Aus dem Deutschen Krebsforschungszentrum (DKFZ) Heidelberg

(Wissenschaftlicher Vorstand: Prof. Dr.med. Michael Baumann)

Abteilung Bewegung, Präventionsforschung und Krebs

(Leitung: Prof. Dr. Karen Steindorf)

Impact of different exercise modalities on tumor relevant Natural killer cell function and their regulation

Inauguraldissertation

zur Erlangung des Doctor scientiarum humanarum (Dr. sc. hum.)

an der Medizinischen Fakultät

der Ruprecht-Karls-Universität Heidelberg

vorgelegt von

Anasua Pal

aus

Kalkutta (Indien)

2020

Dekan: Herr Prof. Dr. Hans-Georg Kräusslich

Doktormutter: Frau Prof. Dr. Karen Steindorf

Table of Contents

List of abbreviations

1-MT	1-methyl tryptophan
1-RM	One-repetition maximum
3-HAA	3-hydroxyanthranilic acid
3HK	3-hydroxykynurenine
AA	Anthranilic acid
ACSM	American College of Sports Medicine
AhR	Aryl hydrocarbon receptor
AIP	AhR-interacting protein
ANCOVA	Analyses of covariance
ANOVA	Analyses of variance
ARNT	AhR nuclear translocator
BEATE	German study acronym for "**B**ewegung und **E**ntspannung **A**ls **T**herapie gegen **E**rschöpfung"
BEST	German study acronym for "**B**ewegung und **E**ntspannung für Brustkrebspatientinnen unter **St**rahlentherapie"
BMI	Body mass index
CD	Cluster of designation
CD	Cluster of differentiation
CNS	central nervous system
CPET	Cardiopulmonary exercise testing
CRP	C-reactive protein

DC	Dendritic cells
DNAM-1	DNAX accessory molecule-1
DRE	DNA replication-related element
ELISA	Enzyme-linked immunosorbent assay
FICZ	6-formylindolo[3,2-b] carbazole
GCN2	General control non-repressed kinase 2
GTP	Guanosine-5'-triphosphate
HHD	Hand-held dynamometry
HR	Heart rate
HRmax	Maximum heart rate
HSP	Heat shock protein
IC50	Half maximal inhibitory concentration
IFN-γ	Interferon gamma
Ig	Immunoglobulin
Il-	Interleukin
IRF	Interferon regulatory factors
JAK	Janus kinase
KA	Kynurenic acid
KAR	Activating immunoglobulin-like killer cell receptors
KAT	Kynurenine aminotransferase
kDa	Kilo Dalton
KIR	Killer-cell immunologlobulin like receptors
KTR	Kynurenine/Tryptophan ratio
KYN	Kynurenine

KYNU	Kynureninase
LAT	Large-neutral amino acid transporter
LPS	Lipopolysaccharides
MHC	Major histocompatibility complex
MIP	Macrophage inflammatory protein
NADPH	Nicotinamide adenine dinucleotide phosphate Hydrogen
NCR	Natural cytotoxic receptors
NCT	National Center for Tumor Diseases
NK	Natural killer
NKCA	NK cell cytotoxic activity
NKG2D	Natural killer group 2D
NMDA	N-methyl-D-aspartate
PAS	Per–Arnt–Sim
PBMC	Peripheral blood mononuclear cell
QA	Quinolinic acid
RCT	Randomized controlled trial
RPE	Rate of perceived exertion
RPM	Revolutions per minute
RT	Resistance training
SLC7a5	System L transporters
STAT	Signal transducer and activator of transcription
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TDO	Tryptophan 2,3-dioxygenase
Th cells	T helper cells

TNF	Tumor necrosis factor
TOP	German study acronym for "Individuelle **T**rainingssteuerung bei **O**nkologischen **P**atienten"
Treg cells	Regulatory T cells
TRP	Tryptophan
UTR	Untranslated region
VO2peak	Peak oxygen consumption
XaA	Xanthurenic acid
α7nACh	α7-nicotinic acetylcholine

List of tables

List of figures

1. INTRODUCTION

1.1 Physical activity/exercise and cancer

The last decade has seen a significant rise in interest in using exercise as medicine. Epidemiologic evidence concludes that those who perform a higher level of physical activity have a reduced likelihood of developing a variety of cancers compared to those who engage in lower levels of physical activity (Brown et al. 2012; Campbell et al. 2019; Meyerhardt et al. 2006; Patel et al. 2019). Cormie et al. in their review had mentioned that regular physical exercise counteracts cancer development and progression and reduces treatment related side effects, such as depressions and fatigue (Cormie et al. 2017). An active lifestyle is associated with reduced breast cancer risk and improved survival in breast cancer patients (Bodai and Tuso 2015). Besides a reduction of chronic inflammation, physical exercise has been shown to regulate hormonal balance including catecholamine, prostaglandins, and cortisol (Keast et al. 1988) (Figure 1). Physical exercise has also been shown to mimic chemotherapy and has an additive effect (Ballard-Barbash et al. 2012; Betof et al. 2015). Although evidence for primary and tertiary prevention in randomized controlled trials is still lacking, preclinical research has shown that exercise has a direct effect on the immune function as shown for the first time in 1989 by Nieman and his team (Nieman et al. 1989). Despite these observational evidences, the underlying mechanisms associated between participation in physical activity, immune modulation and cancer risk reduction remains understudied.
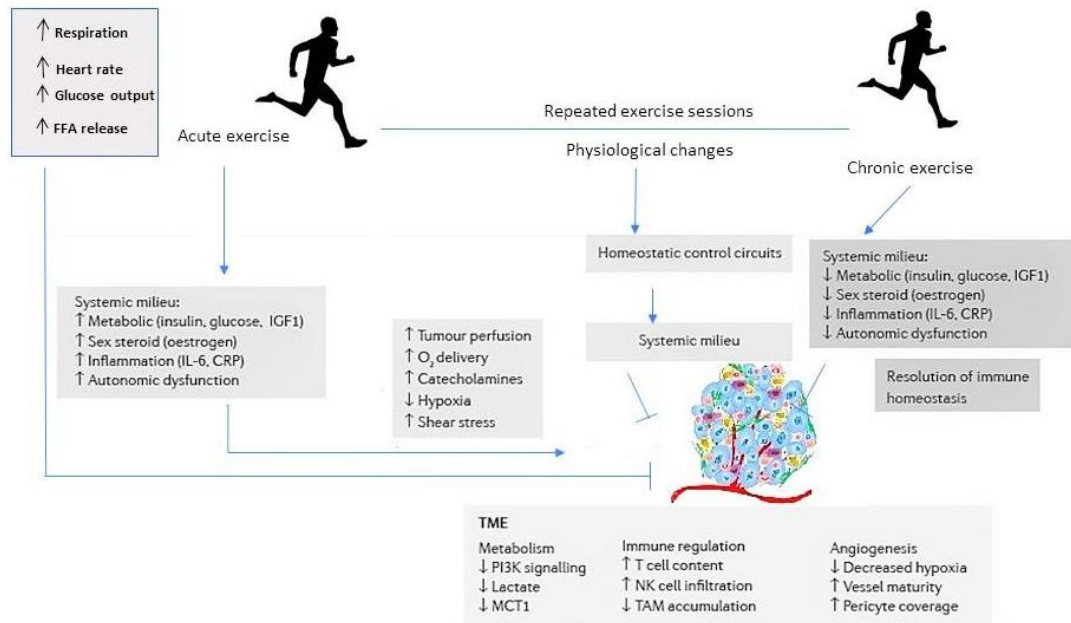
Figure 1: Physiological changes as well as changes observed in the tumor microenvironment due to exercise**:** The schematic diagram reveals improvements in the large-scale vascular (Seok et al.) and microscale physiological microenvironment (bottom) inside an active individual's body. Different physiological changes are observed depending on duration of exercise (acute vs chronic).(compiled with information from (Verma et al. 2009; Wiggins et al. 2018)).

1.2 Overview of the immune system

When exploring possible mechanism by which adaptation to physical exercise could occur, the immune system comes up as one likely candidate of importance. A challenge to the immune system can elicit an immediate and a delayed type response, referred to as innate and adaptive immunity, respectively (Erhard et al. 2000; Janeway 2001). The innate immune system includes epithelial barriers, macrophages, neutrophils, Natural killer cells and cytokines, and responds similarly to repeated infections by the same pathogen. The adaptive immune system (also called specific or acquired) includes lymphocytes (B and T cells) and can, due to its memory function, respond more vigorously to each repeated infection (Erhard et al. 2000) (Figure 2). An immune response is normally defined as the reaction of these cells and molecules to any foreign substance, but in this thesis the term immune response is also used to describe the immunological changes elicited by physical exercise.
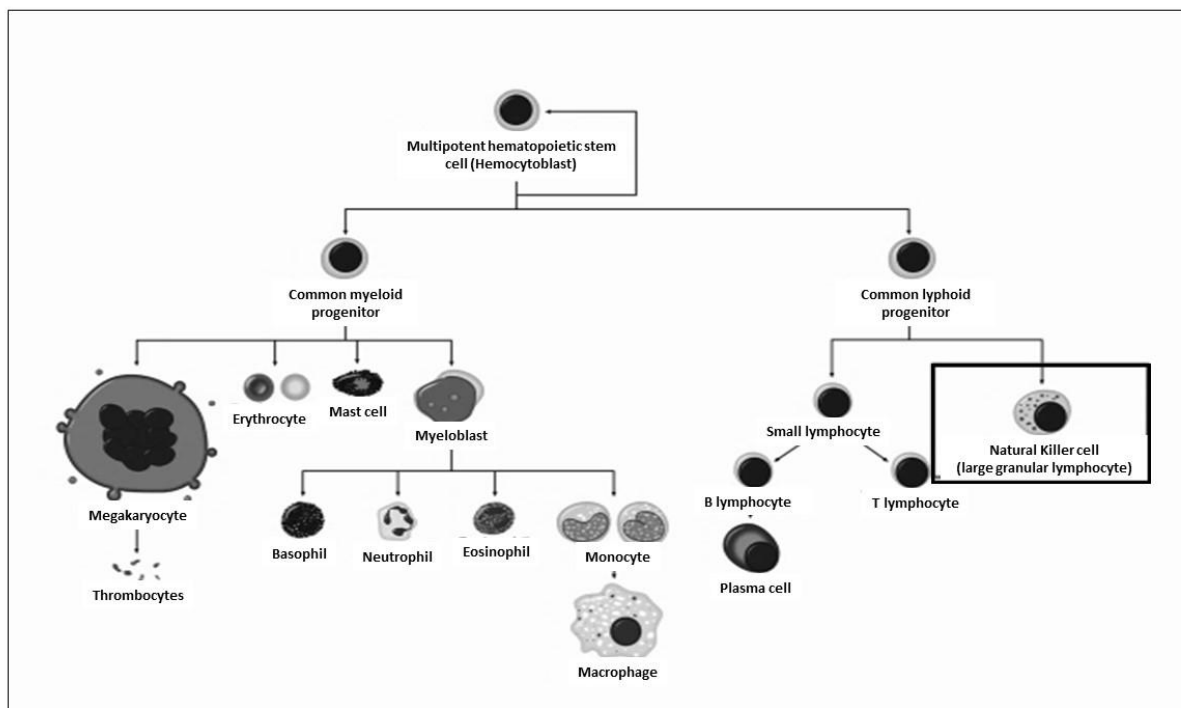
Figure 2: Immune cells lineage: Comprehensive diagram illustrating the formation of various blood cells in both myeloid and lymphoid lineages from haematopoietic stem cells. The box represents the cell of interest for this research: NK cells. (Adapted from (Dudley 1992)).

1.3 NK cell specific immunity

For this research the focus was on the innate branch of immune cells focusing on NK cells. To understand how NK cells are modulated via exercise it is important to understand how NK cells function generally in a human body under physiological conditions.

NK cells are a heterogeneous population that are CD32 and that express characteristic NK cell markers, such as CD16 and CD56 (Abel et al. 2018; Cichocki et al. 2019). NK cells are an important arm of the innate immune response that are directly involved in the recognition and lysis of virus-infected and tumor cells. NK cells express activating and inhibitory receptors that upon engagement by cognate ligands on target tumor cells regulate NK cell antitumor activity (Campbell and Purdy 2011; Kumar 2018) (Figure 3). NK cells induce apoptosis by directly binding with target cell Fas ligand (Zamai et al. 1998). NK cell activity is dependent on the balance between activating and inhibiting receptors (Kumar 2018). The activating receptors belong to C-type Lectin family receptors. The Natural killer group 2 (NKG2) family of receptors identifies as MHC1 non-classical pathway. The prominent inhibitory receptor Killer-cell immunoglobulin like receptors (KIR), belongs to the family of transmembrane glycoproteins. They function by interacting with MHC1 molecules and possess the ability to suppress the cytotoxic ability of NK cells. The maturation of the activating and inhibiting receptors on NK cells through epigenetic modifications enables to strike a balance between effective defence and self-tolerance (Campbell and Purdy 2011; Kumar 2018; Schmiedel and Mandelboim 2018; Zamai et al. 1998). The cytolytic activity of NK cells is enhanced by interferon IFN-α (Une et al. 2000) and interleukin IL-2, whereas certain prostaglandins and immune complexes downregulate the function of NK cells (Paul and Lal 2017).

These data indicate that impaired NK cell antitumor response results from NK cell receptor alterations induced by suppressive factors in the tumor microenvironment, including inflammatory cytokines, growth factors, enzymes and metabolites, as well as by chronic NK cell receptor engagement by the tumor. The established alterations in NK cell receptor expression in cancer patients represent potential disease biomarkers and may aid in choosing therapies that upregulate activating or block inhibitory receptor function (Bassani et al. 2019; Terren et al. 2019).
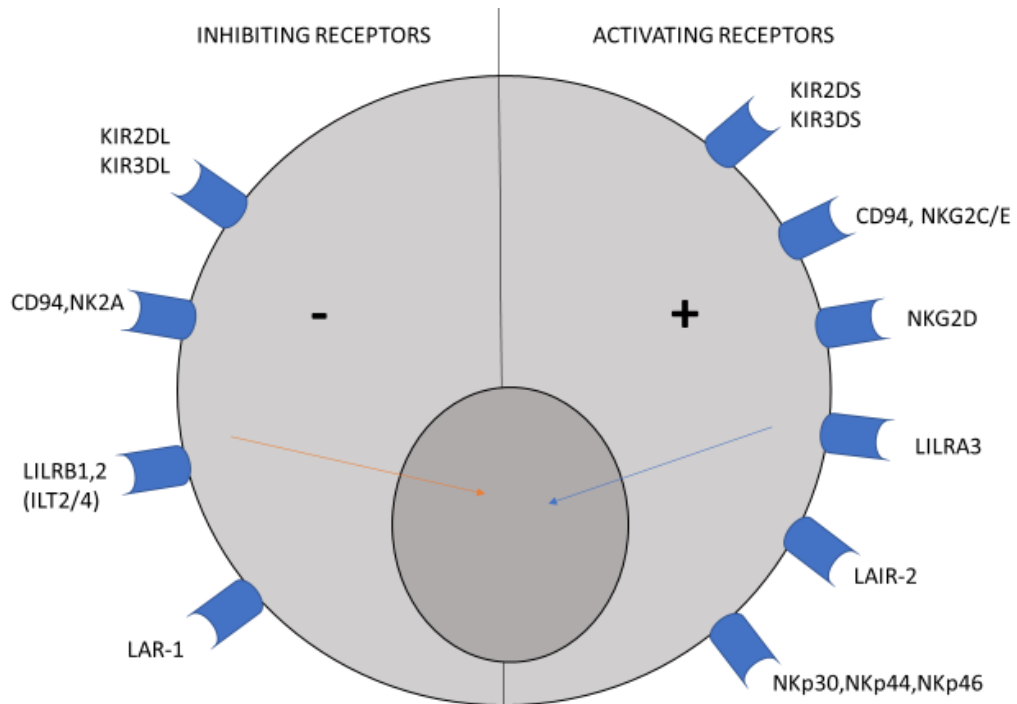
Figure 3: Major receptors expressed on the surface of NK cells. Some important ones include: NKp46, Natural killer cell p46-related protein; NKp44, Natural killer cell p44-related protein; NKp30, Natural killer cell p30-related protein; CD, Cluster of differentiation; NKG2, also known as CD159; KIR, killer-cell immunoglobulin like receptor. The "+" indicates activating and "-" represents inhibiting receptors. (Adapted from (Laperrousaz et al. 2012)).

1.4 Exercise immunology

Research on the effects of exercise on the immune system has grown significantly in the past decade. This remarkable rise in interest in applied immunology and physical activity shows the capacity of the body's immune system being harnessed. Exercise has been known to influence cytokine levels and number as well as proportions and functions of various immune cells. Particularly during moderate intensity aerobic exercise T cell populations transiently rise, (Lancaster 2006) NK cell populations and activity transiently rise, and neutrophil quantity and activity also transiently rise (Nieman and Wentz 2019) (Table 1).

Although these effects are transient during an acute bout of exercise, the repetitive effects may produce a cumulative (training) effect (Nieman and Wentz 2019; Pedersen et al. 1998). Chronic bouts of physical activity have been associated with an inverted 'J-curve' which implies optimal immune function is achieved with moderate intensity physical activity (Nieman and Wentz 2019; Pedersen et al. 1998; Schwellnus et al. 2016; Soligard et al. 2016).

The rapidly emerging field of exercise immunology delves deep into exercise induced immune cell redistribution. Indeed, a recent study demonstrated inhibition of tumor onset and disease progression across a range of tumor models in voluntarily active rodents (Pedersen et al. 2016). In this work, NK cell infiltration was significantly increased in tumors from active versus inactive rodents, leading to the conclusion that the presence of NK cells (but perhaps also T cells) in tumor sites is redeployed by adrenaline during exercise (Melaiu et al. 2019; Pedersen et al. 2016). While these studies are limited to rodents, there is growing evidence that exercise may promote anti-cancer effects in humans. For example, in a key study recently conducted in humans, it was shown that NK cells with a highly mature effector phenotype are preferentially redistributed after exercise and have the capacity to exert augmented cytotoxicity against myeloma and lymphoma cells in vitro (Bigley et al. 2014; Bigley et al. 2015). Considering these results, research is now being conducted to harness the beneficial impact of acute exercise on lymphocyte kinetics for the purposes of cancer immunotherapy (Simpson et al. 2017).

Table 1: Exercise induced changes in different immune cells and cytokines: The table shows the changes in immune cells as well as cytokines during and after exercise. Upward arrow indicates a higher count or expression; downward arrow indicates a lower count or expression. Double upward arrow indicates a marked increase. (Adapted from (Diment et al. 2015; Groer 1995; Nieman 1997)).

	During Exercise	After Exercise
Neutrophil count	↑	↑ ↑
Monocyte count	↑	↑
Lynphocyte count	↑	↓
CD4+ T cell count	↑	↓
CD8+ T cell count	↑	↓
CD19+ B cell count	↑	↓
CD16+56+ NK cell count	↑	↓
Lymphocyte apoptosis	↑	↑
Proliferative response to mitogens	↓	↓
Antibody response in vitro	↓	↓
Saliva IgA	↓	↓
NK cell activity	↑	↓
Lymphokine activated killer cell activity	↑	↓
C-reactive protein	↑	↓
Plasma concentration of TNF-α	↑	↑
Plasma concentration of IL-1	↑	↑
Plasma concentration of IL-6	↑ ↑	↑
Plasma concentration of IL-1ra	↑ ↑	↑
Plasma concentration of IL-10	↑	↑
Plasma concentration of TNF-R	↑	↑
Plasma concentration of MIP-1β,IL-8	↑	↑

Abbreviations: TNF- Tumor necrosis factor, TNF-R- Tumor necrosis factor receptor; IL- Interleukin, MIP-macrophage inflammatory protein.

1.5 Effects of acute exercise on immune system

Despite the apparent health benefits achieved by leading an active lifestyle, which imply that regular physical activity and exercise enhance immune function and regulation, the effect of a single bout of exercise (acute effects) on immune function remains open for discussion.

Physiological responses to acute and long-term adaptations of immunity to exercise have been demonstrated to be dependent on exercise type and dose (type i.e., endurance, resistance or sprint training; dose i.e., low intensity (<40% VO_{2max}), moderate (40-69%VO_{2max}) vigorous (70-90%VO_{2max}), or very high intensity (>90% VO_{2max}) type (Sellami et al. 2018). After a very intense workout, studies have shown that there is a general decrease in immunity for several hours after exercise, termed the "open-window theory of susceptibility to infections" (Sellami et al. 2018).

The foundations of this belief lay as widely discussed in exercise immunology literature: (i) infection risk is increased after an acute bout of prolonged and vigorous aerobic exercise; (ii) acute bouts of vigorous exercise can lead to a temporary reduction to salivary IgA levels culminating in a higher risk of opportunistic infections; and (iii) transient decreases in the number of peripheral blood immune cells, which occurs in the hours following vigorous exercise, represents a period of immune suppression. Even today, the "open window" hypothesis continues to be discussed (Campbell and Turner 2018), despite the existence of contradictory evidence.

Acute exercise impacts on circulation and leaves blood to travel to tissues where it is more likely to encounter infected cells or body cells that have become cancerous. Some studies have reported that the acute effects of exercise can stimulate a mobilization of hematopoietic stem cells from bone marrow and of senescent immune cells from the peripheral tissues to the circulation (Cao Dinh et al. 2017; Pedersen 2000).

Research has also shown that noradrenaline is responsible for the effects of acute exercise on lymphocyte changes, including NK cell and T cell activity (Nieman et al. 1989; Sellami et al. 2018). During long duration acute exercise, increases in catecholamine levels with growth hormones has been reported which mediate the changes in neutrophils levels and control lymphopenia and neutrocytosis. It has been

known that glutamine, the abundant amino acid found in muscles, stimulates *in vitro* lymphocyte proliferation, lymphokine activated killer cell action, and cytokine release (Phaneuf and Leeuwenburgh 2001). Blood and muscle levels of glutamine and glucose continue to decrease during intense exercise, which may be a potential reason for "immunosuppression," despite current evidence that dispute this association between glutamine and immunosuppression (Phaneuf and Leeuwenburgh 2001). According to some research, exercise induced apoptosis accelerates the elimination of damaged cells without causing a pronounced inflammatory status, which may boost the body's function instead (Campbell et al. 2009).

Acute steady state vigorous exercise lasting for 45 minutes elicits the classic biphasic response of lymphocytes characterized by a dramatic lymphocytosis. This response is through a dramatic influx of Natural killer cells, which rise by up to 10-fold, and CD8^{+} T cells which increase to a lesser but still markedly by approximately 2.5-fold (Campbell et al. 2009; Campbell and Turner 2018). For example, among CD8^{+} T cells, subsets that exhibit strong effector function (e.g., CD45RA^{+}CD27^{−}CD28^{−}CCR7^{−}CD62L^{−}CD57^{+}) increase substantially during exercise (Campbell et al. 2009; Turner et al. 2010).

This exercise dependent mobilization is driven partly by increase in shear forces and blood pressure during exercise, causing a non-specific flushing of the marginal pools (Sellami et al. 2018; Shephard 2003). Moreover, it is principally governed by adrenergic stimulation of beta-2-adrenergic receptors on the surface of lymphocytes, arising from adrenaline released during exercise, causing endothelial detachment and subsequent recirculation of lymphocytes into the bloodstream (Benschop et al. 1994; Campbell and Turner 2018).The differential expression of beta-2 adrenergic receptors on the lymphocytes mirrors its mobilization response observed during exercise: NK cells > CD8^{+} T cells > B cells > CD4^{+} T cells, including regulatory T cells (Campbell et al. 2009; Turner et al. 2010). After the exercise session there is a decrease in the frequency of lymphocytes in the bloodstream. This is typically observed approximately 1–2 h post-exercise when the lymphocyte numerical count is lower than pre-exercise levels. Lymphocyte frequency normally returns to pre-exercise levels within 24 h (Kruger et al. 2016; Shek et al. 1995; Shinkai et al. 1992). The lymphopenia that occurs 1–2 h later is dependent on exercise intensity and the most reductions during this period are typically observed among NK cells and CD8^{+} T cells (Shinkai et al. 1992).

Rather than considering it as "exercise induced immunosuppression", a more contemporary viewpoint is that this acute and transient lymphopenia 1–2 h after exercise is beneficial to immune surveillance and regulation. In this highly specialized response, it is proposed that that exercise redeploys immune cells to peripheral tissues (e.g., mucosal surfaces) to conduct immune surveillance. Here, these immune cells are thought to identify and carry out pathogen eradication or eradicate those cells that have become damaged or malignant. This process is known as the acute stress/exercise immune-enhancement hypothesis (Dhabhar 2014). Kruger and colleagues, in their seminal study showed that using fluorescent cell tracking in rodents, T cells are redeployed in large numbers to peripheral tissues including the gut and lungs, and to the bone marrow following exercise (Kruger et al. 2016; Kruger and Mooren 2007). In line with Dhabhar's theory, it is hypothesized that this redistribution reflects heightened immune surveillance in sites where pathogens are likely to be encountered during and after exercise (i.e., lungs, gut). This response has also been proposed to maintain immune homeostasis *via* augmented regulatory activities (Dhabhar 2014). Reports on exercise induced apoptosis and lymphopenia have been interpreted as detrimental. Other studies have reported increased lymphocyte apoptosis immediately after exercise (i.e., as a result of the large mobilization of cells) but not in the hours following exercise during lymphopenia (Mooren et al. 2002; Simpson et al. 2017). Although the extent of lymphocyte apoptosis reported in these studies is dependent on the measurement technique, typically <10% of lymphocytes undergo post-exercise apoptosis (Mars et al. 1998; Mooren et al. 2004). Given the post exercise decrease of 30–60% in lymphocyte numbers (Hansen et al. 1991; Shek et al. 1995; Shephard and Shek 1999) apoptosis could be a small contributor to exercise induced lymphopenia, but this process of cell death is likely to be beneficial given the stimulation of progenitor cells from the bone marrow (Mooren and Kruger 2015).

The most exercise-responsive lymphocyte Natural killer cells—the subset—CD56dim cells are preferentially redeployed rather than their CD56bright counterparts (Campbell et al. 2009). At the same time, T cells also appear to exert heterogeneous but highly coordinated responses to acute exercise. It is consistently observed that discrete populations of CD8^{+} but not CD4^{+} T cell subsets are redeployed by exercise. For some time, there were discussions pertaining to the exact behaviour of CD8^{+} T cells in response to exercise. In studies carried out about a decade ago, it was shown that exercise selectively mobilizes memory CD8^{+} T cells with a phenotypic propensity for

homing to peripheral tissues—characterized for example by CD11, and not CCR7 or CD62L expression—and the distinctive capacity to mount rapid effector functions (Campbell et al. 2009; Simpson et al. 2017; Simpson et al. 2010). This response presumably works in synergy with NK cells facilitating the detection and elimination of neoplastic, stressed or infected cells (Freud and Caligiuri 2006). Aligned with the immune surveillance theory, (Kim et al. 2007), these results imply that exercise induced changes to stress hormones redeploys the immune cells to exert effector functions against neoplastic, stressed, or infected cells after cessation of exercise. This process, which occurs daily in a natural diurnal process (Scheiermann et al. 2013), orchestrated subtly by stress hormones (Dimitrov et al. 2009; Suzuki et al. 2016), appears to be primed in response to exercise, leading to enhanced immune surveillance (Hojman 2017).

Overall, the above-mentioned studies imply that exercise induced lymphocytosis, and the lymphopenia that follows, is beneficial to the immune system's capacity to identify and neutralize damaged and neoplastic cells in peripheral tissues. Furthermore, in the context of neoplastic growth, this process may be directly responsible for reduced incidence of cancer among physically active people across the lifespan (Moore et al. 2016). Further comprehensive discussion of the role of exercise and NK cells can be found in section 1

1.6 Effects of chronic exercise on immune system

Chronic exercise has the potential to exert both positive and deleterious effects on the normal functioning of the immune system. In general, studies on the chronic effects of exercise on immune cells have been focused mostly on healthy young people or athletes to ascertain the effects of overtraining / excessive training. Long-term effect on immune function in the elderly is less debated. In a systematic review by Cao Dinh et al. it was reported that most studies were conducted in young (~20–40 years) and middle-aged (~40–50 years) and showed an increase in activity of NK cells and T lymphocytes occurring without apoptosis (Cao Dinh et al. 2017).

One of the major occurrences in training adaptation in the immune system is the change in catecholamines, with a blunted neuro-endocrine response and downregulation of adrenergic receptors. In fact, previous studies have demonstrated that chronic exercise such as sprint and resistance training may counteract the negative effect of age on catecholamines and growth hormones in 40-year-old men (Sellami et al. 2017; Sellami et al. 2018). As catecholamines modulate immune cell function (Shephard 2003), it is therefore important to highlight the impact of these type of exercises training on immune function.

Chronic regular exercise may reduce the risk of diseases due to its anti-inflammatory effects. Various mechanisms have been proposed to explain how exercise reduces the systemic inflammation (Flynn et al. 2007) (Table 1). Regular exercise reduces visceral fat mass, the accumulation of which results in elevated production of proinflammatory adipokines (Kelly et al. 2014). The release of anti-inflammatory cytokines following a bout of exercise may also contribute to the reduction in systemic inflammation. IL-6 released from skeletal muscle during exercise results in a subsequent increase in IL-10 and IL-1 receptor antagonist, both of which are considered anti-inflammatory (Leal et al. 2018). Further, the hormones released during exercise have anti-inflammatory properties: cortisol acts as an anti-inflammatory mediator (Yeager et al. 2011) and adrenaline downregulates the production of the inflammatory cytokines IL-1β and TNF (Petersen and Pedersen 2005). Exercise also downregulates surface expression of Toll-like receptors (TLRs) on monocytes and macrophages, and in turn mitigates their downstream inflammatory cascades (Gleeson et al. 2006). Exercise may also promote the switching of M1-type

inflammatory macrophages to anti-inflammatory M2-type and reduce the infiltration of macrophages into adipose tissue, resulting in a reduction in the production of inflammatory cytokines (Goh et al. 2016).

Reports from longitudinal training studies showed decline in total NK cells and CD8+ T cells after 12 weeks and 6 months of intensive training in swimmers and cyclists, respectively (Baj et al. 1994; Gleeson et al. 2000). Heavy exercise training has been reported to decrease Neutrophil respiratory burst and NK cell activity (Pyne 1994). Functional declines in adaptive immunity due to prolonged periods of intensive exercise training appeared to be related to alterations in the pro- and anti-inflammatory cytokine balance and elevated plasma stress hormone levels, particularly cortisol (Gunnarsson et al. 2013; Gunzer et al. 2012; Papacosta et al. 2013). Hallmark features of immunosenescence include an inverted CD4+/CD8+ T cell ratio and an increased frequency and proportion of senescent T cells (Huff et al. 2019). It has been hypothesized that regular exercise may facilitate the selective apoptosis of these "older" senescent T cells allowing them to be replaced by "younger" T cells capable of responding to novel antigens (Turner 2016). It is important to note that a common problem with many of the longitudinal studies is that immune function is assessed before and after exercise training in sedentary but otherwise healthy people. It is therefore not surprising that these studies show enhancements in immune function in these healthy subjects.

In context of cancer, numerous trials have tried to reduce low-grade inflammation in cancer survivors, or people at high risk of cancer, through exercise training (Ballard-Barbash et al. 2012; Friedenreich et al. 2016). The conclusions from these studies are that modest reductions in systemic levels of CRP, TNF-α, IL-6, and other pro-inflammatory factors may be obtained with exercise interventions, but these need to be of long duration (Schmidt et al. 2016). The typical 12–16 weeks of exercise training intervention most often fail to regulate systemic low-grade inflammation in cancer survivors, or people at high risk of cancer (Dethlefsen et al. 2017). Despite the large efforts to control systemic low-grade inflammation through exercise training, the evidence that such systemic regulation of pro-inflammatory cytokines should result in control of cancer progression is purely correlational and does not address the underlying causality. In a simplistic experimental design, Dethlefsen and colleagues addressed the effect of a training-dependent reduction in systemic low-grade inflammation, and how it translated into control of cancer cell growth in in vitro settings

(Dethlefsen et al. 2017). To this end, serum obtained from breast cancer survivors, who had participated in a 6-month endurance training intervention study, resulted in marked improvements in fitness levels, as well as significant reductions in the serum levels of the inflammatory cytokines, TNF-α and IL-6 (Dethlefsen et al. 2016). Notwithstanding this reduction in systemic inflammation, the training-conditioned serum had no regulatory effect on breast cancer cell growth, when used in vitro serum incubation studies. In contrast, the study further demonstrated that the systemic changes occurring during a session of exercise, which involves large increases of IL-6 and other cytokines known to be derived from contracting muscles, could, in fact, inhibit cancer cell growth in vitro (Dethlefsen et al. 2016). These findings question whether systemic adaptations to training in inflammatory markers are mediating the beneficial effect of exercise, or instead imply that the accumulative effect of repeated acute exercise responses may lead to control of tumor growth, as consistently reported in observational studies (Schmidt et al. 2016).

1.7 Acute and chronic exercise mediated NK cell changes

One of the most frequently investigated immune cell populations in the context of exercise and cancer are NK cells. As a part of the innate immune system NK cells have the potential to identify and eliminate tumor cells without prior priming (Kumar 2018). Activation of NK cells are dependent on the interplay between its activating and inhibiting receptors. Indeed, higher levels of intra-tumoral NK cell numbers are associated with better prognosis in some types of cancer (Abel et al. 2018; Campbell and Purdy 2011; Cichocki et al. 2019).

Studies have revealed that an acute bout of exercise affects the distribution of NK cell population, which is elevated immediately after exercise, strongly decreases in the subsequent hours and returns to baseline within 24 hours (Nieman 1997), depending on exercise duration and intensity. It was speculated that mobilized NK cells migrate into tumor tissue. Studies have shown that high intensity aerobic exercise preferentially mobilizes highly differentiated (NKG2A-/KIR+) NK cells to the periphery compared to medium differentiated (NKG2A+/KIR+) NK cells, which are mobilized more than low-differentiated (NKG2A+/KIR-) NK cells (Bigley et al. 2014; Bigley et al. 2015). The exercise induced shifts in NK cell subsets may partially explain the observed increase in NKCA/cell (NK cell cytotoxic activity) during recovery from exercise. Recent animal models by Pedersen et al. showed that NK cells were mobilized by epinephrine and blocking the ®-adrenergic signalling blunted training-dependent tumor inhibition (Pedersen et al. 2016). Studies have reported that both epinephrine and norepinephrine drives NK cell mobilization in humans, linking the rapid mobilization of NK cells to exercise intensity dependent responses in catecholamine concentrations (Dimitrov et al. 2009; Kappel et al. 1998; Kappel et al. 1991).

Besides an intermediate exercise induced mobilization of NK cells, it has been suggested that exercise increases NK cell cytotoxicity. However, studies on exercise induced NK cell activity were contradictory. The increased cytotoxic activity of NK cells can be attributed to changes in neuroendocrine status, hematopoiesis, muscle damage, protein synthesis, glucose metabolism, and antioxidant defenses (Fairey et al. 2005). Radom-Aizik et al. reported that a brief bout of exercise affects the gene and miRNA expression pattern in NK cells. They found that the differentially expressed genes were a part of 7 pathways (Radom-Aizik et al. 2013). It was also proposed that

acute activation of NK cells is accompanied by epigenetic modifications (Zimmer et al. 2014). In contrast to acute effects of exercise on NK cell mobilization and cytotoxicity, results of chronic effects of exercise in view of NK cell cytotoxicity, are contradictory.

