggested that targeting this pathway might prevent? immune system evasion by enhancing the recruitment of immune cells into the TME via the CXCR3 axis [49]. In another study, CXCL9 induced cancer cell growth in an in vitro model of human and murine glioma. At the same time, the inhibition of CXCR3 had an anti-tumor effect in this model, similar to our observations [46]. Coherent with our results, CXCL9 was reduced in mice that exercised compared to mice that did not [57].

As cancer cells can produce CXCR3 ligands themselves, it appears plausible that cancer cells utilize this loop to gain cancer stem-cell-like properties, improve proliferation, promote metastasis, and immune system evasion [54]. Along with our pilot study, studies showed that an inhibition of this loop has an inhibitory effect on cancer cell growth, and the CXCR3 axis is proposed to be a target for treatment [56]. This pilot study supports the notion that inhibition of the CXCR3 axis has an inhibitory effect on breast cancer cell growth. Additionally, we showed that an acute endurance exercise decreases CXCL9 concentration in serum. Consequently, exercise may have a direct effect on breast cancer cell growth via the CXCR3 axis, although this was not explicitly demonstrated in our study. However, we could show that serum conditioned by an acute endurance intervention has an inhibitory effect on triple-negative breast cancer cell growth. This indicates that changes in cytokine concentration within the periphery are causing these effects. We suggest further exploration of the CXCR3 axis, its ligands, and exercise in multiple cancer models, as there is a strong link between this pathway and the direct effect of exercise on cancer cells.

Limitations

As our study served as a pilot study, a limitation is the small number of participants. It would be beneficial to apply our findings to a larger population to validate them or to repeat our experiments with serum of cancer patients before and after exercise. Despite choosing two independent researchers and following straightforward guidelines, the interpretation of microscopy images may be subjective. We made an effort to avoid this by calculating the interrater reliability before further analysis. As we explored the overall changes in cytokine concentration via cytokine arrays, these analyses were not as exact as other methods, but as we were trying to investigate a large number of cytokines, it was reasonable for a pilot study. In future studies with more defined research questions, other types of analyses are preferable, and other cytokines should be investigated. Additionally, it is necessary to repeat the experiments on a different cell line to validate the effect of exercise. In our experiments, while the serum used to treat the cells was paired for each individual, the cells themselves were not. This limits our ability to track individual cellular responses. This lack of pairing might result in a loss of contextual information and increased variability. Therefore, statistical power is reduced, requiring larger sample sizes in future studies to verify statistical significances observed in this pilot study. Additionally, since the analysis captures only a fraction of cellular states, identifying dynamic changes remains challenging despite the controlled serum conditions.

4. Materials and Methods

4.1. Participants and Study Design

This study was approved by the ethics committee of the German Sport University Cologne and follows the Declaration of Helsinki on 12 April 2023 under the agreement number 041/2023. All participants were informed about the study and provided written informed consent prior to baseline testing.

The observations of Rundqvist et al. [27] were used to calculate the number of participants required. As we know of no comparable studies, a power of 0.8 and an α-error of 0.05 were assumed for a group size of 12 test subjects, which was initially intended. Compared to Rundqvist et al. [27], two additional subjects were planned to be recruited. This resulted in an effect size of 0.89 and was calculated with G-Power for 12 participants.

A crossover design was chosen, and participants were randomized into one of two groups, A and B, after inclusion. One person withdrew consent, and the data were excluded. This resulted in $N = 6$ in Group A and $N = 5$ in Group B. The inclusion criteria were a physical activity level below 210 min of moderate intensity per week and no history of cancer disease, as well as no other currently treated disease or medication intake. Exclusion criteria were ages below 30 and over 60, presence of any chronic disease, and acute injuries. Participant characteristics are displayed in Table A1.

Upon inclusion, participants completed the Physical Activity Readiness Questionnaire (PAR-Q) and International Physical Activity Questionnaire (IPAQ) in German to validate inclusion criteria [58,59]. Consequently, they performed Cardio Pulmonary Exercise Testing (CPET), utilizing a ramp protocol on a cycle ergometer to determine peak aerobic capacity (VO_{2peak}). The initial load was 20 watts, which increased by 10 watts per minute. The assessment was discontinued when a rating of perceived exertion (RPE) of 20 was reached or upon voluntary termination. All measurements were completed within a four-week time window. The objectives of this study were to identify, if conditioned serum by an acute endurance intervention has a direct effect on breast cancer cell growth in vitro. Furthermore, we aimed to identify relevant cytokines in serum and verify these findings with an inhibitory experiment.

