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Exercise as an adjunct treatment in children with cancer
Feasibility & mechanisms

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I Foreword and Acknowledgements

For a more clear and direct writing, I use active voice throughout this dissertation - as recommended by Nature.

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Hilde and Emil, you are the loves of my life!

„*Alles sollte so einfach wie möglich gemacht werden, aber nicht einfacher.*“

Albert Einstein

II Abstract

Exercise contributes to a healthy lifestyle. Regular physical activity decreases the risk of developing several diseases, including cancer. Exercise not only prevents the development of several types of cancer, but is also beneficial to cancer treatment and its side effects. The research field of exercise oncology analyzes the effects of exercise on cancer. While epidemiological studies conclusively describe these effects, the physiological reasons for their relation remain largely unknown. However, an increasing amount of research has recently been published on potential mechanisms that could explain the beneficial effect of exercise on cancer.

This dissertation contributes to the understanding of two central issues in exercise oncology:

1. Evidence in current exercise oncology is based mainly on research in adult cancer patients. Research in children is scarce and results from adult cancer patients are not necessarily applicable as cancer is a heterogenous, genetic disease and as there are systematic differences between childhood and adult cancer. Despite the fact that cancer is less common in children than in adults, it is still one of the leading causes of death in children. It is therefore essential to conduct independent research in pediatric oncology, in order to utilize the full potential of exercise for childhood cancer patients.

2. Mechanistic research in exercise oncology suggests that catecholamines (adrenaline/noradrenaline) are mediators of the positive effects of exercise on cancer. However, stress studies use the exact same argument to explain negative effects of stress on cancer: catecholamines. Thus, it seems that catecholamines have different effects on cancer cells. It is important to investigate this obvious contradiction, as cancer patients should exercise at high intensities (= increase in catecholamines) only, if this does not increase cancer growth.

This dissertation is based on three studies. 1. My literature analysis showed that exercise with pediatric cancer patients is poorly investigated and that there is a clear need for independent research in pediatric cancer patients. 2. My intervention study with pediatric cancer patients indicates that a single bout of high-intensity exercise is safe, but it is feasible only in a small number of physically fit patients who minimally suffer from treatment-related side effects. While heart rate and lactate increase during the intervention, adrenaline levels remain unaltered. This emphasizes the necessity to analyze physiological effects of exercise specifically in pediatric cancer patients. This might lead to children-specific markers that are influenced by exercise and affect cancer directly. 3. My cell culture experiments provide proof-of-concept for a novel explanation of the contradicting effects of catecholamines on cancer cells. The different expression of adrenergic receptor isoforms can be a reason for different effects. While pediatric sarcoma cells, that express adrenergic receptors, decrease proliferation and migration after

treatment with noradrenaline, pediatric sarcoma cells without these receptors do not respond to the treatment.

In conclusion, the findings of this dissertation suggest that children should receive more attention in exercise oncology research and that the expression of adrenergic receptor isoforms could be an essential mediator of the effect of exercise on cancer.

III Zusammenfassung

Sport und Bewegung sind wesentliche Bestandteile eines gesunden Lebensstils. Wer sich regelmäßig bewegt, beugt damit Krankheiten vor; unter anderem auch Krebs. Sport schützt nicht nur vor der Entstehung einer Reihe von Krebsarten sondern kann auch die Therapie begünstigen und Nebenwirkungen verringern. Dem Zusammenhang von Sport und Krebs widmet sich das Forschungsfeld der Sportonkologie. Während epidemiologische Studien diesen Zusammenhang aussagekräftig darstellen können, sind die physiologischen Gründe für den Zusammenhang weiterhin weitestgehend unklar. Allerdings werden seit einigen Jahren vermehrt Arbeiten publiziert, die physiologische Mechanismen suchen, welche die Wirkung von Sport auf Krebs erklären können.

Die vorliegende Arbeit widmet sich vor diesem Hintergrund zwei Problemstellungen in der Sportonkologie: 1. Der Kenntnisstand der derzeitigen Sportonkologie gründet im Wesentlichen auf Forschung im Erwachsenenbereich. Kinder kommen nur begrenzt in Studien vor und Ergebnisse der Erwachsenenforschung können nicht einfach auf den kindlichen Körper übertragen werden. Zwar ist Krebs bei Kindern deutlich seltener als bei Erwachsenen, nichtsdestotrotz gehört Krebs weiterhin zu den häufigsten Todesursachen im Kindesalter. Es ist daher essentiell, eigenständige Forschung in der Kinderonkologie durchzuführen, um das Potential des Sports auch in diesem Bereich bestmöglich zu nutzen. 2. Die positive Wirkung von Sport auf Krebs wird in mechanistischen Studien häufig mit der Wirkung von Katecholaminen (Adrenalin/Noradrenalin) erklärt. Allerdings erklären Stressstudien die negative Wirkung von Stress auf Krebs mit demselben Argument: Katecholamine. Es zeigt sich also, dass Katecholamine unterschiedliche Wirkung auf Krebszellen zu haben scheinen. Es ist wichtig diesen offensichtlichen Gegensatz zu untersuchen, da Krebspatienten nur dann hochintensiven (= Katecholamin-Anstieg) Sport machen sollten, wenn sichergestellt ist, dass dies das Krebswachstum nicht verstärkt.

Dieser Dissertation liegen drei Studien zugrunde. 1. Unsere Literaturrecherche hat gezeigt, dass Sport mit kindlichen Krebspatienten nur spärlich untersucht ist und dringend eigenständige Forschung benötigt wird. 2. In unserer Interventionsstudie mit krebskranken Kindern konnten wir zeigen, dass eine einmalige hochintensive Sportbelastung zwar sicher ist, jedoch nur mit einer kleinen Gruppe von physisch fitten Patienten, die nur wenig an Nebenwirkungen der Krebsbehandlung leiden, überhaupt durchführbar ist. Während Herzfrequenz und Laktatwerte durch die Intervention ansteigen, bleiben Adrenalinwerte unverändert. Dieser Umstand verdeutlicht die Notwendigkeit, die physiologische Wirkung von Sport spezifisch bei krebskranken Kindern zu untersuchen. Dadurch können womöglich eigene Marker identifiziert werden, welche bei krebskranken Kindern durch Sport beeinflusst werden und sich direkt auf die Erkrankung auswirken können. 3. Mit unseren Zellkultur-Experimenten konnten wir einen neuartigen Ansatz etablieren, weshalb sich

Katecholamine unterschiedlich auf Krebszellen auswirken. Eine Begründung liegt in der Expression andrenerger Rezeptoren. Während kindliche Sarkomzellen mit adrenergen Rezeptoren ihr Proliferations- und Migrationsverhalten durch Noradrenalin verlangsamen, reagieren kindliche Sarkomzellen ohne adrenerge Rezeptoren nicht auf die Behandlung mit Noradrenalin.

Zusammenfassend legen die Ergebnisse dieser Dissertation nahe, dass Kinder in der Sportonkologie stärker in den Fokus rücken sollten und adrenerge Rezeptoren eine Schlüsselrolle bei der Wirkung von Sport auf Krebs haben könnten.

IV List of Publications

First author publications

Kesting, S.*, **Weeber, P.***, Schönfelder, M., Renz, B.W., Wackerhage, H.[+], and von Luettichau, I.[+] (2020). Exercise as a Potential Intervention to Modulate Cancer Outcomes in Children and Adults? Frontiers in Oncology 10. 10.3389/fonc.2020.00196.
Journal Impact Factor: 6.244

Kesting, S.*, **Weeber, P.***, Schönfelder, M., Pfluger, A., Wackerhage, H., and von Luettichau, I. (2022). A Bout of High-Intensity Interval Training (HIIT) in Children and Adolescents during Acute Cancer Treatment—A Pilot Feasibility Study. Cancers 14. 10.3390/cancers14061468.
Journal Impact Factor: 6.639

* Joint first authorsip
[+] Joint last authorship

V Manuscript in Preparation

First author manuscript

Weeber, P., Haferanke, J., Regina, C., Schönfelder, M., Ludwig, C., Wackerhage, H.[+], and von Luettichau, I.[+] (2022). Noradrenaline has Different Effects on Rhabdomyosarcoma and Ewing's Sarcoma Cancer Hallmarks – Implications for Exercise Oncology.

[+] Joint last authorship

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VII List of Figures and Tables

VIII Abbreviations

AI	Artificial Intelligence
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AYAs	Adolescents and young adults
BMI	Body mass index
bpm	Beats per minute
BRCA	BReast CAncer
cAMP	Cyclic adenosine monophosphate
CCLE	Cancer cell line encyclopedia
COVID-19	Coronavirus disease 2019
COX-2	Cyclo-oxidase-2
CSO	Common Scientific Outline
CTCAE	Common Terminology for Adverse Events
DEPC	Diethyl pyrocarbonate
DNA	Deoxyribonucleic acid
e.g.	exempli gratia
EdU	5'-ethynyl-2'-deoxyuridine
ELISA	Enzyme-linked immunosorbent assay
FBS	Fetal bovine serum
fpkm	Fragments per kilobase of transcript per million fragments mapped
H_2O	Water
HCl	Hydrogen chloride
HIIT	High-intensity interval training
Hoechst	bisBenzimide H 33342 trihydrochloride
HR_{max}	Maximal heart rate
i.e.	id est
ICRP	International Cancer Research Partnership
IGF-1	Insulin-like growth factor 1
IGFBP3	insuline-like growth factor-binding protein
KIR2DS4	Killer cell immunoglobulin-like receptor 2DS4
KRAS	Kirsten rat sarcoma virus
mRNA	Messenger ribonucleic acid
n	Number
PBS	Phosphate-buffered saline
PWR	Power-to-weight ratio

PWS	Prader-Willi syndrome
RAS	Rat sarcoma gene
Rb	Retinoblastoma
RNA	Ribonucleic acid
RPE	Rating of perceived exertion
rpm	Rotations per minute
SD	Standard deviation
SRB	Sulforhodamine B
STR	Short Tandem Repeat
TP53	Tumor protein p53
USA	United States of America
VEGF	Vascular Endothelial Growth Factor
VO_{2max}	Maximal oxygen consumption
WHO	World Health Organization
YAP	Yes-associated protein

1 Introduction

Physical activity is generally recognized as beneficial for a healthy lifestyle in all age groups. Therefore, several research fields have now been established that analyze the effect of physical activity on prevention and control of various diseases. One of these fields is termed exercise oncology as it investigates the effects of physical exercise on cancer. Though the research was not always called exercise oncology, *Science* had already published a paper back in the 1940's about this very topic – discussing "Ample Exercise and a Minimum of Food as Measures for Cancer Prevention" (Morris, 1945). In the article, Harold P. Morris discusses that the influence of diet on tumorigenesis has most widely been studied on breast cancer in mice and that obesity seems to correlate with a greater risk of death due to cancer in humans. He points out that the role of exercise in cancer development has barely been investigated, but based on few studies, he speculates that forced exercise in mice might decrease tumor growh. He summarizes however, that the effect of exercise has – even in animals – not been investigated enough to come to a conclusion about its role in preventing cancer. This article shows that almost 80 years ago, researchers began to speculate about the role of exercise in cancer prevention and growth.

Since then, a small number of studies was regularly published on the effect of exercise on cancer, indicating different perspectives on physical activity. In the 1960's, Stukonis and Doll published an epidemiological study regarding the relation between physical activity and gastric cancer (Stukonis and Doll, 1969). They present a correlation analysis and associate physical activity at work in lower social classes with higher mortality from gastric cancer. They speculate that heavy work leads to increased intake of cheap food with high concentrations of carcinogens. A decade later, Gregor et al. describe their discovery that high exercise tolerance in lung cancer patients at the time of diagnosis is associated with improved survival due to a better response to chemotherapy in this cohort compared to less fit patients (Gregor et al., 1979). The authors cannot explain their finding.

Studies with mice in the 1960's presented ambivalent effects of exercise on cancer. While limb exercise was found to promote mestastasis of tumor cells via the lymphatic system (Stoker, 1969), Hoffman et al. published their study with the title "The Influence of Exercise on the Growth of Transplanted Rat Tumors" in the journal *Cancer Research* (Hoffman et al., 1962). They investigated the effect of exercise on tumor growth in Walker 256 tumor transplanted rats (Walker 256 cells were discovered in 1928 in a pregnant albino rat's breast and since then, are one of the most frequently used transplantable tumors in medical research (Shenoy et al., 2016)). Their experiment showed that three weeks of forced daily exercise decreases tumor weight in rats compared to controls with restricted movement possibilities. A similar study with

the same tumor model in rats drew the same conclusion and additionally found that animals which already exercised prior to tumor implantation lived longer compared to controls or animals that only exercised after tumor implantation (Newton, 1965). Comparable study designs with murine models have recently been used. Findings of these studies also indicate that exercise prior to tumor onset is connected with decreased tumor progression in breast (Goh et al., 2014; Hagar et al., 2019), colon (Kelly et al., 2017) and skin cancer (Hojman et al., 2022).

During the 1980's and 90's, researchers investigated protective effects of exercise and a healthy lifestyle from cancer. Blair et al. analyzed physical fitness and risk of all-cause mortality in more than 13,000 humans with an observation period of eight years (Blair et al., 1989). They found lower mortality rates for cancer in higher fitness categories and identified lower physical fitness as a risk factor for cancer mortality. In 1990, R. J. Shepard published a review on the relation between physical activity and cancer in the *International Journal of Sports Medicine* (Shephard, 1990). He summarized that physical activity seems to have a protective effect against colon and breast cancer but that research was not conclusive so far. On the other hand, he also proposed potential negative effects of exercise, i. e. water sports, due to increased exposure to ultra-violet light. Shepard proposes direct and indirect effects of exercise as a potential explanation for its relation to cancer. Indirectly, exercise would encourage an overall healthy lifestyle. Directly, exercise might speed up gastro-intestinal transit or moderate sex hormone levels.

The beginning of the 21st century is associated with a rapid increase of exercise oncology publications. A search on PubMed for "(exercise OR "physical activity" OR sport*) AND (cancer OR tumor OR neoplasia)" resulted in more than 43,000 publications – of which more than 38,000 were published from 2001 on. **Figure 1** shows the number of published papers per year for the aforementioned search on PubMed (date of search: 29.04.2022).

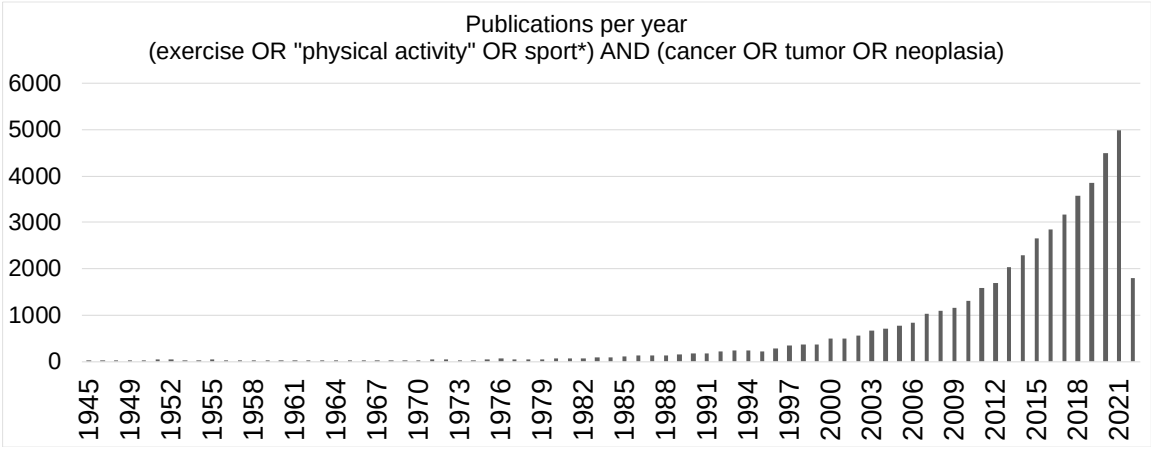

Figure 1: Number of publications per year on the effect of exercise on cancer - as listed on PubMed (29.04.2022).

One of the first papers to use the term “exercise oncology” was published by Lee W. Jones et al. in 2008 in the renowned oncology journal *The Lancet Oncology*. With their systematic review, Jones et al. summarized studies on assessing cardiorespiratory fitness by formal exercise testing in adult cancer patients and concluded with an overall low quality of exercise testing methods (Jones et al., 2008). They provided the reader with guidelines on how to report exercise testing methods and results in clinical oncology research. While this study focused on methodological aspects of exercise testing in clinical oncology, a review in 2012 then reported the safety and positive effects of exercise on treatment-related side effects in cancer patients. The authors summarized that exercise is safe and can be an effective intervention to improve treatment side effects, such as fatigue, cognitive impairments, sleep issues, depression, anxiety, pain, or physical dysfunctions (Mustian et al., 2012). However, the authors also state that the research area was still in its infancy and they raise methodological concerns, which make findings difficult to generalize with regard to the diversity of cancer patients.

With their large meta-analysis in 2016, Moore et al. pooled data from 1.44 million adults in the USA and Europe and associated physical activity with the risk of developing 26 types of cancer. **Figure 2** is adapted from Moore et al., 2016 and shows significant associations between physical exercise and the risk of developing 15 different cancer types. Exercise is associated with a reduced risk of developing 13 cancer types.

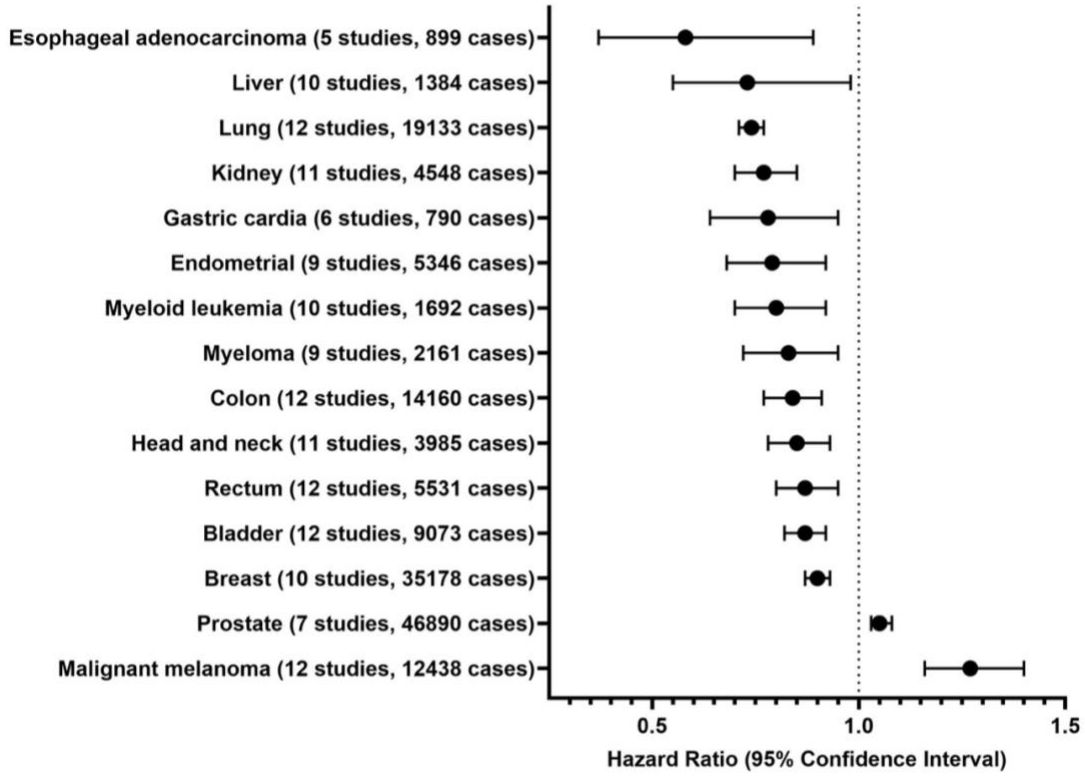

Figure 2: Hazard ratios for 15 types of cancer indicating significant positive or negative effects of exercise on cancer risk. Adapted from Moore et al., 2016 (Moore et al., 2016).

This study represents an important step in the epidemiological analysis of protective effects of exercise from cancer. Moore et al. could show that high levels of physical activity are associated with a lower risk for developing 13 types of cancer: Esophageal adenocarcinoma, liver, lung, kidney, gastric cardia, endometrial, myeloid leukemia, myeloma, colon, head and neck, rectum, bladder, and breast. The risk of developing two types of cancer is associated with high levels of physical activity: Prostate and Malignant melanoma. The authors claim that there is no biological rationale that could explain the association between physical activity and increased prostate cancer risk. However, they speculate that an increased likelihood of receiving digital rectal examinations in active compared to inactive men leads to a screening bias, which could explain the association. The authors explain the association between physical activity and increased melanoma risk similarly to Shepard et al. more than 20 years ago: Frequent outdoor physical activity leads to increased sun exposure.

Recently, Christensen et al. published a comprehensive literature review about the role of exercise in cancer control and treatment (Christensen et al., 2018). They analyzed nearly 700 exercise oncology studies with more than 50,000 cancer patients. And – whilst back in the 1940's, the most frequently analyzed cancer type in nutrition studies was breast cancer (Morris, 1945), this phenomenon still holds true today for exercise oncology studies. The authors summarize that exercise is safe and feasible for cancer patients, and exercise might have indirect as well as direct anti-cancer effects in patients. This detailed literature analysis can be regarded as one of the central studies of current exercise oncology as it shows strengths and limitations of exercise in the cancer continuum, which will be described in "1.3 Anti-cancer effects of exercise and potential mechanisms". The study also forms the basis for the development of the S3-guideline (highest standard of treatment-guidelines in Germany) "Bewegungstherapie bei onkologischen Erkrankungen" [1] (English: Exercise therapy for oncological diseases), which was registered in 2021 and is estimated to be finished in 2024 (Wiskemann and Baumann, 2020).