In addition to the acute effects of exercise on NK cell function, there are chronic effects of exercise that arise from repeated acute bouts of exercise. Studies conducted on chronic exercise effects did not show any change in NK cell cytotoxicity *in vitro* in post-menopausal overweight women compared to the stretching control group (Idorn and Hojman 2016). However, intense training has been reported to alter NK cell subset and reduce NK cell cytotoxicity. It has also been reported that exercise training has no effect (Campbell et al. 2008; Nieman et al. 1993b; Nieman et al. 1990) or a negative effect on NKCA (Suzui et al. 2004). Alternatively, high volume exercise training has been linked to increased NK cell activation and cytotoxicity (Moro-Garcia et al. 2014; Woods et al. 1999), and individuals with high aerobic capacity have greater NKCA compared to their less fit counterparts (Nieman et al. 1993a). High aerobic capacity (Rhind et al. 1994) and intensive exercise training have also been linked to an increased proportion of immunoregulatory CD56bright NK cells (Suzui et al. 2004). Four weeks of hypoxic exercise training has been linked to increased NK cell activating receptor expression, Perforin/Granzyme-B levels, and cytotoxicity (Wang and Weng 2011). Several studies carried out on animals have shown increased NK cell cytotoxicity in vivo with regular exercise, but the underlying mechanisms contributing to this effect is still unclear. The beneficial effects of exercise are not universally agreed upon.

The literature regarding the effects of exercise training on NK cell function in cancer patients is very limited. Na et al. reported a marked increase in NKCA after 2 weeks of supervised aerobic activity in 35 stomach cancer patients after curative surgery (Na et al. 2000). On the other hand, no effect on NK cell count or function by Nieman et al. 1995b has been reported in a study of 16 female breast cancer patients on moderate intensity exercise training, which included both aerobic and anaerobic components (Nieman et al. 1995). Further, a recent study by Saxton et al. on 85 women treated for breast cancer, a 6-month aerobic exercise training regimen combined with a hypocaloric diet had no effect on NK cell count or cytotoxicity (Saxton et al. 2014). While the results discussed above are contradictory, these studies suggest that one of the benefits of regular exercise on cancer prognosis may be enhanced NK cell function.

1.8 Exercise and NK cell gene expression

It is important to understand how the exercise-associated change in the distribution, function and mobilization of NK cells within the circulation could affect the overall expression of the NK cell gene.

Global transcriptional analysis is a powerful approach in providing new insights into the biology of specific subsets of cells. Early studies using this method based on human and mouse NK cells have identified gene sets that were expressed uniquely in NK cells, as well as transcriptional changes involved in NK cell activation (Dybkaer et al. 2007; Obata-Onai et al. 2002). Previous studies have reported gene expression changes in response to exercise in the circulating PBMC and neutrophil population (Connolly et al. 2004; Radom-Aizik et al. 2008). Changes in the expression of the genes are accompanied by alterations in the expression of miRNAs (Radom-Aizik et al. 2013).

Brief exercise, patterned after naturally occurring physical activity, can significantly alter the patterns of NK cells' gene and miRNA expression in circulation (Radom-Aizik et al. 2013). How these changes affect the subsequent patterns of gene and miRNA expression and redistribution of circulating NK cells during recovery from acute exercise is not known. It is worth noting that Shakhar et al. found in murine models that NK cells residing in pulmonary circulation (likely to be released into central circulation with exercise) reacted differently to immuno-stimulation and stress hormone regulation than NK cells residing in the peripheral circulation (Shakhar et al. 2007). In these studies, the pulmonary NK cells were more efficient at killing malignant cells in in vivo in experimental models than the circulating NK cells, which was recently substantiated by Takeda et al. (Takeda et al. 2011). Thus an exercise-associated change from marginal pools in NK cells (or other leukocytes), in which the NK cell gene expression and functional profile vary from the circulation profiles, may prove to be as powerful a biological mechanism as a direct effect of exercise on the gene and functional profile of the circulating cells themselves.

Separate NK cell subsets, as mentioned above, have different functions. The CD3-CD56 dim cells are more cytotoxic than those of CD3-CD56 bright. In comparison, abundant cytokines are produced by the bright NK cells (almost 10 percent of the circulating NK cells) (Cooper et al. 2001). Additionally, there is growing evidence that

the gene and protein expression of NK cell subsets vary (Hanna et al. 2004; Wendt et al. 2006). CD56 dim cells have higher gene expression of cytolytic molecules than CD56bright subsets (with the exception of Granzyme K), while expression of molecules involved in adhesion, migration, and cell-to - cell cross-discussion is generally higher in the CD56bright subset (Radom-Aizik et al. 2013). Radom Aizik reported, expression of KIRs, KIR2DL4, KIR2DS3, and KIR2DS4, increased after exercise, consistent with a higher dim to bright NK cell ratio. In addition, they observed that genes with higher expression at rest in the NK bright population were again de-regulated after exercise (e.g., CXCR3, IL7R, CCR7, GZMK), in line with the change in the ratio of these cells in the post-exercise circulation (Radom-Aizik et al. 2013). Based on these observations, it was suspected that some of the changes in gene expression observed may be mediated by distributional shifts in NK cell subsets.

Radom Aizik also reported, a 1.9-fold decrease in GZMK expression, which is a cell-death-inducing serine protease with trypsin-like protease activity (Radom-Aizik et al. 2013). While they did not detect a significant change in the expression level of TRAIL or Fas ligands, yet they found gene expression alteration in other, functionally related tumor necrosis factor (TNF) family members, e.g. a 2.1-fold increase in TNFAIP3, a programmed cell death inhibitor that plays a role in lymphoid function, a 2-fold decrease in TNFSF13B, and a 3.2-fold decrease in lymphoid function. Additionally, significant exercise-associated changes in gene pathways were identified by them, one of them being the p53 signalling pathway (Radom-Aizik et al. 2013).

Physical activity and exercise are known to cause short- and long-term epigenetic alterations in different cell types and tissues (Abel and Rissman 2013; Alexander and Owens 2012; Hupkes et al. 2011; Radom-Aizik et al. 2012). One may presume that similar mechanisms can also affect NK cells. In this context, the first study was conducted by Nakajima et al. who showed that a six-month endurance exercise program counteracts age-dependent ASC gene DNA demethylation in peripheral blood monocytes that encodes for pro-inflammatory cytokines (Nakajima et al. 2010). Since epigenetic alteration can be cell-specific, these findings are difficult to pass to single immune cell subsets. Against this backdrop, Zimmer et al. have shown reduced H3K9Ac expression in NK cells of B-cell non-hodgkin lymphoma patients who underwent single bout of exercise compared to healthy controls. In addition, a correlation was reported between H4K5Ac and endurance capacity (Zimmer et al. 2014), which links physical activity to NK cell regulation. However, one single bout of

low to moderate endurance exercise did not affect histone acetylation in NK cells, either in patients or in controls. On the contrary, a longer and more vigorous single workout (half-marathon) resulted in a substantial increase in global H4K5ac in both aftercare and controls for cancer patients (Zimmer et al. 2015). Notably, this epigenetic modification is reported to be followed by an elevated expression of NKG2D, which was most pronounced 24 hours after the race. In this interventional review, while global DNA methylation has been unchanged, the authors note that gene-specific alterations cannot be ruled out (Zimmer et al. 2015). These results are consistent with those of Fernandes-Sanchez et al. who have shown that NKG2D expression is regulated by changes in histone and DNA demethylation (Fernandez-Sanchez et al. 2013).

Therefore, it is probable that physical exercise has an effect on NK cell gene expression. Whether these changes are mediated by epigenetics or transcriptional regulation is a question worth investigation. Also, whether these changes are an effect of acute bouts of exercise or chronic training is also important to understand. Finally, as resistance exercise becomes more popular it's imperative to understand whether resistance exercise specifically can elicit a change in NK cell gene expression.

1.9 Immune regulation via Kynurenine pathway

Data from preclinical studies and trials in healthy volunteers indicate that exercise can modulate the levels of Tryptophan metabolites via the Kynurenine pathway (Zimmer et al. 2019b). Kynurenine and its downstream metabolites are known to facilitate cancer progression by inhibiting anti-tumor immune responses and encouraging cancer cell motility (Platten et al. 2014). The decline in resting Kynurenine levels induced by exercise seems to be consistent with some of its immune-modulatory properties. So far, it has been shown that NK cell cytotoxicity is increased in breast cancer patients after engaging in an endurance exercise program (Fairey et al. 2005). Cytotoxicity of NK cells are known to be inhibited by increased levels of Kynurenine (Zimmer et al. 2019b) and can also be regulated by IDO. To understand the role of the Kynurenine pathway and the corresponding IDO axis in immune modulation, it is important to understand how Kynurenine pathway is regulated and how IDO forms an axis with Aryl Hydrocarbon receptor (AhR). In this context, the second and third research interest dives deep into the Kynurenine pathway and the AhR/IDO axis mediated immune regulation.

1.9.1 Kynurenine pathway

Tryptophan is an essential amino acid that the human body cannot synthesize and, therefore, our only source is via nutrition. To maintain the nitrogen balance in the human body (Cervenka et al. 2017) an average daily dose of 3.5 mg per kg of body mass of Tryptophan is required. These can be from different food sources such as chocolate, eggs, fish and dairy products. Majority of Tryptophan stays in the gut whereas the remaining is transported to the brain, heart and skeletal muscle (Agudelo et al. 2014; Cervenka et al. 2017). Moreover, only free plasma Tryptophan can pass through the blood brain barrier via large amino acids transporters (LATs) and is then metabolized into neuro-active substances (Agudelo et al. 2014). The level of free plasma Tryptophan is, however, dependent on the albumin binding rate. This binding,

useful for Tryptophan transport, can be modified with albumin levels and with the availability of binding sites (Curzon et al. 1973). Indeed, free fatty acids can also be bound to albumin subsequently lowering the Tryptophan binding rate and thereby increasing free plasma Tryptophan level (Curzon et al. 1973). Alterations in Tryptophan metabolism are suspected to be involved in the pathogenesis and progression of various diseases including several types of cancer.

Two Tryptophan pathways act competitively: 1) the serotonergic pathway, producing monoaminergic neurotransmitters (e.g. serotonin and melatonin) involved in the control of adaptive responses in the central nervous system (CNS) and linked to alterations in mood, anxiety, or cognition (Badawy 2017) and 2) the Kynurenine pathway which when at a central level comports neuro-protective (e.g Kynurenine, Kynurenic acid) and neuro-toxic metabolites (e.g. quinolinic acid, 3-hydroxykynurenin). In human, the majority of free Tryptophan is degraded through the Kynurenine pathway.

The first step in the Kynurenine pathway for Tryptophan catabolism is the dioxygenation of L-Tryptophan to form L-Kynurenine (Figure 4). This process can be catalysed by one of three enzymes: the indoleamine 2,3-dioxygenases 1 or 2 (IDO1, IDO2) and the Tryptophan 2,3-dioxygenase (TDO). While little is known about IDO2, a vast body of literature suggests that IDO1 production is induced by the presence of viruses, lipopolysaccharides (LPS), and several pro-inflammatory cytokines including interleukins (IL), such as IL-1β and IL6, tumor necrosis factor (TNF)-α, and interferons (IFN-α and IFN-γ) (Talari et al. 2016).

Kynurenine is then either converted to Kynurenic acid by Kynurenine aminotransferases (KATs), or to 3-hydroxyKynurenine (3-HK) by Kynurenine-3-monooxygenase (KMO). Under basal conditions, a large portion of Kynurenine in the brain is used to Kynurenic acid, a N-methyl-D-aspartate (NMDA) and α7-nicotinic acetylcholine (α7nACh) receptor rival (Notarangelo and Pocivavsek 2017). Be that as it may, neuroinflammation and incendiary cytokines pushes Kynurenine digestion through KMO to 3-HK. At that point, further digestion strikes 3-hydroxyanthranilic acid (3-HAA) by Kynureninase (KYNU), and after that 3-HAA is processed to Quinolinic acid (QA). During neuroinflammation, QA is the significant finished result of the

Kynurenine pathway, a metabolite that is a NMDA receptor agonist and an oxidative stressor (Hilmas et al. 2001). This part of metabolites (3-HK, 3-HAA, QA) is viewed as neurotoxic as they can add to oxidative pressure and glutamate excitotoxicity (Hilmas et al. 2001). The final product of the Kynurenine pathway is nicotinamide adenine dinucleotide (NAD$^+$), an important cofactor in cellular reactions linked to energy metabolism (Figure 4). Under certain specific conditions, picolinic acid (PA) is formed instead (Badawy 2017). The other branch of the pathway, leading to the production of Kynurenic acid and xanthurenic acid (Alexander and Owens) from Kynurenine is minor under normal conditions but increases under Tryptophan or Kynurenine loading (Badawy 2017). These ratios change dramatically under different Tryptophan loads and are also influenced by availability of vitamin B6 (Deac et al. 2015). IDO was first reported to lead to foetus survival in maternal–foetus relationship by immune tolerance to the foetus through immunomodulatory activity (Guleria and Sayegh 2007). IDO/TDO overexpression increases the relative concentration of Kynurenine compared to Tryptophan, hence Kynurenine/ Tryptophan ratio can be used as a prognostic clinico-pathological marker to monitor cancer invasiveness and progression. Studies have reported increased systemic Kynurenine / Tryptophan ratio and elevated IDO/TDO activity being associated with poor prognosis and low survival of patients diagnosed with cervical cancer and glioblastoma multiforme (Hascitha et al. 2016). Therefore, IDO/TDO may be a relevant therapeutic target to abrogate immune suppression. Against this backdrop, inhibitors targeting the IDO, TDO or both enzymes represent a promising immunotherapeutical approach for the treatment of cancer. It is reported that PBMCs are potent producers of IDO (Jones et al. 2015). IDO can be inhibited by elevated levels of Tryptophan, which results in channelling the flux of Tryptophan degradation back to TDO (Badawy 2017). Interestingly, the TDO and IDO genes do not share a common ancestor but are an example of functional convergence (Ball et al. 2014).

In spite of the fact that the fundamental mechanisms are not completely comprehended, it has been demonstrated that higher degrees of Kynurenine intercede different inhibitory impacts on the resistant framework including inhibition of T cell expansion, separation of naive T cells into immunosuppressive Treg cells, inhibition

of T$_H$ 17 cells, advancement of tolerogenic dendritic cells, and a disabled capacity of effector cells (NK and cytotoxic T cells) (Munn and Mellor 2016; Routy et al. 2016). In addition to Kynurenine itself, further downstream metabolites of the Kynurenine pathway, namely Kynurenic acid and 3HK have also been reported to inhibit the host antitumor defense (Routy et al. 2016). Finally, QA stimulates the cell cycle of cancer cells and contributes to the acquisition of multidrug resistance against chemotherapeutic agents (Heng et al. 2016).
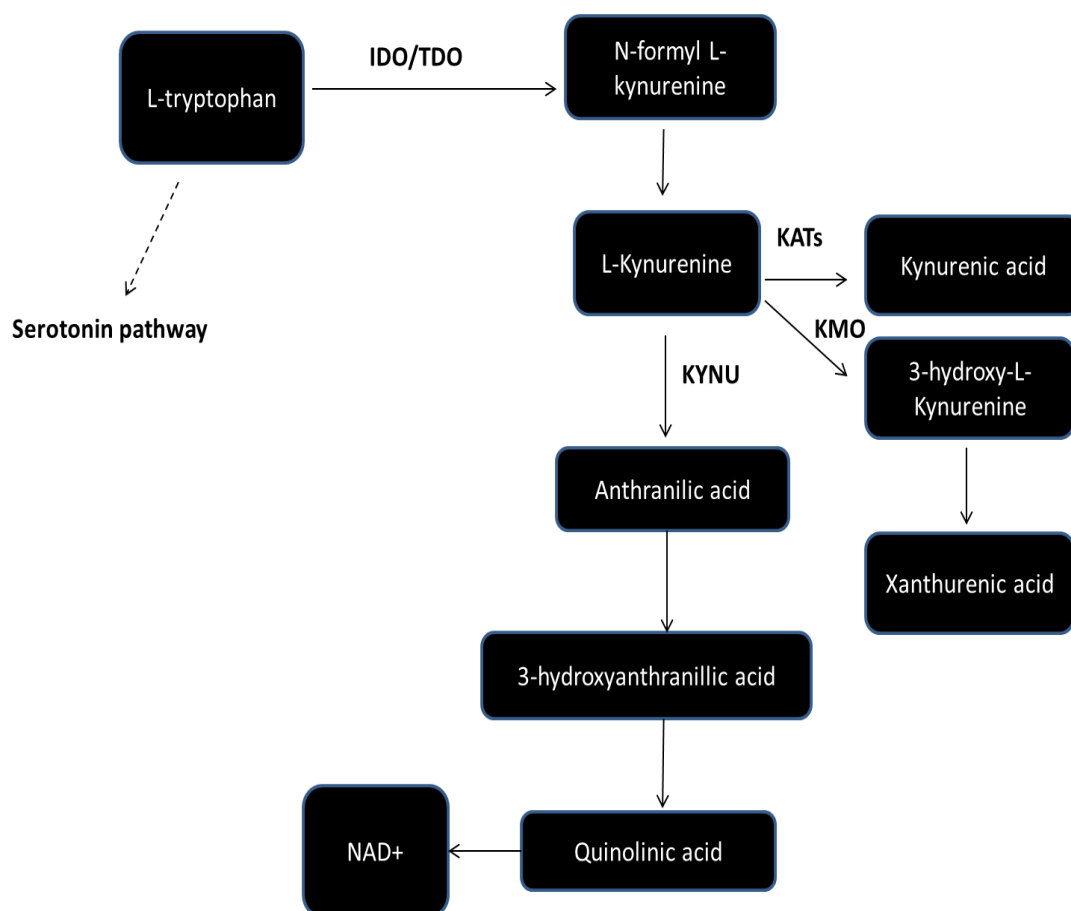

Figure 4: Major metabolites of the Tryptophan metabolism pathway in humans. Abbreviations: IDO - indoleamine 2,3-dioxygenase; TDO - Tryptophan 2,3-dioxygenase; KAT - KYN aminotrasferase I; KMO - KYN monooxygenase; 3-HK-3-hudroxyKynurenine; KYNU – Kynureminase (Pal et al. 2020).

The transcriptional regulation of IDO is mediated by two main pathways, IFN-g-dependent and-independent, which involve transcription factors such as NF-kB, STAT-1, and IRF-1 (Robinson et al. 2003). In section 1.9.3 how Aryl hydrocarbon receptor plays an important role in IDO mediated translational regulation is discussed.

1.9.2 Exercise and Kynurenine pathway

Studies in the context of exercise and the Kynurenine pathway mainly focused on its neurotoxic branch (Joisten et al. 2020; Zimmer et al. 2019a). In brief, QA is known to have several neurotoxic properties, whereas Kynurenic acid is suggested to be neuro protective. Studies in both, rodents and humans have shown that exercise increases the expression of Kynurenine Amino Transferase (KATs) in the skeletal muscle, through activation of PGC1alpha1 signaling (Agudelo et al. 2014). Consequently, peripheral Kynurenine is degraded to Kynurenic acid. In contrast to Kynurenic acid, Kynurenine is not able to cross the blood brain barrier. Therefore, exercise can possibly reduce neurotoxicity and neurological symptoms.

The impact of exercise on the initial step of the Kynurenine pathway, namely IDO/TDO expression and activity is poorly investigated. This is astonishing since exercise has been described to influence systemic inflammation and cortisol levels, thereby targeting main drivers of these enzymes (Basso and Suzuki 2017). Additionally, exercise is known to modify the differentiation, mobilization, and activity of several immune cell subpopulations, such as regulatory T cells, NK cells and cytotoxic T cells [section 1.4]. As already mentioned above all these cell lines are influenced by Kynurenine or its metabolites. In vitro study carried out by Zhang et al., showed that IDO expression was directly proportional to pancreatic cancer progression (Zhang et al. 2017). However, the effects of Kynurenine and its metabolites are not extensively studied especially in context of physical exercise.

Depending on exercise duration and intensity the influence on Kynurenine metabolism pathway varies. As molecular underpinnings, it has been discussed that regular exercise contributes an anti-inflammatory environment (Pedersen 2017). Acute bouts

of exercise were described to mobilize and activate immune cells. In healthy populations it has been shown that after an acute bout of exercise, plasma Tryptophan levels decrease, and plasma Kynurenine levels increases (Areces et al. 2015; Strasser et al. 2016). After single half iron man race in the field, Areces et al. measured a reduction of 7.9 nmol/L (44 ± 9 to 39 ± 11 nmol/L) in plasma Tryptophan levels (Areces et al. 2015). Strasser et al. reported that following 20 min maximal cycling exercise in trained participants, a 12% decrease (65.1 ± 1.87 to 57.1 ± 1.65 µmol/L) in plasma Tryptophan and a 6% increase (1.88 ± 0.08 to 1.99 ± 0.09 µmol/L) in Kynurenine levels (Strasser et al. 2016). Moreover, increased Kynurenine levels have been observed after different endurance activities. An increase of 64% (338 ± 16 to 404 ± 30 nmol/L) and 125% (36 ± 3 to 81 ± 8 nmol/L) were observed following a 150-km cycling time trial and half marathon run in plasma Kynurenic acid levels one hour after exercise completion (Schlittler et al. 2016).

Increase in Kynurenic acid was observed immediately after endurance exercise, however, not after eccentric exercise with the authors speculating that this might be due to the higher metabolic demand of endurance exercise. Prolonged endurance exercise leads to an increase in free fatty acids which are available to bind albumin and thus potentially increases the availability of free Tryptophan in the blood. This may also explain why Kynurenic acid levels were higher in endurance compared with eccentric exercise (Schlittler et al. 2016). This is supported by the study conducted by Lewis et al. reporting a 189% median change (65 to 294 nmol/L) in Kynurenic acid levels from baseline after a marathon (Lewis et al. 2010). The studies presented thus far provide evidence that the Kynurenine pathway is activated in healthy populations following acute aerobic exercise.

The chronic effect of exercise on Tryptophan and Kynurenine metabolism has been sparsely studied. Melancon et al. conducted a study on sixty aged healthy males who averaged 64 years old with a primary focus to investigate the availability of plasma Tryptophan (Melancon et al. 2014). They reported greater plasma Tryptophan availability than at baseline after an acute bout of 30 and 60 min of cycling exercise and following a 16-week supervised exercise training. Interestingly, after chronic exercise, decreased free plasma Tryptophan levels were observed compared to acute

bout of exercise (Melancon et al. 2014). The authors suggested that probably sympathetic and serotonergic activities are increased during exercise to a lower extent than regular exercises.

In clinical settings of disease individuals, some studies have investigated the effects of acute or chronic exercise from low to moderate physical exercise on Tryptophan and Kynurenine levels. These studies have been performed using a variety of clinical patient types including type 2 diabetes (Mudry et al. 2016), depression (Hennings et al. 2013; Millischer et al. 2017), dementia (Kuster et al. 2017) and multiple sclerosis (Bansi et al. 2018). As during exercise, the brain-chained amino acids (isoleucine, leucine and valine) are used in muscle to stimulate protein synthesis (Mero 1999) thereby changing the ratio of competitive amino acids in front of the LAT in favour of Tryptophan to pass through the blood brain barrier. Besides exercise is known to immediately increase cortisol levels (Hayes et al. 2015), subsequently activating the enzyme TDO transforming Tryptophan to Kynurenine at the periphery (Agudelo et al. 2014). Acute exercises seem to increase plasma Kynurenic acid that have not been detected in studies using chronic exercise.

So far, most exercise-studies conducted on the Tryptophan metabolism pathway have explored the neurological aspects (Zimmer et al. 2019a). One study reported that depression was reduced in gastroesophageal junction cancer patients by exercise training, and this effect was associated with an exercise-dependent decrease of the inflammation-induced conversion of Kynurenine to its neurotoxic metabolites (Herrstedt et al. 2019). Agudelo et al. showed that exercise can modulate the skeletal muscle condition through transcription factor PGC-1α1, PPARα/δ, thereby increasing the expression of several Kynurenine aminotransferases (KATs) shifting the peripheral metabolism of stress-induced and exogenous Kynurenine into Kynurenic acid, hence protecting against stress-induced neurotoxicity (Agudelo et al. 2014). Although some studies have explored the consequence of chronic and acute effects of endurance exercise on Multiple Sclerosis and Type II diabetes, studies on cancer population is sparse. These findings support that resistance exercise induces muscle specific conversion of Kynurenine to Kynurenic acid. However, till date, no systematic

resistance training intervention study among pancreatic cancer patients has been reported.

1.9.3 Role of AhR/IDO axis in immune response

Aryl hydrocarbon receptor (AhR) is a ligand-activated member of the Per–Arnt–Sim (PAS) family belonging to basic helix–loop–helix transcription factors. AhR mediates cellular responses to toxins or its ligands, including TCDD, 6-formylindolo[3,2-b] carbazole (FICZ), Kynurenine, and 2-(10H-indole-30-carbonyl)-thiazole-4- carboxylic acid methyl ester (Murray et al. 2014; Nebert 2017). In the cytoplasm AhR forms an active complex with chaperone proteins such as heat shock protein 90 (HSP90), AhR-interacting protein (AIP), and p23 (Pappas et al. 2018). The ligand bound AhR complex translocates to the nucleus and binds AhR nuclear translocator (ARNT). The AhR–ARNT heterodimers bind specific motifs, called dioxin-responsive elements (DREs), in the promoter region of target genes (Murray et al. 2014). As implied by the name for the response elements, AhR was initially studied for its role as a receptor for environmental contaminants and toxins, the most studied of which is 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) (Murray et al. 2014; Pappas et al. 2018).

It has been shown that Aryl hydrocarbon receptor plays an important role in the Kynurenine pathway (Nguyen et al. 2014). It is known that AhR is overexpressed in some cancers and is involved in and production of interleukin-6 (IL6), being an important mediator of pro-tumorigenic properties (Hollingshead et al. 2008). Previous studies suggested the indirect role of AhR signalling in cancer promotion, progression and metastasis by affecting Kynurenine pathway and immune response (Xue et al. 2018). Although the regulatory roles of IDO in Tryptophan metabolism in immune regulation have been widely studied, the mechanisms by which IDO is regulated at the pre-, co-, and post-translational levels are poorly understood.

Kynurenine and Kynurenic Acid have been identified as a potent AhR agonist (DiNatale et al. 2010; Kerkvliet 2012). Recent work showed that the induction of IDO depends on AhR. It has been suggested that miR-203, which is induced by AhR

ligands, have a putative binding site at 3' UTR of IDO (Nguyen et al. 2014). Although no experiments have been carried out to verify the role of miR-203 in IDO, it can be speculated that as it negatively regulates AhR expression in cancer cell lines, it may have a downstream effect on IDO expression. Also, AhR inhibits pro-inflammatory cytokines production such as IL-6 and TNF-a. Therefore, the AhR/IDO axis may have a direct effect on each other's expression. It is also speculated that Kynurenine may activate the AhR for IDO induction in autocrine manner, and form AhR/Kynurenine positive-feedback loop. IDO, together with Tryptophan 2,3-dioxygenase (TDO) and AhR are present in some tumor cells, hence it has been suggested that Kynurenine probably has a double role in promoting cancer invasion and immune escape (Nguyen et al. 2014) (Figure 5). On one hand, activation of cancer cell AhR by Kynurenine increases the expression of genes that promote cell migration (Novikov et al. 2016; Wu et al. 2019) and on the other hand, activated immune cell AhR suppresses effector T cells and increases immune tolerance by targeting dendritic and regulatory B cells (Gutierrez-Vazquez and Quintana 2018).
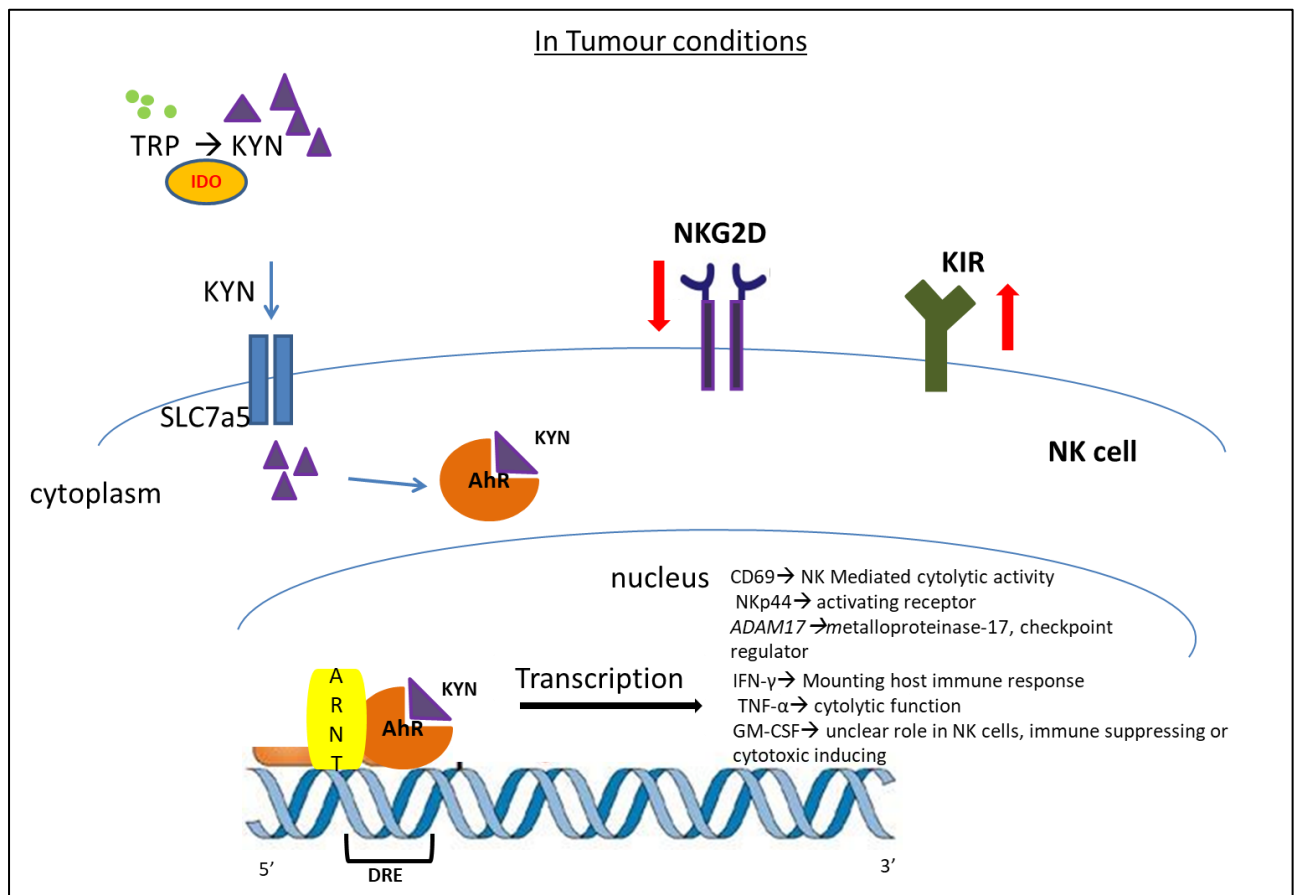
Figure 5: Aryl Hydrocarbon receptor (AhR) and IDO mediated Kynurenine pathway in tumor progression. The figure depicts events in a tumor microenvironment and its consequent effect on NK cell receptors. Upward arrow indicates upregulation and downward arrow indicates down regulation.

AhR is activated in many immune cell types, including T cells, B cells, NK cells, macrophages, and dendritic cells, as well as in epithelial cells, Langerhans cells, innate lymphoid cells, intraepithelial lymphocytes, and microglia (Gutierrez-Vazquez and Quintana 2018). IDO expression is strongly induced in antigen-presenting cells in response to inflammatory signals. Multiple studies have reported that Kynurenine functioning as a ligand for the Aryl hydrocarbon transcription factor complex promotes effector CD4+ T cell differentiation to Foxp3 expressing immunosuppressive regulatory T cells (Xue et al. 2018). Various studies have shown that the AhR/IDO axis

may have a different function depending on cell type and stimulus (Nguyen et al. 2014; Xue et al. 2018).

It is interesting to note that a higher concentration of Kynurenine (micromolar) is needed to activate AhR compared to other ligands (pico molar) (Seok et al. 2018). This can arguably be due to active transport rather than passive diffusion. It is suggested that Kynurenine is transported inside immune cells via neutral amino acid transporters (Seok et al. 2018). Not a lot of research has been conducted on Kynurenine transport inside the immune cells, but it has been suggested that System L transporters SLC7A5 is a critical transporter (Lovelace et al. 2017; Sinclair et al. 2018).

1.9.4 AhR/IDO axis in NK cells

As discussed in previous sections NK cells have a pivotal role in immunosurveillance of cancer cells. Several researchers have revealed that proliferation and function of NK cells can be suppressed by IDO metabolite (Della Chiesa et al. 2006), suggesting there exists a relationship between IDO and decrease in NK cell numbers during inflammatory response. This suggests that regulation of IDO expression could be used as a potential therapeutic starting point for impairment of NK cells in cancer patients.

Several studies have reported that IDO expression leads to generation of reactive oxygen species (ROS) in different cell types (Kim et al. 2014). It is established that oxidative stress, mediated by reactive oxygen species, plays a critical role in immunomodulation. Various studies have recently proposed that reactive oxygen species inhibit anticancer activity of lymphocytes and lead to cell death of cytotoxic lymphocytes (Weinberg et al. 2019). Oxidative stress may lead to malfunction of NK cells as well as decrease in cell numbers in malignant disease conditions (Chan et al. 2014). NK cells are more sensitive to ROS than other lymphocytes (Chan et al. 2014). Song et al. reported that treating NK cells (NK-92 MI) with Kynurenine significantly reduced cell viability through generation of reactive oxygen species (Song et al. 2011). Della Chiesa et al. reported that the IDO metabolite L-Kynurenine downregulates the

expression of NK cell activator molecules, such as NKG2D and NKp46. NKG2D, a C-type-lectin receptor, is expressed by NK cells andCD8+ T cells is triggered by NK cell activation and plays a pivotal role in NK cells function (Della Chiesa et al. 2006). Song et al. also reported that the catabolite of IDO, L-Kynurenine strongly inhibits NKG2D expression on NK cells via JN/KMAPK pathway (Song et al. 2011). Studies conducted on pancreatic cancer cell lines have shown that blocking IDO by 1MT could partially restore NK cell function (Peng et al. 2014). Arum Park 2019 found that thyroid cancer cells suppress NK cell cytotoxicity by regulating IDO and inhibiting the expression of activating receptors, such as NKG2D and NKp46 (Park et al. 2019).

Studies have shown that AhR expression is induced in murine NK cells upon cytokine stimulation (Shin et al. 2013). Conversely it has been shown that activation of AhR with an endogenous Tryptophan derivative, 6-formylindolo[3,2-b] carbazole, potentiates IFN-γ production in NK cell and its cytotoxicity. Also, administration of 6-formylindolo[3,2-b] carbazole in vivo enhances NK cell control of tumors in an NK cell and AhR dependent manner. Finally, similar effects occur with AhR dietary ligands on NK cell potency (Shin et al. 2013). This establishes that activation of AhR receptor is a critical modulator of NK cell antitumor effector functions. It remains to be determined whether endogenous ligands of AhR play a physiologic role in NK cell homeostasis. As discussed above Kynurenine a potent AhR agonist (Nguyen et al. 2014) can inhibit T cells through AhR, it remains to be investigated whether Kynurenine plays a role in activating NK cells within the tumor microenvironment.