4.2. Intervention

The intervention consisted of cycling on an ergometer for a total of 60 min, 20 of which were completed at 50% VO_{2peak} and the remaining 40 min at 60% VO_{2peak} [27]. Venous blood collection was performed directly before ($T0_{intervention}$), after ($T1_{intervention}$), and two hours after ($T2_{intervention}$) the intervention. Participants were advised to remain seated or lying down during the resting period. At the second study appointment, precisely one week after (Group A) or before (Group B), the intervention blood draws were completed at the same time points ($T0_{rest}$, $T1_{rest}$, $T2_{rest}$). Instead of cycling, participants rested for 60 min. Participants were asked to avoid alcohol and strenuous exercise 24 h before testing and caffeine 2 h before and during testing.

Upon collection, serum was isolated from the blood samples and stored at −80 °C until further use.

The MDA-MB-231 is a triple-negative, metastatic breast cancer cell line, which was kindly provided by the American Type Culture Collection (ATCC®, Manassas, VA, USA). The cells were cultured in medium consisting of 89% Dulbecco's Modified Eagle Medium (high glucose, Gibco™ (Thermo Fisher Scientific, Grand Island, NY, USA)), 10% fetal bovine serum (FBS, Gibco™), and 1% Penicillin-Streptomycin (pen/strep, Gibco™). Cells were incubated at 37 °C and 5% CO_2.

4.3. Analysis of the Cytotoxic Effects

We incubated MDA-MB-231 cells with medium containing the serum of individual participants from time points $T0_{intervention}$, $T1_{intervention}$ and $T2_{intervention}$ to test for cytotoxic effects of the endurance intervention.

The MTT assays were performed in 96-well plates (Biofil®, Guangzhou, China) with an area of 0.32 cm^2 per well. For each well, 20,000 cells were seeded in 100 µL medium of each condition from every participant. Each condition was performed twice per participant. After the cells were treated in the incubator for 48 h at 37 °C, 5% CO_2, 10 µL of MTT reagent (10%, R&D Systems®, Minneapolis, MN, USA) was added to each well and incubated for 2 h 30 min in the incubator at 37 °C and 5% CO_2. The medium was then removed, and 100 µL of dimethyl sulfoxide (DMSO) (Thermo Scientific™, Villebon-sur-Yvette, France) was added. After 2 h in darkness at 37 °C, the OD was measured at 570 nm (OD570) and a reference wavelength of 650 nm (OD650) using a multiscan photometer (Thermo Scientific™ Microtiter Plate Photometer Multiskan™ FC). We used a negative control.

4.4. Immunohistochemistry

Immunostaining with the anti-Ki67 antibody (Novus Biologicals®, Centennial, CO, USA) was performed in 24-well plates (Falcon® (Becton, Dickinson and Company), Franklin Lakes, NJ, USA) with 1.09 cm^2 per well. Manufacturer instructions were followed. After coating each well with Aclar platelets, 20,000 MDA-MB-231 cells were seeded in 1 mL medium per well. This resulted in three conditions, T_0, T_1, and T_2 for each participant. The cells were treated with a medium containing conditioned serum for 48 h. The media consisted of 89% Dulbecco's Modified Eagle Medium (high glucose, Gibco™) 10% serum ($T0_{intervention}$, $T1_{intervention}$, $T2_{intervention}$), and 1% pen/strep (Gibco™). The medium was then removed, and the cells were washed twice with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde at 37 °C for 15 min.