[1] https://www.awmf.org/leitlinien/detail/anmeldung/1/ll/032-058OL.html, accessed 30. April 2022

1.1 Cancer and pediatric cancer: Statistics in Germany and worldwide

Cancer is one of the main causes of death worldwide. It is therefore not surprising that globally, more than 4,600 organizations in over 100 countries fund cancer research (Schmutz et al., 2019). One can only speculate about the total amount of cancer research funding. Though in 2021, a detailed economic analysis was published by Rachel Abudu et al., which analyzed cancer research investments between 2006 and 2018 (Abudu et al., 2021). The authors summarized research funding of 120 partner organizations of the International Cancer Research Partnership (ICRP), which is a network of cancer research funding organizations aiming to enhance global collaboration. In 2018, these 120 organizations alone funded cancer research with 8.511 billion US dollars. The funding benefits six research fields, that are shown in **Figure 3** (Abudu *et al.*, 2021).

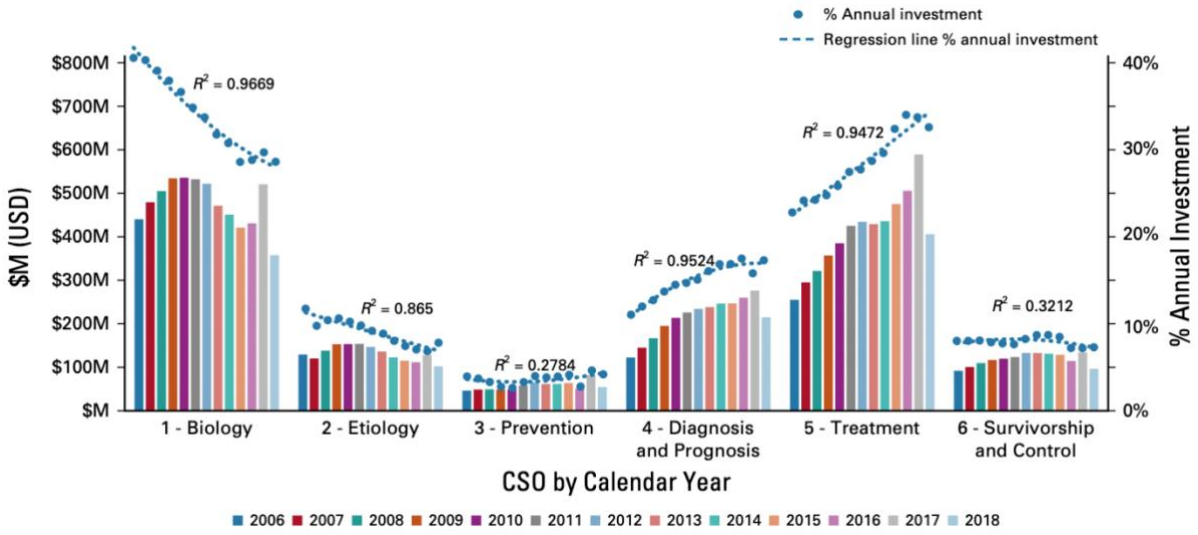

Figure 3: Annual investment of International Cancer Research Partnership (ICRP) organizations in cancer research; sorted by research fields. CSO = Common Scientific Outline.

Image from Abudu et al., 2021 (Abudu *et al.*, 2021) published in *American Society of Clinical Oncology* in *JCO Global Oncology*, CC BY 4.0 https://creativecommons.org/licenses/by/4.0/, download-link: https://ascopubs.org/doi/pdf/10.1200/GO.20.00591.

While annual investments in cancer diagnosis and prognosis as well as treatment increased between 2006 and 2018, investments in cancer biology and etiology decreased. Cancer prevention as well as survivorship and control are unchanged and are the research fields that receive least funding.

In their economic analysis, Abudu et al. also mention childhood cancer research and its funding. In 2018, ICRP organizations funded childhood cancer research with 584 million US dollars. Between 2006 and 2018, according to Abudu et al. childhood cancer research accounted for 5% of total cancer research fundings. **Figure 4** (Abudu *et al.*, 2021) shows ICRP investments in childhood cancer research and distinguishes between cancer types.

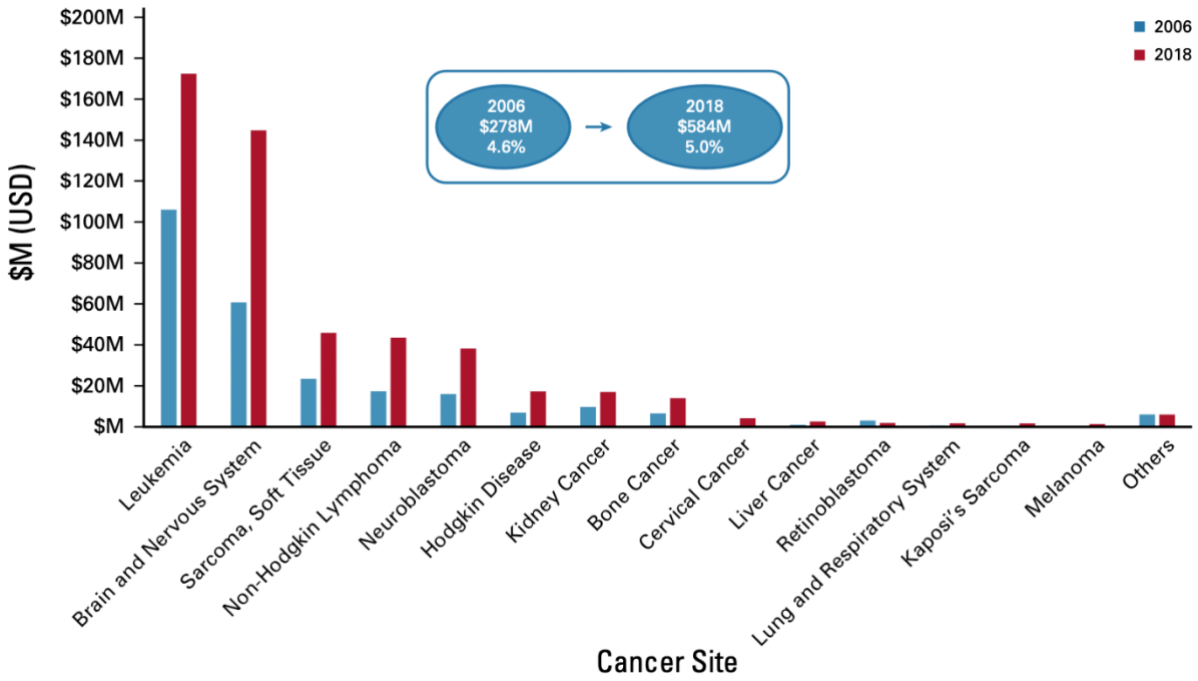

Figure 4: Investment of International Cancer Research Partnership (ICRP) organizations in childhood cancer research; sorted by cancer types. CSO = Common Scientific Outline.

Image from Abudu et al., 2021 (Abudu *et al.*, 2021) published in *American Society of Clinical Oncology* in *JCO Global Oncology*, CC BY 4.0 https://creativecommons.org/licenses/by/4.0/, download-link: https://ascopubs.org/doi/pdf/10.1200/GO.20.00591.

Cancer statistics worldwide

Leukemia and brain and nervous system tumors are the cancer types provided with the most funding in childhood cancer, followed by sarcomas, lymphomas and neuroblastomas. These tumor types are also among the most common childhood cancers. The World Health Organization (WHO) estimates that 400,000 children and adolescents between 0 and 19 years develop cancer each year[2]. Leukemia, lymphomas, brain cancer and solid tumors like neuroblastoma and Wilms tumor belong to the most common childhood cancer types (Steliarova-Foucher et al., 2017). In total, it is estimated that in the year 2020 19.3 million new cancer cases and nearly 10 million cancer deaths occurred (Sung et al., 2021). Among the most common types are breast, lung, prostate and colon cancer (Sung *et al.*, 2021), which are also the types provided with the most funding (Abudu *et al.*, 2021).

[2] https://www.who.int/news-room/fact-sheets/detail/cancer-in-children, accessed 30.04.2022

If the estimates of total cancer cases and childhood cancer cases are combined, childhood cancer makes up around 2.5% of total cancer cases worldwide. These numbers show that cancer incidence is rare in children compared to the adult population.

Cancer statistics in Europe

Despite the low incidence and mortality in children, cancer is still the most common cause of death by disease in children throughout Europe (Kyu et al., 2018). The International Association of Cancer Registries lists 35.000 children and adolescents in Europe that are diagnosed with cancer each year, of which 80% are disease free within five years (International Association of Cancer Registries). In total, the WHO estimates that europewide there are 3.7 million new cancer cases each year[3]. If the estimates of total cancer cases and childhood cancer cases are combined, childhood cancer makes up around 1% of total cancer cases Europewide. Medical and technical progress decreased childhood cancer mortality rates by 2.8% per year between 1990 and 2015 (Bertuccio et al., 2020).

Cancer statistics in Germany

In Germany, there are three cancer types listed in the so-called top ten causes of death: Lung, colorectal and breast cancer (Vos et al., 2020). Every two years, the Robert Koch Institute publishes statistics on cancer incidence and mortality in Germany. The most recent report covers statistics from 2017/2018, which shows that a total of nearly 500.000 new cancer cases were diagnosed in 2018 (Zentrum für Krebsregisterdaten und Gesellschaft der epidemiologischen Krebsregister in Deutschland e. V., 2021). Childhood cancer statistics in Germany are analyzed by the Kinderkrebsregister[4]. Their report for 2018 shows 2255 new cancer cases in children and adolescents younger than 18 years (Erdmann et al., 2020). They also show that leukemia, lymphomas and CNS tumors account for almost 70% of all cancer cases in this cohort. If the estimates of total cancer cases and childhood cancer cases are combined, childhood cancer makes up less than 1% of total cancer cases in Germany.

[3] https://www.euro.who.int/en/health-topics/noncommunicable-diseases/cancer/data-and-statistics, accessed 21.05.2022

[4] https://www.kinderkrebsregister.de, accessed 21.05.2022

1.2 Cancer hallmarks

Cancer is a genetic disease that results from mutations in specific cells-of-origin. More than 175 mutations occur during each cell cycle, even in healthy cells (Nachman and Crowell, 2000). Most mutations are so-called passenger mutations that do not affect cell behavior. Occasionally, driver mutations occur and cause the affected cell to acquire cancer cell behaviors known as the hallmarks of cancer (Hanahan and Weinberg, 2011). Driver mutations are typically spontaneous but sometimes also inherited gain-of-function mutations of oncogenes such as *KRAS* (oncogene in Kirsten RAt Sarcoma virus) or loss-of-function mutations of tumor suppressors such as the tumor protein p53 (gene name: *TP53*). Replication errors (i.e. errors that occur during the doubling of the DNA during the cell cycle) are the main cause of de novo mutations (Tomasetti et al., 2017; Tomasetti and Vogelstein, 2015).

Generally, there are two types of mutations based on the size of the affected DNA: Small mutations are restricted to single bases, called point mutations. In the case that a few bases are affected, these mutations are termed Indels. The second type of mutations are large structural mutations including gains and losses of parts of chromosomes or whole chromosomes (aneuploidy), gene fusions and major genomic rearrangements termed chromothripsis. Recent sequencing efforts have identified large numbers of small and structural mutations affecting hundreds of cancer genes (i.e. oncogenes or tumor suppressors) in both childhood (Gröbner et al., 2018; Ma et al., 2018) and adult cancer (Bailey et al., 2018; Lawrence et al., 2014).

When mutated cancer genes are expressed as mutated cancer-promoting proteins, cell behavior changes from normal to malignant. Rather than responding appropriately to the signals that control normal cell behavior, cancer cells grow and divide in an uncontrolled manner, avoid immune destruction, induce angiogenesis, have a remodeled metabolism, and eventually spread throughout the body. Collectively, these changed behaviors of cancer cells are the described hallmarks of cancer (Hanahan and Weinberg, 2011).

The hallmarks of cancer were first described by Hanahan and Weinberg in 2000 in *Cell* – where they described five abnormal capabilities of cancer cells (Hanahan and Weinberg, 2000). These capabilities seemed to be acquired by cells of all cancer types. A decade later, the authors refined the hallmarks of cancer and present a total of ten abnormalties in cancer cells, that are summarized in **Table 1** (Hanahan and Weinberg, 2011).

Table 1: Hallmarks of cancer as described by Hanahan and Weinberg in 2011 (Hanahan and Weinberg, 2011).

Hallmark	Description
Sustaining proliferative signaling	This is one of the must fundamental hallmarks of a cancer cell. While healthy tissues ensure homeostasis of cell number and therefore maintain normal tissue architecture and function, cancer cells sustain chronic proliferation.
Evading growth suppressors	The healthy human body controls cell proliferation by programs that depend on tumor suppressor genes, such as *Rb* or *TP53*. Cancer cells can circumvent these programs.
Emerging hallmark: Avoiding immune destruction	Cancer cells may avoid immune destruction by disabling components of the body's immune system, which was dispatched to eliminate them.
Enabling replicative immortality	In healthy cells, telomeres at the end of the chromosomes shorten continually during cell division, eventually triggering cell apoptosis. Cancer cells can maintain telomeric DNA, which is often achieved by an upregulation of telomerase expression.
Enabling characteristic: Tumor-promoting inflammation	Tumor-associated inflammation contributes to many hallmark capabilities. Bioactive molecules are supplied into the tumor microenvironment that among others include growth, proliferation and survival factors.
Activating invasion and metastasis	Tumor cells can expand to nearby environments or metastasize to a new location and form a secondary tumor.
Inducing angiogenesis	Just like normal tissues, tumors also require nutrients and oxygen as well as the ability to move out metabolic waste and carbon dioxide. In tumors, angiogenesis is chronically activated, leading to neovasculature.
Enabling characteristic: Genome instability and mutation	Genome instability is regarded as an enabling characteristic, which is associated with a cell's acquisition of hallmark capabilities. Tumor progression is driven by defects in genome maintenance and repair. Specific genome alterations differ between tumors.
Resisting cell death	In healthy cells, programmed cell death serves as protection from cancer. Loss of the tumor suppressor function of *TP53* limits apoptosis in tumor cells.

Emerging hallmark: Deregulating cellular energetics	Chronic and uncontrolled proliferation of tumor cells leads to an adjustment of energy metabolism to fuel cell growth and division. Cancer cells mainly produce energy by glycolysis (Warburg effect).

In 2022, Hanahan published his most recent work on cancer hallmarks in *Cancer Discovery* (Hanahan, 2022). This publication raises phenotypic plasticity and disrupted differentiation as potential hallmark capabilities of cancer cells. Additionally, Hanahan suggests nonmutational epigenetic reprogramming and polymorphic microbiomes as essential enabling characteristics for cancer.

In summary, the generalized loss of growth control exhibited by cancer cells is the net result of cancer gene-affecting mutations which lead to abnormalities in multiple cell regulatory systems and is reflected in several aspects of cell behavior that distinguish cancer cells from their normal counterparts. Research in exercise oncology needs to demonstrate if exercise can influence the hallmarks of cancer e.g. via direct effects on tumor cells or the immune system.

1.3 Anti-cancer effects of exercise and potential mechanisms

Physical exercise can lower the risk of developing cancer and counteract some of the adverse effects of the disease and its treatment. Therefore, exercise can be used as an adjunct treatment for cancer patients. The relationship between exercise and cancer can be divided into indirect and direct effects. Indirect effects of exercise on cancer patients are well established. In 2017, a systematic review of exercise systematic reviews in the cancer literature was published (Stout et al., 2017). This review shows that exercise can improve clinical, functional and survival outcomes in cancer patients. The authors state the overall safety of exercise in patients with all types of cancer but also recommend the screening of patients and precautionary measures. This is not only important in order to utilize the full potential of exercise effects on cancer treatment related side-effects, but also because exercise might affect cancer cells directly. The beneficial relation between physical exercise and cancer growth and progression has been shown in rodents (Eschke et al., 2019). Potential mechanisms that mediate the effects of exercise on cancer were analyzed in a review in 2017 (Thomas et al., 2017). The authors summarize the effects of exercise in the following **Table 2**.

Table 2: Anti-cancer effects of exercise and their mechanisms (Thomas *et al.*, 2017)

Effect of exercise	Mechanism
Cell growth regulation	IGF-1 ↓ , IGFBP3 ↑
Regulation of DNA damage repair proteins	BRCA1 ↑ , BRCA2 ↑
Androgen receptor coactivator regulation	*RAS* family oncogenes ↓
Apoptosis and cell cycle arrest	P53 ↑ , Heat shock proteins ↑
Hormone system regulation	Oestrogen ↓ , testosterone ↓ , vasoactive intestinal peptide ↓ , leptin ↓ , irisin ↑ , resistin ↓
Immune system regulation	Natural killer cells ↑ , white cells ↑
Inflammation	C reactive protein ↓ , interleukin-6, TNFα ↓ , prostaglandins ↓ , COX-2 ↓
Regulation of oxidative stress and antioxidant pathways	Glutathione, ↑ catalase and superoxide dismutase ↑

While the effects above describe physiological adaptions to exercise that influence cancer cell behavior, another potential anti-cancer effect of exercise is the enhancement of treatment efficacy and enhancement of drug tolerance by exercise. These effects have been described in two reviews from 2018 (Christensen *et al.*, 2018; Hojman et al., 2018). A potential explanation of the interaction of exercise with cancer treatment is reduced fat mass and therefore increased metabolically active tissue that can distribute cancer drugs (Christensen *et al.*, 2018). Additionally, improved vascularization from exercise leads to increased intratumoral blood perfusion, which could enhance anti-cancer therapy efficacy (Christensen *et al.*, 2018).

While reviews on the anti-cancer effects of exercise attempt to find mechanistic explanations for the beneficial role of exercise in the cancer continuum, it is important to state the potential bias in the exercise studies used in these reviews and the contrasting effects of catecholamines in particular. Exercise studies generally seem to analyze positive effects of exercise, which might lead to a blind spot regarding potential detrimental effects. This circumstance is best described by the role of catecholamines (adrenaline/noradrenaline) in cancer research. Mechanistic studies in exercise oncology use catecholamines to explain the beneficial effects of exercise for cancer patients. Catecholamines affect a cell via adrenergic receptors as illustrated in **Figure *5***. Adrenergic receptors (three groups: α1, α2 and β) are

G-protein coupled receptors, which can be found on cells throughout the body and also on cancer cells.

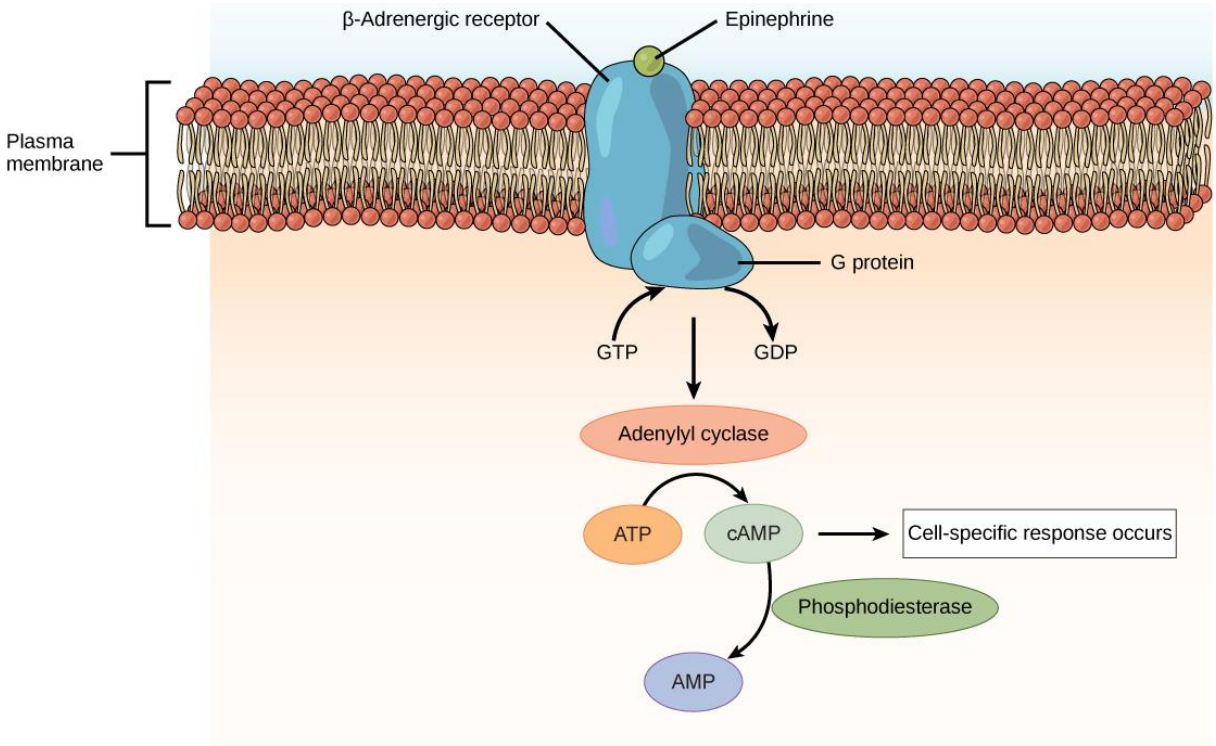

Figure 5: β-adrenergic signaling. Epinephrine binds to a β-receptor which is coupled to a G-protein, that stimulates or inhibits (depending on the G-protein) the enzyme adenylyl cyclase. The enzyme then converts ATP to cAMP, which triggers the cellular response (Hilger et al., 2018). Image from CNX OpenStax, CC BY 4.0 https://creativecommons.org/licenses/by/4.0, via Wikimedia Commons (download-link: https://commons.wikimedia.org/wiki/File:Figure_37_02_02.jpg)

Exercise studies suggest that adrenaline increases cell infiltration by natural killer cells and therefore tumor growth could be decreased (Pedersen et al., 2016). Another mediation of beneficial effects of exercise through catecholamines might be the activation of the hippo tumor suppressor pathway (Dethlefsen et al., 2017). The opposite argument can be found in stress studies. These studies use catecholamines to explain the detrimental effects of stress on cancer. Specifically, stress-induced catecholamines increase the expression of the *VEGF* gene, which enhances tumor vascularization and therefore increases growth and spread of ovarian carcinomas (Thaker et al., 2006). In pancreatic cancer, catecholamines promote tumor innervation via the secretion of neurotrophins, which leads to increased tumor growth (Renz et al., 2018a).