The evidence regarding IDO mediated regulation of NK cells and subsequent receptor modulation is limited. Therefore, in this research work an effort to bridge the possible mechanisms on how IDO changes NK cells receptors especially in context of exercise and cancer were undertaken. Alterations of the exercise induced cellular immune responses are dependent on exercise modalities such as type, duration and intensity and must be taken into consideration when interpreting the outcomes. As discussed above, chronic and acute exercise illicit different responses both in terms of AhR/IDO axis and immune cell regulation.

Nevertheless, no studies have explored acute and chronic effects of different endurance exercise modalities on the AhR/IDO axis and its modulation on NK cells.

In this context, the goal was to combine the previous study results and investigate separately how different endurance exercise modulates the NK cell functions. This research is unique, where the role of AhR along with IDO and NK cell cytotoxicity receptor markers are studied under different exercise regimens in cancer disease background.

1.10 Objectives and goal of this thesis

With the emerging field of 'Exercise Immunology', researchers have been trying to uncover the molecular mechanisms involved behind the regulation of exercise induced immune response. In the last few years, research pertaining to physical exercise and cancer has made tremendous progress. As more evidence comes into light, we understand that a major player in exercise oncology is the immune system. Physical exercise has previously proven to influence NK cells cytotoxicity and migration in intensity- and type-dependent manner.

Against this backdrop, the goal of this thesis could be structured as follows:

1. Whether chronic resistance exercise changes NK cell transcriptome in breast cancer patients undergoing adjuvant therapy (Figure 6).

As NK cells have been shown to be the immune cell population to be most sensitive to exercise, they represent a promising target for further research. Physical exercise has previously proven to influence NK cells cytotoxicity and migration in intensity- and type-dependent manner. As all changes begin at gene level, therefore exercise induced transcriptional regulation provides a comprehensive insight into the mechanisms involved.
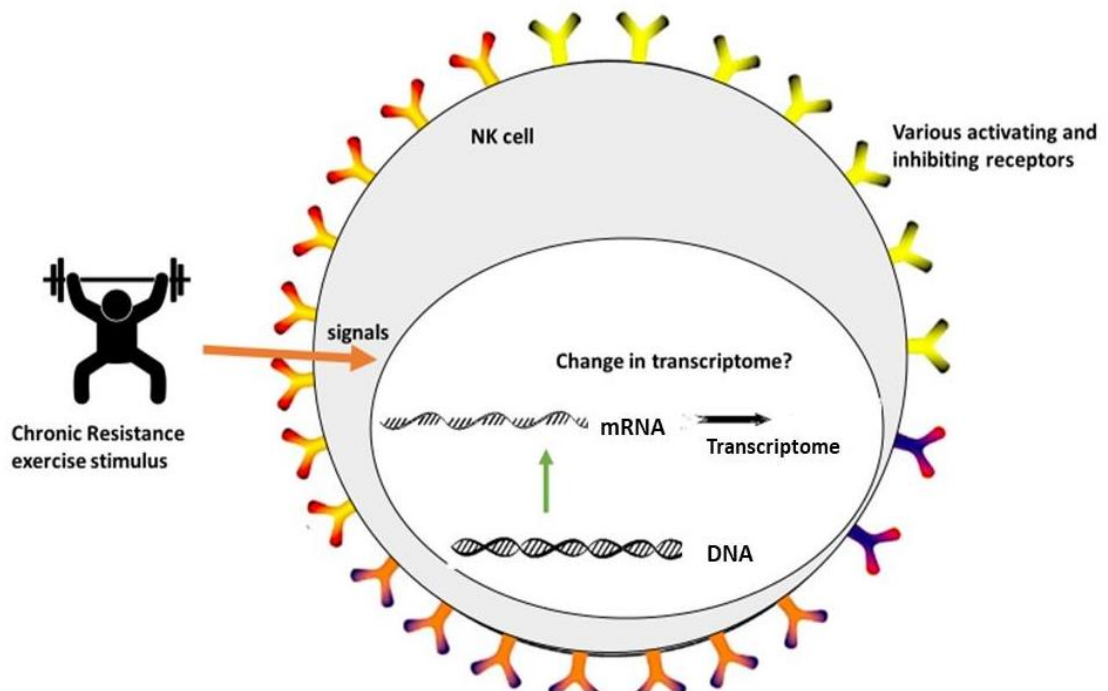
Figure 6: Schematic depicting the hypothesis of the first research question.

2. Investigating whether resistance exercise modulates Kynurenine pathway in pancreatic cancer patients undergoing chemotherapy.

One of the most discussed topics in recent times is the metabolism of Tryptophan via Kynurenine Pathway. The Kynurenine Pathway is balanced under physiological conditions but is upregulated as part of the active immune response. A recent study showed that resistance exercise can counteract radiation associated increase in Kynurenine levels and IDO/TDO activity in breast cancer patients (Zimmer, 2019). This finding supports that resistance exercise induces muscle specific conversion of Kynurenine to Kynurenic acid. However, to date, no systematic resistance training intervention study among pancreatic cancer patients has been reported. Therefore, understanding how the Kynurenine pathway and its enzyme IDO is regulated in cancer patients under resistance training can fill in the puzzle of how exercise regulates this pathway in immune cells.

3. Investigating the following in breast and prostate cancer patients:

> i. Whether AhR and IDO levels are affected by acute single bout of exercise and different endurance training (chronic) modalities

> ii. Whether potential changes in these outcomes are associated with the expression of activating and inhibiting NK cell receptors (as indirect measure of cell function).

The transport of Kynurenine inside the NK cells and thereby its binding with Aryl Hydrocarbon receptor (AhR) and the subsequent regulation in disease backdrop is understudied. It is known that NK cell activating and inhibiting receptors are regulated by transcriptional factors after being activated by AhR complex. The link between intensity and type of exercise training and the corresponding changes in NK cell receptors via Kynurenine pathway has never been investigated. Therefore, whether exercise mediated Kynurenine pathway changes NK cell receptors expression, may elevate our understanding of exercise immunology in cancer patients.

The presented results are based on three publications by the author: Pal et al. (2019): No evidence for effect of exercise on transcriptome of NK cells in breast cancer patients undergoing adjuvant therapy: Results from a pilot study. Frontiers in Physiology, Pal et al. (2020): Resistance exercise modulates Kynurenine pathway in pancreatic cancer patients. International Journal of Sports Medicine, and Pal et al. (2021): Different endurance exercises modulate NK cell activity via AhR/IDO axis. European Journal of Applied Physiology, which resulted from the various randomized controlled intervention studies mentioned in section 2.2.

2. MATERIAL AND METHODS

2.1 Materials

2.1.1 Devices

Table 2: All devices used for the entire research have been listed here along with their manufacturer's information.

Devices	Manufacturer
Bioanalyzer 2100	Agilent Technologies
96-well white plate with opaque bottom	eBioscience ®
Bench centrifuge	Biozymt
Centrifuge	Eppendorf
Confocal Microscope DM 6000 CFS	Leica
Corning® 96 well plates, clear bottom	Sigma Aldrich
Cover slips	Roth
Cryovials	Thermo Scientific
Eppendorf tubes 1.5 mL, 2mL, 5mL	Eppendorf
Flow Cytometer	BD FACSLyric
Forceps	Jeweler's forceps
Glass capillaries	Hirschmann Hematokrit kapillaren
Heat bead sterilizer	Fine Science Tools
HumanHT-12 v4 Expression BeadChip	Illumina technologies®
Incubator Heraeus	Thermo Scientific
iScan array scanner	Illumina technologies®
Laminar Flow	Kendro Laboratory Products

Luna cell counter	Biozymt
Microplate Reader Infinite® 200 PRO	Tecan
Multichannel pipette	Eppendorf
Pipette boy	Eppendorf
Pipette tips	Starlab
Pipettes	Gilson/Eppendorf
TECAN ®	Tecan Group
Vortexer Reax top	Heidolph
Water bath	Memmert

2.1.2 Chemicals, kits and consumables

Table 3: All chemicals, laboratory kits and consumables used for the entire research has been listed here along with their manufacturer's information.

Chemicals, kits and consumables	Manufacturer
Alexa fluor 647 conjugated mouse anti-IDO1	BD Biosciences
Alpha Minimum Essential medium	Sigma Aldrich
BV421 conjugated mouse anti-AhR	BD Biosciences
BV510 conjugated NKG2D	BD Biosciences
Colorimetric Cell Viability Kit III (XTT)	PromoKine
diethyl pyrocarbonate	Sigma Aldrich
Dimethylsulfoxid (DMSO)	Sigma Aldrich
EasySep Human NK cell Enrichment Kit	StemCell Technologies
Ethanol	Emsure Merck
Fetal Bovine Serum (FBS)	Gibco
Ficoll 400	Merck
FITC conjugated KIR2DL1	BD Biosciences
Fix/Via Stain 700	BD Biosciences
Horse serum	Life Technologies
IL-2	Preprotech
IL-6 ELISA kits	R&D Systems
Isopropanol	Sigma Aldrich
Kynurenine ELISA kits	Immundiagnostik GmBH
L-glutamine	Sigma Aldrich

NK-92 Cells	ATCC®
PE-Cy7 conjugated anti-CD56	eBioscience
Phosphate buffered saline (PBS)	Gibco
RNAse free water	Sigma-Adrich
RNeasy Mini Kit	Qiagen
sodium bicarbonate	Sigma Aldrich
TRIzol	Ambion by Life technologies
Trypan blue	Gibco
Trypsin 0.25% EDTA	Gibco
Tryptophan ELISA kits	Immundiagnostik GmBH
B-mercaptoethanol (β-ME)	Aldrich

2.1.3 Software

Table 4: All software used for the entire research has been listed here along with their manufacturer's information.

Software	Manufacturer
SPSS Statistics 25	IBM
ImageJ	Free source software
Prism 8	GraphPad
RStudio	RStudio, Inc.

2.2 Methods

In this section, the methods used of the individual stand-alone studies mentioned in the sections Introduction are discussed here. Therefore, parts can correlate with the corresponding publications **Section 2.2.1:** (Pal et al. 2019), **Section 2.2.2**: (Pal et al. 2020), and **Section 2.2.3**: (Pal et al. 2021).

2.2.1 Resistance exercise and NK cell transcriptome

For investigating the question whether chronic resistance exercise mediates changes in NK cell transcriptome of breast cancer patients undergoing therapy, samples from two randomised controlled trials were used. They are briefly described in the following sections.

2.2.1.1 Research Design

The BEST and BEATE studies were approved by the ethics committee of the University of Heidelberg and registered at ClinicalTrials.gov (NCT01468766, NCT01106820). These were randomized, controlled intervention trials investigating the effects of resistance exercise training in 261 stage 0-III breast cancer patients during adjuvant radiation therapy or adjuvant chemotherapy, respectively. For this research, blood samples were analysed prior to the intervention (baseline, t0), and after the end of intervention (t2). Details of the study designs and primary results are already published (Potthoff et al. 2013; Schmidt et al. 2015; Schmidt et al. 2013; Steindorf et al. 2014). For this thesis, a homogeneous subgroup of n=19 participants from these studies were analysed in order to determine the physiological adaptations of 12-week resistance training.

2.2.1.2 Participants

Both informed and written consent was obtained from all participants. General exclusion criteria for the exercise intervention comprised of contra-indications for progressive resistance training and patients who already performed a systematic intense resistance training (at least twice a week). Ten participants of each trial (BEATE and BEST) were carefully selected for immunological analysis. One participant's transcriptome analysis from the Chemotherapy intervention group (BEATE) was not successful; hence the total sample size is 19. Participants were

selected by similar baseline values of BMI, age and fitness levels between both trials (BEATE and BEST) as well as between participants of the intervention and the control groups. Participants of the exercise groups were only included if they attended more than 80% of training sessions. Additionally, participants who reported acute infections before or during blood sampling were excluded.

2.2.1.3 Exercise intervention protocol

Both interventions were performed under the supervision and guidance of experienced therapists in specific training facilities. The interventions consisted of group-based 60 min resistance training twice weekly over 12 weeks. Exercises comprised of 8 different machine-based progressive resistance exercises (three sets, 8–12 repetitions at 60–80% of one repetition maximum) without any specific aerobic exercise. The control group underwent progressive muscle relaxation according to Jacobson without any aerobic or muscle strengthening exercise (Schmidt et al. 2013).

2.2.1.4 NK cell isolation

PBMCs isolation was done from freshly collected blood. After PBMCs were isolated with Ficoll, the cells were adjusted to a maximum of $5*10^7$ cells/ml and were re-suspended in 1ml PBS (Phosphate buffer solution) and were stored in liquid Nitrogen. NK cells isolation from PBMCs were done using EasySep Human NK cell Enrichment Kit, StemCell Technologies (Cat# 19055).

2.2.1.5 RNA extraction

Total RNA extraction for gene and miRNA analysis was achieved using TRIzol (Ambion by Life technologies). Qiagen-RNeasy Mini Kit was used to purify the extracted RNA for the gene expression study. Re-suspension of RNA pellets was done in diethyl pyrocarbonate-treated water. RNA integrity was measured (before beginning target processing) using Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA).

2.2.1.6 Gene expression microarrays

Illumina HumanHT-12 v4 Expression BeadChip was used for microarray analysis with the extracted RNA of NK cells. Each array on the HumanHT-12 v4 Expression BeadChip consists of more than 47,000 target probes and 31,000 annotated genes derived from the National Centre for Biotechnology Information Reference Sequence (NCBI). The complete list of genes can be found with accession number GPL10558 at NCBI. Strict adherence to protocol provided by Illumina technologies® was maintained.

2.2.1.7 Data analysis

Microarray scanning was done with iScan array scanner. Data extraction was performed for all beads individually. Outliers were removed when the absolute difference to the median was greater than 2.5 times MAD (Median absolute deviation) (2.5 Hampel's method). All remaining bead level data points were then background corrected and quantile normalized (Shi et al. 2010). Student's t-test was used to test for significance on the (log2 scaled) bead expression values of the two groups of interest. When the expression was significant against the background, test for greater than all negative beads for that sample and in the case of comparing separate groups test for inequality of the means of the groups were performed. In the case of comparing groups, we additionally calculated p-values using averaged expression values for each sample in the group. For the differential expression analysis Benjamini-Hochberg correction was applied to the complete set of p-values of all ProbeIDs on the chip. The average expression value was calculated as mean of the measured expressions of beads together with the standard deviation of the beads. The test for baseline-adjusted differences between the exercise vs non-exercise groups after oncological treatment was done using analysis of covariance (ANCOVA) expression after treatment as dependent variable and expression at baseline as well as medical treatment as covariate. In order to control for false positive findings during the testing of several thousands of genes individually it is required to adjust for multiple testing. To adjust the p-values Benjamini-Hochberg method was implemented, which controls the false discovery rate. All statistical analyses were done using the R statistical environment. The statistical analysis was supported by Dr. Manuela Hummel, Division of Biostatistics, German Cancer Research Center (DKFZ), Heidelberg, Germany

2.2.2 Resistance exercise and Kynurenine pathway

For investigating whether the Kynurenine pathway regulated differently in supervised vs. home-based chronic resistance training in pancreatic cancer patients.

2.2.2.1 Research Design

This research is performed as secondary analysis of the SUPPORT (Supervised Progressive Resistance Training for Pancreatic Cancer Patients) randomized, controlled clinical trial which analysed the effects of 6-month resistance training in pancreatic cancer patients within a 3-arm design. The study has been registered at ClinicalTrials.gov (NCT01977066). The outcomes were measured at three time points: baseline (t0), after three months (t1) and after six months (t2).

2.2.2.3 Participants

Between 12/2013 and 12/2015, 65 out of 304 eligible patients were recruited in the study. Both informed and written consent was obtained from all participants. The eligibility criteria included: resectable or non-resectable pancreatic cancer (stage I-IV); age ≥18 years and sufficient German skills. As they receive identical treatments, patients with adenocarcinoma of the distal bile duct (pancreatic biliary) and with ampullary ductal adenocarcinoma were also eligible. Exclusion criteria were: if the patient showed contra-indications for progressive resistance training, including severe pain, heart insufficiency > NYHA III, reduced standing or walking ability, insufficient wound healing, impaired hematological capacity (either hemoglobin value <8g/dl or thrombocytes <50.000), uncontrolled hypertension, severe renal dysfunction (GFR <30%, creatinine >3mg/dl), or any other comorbidities, that precluded their participation. Patients who already performed a systematic intense resistance training (at least twice a week) were excluded. Patients living close to the study center (about <20 km) were randomized to supervised or control group, while patients living further away were assigned to home-based or control group. A 2:1 block randomization, stratified by sex and age, with a random number generator was implemented. Detailed information on participant's recruitment is beyond the scope of this thesis and can be found here (Clauss et al. 2017).

2.2.2.2 Exercise intervention protocol

The 6-month intervention consisted of resistance training twice a week. A time of 12 weeks after surgery was recommended to allow for wound healing. One patient who did not undergo surgery, the training started immediately after randomisation. Both resistance training intervention groups went through corresponding resistance exercises for the major muscle groups of the upper and lower extremities. The difference between the two resistance training interventions was primarily the intensity and mode-of-administration of the intervention. Patients belonging to the supervised resistance training group performed the training on weight machines under therapeutical supervision with exercise intensities of 60-80% of the maximum (1-RM). Patients belonging to the home-based progressive resistance training performed the training unsupervised with an exercise manual by themselves at home with their own body weight and/or resistance bands and with exercise intensities of 14-16 on the Borg Scale of Perceived Exertion *(*Borg 1998*)*. Further details of the exercise protocol are published here (Clauss et al. 2017).

The control group underwent standard usual care in accordance with their cancer treatment. Patients were telephoned by the exercise specialist once a month to ask about possible chemotherapy-related side effects.

2.2.2.4 Outcome Assessment

Data assessments which included anthropometric and clinical information as well as the collection of serum samples, were performed at baseline (t0), after three months (t1) and after six months (t2). The blood marker analyses for this thesis were limited to 32 subjects, including seven participants from supervised, 14 participants from home-based, and 11 participants from control group. Only participants who had viable quantity of serum samples for all three time points as well as serum that has not undergone haemolysis were included in the biomarker analysis

For storage, the serum samples were frozen and stored at -80°C. Serum Tryptophan, Kynurenine and IL-6 levels were measured for all time points by Enzyme-linked Immunosorbent Assay (ELISA) in duplicates according to the manufacturer's instructions using the following ELISA kits: #K7728, #K7730 (Immundiagnostik GmBH, Germany) and #HS600C (R&D Systems, Germany).

The performance characteristics of the ELISAs are listed in Appendix (section 8).

2.2.2.5 Data Analysis

All statistical data analyses were conducted using IBM SPSS Statistics 25. At baseline, anthropometric and clinical outcome variables were compared between the groups, supported by using Pearson Chi-square test or ANOVA. KTR was calculated mathematically as a ratio of Kynurenine and Tryptophan. Additionally, baseline differences for all outcomes (Kynurenine, Tryptophan, KTR, IL-6) were evaluated using one-way ANOVA. To determine changes over time and between the groups, baseline adjusted 3 (groups: supervised, home-based, control group) × 3 (time points: t0, t1, t2) ANCOVA was conducted for each outcome variable. Significant time effects and group x time interactions were further analysed by Bonferroni corrected simple effects analysis. Before performing ANCOVA, possible outliers were identified via Z-scores (> +/– 3 SD). The data was checked for sphericity using Mauchy's test. In case of violation of sphericity, Green House Geisser correction was applied. For all analyses, the level of significance was set at 0.05.

2.2.3 Endurance exercise mediated changes in NK cells via AhR/IDO axis

For investigating how does endurance exercise modulate NK cell function via the AhR/IDO axis in cancer patients. Additionally, whether there is a difference in acute endurance exercise mediated changes in NK cells and how does chronic endurance training of different modalities elicit a change in NK cells.

2.2.3.1 Research Design

For this research question, secondary analyses in the TOP (Individually tailored training prescriptions in cancer patients) randomized clinical trial were performed. This study aimed to identify the most effective training method in terms of enhancing cardiorespiratory fitness in breast and prostate cancer patients. The study has been registered at ClinicalTrials.gov (NCT02883699). The outcomes were measured at four time points: pre-CPET (baseline), post- (immediately after the first cardiopulmonary exercise test (CPET) (=T0), 1h post- (one hour after the first CPET) and after 12 weeks of intervention. Here it is reported on acute single bout of exercise (pre randomization) mediated changes in NK cell receptors and AhR/IDO as well as on chronic effects of standard vs polarized endurance training (after randomization) on NK cell mediated immune response in cancer patients. Data assessments and collection of serum samples took place at pre-CPET, post-CPET, and 1h post-CPET and 12 weeks post-intervention and processed for experimental outcome. The serum samples were frozen and stored at -80°C for analysis. To determine changes in NK cells due to exercise, NK-92 (ATCC® CRL-2407™) cells were incubated with patient exercise serum. The incubated NK-92 cells were analysed via flow cytometry for surface markers as well as internal markers.

2.2.3.2 Participants

In this randomized controlled training intervention study, 60 breast and 60 prostate cancer patients were recruited. Immune analyses were restricted to 21 subjects, including 9 participants from endurance standard group and 12 participants from endurance polarized group due to limited availability of viable quantity of serum samples for all four time points as well as serum that has not undergone haemolysis. Both informed and written consent was obtained from all participants. Criteria for inclusion were: Patients diagnosed with non-metastatic (M0) breast cancer or non-

metastatic or metastatic (M0 or M1, except for bone or brain metastases, with prostate-specific antigen evidence of stable disease) prostate cancer, 6 to 52 weeks after the end of primary therapy (i.e., surgery and/or radiotherapy and/or chemotherapy), 18 to 75 years of age, and no regular vigorous endurance training (>1 session/week) since diagnosis or within the last 6 months. Exclusion criteria included: diagnosis with additional other cancer and severe comorbidities that precluded participation in exercise testing or training (acute infectious diseases, severe cardiac, respiratory, renal or neurological diseases).

2.2.3.3 Exercise intervention protocol

To determine the effects of acute exercise on NK cells all participants performed a CPET to exhaustion according to exercise protocol designed for the study (Schneider et al. 2020).

To determine the effects of chronic exercise on NK cells all participants were randomly assigned to one of the following groups (stratification criteria: cancer type/sex, age and baseline fitness (VO_{2max} in ml/min/kg body weight):

For this research 'n' indicates the number of study participants samples available for biomarker analysis.

(1) Standard endurance training group (n=9): Two times per week 40 min of continuous cycle ergometer exercise at vigorous intensity, slightly below (97%) the individual anaerobic blood lactate threshold (IAT). Intensity corresponding to this point was prescribed as power output (W).

(2) Polarized endurance training group (n=12): Polarized training was modified for cancer survivors as follows: One time per week HIIT 4 times 4 min at 85-95% peak heart rate (HR_{peak}) of the CPET and 3 times 3 min recovery at 70% HR_{peak} and one time per week moderate intensity continuous training (MICT) at the first lactate threshold (LT1) (work-matched to the standard endurance training group, therefore duration was individual for each patient). All training sessions took place on a cycle ergometer.

2.2.3.4 Cell Culture

NK-92 cells (ATCC® CRL-2407™) were grown through five passages, and then aliquoted and stored in liquid nitrogen to minimize target cell differences between assays. Frozen cells were thawed 2-10 days prior to each experiment and maintained in 2 mM L-glutamine and 1.5 g/L sodium bicarbonate enriched Alpha Minimum Essential medium without ribonucleosides and deoxyribonucleosides supplemented with 12.5% FBS and 12.5% Horse serum as mentioned in ATCC guidelines. On every third day, the cells were fed with 50 IU of IL-2 (Prepotech catalogue number 200-02). On the day of NK cell assay, 1 x 10^5 target cells were removed and plated in 96 well (eBioscience, San Diego, CA, USA). Cells were incubated for 1-hour with 50% autologous serum from either endurance polarized or standard intervention collected at four different time points. This concentration of serum corresponds to the proportion of serum in whole blood in vivo and has been used in similar work before (Booth et al. 2010). Following incubation, cells were analysed on a BD FACSLyric Cytometer (Heidelberg, Germany).

2.2.3.5 Flow cytometric analysis of NK cells

NK-92 cells incubated in serum from either endurance or resistance intervention group were labelled with Fix/Via Stain 700(BD 564997) to check for viability. The following antibodies were used in order to detect the markers of choice: PE-Cy7 conjugated anti-CD56- (eBioscience 25-0567-42), Alexa fluor 647 conjugated mouse anti-IDO1 (BD 566648), FITC conjugated KIR2DL1 (BD 556062), BV421 conjugated mouse anti-AhR (BD 565791), BV510 conjugated NKG2D (BD 563266). All antibodies were purchased from BD Biosciences Heidelberg, Germany. Cells were incubated with 5uL of each antibody for 20 min and 30 min for extracellular and intracellular staining respectively in the dark at room temperature, then washed and resuspended in 200 µl PBS prior to analyse on flow cytometry. Due to lack of serum sample volume availability no experimental replicates were performed. Labelled cells were directly analysed with BD FACSLyrics cytometer. Live and dead cells were identified by Fix/Via stain through forward and side scatter characteristics and gated electronically using FlowJo™ software. CD56+ lymphocytes were gated as NK cells. The surface markers were identified by gating for NKG2D+, KIR2DL1+ and intracellular markers by AhR+, IDO+. For fluorescence minus two (FM2) control, one intracellular (IDO) and one extracellular (NKG2D) unstained marker was used to determine the negative population. Representative plots demonstrating gating strategy are given in Figure 7.

2.2.3.6 Flow cytometer settings

The BD FACSLyric™ system comprises a flow cytometer available in different optical configurations with BD FACSuite™ Clinical software, and optional BD FACS™ Universal Loader. By using patented BD™ FC Beads and BD CS&T Beads a universal setup for performance quality control, instrument control, data acquisition/storage, online/offline data analysis, and instrument standardization can be ensured. The system consists of 14 parameters in total, 12 colours across up to 3 Lasers plus the parameter for cell size (FSC) and granularity (Steins Bisschop et al.). The laser configuration is 4 blue, 3 red and 4 violet lasers. The PMT voltages are automatically updated to maintain target MFI values as a part of Quality control every 60 days only. This daily bead-based Setup ensures <0,4% variability across all parameters which leads to reproducible results. The 20 min compensation procedure only needs to be performed every 2 months and the Spill Over Values (SOVs) are automatically updated as part of daily Quality control. The raw data can either be analysed directly on the FACS Lyric by using the BDSuite Software, which is also the measure software of the samples or can be exported as fcs files into the Flow Jo Software (BD).

Figure 7: The methodology and parameters used for flow cytometry data acquisition using FACS Lyric and Flow Jo software (BD).

Full stained sample
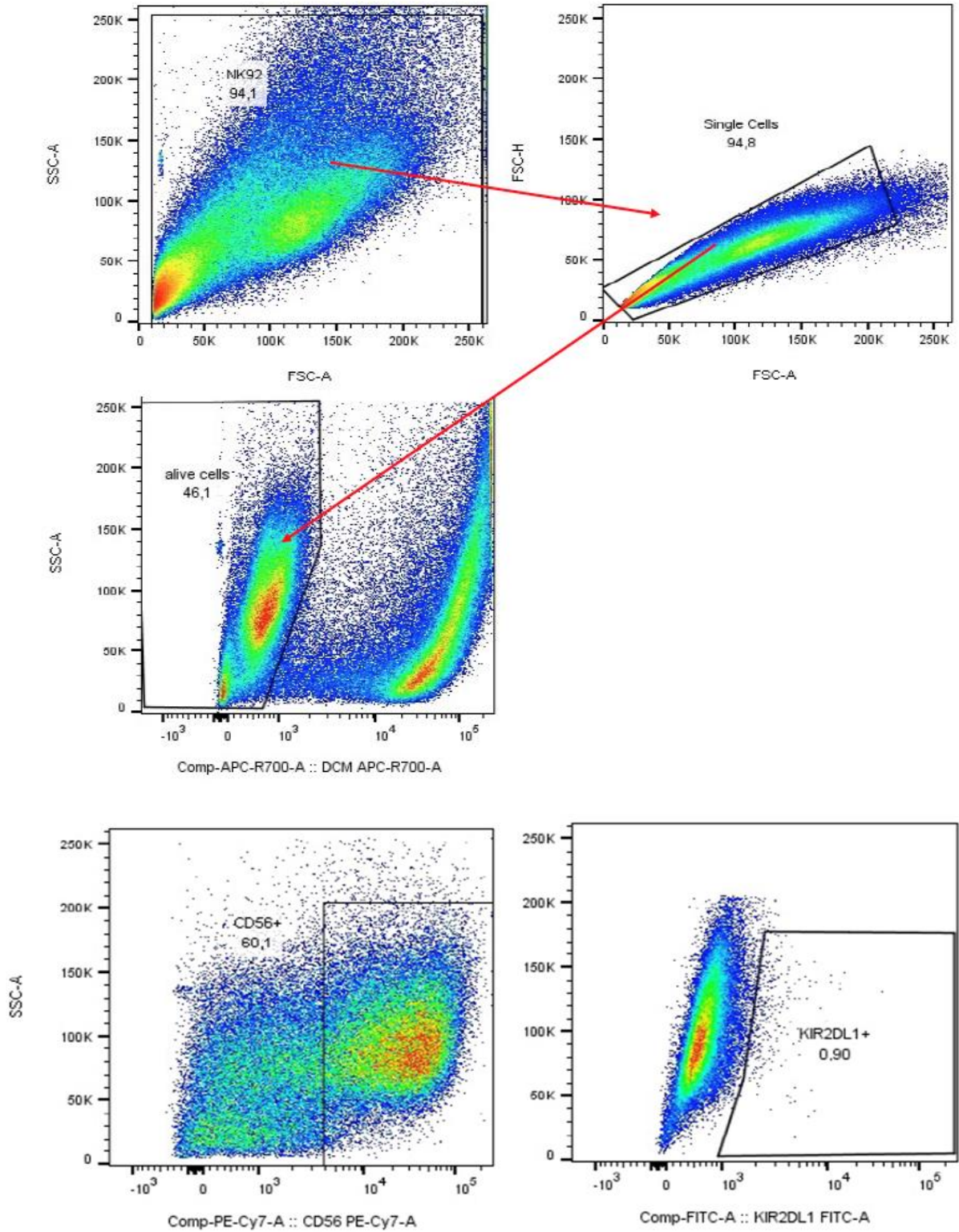
(Figure 7 continued)
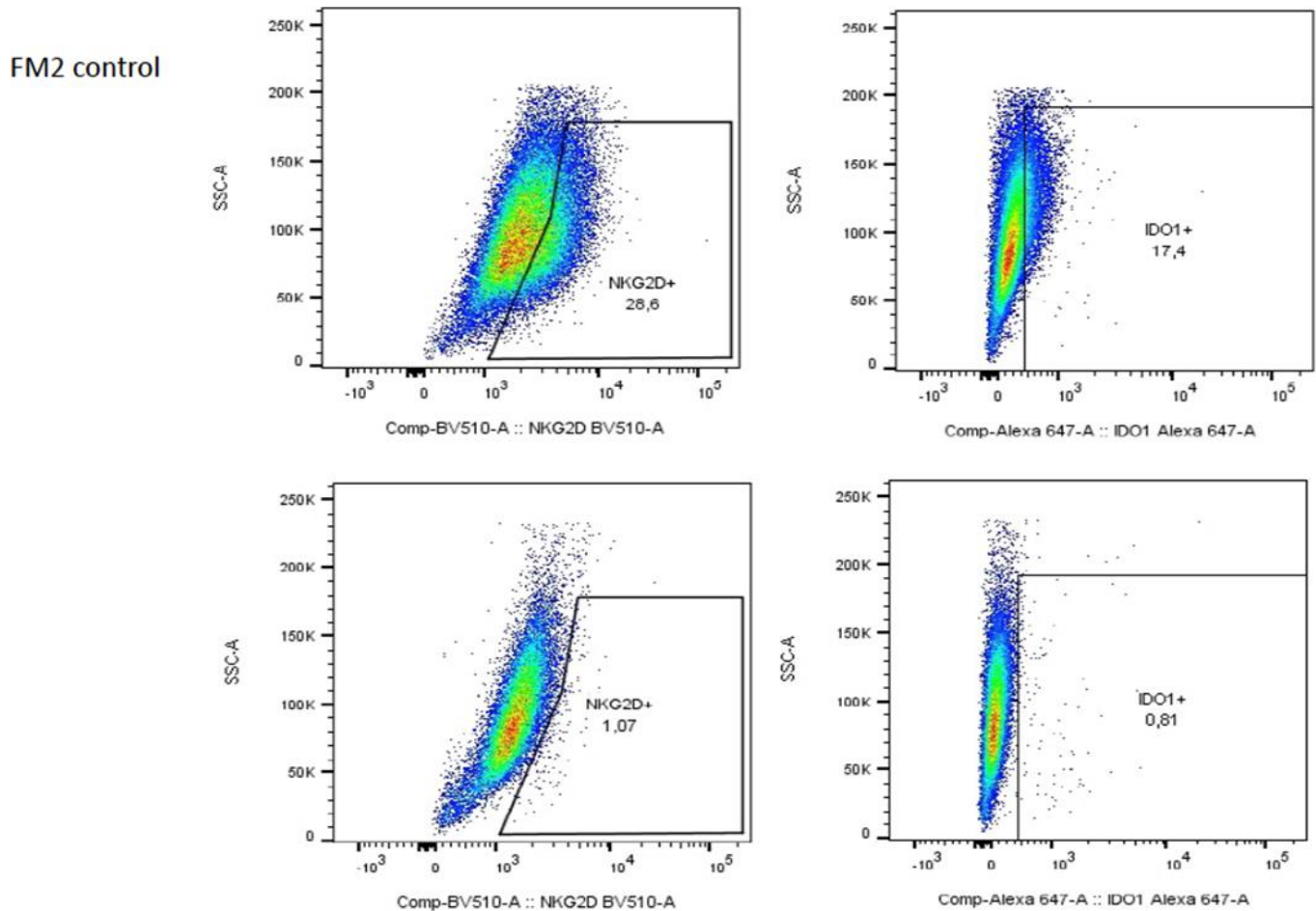
2.2.3.7 Data Analysis

All statistical data analyses were performed using IBM SPSS Statistics 25. At baseline, anthropometric and clinical outcome variables were compared between the groups, supported by using Pearson Chi-square test or unpaired t-test. Data were analysed for normality by checking for skewness and kurtosis and were found to be normally distributed. To determine acute (CPET) effects over time, repeated measures ANOVA (pre-CPET, post-CPET and 1h post-CPET) was conducted for expression of each outcome variable for all 21 patients. Significant time effects were further analysed by Bonferroni corrected post-hoc analysis. To determine between group and within group

effects of different chronic endurance exercise over time, expressions were compared after randomisation using a baseline adjusted analysis of covariance (ANCOVA) for pre-CPET and 12 weeks after intervention for each group. Sex, age and treatments were not included as a covariate because initial analyses indicated they did not significantly contribute to variation. Prior to statistical testing, Z-scores were calculated to identify possible outliers ($> +/- 3$ SD). Data were checked for sphericity using Mauchly's test. In case of violation of sphericity, Greenhouse-Geisser correction was applied. For all analyses, the significance level was set at 0.05.

3. RESULTS

In this section, the results of the individual stand-alone studies mentioned in the sections Introduction and Methods will be described. Therefore, parts can correlate with the corresponding publications **Section 3.1:** (Pal et al. 2019), **Section 3.2**: (Pal et al. 2020), and **Section 3.3**: (Pal et al. 2021).

3.1 Resistance exercise mediated changes in NK cell transcriptome

The investigation here focused on whether chronic resistance exercise mediate changes in NK cell transcriptome of breast cancer patients undergoing therapy. Participant's clinical and anthropometric characteristics are shown in Table 5. The mean age of the breast cancer patients undergoing therapy was 51.1 years, with the exercise group at 51.8 years and control group at 50.2 years. They showed on average a normal weight (mean BMI 23.8 kg/m^2, exercise: 24.5 kg/m^2, control: 22.9 kg/m^2. The majority of the patients were diagnosed with stage I breast cancer.