They were washed three times with PBS and stored for 7 days at 4 °C with 1 mL PBS per well. Each well was washed with 0.05 M TRIS-buffered saline (TBS), then incubated for 20 min with 3% hydrogen peroxide in methanol and washed again with 0.05 M TBS. After 10 min with 0.5 M ammonium chloride and 0.25% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) in 0.05 M TBS and washing with 0.05 M TBS, 5% bovine albumin (BSA) in 0.05 M TBS was added for 60 min. The cells were incubated overnight with the anti-Ki-67-Rb antibody (NB500-170,j 1:250 in 1% BSA; Novus Biologicals®) at 4 °C and washed with 0.05 M TBS. A Goat anti-rabbit antibody (rabbit polyclonal to Ki-67, 1:400 dilution in 0.05 M TBS, Novus Biologicals®) was added to the cells for 60 min. After multiple washes with 0.05 M TBS, the cells were incubated for 60 min with the Horseradish peroxidase complex (1:400 dilution in 0.05 M TBS, Sigma-Aldrich) and washed again with 0.05 M TBS. The 3,3′Diaminobenzidine (DAB) solution mixture (150 µL DAB, 150 µL ammonium chloride, 300 µL nickel sulfate, 300 µL glucose, 5 µL glucose oxidase in 15 mL 0.1 M Pb, pH 7.4) was then added to the cells for 20 min. The reaction was stopped by removing the DAB solution and adding 0.05 M TBS. Finally, methyl green staining was performed. For this purpose, 200 µL of methyl green zinc chloride (1%, Sigma-Aldrich) was added to each well for 8 min and then washed with 0.05 M TBS and 96% ethanol. The water was removed using a descending series of alcohols (96%, 100% ethanol, and 100% xylene), and the glass plates with the cells were then transferred to slides.

Slides were analyzed under a light microscope. One researcher photographed them, while two others independently counted 100 cells per condition per participant. A fourth researcher evaluated interrater reliability by assessing the ratio of inactive to total cells for each condition and time point.

4.5. Cytokine Array

Cytokines in serum samples were screened using the RayBio® Human Cytokine Array C1000 Series (RayBiotech, Inc., Peachtree Corners, GA, USA.). Undiluted pooled samples for $T0_{intervention}$, $T1_{intervention}$, $T2_{intervention}$, $T0_{restl}$, $T1_{rest}$, and $T2_{rest}$ were processed following the manufacturer's instructions with minimal incubation times. Chemiluminescence was detected using the ChemiDoc™ MP Imaging System (Bio-Rad Laboratories, GmbH, Feldkirchen, Germany). Cytokines with visually distinct concentrations across time points were further analyzed via 2-D densitometry in ImageJ.

4.6. Lactate Concentration

Lactate concentration was measured in 20 µL of serum per participant per time point using Eppendorf vessels and analyzed with the EKF Biosen C-line.

4.7. Blocking CXCL9

The previous experiments revealed a decrease of Chemokine (C-X-C motif) ligand 9 (CXCL9) in serum as portrayed by a protein array, along with a reduction in cell proliferation as determined by immunohistochemistry with Ki-67. Cell growth and vitality, as shown in the MTT assay and immunohistochemistry, differed significantly from the time points T0 to T1, and these differences were also significant in the intervention condition vs. the control condition.

As CXCL9 is associated with changes in the tumor growth, we further investigated if the reduction in cell activity is caused by changes in CXCL9 concentration in serum [33]. As we observed a reduction in CXCL9, we used AMG 487 (Biomol, Hamburg, Germany), a CXC chemokine receptor 3 (CXCR3) antagonist, to block the CXCR9 pathway. We replicated the MTT assay and immunohistochemistry with serum from the time points T0 and T0 + IH. We expected to observe similar results to previous experiments.

Tumor cells were seeded as previously described for the MTT assay and immunohistochemistry, and consequently treated with medium containing T0 serum or medium with serum from T0 containing 1 µmol/L AMG 487 for 48 h at 37 °C and 5% CO_2 to compare the results to our previous ones [60].

AMG 487 was prepared as a 0.2 mM stock with DMSO according to the manufacturer's instructions.

The MTT assay and immunohistochemistry with Ki-67 were performed as previously described using controls with FBS and DMSO.

4.8. Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics (Version 29).

For the cell proliferation assay, we first calculated the interrater correlation coefficient. We looked at the ratio of Ki-67-negative to Ki-67-positive cells and the changes from $T0_{intervention}$ to $T1_{intervention}$, $T1_{intervention}$ to $T2_{intervention}$, and $T0_{intervention}$ to $T2_{intervention}$. Two independent researchers counted 100 cells per participant at each time point, and the proportion of negative cells was used to calculate the intraclass correlation coefficient (ICC) with a two-way mixed model, assuming systematic error [61]. The results are shown in Table 1.