In summary, there are promising suggestions for potential direct anti-cancer effects of exercise. However, there is an obvious contradiction with regard to catecholamines and their role in

cancer cells. This emphasizes the need for further research. We term this contradiction the Cancer Catecholamine Conundrum (Wackerhage et al., 2021), which is one of the two rationales for our studies. The second rationale is the neglect of children in exercise oncology research.

1.4 Knowledge gap between adult and pediatric exercise oncology

Cancer in adults and children differs greatly in various aspects. Error! Not a valid bookmark self-reference. shows the characteristics of childhood and adult cancers and emphasizes the differences. It is important to note these differences, particularly if research on childhood cancer is based on current evidence regarding exercise-induced mechanisms in adults.

Table 3: Comparison of basic characteristics of childhood and adult cancer.

Aspect	Childhood cancer	Adult cancer	Differences
Cancer type	• Cells of origin: In all three germ layers of the embryo, mainly in mesodermal cells (i.e. blood, muscle, bones, kidneys and gonads) (Chen et al., 2015). • Highly specific to children: e.g., medulloblastoma, neuroblastoma and nephroblastoma (Kaatsch P, 2019).	• Cells of origin: Mostly epithelial cells (Downing et al., 2012). • Mesenchymal tumors (e.g. Sarcomas) less frequent • Highly specific to adults: carcinomas, particularly, breast, prostate, colon, and lung (Siegel et al., 2019).	→ Varying originate tissues → Incidence of cancer types depending on age
Survival	• 5-year survival rate: > 80% in developed countries, ranging from 47% (mixed and unspecified gliomas, neuroepithelial glial tumors of uncertain origin, oligodendrogliomas) to 99%	• 5-year survival rate of almost 70%, ranging from ≈9% in pancreatic cancer to ≈98% in prostate cancer or 90% in breast	→ Higher overall survival rates in childhood cancer patients

	(Hodgkin Lymphoma) (Kaatsch P, 2019).	cancer (Siegel *et al.*, 2019).	
Long-term effects	• Childhood cancer survivors are at high risk for treatment-related adverse long-term health outcomes (e.g. impaired pulmonary, auditory, endocrine-reproductive, cardiac and neurocognitive function) (Hudson et al., 2013). • Long period of survival and development of negative health outcomes increasing with age (Hudson *et al.*, 2013).	• Most common long-term effects are fatigue and mental health issues (Gegechkori et al., 2017).	→ Longer period of survival in survivors of childhood cancer → Focus on quality of survival and reduction on disease- and therapy-related negative long-term effects in children
Mutational rates	• Mutational rate: lower average mutational burden (Filbin and Monje, 2019; Gröbner *et al.*, 2018) due to fewer replication-dependent mutations (Nachman and Crowell, 2000).	• Mutational rate: higher average mutational burden (Gröbner *et al.*, 2018) due to mutagens (e.g. tobacco smoke) (Alexandrov et al., 2016), mutation of DNA repair genes (Campbell et al., 2017).	→ Lower mutational rate in children → Higher mutational rate and increased cancer risk in adults

The success in survival rates regarding childhood cancer has been achieved by a better understanding of the biology, improved diagnostics, stratification based on biomarkers, monitoring of disease and treatment (Hudson et al., 2012), and by the high adherence rate to clinical studies (Downing *et al.*, 2012). The drawbacks of the success rate are major long-term side effects (Filbin and Monje, 2019). The International Guideline Harmonization Group for

Late Effects of Childhood Cancer[5] studies long-term effects to optimize the quality of care and to improve the quality of life for childhood cancer survivors. Currently, around 37.000 survivors of childhood cancer participate in long-term follow-up programs in Germany (Kaatsch P, 2019), and more than 430.000 survivors of childhood cancer are living in the USA[6], representing 0.05% and 0.13% of the whole population, respectively.

In summary, both childhood and adult cancer are often initiated when cancer genes are exposed to driver mutations that further promote cancer hallmarks. However, cancer type, frequency, survival rates, mutation rates and mutated driver genes differ considerably between childhood and adult cancer. These age-specific distinctions need to be considered in mechanistic research.

[5] www.ighg.org, accessed 01.05.2022
[6] https://www.cancer.gov/types/childhood-cancers/ccss, accessed 01.05.2022

1.5 Aim of the research

Exercise oncology is the research field that analyzes effects of exercise on cancer. There is solid epidemiological evidence that exercise can lower the risk of developing several cancer types. Furthermore, exercise can counteract some of the adverse effects of cancer and its treatment and improve survival in cancer patients. This association between exercise and cancer does not necessarily indicate causality, which is why recently more research has been conducted on physiological mechanisms that mediate this relationship between exercise and cancer.

This dissertation contributes to awareness and addresses possible solutions to two central issues in the current field of exercise oncology.

1. There is a lack of evidence in children. Most exercise oncology research is conducted in murine models or adult cancer patients. Results of these cannot simply be transferred to the growing organisam of childhood cancer patients.
   The aim of my research is therefore to analyze differences between adult and pediatric exercise oncology and to investigate safety and physiological effects of exercise specifically in pediatric cancer patients.
2. Catecholamines seem to have contradicting roles in exercise and stress studies. While it is suggested that catecholamines mediate the positive effects of exercise on cancer, stress studies suggest catecholamines as mediators of the detrimental effect of stress on cancer. These contradicting effects of the same molecule warrant further research, as this might reflect an expectation bias in exercise oncology.
   The aim of my research is therefore to analyze whether the expression of adrenergic receptors mediate the effects of catecholamines on cancer cells.

To pursue the issues raised, this dissertation is based on three studies.

Study 1: Review on pediatric exercise oncology

I conducted a literature analysis to evaluate the differences between adult and pediatric exercise oncology in a narrative review. Specifically, I summarized psychosocial and physiological effects of exercise in adult and pediatric cancer patients. The aim of the study is to discuss potential mechanisms by which exercise might directly affect cancer cell behavior in adults and children and to point out research needs specifically in pediatric cancer patients.

Study 2: High-intensity exercise intervention with pediatric cancer patients

I conducted this intervention study to evaluate the safety and feasibility of a high-intensity exercise intervention in pediatric cancer patients. Furthermore, I analyzed the physiological effects of this intervention, which gave novel insights about this group. The aim of the study is to show opportunities and limitations of pediatric exercise oncology.

Study 3: Effects of noradrenaline on hallmarks in pediatric sarcoma cell lines

I conducted this cell culture study to evaluate the effects of noradrenaline on proliferation, migration and signaling in pediatric sarcoma cell lines. Specifically, we suggest adrenergic receptor expression of tumor cells to mediate the cellular response to noradrenaline. Therefore, I used cells with and without expression of adrenergic receptors. The aim of the study is to prove the concept of adrenergic receptor expression as a contribution to solving the Cancer Catecholamine Conundrum.

2 Methods

2.1 Cell culture and laboratory experiments

2.1.1 Laboratory kits

I used the following kits to conduct the experiments for this thesis.

Kit	Manufacturer	Reference
Adrenaline Competitive ELISA	LSBio	Cat # LS-F5372
Cyclic AMP Competitive ELISA	Thermo Fisher	Cat # EMSCAMPL
EdU cell proliferation HTS Kit	baseclick	Cat # BCK-HTS555-2
Culture-Insert 2 Well in µ-Dish 35 mm	ibidi	Cat # 81176

2.1.2 Chemicals and reagents

In addition to the provided chemicals and reagents in laboratory kits, I used the following reagents.

Reagent	Manufacturer	Reference
bisBenzimide H 33342 trihydrochloride	Sigma-Aldrich	Cat # B2261
Bovine Serum Albumin	Sigma-Aldrich	Cat # A9418
DEPC-treated Water	Thermo Fisher	Cat # 11597065
Dulbecco's Modified Eagle Medium	Thermo Fisher	Cat # 11965092
Fetal Bovine Serum, heat inactivated	Thermo Fisher	Cat # 10082147
Norepinephrine hydrochloride	Sigma-Aldrich	Cat # A7256
Penicillin-Streptomycin	Thermo Fisher	Cat # 15140122
Phosphate Buffered Saline	Thermo Fisher	Cat # 14190144
RPMI 1640 Medium	Thermo Fisher	Cat # 21875091
Sulforhodamine B	Thermo Fisher	Cat # S1307
Trichloroacetic acid	Sigma-Aldrich	Cat # T6399
Tris(hydroxymethyl)aminomethane	Sigma-Aldrich	Cat # 1.08382
Triton X-100	Thermo Fisher	Cat # 11498696
Trizol Reagent	Thermo Fisher	Cat # 15596018
Trypan Blue Solution, 0.4%	Thermo Fisher	Cat # 15250061
Trypsin-EDTA, 0.25% (1X)	Thermo Fisher	Cat # 11560626

2.1.3 Cell lines

For our cell culture experiments, we identified tumor cell lines from the same tumor type but with different adrenergic receptor expression. Based on expression data from the cancer cell line encyclopedia (CCLE), we chose the two sarcoma cell lines A673 and RD. While A673 cells express isoforms of all three adrenergic receptor groups, RD cells do not express adrenergic

receptors. We validated these expression data by RNA-Seq analysis. The A673 cell line was established from a patient with a Ewing-related tumor and shows fusion of genes *EWS* and *FLI1* (Martínez-Ramírez et al., 2003). The rhabdomyosarcoma cell line RD has mutations of *NRAS* and *TP53* genes and an amplification of the *MYC* gene (Hinson et al., 2013).

The cell lines we used for our experiments were authenticated by Eurofins Genomics using Applied Biosystems™ AmpFLSTRTM Identifiler™ Plus PCR Amplification Kit with 16 markers. **Table 4** shows results on Short Tandem Repeat (STR) DNA profiling, which authenticated the cell lines.

Table 4: STR DNA profiles authenticating A673 and RD cell lines

Markers of STR profile	A673 cell line	RD cell line
D8S1179	11,13	11,15
D21S11	29,30.2	28,29
D7S820	10,12	8,12
CSF1PO	11,12	10,11
D3S1358	14,14	15,17
TH01	9.3,9.3	9.3,9.3
D13S317	8,13	13,13
D16S539	11,11	10,11
D2S1338	16,21	17,23
D19S433	13,14	11,14
vWA	15,18	18,18
TPOX	8,8	9,9
D18S51	13,16	13,18
AMEL	X,X	X,X
D5S818	11,12	11,11
FGA	19,20	20,21

2.1.4 Noradrenaline

The noradrenaline concentration for our experiments was chosen based on a preliminary cell number assay. We treated A673 and RD cells in triplicates for 24, 48 and 72 hours with a 5x dilution series of noradrenaline concentrations (0.3 mM, 60 µM, 12 µM, 2.4 µM, 0.48 µM and 96 nM) or growth medium only as a negative control. Cell numbers were quantified using the sulforhodamine B (SRB) assay. Since A673 cells showed decreased cell numbers after being treated with 60 µM of noradrenaline, we used this concentration for further experiments.

We prepared a noradrenaline stock solution of 3 mM by dissolving 12.3 mg noradrenaline in 20 mL DEPC-treated water. We froze 1 mL aliquots of stock solutions at -20°C no longer than three months until they were used for experiments. We used growth medium to dilute the noradrenaline stock solution to the desired concentration. Growth medium for controls contained DEPC-treated water in the same ratio as the noradrenaline solution.

2.1.5 Effect of noradrenaline on cyclic AMP concentration in sarcoma cells

To analyze the effect of noradrenaline on cyclic adenosine monophosphate (cAMP) concentrations in the two cell lines A673 and RD, we seeded 350,000 cells per well in a 24-well-plate. After 24 hours, we treated the cells with 60 µM and 20 nM of noradrenaline for 5 and 30 minutes. Control samples were treated with growth medium and vehicle only. We induced cell lysis with 500 µL 0.1 M hydrogen chloride (HCl) and 1% Triton X-100. After 10 minutes, we centrifuged the plates at 600 x g at room temperature for 10 minutes and pipeted the supernatant in 1,5 mL Eppendorf tubes. Samples were frozen at -20°C and used for further analysis within one week. To quantify cAMP concentrations in all samples, we used the cAMP competitive enzyme-linked immunsorbent assay (ELISA) kit from Thermo Fisher™ with acetylating reagent. The acetylating reagent was prepared by diluting 0.5 mL of acetic anyhydride in 1 mL of triethylamine. We prepared a 4 x dilution series of acetylated cAMP standards in labeled glass tubes with cAMP concentrations of 20, 5, 1.25, 0.312 and 0.078 pmol/mL dissolved in 0.1 M HCl. The ELISA was done with at least two replicates per standard and sample and in accordance with the manufacturer's instructions. We analyzed optical density values using a Tecan Infinite™ M200 plate-reader at 405 nm with correction at 580 nm and calculated cAMP concentrations by data interpolation based on the standard curve's exponential function in Microsoft Excel™.

2.1.6 Effect of noradrenaline on cancer hallmarks in sarcoma cells

We analyzed the effect of noradrenaline on cell proliferation and migration in A673 and RD cells. To measure cell proliferation, we used 5'-ethynyl-2'-deoxyuridine (EdU) incorporation of the EdU Cell Proliferation Kit for Imaging (EdU-Click 555) during the last four hours of a 72 hour incubation period. Cells were additionally dyed with bisBenzimide H 33342 trihydrochloride to visualize living cells and we counted at least 2,000 cells in three random view fields per experiment. We used 35 mm Culture-Insert 2 Well in µ-Dishes from ibidi to measure the effect of noradrenaline on migration of A673 and RD cells. We first seeded 50,000 cells in the two chambers of the silicone insert. After 24 hours, cells reached confluence of up to 100% and we removed the silicone insert to uncover a 500 µm cell free gap. We then treated the cells with 60 µM of noradrenaline or growth medium (control) and took microscopy images using a Zeiss Axiovert 100 at 10x magnification directly after treatment as well as 24,

48 and 72 hours later. Images were analyzed calculating the percentage of scratch open area using the ibidi analysis software FastTrack AI.

2.1.7 Transcriptomics and proteomics analyses

To analyze gene expression changes induced by noradrenaline, which could explain the effect of noradrenaline on cancer hallmarks in A673 cells, we performed transcriptomic and proteomic analyses. We seeded 2 x 10^6 cells per petri dish (150 mm) in quadruplicates and incubated the dishes at 37°C and 5% CO_2 for 24 hours. After cells adhered to the plastic, we treated the cells with 60 µM of noradrenaline for 24 or 48 hours or with growth medium for 48 hours (control). We obtained each sample for RNA-sequencing and proteomics analysis from the same petri dish. After washing with phosphate-buffered saline (PBS), we pelleted the cells with a cell scraper. Proteomics analysis was conducted by the Bavarian Center for Biomolecular Mass Spectrometry (Freising, Germany). For RNA-sequencing, we extracted RNA with Trizol and the RNA was sequenced by Novogene (Cambridge, UK).

2.1.8 Statistics

Data was analyzed using the SigmaPlot v13 software tool and illustrated using the PRISM 9.2.0 tool (GraphPad, San Diego, California, USA). In our studies we used descriptive and inferential statistics. Pre/post exercise differences between average values were calculated using a two-tailed paired *t*-test. Two-way ANOVA with multiple comparison Turkey test or unpaired t-test with Holm-Šídák method were used to calculate *p*-values for differences between treated and untreated cells. Normality was proven by the Shapiro–Wilk Test. Significant difference was determined by $p < 0.05$.

3 Results

In this section, I present the three studies I conducted together with my co-authors to analyze physical activity and its opportunities in childhood cancer patients.

In the first publication, I discuss the current state of literature in pediatric exercise oncology. Adult cancer research suggests beneficial effects of exercise on cancer patients and potential direct effects on cancer cells. Research in pediatric exercise oncology is scarce, which is why I conducted the literature analysis as a narrative review and summarize psychosocial and physiological effects of exercise in adult and childhood cancer.

In my second publication, I analyze safety and feasibility as well as physiological effects of a high-intensity interval exercise intervention in childhood cancer patients. This is to my knowledge the first study of its kind. Since children are not simply smaller adults, it is important to conduct independent exercise research in this group and use findings from adult exercise oncology as a basis. My study shows opportunities and limitations of this approach.

In my third study, I focused on the effect of noradrenaline – which is released during exercise and is associated with positive effects of exercise on cancer in adults – on pediatric tumor cell behavior. I conducted cell culture experiments and analyzed cell proliferation, migration and signaling as well as gene expression responses to noradrenaline. My research can be a basis for the development of tailored exercise prescriptions, as it provides proof-of-concept that adrenergic receptor expression may be a reason for variable effects of catecholamines in childhood cancer patients.

3.1 Study 1: Exercise as a Potential Intervention to Modulate Cancer Outcomes in Children and Adults?

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Conflict of Interest:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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This review is dedicated to Pernille Hojman who recently passed away. She has been a leader in the exercise oncology field and has greatly inspired and influenced our research.

Individual Contribution:

I am the first author of this paper together with Sabine Kesting. This work resulted from a collaboration of the chair of exercise biology (TUM) with the Klinikum Munich Schwabing. I did the main work on the comprehensive literature research together with Sabine Kesting, we extracted data from publications and wrote the first draft of the manuscript together with Henning Wackerhage and Irene von Lüttichau. Myself together with Sabine Kesting managed the writing process. Henning Wackerhage and Irene von Lüttichau conceptualized the idea for this manuscript. Myself together with Sabine Kesting managed the submission and review process. All authors read and approved the final manuscript.

Abstract

Exercise is recommended for the healthy population as it increases fitness and prevents diseases. Moreover, exercise is also applied as an adjunct therapy for patients with various chronic diseases including cancer. Childhood cancer is a rare, heterogeneous disease that differs from adult cancer. Improved therapeutic strategies have increased childhood cancer survival rates to above 80% in developed countries. Although this is higher than the average adult cancer survival rate of about 50%, therapy results often in substantial long-term side effects in childhood cancer survivors. Exercise in adult cancer patients has many beneficial effects and may slow down tumor progression and improve survival in some cancer types, suggesting that exercise may influence cancer cell behavior. In contrast to adults, there is not much data on general effects of exercise in children. Whilst it seems possible that exercise might delay cancer progression or improve survival in children as well, there is no reliable data yet to support this hypothesis. Depending on the type of cancer, animal studies of adult cancer types show that the exercise-induced increase of the catecholamines epinephrine and norepinephrine, have suppressive as well as promoting effects on cancer cells. The diverse effects of exercise in adult cancer patients require investigating whether these results can be achieved in children with cancer.

Introduction and Background

Childhood cancer contributes about 1% to all malignant diseases worldwide (Bhakta et al., 2019) and the incidence rate of cancer in children aged 0-19 years in the USA from 1975 to 2016 varies between 14.7-19.3 cases per 100.000 per year with an increasing trend (Noone et al., 2018). Thanks to more effective therapies, the 5-year survival has increased to 84.8% in the USA in 2016 in children aged 0-19 years (Noone *et al.*, 2018). The success in survival rates regarding childhood cancer has been achieved by a better understanding of the biology, improved diagnostics, stratification based on biomarkers as well as monitoring of disease and treatment (Hudson *et al.*, 2013) and by the high adherence rate to clinical studies (Downing *et al.*, 2012). The drawback of the success rate are major long-term side effects (Filbin and Monje, 2019). The International Guideline Harmonization Group for Late Effects of Childhood Cancer studies long-term effects to optimize the quality of care and to improve the quality of life for childhood cancer survivors (www.ighg.org). Currently, around 37.000 survivors of childhood cancer are subjected to long-term follow-up programs in Germany (Kaatsch P, 2019) and more than 430.000 survivors of childhood cancer are living in the USA (National Cancer Institute, 2018), representing 0.05% and 0.13% of the whole population, respectively. The incidence of childhood cancer is similar in Germany and the 15-year survival rate under the age of 15 has reached 82% (Kaatsch P, 2019). Worldwide, the incidence of childhood (0-14 years) cancer is 141 per million person years (Steliarova-Foucher *et al.*, 2017). This means that 215.000

children under the age of 15 and 80.000 adolescents between the age of 15 and 19 are globally diagnosed with cancer per year (Steliarova-Foucher *et al.*, 2017). Adult and childhood cancer not only differs regarding incidence and survival, but also with respect to biological features (Tricoli and Bleyer, 2018). This needs to be considered when transferring exercise oncology evidence from adults to children.

Physical activity and structured exercise programs not only help to prevent many chronic diseases, but exercise can also be an effective additive treatment strategy (Pedersen and Saltin, 2015). This might also be true for cancer. An analysis of 1.44 million adults demonstrates an association between physical activity and reduced risk for at least 13 types of cancer (Moore *et al.*, 2016). Exercise is a safe and feasible intervention and improves the psychosocial and physiological wellbeing of cancer patients. Moreover, observational studies suggest that exercise may slow down tumor progression and increase survival in specific types of cancer (Christensen *et al.*, 2018; Hojman *et al.*, 2018).

Whilst the effects of exercise on adult cancer patients are increasingly characterized (Christensen *et al.*, 2018), far less is known about the effects of exercise in children with cancer (Baumann et al., 2013; Braam et al., 2016; Morales et al., 2018; Rustler et al., 2017; West et al., 2019). In this narrative review, we summarize the psychosocial and physiological effects of exercise in adults and childhood cancer patients. Eventually, we discuss potential mechanisms by which exercise may directly affect cancer cell behavior. Because there is hardly any research published on mechanisms by which exercise affects childhood cancer cells, we also point out known mechanisms that could potentially be exploited to target childhood cancer.

Effects of Exercise Training in Cancer Patients

Exercise not only improves fitness and prevents disease but is also an effective treatment strategy for many chronic diseases (Pedersen and Saltin, 2015). In their comprehensive review, the authors summarize the evidence for the prescription of exercise as therapy in 26 different diseases including psychiatric, neurological, metabolic and cardiovascular diseases, musculo-skeletal disorders and cancer. In recent years exercise training has been increasingly utilized in cancer patients and in a position statement the Clinical Oncology Society of Australia (COSA, 2018) now recommends that exercise should be embedded as part of standard practice in cancer care. We divide the effects of exercise in cancer patients before, during and after treatment in two categories:

General Effects: Exercise has widely been shown to influence quality of life as well as physical health. Examples for general effects of exercise training are an increase of cardiorespiratory fitness, increased muscular mass or a decreased cancer-induced fatigue (Christensen *et al.*, 2018).