Table 5: Clinical and anthropometric characteristics of participants. n= number of participants, SD= standard deviation (Pal et al. 2019).

		Total		Exercise		Control	
Total n, %		19	100%	10	100%	9	100%
Age, mean (SD)(years)		51.10	(5.81)	51.80	(7.82)	50.20	(1.91)
BMI, mean (SD)(kg/m$^{2)}$)		23.80	(3.30)	24.50	(4.11)	22.90	(2.01)
VO2peak [ml/min], mean (SD)		1570	(347.0)	1615	(405.50)	1522	(282.70)
Treatments, n (%)	**Chemotherapy**	9	45.50%	6	66.70%	3	33.33%
	Radiation therapy	10	54.50%	4	40.00%	6	60.00%
stage, n (%)	0	1	4.50%	1	8.30%	0	0%
	1	10	45.50%	3	25.00%	7	70.50%
	2	8	36.40%	6	50.00%	2	20.60%
	3	3	13.60%	2	16.40%	1	10.00%

The results after microarray analysis using HumanHT-12 v4 Expression BeadChip is graphically represented on a heatmap in Figure 8. The exercise group is colour coded as green and the non-exercise group denoted as 'control' is colour coded as grey. The scaled expression levels of each gene of interest with documented function and possible role in NK cells are mapped in each row. The expression levels of each analysed breast cancer patient undergoing chemotherapy or radiation therapy is mapped in each column. The patient IDs and the type of therapy that the individual patient went through is additionally mentioned with its corresponding expression levels.

Microarray analysis revealed no significant group x time interactions after correcting for multiple testing (all adjusted *p-values >0.9*). However, it is of interest to explore the top candidates, e.g., the attached heat map contains the genes with raw *p < 0.01* and a cutoff fold change of 0.5.

One of the interesting candidates is the TET1 gene (Ten-eleven translocation methyl cytosine dioxygenase 1). The protein encoded by this gene is a TET (ten-eleven translocation) family demethylase. TET protein family members play an important role in DNA methylation and gene activation. After treatment the expression levels tend to be higher in the exercise group in comparison to the non-exercise group (*raw p< 0.01 adjusted p=0.99*). The higher expression after exercise is mainly observed in two patients with radiation therapy. One patient with chemotherapy had lower expression after exercise. All other subjects did not change TET1 expression considerably irrespective of exercise or non-exercise status.

FXYD4 is an ion transport regulator gene, which encodes a member of a family of small membrane proteins that share a 35-amino acid signature sequence domain. The function lies in transmembrane transport of ions like Sodium and Potassium. FXYD4 has a significant difference in expression between exercise and non-exercise groups with exercise group showing a higher expression (*raw p< 0.01, adjusted p=0.99)*. The higher expression levels after exercise can be attributed mostly to the patients who underwent radiation therapy. Contrastingly, the lower expression in non-exercise group can be attributed mostly to the patients who received chemotherapy.

VAPA, Homo sapiens VAMP (vesicle-associated membrane protein)-associated protein A encodes a type IV membrane protein. It is present in the plasma membrane and intracellular vesicles. It may also be associated with the cytoskeleton. This protein may function in vesicle trafficking, membrane fusion, protein complex assembly and cell motility. The expression of VAPA, revealed a higher expression in the exercise group in comparison to the non-exercise group after treatment (*raw p< 0.01, adjusted p=0.99*). The higher expression is consistently observed in the exercise group irrespective of radiation or chemotherapy. The lower expression levels of VAPA in the non-exercise group was witnessed in both patients undergoing chemotherapy and radiation therapy.

Homo sapiens WD repeat domain 68 (WDR68) encodes WD repeats. WD repeats are minimally conserved regions of approximately 40 amino acids typically bracketed by gly-his and trp-asp (GH-WD), which may facilitate formation of heterotrimeric or multi-protein complexes. The encoded protein interacts with serine/threonine kinase 11 and has one of its functions in cell cycle arrest. The expression levels of WDR68 after treatment tend to be higher in the exercise group when compared to the non-exercise

group. This effect was still visible when analysis was carried out without the "outlier" sample in the non-exercise group with very low expression before treatment (*raw p< 0.01, adjusted p=0.99*). The expression levels of WDR68 were similar under radiation or chemotherapy. Although the expression levels were not markedly higher in the exercise group, the non-exercise group seems to have a curbed WDR68 expression.

Homo sapiens chromosome 20 open reading frame 199 (C20orf199) showed a significantly higher expression in the exercise group compared to the non-exercise group after treatment (raw *p< 0.01, adjusted p=0.99*). In the exercise group both patients undergoing radiation or chemotherapy had a consistent higher expression of C20orf199.

SLC22A10, Solute carrier family 22 member 10 is a gene encoding for a protein responsible for transmembrane anion transport. The gene expression of SLC22A10 revealed a significantly higher expression in the exercise group when compared to the non-exercise group after treatment (*raw p< 0.01, adjusted p=0.99*). The higher expression is contributed by both chemotherapy and radiation therapy patients in the exercise group. Within the small sample size, four patients remained unchanged in their expression in the non-exercise group. (Figure 8) (Pal et al. 2019).
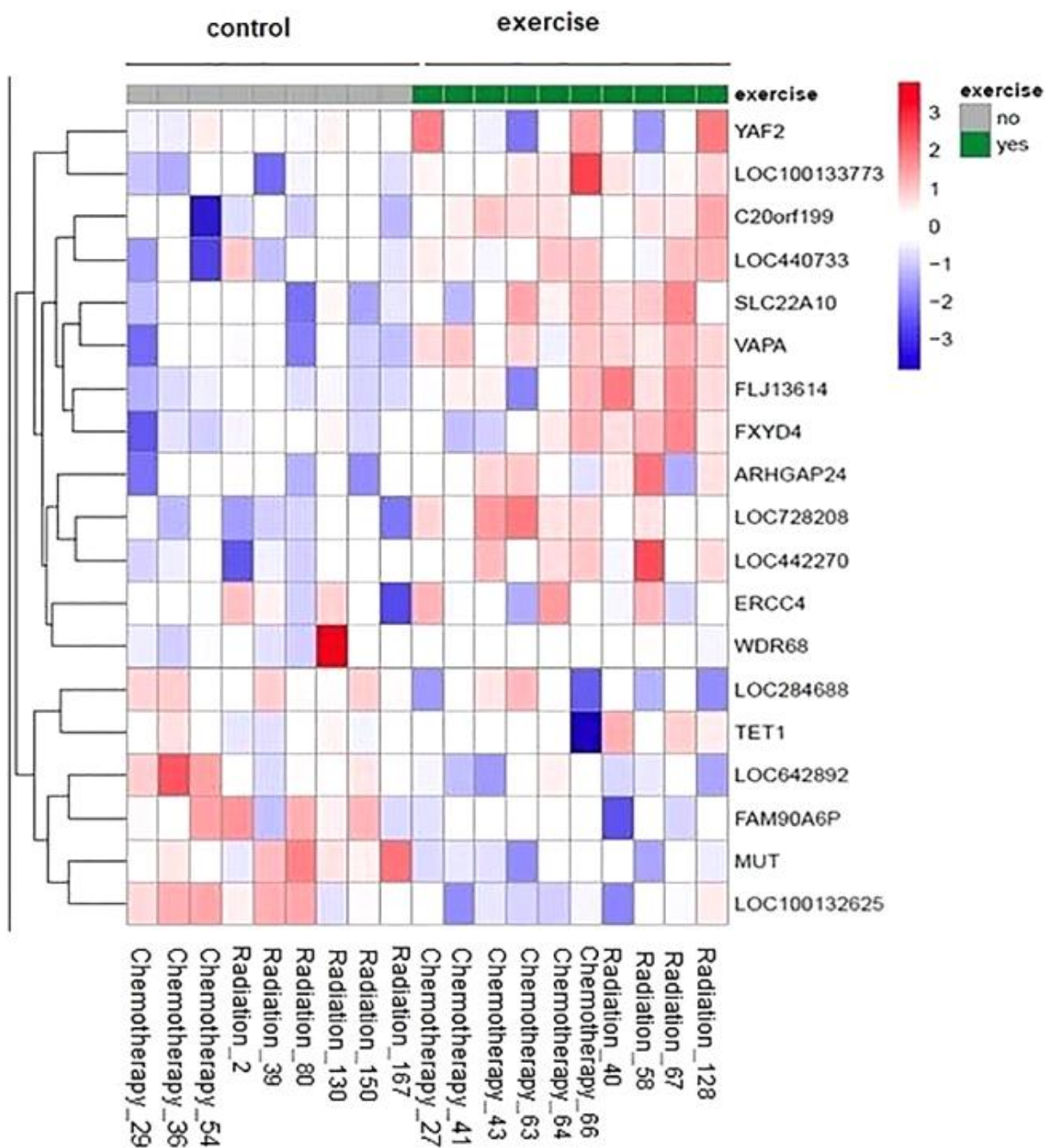
Figure 8: Impact of physical exercise on NK cell gene expression using microarray analysis with Illumina HumanHT-12 v4 Expression BeadChip: The heat map represents the scaled expression levels of genes with raw p < 0.01 and fold change cutoff 0.5. The rows represent genes identified by the name on the right of the figure. The individual patient samples are shown as columns (1 column per sample). The colour Red represents high expression, while blue represents low expression. The heat map is combined with clustering where genes are grouped together based on the similarity of their gene expression pattern (Pal et al. 2019). The results contain a single experimental outcome.

3.2 Supervised vs home-based resistance exercise mediated changes in Kynurenine pathway

The research question here deals with whether the Kynurenine pathway is regulated differently in supervised vs. home-based chronic resistance training in pancreatic cancer patients.

All 32 patients completed baseline assessments for muscle strength and cardiorespiratory fitness. Assessments were performed at pre-intervention, after 3 months and after 6 months of intervention. Blood marker analysis was performed using Enzyme linked Immunosorbent Assay (ELISA) for these patients. Here we explore the findings of serum blood marker analyses of the Kynurenine pathway.

The baseline demographic and clinical characteristics of the study population are listed in Table 6. Mean age of the pancreatic cancer patients undergoing supervised training was 61.1 years, home-based training was 59.3 years and control group was 61.3 years. They showed on average a normal weight (mean BMI supervised: 23.1 kg/m^2, home-based: 22.4 kg/m^2, control: 26.9 kg/m^2). The majority of the patients were diagnosed with stage IIB cancer (Pal et al, 2020).

Table 6: Demographic and clinical outcomes of study participants at baseline (Pal et al. 2020).

		Group					
		Supervised		**Home-based**		**Control**	
TOTAL (n)		7		14		11	
Age, mean (SD)		61.1	(5.8)	59.3	(9.86)	61.3	(10.5)
BMI, mean (SD)		23.1	(3.25)	22.4	(2.8)	26.9	(5.94)
Days since surgery, mean (SD)		100.1	(20.9)	88.0	(19.3)	97.4	(24.3)
Days since first chemotherapy, mean (SD)		44.9	(19.9)	44.4	(21.9)	41.7	(20.0)
Haemoglobin [g/dl], mean (SD)		11.8	(1.6)	11.3	(1.1)	11.6	(1.3)
Adenocarcinoma Type, n (%)	Pancreatic ductal	6	85.70%	13	92.80%	9	81.80%
	Distal bile duct [1]	1	14.30%	1	7.20%	1	9.00%
	Ampullary ductal					1	9.00%
Stage, n (%)	IA					1	9.00%
	IB	1	14.90%			1	9.00%
	IIA	2	28.60%	3	21.40%	5	45.40%
	IIB	4	57.10%	10	78.60%	4	36.40%
	IV			1	5.00%		
Treatment, n (%)	Surgery, adj. CT	6	85.70%	10	71.40%	11	100.00%
	Neoadj. CT, Surgery			2	14.90%		
	Neoadj. CT, Surgery, adj. CT	1	14.30%	1	7.10%		
	CT			1	7.10%		
Operative procedures, n (%)	None			1	7.10%		
	Total pancreatectomy			3	21.40%	1	9.00%
	Distal pancreatectomy	2	28.60%	2	14.20%	1	9.00%
	Pancreaticoduodenectomy	4	57.10%	4	28.40%	4	36.70%
	PD pylorus-preserving	1	14.30%	4	28.40%	5	45.50%

SD= standard deviation; BMI = Body Mass Index; PD= Pancreaticoduodenectomy; CT = Chemotherapy; [1] pancreatic biliary.

Baseline comparison between different exercise intervention groups and control

No baseline differences for any of the blood marker outcomes as well as the anthropometric and clinical/disease characteristics between the three groups were detected (age: *p=0.85*, BMI: *p= 0.10*, days since surgery: *p= 0.43*, days since chemotherapy: *p=0.23*).

Impact of resistance exercise intervention on blood marker outcomes

After analysing the ELISA data using repeated measures analysis of covariance, the results are plotted in Figure 9 for visual representation.

Kynurenine showed after ANCOVA a significant group x time interaction (*p=0.01, F=3.83, df=3.33*) as well as a significant time effect (*p<0.01, F=6.48, df=1.66*). Bonferroni corrected *post-hoc* analysis showed for Kynurenine a potential difference at t2 between supervised and home-based (*p=0.07)*. For home-based there was a significant increase from t0 to t2 (*p=0.02)* and from t1 to t2 (*p=0.04*) (Figure 9A). On the contrary, the supervised exercise group had a decreased Kynurenine level from t1 to t2. The results depict that in the time frame of t1 to t2, Kynurenine levels diverge in opposite direction between home-based and supervised exercise groups. Over the time course, Kynurenine levels remained constant for the control group.

Regarding Tryptophan, ANCOVA revealed no significant group x time interaction (*p=0.10, F=2.07, df=3.92*) but a significant time effect was observed (*p<0.01, F=15.85, df=2*). Bonferroni corrected *post-hoc* analysis showed a tendency for difference between supervised and home-based at t2 (*p=0.05*) and no statistical significance change over time (Figure 9B). Although not statistically significant, for both supervised and home-based exercise groups Tryptophan levels tended to decrease over three months' time. In the supervised exercise group, Tryptophan levels increased from t1 to t2. On the other hand, Tryptophan levels decreased in the home-based group from

t1 to t2. Almost constant Tryptophan levels were observed in the control group from t1 to t2.

Analysis of KTR (Kynurenine/Tryptophan ratio -Serum IDO/TDO), showed a significant group x time interaction (*p<0.01, F=4.42, df=3.59*) as well as a significant time effect (*p<0.01, F=11.63, df=1.80*). Bonferroni corrected *post-hoc* analysis showed a significant group difference between supervised and home-based at t2 (p=0.01). For home-based a significant increase was observed when comparing t2 to t0, and t2 to t1 (*p<0.01; p=0.02* respectively) (Figure 9C). Supervised, although not statistically significant, decreased over time compared to the control group *(t2: p=1.00;* t3: *p=0.67)*. KTR levels for the control group decreased from t0 to t1 and then remained constant over t1 to t2.

After analysis, IL-6 did not show a group x time interaction (*p=0.31, F=1.22, df=2.17*), but showed an almost significant time effect (*p=0.06, F=3.90, df=1.08*). It is interesting to note that *Bonferroni* corrected *post-hoc* analysis showed no significant result in between group effect but had a significant time effect for all three groups (supervised, home-based and control group) from t0 to t1 (*supervised: p<0.01, home-based: p<0.01, control group: p<0.01*). From t1 to t2, both supervised and home-based exercise groups remained constant in IL-6 expression. In contrast, the control group first decreased IL6 expression from time period of t0 to t1 and then showed a non-significant higher expression from t1 to t2 (Figure 9D) (Pal et al. 2020).

Mean data and time courses of all outcome measures as well as Bonferroni corrected post-hoc ANCOVA results are shown in Figure 9 and Table 7.
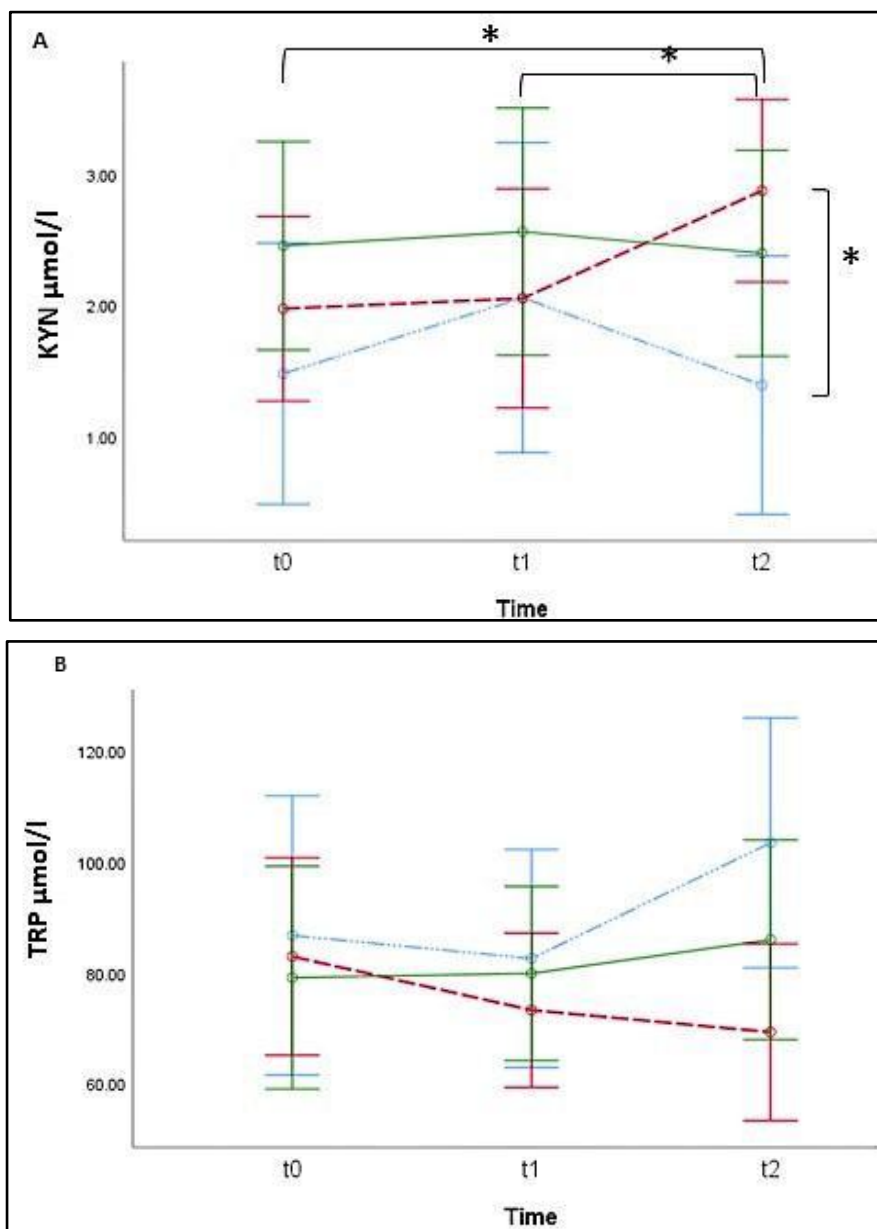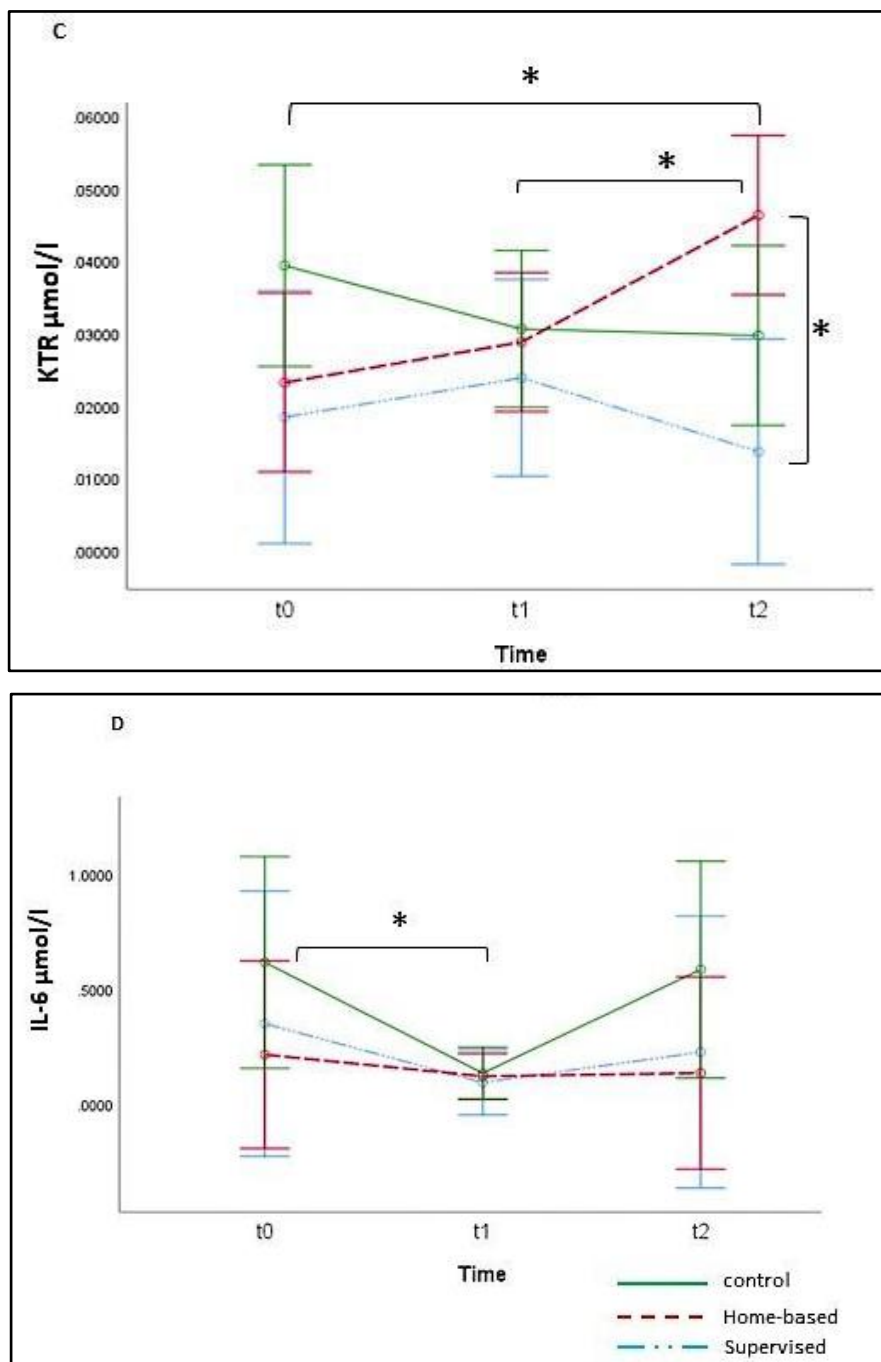
Figure 9: Change in expression of blood marker outcomes during intervention at t0 (baseline), t1 (three months) and t2 (six months) using Enzyme linked Immunosorbent Assay (ELISA). Data from double experimental replicates are presented as means with 95% confidence intervals. Statistical significance was calculated using ANCOVA. (Supervised exercise n=7, home-based exercise n=14, control group n=11). (A) Kynurenine (KYN), (B) Tryptophan (TRP), (C) KTR (Kynurenine/Tryptophan ratio), (D) IL-6 (Pal et al. 2020). * Indicating results of statistical significance of Bonferroni corrected post-hoc analyses.

Table 7: Mean values and baseline adjusted ANCOVA results of analysed serum outcomes using Enzyme linked Immunosorbent Assay (ELISA). (F-values, degrees of freedom (df) and p-values) for Kynurenine (KYN), Tryptophan (TRP), KTR (Kynurenine/Tryptophan ratio) and IL-6 are listed. Bold font indicates significant group difference (p < 0.05); SD-Standard deviation (Pal et al. 2020).

Outcome	Group	n	T0, mean (SD)	T1, mean (SD)	T2, mean (SD)	ANCOVA time			ANCOVA group x time		
						F	df	P	F	df	P
KYN (µmol/l)	control	7	2.45 (1.42)	2.56 (1.65)	2.40 (1.20)						
	supervised	14	1.47 (0.75)	2.06 (1.30)	1.39 (0.66)	6.48	1.66	**<0.01**	3.83	3.33	**0.01**
	home-based	11	1.97 (1.26)	2.05 (1.38)	2.88 (1.42)						
TRP (µmol/l)	control	7	79.06 (29.73)	79.81 (16.42)	85.90 (19.57)						
	supervised	14	86.67 (27.69)	82.51 (26.29)	103.43 (36.26)	15.85	2.00	**<0.01**	2.07	3.92	0.10
	home-based	11	82.87 (33.65)	73.18 (28.12)	69.19 (28.50)						
KTR (µmol/l)	control	7	0.04 (0.03)	0.03 (0.02)	0.03 (0.02)						
	supervised	14	0.02 (0.01)	0.02 (0.01)	0.01 (0.01)	11.63	1.80	**<0.01**	4.42	3.59	**<0.01**
	home-based	11	0.02 (0.02)	0.03 (0.02)	0.05 (0.02)						
IL-6 (µmol/l)	control	7	0.62 (1.04)	0.14 (0.10)	0.59 (1.17)						
	supervised	14	0.35 (0.50)	0.09 (0.10)	0.22 (0.32)	3.90	1.08	0.06	1.22	2.17	0.31
	home-based	11	0.22 (0.43)	0.12 (0.24)	0.14 (0.29)						

3.3 Endurance exercise mediated changes in NK cells via AhR/IDO axis

The research question investigated here involves whether endurance exercise can modulate NK cell function via AhR/IDO axis in cancer patients. Additionally, whether there is a difference in acute endurance exercise mediated changes in NK cells and how does chronic endurance training of different modalities elicit a change in NK cells.

Serum samples from all patients' were analysed for their ability to impact NK cell receptors as well as AhR and IDO levels. The surface markers were identified by gating for NKG2D+, KIR2DL1+ and intracellular markers by AhR+, IDO+ cells (Figure 10 representative data of one patient).

Baseline comparison between different endurance exercise groups

Analysis of baseline differences between age ($p=0.72$), weight ($p=0.15$), BMI ($p=0.25$) and cancer type ($p=0.55$) between endurance standard and endurance polarized groups did not show any significance (mean data listed in Table 8). Mean age of the study participants were 60.67 years in endurance polarized group and 59.11 years in endurance standard group. They showed on average a slightly higher weight in both groups (mean BMI polarized:26.14 kg/m^2, standard: 28.53 kg/m^2). The study participants include both breast and prostate cancer. The majority of the patients were diagnosed with stage I cancer (Pal et al. 2021).

Table 8: Anthropometric and clinical parameters of patient population *(Pal et al. 2021)*.
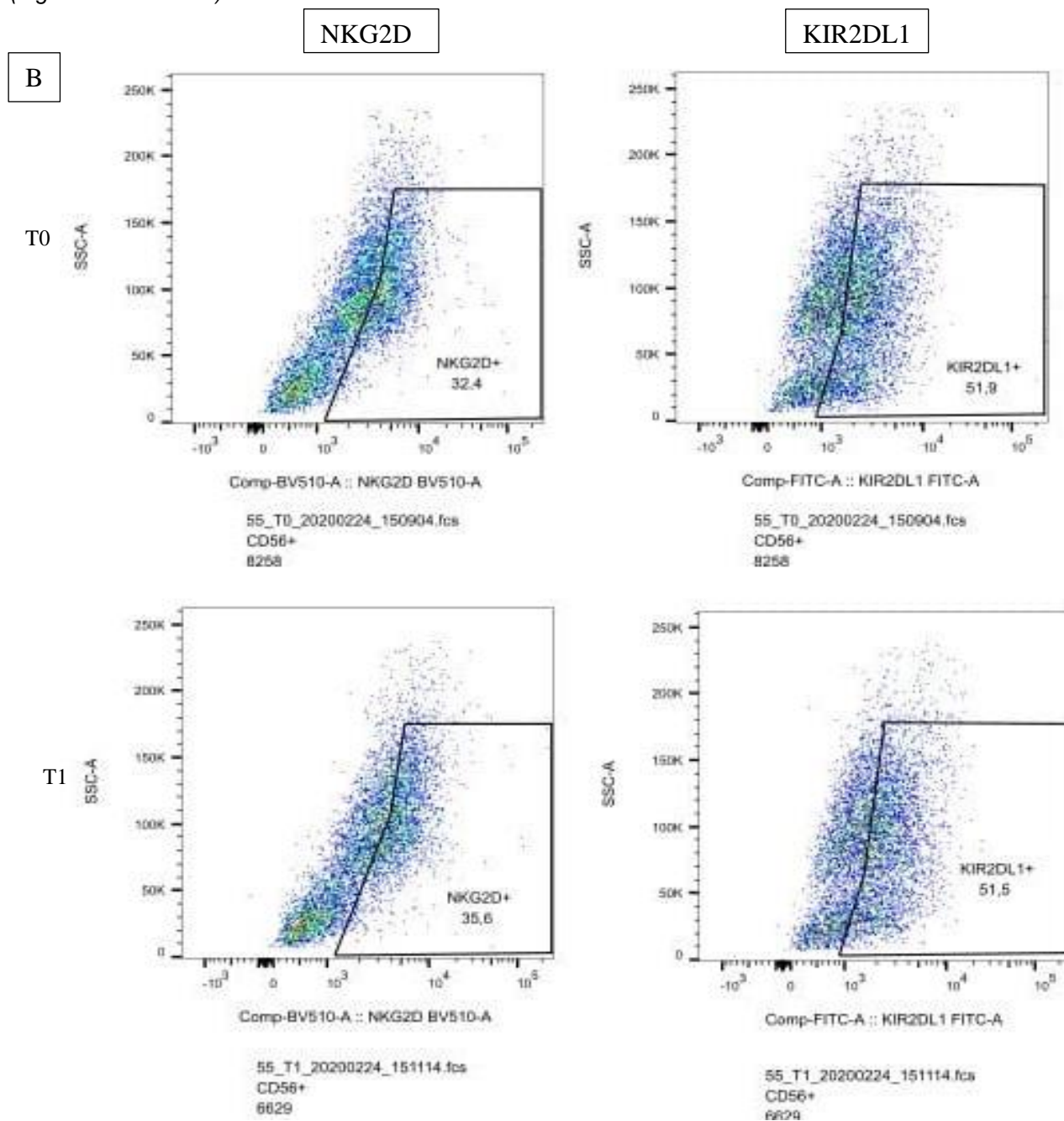
Study	Endurance POLY	Endurance STD
TOTAL (n)	12	9
Age, mean (SD)	60.67 (8.70)	59.11 (9.87)
BMI, mean (SD)	26.14 (3.53)	28.53 (4.01)
VO2 max, mean (SD)	23.03 (3.68)	21.6 (4.71)
Breast Cancer, n (%)	7 (58.33%)	4 (44.44%)
Prostate Cancer, n (%)	5 (41.66%)	5 (55.55%)
Stage, n (%)		
I	9 (75%)	7 (77.77%)
II	3 (25%)	2 (22.22%)
III	0 (0%)	0 (0%)
IV	0 (0%)	0 (0%)
Treatment, n (%)		
Adjuvant Hormone Therapy	7 (58.33%)	5 (55.55%)
Antibody Therapy	1 (8.3%)	1 (11.11%)
Neo-Adjuvant Chemotherapy	0 (0%)	2 (22.22%)
Intra-Radiation Therapy	1 (8.3%)	0 (0%)
Radiation Therapy	9 (75%)	6 (66.66%)
Operative procedures, n (%)	10 (83.33%)	8 (88.88%)

n= number of participants, SD= Standard Deviation; BMI = Body Mass Index

Figure 10 Representative plot of gating strategy for a single patient. A-Histogram, B- dot plots. NK-92 cells were analysed by flow cytometry, gating by forward/side scatter. The markers used: NKG2D, KIR2DL1, AhR and IDO

(Figure 10 continued)

B

| NKG2D | KIR2DL1 |

T0

Comp-BV510-A :: NKG2D BV510-A

NKG2D+
32.4

55_T0_20200224_150904.fcs
CD56+
8258

Comp-FITC-A :: KIR2DL1 FITC-A

KIR2DL1+
51,9

55_T0_20200224_150904.fcs
CD56+
8258

T1

Comp-BV510-A :: NKG2D BV510-A

NKG2D+
35,6

55_T1_20200224_151114.fcs
CD56+
6629

Comp-FITC-A :: KIR2DL1 FITC-A

KIR2DL1+
51,5

55_T1_20200224_151114.fcs
CD56+
6629

(Figure 10 continued)

T2

T3
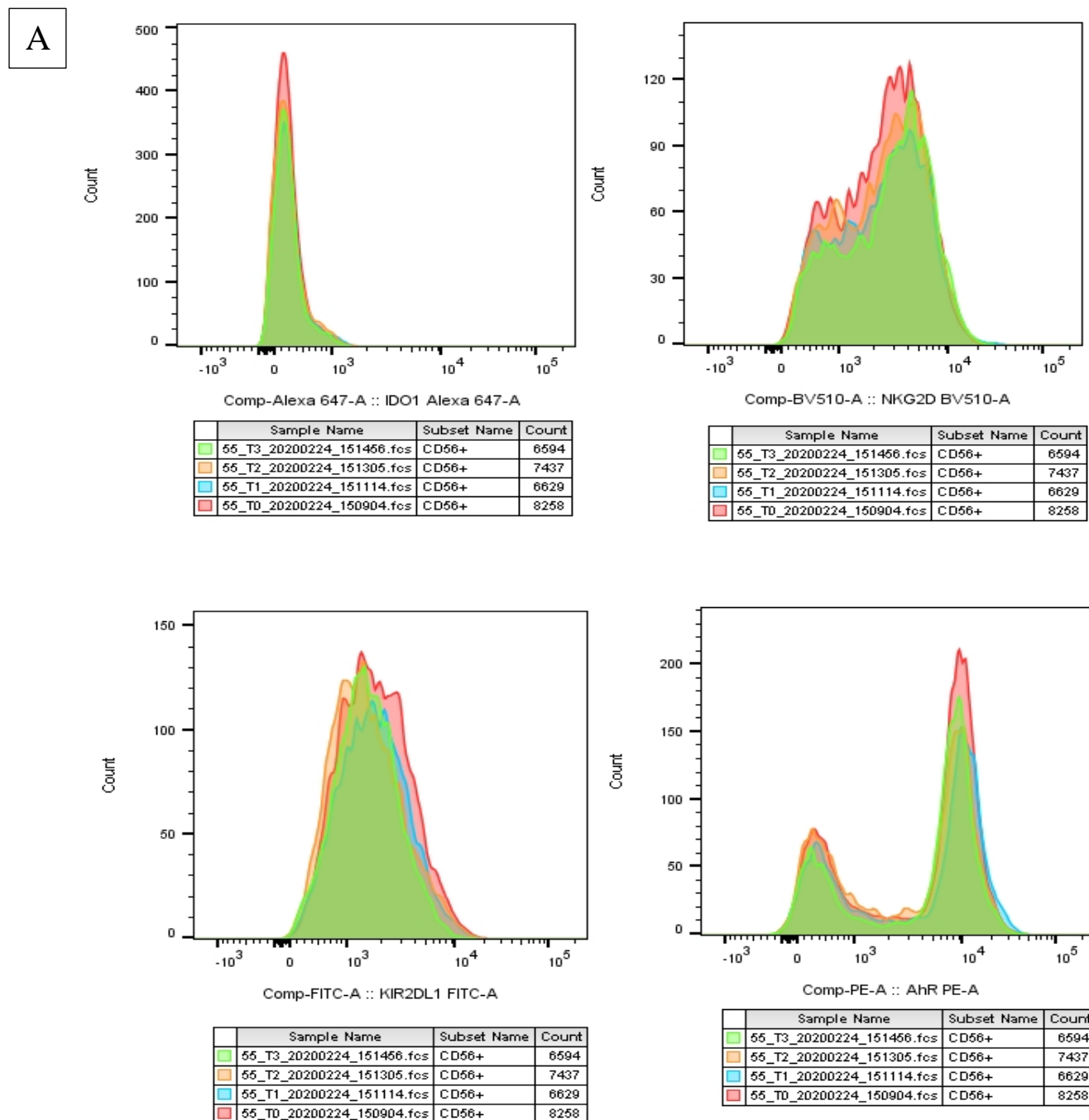
(Figure 10 continued)