We tested the data for normality using a Shapiro–Wilk test and found they were not normally distributed. Therefore, we used a Friedman test to compare time points and performed a post hoc analysis with Bonferroni correction (significance level $\alpha = 0.05$). We also calculated effect sizes. For the cytotoxicity analysis, normality was tested with a Shapiro–Wilk test, and sphericity with Mauchly's test followed. Since the data from the cytokine array densitometry analysis were not normally distributed, we used a Friedman test to test if differences between T0–T1, T1–T2, and T0–T2 were due to exercise or other factors. We compared absolute densitometry values and performed a post hoc test with Bonferroni correction. For the cell proliferation essay, after blocking with AMG 487, we followed a similar approach, calculating ICCs. Both ICCs are good or very good (*T0* = 0.819, *T0* + IH = 0.652) [61]. We tested the data of the inhibitor experiments for normality with a Shapiro–Wilk test and compared the two conditions with a two-sided paired *t*-test. A similar *t*-test was also used to compare the conditions in the cytotoxicity analysis.

Table 1. ICC for observation of immunohistochemistry. This table presents the results of the intraclass correlation analysis (ICC), which was conducted to assess the agreement between researchers in their observations of immunohistochemistry images.

Time Point	ICC [1]	95% Confidence Intervals (Lower/Upper)	
T0	0.905	0.488	0.977
T1	0.802	0.319	0.946
T2	0.815	0.359	0.949

[1] Intraclass correlation coefficient.

5. Conclusions

There are multiple known factors, like the immune and capillary systems, as well as cytokines and metabolites, which have an influence on tumor growth. In our pilot study, we show that there are variable components in serum conditioned by an acute endurance intervention. These factors in conditioned serum inhibited triple-negative breast cancer cell growth in vitro. Moreover, we identified a cytokine, CXCL9, which is likely regulated by exercise and may have a direct effect on cancer cell growth. These results align with the current literature on this topic. While chronic exercise interventions benefit overall health, the cumulative effects of individual exercise sessions may have the most significant impact on reducing tumor growth [40]. Our results support the theory that a single endurance intervention has an inhibitory effect on triple-negative breast cancer cell growth in vitro. Here, more highly standardized and homogenous studies investigating chronic vs. acute effects of different exercise modalities and different cancer cell lines are required to gain perspective. We could also support the finding that the CXCR3 axis has an inhibitory effect on tumor cell growth. Some studies also demonstrate the involvement of this axis in the TME, while other findings are contrasting [33]. More studies are required to understand this axis and the specific influence of exercise on it, as well as its subsequent impact on cancer. More factors should be analyzed individually and in combination, especially in cancer patient populations.

Author Contributions: N.G. designed the study, carried out the statistical analyses, supported the data collection, and wrote the manuscript in consultation with D.C. and W.B., K.S., P.N.-G., K.F. and A.V. carried out the experiments and contributed to data analysis. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was approved by the Institutional Review Board (IRB) of the German Sport University Cologne on 12 April 2023 under 041/2023. The study and all protocols used are in accordance with the Declaration of Helsinki.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Anonymous raw data can be provided upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

Natural killer cells	NK cells
Tumor microenvironment	TME
Myeloid-derived suppressor cells	MDSCs
Interleukin-6	IL-6
Vascular endothelial growth factor	VEGF
Matrix metalloprotease	MMP
Physical Activity Readiness Questionnaire	PAR-Q
International Physical Activity Questionnaire	IPAQ
Cardio Pulmonary Exercise Testing	CEPT
Peak aerobic capacity	VO2peak
Rate of perceived exertion	RPE
Phosphate-buffered saline	PBS
TRIS-buffered saline	TBS
Bovine albumin	BSA
3,3′Diaminobenzidine	DAB
Chemokine C-X-C motif ligand 9	CXCL9
CC-chemokine ligand	15 CCL15
Intraclass correlation coefficient	ICC
Inhibitor	IH
C-X-C motif chemokine 10	CXCL10
C-X-C motif chemokine 11	CXCL11

Appendix A

Table A1. Participant characteristics.