Direct Effects: Exercise-regulated factors may directly affect features of cancer cells, such as self-sufficiency in growth signals, evading apoptosis or inducing angiogenesis, which are known as the hallmarks of cancer (Hanahan and Weinberg, 2011) or they may contribute to recruiting immune cells towards the tumor (Pedersen *et al.*, 2016).
Research in exercise oncology has traditionally focused on the general effects of exercise in cancer patients. However, recent mechanistic and functional studies have reported direct effects of exercise on cancer cells (Hojman *et al.*, 2018; Ruiz-Casado et al., 2017; Thomas *et al.*, 2017). These direct effects will be described in chapter 4 “Direct effects of exercise on cancer cells” of this review. The exercise oncology literature has recently been summarized in a systematic review of 679 exercise intervention studies of 50.112 mainly adult patients suffering from 25 different types of cancer (Christensen *et al.*, 2018). This review and other reviews on exercise and cancer (Bourke et al., 2015; Fuller et al., 2018; Furmaniak et al., 2016; Scott et al., 2018) conclude that:

- Exercise training is a safe and feasible intervention in different types of cancer during and after cancer therapy.
- Exercise training can improve body composition, cardiovascular fitness, muscle strength, psychological wellbeing, health-related quality of life, and can reduce depression incidence, fatigue and helps to prevent or treat cancer cachexia (Lira et al., 2014; Re Cecconi et al., 2019). Exercise can also positively influence long-term adverse events of treatment in cancer patients such as weight gain, metabolic dysfunction, endocrine disturbances, cardiotoxicity and the risk of developing cardiovascular diseases.

General Effects of Exercise Training in Children and Adults with Cancer

Because childhood cancer is a rare and heterogeneous disease, there are few studies typically with fewer subjects, mixed cancer types and of a lower methodological quality on the effect of exercise in children with cancer when compared to studies in adult patients. Specifically, in the review of Christensen et al., only 32 out of 679 of the analyzed studies were conducted in children with cancer (Christensen *et al.*, 2018). Four reviews have summarized the findings on exercise in children with cancer based on just 30 studies covering all major childhood cancer types (Baumann *et al.*, 2013; Braam *et al.*, 2016; Morales *et al.*, 2018; Rustler *et al.*, 2017). Most of the studies were conducted in patients treated for acute lymphoblastic leukemia

representing the most often diagnosed childhood cancer. The conclusions of these four reviews for exercise in children are described together with findings in adult cancer patients. By presenting current evidence existing in adult versus childhood cancer patients together, we aim to facilitate the assessment of differences.

Safety and Feasibility: Similar to adults with cancer across the broad range of entities and phases of therapy (Christensen *et al.*, 2018), exercise is a safe and feasible intervention in children during acute inpatient care (Rustler *et al.*, 2017) and after cancer treatment (Braam *et al.*, 2016). This conclusion is based on the finding that none of the included studies reported any adverse events (Baumann *et al.*, 2013; Morales *et al.*, 2018).

Physical Outcomes: A meta-analysis of 34 RCTs including 4.519 adult patients with cancer shows that exercise significantly improves physical fitness both during and after treatment (Buffart et al., 2017). Different demographic or clinical characteristics did not affect the effectiveness of exercise on physical fitness. In children with cancer, exercise may positively influence the physical fitness, but mainly in patients with acute lymphoblastic leukemia, within an age range of one to 18 years (Baumann *et al.*, 2013; Braam *et al.*, 2016). Three out of five studies reported a significant increase of muscle strength after combined exercise training including mobility, resistance and aerobic training in patients aged four to 16 years during treatment (Fiuza-Luces et al., 2017; Marchese et al., 2004; Morales *et al.*, 2018; Tanir and Kuguoglu, 2013). Similarly, cardiorespiratory fitness assessed for example through a 9-minute walking test significantly increased in two out of five studies in patients with acute lymphoblastic leukemia, aged five to 12 years (Morales *et al.*, 2018; Moyer-Mileur et al., 2009; Tanir and Kuguoglu, 2013). There is a trend that a combined exercise training intervention including mobility, aerobic and resistance training increases flexibility in joints and muscles in patients aged one to 17 years and during treatment of acute lymphoblastic leukemia, but the results are not significant (Braam *et al.*, 2016; Morales *et al.*, 2018). Exercise has no clear effect on body composition. Hartman et al. reported a favorable reduction of the body mass index and body fat in the exercise versus the control group in a 2-year supervised and home-based exercise program including mobility, endurance and functional training during treatment for acute lymphoblastic leukemia aged one to 17 years, but this was also not significant (Hartman et al., 2009). Morales et al. (Morales *et al.*, 2018) found that three other studies did not show any beneficial impact of a combined aerobic and resistance training on the body mass index or bone mineral density in patients aged one to 19 years treated for acute lymphoblastic leukemia. In summary, exercise can somewhat improve the physical fitness of children with cancer. Presumably, to achieve measurable training adaptations, exercise interventions must

go beyond playful low intensity activities and include longer periods of moderate or vigorous intensity, which might be a challenge when dealing with children with cancer in a hospital.

Psychosocial Outcomes: Comprehensive reviews and meta-analyses have shown that exercise has small but significant effects on health-related quality of life, depression and fatigue in adult cancer patients (Buffart *et al.*, 2017; Christensen *et al.*, 2018; Mishra et al., 2012). Mainly breast cancer patients were studied, but effects are also convincing in other types of cancer, such as prostate and hematological cancer (Christensen *et al.*, 2018). In contrast, there are no clear effects of exercise on psychosocial variables in children with cancer. Baumann et al. (Baumann *et al.*, 2013) concluded in their review regarding childhood cancer patients that there are positive effects of exercise on fatigue and health-related quality of life during treatment and survivorship. Braam et al. (Braam *et al.*, 2016), who concluded that exercise does not significantly affect fatigue nor health-related quality of life in the reviewed randomized control trials in childhood cancer patients, contrast these data. Thus, at this point we need more experimental data to substantiate the evidence on this topic in children.

Mortality and Relapse: Physical activity is associated with improved survival (Ballard-Barbash et al., 2012) and reduced recurrence of breast (Holmes et al., 2005), prostate (Kenfield et al., 2011) and colorectal cancer (Meyerhardt et al., 2006) in adults. In children, not enough cases have been studied to reliably judge whether exercise has an effect on survival or relapse. In a systematic review and meta-analysis of eight randomized control trials including 283 childhood cancer patients, the authors stated that exercise has neither positive nor negative effects on mortality or relapse risk (Morales *et al.*, 2018).

In summary, exercise in children with cancer is understudied and there is an urgent need for high quality basic and translational research to exploit the effects of exercise in childhood cancer, thereby hopefully gaining better evidence on this topic. Currently, conclusions whether exercise in children with cancer has similar general effects as in adult cancer patients cannot be drawn. Moreover, it is important to identify joyous exercise programs specifically for in-patients that deliver a sufficiently high dose of resistance and endurance exercise for biological adaptions in children. These programs need to be playful and motivating using e.g. balls, obstacles and suitable equipment for smaller children as well as appropriate tools for adolescents, e.g. dumb bells, cable pull and ergometers for short bouts of high intensity training. The differentiation and age-appropriate adaption of exercise programs between children and adolescents might be beneficial. The group of adolescents and young adults (so called AYAs) is still understudied and robust evidence regarding specific and health-beneficial exercise programs is lacking (Munsie et al., 2019). In adults, exercise seems to have a dose-dependent

effect on cancer-specific mortality; however, the optimal dose of exercise is not known for adults nor for children (Patel et al., 2019). A special focus of future childhood exercise oncology studies should be to investigate whether exercise can be employed to reduce the often life-long consequences that are caused by highly toxic or mutilating treatments such as chemotherapy, surgery and radiotherapy delivered to a growing organism (Downing *et al.*, 2012). Comprehensive and confirmatory long-term studies with childhood cancer patients subjected to exercise programs already during treatment are still missing due to the short period of implementation. Based on these investigations, clear training recommendations regarding the different types of exercise to target specific toxicities are required. The first promising results of an exploratory analysis suggest a mitigation of cardiovascular diseases and an improvement of bone mineral density in childhood cancer survivors due to adequate levels of physical activity (Sloof et al., 2019).

Direct Effects of Exercise on Cancer Cells

Much of the early research in exercise oncology has focused on the general effects of exercise in cancer patients. In contrast, recent basic research studies aim to identify molecular and cellular mechanisms by which physical activity or exercise training might directly change cancer cell behavior. These studies all investigate adult cancer types.

Animal Models

Observational studies in animal models suggest that exercise has direct effects on cancer cells. Generally, the exercising muscles and other organs will alter the molecular and cellular composition of blood and thereby potentially influence tumor cells. Additionally, effects of exercise may be directly mediated by the nervous system, a part of the tumor microenvironment gaining increasing attention in cancer research (Hayakawa et al., 2017; Magnon et al., 2013; Renz *et al.*, 2018a; Renz et al., 2018b; Zhao et al., 2014). A recent systematic review analyzed mechanisms of aerobic exercise on cancer initiation, progression, and metastasis in animal models (Ashcraft et al., 2016). They included a total of 53 studies and reported a considerable methodological heterogeneity, which impedes reliable comparison. Most of the reviewed studies worked with voluntary running, forced running or swimming. The majority of studies analyzed incidence and growth and found that exercise inhibits tumor initiation as well as growth in most instances. Three studies used models where metastases arose from primary tumors, that reported non-significant tumor inhibition in two cases (Jones et al., 2012; Yan and Demars, 2011) and in one case accelerated tumor growth (Gershbein et al., 1974) with exercise. The authors conclude that the variety of outcomes is a result of poor methodological consistency and therefore they provide methodological and data reporting standards for preclinical studies in exercise oncology.

Human Studies

In humans, exercise not only increases the concentration of many metabolites e.g. lactate (Manaf et al., 2018), proteins (Schild et al., 2016), packed proteins in extracellular vesicles (Whitham et al., 2018) or hormones such as catecholamines (Von Euler and Hellner, 1952; Zouhal et al., 2008). Exercise also affects blood cell numbers, especially those of immune cells, possibly altering immune responses in relation to time and intensity of exercise (Nieman and Wentz, 2019; Pedersen and Hoffman-Goetz, 2000). However, there is limited data on the effects of exercise on the immune system in children with cancer (Baumann *et al.*, 2013). One study demonstrated a significant increase of natural killer cell cytotoxicity in children with mixed tumor entities undergoing an allogeneic hematopoietic stem cell transplantation subjected to a 10-week exercise program with 60 minutes of combined aerobic and strength training with moderate-to-vigorous intensity (Chamorro-Vina et al., 2017). Another study only found a trend toward an interaction effect for natural killer cells expressing the immunoglobulin-like receptor KIR2DS4 in children with solid tumors during chemotherapy treatment conducting an exercise program including aerobic and strength training of at least 60 minutes with moderate-to-vigorous intensity for 20 weeks on average (Fiuza-Luces *et al.*, 2017). Finally, exercise affects systemic and organ blood flow and body core temperature (Hawley et al., 2014). This conceptual framework for the direct effects of exercise on cancer is illustrated in **Figure 6**.

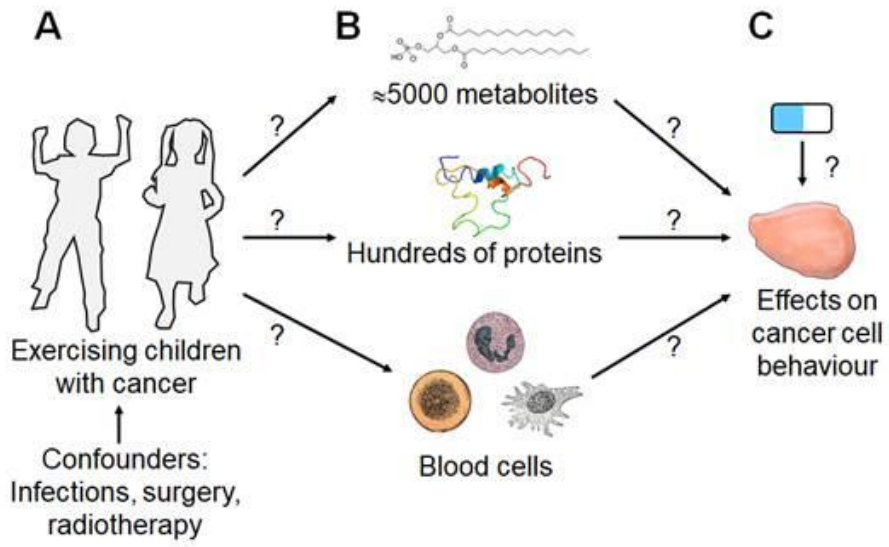

Figure 6: Conceptual framework of exercise-induced, blood-mediated effect on cancer cell behavior. **A** Structured exercise training of a child will affect the concentration of **B** blood components such as metabolites, proteins and blood cells. **C** Whilst blood changes will not correct the driver mutations that cause cancer, there is evidence that exercise-conditioned blood can affect the behaviors or hallmarks of cancer cells and may alter the responsiveness of cancer cells to anti-cancer drugs. [Blood cell images are from Gray's anatomy and in the public domain. The IGF-1 protein structure, shown above the "Hundreds of proteins" is from https://commons.wikimedia.org/w/index.php?curid=8820088. All other figures are drawn by HW].

The molecular mechanisms by which exercise can affect adult cancer cell behavior have been discussed extensively in several recent reviews. Direct cancer effects in adult patients include influence on proliferation, signal transduction, cancer metabolism, inflammation and cancer-immune system interactions (Hojman *et al.*, 2018; Ruiz-Casado *et al.*, 2017; Thomas *et al.*, 2017). Here, we describe an example of direct cancer effects of exercise with potential importance for childhood cancer.

Direct Effects of Exercise on Cancer Cells

Human Studies

The exercise-induced stress hormones epinephrine (British English: adrenaline) and norepinephrine (British English: noradrenaline) not only regulate many acute adaptations to exercise but can also affect cancer cell signaling and behavior. The stimulation of catecholamines by exercise was first described in the early 1950s (Von Euler and Hellner, 1952). Generally, the blood concentrations of epinephrine and norepinephrine increase with the intensity and duration of exercise (Zouhal *et al.*, 2008), in both children and adolescents (Lehmann et al., 1982). There are no major differences in the catecholamine response to exercise in children and adults (Riddell, 2008). Epinephrine and norepinephrine bind to α1,2 or β1,2,3 adrenergic receptor isoforms (Cole and Sood, 2012). Notably, many cancer cells express adrenergic receptor isoforms. Catecholamines binding to their receptors can potentially modulate inflammation, angiogenesis, tissue invasion, epithelial-to-mesenchymal transformation and affect cellular immune responses in cancer cells (Cole and Sood, 2012). Evidence that catecholamine signaling is important in cancer originates in epidemiological studies that report an association between β-blocker medication and reduced tumor progression (Cole and Sood, 2012). In children, β-blockers are used as a first-line treatment for infantile hemangioma (Bayart and Brandling-Bennett, 2015).

Animal Models and Cell Culture Studies

In addition to humans, Pedersen et al. demonstrated that voluntary running of mice before but not after injection of B16, melanoma cells reduced the growth and volume of the developing tumors. Moreover, there were more natural killer cells in the xenotransplant tumors of the mice that had exercised. Further experiments demonstrated that this was dependent on catecholamines as receptor blockade with the unselective β-blocker propranolol prevented this effect (Pedersen *et al.*, 2016). In a further cell culture based study, the Hojman group published data suggesting that catecholamines can inhibit Hippo effector YAP (yes-associated protein) in breast cancer cells, a known regulator of proliferation (Yu et al., 2013; Yu et al., 2012). The Copenhagen team who provided evidence that catecholamines may mediate some of the

direct exercise effects in breast cancer followed this up (Dethlefsen *et al.*, 2017). Together, this data suggests that exercise-induced modulation of catecholamines can directly inhibit cancer cell seeding and growth.

In contrast, Renz et al., who found that chronic restraint stress - not exercise-induced -, promoted the release of catecholamines in *KRAS*-mutant mice thereby significantly increasing incidence of pancreatic adenocarcinomas. Moreover, they also reported observational data that pancreatic adenocarcinoma patients treated with unselective beta-blockers for other reasons survived significantly longer than patients that did not receive this specific medication (Renz *et al.*, 2018a). These data suggest that catecholamines may have adverse effects on a subgroup of malignant tumors or at least in different circumstances. This finding is in line with other basic and clinical studies that report an association between β-blocker medication and better survival in ovarian cancer in mouse models (Thaker *et al.*, 2006; Watkins et al., 2015).

In summary, current evidence suggests that catecholamines can have both positive and negative effects on cancer cells presumably depending on circumstance as well as on the type of cancer. The fact that exercise reduces cancer risk, may reduce its progression and improve survival in humans (Christensen *et al.*, 2018; Hojman *et al.*, 2018; Moore *et al.*, 2016) suggests that either catecholamines inhibit cancer progression in most cases or that tumor promoting effects of catecholamines are compensated by other exercise-induced factors. For example, in a B16F10 melanoma xenotranplant mouse the tumors grow significantly more in the exercising than in the control mice (Schadler et al., 2016). A key research focus in childhood cancer should therefore be to study the effect of catecholamines on childhood tumors. Furthermore, mechanistic research should divide the group of adults according to their age, since exercise seems to have different effects in different age-groups (Zouhal *et al.*, 2008).

Concluding Remarks

This narrative review summarizes the effects of exercise as an adjunct therapy in cancer patients. In adult cancer patients, exercise is an effective intervention to trigger many general, indirect effects that improve the condition and physical performance of the patient. However, this evidence is mainly based on studies with breast cancer patients. Therefore, further investigations are needed for other cancer types and the optimal dose of exercise. Moreover, there is increasing evidence that exercise also directly affects cancer cells. In children, these issues have not been sufficiently addressed.

Recent publications have shown that exercise can improve patient outcome in many different ways. The idea of using exercise to put a cancer patient into a “sweet spot” where drug

effectiveness may be increased, toxicity reduced and life quality maintained or improved has not been explored extensively so far and therefore may be worth testing.

Consequently, the following issues should be a focus for researchers working in the field of childhood exercise oncology to gain knowledge especially regarding mechanisms by which exercise may influence childhood cancer cell lines.

Future Research Needs

- Based on the finding that the general effects of exercise in children with cancer are small, a focus of future research should be to design and evaluate specific, joyous exercise interventions that allow delivering a higher dose of moderate-to-vigorous exercise to trigger clinically meaningful general adaptations in children with cancer.
- Future studies should also seek to determine whether exercise interventions can be used to protect children from the negative long-term consequences that result from treating a growing organism with aggressive therapies.
- Large, multi-centre high quality studies should be performed to confirm whether exercise slows cancer progression and improves survival in children as has been shown in some adult tumors (Christensen *et al.*, 2018; Hojman *et al.*, 2018).
- As reliable epidemiological data on exercise and childhood cancer will take a long time due to the low case numbers, one strategy could be to use childhood cancer cell lines or childhood patient-derived murine xenotransplant models to study how exercise, exercise-conditioned sera or specific exercise-induced blood factors alter the behavior of childhood cancer cells.
- Research on childhood cancer and exercise may even play an exemplary role in some respects regarding cancer specific processes. Due to a less complex mutational landscape and a less immunogenic character of childhood cancer entities compared to adult cancer, especially the influence of exercise on the immune system should be investigated further to reveal novel insights.
- Some tumors (e.g. cerebellar tumors) already originate during early embryonal development (Jessa et al., 2019; Vladoiu et al., 2019). Therefore, in children, cancer prevention through lifestyle changes should not be the main focus. However, considering the emerging evidence it could be assumed that exercise induced manipulation of tumor cells by influencing the metabolome and proteome could potentially be implemented to reduce relapse, mitigate late effects and facilitate therapy.

3.1.1 Discussion of Study 1

The aim of my review was to provide an overview of exercise oncology research with a focus on childhood cancer patients. My literature research revealed a considerable knowledge gap in this cohort compared to adult cancer research. I found that exercise can improve the physical fitness of children with cancer. In adults, exercise has been shown to have beneficial effects on treatment-related side effects and might even affect cancer cells directly. However, research is not sufficient to determine whether exercise has similar beneficial effects in children.

A major aim of pediatric exercise oncology is the support for survivors of childhood cancer and the reduction of longterm adverse effects of cancer treatment. Children have a high probability of surviving the disease but this comes with the drawback of many longterm side-effects. The important role of exercise in the aftercare of childhood cancer survivors has recently been summarized (Morales et al., 2021).

This first study served as a foundation for my subsequent studies. My review confirms that more research is needed specifically in childhood cancer patients. While research in pediatric exercise oncology is conclusive about the overall safety of physical activity programs, it is uncertain whether higher intensities of exercise are also feasible in this cohort and how such an intervention would affect the physiology of these patients. Therefore, I then conducted an intervention study with a high-intensity interval exercise to analyze safety and feasibility of such a program and to assess physiological parameters such as heart rate, lactate and adrenaline.

My cell culture experiments build upon the effects of catecholamines on cancer cells, that I also mention in my review. Outside the exercise oncology field, stress studies indicate that catecholamines might be mediators of the detrimental effect of stress on cancer (Renz *et al.*, 2018a; Thaker *et al.*, 2006). This contradicting role of catecholamines is what we term the Cancer Catecholamine Conundrum (Wackerhage *et al.*, 2021). Since I found very little mechanistic research in pediatric exercise oncology for my review, I used pediatric tumor cell lines for my cell culture experiments to contribute to a better understanding of physiological mechanisms of exercise specifically in children with cancer.

3.2 Study 2: A Bout of High-Intensity Interval Training (HIIT) in Children and Adolescents during Acute Cancer Treatment—A Pilot Feasibility Study

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Conflict of Interest:
The authors declare that they have no competing interests. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Institutional Review Board Statement:

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the School of Medicine of the Technical University of Munich (protocol code 535/17 S; 16 February 2018 and Amendment 25 March 2019).