(Figure 10 continued)

Prior to the samples being analysed by flow cytometry, compensation was performed for each individual parameters using NK92 cell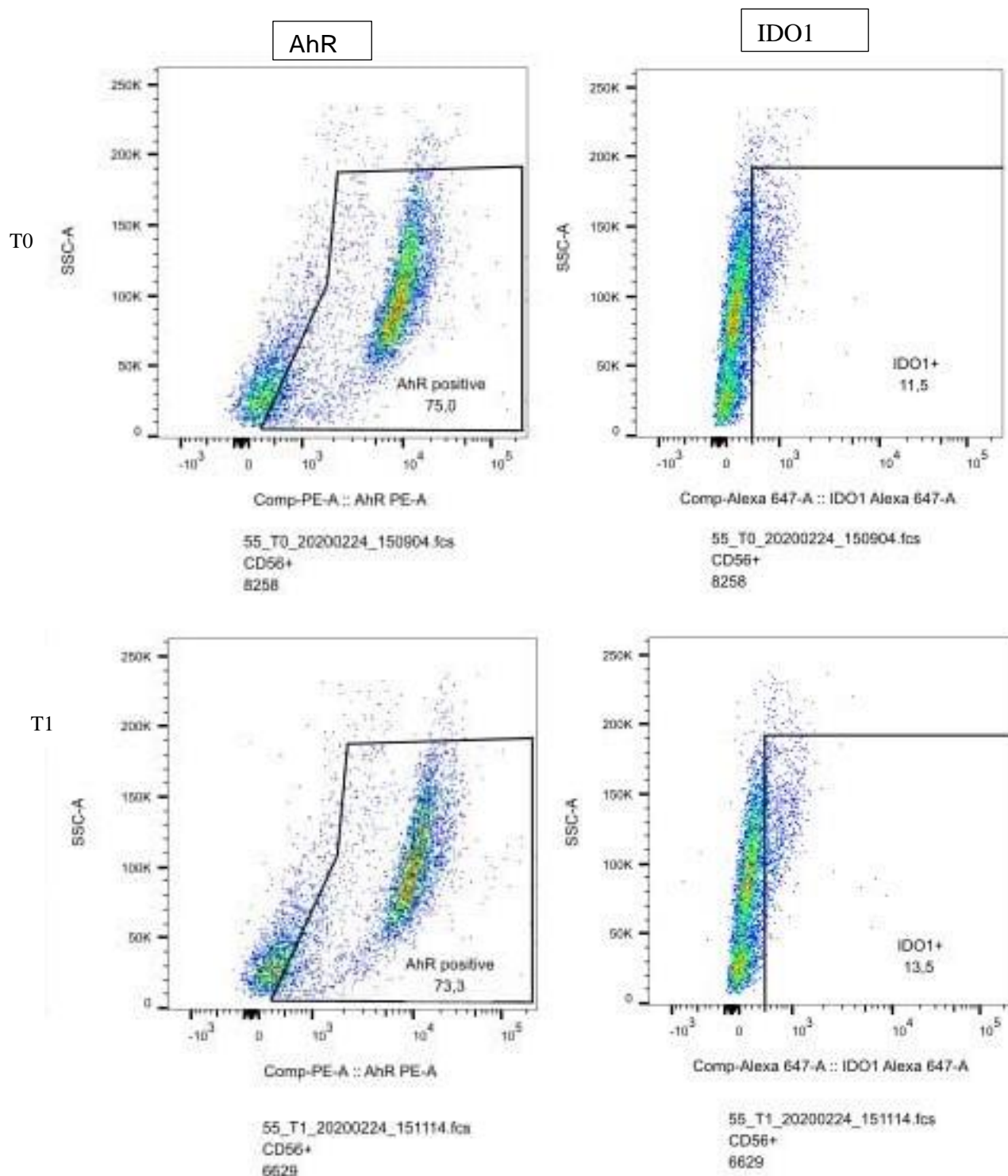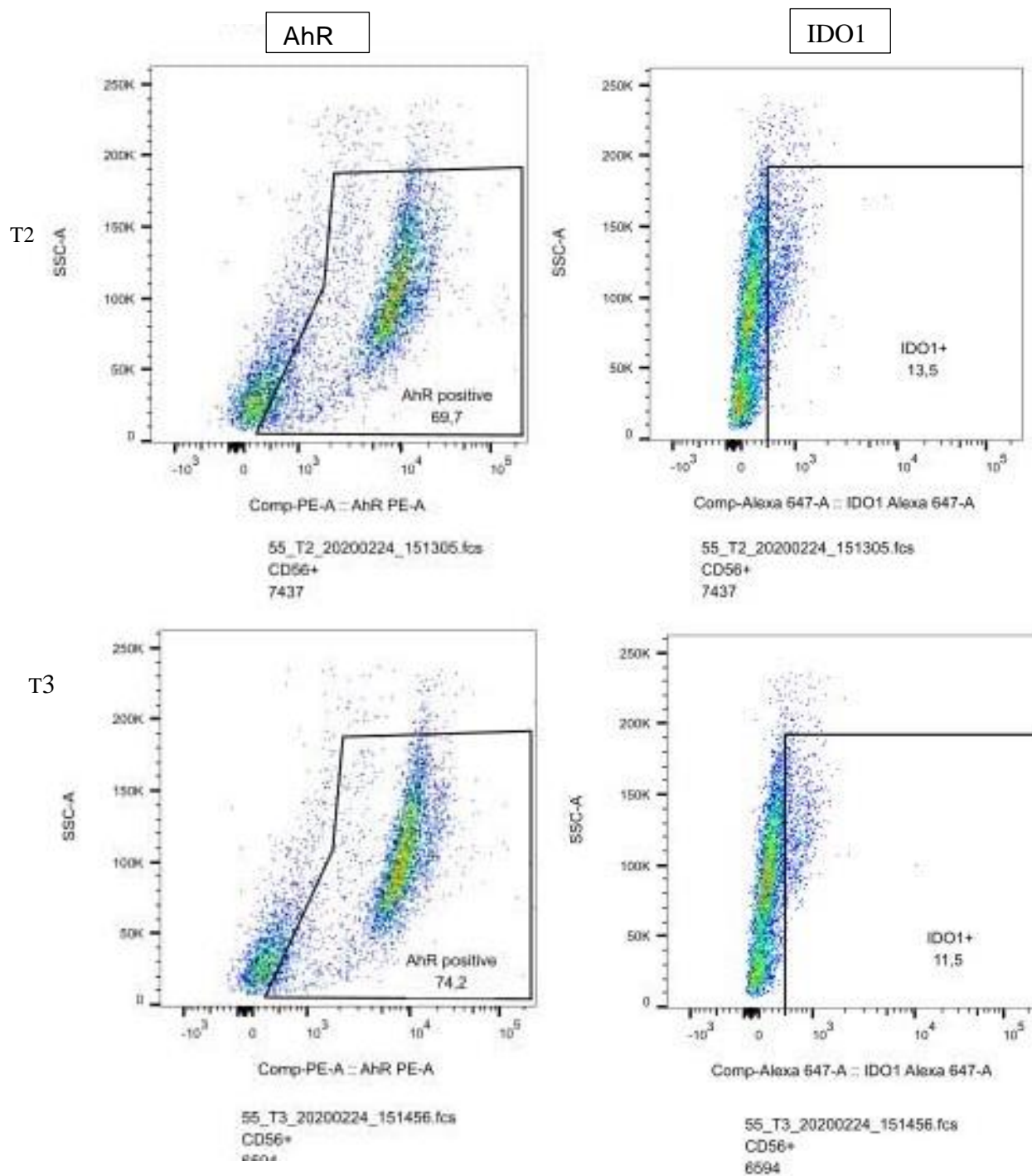s. SSC-A and FSC-A voltages were adjusted according to the observed population of cells. Flow cytometry data revealed distinct shifts in NKG2D+, KIR2DL1+, AhR+ and IDO+ cells. The dot plot showed a distinct population of AhR+ cells. The marked decrease in KIR2DL1+ cells proportion at 1h post-CPET (T2) was observed in all patient's serum incubated with NK cells. After randomization into 2 groups of either polarized or standard endurance training for 12 weeks, KIR2DL1 levels rose slightly for both groups in comparison to 1h post-CPET levels. Similarly, NKG2D expressing positive NK cells number were also slightly higher after 12 weeks of randomization when compared to pre-CPET, post-CPET or 1h post-CPET. IDO+ cells were detected low in number but a shift in cell population of IDO+ cells were nonetheless detected.

Visual representation of both acute effects and chronic effects of endurance exercise after flow cytometry are represented in Figure 11 and 12 respectively.

3.3.1 Acute effects of endurance training on NK cell

The receptor AhR, although expression reduced post-CPET and was back to pre-CPET values after 1h ANOVA revealed no significant change over time ($p=0.13$, $F=2.11$, $df=1.78$) (Figure 11A). For IDO, a significant change over time was observed ($p=0.02$, $F=4.63$, $df=1.52$). Bonferroni corrected post-hoc analysis showed statistically significant increase between following time points post-CPET and 1h post-CPET ($p=0.03$) (Figure 11B). IDO levels initially decreased immediately after CPET. This result was however of no significance. Although not significant, IDO levels also increased from pre-CPET levels to 1h post-CPET. KIR2DL1 expression decreased significantly over time ($p<0.01$, $F=6.15$, $df=1.60$) (Figure 11C). Bonferroni corrected post-hoc analysis showed statistically significant decrease between pre-CPET and 1h post-CPET ($p=0.03$) (Figure 11C). From pre-CPET levels to post-CPET levels, the expression of KIR2DL1 also tended to decrease (non-significant). For NKG2D, the expression remained almost constant at all three time points without any statistical significance ($p=0.31$, $F=1.20$, $df=1.69$) (Figure 11D) (Pal et al. 2021).

Mean data and time courses of all outcome measures as well as Bonferroni corrected post-hoc ANOVA results are shown in Figure 11 and listed in Table 9.
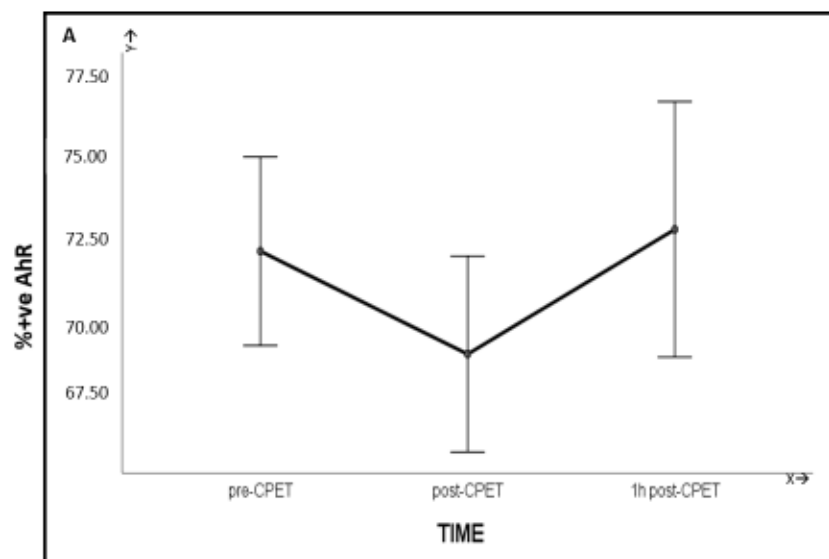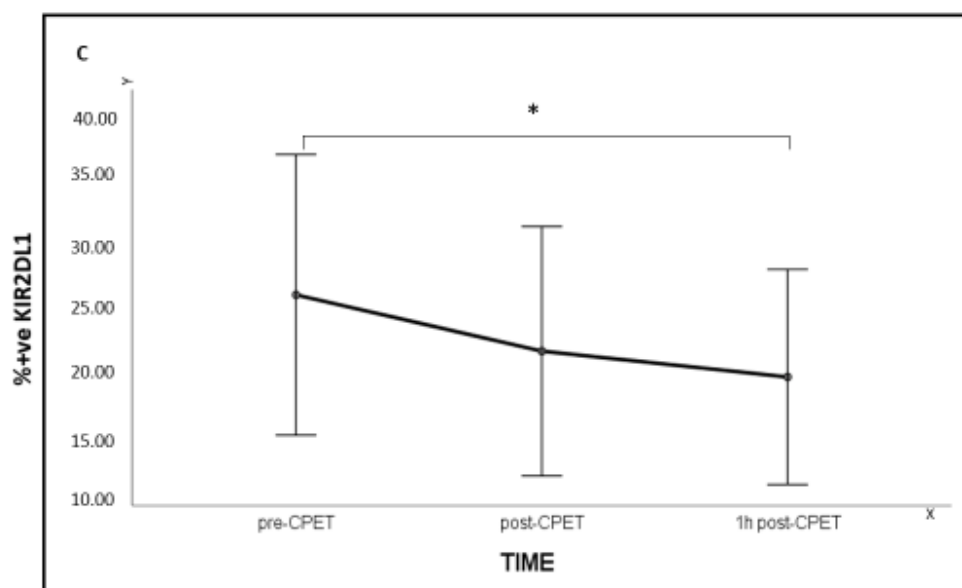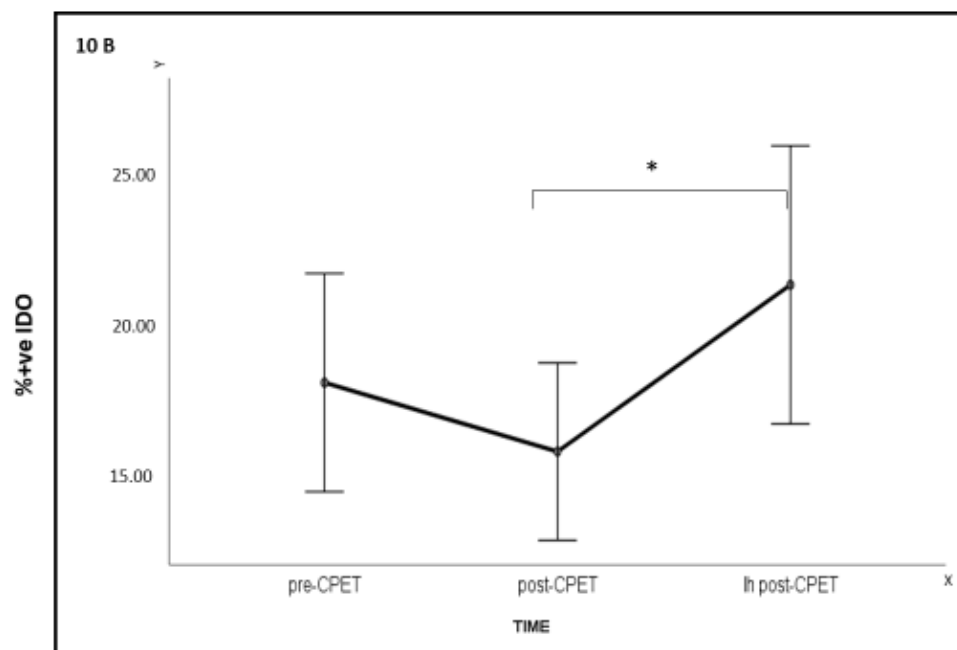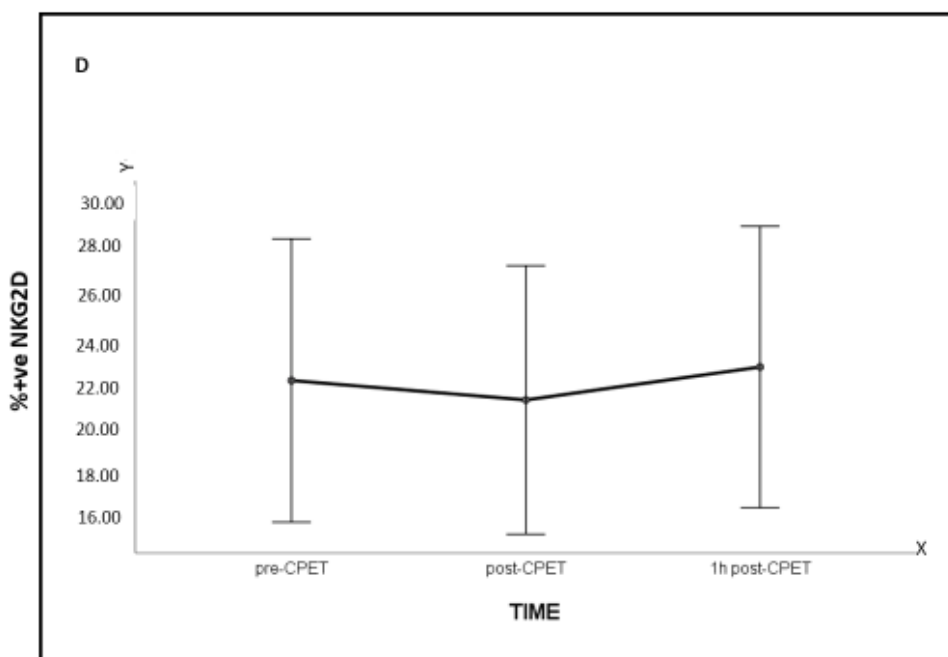
Figure 11: Acute effects of single bout of endurance exercise: Mean expression of AhR, IDO and NK cell receptors KIR2DL1 and NKG2D are plotted at pre-CPET, post-CPET and 1h post- CPET after Flow cytometry assay (FACS) using patient's serum incubated NK cells. Data are presented as means with 95% confidence intervals. (A) AhR (B) IDO (C) KIR2DL1, (D) NKG2D. X-axis: time, Y-axis: % positive cells. * Indicating results of statistical significance.

Table 9: Mean values and ANOVA results (F-values, degrees of freedom (df) and p-values) for AhR, IDO, KIR2DL1 and NKG2D in NK cells are listed. Bold font indicates significant group difference (p < 0.05); SD-Standard deviation (Pal et al. 2020)

Outcome	n	pre-CPET, mean (SD)	post-CPET, mean (SD)	1h post-CPET, mean (SD)	ANOVA		
					F	*df*	*P*
AhR	21	71.95 (6.32)	68.73 (6.56)	72.63 (8.56)	2.1	1.78	0.13
IDO	21	18.00 (7.92)	15.67 (6.44)	21.32 (10.09)	4.6	1.52	**0.02**
KIR2DL1	21	25.87 (22.94)	21.58 (20.37)	19.61 (17.58)	6.2	1.6	**<0.01**
NKG2D	21	22.06 (13.33)	21.20 (12.60)	22.65 (13.24)	1.2	1.69	0.31

3.3.2 Chronic effects of different endurance training modalities on NK cells

The results here are baseline adjusted to assess the difference of how two different endurance training modalities affect the expression of NK cells receptors and AhR and IDO levels. The receptor AhR revealed no significant group x time interaction *(p=0.27, F=3.59, df=1.00)* and no significant time effect *(p=0.07, F=30.35, df=1.00)* for chronic effects of different endurance exercise modalities (Figure 12A). Although not significant, AhR levels seemed to increase for the endurance standard group after 12 weeks of intervention. In contrast, the endurance polarized group remained almost the same after 12 weeks of intervention. The standard deviation of both groups is large and therefore these results should be considered exploratory.

For IDO, there was a significant group x time interaction *(p=0.01, F=10.66, df=1.00)* as well as a significant time effect (*p=0.01, F=10.03, df=1.00)* (Figure 12B.) After 12 weeks of intervention, a Bonferroni corrected post-hoc analysis revealed significantly higher IDO expression levels in the endurance standard group compared to the endurance polarized group *(p=0.01)*, but no significant within group differences *(p=0.17)*.

KIR2DL1, on the other hand, showed no significant group x time interaction *(p=0.92, F=0.01, df=1)* or time effect *(p=0.92, F=0.01, df=1)* (Figure 12C). Although the standard deviation is large, the expression of KIRDL1 seems to be decreasing for both endurance groups after 12 weeks of intervention.

 A statistically significant group x time interaction for NKG2D *(p=0.02, F=6.45, df=1.00)* as well as a significant time effect *(p=0.03, F=6.00, df=1.00)* was found. However, Bonferroni corrected post-hoc analysis revealed only a significant difference between groups after 12 weeks *(p=0.02)* and no significant differences within groups *(p=0.27)* (Figure 12D) (Pal et al. 2020b). NKG2D levels seemed to diverge at 12 weeks time point between the endurance polarized and standard group. The endurance polarized group revealed a higher expression from pre-CPET values after 12 weeks. On the other hand, in the endurance standard group NKG2D levels showed a decreased expression after 12 weeks when compared to pre-CPET levels.

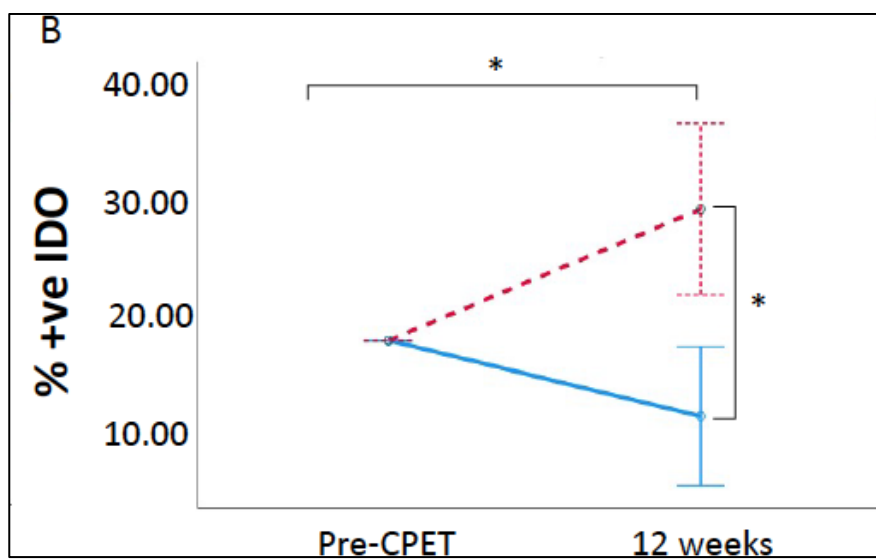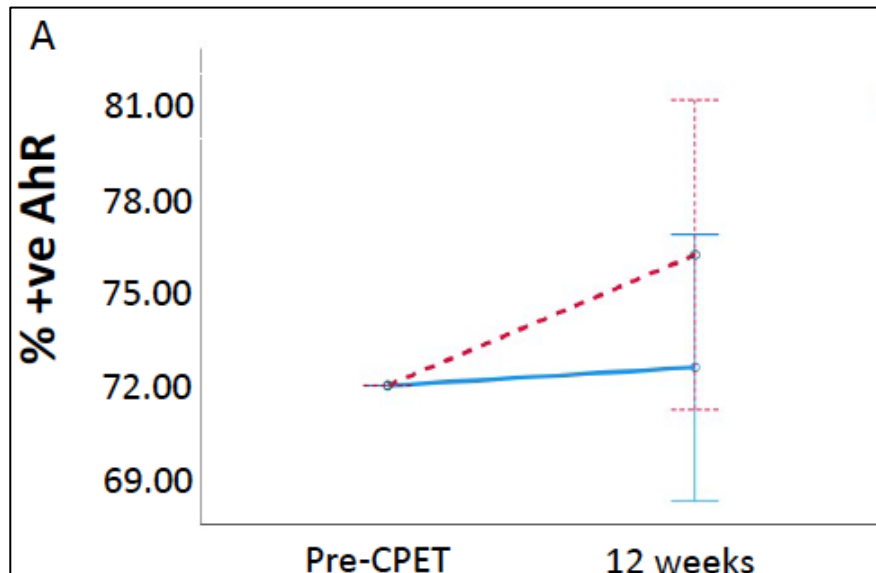Baseline adjusted mean data of all outcome measures using ANCOVA at pre-CPET and after 12 weeks are shown in Figure 12 and listed in Table 10.
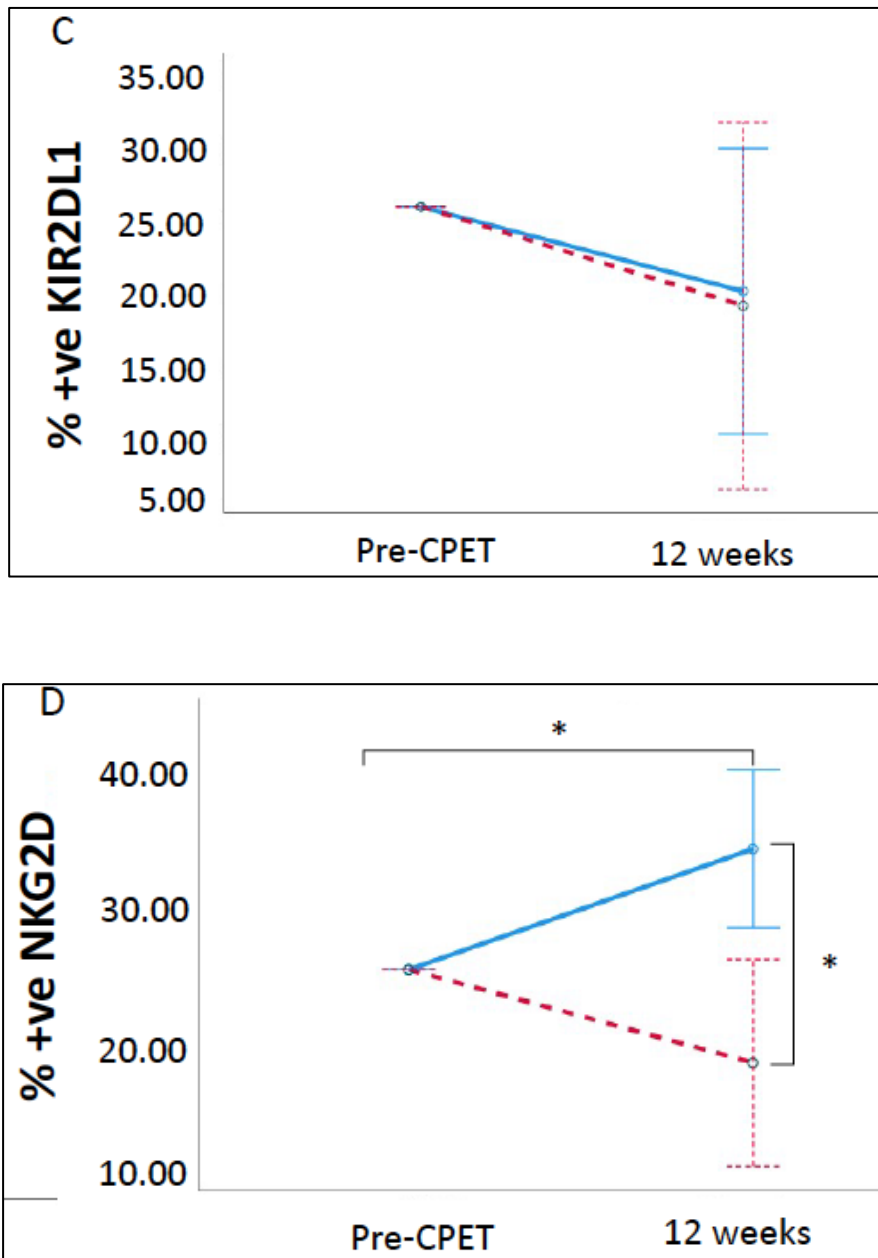
Figure 12: Baseline adjusted mean expression of AhR, IDO and NK cell receptors are plotted at pre-CPET and after 12 weeks of chronic exercise training modalities after flow cytometry assay using patient's serum incubated NK cells. Data are presented as means with 95% confidence intervals. (Endurance polarized: blue n=12, endurance standard: dashed red n=9). (A) AhR, (B) IDO, (C) KIR2DL1, (D) NKG2D (Data listed in Supplementary Table 2). X-axis: time, Y-axis: % positive cells. *
Indicating results of statistical significance.

Table 10: Chronic endurance exercise effects on NK cell outcomes between pre-CPET and 12 weeks. Mean values and standard deviations (SD) and baseline adjusted ANCOVA results are listed for all outcomes**.** (F-values, degrees of freedom (df) and p-values) for AhR, IDO, KIR2DL1 and NKG2D. Bold font indicates significant group difference ($p < 0.05$); SD-Standard deviation (Pal et al 2021).

Outcome	Group	n	pre-CPET, mean (SD)	12 weeks, mean (SD)	ANCOVA time			ANCOVA group X time		
					F	df	P	F	df	P
AhR	Endurance STD	9	74.21 (5.57)	77.40 (6.47)	30.35	1	0.07	3.59	1	0.27
	Endurance POLY	12	70.26 (6.32)	71.59 (7.71)						
IDO	Endurance STD	9	25.29 (6.63)	29.01 (9.58)	10.03	1	**<0.01**	10.66	1	**<0.01**
	Endurance POLY	12	12.55 (2.70)	11.70 (3.81)						
KIR2DL1	Endurance STD	9	1.29 (0.17)	1.01 (0.10)	0.01	1	0.92	0.01	1	0.92
	Endurance POLY	12	44.3 (11.29)	33.63 (13.26)						
NKG2D	Endurance STD	9	7.15 (2.95)	7.41 (2.17)	6.00	1	**0.03**	6.45	1	**0.02**
	Endurance POLY	12	33.63 (13.26)	36.73 (4.48)						

***Although all the experiments were performed and analysed simultaneously, there was some clustering observed in KIR2DL1 and NKG2D markers. This is hard to explain as no other marker showed any clustering or batch effect. Therefore, the analysis should be considered exploratory, and this should be taken into consideration while interpreting the results.*

4. DISCUSSION

In this section, the discussion on different endurance and resistance training modalities and intensities on cancer patient population's NK cells and Kynurenine pathway will be explored. Therefore, parts can correlate with the corresponding publications **Section 4.1**: (Pal et al. 2019), **Section 4.2**: (Pal et al. 2020), **Section 4.2:** (Pal et al. 2021).

For the first time in the field of exercise immunology research, a direct investigation into exercise induced modulation of NK cells via Kynurenine pathway has been conducted. The research at hand shows that resistance and endurance exercise impact the kynurenine pathway differently, especially in context of NK cells. However, this research also shows that although resistance exercise does not change NK cell transcriptome but it can influence the Kynurenine pathway. Additionally, endurance exercise has the potential to impact the AhR/IDO axis and consequently change NK cell receptor (activating and inhibiting) expressions. The main finding is that irrespective of modalities and intensities, exercise has the potential to modulate the immune system in different cancer patient populations.

4.1 Resistance exercise mediated changes in NK cell transcriptome

Our pilot study investigating the chronic effects of resistance training on NK cell gene expression profile in breast cancer patients undergoing adjuvant therapy showed, after 12-week exercise intervention, numerous genes with higher expression in comparison to the control group. However, after adjusting for multiple testing, there had been no considerable significance in differences in expression observed.

Research on chronic effect of resistance exercise training on differential gene expression of NK cells is sparse. Dias et al. reported a change in 211 gene transcripts involved in cell cycle regulation, proliferation and development of immune cells in PBMCs after 18 weeks of aerobic endurance training (Dias et al. 2015). A study conducted by Liu et al. identified 72 transcripts in PBMCs concerned in encoding

ribosomal proteins and oxidative phosphorylation. The study was conducted on young athletes compared to non-athletes with a false discovery rate < 0.05 (Liu et al. 2017). However, it should be noted that PBMCs are a heterogeneous mix of immune cells.

Observed variations in gene expression over time may be attributed to alterations of cell proportions. Shephard RJ. et al. showed that exercise training results in changes in immune cell subsets (Shephard 1996). Additionally, the various exercise routines (aerobic vs. strength) that are applied can also explain differences in performance. Most studies investigating the impact of training on cytotoxicity of NK cells documented no change (Campbell et al. 2008; Zimmer et al. 2015).

Studies indicate that acute exercise causes chromatin remodelling in NK cells relative to chronic effects of exercise and can alter the expression of NK cell receptors (Timmons and Cieslak 2008; Zimmer et al. 2015). Zimmer et al. also showed that the cytokine levels which subsequently affect the epigenome of NK cells could be impacted by a single bout of exercise (Zimmer et al. 2014). Few studies that have recorded patterns of NK cell gene expression, migration potential, cytotoxicity and change in the population of NK cell subsets are in context of acute exercise, data on chronic exercise are scarce and conflicting.

The study results showed negligible effects of the exercise intervention on the gene expression. The TET1 transcript is responsible for DNA methylation, and its expression appears to be somewhat higher after exercise. The TET1 gene (Ten-eleven translocation methyl-cytosine dioxygenase 1) is an epigenetic marker responsible for the conversion of the modified DNA base 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) and is the initial step for active DNA demethylation in mammals. Lower TET1 levels were associated with higher levels of proliferation (Weber-Boyvat et al. 2015). The other interesting candidates were: FXYD4, a regulator for ion transport. VAPA, Homo sapiens VAMP (vesicle-associated membrane protein)-associated protein. This has a role in signalling RRAS, which in turn diminishes the activation of integrin beta-1 (ITGB1) at the cell surface (Genevini et al. 2019). Homo sapiens WD repeat domain 68 (WDR68) interacts with other enzymes and can play a significant role in controlling cell proliferation via a signalling pathway (Gangula and Maddika 2013). Homo sapiens chromosome 20 open reading frame 199 (C20orf199), SLC22A10 is a solute carrier family protein. In addition to

TET1, the genes listed above are well known but may not have a specific role with respect to the function of NK cells or its cytotoxicity. Their role is still to be elucidated in NK cells. The other transcripts with a significant P value before multiple test changes are hypothetical potential protein coding genes, and literature review found no specific known function. They do not communicate with each other, nor form a pattern of pathways. Therefore, the results of this study do not provide evidence that the transcriptomic profile of NK cells in breast cancer patients is affected by chronic resistance exercise.

The immune system's equilibrium is undoubtedly disrupted by physical activity. However, the findings suggest that acute rather than chronic exercise impacts are likely to be responsible for these alterations. Furthermore, in breast cancer patients undergoing medical treatment, chronic exercise may not elicit any changes in NK-cell gene expression.

One possible explanation for not finding significant changes in NK cell gene expression after chronic resistance training is that medical treatments (chemotherapy or radiation therapy) have a considerable influence on gene expression in diverse tissues (Klebanoff et al., 2005), therefore can change NK cell gene expression patterns too. The lack of a healthy control group to compare these findings is unfortunately, a weakness of this study.

It's worth noting that, like other studies in this field, this study was constrained by a small number of participants and should thus be regarded as a pilot study. Another drawback is that NK cell functions or the change in NK cell or proportions were not assessed (Pal et al, 2019).