Variable	Mean	SD [5]
Age in *years*	46.4	9.4
Weight in *kg*	66.2	10.2
Height in *cm*	167.9	7.3
IPAQ-SF category [1]	Minimally active N = 8 Inactive N = 3	
PAR-Q score [2]	0.1	0.3
VO_{2peak} in *mL/min/kg* [3]	31.1	5.66
65% VO_{2peak} in *mL/min/kg*	20.2	3.68
50% VO_{2peak} in *mL/min/kg*	15.6	2.83
RPE_{max} [4]	19.7	0.45
$Watt_{max}$	155.9	27.49
$Watt_{65\%}$	101.3	17.87
$Watt_{50\%}$	78.0	13.74

[1] International Physical Activity Questionnaire—short form, [2] Physical Activity Readiness Questionnaire, [3] Peak oxygen consumption, [4] Rate of Perceived Exertion, [5] Standard deviation.

Table A2. Densitometry results.

Protein	difT0-T1	difT1-T2	difT0-T2	difRT0-T1	difRT1-T2	difRT0-T2
Leptin	1466.07	0.31	453.36	0.943	667,987	629,833
PDGF-BB	1875.27	0.66	1233.71	702,465	0.716	502,653
CCL5	1.21	0.66	0.80	713,666	1.044	744,822
MCP1	1.42	0.64	0.91	793,319	0.773	613,088
IL-15	0.63	0.87	0.55	1,061,224	0.893	947,585
Angiogenin	0.80	0.95	0.77	689,367	1.113	767,531
BDNF	0.78	0.74	0.57	550,514	1.014	558,105
TNF alpha	0.72	0.68	0.49	1,608	1.084	1.743
MIG (CX CL9)	0.92	0.72	0.66	0.497	1.332	0.662
IL-3	0.59	0.74	0.43	0.658	1.145	0.754
MIP-1delta (CCL 15)	0.92	0.65	0.60	0.700	1.565	1.096
IGFBP-1	1.26	1.05	1.33	0.721	1.070	0.771
NAP-2	1.27	0.49	0.62	0.996	1.096	1.092
EGF	1.25	1.31	1.63	0.602	0.704	0.424
IGFBP-2	1.58	0.80	1.26	0.689	1.027	0.708
Eotaxin-1 (CCL11)	0.72	1.66	1.20	0.741	1.017	0.754
ICAM-1(CD54)	0.99	1.50	1.48	2.523	0.853	2.152
TIMP-1	1.62	0.89	1.43	0.454	1.189	0.540
EGFR	1.43	0.71	1.02	0.761	0.948	0.721
TIMP-2	1.46	0.84	1.23	0.782	1.091	0.853
ENA-78 (CXCL5)	1.75	0.56	0.98	0.763	1.029	0.785
IGFBP-6	1.45	0.92	1.33	0.880	0.875	0.770
MIP-1-beta	1.61	0.81	1.31	0.654	0.658	0.430
TRAILR3	0.07	0.48	0.04	1.355	1.032	1.398
Adiponectin	19.78	1.04	20.50	0.854	0.997	0.852
MIP-3-beta	2.05	0.81	1.67	0.562	0.939	0.528
MSP	1.44	0.85	1.22	0.497	0.951	0.473
uPAR	1.35	0.70	0.95	1.016	0.615	0.625
ANGPT2	1.22	1.49	1.82	0.649	1.396	0.907
OPG	0.59	0.99	0.58	0.580	1.096	0.636
GRO	0.54	1.17	0.64	0.605	0.985	0.596
gp130	2.35	0.96	2.26	0.387	0.965	0.374
HCC-4	0.32	1.39	0.44	0.585	0.847	0.496
Il-6 R	2788.88	1.19	3321.07	0.878	0.916	0.804

The results of the densitometry analysis are presented as concentration increases and decreases between the time points. Values above one indicate a concentration increase, while values below one indicate a concentration decrease. The columns difT0-T1, difT1-T2, and difT0-T2 refer to the exercise condition; difRT0-RT1, difRT1-T2, and difRT0-T2 refer to the control condition.

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