Informed Consent Statement:

Informed consent was obtained from all subjects involved in the study and their legal guardian in the case of underaged participants.

Individual Contribution:

I am the first author of this paper together with Sabine Kesting. This work resulted from a collaboration of the chair of exercise biology (TUM) with the Klinikum Munich Schwabing. Myself together with Sabine Kesting and Anja Pfluger supervised the patients during the study's exercise intervention. I processed blood from patients to plasma and serum samples, and analzyed the adrenaline as well as latate levels. I prepared the samples for metabolomics analysis. I analyzed the data together with Sabine Kesting, Martin Schönfelder and Anja Pfluger. Myself and Sabine Kesting wrote the first draft of the manuscript. Sabine Kesting, Henning Wackerhage and Irene von Lüttichau conceptualized the idea for this manuscript. Myself together with Sabine Kesting managed the submission and review process. All authors read and approved the final manuscript.

Abstract

Low- and moderate-intensity exercise is safe and feasible during childhood cancer treatment. The feasibility of a bout of high-intensity interval training (HIIT) in this population has not been analyzed to date. Pediatric cancer patients aged between 6 and 18 years were selected based on clinical conditions to perform ten sets of 15 s HIIT (>90% of estimated maximal heart rate (HR_{max})) and 1 min active recovery on a bicycle ergometer within the first three chemotherapy courses. We assessed safety and feasibility criteria and the following parameters: perceived exertion rate, heart rate, and lactate and adrenaline concentrations. Out of 212 eligible patients, 11 patients aged 13.9 ± 3.6 years (n = 7 ♂) with lymphoma, leukemia, rhabdomyosarcoma, nephroblastoma, and synovial sarcoma completed the bout of HIIT without serious adverse events. During exercise, patients reached a BORG value maxima of 16 ± 1.2, and their heart rates rose from 78 ± 17 beats per minute (bpm) at rest to 178 ± 12 bpm after exercise (90 ± 6% estimated HR_{max}). The power-to-weight ratio was 2 ± 0.5 W/kg (watt per kilogram). Blood lactate concentrations increased from 1.09 ± 0.50 mmol/L (millimole per liter) at rest to 5.05 ± 1.88 mmol/L post-exercise. Our preliminary data suggest that HIIT is applicable only in a small number of childhood cancer patients. Individually adapted exercise protocols for patients with multiple impairments are needed.

Introduction

Cancer is rare in children, and even though disease mortality is low in childhood, cancer is still the most common cause of death by disease in children throughout Europe (Kyu *et al.*, 2018). According to the International Association of Cancer Registries, in Europe, 35.000 children and adolescents are diagnosed with cancer each year. Of these, 80% are disease free within 5 years after diagnosis (International Association of Cancer Registries). Both adult and childhood cancer are primarily treated with chemotherapy, surgery, and/or radiation. In recent years, exercise has additionally been utilized in supportive cancer care. Exercise not only improves wellbeing and fitness, but it may also have direct anti-cancer effects. Because of these benefits, some cancer associations, such as the Clinical Oncology Society of Australia (Clinical Oncology Society of Australia (COSA)) and the American College of Sports Medicine, now recommend exercise for all cancer patients (Patel *et al.*, 2019). For childhood cancer, the German Network ActiveOncoKids recently published a guideline for physical activity promotion during and after treatment (AWMF - Das Portal der wissenschaftlichen Medizin). In adult cancer patients, exercise is safe and feasible; can improve physical functioning; increase quality of life; and reduce side effects, such as chemotherapy-induced toxicities and cancer fatigue (Buffart *et al.*, 2017; Christensen *et al.*, 2018). In addition, high-intensity interval training (HIIT) has been rendered applicable in adult cancer patients (Großek et al., 2021), as well as in adult cancer survivors (Klika, 2021). Several lines of evidence suggest that physical activity

and exercise directly affect cancer, and this, in turn, may affect cancer risk, survival, and recurrence. A large meta-analysis pooling data from 1.44 million adults found leisure-time physical activity to lower the risk of developing 13 different adult cancer types (Moore *et al.*, 2016). Moreover, observational studies report improved survival and reduced recurrence in patients with breast (Holmes *et al.*, 2005), prostate (Kenfield *et al.*, 2011), or colorectal cancer (Meyerhardt *et al.*, 2006) who are more physically active. Serum obtained from healthy sedentary adults after nine weeks of high-intensity endurance cycling was found to reduce viability and proliferative capacity in tumor cells in vitro (Baldelli et al., 2020). The amino acid glutamine supports growth in cells that have high energy demands and synthesize large amounts of proteins and nucleic acids. The reduction in serum glutamine by acute exercise has been shown to inhibit cancer cell growth (Pedersen et al., 2020).

In children, physical activity generally plays a vital role in physical and social development (Eime et al., 2013; Strong et al., 2005). Recent studies have demonstrated that physical activity programs for children with cancer are safe and feasible (Baumann *et al.*, 2013; Rustler *et al.*, 2017) and that they contribute to their functional mobility (Morales *et al.*, 2018). Positive effects have been found for physical and cardiorespiratory fitness, muscle strength, body composition, flexibility, and health-related quality of life (Braam *et al.*, 2016). However, there is a lack of knowledge about whether exercise affects tumor cells directly in pediatric cancer patients and about the potential mechanisms (Kesting et al., 2020; Ruiz-Casado *et al.*, 2017).

HIIT is a time-efficient form of training that may improve VO_{2max} (maximal oxygen consumption) and provide health-related benefits within 10 min. These effects are similarly achieved by training for one hour at moderate intensity (Gibala et al., 2012). In addition, cancer-modulating hormones, such as catecholamines adrenaline and noradrenaline, increase at a more pronounced rate after high-intensity exercise (Wackerhage *et al.*, 2021) compared to after low-intensity exercise. Catecholamines have been linked to some anti-cancer effects of exercise, such as the accumulation of natural killer cells (Pedersen *et al.*, 2016), an increase in antitumor immunity, and more CD8+ T cell tumor infiltration (Liu et al., 2021), as well as the activation of the Hippo tumor suppressor pathway (Yu *et al.*, 2012). It is unclear though if HIIT is safe and feasible in children with cancer and whether children are able and motivated to perform such exercise. The scientific community agrees that there is a need for age- and health-appropriate exercise prescriptions in the childhood cancer population.

The aims of our preliminary study were therefore to evaluate the safety and feasibility of a single bout of HIIT (10 × 15 s) in pediatric cancer patients. The outcome variables were chosen with respect to the following aspects: We focused on feasibility and safety criteria to ensure

practicability and safe application of this intervention as required for follow-up studies. We observed physiological parameters to evaluate the responsiveness in this particular situation. Moreover, we analyzed the concentrations of lactate and adrenaline as objective parameters for exercise load. Additionally, adrenaline has been linked to anti-cancer effects in adults. If our protocol proves feasible and results in physiological responses, this will emphasize the need for mechanistic research and provide conditions for studies specifically focused on childhood cancer patients.

Materials and Methods

Study Design and Supervised HIIT Intervention

We conducted this cross-sectional monocentric study between March 2018 and April 2021 at the Department of Pediatrics and Children's Cancer Research Center at the Technical University of Munich. Participation was voluntary, and informed written consent, including all information about study procedures, was signed by each participant, as well as by their legal guardian. All collected data were encoded (pseudonym) and in accordance with privacy policy standards. The Ethics Committee of the School of Medicine of the Technical University of Munich approved the study protocol (protocol code 535/17 S; 16 February 2018 and amendment 25 March 2019) and gave approval to the informed consent.

Participants performed a single supervised HIIT protocol with individual workloads on a bicycle ergometer as shown in **Figure 7**. To the best of our knowledge, no protocol for HIIT interventions in childhood cancer patients has been published to date. Regarding the feasibility character of our study and the vulnerability of our group of patients, we decided to use a protocol with 10 intervals of 15 s followed by 1 min of active recovery. We intended to reach an intensity of >90% of estimated HR_{max} (maximal heart rate) (Tanaka et al., 2001) by increasing the workload individually during the intervals. This protocol is based on the characteristics of HIIT interventions in children and adolescents summarized in the systematic review of Eddolls and colleagues (Eddolls et al., 2017) in combination with an expert discussion, and it was created with respect to the presumably impaired physical capacity of childhood cancer patients during acute treatment.

Following recruitment and prior to the intervention date, participants were informed again about the study procedures via telephone call. Participants were told not to exercise intensely the day before the intervention and to have a light breakfast, to avoid caffeine and high sugar-containing foods (e.g., chocolate and bananas), and to fast two hours before. These precautions were taken to avoid the potential influence of these foods on adrenaline levels according to the manufacturer's specifications of the adrenaline measurement kit.

On the day of the intervention, participants came for a regular visit to the outpatient-clinic. First, participants underwent routine care procedures in the outpatient clinic, and the attending physician gave medical consent to perform the exercise intervention. Anthropometric data (height, weight) and vital parameters (heart rate, blood pressure) were collected. Second, after ten minutes of rest in a seated position, staff took the first blood sample via the central venous catheter following standardized clinical procedures. Third, the exercise physiologist adjusted the heart rate monitor and guided each participant through a low-intensity warm-up, mobilizing the joints for two minutes (see Section “Objective and Subjective Exhaustion Parameter”). Fourth, the bicycle ergometer was adjusted to the participants’ height. For participants shorter than 1.40 m, we used an electromagnetically braked children’s bicycle ergometer (Corival Pediatric, Lode, Groningen, the Netherlands), and for participants taller than 1.40 m, we used an adult ergometer with an induction brake (Kettler Ergometer Tour 300, Trisport AG, Hüneburg, Switzerland). Prior to the first interval, participants cycled for one minute at the lowest level of intensity (children ergometer: 0 watt, adult ergometer: 25 watt). The exercise physiologist announced the start of the HIIT, and participants were encouraged to cycle as fast as possible against the resistance, at a minimum rate of 60 rotations per minute (rpm). For the first interval, a low and individual workload was chosen with respect to the patients’ weight and height. Participants performed the intervention in a seated position without a face mask. Our protocol consisted of ten sets of 15 s HIIT (**Figure 7**). After each interval, participants cycled during an active recovery period of one minute with a low-intensity burden of 25 watt on the adult ergometer and 0 watt on the children’s ergometer. During active recovery, patients were told not to stop cycling but to continue pedaling. Immediately after each interval, the participants were asked to state their subjective rate of perceived exertion according to an adapted BORG scale (6–20), appropriate for children (Borg 1962, **Figure 8**). The next interval was adjusted according to the named number on the scale. During the intervention, we aimed to achieve a subjective exertion rate of 14–16 (yellow-red section on the scale), and participants were informed about this aim prior to the intervention. The load for each interval was individually increased in increments of 10–50 watts according to the participants’ feedback on the BORG scale, as long as the red section was not reached. Immediately after the last interval, participants laid down on the examination couch, and the second blood sample was taken within two to three minutes after finishing the last interval. Following the intervention, participants filled out the physical activity questionnaire ActiOn (see Section “Physical Activity Pre-Diagnosis”).

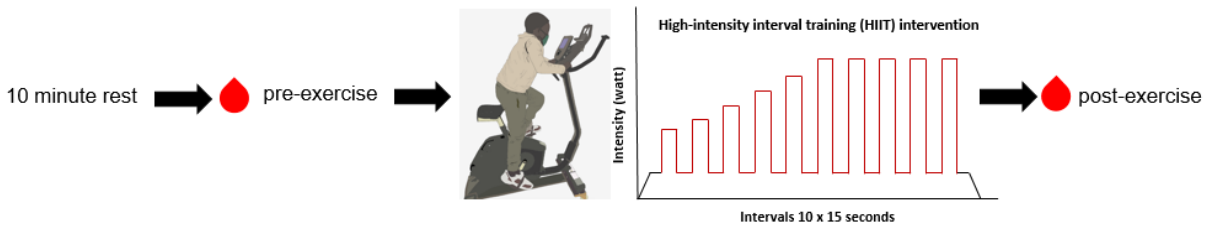

Figure 7: High-intensity interval training (HIIT) intervention protocol, including point of time for blood samples.

Vital parameters were measured prior to the intervention. Patients rested 10 min in a sitting position prior to the collection of blood samples. Processing of blood samples was conducted immediately.

How exhausting is your exercise?		
6		
7	very, very light	
8		
9	very light	
10		
11	quite light	
12		
13	somewhat exhausting	
14		
15	exhausting	
16		
17	very exhausting	
18		
19	very, very exhausting	
20		

Figure 8: Scale for rating of perceived exertion (RPE scale, modified version of the BORG scale) (Borg 1962). The scale shows a range from 6 to 20. Participants were asked to estimate their subjectively per-ceived rate of exertion. For children and adolescents, the scale was modified by adding faces that visually reflect the level of exertion.

Participants

We screened all newly diagnosed patients at our institution according to the following inclusion criteria: (1) diagnosed with pediatric cancer (new diagnosis or relapse), (2) aged between 6 and 18 years, (3) planned or implanted central venous catheter, (4) ability to follow study instructions in German, and (5) signed informed consent by patient and legal guardians and medical consent by the attending physician. The following exclusion criteria were applied: (1) medical contraindication regarding an intense exercise intervention (e.g., suspicious heart

echocardiography, increasing pain during exercise, orthopedic impairments of the lower extremities) and parameters regarding an acute infection (temperature > 38 °C), blood counts below a certain level (platelet levels < 50.000 per µL, neutrophil count < 500 cells per µL, and hemoglobin < 8 g/dL) (Perondi et al., 2012), and blood pressure values at rest according to age to ensure circulatory stability; (2) intellectual disability causing inability to follow study instructions; (3) inability to follow study instructions in German; (4) no signed informed consent; and (5) participation in another clinical exercise trial at the same time. Recruitment and intervention were conducted as early as possible following diagnosis and within the first three cycles of chemotherapy.

Procedures and Trial Endpoints

Feasibility and Safety

We planned and conducted this project as a feasibility study referring to Thabane's and colleagues' review on pilot studies (Thabane et al., 2010). To define feasibility of our intervention, the following criteria were determined: recruitment, acceptance, practicability, and data acquisition. Recruitment is rated as feasible if at least 50% of eligible patients addressed agree with participation in the study. Acceptance is rated as given if at least 50% of participants finish the intervention. Practicability is defined as the realization of the intervention with respect to this specific cohort and the strict inclusion criteria within the estimated time investment of 6 h per participant on average (including preparations and data analysis). Data acquisition is rated as given if applicable data of all participants for analysis are collected (subdivided into subjective and objective data). Safety is defined as no serious adverse events and only adverse events of grade 1 (without consequences) occurring during the intervention (Gauß et al., 2021). This was also defined by the CTCAE (Common Terminology for Adverse Events) (Common Terminology Criteria for Adverse Events (CTCAE)). In order to minimize risks and to ensure safety during HIIT, we adhered closely to the following preventive guidelines:

- The attending physician gives medical consent regarding the capability to participate (see also Section exclusion criteria) and appropriate blood values to perform HIIT;
- The intervention is conducted in a room within the outpatient clinic with medical supervision during and after the intervention;
- Low-intensity warm-up, including mobilization of the joints;
- Participants perform the intervention in a seated position to avoid tangling of the central venous catheter with the bike's handlebar;
- Follow-up observation of every participant for 30 min after finishing the intervention;
- At least two individuals in the room during the intervention in case of an emergency;
- Close observation of the participant, including objective signs of exertion (e.g., flushed cheeks, paleness, intense breathing, quality of coordination and movement).

Lactate and Adrenaline Concentrations

Blood samples were taken prior to and directly after the intervention within two to three minutes. From each whole blood sample, 20 µL was pipetted into 2.0 mL safelock microcentrifuge lactate tubes pre-filled with hemolytic solution (EKF Diagnostics, Magdeburg, Germany). Lactate samples were frozen for no longer than 14 days at −20 °C until measurement using a Biosen C-Line device (EKF Diagnostics, Magdeburg, Germany). Serum samples were established with 2.2 mL of whole blood collected in a 2.7 mL S-Monovette® (Sarstedt, Nümbrecht, Germany). After 25 min of coagulation in an upright position, collection tubes were centrifuged for ten minutes at 2260 g and 20 °C. After centrifugation, serum was aliquoted by 300 µL into 1.5 mL Eppendorf® safelock microcentrifuge tubes, immediately snap-frozen on dry ice, and stored until analysis for maximum of one year at −80 °C. Plasma samples were established from 2.7 mL of whole blood collected in a 2.7 mL EDTA S-Monovette® (Sarstedt, Nümbrecht, Germany). Plasma samples were centrifuged within five minutes after drawing and were processed using the same method used for the serum samples after centrifugation. Adrenaline concentrations were measured in plasma samples using the all-species adrenaline enzyme-linked immunosorbent assay (ELISA) kit (cat. No. LS-F5372, LifeSpan BioSciences Inc., Seattle, WA, USA) according to the manufacturer's protocol. Plasma adrenaline concentrations were measured in pre- and post-exercise samples using a Tecan Sunrise plate reader and Magellan Software tool (Tecan, Männedorf, Switzerland). Adrenaline was measured in duplicate, and mean values were subjected to statistical analysis.

Objective and Subjective Exhaustion Parameter

The rate of perceived exertion (modified BORG scale) is a common subjective parameter used in pediatric exercise oncology (Beulertz et al., 2016; Stössel et al., 2020; Wallek et al., 2018). Heart rate was measured using a chest strap (Polar RS 800CX, Kempele, Finland) as an objective measurement of intensity at the moment of the intervention and of the level of the participants' exhaustion. Additionally, the exercise physiologist observed the participants closely for the usual visible signs of exertion (e.g., flushed cheeks, paleness, intense breathing, quality of coordination, and movement). The retrospective evaluation of exertion was measured by the level of lactate in the blood samples collected prior to and following the intervention.

Physical Activity Pre-Diagnosis

We assessed physical activity with the pre-diagnosis version of the ActiOn questionnaire to compare the patients' level of physical activity to that of healthy children and adolescents. This tool was recently developed by exercise physiologists with profound expertise in pediatric exercise oncology from the German Network ActiveOncoKids for childhood cancer patients

and survivors (Network ActiveOncoKids). It is based on validated physical activity questionnaires for children and adolescents and retrospectively assesses the estimated physical activity level before diagnosis. Validation of this questionnaire is pending. Questions about the number of days with at least 60 min of physical activity during a regular week and the number of days with moderate-to-vigorous physical activity are included. These questions reflect the 2020 recommendations of the World Health Organization for children and adolescents (WHO Guidelines on physical activity and sedentary behaviour). Reference data from the German Health Interview and Examination Survey for Children and Adolescents (KiGGS Wave 2, *n* = 6532 girls and 6449 boys between 2014–2017; Finger et al. 2018) were used for comparison with our data.

Statistical Analysis

All aspects and results regarding feasibility and safety are provided descriptively. Physiological endpoints were analyzed with inferential statistics. To test the pre/post differences between the average values of two data sets of repeated measures, we used a two-tailed paired *t*-test. Normality was proven by the Shapiro–Wilk Test. Significant difference was determined by $p < 0.05$. Statistical analysis was performed using SigmaPlot v13 software tool and illustrated by PRISM 9.2.0 tool (GraphPad, San Diego, California, USA). Statistical evaluation was performed in consultation with the Institute of Medical Informatics, Statistics, and Epidemiology of the Technical University of Munich.

Results

The purposes of the present study were to evaluate the feasibility and safety of a bout of HIIT in a sample of childhood cancer patients, and to document the physiological response. Only 11 (5%) out of 212 eligible childhood cancer patients could be recruited. A total of 195 patients did not meet the inclusion criteria. The response of a single set of ten high-intensity interval bouts on outcomes of heart rate, blood lactate, rate of perceived exertion, and blood adrenaline concentration was analyzed in the participants.

Patient Recruitment and Characteristics

Out of the 212 newly diagnosed and screened patients with a pediatric malignancy, 14 patients were included in our study within a recruitment period of three years. The majority (*n* = 195) did not meet the strict inclusion criteria that were applied for safety aspects. A huge number of 83 patients were aged zero–five years, and HIIT intervention was not applicable due to body height, suitable bicycle ergometers, and compliance. Patients with bone tumors located at the lower (*n* = 20) or upper extremity (*n* = 6) had to be excluded due to a prohibition of weight bearing of the affected limb and the risk of a pathologic fracture. Another 25 patients already

participated in a pre-existing randomized controlled exercise intervention study during the whole period of acute treatment (ClinicalTrials.gov Identifier NCT03934060), and they were not included in this study due to good scientific practice. In 23 patients, language barriers hampered the procedures of recruitment and the following of instructions during the intervention. Another 23 patients were excluded due to medical reasons according to the attending physician (i.e., severely reduced capacity (*n* = 13), treatment at intensive care unit (*n* = 3), osteoporotic vertebral compression fractures (*n* = 3), inability to sit/walk (*n* = 2), ventilated (*n* = 1), and severe comorbidity (*n* = 1)). A burdensome palliative situation and recently conducted brain surgery led to the exclusion of seven and five patients, respectively, and intense exercise was not possible. Three patients did not receive a central venous catheter, and due to ethical reasons, peripheral blood collection for study purposes only was not approved by the Ethics Committee. Another three eligible patients could not be included due to the restriction of study procedures, including intense physical activity, during the COVID-19 pandemic. One patient declined participation prior to the intervention due to personal reasons. One recruited participant did not receive the intervention due to an infection. Another one started the intervention but discontinued after the first interval due to treatment-related nausea and was not able to continue. This patient suffered from pre-existing treatment-related nausea during hospital stays but, after physician approval, had agreed to perform the intervention despite this problem. A total of 11 (5%) participants completed the intervention. **Figure 9** shows a flowchart of the recruitment and study participation.