4.2 Supervised vs home-based resistance exercise mediated changes in Kynurenine pathway

This is the first research to investigate the effects of resistance exercise training in patients with pancreatic cancer in the light of the Kynurenine metabolism pathway. The findings show that supervised resistance exercise training can reduce serum KTR (indication of IDO / TDO enzyme) levels over time compared to home-based group. The findings are consistent with the previous analysis of breast cancer patients showing that chronic resistance exercise counteracts a spike in Kynurenine levels and activity of KTR (IDO / TDO) during adjuvant radiotherapy (Zimmer et al. 2019b). As IDO / TDO is currently being evaluated as drug targets for different cancer therapies, this could be a positive outcome in relation to cancer prognosis (Qian et al. 2016).

Improved serum KTR levels are often seen in patients with diseases that cause immune activation and inflammation such as cancers, autoimmune syndromes or malignant tumors (Lemos et al. 2019). So far there is scarce information available on the effect of resistance exercise on inflammation and its relationship to IDO/ TDO activity and Kynurenine pathway in humans. In this study, the supervised exercise group reacted according to the hypothesis of the study, i.e. showing downregulation of Kynurenine and KTR levels. The unsupervised home-based exercise group, by comparison, showed opposite results with increased levels of Kynurenine and KTR. The disparity in outcome in supervised and home-based exercise groups can possibly be explained by the fact that the supervised group had more responsibility to exercise with prescribed training intensities under clinical supervision. The training modes also differed between the two intervention groups. The home-based group used Thera-bands ® and performed bodyweight workouts at home while the supervised group implemented a comprehensive machine-based training system. It can also be inferred that the supervised group conducted the exercises at a higher intensity under supervision. The adherence to training also decreased in the home-based group compared to the supervised group. Notably, the results of the supervised resistance exercise on KTR described here are inconclusive of whether it is due to IDO or TDO activity (Courneya et al. 2003). It is important to note that there are slightly more patients in the home-based exercise group who are in late-stage pancreatic

adenocarcinoma (10 patients in stage IIB), whereas the supervised exercise group has the fewest late stage patients (4 patients in stage IIB). This could explain the deterioration of several outcomes in the home-based group compared to the supervised group. It is also worth mentioning that the absence of significant baseline differences for any of the blood marker outcomes as well as the anthropometric and clinical/disease characteristics between the three groups could be attributed to a rather small sample size (n=32).

Given all the above points, it is beyond comprehension as to why the analysed outcomes of the unsupervised home-based exercise group worsens compared to the control group. Previously published study findings on the intervention impact on muscle strength measured by Handheld Dynamometry (HHD) (elbow flexors, knee flexors, hip flexors, hip abductors) revealed a non-statistically significant decrease in isometric muscle strength in the home-based group compared to the control group (Hardee et al. 2019), also increasing the likelihood that the control group did perform exercises. Such contamination effects in the control group have also been documented in other exercise intervention studies: because no exercise regimen is recommended for the control group, it is common for them to gather information on the benefits of exercise and may begin to exercise themselves (Courneya et al. 2003; Steins Bisschop et al. 2015). One way of circumventing this problem would be to use wearable trackers for all groups, which have already been implemented for ongoing studies.

Earlier studies have shown that exercise decreases resting levels of inflammatory markers, such as IL-6 (Schmidt et al. 2016). The result for IL-6 tends to decrease in both intervention groups (supervised and home-based) over the first three months. Interestingly, the IL-6 levels also decreased for the control group in the first three months. The relation between IDO / TDO and cytokines is very complex, particularly when the system is disturbed by external treatment such as chemotherapy. IL-6 may not be the key reason for the variations in the sample and the results that may be observed can also be triggered by other pro-inflammatory cytokine such as IFN-ɣ or TNF-α (Fujigaki et al. 2001). Further factors including peripheral levels of cortisol that can induce IDO / TDO activity should also be considered (O'Leary and Hackney 2014). The physiological concentrations of cortisol may induce IDO1 in PBMCs (Da Pozzo et

al. 2018). In addition, the hormonal influence of oestrogen and testosterone should also be taken into consideration since IDO / TDO activity has been reported to be regulated (Catena-Dell'Osso et al. 2011).

As noted in the introduction, after a week of unsupervised "increased physical activity" (daily fitness / stretching) (Hennings et al. 2013), no changes were noted in the Kynurenine levels or inflammatory markers among study participants with depression. Unfortunately, there has been no data on KTR available. Therefore, supervised resistance exercise might positively regulate the Kynurenine pathway and downregulate the Kynurenine/Tryptophan (indicative of IDO/TDO enzyme) levels, hence modulating the immune system.

The findings of this study should be interpreted in terms of its strengths and limitations. This is the first resistance exercise trial addressing the metabolites of the Kynurenine pathway in pancreatic cancer patients, despite the small sample size (n = 32). A bigger sample size is necessary to further understand how various intensities of exercise may affect Kynurenine metabolism. Other (anti) inflammatory markers including IFN-γ and IL-10, as well as cortisol, could be explored further to better understand the influence of exercise on the Kynurenine pathway.

Further research into the effects of Kynurenine metabolites on diverse immune cells, such as NK cells and T cells, could fill in a gap in exercise immunology. Finally, given that IDO/TDO is emerging as a new potential pharmacological target for cancer treatment, it will be fascinating to investigate if they have a direct impact on cancer cell division and its outcome when it is inhibited (Pal et al, 2020).

4.3 Endurance exercise mediated changes in NK cells via AhR/IDO axis

This is the first study to examine the acute and long-term effects of endurance exercise on alterations in NK cell receptor expression mediated by the AhR/IDO axis in patients with breast and prostate cancer. The study's main findings are that acute exercise causes considerable increases in IDO expression, as well as significant decreases in the expression of the inhibitory NK cell receptor KIR2DL1. When comparing the two different training programs, polarized endurance exercise results in significantly lower IDO expression levels than standard endurance exercise after 12 weeks. Intriguingly, the polarized group's chronically reduced IDO expression was accompanied by a significant increase in the expression of the activating NK cell receptor NKG2D, whereas the standard group showed the opposite trend.

Although not significant, the decreased levels of AhR immediately after CPET might be of importance as recent findings have revealed induction of IDO depends on AhR expression (Nguyen et al. 2014). Consequently, IDO-mediated Tryptophan catabolism and Kynurenine formation, which is an AhR agonist is an important immune-regulatory mechanism underlying immunosuppression and tolerance (Park et al. 2019). Despite NK cells' ability to directly eliminate cancer cells, they are inhibited by several factors, one such factor being IDO mediated suppression (Wang et al 2012) (Hornyak et al 2018). Therefore, acute single bout of endurance exercise may reduce AhR levels as well as IDO levels in NK cells. It can be worth speculating whether the detected decrease in AhR levels in the cytoplasm post-CPET is due to lower expression or whether AhR translocates more into the nucleus forming the transcriptional activation complex. The similar expression pattern of both AhR and IDO is indicative of the inter-dependent relationship between them. The restored levels of AhR and IDO after 1h of CPET can indicate to the AhR and IDO feedback loop as IDO levels correspond to the AhR expression pattern forming the AhR/IDO axis.

We looked for surface markers of NKG2D and KIR2DL1 to see if changes in AhR and IDO expression directly alter NK cell activating and inhibitory receptors. The absence of an NK cell-specific inhibitory signalling cascade would be indicated by the significantly lower expression of the inhibiting receptor KIR2DL1 as well as the

significant reduction between pre-CPET and 1h post-CPET levels. Although the activating receptor NKG2D expression is affected in a dose-response manner (shown previously by Zimmer et al. 2015), it is interesting to note that the expression does not change. It is plausible that the single bout of endurance training did not provide enough stimuli to change receptor expression. It should be noted that while we assessed NK cell receptors, an NK cell cytotoxic assay should have been performed in order to establish clinical relevance on NK cell cytotoxicity.

Our results on the chronic effects of endurance training over 12 weeks were equally pronounced after randomization into two different endurance training modalities. After 12 weeks of polarized or standard endurance training, the expression of AhR levels did not change significantly. It is worth noting that, while the difference was not statistically significant, the change in AhR levels over 12 weeks was slightly higher in the endurance standard group compared to the endurance polarized group. It's noteworthy that IDO levels after 12 weeks of training differed significantly between the two groups, with the endurance standard group having a higher expression. After 12 weeks of intervention, the expression of KIR2DL1 was non-significantly decreased in both the endurance polarized and standard groups, possibly interjecting the inhibition cascade. After 12 weeks of intervention, NKG2D expression was significantly higher in the endurance polarized group compared to the endurance standard group. This demonstrates that endurance polarized exercise, which incorporates high intensity interval training, has different effects than standard training.

Therefore, it may be concluded that irrespective of acute or chronic phases, KIR2DL1 expression might be lowered by endurance training. In comparison to chronic exercise, acute exercise has a more pronounced effect on all outcomes studied. Chronic polarized exercise has the potential to downregulate the AhR/IDO axis and activate cytotoxicity in NK cells. It may be possible to reckon that endurance polarized training may be a more potent stimulus for AhR/IDO mediated NK cell receptor changes than standard endurance exercise. Our findings suggest that after acute endurance exercise, IDO levels decrease immediately after CPET but significantly increase 1 hour later. Previous studies have reported that IDO levels increase with acute exercise and decrease after chronic exercise (Metcalfe, et al 2018). This is possible since these studies use IDO as an indirect measure of the Kynurenine and

Tryptophan ratio. Following a vigorous exercise session, it is possible that free Tryptophan is being used for protein synthesis. Repeated bouts of intense exercise increase the demand for protein synthesis, reducing the amount of available Tryptophan to be degraded into Kynurenine and, as a result, lowering the Kynurenine/Tryptophan ratio. Since no studies have been conducted to date on the effects of endurance exercise in both acute and chronic phases, it is possible that the Tryptophan breakdown is halted under higher energy demand.

These findings are a first step in understanding how the AhR/IDO axis may modulate NK cell function via exercise. It has already been established that IDO converts Tryptophan to Kynurenine within tumor cells. Increased Kynurenine levels in the microenvironment can enter NK cells and act as a potent internal AhR agonist. The AhR-Kynurenine complex translocates into the nucleus upon ligand binding and binds to the DREs (Dioxin responsive elements). This acts as a transcriptional regulator, causing more IDO to be produced while also increasing surface KIR2DL1 (inhibitory) and decreasing NKG2D (activating) expression. Endurance exercise, according to these findings, may lower IDO levels and decrease AhR agonist Kynurenine production. This inhibits the downstream cascade of AhR ligand binding and its subsequent transcriptional activity. Thereby, expressing lower KIR2DL1 and higher NKG2D levels, leading to enhanced NK cell cytotoxic potential.

The current study's findings should be interpreted in light of its strengths and limitations. Despite the small sample size (n=21), this is a one-of-a-kind study that investigates the effects of different endurance training modalities on NK cell activation via the AhR/IDO axis in cancer patients. Another limitation is clustering in the KIR2DL1 and NKG2D markers, so the analysis should be regarded as exploratory. Protein quantification techniques may also provide useful information in understanding the expression of these markers. Further research into Kynurenine transporters and other Kynurenine pathway metabolites is required to understand how the AhR/IDO axis functions specifically in NK cells. It would also be worth looking for AhR levels in the nucleus to see if exercise causes AhR translocation from the cytoplasm. Additionally, it would be interesting to look into whether different resistance exercise modalities have a similar ability to modulate the AhR/IDO axis, which leads to NK cell function.

We reasonably assume that both acute and chronic endurance training can influence NK cell function via the AhR/IDO axis. This is clinically important because promising cancer therapeutics based on AhR/IDO axis intervention are already being researched extensively (Bianchi-Smiraglia et al 2018) (Nguyen et al 2014) (Pal et al. 2021).

4.4 Strengths and limitations

The presented work should be read within its context of strengths and limitations. It is important to note that this research was limited by small sample sizes, a feature similar to other investigations in this area and should therefore be considered as pilot research projects. The research is also limited by its study designs as only samples from specific studies are available for biological analysis. Therefore, a combination of resistance training and endurance training from different studies were analysed for the entire research work. Additionally, a specific cancer population for analyses was not available. So, the hypotheses were tested on breast, pancreatic as well as prostate cancer patients.

The randomized trials of the studies were developed in compliance with the practice guidelines for cancer survivors of the American College of Sports Medicine (ACSM) (Schmitz et al. 2010), which should be considered as a major strength of this research.

For the first research question, two studies under different cancer treatments, chemotherapy and radiation therapy, were available to understand the changes in breast cancer patients undergoing resistance training. Comparing exercise vs non-exercise intervention among these patients is a strength of this study. Another major strength lies in including relevant cofactors in the analysis. Microarray is the current gold standard for determining gene expression changes. The resistance training program implemented is of gold standard. As mentioned above, the limitation lies in small sample size (n=19). Additionally, the distribution of patients in exercise and non-exercise groups were uneven. Also, the distribution among radiation therapy and chemotherapy was uneven. For the samples analysed, the study also lacked a healthy control group without any physical exercise intervention.

For the second research question, two separate exercise intervention modes were implemented as supervised and home-based forms and were compared against each other and against a usual care control group. The research gave initial impressions about the changes in Kynurenine pathway in both controlled and home-based resistance trainings in patients with pancreatic cancer. Another strength of the presented work was the use of gold standard measurements to collect comprehensive data on anthropometric and clinical parameters. The laboratory assays used in protein quantification were extremely sophisticated and used widely for these kinds of experiments. Additionally, exploration of the Kynurenine pathway and its modulation via both resistance exercises was unique. A general drawback of the presented study was that the data obtained were from a somewhat heterogeneous group of pancreatic cancer patients undergoing different cancer treatments (n=32). The control group was not monitored on their physical activity levels hence, had probably exercised on their own.

For the third research question, a single bout of exercise as well as chronic training effects on NK cell receptors and AhR/IDO axis were tested. This is a unique approach to unearth the molecular mechanisms behind immune regulation via exercise. The implementation of different endurance exercise modalities (polarized and standard) is a strength of this analysis. The use of flow cytometry to assess internal and external biomarkers is gold standard. The use of patient's autologous serum in NK cell culture is novel. A limitation in this study includes a small study sample size (n=21). Further, the study lacks a control group without exercise intervention. Additionally, the study included both breast and prostate cancer patients, hence a heterogenous cancer population is analysed. The patients also underwent different cancer treatments. Furthermore, due to limited amount of patients' serum availability the experiments were performed only once. Finally, as some clustering in KIR2DL1 and NKG2D markers were observed, therefore the analysis should be considered exploratory. The reason behind the clustering is speculative and can be attributed to the cell line or experimental bias.

All these strengths and limitations should be kept in mind while interpreting the results. Therefore, it is imperative to mention that the viability of these results are not conclusive. Nevertheless, this is the first novel research to investigate the impact of

different physical exercise on NK cells and immune regulation mechanisms in a varied population of cancer background.

4.5 Outlook

This research has been a first step in a course of many other steps within the complex pathways that are worth studying in a similar setting. It is important to note that human physiology is extremely complex and intricate. With the emerging field of exercise immunology, research to uncover the molecular mechanisms involved in the regulation of exercise induced immune response is gaining attention. The cell signalling pathways and their consecutive immune regulation is extremely fine-tuned. It is difficult to pinpoint one marker or pathway to the visible changes. As NK cells emerge to be the key in immune monitoring, further investigation into its subtle switches would give more insight into how to optimize its full potential in context of cancer. Therefore, a lot more research in this direction is imperative.

A greater sample size and exercise monitoring are recommended for further study into exercise induced NK cell changes. It is also of interest to see how other forms of exercise (different resistance and aerobic) affect Kynurenine metabolism. A closer look at other (anti) inflammatory factors, including IFN- ɣ, IL-10 or cortisol could also be useful in order to clearly understand the impact of exercise on the Kynurenine pathway. Given that IDO / TDO appears to be a new possible drug target for cancer treatment, it is important to see if they have a direct effect on and consequence of cancer cell division when inhibited. It is also worth investigating whether Kynurenine and Tryptophan concentrations decrease in tumor microenvironment. Similarly, as the Kynurenine pathway is extremely complex, its other metabolites and enzymes could provide more information on how the pathway is regulated via exercise. A deeper look into NAD+ mediated energy production can possibly reveal how the alternate metabolism is utilized for energy during exercise.

In order to understand how the AhR/IDO axis functions specifically in NK cells, further analyses of Kynurenine transporters and other Kynurenine pathway metabolites are necessary. Large amino acid transporters could be of interest, namely the SLC7a5. It will be interesting to evaluate the levels of AhR in the nucleus and see whether exercise contributes to the translocation of AhR from the cytoplasm to the nucleus. Different AhR agonists and their corresponding roles could also provide an insight into

the AhR/IDO feedback loop. Additionally, NK cell cytotoxicity assay can provide a comprehensive understanding on how NK cells function changes in response to exercise.

4.6 Conclusion

Exercise is recommended by experts worldwide for cancer survivors because of its numerous beneficial effects (Campbell et al. 2019; Meyerhardt et al. 2006; Schmitz et al. 2010). Physical activity disturbs homeostasis in the immune system. The immune system is very sensitive to exercise, with the intensity and duration of exercise representing the degree of physiological stress that the workload imposes. The earliest immunology research experiments (1900–1979) focused on exercise induced improvements in the counts and function of specific immune cells. Other focus areas were added to the field of exercise immunology during the period 1990 to 2009, including the interactive impact of nutrition, effects of aging on the immune system and influences on the inflammatory cytokines (Nieman and Wentz 2019). In addition, acute and chronic exercise induced immune changes are now being identified as important mechanistic pathways among the physically active for elucidating reduced risk of cancer and heart disease.

Acute exercise activates the change in subpopulations of different cells and components of the innate immune system between the lymphoid tissues and the blood compartment. While temporary, the effects accumulate over time leading to enhanced immunosurveillance and reduced systemic inflammation (chronic effects). With development of sophisticated technologies and laboratory methods, the investigation into exercise immunology focused more on molecular mechanisms behind these changes.

This research shows that these shifts in immune cells are likely caused by consequences of acute rather than chronic exercise. The changes in immune cells subsets are mediated by changes in gene expression. These changes can be elicited by epigenetic changes, transcriptional regulation or translational regulation. This research focused on NK cells and the mechanisms behind its changes. The results show that chronic resistance exercise in breast cancer patients receiving medical treatment may not cause any changes in the expression of the NK cell genes (Pal et al. 2019).

Second analysis on supervised vs home-based resistance exercise suggest that supervised resistance exercise can downregulate the levels of IDO/ TDO in pancreatic

cancer patients undergoing chemotherapy and can therefore decrease potential disease progression (Pal et al. 2020).

After the investigation of endurance exercise and its AhR/IDO mediated changes in NK cells a possible conclusion is that both acute and chronic endurance training can regulate NK cell function. The reduced expression of inhibitory receptor KIR2DL1 in acute phases of endurance exercise points to the fact that in absence of inhibitory signalling, endurance exercise has the potential to enhance NK cell cytotoxicity. The lower IDO levels immediately post-CPET (acute load), and its further normalization to pre-CPET levels after 1h is in line with other studies in this field. The similar pattern observed in expression of AhR although not significant indicates the role of AhR/IDO axis and the IDO feedback loop in regulating AhR expression (Pal et al. 2021). This is clinically important, as promising therapeutics focused on the intervention of the AhR/IDO axis are already under comprehensive investigation.

Chronic endurance polarized exercise seemed significantly efficient in reducing IDO levels after 12 weeks compared to standard endurance exercise. Similarly, endurance polarized exercise increased activating receptor NKG2D expression significantly after 12 weeks compared to endurance standard exercise group. This indicates that polarized endurance training might have the potential to enhance NK cell cytotoxicity and function.

Understanding the different effects of acute and chronic exercise as well as the different effects of resistance and endurance training can help us in improving the "exercise dose" in cancer patients. The three questions explored in this research binds together the implication of different physical exercise training in different cancer populations, thereby bringing forward the role of exercise in cancer. Taking advantage of the body's exercise ability can imitate immunotherapy which can contribute to improved health outcomes.

5. SUMMARY

An active lifestyle is associated with reduced cancer risk and improved survival in cancer patients, but mechanisms involved behind such effects are not investigated enough. Exercise has been shown to mobilize and impact NK cell function in intensity- and type- dependent manner. But evidence regarding how exercise impacts NK cell gene expression is sparse. Furthermore, the Kynurenine pathway and the corresponding AhR/IDO axis is known to have a role in cancer progression and immunosuppression and therefore may have a role in NK cell regulation. In this context the following research questions were investigated: 1) whether chronic resistance exercise mediate changes in NK cell transcriptome of breast cancer patients undergoing therapy 2) whether the Kynurenine pathway is regulated differently in supervised vs. home-based chronic resistance training in pancreatic cancer patients 3) whether different endurance exercise modalities and intensities modulate NK cell function via AhR/IDO axis in cancer patients.

For the first research question patients with breast cancer were randomly allocated to either a 12-week resistance exercise program or an adjuvant therapy adjunct relaxation control group. In a subsample of 19 participants, RNA was extracted from isolated NK cells and then analysed for differential gene expression before and after intervention. After chronic exercise intervention several genes showed higher differential expression compared to the control group. However, after correction for multiple testing, baseline-adjusted analyses of covariance (ANCOVA) indicated no significant differences between the intervention and the control group with regard to the gene expression profile. Therefore, these results did not indicate that resistance exercise of 12 weeks in breast cancer patients undergoing adjuvant therapy alter the gene expression profile of NK cells over the long term.

For the second research question adult pancreatic cancer patients were randomized to intervention programs of 6-month (1) a supervised moderate-to-high-intensity progressive resistance training or (2) unsupervised home-based resistance training, or (3) to a standard care patient control group. Serum levels Kynurenine, Tryptophan and IL-6 were assessed before, after three months and after six months of exercise

intervention. Patients in the supervised training group showed decreased levels of serum Kynurenine and Kynurenine/Tryptophan ratio *(p=0.07; p=0.01 respectively)* as well as increased Tryptophan levels (*p=0.05*) in comparison to home-based and control group over time. The home-based exercise group had significant increased Kynurenine and Kynurenine/Tryptophan ratio levels. IL-6 levels decreased over the first three months for both intervention groups as well as the control group (*supervised: p<0.01, home-based: p<0.010, control group: p<0.01*). The results suggest that supervised resistance exercise might positively regulate the Kynurenine pathway and downregulate the Kynurenine/Tryptophan (indicative of IDO/TDO enzyme) levels, hence modulating the immune system.

For the third research question, as chronic resistance exercise did not show any major changes in NK cell gene expression in the first part of this thesis, it was important to establish whether different intensities of endurance exercise and its modalities have the ability to change NK cell receptors and whether AhR/IDO axis plays a role in it. This was achieved by incubating NK cell line in exercised patients' autologous serum and assessing NK cell receptors, AhR and IDO expression via flow cytometry. For acute effects, pre-CPET, post-CPET and 1h post-CPET values were assessed. For chronic effects after randomization into 1) polarized or 2) standard endurance training, changes were compared between pre-CPET and 12 weeks after training. In acute endurance exercise, AhR (non-significant) and IDO levels (significant), reduced post-CPET and was almost restored back to pre-CPET levels after 1h (*p=0.13, p=0.02 respectively).* KIR2DL1 levels significantly decreased over time (*p<0.01).* NKG2D levels remained constant without significance (*p=0.31).* For Chronic endurance exercise, significant differences were observed for IDO and NKG2D with lower and higher expression in endurance polarized group *(both p<0.01)* respectively*.* AhR and KIR2DL1 levels revealed no significant differences. The results indicate that both acute and chronic endurance exercise may positively regulate the AhR/IDO axis and can consequently change NK cell receptor expression. Hence, we may conclude that NK cell regulation is possibly a property of acute and not chronic resistance exercise. Furthermore, Chronic resistance exercise has the ability to modulate Kynurenine pathway. Finally, irrespective of acute or chronic, endurance exercise modalities have the potential to influence NK cell receptors via the AhR/IDO axis.

The mechanisms behind exercise induced immune regulation are complex and further research is needed in this direction. This was a first attempt to understand on how to utilize the body's own resources to combat cancer and modulate the immune system. We have not yet reached that goal, just taken the first few steps.

ZUSAMMENFASSUNG

Ein aktiver Lebensstil ist mit einem verringerten Krebsrisiko und einem verbesserten Überleben bei Krebspatienten verbunden, wobei die Mechanismen, die hinter solchen Effekten stehen, bisher unzureichend untersucht sind. Als ein potentieller Mechanismus wird eine sportinduzierte Mobilisierung und Aktivierung von NK-Zellen diskutiert. Darüber hinaus ist bekannt, dass bestimmte sportliche Aktivitäten den Tryptophanmetabolismus und insbesondere den sogenannten Kynureninpfad beeinflussen, der seinerseits über die AhR / IDO-Achse eine zentrale Rolle bei der Regulation von Tumorzellen und diverser Immunzellen spielt. Neuere Arbeiten weisen darauf hin, dass auch NK-Zellen durch Kynureninpfadmetabolite reguliert werden. Vor diesem Hintergrund wurden im Rahmen dieser Arbeit folgende Forschungsfragen untersucht: 1) Provoziert ein mehrwöchiges Krafttraining Veränderungen im NK-Zelltranskriptom von Brustkrebspatientinnen? 2) Provoziert ein mehrwöchiges Krafttraining Veränderungen im Kynureninpfad bei Menschen mit Bauchspeicheldrüsenkrebs? 3) Provozieren unterschiedliche Ausdauertrainingsmodalitäten die NK-Zellfunktion über die AhR / IDO-Achse bei Menschen mit Bruts- bzw. Prostatakrebserkrankungen.

Für die erste Forschungsfrage wurden eine Teilstichprobe von Brustkrebspatientinnen aus zwei RCTs , die zufällig einem 12-wöchigen Krafttrainingsprogramm oder einer Kontrollgruppe zugeordnet wurden, untersucht. Von 19 Teilnehmerinnen wurde RNA aus isolierten NK-Zellen extrahiert und vor und nach der Intervention auf differentielle Genexpression analysiert. Nach einer chronischen Belastung zeigten mehrere Gene im Vergleich zur Kontrollgruppe eine höhere differentielle Expression. Nach Korrektur für multiples Testen zeigte die für die Baseline adjustierte Kovarianzanalyse (ANCOVA) jedoch keine signifikanten Unterschiede hinsichtlich des Genexpressionsprofils zwischen der Interventions- und der Kontrollgruppe. Diese ersten Ergebnisse weisen darauf hin, dass ein 12-wöchiges Krafttraining bei Brustkrebspatientinnen, die sich einer adjuvanten Therapie unterziehen, zu keiner dauerhaften Veränderung des NK-Zell Genexpressionsprofils führt.

Für die zweite Forschungsfrage wurden bereits operierte Pankreaskrebspatienten in drei Gruppen randomisiert: (1) ein 6-monatiges betreutes Krafttraining bei mittlerer bis hoher Intensität, (2) ein 6-monatiges nicht supervidiertes home-based Krafttraining

oder (3) eine Kontrollgruppe, die die Standardversorgung erhielt. Die Serumspiegel von Kynurenin, Tryptophan und IL-6 wurden vor, nach drei Monaten und nach Beendigung des Trainings nach sechs Monaten bestimmt. Patienten in der betreuten Trainingsgruppe zeigten im Vergleich zur Heimtraining- und Kontrollgruppe im Laufe der Zeit verringerte Serumspiegel von Kynurenin und der Kynurenin / Tryptophan Ratio ($p = 0{,}07$; $p = 0{,}01$) sowie erhöhte Tryptophanspiegel ($p = 0{,}05$). Die Heimtrainingsgruppe hatte signifikant erhöhte Kynurenin- und Kynurenin / Tryptophan-Verhältnisse. Die IL-6-Spiegel nahmen in den ersten drei Monaten sowohl für die Interventionsgruppen als auch für die Kontrollgruppe ab (betreutes Training: $p < 0{,}01$, zu Heimtraining: $p < 0{,}010$, Kontrollgruppe: $p < 0{,}01$). Die Ergebnisse legen nahe, dass betreutes Krafttraining den Kynurenin-Weg positiv regulieren und die Kynurenin / Tryptophan-Spiegel (ein Hinweis auf das IDO / TDO-Enzym) herunterregulieren könnte, was potentiell positive Effekte auf das Immunsystem hat.

Um die dritte Forschungsfrage zu beantworten wurde im Rahmen einer dritten Studie, der Einfluss verschiedener Ausdauertrainingsarten auf akute und dauerhafte Veränderungen der NK-Zellrezeptorexpression und die AhR / IDO Achse untersucht. Dazu wurde eine NK-Zelllinie im autologen Serum der trainierten Patienten inkubiert und die NK-Zellrezeptoren, die AhR- und IDO-Expression mittels Durchflusszytometrie analysiert. Für akute Effekte wurden Vor-Spiroergometrie-, Nach-Spiroergometrie- und 1-Stunden-Nach-Spiroergometrie-Daten erhoben. Für chronische Effekte bzw. Trainingsanpassungen wurden die Proband*innen in 1) polarisierte oder 2) Standard-Ausdauertrainingsgruppen randomisiert. Anschließend wurden Veränderungen der o.g. Parameter von vor und nach der 12-wöchigen Intervention verglichen. Nach einer akuten maximalen Belastung (Spiroergometrie) verringerten sich die AhR-Expression (nicht signifikant) und IDO-Werte (signifikant) und erreichten eine Stunde nach Belastung wieder die Ausgangswerte ($p = 0{,}13$ bzw. $p = 0{,}02$). Die KIR2DL1-Spiegel nahmen zu beiden Zeitpunkten signifikant ab ($p < 0{,}01$). Die NKG2D-Spiegel blieben unverändert ($p = 0{,}31$). Bei chronischem Ausdauertraining wurde eine Abnahme der Expression von vor der Spiroergometrie im Vergleich zu 12 Wochen danach für IDO in der Polarisierten Gruppe und eine Zunahme in der Standardgruppe (beide $p < 0{,}01$) beobachtet. Die NKG2D-Spiegel nahmen in der Standard und den Ausdauergruppen signifikant ab ($p < 0{,}01$). AhR- und KIR2DL1-Spiegel zeigten keine signifikante Änderung ($p = 0{,}27$; $p = 0{,}92$). Die

Ergebnisse zeigen, dass sowohl akutes als auch chronisches Ausdauertraining die AhR / IDO-Achse positiv regulieren und folglich die Expression des aktivierenden NK-Zellrezeptors verändern können. Wir schließen daraus, dass die Regulation von NK-Zellen v.a. auf Akuteffekten beruht. Darüber hinaus kann chronisches Krafttraining den Kynurenin-Signalweg modulieren. Unabhängig von akuten oder chronischen Faktoren können verschiedene Arten von Ausdauertrainings die NK-Zellrezeptoren über die AhR / IDO-Achse beeinflussen.

Die Mechanismen hinter der durch körperliche Betätigung induzierten Immunregulation sind komplex und weitere Forschung in dieser Richtung ist erforderlich. Dies war ein erster Versuch zu verstehen, wie die körpereigenen Ressourcen zur Bekämpfung von Krebs und zur Modulation des Immunsystems genutzt werden können. Wir haben dieses Ziel noch nicht erreicht, sondern nur die ersten Schritte unternommen.

6. REFERENCES

Abel, A. M., Yang, C., Thakar, M. S. and Malarkannan, S. (2018). **Natural Killer Cells: Development, Maturation, and Clinical Utilization**. Front Immunol *9,* 1869, doi: 10.3389/fimmu.2018.01869.

Abel, J. L. and Rissman, E. F. (2013). **Running-induced epigenetic and gene expression changes in the adolescent brain**. Int J Dev Neurosci *31 (6),* 382-390, doi: 10.1016/j.ijdevneu.2012.11.002.

Agudelo, L. Z., Femenia, T., Orhan, F., Porsmyr-Palmertz, M., Goiny, M., Martinez-Redondo, V., Correia, J. C., Izadi, M., Bhat, M., Schuppe-Koistinen, I., Pettersson, A. T., Ferreira, D. M. S., Krook, A., Barres, R., Zierath, J. R., Erhardt, S., Lindskog, M. and Ruas, J. L. (2014). **Skeletal muscle PGC-1alpha1 modulates kynurenine metabolism and mediates resilience to stress-induced depression**. Cell *159 (1),* 33-45, doi: 10.1016/j.cell.2014.07.051.

Alexander, M. R. and Owens, G. K. (2012). **Epigenetic control of smooth muscle cell differentiation and phenotypic switching in vascular development and disease**. Annu Rev Physiol *74,* 13-40, doi: 10.1146/annurev-physiol-012110-142315.

Areces, F., Gonzalez-Millan, C., Salinero, J. J., Abian-Vicen, J., Lara, B., Gallo-Salazar, C., Ruiz-Vicente, D. and Del Coso, J. (2015). **Changes in Serum Free Amino Acids and Muscle Fatigue Experienced during a Half-Ironman Triathlon**. PLoS One *10 (9),* e0138376, doi: 10.1371/journal.pone.0138376.

Badawy, A. A. (2017). **Kynurenine Pathway of Tryptophan Metabolism: Regulatory and Functional Aspects**. Int J Tryptophan Res *10,* 1178646917691938, doi: 10.1177/1178646917691938.

Baj, Z., Kantorski, J., Majewska, E., Zeman, K., Pokoca, L., Fornalczyk, E., Tchorzewski, H., Sulowska, Z. and Lewicki, R. (1994). **Immunological status of competitive cyclists before and after the training season**. Int J Sports Med *15 (6),* 319-324, doi: 10.1055/s-2007-1021067.

Ball, H. J., Jusof, F. F., Bakmiwewa, S. M., Hunt, N. H. and Yuasa, H. J. (2014). **Tryptophan-catabolizing enzymes - party of three**. Front Immunol *5,* 485, doi: 10.3389/fimmu.2014.00485.

Ballard-Barbash, R., Friedenreich, C. M., Courneya, K. S., Siddiqi, S. M., McTiernan, A. and Alfano, C. M. (2012). **Physical activity, biomarkers, and disease outcomes in cancer survivors: a systematic review**. J Natl Cancer Inst *104 (11),* 815-840, doi: 10.1093/jnci/djs207.

Bansi, J., Koliamitra, C., Bloch, W., Joisten, N., Schenk, A., Watson, M., Kool, J., Langdon, D., Dalgas, U., Kesselring, J. and Zimmer, P. (2018). **Persons with secondary progressive and relapsing remitting multiple sclerosis reveal different responses of tryptophan metabolism to acute endurance exercise and training**. J Neuroimmunol *314*, 101-105, doi: 10.1016/j.jneuroim.2017.12.001.

Bassani, B., Baci, D., Gallazzi, M., Poggi, A., Bruno, A. and Mortara, L. (2019). **Natural Killer Cells as Key Players of Tumor Progression and Angiogenesis: Old and Novel Tools to Divert Their Pro-Tumor Activities into Potent Anti-Tumor Effects**. Cancers (Basel) *11 (4)*, doi: 10.3390/cancers11040461.

Basso, J. C. and Suzuki, W. A. (2017). **The Effects of Acute Exercise on Mood, Cognition, Neurophysiology, and Neurochemical Pathways: A Review**. Brain Plast *2 (2)*, 127-152, doi: 10.3233/BPL-160040.

Benschop, R. J., Nijkamp, F. P., Ballieux, R. E. and Heijnen, C. J. (1994). **The effects of beta-adrenoceptor stimulation on adhesion of human natural killer cells to cultured endothelium**. Br J Pharmacol *113 (4)*, 1311-1316, doi: 10.1111/j.1476-5381.1994.tb17141.x.

Betof, A. S., Lascola, C. D., Weitzel, D., Landon, C., Scarbrough, P. M., Devi, G. R., Palmer, G., Jones, L. W. and Dewhirst, M. W. (2015). **Modulation of murine breast tumor vascularity, hypoxia and chemotherapeutic response by exercise**. J Natl Cancer Inst *107 (5)*, doi: 10.1093/jnci/djv040.