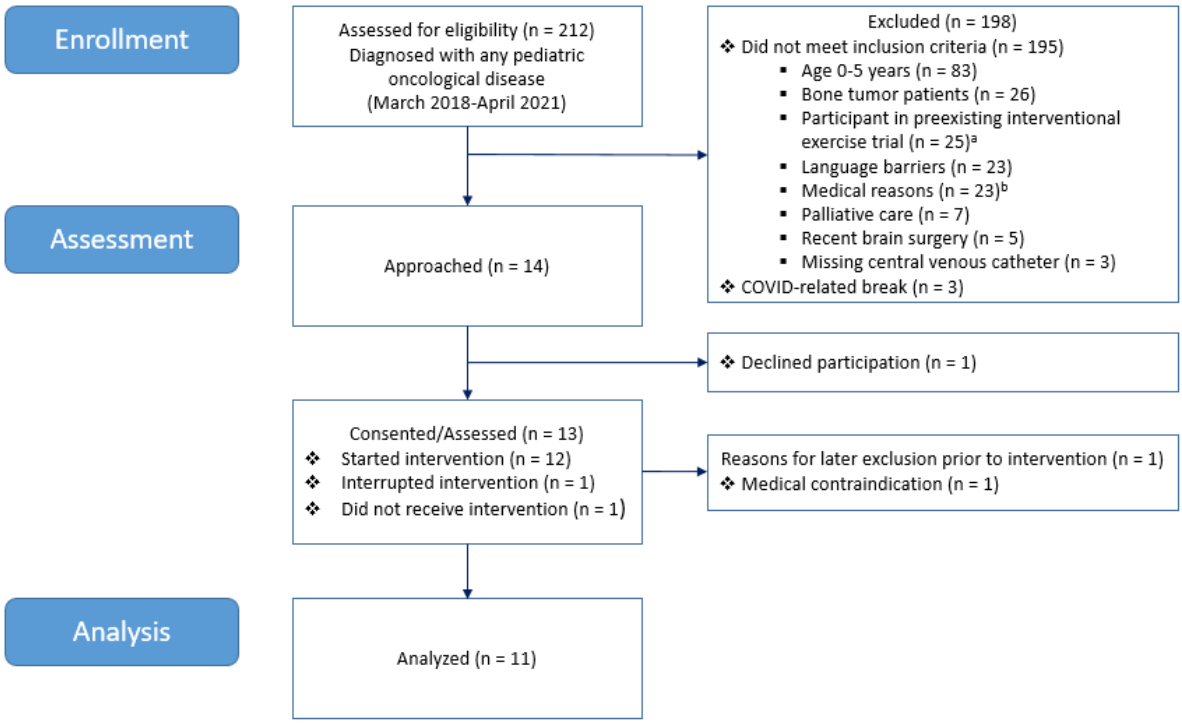

Figure 9: Flowchart of recruitment and study participation. *n*, number. [a] pre-existing exercise intervention study (ClinicalTrials.gov Identifier NCT03934060). [b] medical reasons for exclusion: severely reduced capacity (*n* = 13), treatment at intensive care unit (*n* = 3), osteoporotic

vertebral compression fractures (*n* = 3), inability to sit/walk (*n* = 2), ventilated (*n* = 1), and severe comorbidity (*n* = 1).

The eleven participants were 13.9 ± 3.4 years old and had been diagnosed with different types of childhood malignancies (lymphoma, leukemia, rhabdomyosarcoma, nephroblastoma, and synovial sarcoma). A total of eleven participants finished the intervention and were analyzed. **Table 5** provides further details about the participants' medical and anthropometrical characteristics. Ten participants performed the HIIT on the adult ergometer and one participant (7 years, 1.25 m) used the children's bicycle ergometer.

Table 5: Participant characteristics.

Characteristics	**Study Group (Analyzed), *n* = 11**
Age (mean ± SD, median, [range], years)	
At diagnosis	13.8 ± 3.4, 15 [7–18]
At intervention	13.9 ± 3.6, 15 [7–18]
Age group (*n*)	
Children (6–11 years) [a]	3
Adolescents (12–17 years)	6
Young adults (>17 years)	2
Sex (% male)	
Male	64
Female	36
Time since Diagnosis (mean ± SD, median [range], days)	55 ± 11, 54, [34–74]
Cancer type	
Leukemia	2
Lymphoma [b]	6
Other solid tumor [c]	3
Anticancer treatment received until examination	
Chemotherapy	12
Radiotherapy	0
Surgery [d]	0
Last application of chemotherapy before the intervention (days) [e]	9 ± 6 [2–20]

Anthropometrical variables	
(mean ± SD, median [range])	
Body weight (kg)	61.6 ± 20.2, 59.0 (21.6–90.0)
BMI (kg/m^2)	20.7 ± 4.1, 20.6 (13.8–27.7)
Body surface (m^2)	1.69 ± 0.37, 1.72 (0.85–2.18)
Physiological parameters (at rest)	
(mean ± SD, median [range])	
Heart rate (bpm)	78 ± 13, 78 [55–107]
Blood pressure systolic (mmHg)	117 ± 11, 111 [104–135]
Blood pressure diastolic (mmHg)	80 ± 9, 80 [68–97]
Physical activity pre-diagnosis [f]	
(mean ± SD, days)	
Days with physical activity ≥ 60 min per day	4 ± 3, 3, [0–7]
Days with moderate-to-vigorous intensity	4 ± 3, 3 [0–7]

n, number; SD, standard deviation; kg, kilogram; BMI, body mass index; m^2, square meters; bpm, beats per minute; mmHg, millimeters of mercury; min, minute. [a] only one child at the age of 7 years with a body height of 1.25 m performed the HIIT on the children's bicycle ergometer. [b] non-Hodgkin lymphoma and Morbus Hodgkin; *n* = 1 early relapse of Morbus Hodgkin. [c] rhabdomyosarcoma, nephroblastoma, synovial sarcoma. [d] indicates a surgical intervention other than implantation of a central catheter or biopsy for diagnostic reasons. [e] if cardiotoxic chemotherapeutical treatment was applied, period before HIIT was at least 7 days. [f] days with ≥ 60 min of any physical activity per week (normal week) prior to diagnosis and days with moderate-to-vigorous physical activity (normal week) prior to diagnosis.

Endpoints

Feasibility and Safety

Criteria for safety and feasibility were defined prior to the start of the study (Section "Feasibility and Safety"). **Table 6** summarizes the feasibility criteria and provides information about the fulfillment rate.

Table 6: Feasibility criteria and rate of fulfillment.

Feasibility Criteria	**Objective**
Recruitment	>50% of addressed participants
Acceptance	>50% finish the intervention (83%; 10/12) *

Practicability	Realizable for a specific group of participants with respect to strict inclusion criteria and an average time investment of 6 h per participant as estimated, and participants achieve the intended level of intensity (subjectively via BORG scale and objectively via lactate concentrations)
Data acquisition	Applicable data of all participants Subjective data: • Physical activity questionnaire (100%; 11/11) • Rate of perceived exertion (BORG scale) (100%; 11/11) Objective data: • Heart rate (64%; 7/11) • Blood samples for lactate and adrenaline concentrations (100%; 11/11)

n, number. * one participant interrupted after the first interval due to pre-existing treatment-related nausea and therefore could not be included in the final analysis; one participant interrupted after the eighth interval due to muscular tiredness of the legs and was included in the final analysis.

No serious adverse events and no adverse events with consequences occurred during the intervention (Common Terminology Criteria for Adverse Events (CTCAE)). One participant had to stop after the first interval due to pre-existing nausea in association with chemotherapy. Another participant had slight nose bleeding after the ninth interval, but this was stated to be a regular occurrence, even before cancer diagnosis, and they finished the intervention.

Physical Parameters

To characterize how much the bout of intense exercise affected the cardiovascular system, metabolism, and the cancer modulator adrenaline, heart rate was measured during the intervention, and blood lactate and adrenaline were measured at rest within three minutes post-exercise. **Figure 10a,b** show the continuous increase in power and perceived exertion in all analyzed patients (*n* = 11). Mean heart rate increased from 78 ± 17 bpm at rest to a HR_{max} of 178 ± 12 bpm post-exercise (**Figure 10c**) in *n* = 7/11 patients. This corresponds to 90% of the estimated HR_{max}, as calculated using the Tanaka equation (HR_{max} = 208 − 0.7 x age), in children and adolescents (Mahon et al., 2010; Tanaka *et al.*, 2001). In four patients, the measurement of heart rate was impaired due to technical difficulties with the chest strap. After exercise, blood lactate reached a maximum concentration of 5.05 ± 1.88 mmol/L (**Figure 11a**). Adrenaline concentration did not change from rest to post-exercise (**Figure 11b**).

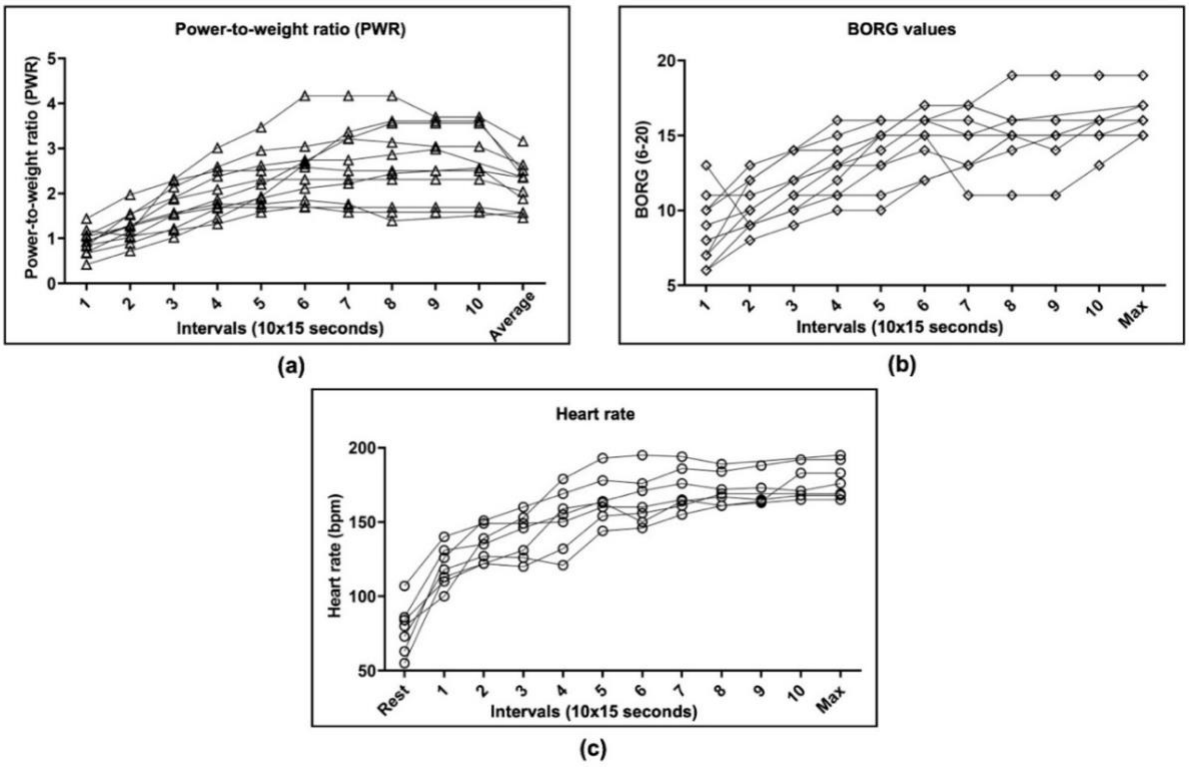

Figure 10: High-intensity interval training (HIIT) was performed with 11 childhood cancer patients after completing a two-minute warm-up. Between each interval, there was one minute of active recovery, during which the patients stated their estimated exertion level. (**a**) Power-to-weight ratio indicates the ratio of body weight to power output for ten intervals for all eleven childhood cancer patients. Average values are presented, excluding active recovery periods, as the mean of the 10 intervals per patient, which range from 1.46 to 3.16 W/kg. The maximum power levels reached range between 65 watts and 300 watts. (**b**) Patients provided information on their exertion level based on the BORG scale ranging from 6 to 20. After warm-up, BORG values were stated to be between 6 and 7, indicating a very, very low perceived exertion level. Maximum BORG values range from 15 to 19, indicating the perceived exertion level to be high and very high, respectively. (**c**) Heart rate, evaluated in only seven of eleven patients due to technical difficulties with the chest strap, increased significantly during exercise from 78 ± 17 bpm at rest to a maximum of 178 ± 12 bpm using a paired t-test ($p < 0.0001$). n, number; W, watt; kg, kilogram; PWR, power-to-weight ratio; bpm, beats per minute; max, maximal.

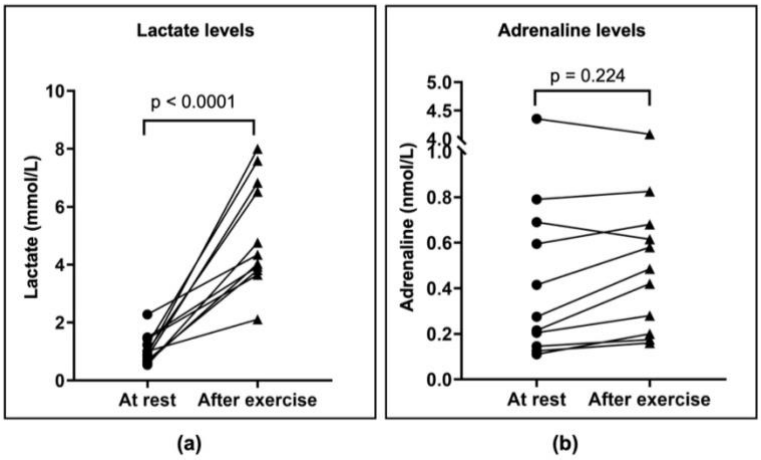

Figure 11: Lactate and plasma adrenaline concentrations were measured in blood samples obtained prior to and directly after exercise intervention. (**a**) There was a significant increase

in lactate concentrations when comparing resting (1.09 ± 0.50 mmol/L) and post-exercise values (5.05 ± 1.88 mmol/L) using a paired t-test ($p < 0.0001$). (**b**) There was no significant change in adrenaline concentrations when comparing resting and post-exercise values, despite adrenaline concentrations showing high variability within the tested group. Low adrenaline concentrations of 0.2 nmol/L (nanomole per liter) are in line with available data in healthy children (Rubin et al., 2015). mmol/L, millimole per liter; nmol/L, nanomole per liter.

Physical Activity Pre-Diagnosis

Participants reported 60 min of moderate-to-vigorous physical activity in a regular week pre-diagnosis on 3.5 ± 2.5 days (median: 3 days, range: 0–7 days). Two participants met the WHO recommendations of 60 min of physical activity every day, and one was active for 60 min on six days in a normal week. These data are in accordance with the healthy reference population of the KiGGS study's Wave 2, with 22.4% of girls and 29.4% of boys achieving the recommendations (Finger et al., 2018). Five patients attended sport club activities regularly before diagnosis, eight engaged in leisure-time exercise activities, and all participated in physical education at school.

Discussion

The first major finding of this pilot study suggests that our HIIT protocol is not suitable for the majority of children during the first weeks of cancer treatment. We conclude this since only 14 of the 212 childhood cancer patients could be recruited for the study due to the restrictive inclusion criteria. Of these, 11 patients were able to finish a bout of HIIT. The second major finding is that the exercise intervention is feasible and safe for patients who suffer from only mild treatment side effects. The remaining 11 patients who completed a bout of HIIT demonstrate this finding: their heart rates increased to 178 ± 12 bpm and their blood lactate concentrations to 5.05 ± 1.88 mmol/L. Surprisingly, adrenaline did not change significantly but varied greatly inter-individually.

Between March 2018 and April 2021, a total of 212 children were hospitalized, but recruitment was challenging. The COVID-19 pandemic further hampered recruitment due to strict hygienic measures and a restriction of study procedures involving intense physical activity. Because of these measures, a large proportion of newly diagnosed patients under the age of six years were excluded from the intervention. Typically, all patients older than two years of age are offered participation in an individually adapted physical activity program. Our HIIT protocol is feasible with a very small number of physically fit childhood cancer patients; however, it cannot be applied as a general approach for this group. We observed no serious adverse events with consequences in our study. Only experienced staff attended to the patients during the study while adhering to specific safety prevention strategies (Saultier et al., 2021) in order to minimize risk. Excluding one, all participants performed the intervention on the adult bicycle

ergometer. One patient was analyzed on the children's ergometer due to body height. This seven-year-old child was able and motivated to perform our HIIT protocol. However, the overall workload and recovery load are not comparable to the adult-sized ergometer. In general, exercise interventions are known to be feasible and safe during and after childhood cancer treatment (Braam *et al.*, 2016; Rustler *et al.*, 2017), but research and data regarding HIIT during treatment have, to the best of our knowledge, not been published to date.

In addition to the careful selection of study participants for our HIIT intervention, the setting must also be considered. Recruitment within the first three cycles of chemotherapy treatment is challenging due to the short time frame and known treatment-related side effects (e.g., nausea, fever, infections, and mucositis). Taking preventive safety strategies (e.g., blood count, and cycles of chemotherapy) into account, patients' and physicians' approval to participate, and the insertion of a central venous catheter to take blood samples, timing is central to the success of the intervention. The demonstrated feasibility of an early HIIT intervention will allow for the measurement of potential tumor-influencing parameters early in the course of disease in future studies.

Our HIIT protocol caused an increased heart rate in the participating patients, which is in accordance with published data in healthy children and adolescents (Gelbart et al., 2017). To the best of our knowledge, there are no existing data about adrenaline or lactate concentrations in childhood cancer patients following exercise. Surprisingly, the blood concentration of adrenaline did not increase when comparing it at rest to post-exercise. This finding is in contrast to published data in adults (Wackerhage *et al.*, 2021) and healthy children. We do not assume chemotherapeutical interference in adrenaline accumulation in childhood cancer patients. Although we know that the two- to three-minute half-life period of adrenaline is rather short, we managed to take blood samples within this time frame, while also adhering to hygienic guidelines. It is possible that the overall workload throughout our protocol could be too low to produce an increased blood concentration of adrenaline. Since adrenaline concentration can increase during HIIT in relation to mean exercise intensity (Moser et al., 2015), an extension of our HIIT protocol might have resulted in increased adrenaline levels.

As expected, we found a significant increase in blood lactate in the immediate post-exercise period, which validates the subjectively perceived exertion levels stated by the patients. There was high variability in power values (watt), which can be explained by the heterogeneity of our test subjects regarding age and fitness level. The power-to-weight ratios show comparable burdens throughout our studied group. Low power values (watt) also led to an increase in lactate, which shows that the individual watt adaption was successful in achieving high intensity.

Consequently, we have planned a follow-up study to observe one group of physically fit childhood cancer patients performing a HIIT intervention with more intervals compared to one group of less physically fit childhood cancer patients performing a less intensive protocol. Our aim is to define a minimum feasible exercise burden that leads to a physiological response on a molecular level for the majority of childhood cancer patients.

The questionnaire data show that most children and adolescents in our study did not meet the WHO physical activity recommendations of at least 60 min of mostly aerobic physical activity prior to diagnosis. However, they were similarly physically active in comparison to their healthy peers (Finger *et al.*, 2018). The study participants could participate in a single bout of HIIT because they only experienced minor treatment side effects at the time of the intervention.

Conclusions and Perspective

The main finding of this study is that our HIIT protocol is safe and feasible in a small and physically fit group of childhood cancer patients. However, given that only about 5% of the eligible children and adolescents with cancer were capable of participating in this intervention due to our strict inclusion criteria, our HIIT protocol seems to be unsuitable for inclusion in the treatment and supportive care regimen for childhood patients with cancer on a regular basis. Our findings contribute to a better understanding of the applicability of HIIT in the field of pediatric exercise oncology. There is growing interest in mechanistic research on the effect of exercise; however, the focus of this research remains on adult cancer patients. We aim to expand our studies with the goal of determining the effects of exercise at the molecular level in childhood cancer patients. This represents an essential new research perspective beyond current research programs on soft but important outcome parameters (e.g., quality of life and physical fitness). Specifically, we propose the following key aspects for future research in this field:

- The adaption of exercise tests specifically for childhood cancer patients is needed to define standardized workloads for training protocols.
- The development of individually adapted exercise protocols (low-intensity exercise training) for patients with multiple impairments and health restrictions due to their underlying disease and cancer treatment.
- The application of repeated HIIT interventions and analysis of physiological parameters during the entire course of chemotherapy treatment.
- The initiation of multicenter studies to generate a greater sample size and increase informative value.
- The analysis of metabolite concentration changes in exercise-conditioned serum to detect relevant exercise and cancer-related metabolites in children.

3.2.1 Discussion of Study 2

The aim of my intervention study was to evaluate safety and feasibility of a single bout of HIIT in children during cancer treatment. Additionally, I aimed to assess physiological effects of HIIT in this cohort, which has not been shown before. Specifically, I observed effects of HIIT on heart rate, lactate and adrenaline concentrations. My interest in adrenaline concentrations is based on adult cancer research. Catecholamines are potential mediators of the effect of exercise on cancer (Dethlefsen *et al.*, 2017; Pedersen *et al.*, 2016). It is therefore important to understand, whether an intense exercise intervention in childhood cancer patients can lead to an increase in adrenaline concentrations, which in turn might affect cancer growth.

My intervention study showed that HIIT is safe and feasible only in a small number of physically fit childhood cancer patients. HIIT leads to an increase of heart rate and lactate. Adrenaline levels are not affected by HIIT and baseline concentrations are heterogenous in my sample. In adults, HIIT is suggested to be a time-efficient form of exercise throughout patients of all stages of cancer treatment (Mugele et al., 2019). There is no literature in childhood cancer patients about catecholamine levels after HIIT. A publication on exercise with obese and Prader-Willi syndrome (PWS is a genetic disease often leading to obesity) children showed that endurance exercise leads to an adrenaline increase only in healthy children but not in obese and PWS groups (Rubin *et al.*, 2015). Exercise in adult cancer patients leads to a less pronounced adrenaline increase compared to healthy adults (Hanson et al., 2018). The authors suggest altered adrenal function as an explanation. This shows that generally an adrenaline increase after exercise could be more pronounced in healthy groups and that adrenaline might not be a relevant marker in childhood cancer patients. It is possible though, that there are specific markers in childhood cancer patients which are altered via exercise and might affect cancer cells directly. We therefore used the blood samples of our 11 patients to conduct a preliminary untargeted metabolomics analysis. Further analyses of significantly increased or decreased metabolites after exercise could identify exercise markers specific to childhood cancer patients. For example, we found putrescine to be increased in all patients after exercise. The following analysis could be an example of how to analyze all altered metabolites and use the information to identify specific exercise markers.

In April 2020, I searched PubMed for putrescine (+ synonyms) and its effect on cancer:

(((putrescine[Title/Abstract] OR "1,4-diaminobutane"[Title/Abstract] OR "1,4 butanediamine"[Title/Abstract] OR "butane-1,4-diamine"[Title/Abstract] OR tetramethylenediamine[Title/Abstract] OR Butylenediamine[Title/Abstract])) AND cancer[Title/Abstract]) AND (proliferation[Title/Abstract] OR signalling[Title/Abstract])

and retrieved publications from the past 10 years. This resulted in 43 publications (meanwhile 12 more studies have been published, 22.05.2022). I excluded 25 publications, as they added no information, had no information on proliferation or signaling, or had another focus. The metabolite putrescine is a small cationic molecule that takes great part in cell proliferation and differentiation, DNA stabilization, ion channel function and also regulation of gene expression (Irecta-Najera et al., 2017; Ramani et al., 2014). It is present at micro-millimolar concentrations in eukaryotic and prokaryotic cells and in mammalian cells it is formed exclusively from L-ornithine (Hyvonen et al., 2020). Putrescine belongs to the family of polyamines and binds the transferase deoxyhypusine synthase (DHS) (Wator et al., 2020). Polyamines generally play important roles in regard to cell development, amino acid and protein synthesis, oxidative DNA damage, proliferation, and cellular differentiation (Latour et al., 2020). With ageing, a decline in polyamine levels occurs in humans (Handa et al., 2018). High levels of polyamines are related to cancer progression and an overall poor prognosis in cancer patients (Dai et al., 2017; Gurkan et al., 2013). Polyamines particularly play a role in rapidly dividing cells such as in the immune system and digestive tract (Ramani *et al.*, 2014). It is unclear, how long putrescine levels are increased after exercise in our cohort. But this analysis shows that exercise induces changes in the metabolome of childhood cancer patients, which might be associated with cancer. A holistic research approach is needed to establish metabolic effects of exercise and its effect on cancer.

The sample size of 11 patients for my study might be seen as a limitation. However, as I also discuss in the publication, it should be considered that incidence of cancer is lower in children than in adults and therefore comparable sample sizes cannot be expected. Additionally, conducting this kind of intervention with childhood cancer patients during their treatment schedule is challenging and time consuming. It is important to take this first step in pediatric exercise oncology in order to contribute to a better understanding of the mechanisms of exercise, specifically in this population. However, the long publication process for this study showed that the importance of sample size needs to be carefully discussed. Therefore, multicenter studys are a logical next step for further analyses.

3.3 Study 3: Noradrenaline has Different Effects on Rhabdomyosarcoma and Ewing's Sarcoma Cancer Hallmarks – Implications for Exercise Oncology

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Keywords:

catecholamines, noradrenaline, tumor cells, childhood cancer, exercise, molecular mechanisms, adrenergic receptors

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Conflict of Interest:

The authors declare that they have no competing interests. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Individual Contribution:

I am the first author of this manuscript that is in final preparation for peer-review submission. This work resulted from a collaboration of the chair of exercise biology (TUM) with the Klinikum Munich Schwabing. I coordinated the lab work and conducted all experiments assisted by Jonas Haferanke. I managed the transcriptomics and proteomics analyses process for this project. Myself and Carla Regina assisted by Jonas Haferanke analyzed the data. I am the main writer of the manuscript. Henning Wackerhage and Irene von Lüttichau conceptualized

the idea for this study. I manage the submission and review process and coordinate editing with all authors

Abstract

Exercise has beneficial effects on cancer and its treatment. While epidemiological data on this relation is strong, the physiological mechanisms that mediate this relation remain largely unknown. A key mediating role in exercise oncology research, however, is attributed to catecholamines. The stress hormones adrenaline and noradrenaline redistribute natural killer cells or activate the hippo pathway, which both has anti-cancer effects. Other studies indicate that catecholamines mediate the detrimental effects of stress via increased tumor vascularization or tumor innervation. We term this contradicting role of catecholamines the Cancer Catecholamine Conundrum. This study provides proof-of-concept that the effect of catecholamines on cancer is mediated by the expression of adrenergic receptors. We cultured two pediatric sarcoma cell lines that express (A673 cel lline) or do not express (RD cell line) adrenergic receptors. Our results show that noradrenaline increases cAMP signaling and decreases cell proliferation and migration only in the A673 cells, which express adrenergic receptors. RD cells are not affected by noradrenaline treatment. Our results might explain why catecholamines have different effects in different tumor cells. Further research on adrenergic receptor expression in tumor cells is necessary, as this could contribute to individualized exercise prescriptions for cancer patients.

Introduction

Phyiscal activity reduces the risk to develope cancer (Moore *et al.*, 2016), improves survival of breast, colorectal and prostate cancer (McTiernan et al., 2019), supports treatment side-effects management (Stout *et al.*, 2017), and increases overall fitness and well-being of cancer patients. For these reasons, cancer associations such as the Clinical Oncology Society of Australia (Clinical Oncology Society of Australia (COSA)) and the American College of Sports Medicine (Patel *et al.*, 2019) recommend exercise for all cancer patients. An increasing amount of evidence suggests that exercise not only benefits cancer patients' general health, but also affects cancer and its treatment directly (Christensen *et al.*, 2018). The mechanisms by which exercise affects the behavior of tumor cells and cells in the tumor microenvironment are little understood. Generally, exercise changes concentrations of hormones (Copeland et al., 2002), myokines (Leal et al., 2018), metabolites (Schranner et al., 2021) and immune cells (Idorn and Hojman, 2016) in the blood. The exercise-conditioned blood may influence the tumor directly if it has receptors or targets for the molecules that change their concentration during exercise or if e.g. metabolites or immune cells enter the tumor or tumor cells.

The medulla of the adrenal gland synthesizes and secrets adrenaline and noradrenaline (Tank and Lee Wong, 2015). During exercise, the concentrations of adrenaline and noradrenaline, respectively, increase from 0.6 nmol/L or 1.7 nmol/L at rest to 4 nmol/L or 25 nmol/L after

10 intervals of 6 second sprints (Bracken et al., 2009). Exercise-released adrenaline and noradrenaline can promote the accumulation of natural killer cells (Pedersen *et al.*, 2016), an increase of antitumor immunity and more CD8+ T cell tumor infiltration (Liu *et al.*, 2021) as well as the activation of the Hippo tumor suppressor pathway (Yu *et al.*, 2012). In addition, catecholamine concentrations increase when we are stressed. Surprisingly, while exercise studies report beneficial anti-cancer effects of catecholamines, stress studies generally find that catecholamines worsen cancer (Cui et al., 2019; Renz *et al.*, 2018a). We term the contradiction that catecholamines can have both positive and negative effects on cancer the cancer catecholamine conundrum (Wackerhage *et al.*, 2021) which is little explained.

The aim of the present study was to investigate whether differences in adrenergic receptor isoform expression can explain different cancer hallmark responses to catecholamines. We compared the A673 Ewing's sarcoma cell line, that genereally expresses high amounts of α1-, α2- and β-adrenergic receptors with the RD rhabdomyosarcoma cell line that expresses low levels of adrenergic receptors. Specifically, we aimed to address two research questions:

1) Does the cAMP, proliferation, and migration response to noradrenaline differ between A673 and RD cells?
2) Does the expression of adrenergic receptor isoforms and other catecholamine signalling molecules in A673 and RD cells explain the cAMP, proliferation, and migration responses of these cells to noradrenaline?

Methods

Cell Lines and Culturing

We selected Ewing Sarcoma cell line A673 and rhabdomyosarcoma cell line RD based on their adrenergic receptor expression in the cancer cell line encyclopedia (CCLE, https://sites.broadinstitute.org/ccle/). Both cell lines belong to the group of pediatric sarcomas and express adrenergic receptors differently. While according to expression data in the CCLE A673 cells show high relative expression of all three groups of adrenergic receptors (α1, α2 and β), RD cells show low relative expression of all three groups of adrenergic receptors (Ghandi et al., 2019). Specifically, A673 cells show high relative expression in α1D-, α2C-, β1 and β3-receptors. RD cells show low relative expression in all adrenergic receptors.

We cultured A673 and RD cell lines (DSMZ; Braunschweig, Germany) in RPMI 1640 or DMEM/F12 medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin (all obtained from Life Technologies, Grand Island, NY, USA). Cells were authenticated (Eurofins Genomics Europe Applied Genomics GmbH; Ebersberg,

Germany) and regularly tested to be free of mycoplasma contamination. We sub-cultured cells twice every week at 80% confluence and used cells for experiments up to passage 30.

For the treatment of cells, we prepared a noradrenaline stock solution of 3 mM (noradrenaline -hydrochlorid in crystalline form was optained from Merck; Darmstadt, Germany) by dissolving 12.3 mg noradrenaline in 20 mL DEPC-treated H_2O (Invitrogen™, Thermo Fisher Scientific; Waltham, MA, USA). Stock solution 1 mL aliquots were kept at -20°C no longer than three months until use for experiments. We diluted the noradrenaline stock solution to the desired concentration in growth medium. Growth medium used for controls contained DEPC-treated H_2O in the same ratio as the noradrenaline solution did.

Sulforhodamine B Assay

To measure the effect of noradrenaline on cell number in the two cell lines A673 and RD, we treated the cells for 24, 48 and 72 hours with different concentrations of noradrenaline or growth medium only (control) and quantified cell numbers using the sulforhodamine B (SRB) assay. This assay is commonly used in cancer cell lines to test drug-toxicity (Vichai and Kirtikara, 2006). We seeded A673 and RD cells in triplicates in three 24-well plates each; 50.000 cells per well. After 24 hours, when cells adhered to the plastic, we tested different concentrations of noradrenaline using the following concentrations in a 5x dilution series: 0.3 mM, 60 µM, 12 µM, 2.4 µM, 0.48 µM and 96 nM. We fixed the cells after 24, 48 and 72 hours to the tissue-culture plates with 10% trichloroacetic acid and incubated plates for at least one hour at 4°C. After dyeing the cells with 0.05% SRB, eventual washing with 1% acetic acid and air-drying, we added 10 mM Tris base solution to each well to solubilize the protein-bond dye. Photometric measurement was conducted using Tecan Infinite™ M200 plate-reader (Tecan Trading AG, Switzerland) at 530 nm. We conducted the experiment three times and present the results of one run representative for all runs.

Proliferation Assay

To measure the effect of noradrenaline on proliferation in the two cell lines A673 and RD, we treated cells for 72 hours with 60 µM of noradrenaline. We based treatment and duration in this proliferation assay on results of the preliminary SRB assay. After seeding 20.000 cells per well in duplicates in a 24-well plate, we incubated the cells for 24 hours until cells adhered to the plastic. Proliferation was assessed by 5'-ethynyl-2'-deoxyuridine (EdU) incorporation using the EdU Cell Proliferation Kit for Imaging (EdU-Click 555, baseclick GmbH; Neuried, Germany) according to the manufacturer's protocol. We added the EdU solution to the medium during the last 4 hours of the 72 hour incubation period. Further, we dyed cells with bisBenzimide H 33342 trihydrochloride (B2261, Sigma-Aldrich) to visualize living cells and took fluorescence

microscopy images with a Zeiss Axiovert 100 at 10x magnification. In each image, we defined three random view fields and counted at least 2,000 cells per experiment.

Migration Assay

We assessed the effect of noradrenaline on cell migration in the two cell lines A673 and RD using 35 mm Culture-Insert 2 Well in µ-Dishes (ibidi GmbH; Gräfelfing, Germany) and based the treatment concentration on results of the preliminary SRB assay. First, we seeded cells at a density of 7 x 10^5 per mL (70 µL volume) in each of the two chambers of the silicone insert and incubated the dishes at 37°C and 5% CO_2 for 24 hours. After cells adhered to the plastic and reached up to 100% confluence, we removed the silicone insert to uncover the 500 µm cell free gap. Second, we treated the cells with 60 µM of noradrenaline or growth medium (control) and took microscopy images with a Zeiss Axiovert 100 at 10x magnification immediately after treatment as baseline. Third, we took microscopy images after 24, 48 and 72 hours of treatment in each dish and analyzed the images by the percentage of scratch open area using the web-based quantitative image analysis software FastTrack AI (ibidi GmbH; Gräfelfing, Germany). Cells were seeded at three different dates with three technical replicates each. We present results of one run representative for all runs.

cAMP Signalling Assay

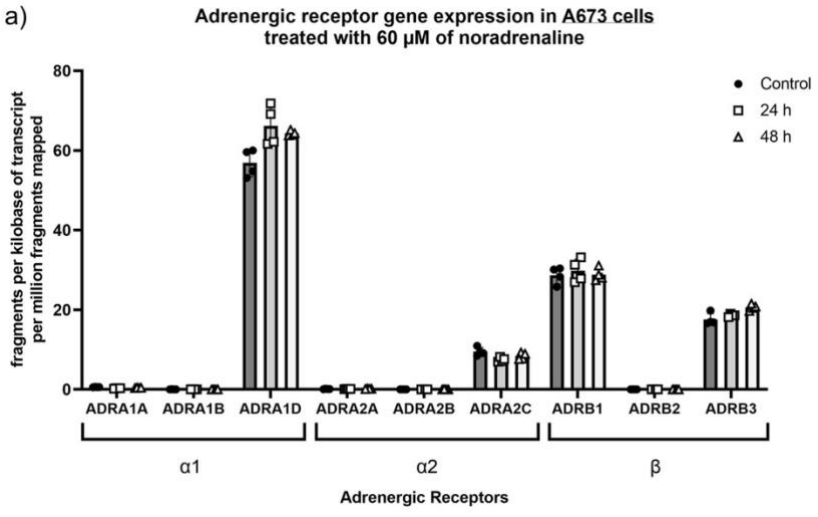
To analyze the effect of noradrenaline on signalling in the two cell lines A673 and RD, we assessed the cellular concentration of cyclic adenosine monophosphate (cAMP) after treating cells with 60 µM or 20 nM of noradrenaline or growth medium (control). We quantified cAMP concentrations using cAMP competitive enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen™, Thermo Fisher Scientific; Waltham, MA, USA). In brief, we seeded 3,5 x 10^5 cells per well in 24-well plates (one plate per cell line) and incubated the cells at 37°C and 5% CO_2 for 24 hours. After cells adhered to the plastic, we treated the cells with 60 µM or 20 nM of noradrenaline or with growth medium for 5, 15, and 30 minutes. Cell lysis was then induced by 0.1 M hydrogen chloride (HCl) and 1% Triton X-100. After ten minutes incubation at room temperature, we centrifuged the plates at 600 x g at room temperature for another ten minutes and used the supernatant duplicates in the assay according to the manufacturer's protocol. Optical density values were analyzed using Tecan Infinite™ M200 plate-reader (Tecan Trading AG, Switzerland) at 405 nm with correction at 580 nm. We calculated cAMP concentrations by data interpolation based on the standard curve's exponential function in Microsoft Excel™ (Microsoft; Redmond, WA, USA).

RNA-Sequencing and Proteomics Analysis

We analyzed the effect of noradrenaline on gene expression (by RNA-sequencing) and the proteome (by proteomics analysis) in the two cell lines A673 and RD to assess potential explanations for noradrenaline-associated changes in cell behavior. Therefore, we seeded 2 x 10^6 cells per petri dish (150 mm) in quadruplicates and incubated the dishes at 37°C and 5% CO_2 for 24 hours. After cells adhered to the plastic, we treated the cells with 60 µM of noradrenaline for 24 or 48 hours or with growth medium for 48 hours (control). We obtained each sample for RNA-sequencing and proteomics analysis from the same petri dish. After washing with phosphate-buffered saline (PBS), we pelleted the cells with a cell scraper. Proteomics analysis was conducted by the Bavarian Center for Biomolecular Mass Spectrometry (Freising, Germany). For RNA-sequencing, we extracted RNA with Trizol (Invitrogen™, Thermo Fisher Scientific; Waltham, MA, USA) and the RNA was sequenced by Novogene (Cambridge, UK).

Results

To identify two pediatric sarcoma cell lines that systematically differ in their adrenergic receptor isoform expression, we retrieved adrenergic receptor isoform expression data for 1019 cell lines from the cancer cell line encyclopedia (Barretina et al., 2012). Among the sarcoma cell lines, A673 expresses α1D-, α2C-, β1 and β3-receptors of Z-transformed expression levels of above 4.04 in each group, whereas the RD cell line has Z-transformed adrenergic receptor isoform expression levels of below 0.4. This means that the A673 Ewing sarcoma cell line is a cell line with a high expression of all three groups of adrenergic receptors, whereas the RD rhabdomyosarcoma cell line is a low expressing cell line. Both cell lines were authenticated by Eurofins by STR/DNA profiling. The CCLE adrenergic receptor isoform expression was confirmed by our RNA-Seq analysis (**Figure 12**).



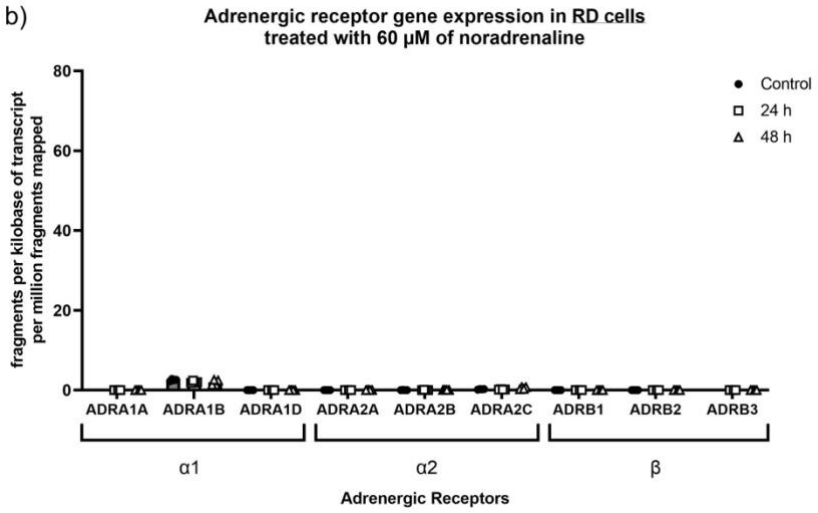


Figure 12: Adrenergic receptor isoform mRNA expression in a) A673 and b) RD tumor cell line. Expression is shown as fragments per kilobase of transcript per million fragments mapped (fpkm) in control samples (treated only with growth medium) as well as noradrenaline-treated samples.

The aim of our study was to find out whether the large difference in adrenergic receptor expression between the two cell lines is associated with different proliferation, migration and signalling response of the cells to noradrenaline (targets all nine adrenergic receptor isoforms). Therefore, we first tested cytotoxicity of different treatment durations and concentrations of noradrenaline on the two cell lines A673 and RD.

Effect of Noradrenaline on cAMP Signalling in A673 and RD Sarcoma Cell Lines

We first investigated whether noradrenaline alters the concentration of the classical second messenger cyclic adenosine monophosphate (cAMP) of catecholamine signalling. cAMP is a second messenger regulated by G-proteins and therefore cAMP plays an important role in a cell's response to catecholamines (which bind to G-protein-coupled adrenergic receptors). cAMP regulates signalling pathways associated with cancer cell growth, migration, invasion and metabolism (Zhang et al., 2020). Since we found noradrenaline at a concentration of 60 µM to affect cell proliferation in A673 but not RD sarcoma cells, we measured cAMP concentrations via an enzyme-linked immunosorbent assay (ELISA) in both cell lines directly after treatment with noradrenaline (**Figure 13**). We treated cells with 60 µM or 20 nM of noradrenaline or growth medium (control) for 5 and 30 minutes. In A673 cells, five minute treatment with 60 µM of noradrenaline significantly increases cAMP levels 38-fold from 0.78 ± 0.13 pmol/mL to 29.15 ± 1.09 pmol/mL. Thirty minutes after 60 µM noradrenaline treatment, cAMP levels in A673 cells are still similarly increased at 29.80 ± 1.02 pmol/mL. The same treatment does not affect cAMP levels in RD cells with a cAMP baseline concentration

(control) of 3.46 ± 0.14 pmol/mL. Neither of the two cell lines' cAMP concentrations is affected by treatment with a physiological noradrenaline concentration of 20 nM.

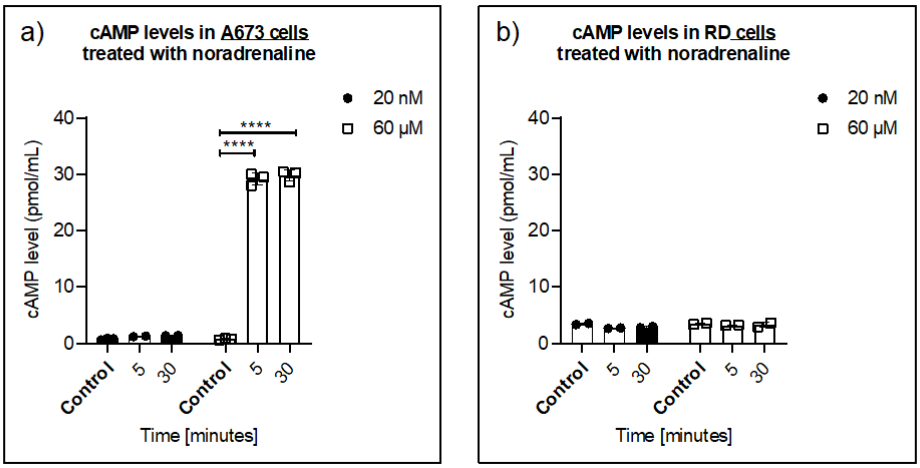


Figure 13: Noradrenaline treatment affects A673 and RD sarcoma cell signalling differently with measurable treatment-effects only in A673 cells and only with a noradrenaline concentration of 60 µM but not of 20 nM. We used a competitive enzyme-linked immunosorbent assay (ELISA) kit to detect cAMP concentrations after we treated A673 and RD cells with 60 µM or 20 nM of noradrenaline or with growth medium (control) for 5 or 30 minutes. For the experiment, we used at least two technical replicates per sample and three technical replicates in the A673 cells treated with 60 µM of noradrenaline. Each data point as well as mean ± SD are presented in the figure. a) We found significant differences in cAMP concentrations in A673 cells after 5 and 30 minutes of 60 µM noradrenaline treatment compared to control. Treatment of A673 cells with 20 nM of noradrenaline did not affect cAMP concentrations. b) cAMP levels in RD cells were not affected by noradrenaline treatment. Two-way ANOVA with multiple comparison Turkey test was used to calculate p values. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.001$.