Bigley, A. B., Rezvani, K., Chew, C., Sekine, T., Pistillo, M., Crucian, B., Bollard, C. M. and Simpson, R. J. (2014). **Acute exercise preferentially redeploys NK-cells with a highly-differentiated phenotype and augments cytotoxicity against lymphoma and multiple myeloma target cells**. Brain Behav Immun *39*, 160-171, doi: 10.1016/j.bbi.2013.10.030.

Bigley, A. B., Rezvani, K., Pistillo, M., Reed, J., Agha, N., Kunz, H., O'Connor, D. P., Sekine, T., Bollard, C. M. and Simpson, R. J. (2015). **Acute exercise preferentially redeploys NK-cells with a highly-differentiated phenotype and augments cytotoxicity against lymphoma and multiple myeloma target cells. Part II: impact of latent cytomegalovirus infection and catecholamine sensitivity**. Brain Behav Immun *49*, 59-65, doi: 10.1016/j.bbi.2014.12.027.

Bodai, B. I. and Tuso, P. (2015). **Breast cancer survivorship: a comprehensive review of long-term medical issues and lifestyle recommendations**. Perm J *19 (2)*, 48-79, doi: 10.7812/TPP/14-241.

Booth, S., Florida-James, G. D., McFarlin, B. K., Spielmann, G., O'Connor, D. P. and Simpson, R. J. (2010). **The impact of acute strenuous exercise on TLR2, TLR4 and HLA.DR expression on human blood monocytes induced by autologous serum**. Eur J Appl Physiol *110 (6)*, 1259-1268, doi: 10.1007/s00421-010-1616-2.

Brown, J. C., Winters-Stone, K., Lee, A. and Schmitz, K. H. (2012). **Cancer, physical activity, and exercise**. Compr Physiol *2 (4)*, 2775-2809, doi: 10.1002/cphy.c120005.

Campbell, J. P., Riddell, N. E., Burns, V. E., Turner, M., van Zanten, J. J., Drayson, M. T. and Bosch, J. A. (2009). **Acute exercise mobilises CD8+ T lymphocytes exhibiting an effector-memory phenotype**. Brain Behav Immun *23 (6)*, 767-775, doi: 10.1016/j.bbi.2009.02.011.

Campbell, J. P. and Turner, J. E. (2018). **Debunking the Myth of Exercise-Induced Immune Suppression: Redefining the Impact of Exercise on Immunological Health Across the Lifespan**. Front Immunol *9*, 648, doi: 10.3389/fimmu.2018.00648.

Campbell, K. L., Winters-Stone, K. M., Wiskemann, J., May, A. M., Schwartz, A. L., Courneya, K. S., Zucker, D. S., Matthews, C. E., Ligibel, J. A., Gerber, L. H., Morris, G. S., Patel, A. V., Hue, T. F., Perna, F. M. and Schmitz, K. H. (2019). **Exercise Guidelines for Cancer Survivors: Consensus Statement from International Multidisciplinary Roundtable**. Med Sci Sports Exerc *51 (11)*, 2375-2390, doi: 10.1249/MSS.0000000000002116.

Campbell, K. S. and Purdy, A. K. (2011). **Structure/function of human killer cell immunoglobulin-like receptors: lessons from polymorphisms, evolution, crystal structures and mutations**. Immunology *132 (3)*, 315-325, doi: 10.1111/j.1365-2567.2010.03398.x.

Campbell, P. T., Wener, M. H., Sorensen, B., Wood, B., Chen-Levy, Z., Potter, J. D., McTiernan, A. and Ulrich, C. M. (2008). **Effect of exercise on in vitro immune function: a 12-month randomized, controlled trial among postmenopausal women**. J Appl Physiol (1985) *104 (6)*, 1648-1655, doi: 10.1152/japplphysiol.01349.2007.

Cao Dinh, H., Beyer, I., Mets, T., Onyema, O. O., Njemini, R., Renmans, W., De Waele, M., Jochmans, K., Vander Meeren, S. and Bautmans, I. (2017). **Effects of Physical Exercise on Markers of Cellular Immunosenescence: A Systematic Review**. Calcif Tissue Int *100 (2)*, 193-215, doi: 10.1007/s00223-016-0212-9.

Catena-Dell'Osso, M., Bellantuono, C., Consoli, G., Baroni, S., Rotella, F. and Marazziti, D. (2011). **Inflammatory and neurodegenerative pathways in depression: a new avenue for antidepressant development?** Curr Med Chem *18 (2)*, 245-255, doi: 10.2174/092986711794088353.

Cervenka, I., Agudelo, L. Z. and Ruas, J. L. (2017). **Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health**. Science *357 (6349)*, doi: 10.1126/science.aaf9794.

Chan, C. J., Smyth, M. J. and Martinet, L. (2014). **Molecular mechanisms of natural killer cell activation in response to cellular stress**. Cell Death Differ *21 (1)*, 5-14, doi: 10.1038/cdd.2013.26.

Cichocki, F., Grzywacz, B. and Miller, J. S. (2019). **Human NK Cell Development: One Road or Many?** Front Immunol *10*, 2078, doi: 10.3389/fimmu.2019.02078.

Clauss, D., Tjaden, C., Hackert, T., Schneider, L., Ulrich, C. M., Wiskemann, J. and Steindorf, K. (2017). **Cardiorespiratory fitness and muscle strength in pancreatic cancer patients**. Support Care Cancer *25 (9)*, 2797-2807, doi: 10.1007/s00520-017-3694-8.

Connolly, P. H., Caiozzo, V. J., Zaldivar, F., Nemet, D., Larson, J., Hung, S. P., Heck, J. D., Hatfield, G. W. and Cooper, D. M. (2004). **Effects of exercise on gene expression in human peripheral blood mononuclear cells**. J Appl Physiol (1985) *97 (4)*, 1461-1469, doi: 10.1152/japplphysiol.00316.2004.

Cooper, M. A., Fehniger, T. A. and Caligiuri, M. A. (2001). **The biology of human natural killer-cell subsets**. Trends Immunol *22 (11)*, 633-640, doi: 10.1016/s1471-4906(01)02060-9.

Cormie, P., Zopf, E. M., Zhang, X. and Schmitz, K. H. (2017). **The Impact of Exercise on Cancer Mortality, Recurrence, and Treatment-Related Adverse Effects**. Epidemiol Rev *39 (1)*, 71-92, doi: 10.1093/epirev/mxx007.

Courneya, K. S., Friedenreich, C. M., Quinney, H. A., Fields, A. L., Jones, L. W. and Fairey, A. S. (2003). **A randomized trial of exercise and quality of life in colorectal cancer survivors**. Eur J Cancer Care (Engl) *12 (4)*, 347-357, doi: 10.1046/j.1365-2354.2003.00437.x.

Curzon, G., Friedel, J. and Knott, P. J. (1973). **The effect of fatty acids on the binding of tryptophan to plasma protein**. Nature *242 (5394)*, 198-200, doi: 10.1038/242198a0.

Da Pozzo, E., Giacomelli, C., Cavallini, C. and Martini, C. (2018). **Cytokine secretion responsiveness of lymphomonocytes following cortisol cell exposure: Sex differences**. PLoS One *13 (7)*, e0200924, doi: 10.1371/journal.pone.0200924.

Deac, O. M., Mills, J. L., Shane, B., Midttun, O., Ueland, P. M., Brosnan, J. T., Brosnan, M. E., Laird, E., Gibney, E. R., Fan, R., Wang, Y., Brody, L. C. and Molloy, A. M. (2015). **Tryptophan catabolism and vitamin B-6 status are affected by gender and lifestyle factors in healthy young adults**. J Nutr *145 (4)*, 701-707, doi: 10.3945/jn.114.203091.

Della Chiesa, M., Carlomagno, S., Frumento, G., Balsamo, M., Cantoni, C., Conte, R., Moretta, L., Moretta, A. and Vitale, M. (2006). **The tryptophan catabolite L-kynurenine inhibits the surface expression of NKp46- and NKG2D-activating receptors and regulates NK-cell function**. Blood *108 (13)*, 4118-4125, doi: 10.1182/blood-2006-03-006700.

Dethlefsen, C., Lillelund, C., Midtgaard, J., Andersen, C., Pedersen, B. K., Christensen, J. F. and Hojman, P. (2016). **Exercise regulates breast cancer cell viability: systemic training adaptations versus acute exercise responses**. Breast Cancer Res Treat *159 (3)*, 469-479, doi: 10.1007/s10549-016-3970-1.

Dethlefsen, C., Pedersen, K. S. and Hojman, P. (2017). **Every exercise bout matters: linking systemic exercise responses to breast cancer control**. Breast Cancer Res Treat *162 (3)*, 399-408, doi: 10.1007/s10549-017-4129-4.

Dhabhar, F. S. (2014). **Effects of stress on immune function: the good, the bad, and the beautiful**. Immunol Res *58 (2-3)*, 193-210, doi: 10.1007/s12026-014-8517-0.

Dias, R. G., Silva, M. S., Duarte, N. E., Bolani, W., Alves, C. R., Junior, J. R., da Silva, J. L., de Oliveira, P. A., Alves, G. B., de Oliveira, E. M., Rocha, C. S., Marsiglia, J. D., Negrao, C. E., Krieger, E. M., Krieger, J. E. and Pereira, A. C. (2015). **PBMCs express a transcriptome signature predictor of oxygen uptake responsiveness to endurance exercise training in men**. Physiol Genomics *47 (2)*, 13-23, doi: 10.1152/physiolgenomics.00072.2014.

Diment, B. C., Fortes, M. B., Edwards, J. P., Hanstock, H. G., Ward, M. D., Dunstall, H. M., Friedmann, P. S. and Walsh, N. P. (2015). **Exercise Intensity and Duration Effects on In Vivo Immunity**. Med Sci Sports Exerc *47 (7)*, 1390-1398, doi: 10.1249/MSS.0000000000000562.

Dimitrov, S., Benedict, C., Heutling, D., Westermann, J., Born, J. and Lange, T. (2009). **Cortisol and epinephrine control opposing circadian rhythms in T cell subsets**. Blood *113 (21)*, 5134-5143, doi: 10.1182/blood-2008-11-190769.

DiNatale, B. C., Murray, I. A., Schroeder, J. C., Flaveny, C. A., Lahoti, T. S., Laurenzana, E. M., Omiecinski, C. J. and Perdew, G. H. (2010). **Kynurenic acid is a potent endogenous aryl hydrocarbon receptor ligand that synergistically induces interleukin-6 in the presence of inflammatory signaling**. Toxicol Sci *115 (1)*, 89-97, doi: 10.1093/toxsci/kfq024.

Dudley, D. J. (1992). **The immune system in health and disease**. Baillieres Clin Obstet Gynaecol *6 (3)*, 393-416, doi: 10.1016/s0950-3552(05)80003-3.

Dybkaer, K., Iqbal, J., Zhou, G., Geng, H., Xiao, L., Schmitz, A., d'Amore, F. and Chan, W. C. (2007). **Genome wide transcriptional analysis of resting and IL2 activated human natural killer cells: gene expression signatures indicative of novel molecular signaling pathways**. BMC Genomics *8*, 230, doi: 10.1186/1471-2164-8-230.

Erhard, M. H., Ozpinar, H., Bilal, T., Abas, I., Kutay, C., Eseceli, H. and Stangassinger, M. (2000). **The humoral immune response and the productivity of laying hens kept on the ground or in cages**. Altern Lab Anim *28 (5)*, 699-705, doi: 10.1177/026119290002800504.

Fairey, A. S., Courneya, K. S., Field, C. J., Bell, G. J., Jones, L. W. and Mackey, J. R. (2005). **Randomized controlled trial of exercise and blood immune function in postmenopausal breast cancer survivors**. J Appl Physiol (1985) *98 (4)*, 1534-1540, doi: 10.1152/japplphysiol.00566.2004.

Fernandez-Sanchez, A., Baragano Raneros, A., Carvajal Palao, R., Sanz, A. B., Ortiz, A., Ortega, F., Suarez-Alvarez, B. and Lopez-Larrea, C. (2013). **DNA demethylation and histone H3K9 acetylation determine the active transcription of the NKG2D gene in human CD8+ T and NK cells**. Epigenetics *8 (1)*, 66-78, doi: 10.4161/epi.23115.

Flynn, M. G., McFarlin, B. K. and Markofski, M. M. (2007). **The Anti-Inflammatory Actions of Exercise Training**. Am J Lifestyle Med *1 (3)*, 220-235, doi: 10.1177/1559827607300283.

Freud, A. G. and Caligiuri, M. A. (2006). **Human natural killer cell development**. Immunol Rev *214*, 56-72, doi: 10.1111/j.1600-065X.2006.00451.x.

Friedenreich, C. M., O'Reilly, R., Shaw, E., Stanczyk, F. Z., Yasui, Y., Brenner, D. R. and Courneya, K. S. (2016). **Inflammatory Marker Changes in Postmenopausal Women after a Year-long Exercise Intervention Comparing High Versus Moderate Volumes**. Cancer Prev Res (Phila) *9 (2)*, 196-203, doi: 10.1158/1940-6207.CAPR-15-0284.

Fujigaki, S., Saito, K., Sekikawa, K., Tone, S., Takikawa, O., Fujii, H., Wada, H., Noma, A. and Seishima, M. (2001). **Lipopolysaccharide induction of indoleamine 2,3-dioxygenase is mediated dominantly by an IFN-gamma-independent mechanism**. Eur J Immunol *31 (8)*, 2313-2318, doi: 10.1002/1521-4141(200108)31:8<2313::aid-immu2313>3.0.co;2-s.

Gangula, N. R. and Maddika, S. (2013). **WD repeat protein WDR48 in complex with deubiquitinase USP12 suppresses Akt-dependent cell survival signaling by stabilizing PH domain leucine-rich repeat protein phosphatase 1 (PHLPP1)**. J Biol Chem *288 (48)*, 34545-34554, doi: 10.1074/jbc.M113.503383.

Genevini, P., Colombo, M. N., Venditti, R., Marcuzzo, S., Colombo, S. F., Bernasconi, P., De Matteis, M. A., Borgese, N. and Navone, F. (2019). **Correction: VAPB depletion alters neuritogenesis and phosphoinositide balance in motoneuron-like cells: relevance to VAPB-linked amyotrophic lateral sclerosis (doi:10.1242/jcs.220061)**. J Cell Sci *132 (12)*, doi: 10.1242/jcs.235176.

Gleeson, M., McDonald, W. A., Pyne, D. B., Clancy, R. L., Cripps, A. W., Francis, J. L. and Fricker, P. A. (2000). **Immune status and respiratory illness for elite swimmers during a 12-week training cycle**. Int J Sports Med *21 (4)*, 302-307, doi: 10.1055/s-2000-313.

Gleeson, M., McFarlin, B. and Flynn, M. (2006). **Exercise and Toll-like receptors**. Exerc Immunol Rev *12*, 34-53.

Goh, J., Goh, K. P. and Abbasi, A. (2016). **Exercise and Adipose Tissue Macrophages: New Frontiers in Obesity Research?** Front Endocrinol (Lausanne) *7*, 65, doi: 10.3389/fendo.2016.00065.

Groer, M. (1995). **Exercise and immunity**. Image J Nurs Sch *27 (2)*, 90, doi: 10.1111/j.1547-5069.1995.tb00826.x.

Guleria, I. and Sayegh, M. H. (2007). **Maternal acceptance of the fetus: true human tolerance**. J Immunol *178 (6)*, 3345-3351, doi: 10.4049/jimmunol.178.6.3345.

Gunnarsson, T. P., Christensen, P. M., Thomassen, M., Nielsen, L. R. and Bangsbo, J. (2013). **Effect of intensified training on muscle ion kinetics, fatigue development, and repeated short-term performance in endurance-trained cyclists**. Am J Physiol Regul Integr Comp Physiol *305 (7)*, R811-821, doi: 10.1152/ajpregu.00467.2012.

Gunzer, W., Konrad, M. and Pail, E. (2012). **Exercise-induced immunodepression in endurance athletes and nutritional intervention with carbohydrate, protein and fat-what is possible, what is not?** Nutrients *4 (9)*, 1187-1212, doi: 10.3390/nu4091187.

Gutierrez-Vazquez, C. and Quintana, F. J. (2018). **Regulation of the Immune Response by the Aryl Hydrocarbon Receptor**. Immunity *48 (1)*, 19-33, doi: 10.1016/j.immuni.2017.12.012.

Hanna, J., Bechtel, P., Zhai, Y., Youssef, F., McLachlan, K. and Mandelboim, O. (2004). **Novel insights on human NK cells' immunological modalities revealed by gene expression profiling**. J Immunol *173 (11)*, 6547-6563, doi: 10.4049/jimmunol.173.11.6547.

Hansen, J. B., Wilsgard, L. and Osterud, B. (1991). **Biphasic changes in leukocytes induced by strenuous exercise**. Eur J Appl Physiol Occup Physiol *62 (3)*, 157-161, doi: 10.1007/BF00643735.

Hardee, J. P., Counts, B. R. and Carson, J. A. (2019). **Understanding the Role of Exercise in Cancer Cachexia Therapy**. Am J Lifestyle Med *13 (1)*, 46-60, doi: 10.1177/1559827617725283.

Hascitha, J., Priya, R., Jayavelu, S., Dhandapani, H., Selvaluxmy, G., Sunder Singh, S. and Rajkumar, T. (2016). **Analysis of Kynurenine/Tryptophan ratio and expression of IDO1 and 2 mRNA in tumour tissue of cervical cancer patients**. Clin Biochem *49 (12)*, 919-924, doi: 10.1016/j.clinbiochem.2016.04.008.

Hayes, L. D., Sculthorpe, N., Herbert, P., Baker, J. S., Hullin, D. A., Kilduff, L. P. and Grace, F. M. (2015). **Resting steroid hormone concentrations in lifetime exercisers and lifetime sedentary males**. Aging Male *18 (1)*, 22-26, doi: 10.3109/13685538.2014.977246.

Heng, B., Lim, C. K., Lovejoy, D. B., Bessede, A., Gluch, L. and Guillemin, G. J. (2016). **Understanding the role of the kynurenine pathway in human breast cancer immunobiology**. Oncotarget *7 (6)*, 6506-6520, doi: 10.18632/oncotarget.6467.

Hennings, A., Schwarz, M. J., Riemer, S., Stapf, T. M., Selberdinger, V. B. and Rief, W. (2013). **Exercise affects symptom severity but not biological measures in depression and somatization - results on IL-6, neopterin, tryptophan, kynurenine and 5-HIAA**. Psychiatry Res *210 (3)*, 925-933, doi: 10.1016/j.psychres.2013.09.018.

Herrstedt, A., Bay, M. L., Simonsen, C., Sundberg, A., Egeland, C., Thorsen-Streit, S., Djurhuus, S. S., Magne Ueland, P., Midttun, O., Pedersen, B. K., Bo Svendsen, L., de Heer, P., Christensen, J. F. and Hojman, P. (2019). **Exercise-mediated improvement of depression in patients with gastro-esophageal junction cancer is linked to kynurenine metabolism**. Acta Oncol *58 (5)*, 579-587, doi: 10.1080/0284186X.2018.1558371.

Hilmas, C., Pereira, E. F., Alkondon, M., Rassoulpour, A., Schwarcz, R. and Albuquerque, E. X. (2001). **The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: physiopathological implications**. J Neurosci *21 (19)*, 7463-7473.

Hojman, P. (2017). **Exercise protects from cancer through regulation of immune function and inflammation**. Biochem Soc Trans *45 (4)*, 905-911, doi: 10.1042/BST20160466.

Hollingshead, B. D., Beischlag, T. V., Dinatale, B. C., Ramadoss, P. and Perdew, G. H. (2008). **Inflammatory signaling and aryl hydrocarbon receptor mediate synergistic induction of interleukin 6 in MCF-7 cells**. Cancer Res *68 (10)*, 3609-3617, doi: 10.1158/0008-5472.CAN-07-6168.

Huff, W. X., Kwon, J. H., Henriquez, M., Fetcko, K. and Dey, M. (2019). **The Evolving Role of CD8(+)CD28(-) Immunosenescent T Cells in Cancer Immunology**. Int J Mol Sci *20 (11)*, doi: 10.3390/ijms20112810.

Hupkes, M., Jonsson, M. K., Scheenen, W. J., van Rotterdam, W., Sotoca, A. M., van Someren, E. P., van der Heyden, M. A., van Veen, T. A., van Ravestein-van Os, R. I., Bauerschmidt, S., Piek, E., Ypey, D. L., van Zoelen, E. J. and Dechering, K. J. (2011). **Epigenetics: DNA demethylation promotes skeletal myotube maturation**. FASEB J *25 (11)*, 3861-3872, doi: 10.1096/fj.11-186122.

Idorn, M. and Hojman, P. (2016). **Exercise-Dependent Regulation of NK Cells in Cancer Protection**. Trends Mol Med *22 (7)*, 565-577, doi: 10.1016/j.molmed.2016.05.007.

Janeway, C. A., Jr. (2001). **How the immune system protects the host from infection**. Microbes Infect *3 (13)*, 1167-1171, doi: 10.1016/s1286-4579(01)01477-0.

Joisten, N., Kummerhoff, F., Koliamitra, C., Schenk, A., Walzik, D., Hardt, L., Knoop, A., Thevis, M., Kiesl, D., Metcalfe, A. J., Bloch, W. and Zimmer, P. (2020). **Exercise and the Kynurenine pathway: Current state of knowledge and results from a randomized cross-over study comparing acute effects of endurance and resistance training**. Exerc Immunol Rev *26*, 24-42.

Jones, S. P., Franco, N. F., Varney, B., Sundaram, G., Brown, D. A., de Bie, J., Lim, C. K., Guillemin, G. J. and Brew, B. J. (2015). **Expression of the Kynurenine Pathway in Human Peripheral Blood Mononuclear Cells: Implications for Inflammatory and Neurodegenerative Disease**. PLoS One *10 (6)*, e0131389, doi: 10.1371/journal.pone.0131389.

Kappel, M., Poulsen, T. D., Galbo, H. and Pedersen, B. K. (1998). **Effects of elevated plasma noradrenaline concentration on the immune system in humans**. Eur J Appl Physiol Occup Physiol *79 (1)*, 93-98, doi: 10.1007/s004210050479.

Kappel, M., Tvede, N., Galbo, H., Haahr, P. M., Kjaer, M., Linstow, M., Klarlund, K. and Pedersen, B. K. (1991). **Evidence that the effect of physical exercise on NK cell activity is mediated by epinephrine**. J Appl Physiol (1985) *70 (6)*, 2530-2534, doi: 10.1152/jappl.1991.70.6.2530.

Keast, D., Cameron, K. and Morton, A. R. (1988). **Exercise and the immune response**. Sports Med *5 (4)*, 248-267, doi: 10.2165/00007256-198805040-00004.

Kelly, K. R., Navaneethan, S. D., Solomon, T. P., Haus, J. M., Cook, M., Barkoukis, H. and Kirwan, J. P. (2014). **Lifestyle-induced decrease in fat mass improves adiponectin secretion in obese adults**. Med Sci Sports Exerc *46 (5)*, 920-926, doi: 10.1249/MSS.0000000000000200.

Kerkvliet, N. I. (2012). **TCDD: an environmental immunotoxicant reveals a novel pathway of immunoregulation--a 30-year odyssey**. Toxicol Pathol *40 (2)*, 138-142, doi: 10.1177/0192623311427710.

Kim, H. R., Lee, A., Choi, E. J., Hong, M. P., Kie, J. H., Lim, W., Lee, H. K., Moon, B. I. and Seoh, J. Y. (2014). **Reactive oxygen species prevent imiquimod-induced psoriatic dermatitis through enhancing regulatory T cell function**. PLoS One *9 (3)*, e91146, doi: 10.1371/journal.pone.0091146.

Kim, R., Emi, M. and Tanabe, K. (2007). **Cancer immunoediting from immune surveillance to immune escape**. Immunology *121 (1)*, 1-14, doi: 10.1111/j.1365-2567.2007.02587.x.

Kruger, K., Alack, K., Ringseis, R., Mink, L., Pfeifer, E., Schinle, M., Gindler, K., Kimmelmann, L., Walscheid, R., Muders, K., Frech, T., Eder, K. and Mooren, F. C. (2016). **Apoptosis of T-Cell Subsets after Acute High-Intensity Interval Exercise**. Med Sci Sports Exerc *48 (10)*, 2021-2029, doi: 10.1249/MSS.0000000000000979.

Kruger, K. and Mooren, F. C. (2007). **T cell homing and exercise**. Exerc Immunol Rev *13*, 37-54.

Kumar, S. (2018). **Natural killer cell cytotoxicity and its regulation by inhibitory receptors**. Immunology *154 (3)*, 383-393, doi: 10.1111/imm.12921.

Kuster, O. C., Laptinskaya, D., Fissler, P., Schnack, C., Zugel, M., Nold, V., Thurm, F., Pleiner, S., Karabatsiakis, A., von Einem, B., Weydt, P., Liesener, A., Borta, A., Woll, A., Hengerer, B., Kolassa, I. T. and von Arnim, C. A. F. (2017). **Novel Blood-Based Biomarkers of Cognition, Stress, and Physical or Cognitive Training in Older Adults at Risk of Dementia: Preliminary Evidence for a Role of BDNF, Irisin, and the Kynurenine Pathway**. J Alzheimers Dis *59 (3)*, 1097-1111, doi: 10.3233/JAD-170447.

Lancaster, T. (2006). **Review: Secondary prevention programmes with and without exercise reduce all cause mortality and recurrent MI**. Evid Based Med *11 (3)*, 87, doi: 10.1136/ebm.11.3.87.

Laperrousaz, S., Tiercy, S., Villard, J. and Ferrari-Lacraz, S. (2012). **HLA and non-HLA polymorphisms in renal transplantation**. Swiss Med Wkly *142*, w13668, doi: 10.4414/smw.2012.13668.

Leal, L. G., Lopes, M. A. and Batista, M. L., Jr. (2018). **Physical Exercise-Induced Myokines and Muscle-Adipose Tissue Crosstalk: A Review of Current Knowledge and the Implications for Health and Metabolic Diseases**. Front Physiol *9,* 1307, doi: 10.3389/fphys.2018.01307.

Lemos, H., Huang, L., Prendergast, G. C. and Mellor, A. L. (2019). **Immune control by amino acid catabolism during tumorigenesis and therapy**. Nat Rev Cancer *19 (3)*, 162-175, doi: 10.1038/s41568-019-0106-z.

Lewis, G. D., Farrell, L., Wood, M. J., Martinovic, M., Arany, Z., Rowe, G. C., Souza, A., Cheng, S., McCabe, E. L., Yang, E., Shi, X., Deo, R., Roth, F. P., Asnani, A., Rhee, E. P., Systrom, D. M., Semigran, M. J., Vasan, R. S., Carr, S. A., Wang, T. J., Sabatine, M. S., Clish, C. B. and Gerszten, R. E. (2010). **Metabolic signatures of exercise in human plasma**. Sci Transl Med *2 (33)*, 33ra37, doi: 10.1126/scitranslmed.3001006.

Liu, D., Wang, R., Grant, A. R., Zhang, J., Gordon, P. M., Wei, Y. and Chen, P. (2017). **Immune adaptation to chronic intense exercise training: new microarray evidence**. BMC Genomics *18 (1)*, 29, doi: 10.1186/s12864-016-3388-5.

Lovelace, M. D., Varney, B., Sundaram, G., Lennon, M. J., Lim, C. K., Jacobs, K., Guillemin, G. J. and Brew, B. J. (2017). **Recent evidence for an expanded role of the kynurenine pathway of tryptophan metabolism in neurological diseases**. Neuropharmacology *112 (Pt B)*, 373-388, doi: 10.1016/j.neuropharm.2016.03.024.

Mars, M., Govender, S., Weston, A., Naicker, V. and Chuturgoon, A. (1998). **High intensity exercise: a cause of lymphocyte apoptosis?** Biochem Biophys Res Commun *249 (2)*, 366-370, doi: 10.1006/bbrc.1998.9156.

Melaiu, O., Lucarini, V., Cifaldi, L. and Fruci, D. (2019). **Influence of the Tumor Microenvironment on NK Cell Function in Solid Tumors**. Front Immunol *10*, 3038, doi: 10.3389/fimmu.2019.03038.

Melancon, M. O., Lorrain, D. and Dionne, I. J. (2014). **Changes in markers of brain serotonin activity in response to chronic exercise in senior men**. Appl Physiol Nutr Metab *39 (11)*, 1250-1256, doi: 10.1139/apnm-2014-0092.

Mero, A. (1999). **Leucine supplementation and intensive training**. Sports Med *27 (6)*, 347-358, doi: 10.2165/00007256-199927060-00001.

Meyerhardt, J. A., Giovannucci, E. L., Holmes, M. D., Chan, A. T., Chan, J. A., Colditz, G. A. and Fuchs, C. S. (2006). **Physical activity and survival after colorectal cancer diagnosis**. J Clin Oncol *24 (22)*, 3527-3534, doi: 10.1200/JCO.2006.06.0855.

Millischer, V., Erhardt, S., Ekblom, O., Forsell, Y. and Lavebratt, C. (2017). **Twelve-week physical exercise does not have a long-lasting effect on kynurenines in plasma of depressed patients**. Neuropsychiatr Dis Treat *13*, 967-972, doi: 10.2147/NDT.S131746.

Moore, S. C., Lee, I. M., Weiderpass, E., Campbell, P. T., Sampson, J. N., Kitahara, C. M., Keadle, S. K., Arem, H., Berrington de Gonzalez, A., Hartge, P., Adami, H. O., Blair, C. K., Borch, K. B., Boyd, E., Check, D. P., Fournier, A., Freedman, N. D., Gunter, M., Johannson, M., Khaw, K. T., Linet, M. S., Orsini, N., Park, Y., Riboli, E., Robien, K., Schairer, C., Sesso, H., Spriggs, M., Van Dusen, R., Wolk, A., Matthews, C. E. and Patel, A. V. (2016). **Association of Leisure-Time Physical Activity With Risk of 26 Types of Cancer in 1.44 Million Adults**. JAMA Intern Med *176 (6)*, 816-825, doi: 10.1001/jamainternmed.2016.1548.

Mooren, F. C., Bloming, D., Lechtermann, A., Lerch, M. M. and Volker, K. (2002). **Lymphocyte apoptosis after exhaustive and moderate exercise**. J Appl Physiol (1985) *93 (1)*, 147-153, doi: 10.1152/japplphysiol.01262.2001.

Mooren, F. C. and Kruger, K. (2015). **Apoptotic lymphocytes induce progenitor cell mobilization after exercise**. J Appl Physiol (1985) *119 (2)*, 135-139, doi: 10.1152/japplphysiol.00287.2015.

Mooren, F. C., Lechtermann, A. and Volker, K. (2004). **Exercise-induced apoptosis of lymphocytes depends on training status**. Med Sci Sports Exerc *36 (9)*, 1476-1483, doi: 10.1249/01.mss.0000139897.34521.e9.

Moro-Garcia, M. A., Fernandez-Garcia, B., Echeverria, A., Rodriguez-Alonso, M., Suarez-Garcia, F. M., Solano-Jaurrieta, J. J., Lopez-Larrea, C. and Alonso-Arias, R. (2014). **Frequent participation in high volume exercise throughout life is associated with a more differentiated adaptive immune response**. Brain Behav Immun *39*, 61-74, doi: 10.1016/j.bbi.2013.12.014.

Mudry, J. M., Alm, P. S., Erhardt, S., Goiny, M., Fritz, T., Caidahl, K., Zierath, J. R., Krook, A. and Wallberg-Henriksson, H. (2016). **Direct effects of exercise on kynurenine metabolism in people with normal glucose tolerance or type 2 diabetes**. Diabetes Metab Res Rev *32 (7)*, 754-761, doi: 10.1002/dmrr.2798.

Munn, D. H. and Mellor, A. L. (2016). **IDO in the Tumor Microenvironment: Inflammation, Counter-Regulation, and Tolerance**. Trends Immunol *37 (3)*, 193-207, doi: 10.1016/j.it.2016.01.002.

Murray, I. A., Patterson, A. D. and Perdew, G. H. (2014). **Aryl hydrocarbon receptor ligands in cancer: friend and foe**. Nat Rev Cancer *14 (12)*, 801-814, doi: 10.1038/nrc3846.

Na, Y. M., Kim, M. Y., Kim, Y. K., Ha, Y. R. and Yoon, D. S. (2000). **Exercise therapy effect on natural killer cell cytotoxic activity in stomach cancer patients after curative surgery**. Arch Phys Med Rehabil *81 (6)*, 777-779, doi: 10.1016/s0003-9993(00)90110-2.

Nakajima, K., Takeoka, M., Mori, M., Hashimoto, S., Sakurai, A., Nose, H., Higuchi, K., Itano, N., Shiohara, M., Oh, T. and Taniguchi, S. (2010). **Exercise effects on methylation of ASC gene**. Int J Sports Med *31 (9)*, 671-675, doi: 10.1055/s-0029-1246140.

Nebert, D. W. (2017). **Aryl hydrocarbon receptor (AHR): "pioneer member" of the basic-helix/loop/helix per-Arnt-sim (bHLH/PAS) family of "sensors" of foreign and endogenous signals**. Prog Lipid Res *67*, 38-57, doi: 10.1016/j.plipres.2017.06.001.

Nguyen, N. T., Nakahama, T., Le, D. H., Van Son, L., Chu, H. H. and Kishimoto, T. (2014). **Aryl hydrocarbon receptor and kynurenine: recent advances in autoimmune disease research**. Front Immunol *5*, 551, doi: 10.3389/fimmu.2014.00551.

Nieman, D. C. (1997). **Immune response to heavy exertion**. J Appl Physiol (1985) *82 (5)*, 1385-1394, doi: 10.1152/jappl.1997.82.5.1385.

Nieman, D. C., Berk, L. S., Simpson-Westerberg, M., Arabatzis, K., Youngberg, S., Tan, S. A., Lee, J. W. and Eby, W. C. (1989). **Effects of long-endurance running on immune system parameters and lymphocyte function in experienced marathoners**. Int J Sports Med *10 (5)*, 317-323, doi: 10.1055/s-2007-1024921.

Nieman, D. C., Cook, V. D., Henson, D. A., Suttles, J., Rejeski, W. J., Ribisl, P. M., Fagoaga, O. R. and Nehlsen-Cannarella, S. L. (1995). **Moderate exercise training and natural killer cell cytotoxic activity in breast cancer patients**. Int J Sports Med *16 (5)*, 334-337, doi: 10.1055/s-2007-973015.

Nieman, D. C., Henson, D. A., Gusewitch, G., Warren, B. J., Dotson, R. C., Butterworth, D. E. and Nehlsen-Cannarella, S. L. (1993a). **Physical activity and immune function in elderly women**. Med Sci Sports Exerc *25 (7)*, 823-831, doi: 10.1249/00005768-199307000-00011.

Nieman, D. C., Miller, A. R., Henson, D. A., Warren, B. J., Gusewitch, G., Johnson, R. L., Davis, J. M., Butterworth, D. E. and Nehlsen-Cannarella, S. L. (1993b). **Effects of high- vs moderate-intensity exercise on natural killer cell activity**. Med Sci Sports Exerc *25 (10)*, 1126-1134.

Nieman, D. C., Nehlsen-Cannarella, S. L., Markoff, P. A., Balk-Lamberton, A. J., Yang, H., Chritton, D. B., Lee, J. W. and Arabatzis, K. (1990). **The effects of moderate exercise training on natural killer cells and acute upper respiratory tract infections**. Int J Sports Med *11 (6)*, 467-473, doi: 10.1055/s-2007-1024839.

Nieman, D. C. and Wentz, L. M. (2019). **The compelling link between physical activity and the body's defense system**. J Sport Health Sci *8 (3)*, 201-217, doi: 10.1016/j.jshs.2018.09.009.

Notarangelo, F. M. and Pocivavsek, A. (2017). **Elevated kynurenine pathway metabolism during neurodevelopment: Implications for brain and behavior**. Neuropharmacology *112 (Pt B)*, 275-285, doi: 10.1016/j.neuropharm.2016.03.001.

Novikov, O., Wang, Z., Stanford, E. A., Parks, A. J., Ramirez-Cardenas, A., Landesman, E., Laklouk, I., Sarita-Reyes, C., Gusenleitner, D., Li, A., Monti, S., Manteiga, S., Lee, K. and Sherr, D. H. (2016). **An Aryl Hydrocarbon Receptor-Mediated Amplification Loop That Enforces Cell Migration in ER-/PR-/Her2- Human Breast Cancer Cells**. Mol Pharmacol *90 (5)*, 674-688, doi: 10.1124/mol.116.105361.

O'Leary, C. B. and Hackney, A. C. (2014). **Acute and chronic effects of resistance exercise on the testosterone and cortisol responses in obese males: a systematic review**. Physiol Res *63 (6)*, 693-704.

Obata-Onai, A., Hashimoto, S., Onai, N., Kurachi, M., Nagai, S., Shizuno, K., Nagahata, T. and Matsushima, K. (2002). **Comprehensive gene expression analysis of human NK cells and CD8(+) T lymphocytes**. Int Immunol *14 (10)*, 1085-1098, doi: 10.1093/intimm/dxf086.

Pal, A., Schneider, J., Schlüter, K. *et al.* **Different endurance exercises modulate NK cell cytotoxic and inhibiting receptors**. Eur J Appl Physiol (2021). doi.org/10.1007/s00421-021-04735-z

Pal, A., Zimmer, P., Clauss, D., Schmidt, M. E., Ulrich, C. M., Wiskemann, J. and Steindorf, K. (2020). **Resistance Exercise Modulates Kynurenine Pathway in Pancreatic Cancer Patients**. Int J Sports Med, doi: 10.1055/a-1186-1009.

Pal, A., Zimmer, P., Schmidt, M. E., Hummel, M., Ulrich, C. M., Wiskemann, J. and Steindorf, K. (2019). **No Evidence for Effect of Exercise on Transcriptome of NK Cells in Breast Cancer Patients Undergoing Adjuvant Therapy: Results From a Pilot Study**. Front Physiol *10*, 959, doi: 10.3389/fphys.2019.00959.

Papacosta, E., Gleeson, M. and Nassis, G. P. (2013). **Salivary hormones, IgA, and performance during intense training and tapering in judo athletes**. J Strength Cond Res *27 (9)*, 2569-2580, doi: 10.1519/JSC.0b013e31827fd85c.

Pappas, B., Yang, Y., Wang, Y., Kim, K., Chung, H. J., Cheung, M., Ngo, K., Shinn, A. and Chan, W. K. (2018). **p23 protects the human aryl hydrocarbon receptor from degradation via a heat shock protein 90-independent mechanism**. Biochem Pharmacol *152*, 34-44, doi: 10.1016/j.bcp.2018.03.015.

Park, A., Yang, Y., Lee, Y., Kim, M. S., Park, Y. J., Jung, H., Kim, T. D., Lee, H. G., Choi, I. and Yoon, S. R. (2019). **Indoleamine-2,3-Dioxygenase in Thyroid Cancer Cells Suppresses Natural Killer Cell Function by Inhibiting NKG2D and NKp46 Expression via STAT Signaling Pathways**. J Clin Med *8 (6)*, doi: 10.3390/jcm8060842.

Patel, A. V., Friedenreich, C. M., Moore, S. C., Hayes, S. C., Silver, J. K., Campbell, K. L., Winters-Stone, K., Gerber, L. H., George, S. M., Fulton, J. E., Denlinger, C., Morris, G. S., Hue, T., Schmitz, K. H. and Matthews, C. E. (2019). **American College of Sports Medicine Roundtable Report on Physical Activity, Sedentary Behavior, and Cancer Prevention and Control**. Med Sci Sports Exerc *51 (11)*, 2391-2402, doi: 10.1249/MSS.0000000000002117.

Paul, S. and Lal, G. (2017). **The Molecular Mechanism of Natural Killer Cells Function and Its Importance in Cancer Immunotherapy**. Front Immunol *8*, 1124, doi: 10.3389/fimmu.2017.01124.

Pedersen, B. K. (2000). **Special feature for the Olympics: effects of exercise on the immune system: exercise and cytokines**. Immunol Cell Biol *78 (5)*, 532-535, doi: 10.1111/j.1440-1711.2000.t01-11-.x.

Pedersen, B. K. (2017). **Anti-inflammatory effects of exercise: role in diabetes and cardiovascular disease**. Eur J Clin Invest *47 (8)*, 600-611, doi: 10.1111/eci.12781.

Pedersen, B. K., Rohde, T. and Ostrowski, K. (1998). **Recovery of the immune system after exercise**. Acta Physiol Scand *162 (3)*, 325-332, doi: 10.1046/j.1365-201X.1998.0325e.x.

Pedersen, L., Idorn, M., Olofsson, G. H., Lauenborg, B., Nookaew, I., Hansen, R. H., Johannesen, H. H., Becker, J. C., Pedersen, K. S., Dethlefsen, C., Nielsen, J., Gehl, J., Pedersen, B. K., Thor Straten, P. and Hojman, P. (2016). **Voluntary Running Suppresses Tumor Growth through Epinephrine- and IL-6-Dependent NK Cell Mobilization and Redistribution**. Cell Metab *23 (3)*, 554-562, doi: 10.1016/j.cmet.2016.01.011.

Peng, Y. P., Zhang, J. J., Liang, W. B., Tu, M., Lu, Z. P., Wei, J. S., Jiang, K. R., Gao, W. T., Wu, J. L., Xu, Z. K., Miao, Y. and Zhu, Y. (2014). **Elevation of MMP-9 and IDO induced by pancreatic cancer cells mediates natural killer cell dysfunction**. BMC Cancer *14*, 738, doi: 10.1186/1471-2407-14-738.

Petersen, A. M. and Pedersen, B. K. (2005). **The anti-inflammatory effect of exercise**. J Appl Physiol (1985) *98 (4)*, 1154-1162, doi: 10.1152/japplphysiol.00164.2004.

Phaneuf, S. and Leeuwenburgh, C. (2001). **Apoptosis and exercise**. Med Sci Sports Exerc *33 (3)*, 393-396, doi: 10.1097/00005768-200103000-00010.

Platten, M., von Knebel Doeberitz, N., Oezen, I., Wick, W. and Ochs, K. (2014). **Cancer Immunotherapy by Targeting IDO1/TDO and Their Downstream Effectors**. Front Immunol *5*, 673, doi: 10.3389/fimmu.2014.00673.

Potthoff, K., Schmidt, M. E., Wiskemann, J., Hof, H., Klassen, O., Habermann, N., Beckhove, P., Debus, J., Ulrich, C. M. and Steindorf, K. (2013). **Randomized controlled trial to evaluate the effects of progressive resistance training compared to progressive muscle relaxation in breast cancer patients undergoing adjuvant radiotherapy: the BEST study**. BMC Cancer *13*, 162, doi: 10.1186/1471-2407-13-162.

Pyne, D. B. (1994). **Regulation of neutrophil function during exercise**. Sports Med *17 (4)*, 245-258, doi: 10.2165/00007256-199417040-00005.

Qian, S., He, T., Wang, W., He, Y., Zhang, M., Yang, L., Li, G. and Wang, Z. (2016). **Discovery and preliminary structure-activity relationship of 1H-indazoles with promising indoleamine-2,3-dioxygenase 1 (IDO1) inhibition properties**. Bioorg Med Chem *24 (23)*, 6194-6205, doi: 10.1016/j.bmc.2016.10.003.

Radom-Aizik, S., Zaldivar, F., Haddad, F. and Cooper, D. M. (2013). **Impact of brief exercise on peripheral blood NK cell gene and microRNA expression in young adults**. J Appl Physiol (1985) *114 (5)*, 628-636, doi: 10.1152/japplphysiol.01341.2012.

Radom-Aizik, S., Zaldivar, F., Jr., Leu, S. Y., Adams, G. R., Oliver, S. and Cooper, D. M. (2012). **Effects of exercise on microRNA expression in young males peripheral blood mononuclear cells**. Clin Transl Sci *5 (1)*, 32-38, doi: 10.1111/j.1752-8062.2011.00384.x.

Radom-Aizik, S., Zaldivar, F., Jr., Leu, S. Y., Galassetti, P. and Cooper, D. M. (2008). **Effects of 30 min of aerobic exercise on gene expression in human neutrophils**. J Appl Physiol (1985) *104 (1)*, 236-243, doi: 10.1152/japplphysiol.00872.2007.

Rhind, S. G., Shek, P. N., Shinkai, S. and Shephard, R. J. (1994). **Differential expression of interleukin-2 receptor alpha and beta chains in relation to natural killer cell subsets and aerobic fitness**. Int J Sports Med *15 (6)*, 311-318, doi: 10.1055/s-2007-1021066.

Robinson, C. M., Shirey, K. A. and Carlin, J. M. (2003). **Synergistic transcriptional activation of indoleamine dioxygenase by IFN-gamma and tumor necrosis factor-alpha**. J Interferon Cytokine Res *23 (8)*, 413-421, doi: 10.1089/107999003322277829.

Routy, J. P., Routy, B., Graziani, G. M. and Mehraj, V. (2016). **The Kynurenine Pathway Is a Double-Edged Sword in Immune-Privileged Sites and in Cancer: Implications for Immunotherapy**. Int J Tryptophan Res *9*, 67-77, doi: 10.4137/IJTR.S38355.

Saxton, J. M., Scott, E. J., Daley, A. J., Woodroofe, M., Mutrie, N., Crank, H., Powers, H. J. and Coleman, R. E. (2014). **Effects of an exercise and hypocaloric healthy eating intervention on indices of psychological health status, hypothalamic-pituitary-adrenal axis regulation and immune function after early-stage breast cancer: a randomised controlled trial**. Breast Cancer Res *16 (2)*, R39, doi: 10.1186/bcr3643.

Scheiermann, C., Kunisaki, Y. and Frenette, P. S. (2013). **Circadian control of the immune system**. Nat Rev Immunol *13 (3)*, 190-198, doi: 10.1038/nri3386.

Schlittler, M., Goiny, M., Agudelo, L. Z., Venckunas, T., Brazaitis, M., Skurvydas, A., Kamandulis, S., Ruas, J. L., Erhardt, S., Westerblad, H. and Andersson, D. C. (2016). **Endurance exercise increases skeletal muscle kynurenine aminotransferases and plasma kynurenic acid in humans**. Am J Physiol Cell Physiol *310 (10)*, C836-840, doi: 10.1152/ajpcell.00053.2016.

Schmidt, M. E., Meynkohn, A., Habermann, N., Wiskemann, J., Oelmann, J., Hof, H., Wessels, S., Klassen, O., Debus, J., Potthoff, K., Steindorf, K. and Ulrich, C. M. (2016). **Resistance Exercise and Inflammation in Breast Cancer Patients Undergoing Adjuvant Radiation Therapy: Mediation Analysis From a Randomized, Controlled Intervention Trial**. Int J Radiat Oncol Biol Phys *94 (2)*, 329-337, doi: 10.1016/j.ijrobp.2015.10.058.

Schmidt, M. E., Wiskemann, J., Armbrust, P., Schneeweiss, A., Ulrich, C. M. and Steindorf, K. (2015). **Effects of resistance exercise on fatigue and quality of life in breast cancer patients undergoing adjuvant chemotherapy: A randomized controlled trial**. Int J Cancer *137 (2)*, 471-480, doi: 10.1002/ijc.29383.

Schmidt, M. E., Wiskemann, J., Krakowski-Roosen, H., Knicker, A. J., Habermann, N., Schneeweiss, A., Ulrich, C. M. and Steindorf, K. (2013). **Progressive resistance versus relaxation training for breast cancer patients during adjuvant chemotherapy: design and rationale of a randomized controlled trial (BEATE study)**. Contemp Clin Trials *34 (1)*, 117-125, doi: 10.1016/j.cct.2012.10.006.

Schmiedel, D. and Mandelboim, O. (2018). **NKG2D Ligands-Critical Targets for Cancer Immune Escape and Therapy**. Front Immunol *9*, 2040, doi: 10.3389/fimmu.2018.02040.

Schmitz, K. H., Courneya, K. S., Matthews, C., Demark-Wahnefried, W., Galvao, D. A., Pinto, B. M., Irwin, M. L., Wolin, K. Y., Segal, R. J., Lucia, A., Schneider, C. M., von Gruenigen, V. E., Schwartz, A. L. and American College of Sports, M. (2010). **American College of Sports Medicine roundtable on exercise guidelines for cancer survivors**. Med Sci Sports Exerc *42 (7)*, 1409-1426, doi: 10.1249/MSS.0b013e3181e0c112.

Schneider, J., Schluter, K., Sprave, T., Wiskemann, J. and Rosenberger, F. (2020). **Exercise intensity prescription in cancer survivors: ventilatory and lactate thresholds are useful submaximal alternatives to VO2peak**. Support Care Cancer, doi: 10.1007/s00520-020-05407-y.

Schwellnus, M., Soligard, T., Alonso, J. M., Bahr, R., Clarsen, B., Dijkstra, H. P., Gabbett, T. J., Gleeson, M., Hagglund, M., Hutchinson, M. R., Janse Van Rensburg, C., Meeusen, R., Orchard, J. W., Pluim, B. M., Raftery, M., Budgett, R. and Engebretsen, L. (2016). **How much is too much? (Part 2) International Olympic Committee consensus statement on load in sport and risk of illness**. Br J Sports Med *50 (17)*, 1043-1052, doi: 10.1136/bjsports-2016-096572.

Sellami, M., Ben Abderrahman, A., Kebsi, W., De Sousa, M. V. and Zouhal, H. (2017). **Original Research: Effect of sprint and strength training on glucoregulatory hormones: Effect of advanced age**. Exp Biol Med (Maywood) *242 (1)*, 113-123, doi: 10.1177/1535370216662711.

Sellami, M., Gasmi, M., Denham, J., Hayes, L. D., Stratton, D., Padulo, J. and Bragazzi, N. (2018). **Effects of Acute and Chronic Exercise on Immunological Parameters in the Elderly Aged: Can Physical Activity Counteract the Effects of Aging?** Front Immunol *9*, 2187, doi: 10.3389/fimmu.2018.02187.

Seok, S. H., Ma, Z. X., Feltenberger, J. B., Chen, H., Chen, H., Scarlett, C., Lin, Z., Satyshur, K. A., Cortopassi, M., Jefcoate, C. R., Ge, Y., Tang, W., Bradfield, C. A. and Xing, Y. (2018). **Trace derivatives of kynurenine potently activate the aryl hydrocarbon receptor (AHR)**. J Biol Chem *293 (6)*, 1994-2005, doi: 10.1074/jbc.RA117.000631.

Shakhar, G., Abudarham, N., Melamed, R., Schwartz, Y., Rosenne, E. and Ben-Eliyahu, S. (2007). **Amelioration of operation-induced suppression of marginating pulmonary NK activity using poly IC: a potential approach to reduce postoperative metastasis**. Ann Surg Oncol *14 (2)*, 841-852, doi: 10.1245/s10434-006-9078-9.

Shek, P. N., Sabiston, B. H., Buguet, A. and Radomski, M. W. (1995). **Strenuous exercise and immunological changes: a multiple-time-point analysis of leukocyte subsets, CD4/CD8 ratio, immunoglobulin production and NK cell response**. Int J Sports Med *16 (7)*, 466-474, doi: 10.1055/s-2007-973039.

Shephard, R. J. (1996). **Exercise and cancer: linkages with obesity?** Crit Rev Food Sci Nutr *36 (4)*, 321-339, doi: 10.1080/10408399609527728.

Shephard, R. J. (2003). **Adhesion molecules, catecholamines and leucocyte redistribution during and following exercise**. Sports Med *33 (4)*, 261-284, doi: 10.2165/00007256-200333040-00002.

Shephard, R. J. and Shek, P. N. (1999). **Effects of exercise and training on natural killer cell counts and cytolytic activity: a meta-analysis**. Sports Med *28 (3)*, 177-195, doi: 10.2165/00007256-199928030-00003.

Shi, W., Oshlack, A. and Smyth, G. K. (2010). **Optimizing the noise versus bias trade-off for Illumina whole genome expression BeadChips**. Nucleic Acids Res *38 (22)*, e204, doi: 10.1093/nar/gkq871.

Shin, J. H., Zhang, L., Murillo-Sauca, O., Kim, J., Kohrt, H. E., Bui, J. D. and Sunwoo, J. B. (2013). **Modulation of natural killer cell antitumor activity by the aryl hydrocarbon receptor**. Proc Natl Acad Sci U S A *110 (30)*, 12391-12396, doi: 10.1073/pnas.1302856110.

Shinkai, S., Shore, S., Shek, P. N. and Shephard, R. J. (1992). **Acute exercise and immune function. Relationship between lymphocyte activity and changes in subset counts**. Int J Sports Med *13 (6)*, 452-461, doi: 10.1055/s-2007-1021297.

Simpson, R. J., Bigley, A. B., Agha, N., Hanley, P. J. and Bollard, C. M. (2017). **Mobilizing Immune Cells With Exercise for Cancer Immunotherapy**. Exerc Sport Sci Rev *45 (3)*, 163-172, doi: 10.1249/JES.0000000000000114.

Simpson, R. J., Cosgrove, C., Chee, M. M., McFarlin, B. K., Bartlett, D. B., Spielmann, G., O'Connor, D. P., Pircher, H. and Shiels, P. G. (2010). **Senescent phenotypes and telomere lengths of peripheral blood T-cells mobilized by acute exercise in humans**. Exerc Immunol Rev *16*, 40-55.

Sinclair, L. V., Neyens, D., Ramsay, G., Taylor, P. M. and Cantrell, D. A. (2018). **Single cell analysis of kynurenine and System L amino acid transport in T cells**. Nat Commun *9 (1)*, 1981, doi: 10.1038/s41467-018-04366-7.

Soligard, T., Schwellnus, M., Alonso, J. M., Bahr, R., Clarsen, B., Dijkstra, H. P., Gabbett, T., Gleeson, M., Hagglund, M., Hutchinson, M. R., Janse van Rensburg, C., Khan, K. M., Meeusen, R., Orchard, J. W., Pluim, B. M., Raftery, M., Budgett, R. and Engebretsen, L. (2016). **How much is too much? (Part 1) International Olympic Committee consensus statement on load in sport and risk of injury**. Br J Sports Med *50 (17)*, 1030-1041, doi: 10.1136/bjsports-2016-096581.

Song, H., Park, H., Kim, Y. S., Kim, K. D., Lee, H. K., Cho, D. H., Yang, J. W. and Hur, D. Y. (2011). **L-kynurenine-induced apoptosis in human NK cells is mediated by reactive oxygen species**. Int Immunopharmacol *11 (8)*, 932-938, doi: 10.1016/j.intimp.2011.02.005.

Steindorf, K., Schmidt, M. E., Klassen, O., Ulrich, C. M., Oelmann, J., Habermann, N., Beckhove, P., Owen, R., Debus, J., Wiskemann, J. and Potthoff, K. (2014). **Randomized, controlled trial of resistance training in breast cancer patients receiving adjuvant radiotherapy: results on cancer-related fatigue and quality of life**. Ann Oncol *25 (11)*, 2237-2243, doi: 10.1093/annonc/mdu374.

Steins Bisschop, C. N., Courneya, K. S., Velthuis, M. J., Monninkhof, E. M., Jones, L. W., Friedenreich, C., van der Wall, E., Peeters, P. H. and May, A. M. (2015). **Control group design, contamination and drop-out in exercise oncology trials: a systematic review**. PLoS One *10 (3)*, e0120996, doi: 10.1371/journal.pone.0120996.

Strasser, B., Geiger, D., Schauer, M., Gatterer, H., Burtscher, M. and Fuchs, D. (2016). **Effects of Exhaustive Aerobic Exercise on Tryptophan-Kynurenine Metabolism in Trained Athletes**. PLoS One *11 (4)*, e0153617, doi: 10.1371/journal.pone.0153617.

Suzui, M., Kawai, T., Kimura, H., Takeda, K., Yagita, H., Okumura, K., Shek, P. N. and Shephard, R. J. (2004). **Natural killer cell lytic activity and CD56(dim) and CD56(bright) cell distributions during and after intensive training**. J Appl Physiol (1985) *96 (6)*, 2167-2173, doi: 10.1152/japplphysiol.00513.2003.

Suzuki, K., Hayano, Y., Nakai, A., Furuta, F. and Noda, M. (2016). **Adrenergic control of the adaptive immune response by diurnal lymphocyte recirculation through lymph nodes**. J Exp Med *213 (12)*, 2567-2574, doi: 10.1084/jem.20160723.

Takeda, K., Nakayama, M., Sakaki, M., Hayakawa, Y., Imawari, M., Ogasawara, K., Okumura, K. and Smyth, M. J. (2011). **IFN-gamma production by lung NK cells is critical for the natural resistance to pulmonary metastasis of B16 melanoma in mice**. J Leukoc Biol *90 (4)*, 777-785, doi: 10.1189/jlb.0411208.

Talari, N. K., Panigrahi, M., Madigubba, S., Challa, S. and Phanithi, P. B. (2016). **Altered tryptophan metabolism in human meningioma**. J Neurooncol *130 (1)*, 69-77, doi: 10.1007/s11060-016-2225-7.

Terren, I., Orrantia, A., Vitalle, J., Zenarruzabeitia, O. and Borrego, F. (2019). **NK Cell Metabolism and Tumor Microenvironment**. Front Immunol *10*, 2278, doi: 10.3389/fimmu.2019.02278.

Timmons, B. W. and Cieslak, T. (2008). **Human natural killer cell subsets and acute exercise: a brief review**. Exerc Immunol Rev *14*, 8-23.

Turner, J. E. (2016). **Is immunosenescence influenced by our lifetime "dose" of exercise?** Biogerontology *17 (3)*, 581-602, doi: 10.1007/s10522-016-9642-z.

Turner, J. E., Aldred, S., Witard, O. C., Drayson, M. T., Moss, P. M. and Bosch, J. A. (2010). **Latent cytomegalovirus infection amplifies CD8 T-lymphocyte mobilisation and egress in response to exercise**. Brain Behav Immun *24 (8)*, 1362-1370, doi: 10.1016/j.bbi.2010.07.239.

Une, C., Andersson, J., Eloranta, M. L., Sunnemark, D., Harris, R. A. and Orn, A. (2000). **Enhancement of natural killer (NK) cell cytotoxicity and induction of NK cell-derived interferon-gamma (IFN-gamma) display different kinetics during experimental infection with Trypanosoma cruzi**. Clin Exp Immunol *121 (3)*, 499-505, doi: 10.1046/j.1365-2249.2000.01318.x.

Verma, V. K., Singh, V., Singh, M. P. and Singh, S. M. (2009). **Effect of physical exercise on tumor growth regulating factors of tumor microenvironment: implications in exercise-dependent tumor growth retardation**. Immunopharmacol Immunotoxicol *31 (2)*, 274-282, doi: 10.1080/08923970802562042.

Wang, J. S. and Weng, T. P. (2011). **Hypoxic exercise training promotes antitumour cytotoxicity of natural killer cells in young men**. Clin Sci (Lond) *121 (8)*, 343-353, doi: 10.1042/CS20110032.

Weber-Boyvat, M., Kentala, H., Lilja, J., Vihervaara, T., Hanninen, R., Zhou, Y., Peranen, J., Nyman, T. A., Ivaska, J. and Olkkonen, V. M. (2015). **OSBP-related protein 3 (ORP3) coupling with VAMP-associated protein A regulates R-Ras activity**. Exp Cell Res *331 (2)*, 278-291, doi: 10.1016/j.yexcr.2014.10.019.

Weinberg, F., Ramnath, N. and Nagrath, D. (2019). **Reactive Oxygen Species in the Tumor Microenvironment: An Overview**. Cancers (Basel) *11 (8)*, doi: 10.3390/cancers11081191.

Wendt, K., Wilk, E., Buyny, S., Buer, J., Schmidt, R. E. and Jacobs, R. (2006). **Gene and protein characteristics reflect functional diversity of CD56dim and CD56bright NK cells**. J Leukoc Biol *80 (6)*, 1529-1541, doi: 10.1189/jlb.0306191.

Wiggins, J. M., Opoku-Acheampong, A. B., Baumfalk, D. R., Siemann, D. W. and Behnke, B. J. (2018). **Exercise and the Tumor Microenvironment: Potential Therapeutic Implications**. Exerc Sport Sci Rev *46 (1)*, 56-64, doi: 10.1249/JES.0000000000000137.

Woods, J. A., Ceddia, M. A., Wolters, B. W., Evans, J. K., Lu, Q. and McAuley, E. (1999). **Effects of 6 months of moderate aerobic exercise training on immune function in the elderly**. Mech Ageing Dev *109 (1)*, 1-19, doi: 10.1016/s0047-6374(99)00014-7.

Wu, P. Y., Yu, I. S., Lin, Y. C., Chang, Y. T., Chen, C. C., Lin, K. H., Tseng, T. H., Kargren, M., Tai, Y. L., Shen, T. L., Liu, Y. L., Wang, B. J., Chang, C. H., Chen, W. M., Juan, H. F., Huang, S. F., Chan, Y. Y., Liao, Y. F., Hsu, W. M. and Lee, H. (2019). **Activation of Aryl Hydrocarbon Receptor by Kynurenine Impairs Progression and Metastasis of Neuroblastoma**. Cancer Res *79 (21)*, 5550-5562, doi: 10.1158/0008-5472.CAN-18-3272.

Xue, P., Fu, J. and Zhou, Y. (2018). **The Aryl Hydrocarbon Receptor and Tumor Immunity**. Front Immunol *9*, 286, doi: 10.3389/fimmu.2018.00286.

Yeager, M. P., Pioli, P. A. and Guyre, P. M. (2011). **Cortisol exerts bi-phasic regulation of inflammation in humans**. Dose Response *9 (3)*, 332-347, doi: 10.2203/dose-response.10-013.Yeager.

Zamai, L., Ahmad, M., Bennett, I. M., Azzoni, L., Alnemri, E. S. and Perussia, B. (1998). **Natural killer (NK) cell-mediated cytotoxicity: differential use of TRAIL and Fas ligand by immature and mature primary human NK cells**. J Exp Med *188 (12)*, 2375-2380, doi: 10.1084/jem.188.12.2375.

Zhang, T., Tan, X. L., Xu, Y., Wang, Z. Z., Xiao, C. H. and Liu, R. (2017). **Expression and Prognostic Value of Indoleamine 2,3-dioxygenase in Pancreatic Cancer**. Chin Med J (Engl) *130 (6)*, 710-716, doi: 10.4103/0366-6999.201613.

Zimmer, P., Baumann, F. T., Bloch, W., Schenk, A., Koliamitra, C., Jensen, P., Mierau, A., Hulsdunker, T., Reinart, N., Hallek, M. and Elter, T. (2014). **Impact of exercise on pro inflammatory cytokine levels and epigenetic modulations of tumor-competitive lymphocytes in Non-Hodgkin-Lymphoma patients-randomized controlled trial**. Eur J Haematol *93 (6)*, 527-532, doi: 10.1111/ejh.12395.

Zimmer, P., Bloch, W., Schenk, A., Zopf, E. M., Hildebrandt, U., Streckmann, F., Beulertz, J., Koliamitra, C., Schollmayer, F. and Baumann, F. (2015). **Exercise-induced Natural Killer Cell Activation is Driven by Epigenetic Modifications**. Int J Sports Med *36 (6)*, 510-515, doi: 10.1055/s-0034-1398531.

Zimmer, P., Joisten, N., Schenk, A. and Bloch, W. (2019a). **Impact of physical exercise on the kynurenine pathway in patients with cancer: current limitations and future perspectives**. Acta Oncol *58 (8)*, 1116-1117, doi: 10.1080/0284186X.2019.1599139.

Zimmer, P., Schmidt, M. E., Prentzell, M. T., Berdel, B., Wiskemann, J., Kellner, K. H., Debus, J., Ulrich, C., Opitz, C. A. and Steindorf, K. (2019b). **Resistance Exercise Reduces Kynurenine Pathway Metabolites in Breast Cancer Patients Undergoing Radiotherapy**. Front Oncol *9*, 962, doi: 10.3389/fonc.2019.00962.

7. PUBLICATIONS

Pal, A., Zimmer, P., Schmidt, M. E., Hummel, M., Ulrich, C. M., Wiskemann, J. and Steindorf, K. (2019). **No evidence for effect of exercise on transcriptome of NK cells in breast cancer patients undergoing adjuvant therapy: Results from a pilot study**. Front Physiol *10*, 959, doi: 10.3389/fphys.2019.00959.

Pal, A., Zimmer, P., Clauss, D., Schmidt, M. E., Ulrich, C. M., Wiskemann, J. and Steindorf, K. (2020). **Resistance exercise modulates Kynurenine pathway in pancreatic cancer patients**. Int J Sports Med, doi: 10.1055/a-1186-1009.

Pal, A., Schneider, J., Schlüter, K. *et al.* **Different endurance exercises modulate NK cell cytotoxic and inhibiting receptors**. Eur J Appl Physiol (2021). doi.org/10.1007/s00421-021-04735-z

Oral presentations

Pal, A., Zimmer, P., Clauss, D., Schmidt, M. E., Ulrich, C. M., Wiskemann, J. and Steindorf, K. (2020). **Resistance exercise modulates Kynurenine pathway in pancreatic cancer patients**.*– NCT retreat, May 2019, Heidelberg.*

Poster presentations

1. Pal, A., Zimmer, P., Clauss, D., Schmidt, M. E., Ulrich, C. M., Wiskemann, J. and Steindorf, K. (2020). **Does resistance exercise modulate Kynurenine pathway in pancreatic cancer patients?**. *-NCT retreat, May 2020, Heidelberg.*

2*.* Pal, A., Zimmer, P., Clauss, D., Schmidt, M. E., Ulrich, C. M., Wiskemann, J. and Steindorf, K. (2020). **Resistance exercise modulates Kynurenine pathway in pancreatic cancer patients**. *– DKFZ poster presentation, November 2018,2019, Heidlberg.*

3*.* Pal, A., Zimmer, P., Clauss, D., Schmidt, M. E., Ulrich, C. M., Wiskemann, J. and Steindorf, K. (2020). **The Kynurenine pathway in pancreatic cancer patients can be affected by resistance exercise***- Frontiers in Cancer Research, October 2019. Heidelberg.*

8. APPENDIX

<u>Appendix 1: Performance characteristics of the ELISAs</u>

#K7728

<u>Precision and Reproducibility for L-kynurenine</u>

<u>Intra-Assay (n = 14)</u>

Sample	L-kynurenine µmol/l]	CV [%]
1	0.82	7.6
2	2.86	6.2

<u>Inter-Assay (n = 8)</u>

Sample	L-kynurenine [µmol/l]	CV [%]
1	0.80	
2	2.80	6.2

<u>Spiking Recovery</u>

Three samples were spiked with different L-kynurenine concentrations and measured in this assay (n= 2). The mean recovery rate was 102.5%.

<u>Dilution Recovery</u>

Two spiked samples were diluted and analysed. The mean recovery rate was 100.3% (n = 2).

<u>Analytical Sensitivity</u>

Limit of detection, LoD 0.12 µmol/l

#K7730

Precision and Reproducibility for L-tryptophan

<u>Intra-assay (n = 14)</u>

Sample	L-tryptophan [µmol/l]	CV [%]
1	51.4	4.3
2	105.7	6.9

Inter-assay (n = 7)

Sample	L-tryptophan [µmol/l]	CV [%]
1	63.7	8.4
2	60.6	9.1

Spiking Recovery

Three serum samples were spiked with different L-tryptophan concentrations and measured in this assay (n = 2). The mean recovery rate for all concentrations was 97.2%.

Dilution Recovery

Two serum samples were diluted and measured in this assay. The mean recovery was 96.8% (n = 2).

Analytical Sensitivity

Limit of detection, LoD 8.0 µmol/l

#HS600C

Precision and Reproducibility for IL-6

Intra-assay (n = 20)

Sample	IL-6 [µmol/l]	CV [%]
1	0.527	4.7
2	2.75	3.6

Inter-assay (n = 20)

Sample	IL-6 [µmol/l]	CV [%]
1	0.52	10.8
2	2.75	4.9

Spiking Recovery

Serum samples were spiked with different IL-6 concentrations and measured in this assay. The mean recovery rate was 97.0% (n = 4).

Dilution Recovery

Four serum samples were diluted with calibration buffer and measured in this assay. The mean recovery rate was 97% -107% (n = 4).

Analytical Sensitivity

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of human IL-6 ranged from 0.007-0.090 pg/mL. The mean MDD was 0.031 pg/mL.

Analysis of ELISA values were performed with the Magellan software V 7.2 (Tecan Austria GmbH). The Kynurenine/Tryptophan ratio (here denoted as KTR) was calculated to indirectly assess enzyme activity for Tryptophan degradation into Kynurenine by IDO/TDO enzyme.

9. CURRICULUM VITAE

Personal information

First name(s) / Surname(s)	**ANASUA PAL**
Permanent Address	IA-294, Sector-3, Saltlake city, Kolkata-700097, India
Current Address	Hollandtstraße 38, 48161, Münster, Germany
Telephone(s)	+4915214437211
E-mail	mail.anasua@gmail.com
Nationality	Indian
Date of Birth	29.07.1991
Gender	Female

EDUCATION

1. Dates	1.07.2017-30.08.2020
Title of qualification	**PhD**
Department	Physical activity, prevention and cancer
Name of Institution	German Cancer Research Centre (DKFZ)
Grades	Ongoing

2. Dates	1.10.2014-13.12.2016
Title of Qualification	**MSc**.
Principal Subject	Biochemistry and Molecular Biology
Thesis	Investigation of Hippo Kinase LATS2 in pancreatic beta cells
Name of Institution	Universität Bremen
Grades	**1.61** (German scale 1-Best; 5-Worst)

3. Dates	9.07.2010-9.06.2014
Title of qualification	**B-Tech** (Bachelors in Technology)
Principal Subject	Biotechnology
Name of Institution	Bengal Institute of Technology, India
Grades	**84.9%**
4. Dates	2010-CBSE-**12TH** STANDARD SCHOOL
Grades	**80.2%**
5. Dates	2008-ICSE-**10th** STANDARD SCHOOL
Grades	**86.8%**

10. ACKNOWLEDGEMENTS

EIDESSTATTLICHE VERSICHERUNG

1. Bei der eingereichten Dissertation zu dem Thema

Impact of different exercise modalities on tumor relevant Natural killer cell function and their regulation

handelt es sich um meine eigenständig erbrachte Leistung.

2. Ich habe nur die angegebenen Quellen und Hilfsmittel benutzt und mich keiner unzulässigen Hilfe Dritter bedient. Insbesondere habe ich wörtlich oder sinngemäß aus anderen Werken übernommene Inhalte als solche kenntlich gemacht.

3. Die Arbeit oder Teile davon habe ich bislang nicht an einer Hochschule des In- oder Auslands als Bestandteil einer Prüfungs- oder Qualifikationsleistung vorgelegt.

4. Die Richtigkeit der vorstehenden Erklärungen bestätige ich.

5. Die Bedeutung der eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unrichtigen oder unvollständigen eidesstattlichen Versicherung sind mir bekannt. Ich versichere an Eides statt, dass ich nach bestem Wissen die reine Wahrheit erklärt und nichts verschwiegen habe.

Ort und Datum   Unterschrift