With this cAMP competitive ELISA, we could show that in vitro cell signalling is not affected by a physiological concentration of 20 nM of noradrenaline but a concentration of 60 µM does increase cAMP levels significantly in A673 cells. To analyze the effect of 60 µM of noradrenaline on the metastasis potential in sarcoma cell lines, we did a migration assay with A673 and RD cells.

Effect of Noradrenaline on A673 and RD Sarcoma Cell Numbers

Next, we treated A673 and RD cells with a 5x dilution series of noradrenaline or with growth medium (control) for 24, 48 or 72 hours and measured the effect of noradrenaline on cell numbers via a sulforhodamine B (SRB) assay. We found that concentrations of 0.096, 0.48, 2.4 and 12 µM do not affect cell number in both cell lines (**Figure 14**). Treatment of A673 cells with a concentration of 60 µM of noradrenaline decreases cell number significantly by 61.89 ± 10.36% after 72 hours compared to control cells. The same treatment does not affect

cell number in RD cells. Concentrations of 300 µM of noradrenaline decreases cell number by more than 75% after 24 hours and by 95% after 48 hours in both cell lines.

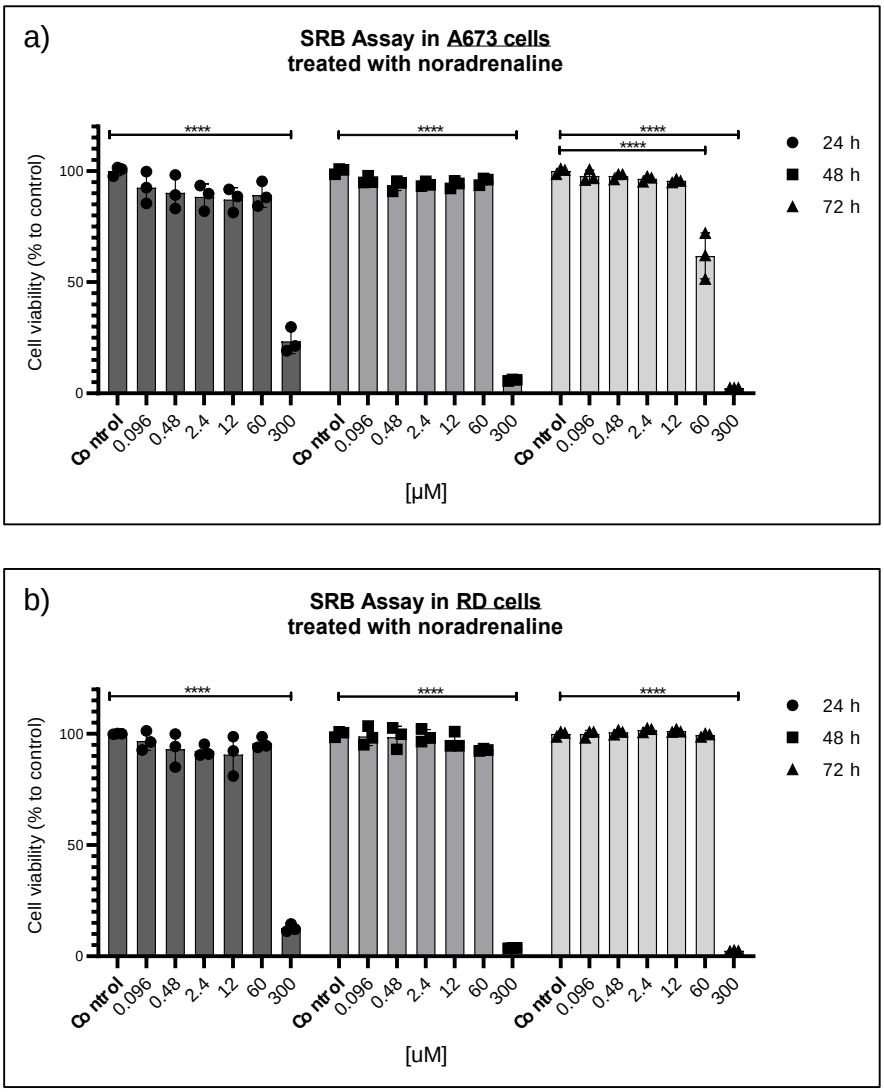


Figure 14: Noradrenaline treatment affects A673 and RD sarcoma cell number differently. We treated a) A673 and b) RD cells with a 5x dilution series of noradrenaline from 300 µM to 0.096 µM and measured cell number via sulforhodamine B (SRB) staining after 24, 48 and 72 hours. Cell number is shown as percentage of cell number in each sample compared to control. Each data point as well as mean ± SD are presented in the figure. We found significant differences in cell number in both cell lines after treatment with 300 µM for 24 hours. Treatment of A673 cells with 60 µM of noradrenaline for 72 hours affected cell number significantly, while RD cells were not affected by the same treatment. Two-way ANOVA with multiple comparison Turkey test was used to calculate p values. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.001$.

We found a concentration of 60 µM of noradrenaline to affect cell number in A673 cells after 72 hours but not in RD cells. Next, we analyzed whether the reduced cell number in A673 cells treated with 60 µM of noradrenaline is associated with reduced proliferation rates in these cells.

Effect of Noradrenaline on Proliferation in A673 and RD Sarcoma Cell Lines

To study the effect of noradrenaline on proliferation, we treated A673 and RD cells with 60 µM of noradrenaline or growth medium (control) and measured cell proliferation after 72 hours via 5'-ethynyl-2'-deoxyuridine (EdU) incorporation and fluorescence microscopy (**Figure 15**). In A673 cells, treatment significantly decreases cell proliferation by 15.88% from 49.89 ± 5.99% in controls to 34.01 ± 1.88% in treated cells. The treatment does not affect proliferation rate in RD cells with 34.20 ± 2.15% proliferating cells in control and 35.06 ± 4.15% in treated samples.

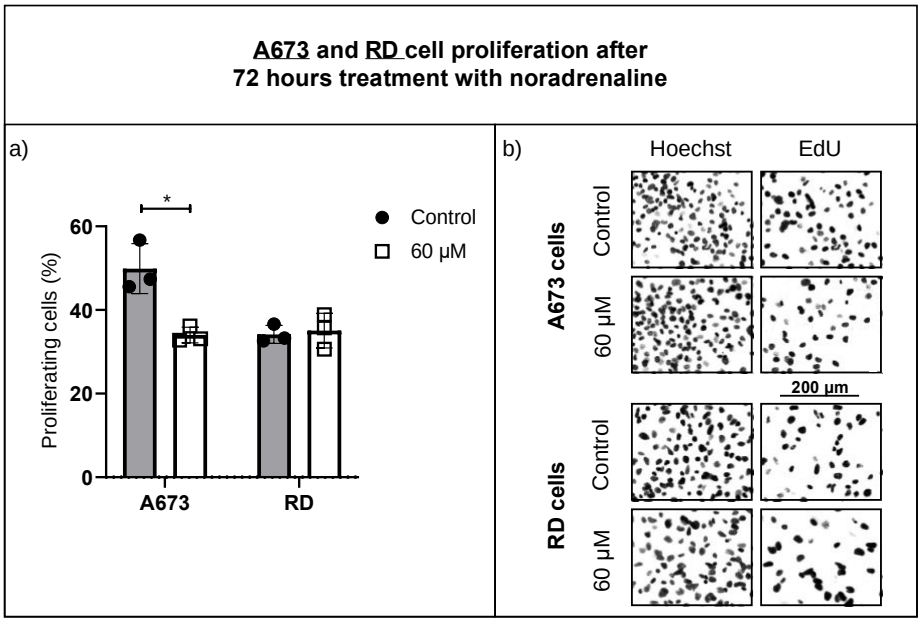


Figure 15: Noradrenaline treatment affects A673 and RD sarcoma cell proliferation differently with measurable treatment-effects only in A673 cells. To detect cell proliferation, we incorporated 5'-ethynyl-2'-deoxyuridine (EdU) in the cells during the last four hours of the 72 hour treatment period. Additionally, we stained nuclei using bisBenzimide H 33342trihydrochloride (Hoechst). Per sample, three images were taken with a fluorescence microscope (Zeiss Axiovert 100). Cells were counted in three random view fields per image with more than 2,000 cells counted per experiment. a) While in A673 cells treatment with 60 µM of noradrenaline decreased cell proliferation by 15.88 ± 6.76% after 72 hours, RD cells were not affected by the same treatment. Unpaired t-test with Holm-Šídák method was used to calculate p-values. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.001$. b) Representative images of view fields of fluorescence microscopy in both cell lines and treatment are shown.

With this EdU assay, we validated that noradrenaline reduces cell number (as measured in the preliminary SRB-assay) in A673 cells but not in RD cells by reduced cell proliferation. To analyze noradrenaline signalling in our cells, we tested the effect of 60 µM of noradrenaline as well as a more physiological concentration of 20 nM of noradrenaline (Bracken *et al.*, 2009) on cAMP in both cell lines.

Effect of Noradrenaline on Cell Migration in A673 and RD Sarcoma Cell Lines

Cell migration is an important marker for the metastasis potential of cancer cells. Therefore, we analyzed the effect of noradrenaline on cell migration in A673 and RD cell lines. Cells were treated with 60 µM of noradrenaline or growth medium (control) for 72 hours and microscopic images of the cell free gap were taken after 0, 24, 48 and 72 hours of treatment (**Figure 16**). In A673 cells, noradrenaline treatment results in a significant reduction of cell migration after 24 hours compared to control. RD cell migration is not affected by noradrenaline treatment.

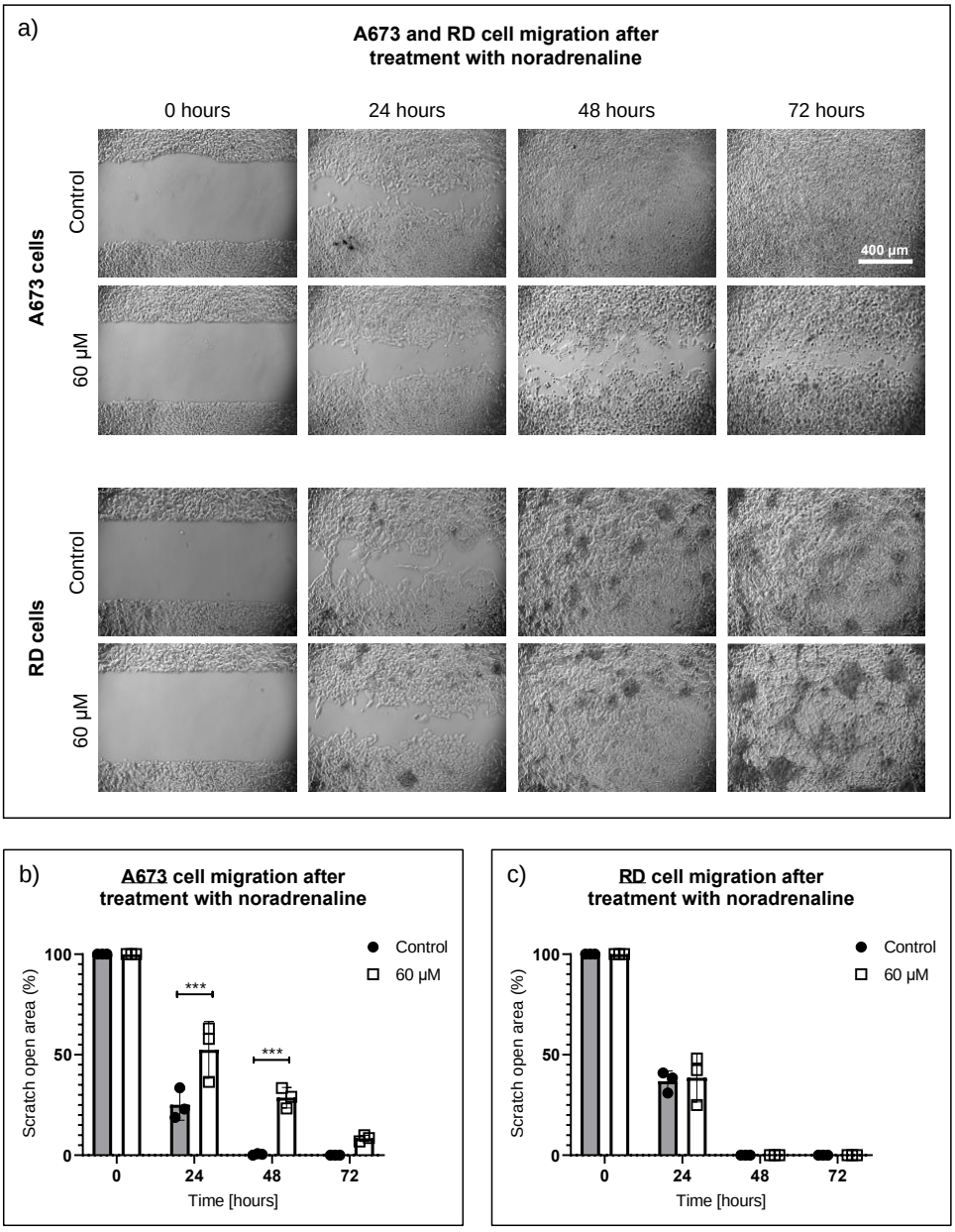


Figure 16: Noradrenaline treatment affects A673 and RD sarcoma cell migration differently with measurable treatment-effects only in A673 cells. We used ibidi™ migration assay dishes with a removable silicone insert that leaves 500 µm of a cell free gap. a) Microscopic images were taken after 0, 24, 48 and 72 hours of 60 µM noradrenaline or growth medium (control) treatment. Percentage of scratch open area was calculated using the ibidi wound healing analysis FastTrack AI. The experiment was conducted three times with three technical replicates each. Results are shown for b) A673 and c) RD cells from one representative

experiment with each data point presenting one technical replicate. We found significant migration differences between 60 µM noradrenaline treatment and control in A673 cells after 24 hours. While after 24 hours in noradrenaline treated A673 cells there is a scratch open area of 52.47 ± 14.08% left, control A673 cells show a scratch open area of 25.09 ± 7.64%. After 48 hours, control A673 cells completely closed the gap, whereas noradrenaline treated cells still display an open area of 28.59 ± 5.07%. Cell migration was not affected by noradrenaline in RD cells. Two-way ANOVA with Šídák's multiple comparisons test was used to calculate p values. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.001$.

Discussion

Our study revealed, that A673 Ewing sarcoma and RD rabdomyosarcoma cells show differential expression of adrenergic receptor isoforms and presumably as aconsequence respond differently to noradrenaline. This supports the hypothesis that the response of cancer cells to catecholamines depends at least in part on the adrenergic receptor expression. While A673 cells express α1D-, α2C-, β1 and β3-receptors and respond to noradrenaline with altered cell behavior, RD cells barely express adrenergic receptors and do not respond to noradrenaline. This could be an explanation for different effects of catecholamines on cancer in stress and exercise studies as described in a recent review of our group (Wackerhage *et al.*, 2021). Catecholamines have been associated with beneficial effects of exercise in cancer prevention and progression (Dethlefsen *et al.*, 2017; Pedersen *et al.*, 2016). However catecholamines have also been linked to the detrimental effects of stress on cancer (Cui *et al.*, 2019; Partecke et al., 2016; Renz *et al.*, 2018a; Thaker *et al.*, 2006). It has already been reported that cAMP, which is a second messenger in a cell's response to catecholamines, may have a tumor-suppressive as well as tumor promoting role, depending on tumor type and context (Zhang *et al.*, 2020). In a recent review on mechanisms that link stress to cancer, Eckerling et al. discussed the contrasting beneficial and deleterious effects of stress-factors induced by exercise or stress, respectively. As potential explanations, they mention the temporary adrenergic response to exercise and inhibited stress responses after exercise. Our study adds adrenergic receptor expression as another potential explanation for the described seemingly contradictory cell responses to the same stimulus.

Our findings are in contrast to published literature with regard to the effect of noradrenaline on cancer cell proliferation. Noradrenaline has been shown to enhance proliferation in gastric (Zhi et al., 2019), breast (Zhou et al., 2020) and colon (Han et al., 2020) cancer cells, which all express β2 adrenergic receptors. A potential explanation for the proliferation inhibiting effect of noradrenaline in A673 cells could be that these cells barely express β2-receptors (z-transformed relative expression of -5.07). Especially β2 signalling is associated with increased tumor cell proliferation (Kaira et al., 2019). Since RD cells barely express adrenergic receptors, we did not expect altered cell proliferation after noradrenaline treatment.

Another important cancer hallmark besides proliferation is the ability of cells to migrate – which is a marker for the metastatic potential of malignant cells (Chaffer and Weinberg, 2011). While A673 cells decrease migration RD cells do not respond to the treatment. This finding goes beyond published literature on the effect of noradrenaline on cancer cell migration. Noradrenaline has been shown to enhance migration in breast (Drell et al., 2003), glioma (Wang et al., 2022) colon (Han *et al.*, 2020) and prostate (Barbieri et al., 2015) cancer cells. Glioblastoma (Zhong et al., 2021) and oral (Bravo-Calderón et al., 2020) cancer cells have been shown to decrease migration after noradrenaline treatment. Migration of pancreatic cancer cells has been shown to increase (Huang et al., 2012) or decrease (Stock et al., 2013) after noradrenaline treatment. Generally, increased cell migration is associated with β2 signaling (Gruet et al., 2020) and A673 cells do not express β2 receptors, which might explain the effects onserved in our analysis. However, cells in oral cancer express β2 adrenergic receptors and were reported to decrease migration after noradrenaline treatment (Bravo-Calderón *et al.*, 2020). Taken together, noradrenaline has different effects on migration in various cell lines. Since RD cells barely express adrenergic receptors, we did not expect altered cell proliferation after noradrenaline treatment.

The first limitation of our study is that we analyzed effects of noradrenaline on cell proliferation, migration and signaling in a total of two cell lines. The cell lines were chosen based on their adrenergic receptor expression to test our hypothesis that cells respond to noradrenaline based on their adrenergic receptors. While our findings support our hypothesis, there is a need for further research with a larger number of cell lines to analyze adrenoreceptor-specific responses of cancer cells to noradrenaline. The second limitation of our study is the high noradrenaline concentration that was needed to trigger cellular responses. While physiological concentrations of 20 nM (Bracken *et al.*, 2009) did not lead to increased cAMP levels in vitro, we could show that 60 µM of noradrenaline led to a 38-fold increase in cAMP levels from 0.78 ± 0.13 pmol/mL to 29.15 ± 1.09 pmol/mL 5 minutes after treatment. This shows that in vitro higher treatment concentrations are needed to trigger a cell response compared to in vivo. The third limitation of our study is that we could show adrenergic receptor expression via RNA-seq but not in proteomics analysis. However, mRNA expression of adrenergic receptors measured in our analysis is in line with adrenergic receptor expression in the CCLE.

In conclusion, our study shows that the same treatment with noradrenaline in the two sarcoma cell lines A673 and RD leads to different proliferation, migration and signalling responses. This indicates that adrenergic receptor expression of a tumor cell determines how a cell responds to catecholamines. For exercise in oncology this means that adrenergic receptor expression might be a potential marker for exercise prescriptions. However, more studies with more cell

lines need to be conducted. Additionally, studying exercise-conditioned sera in vitro can be a next step to measure effects of more than one metabolite on cancer hallmarks. These efforts can eventually lead to highly individualized exercise prescriptions for cancer patients based on molecular findings.

3.3.1 Discussion of Study 3

The aim of this study was to provide proof-of-concept that the expression of adrenergic receptors determines the response of a tumor cell to catecholamines. This would contribute to solving the Cancer Catecholamine Conundrum, as catecholamines have been shown to affect tumor cells differently in different studies. We chose noradrenaline for our experiment as it binds to all adrenergic receptors. My cell culture studies showed that noradrenaline decreases cell proliferation and migration and increases cAMP signaling only in A673 cells, which express adrenergic receptors, but not in RD cells, which do not express adrenergic receptors. This novel approach could explain different effects of catecholamines in different studies. Future studies evaluating the effect of catecholamines on cancer should consider the adrenergic receptor expression in their specific tumor cells.

For exercise oncology, the results of this study show that exercise-induced noradrenaline affects tumor cells based on their adrenergic receptor expression. Hence, if exercise would affect tumor growth via increased noradrenaline concentrations, the effect will not be seen in all tumor cells. More importantly, further research in exercise oncology should focus on specific adrenergic receptor signaling and its effect on cancer hallmarks. This means that specific receptors might trigger different and even opposite effects. If so, precautions should be taken when exercising at high intensities with specific cancer patients.

For this study, I isolated RNA using Trizol and Novogene in Cambridge sequenced the RNA. This way, I could show gene expression of the nine adrenergic receptors *ADRA1A*, *ADRA1B*, *ADRA1D*, *ADRA2A*, *ADRA2B*, *ADRA2C*, *ADRB1*, *ADRB2* and *ADRB3*. Unfortunately, untargeted proteomics analyses could not detect the translated proteins of adrenergic receptors. A subsequent correlation analysis between mRNA and protein levels in my sample will reveal the general informative value of mRNA expression for protein levels. The obtained transcriptome for both cell lines after noradrenaline treatment could also give indications about gene expression changes induced by noradrenaline treatment, which will be part of further analyses.

4 Summary

All in all, the beginning of exercise oncology dates back to long before the current century. However, research interest substantially increased with the beginning of the 21st century. New omics-approaches now allow researchers to analyze why exercise has an effect on cancer at the molecular level. It is well established that exercise has protective effects for various cancer types and can also improve outcomes of cancer therapy. The three studies of this dissertation contribute to awareness and address possible solutions to two central issues in the current field of exercise oncology: 1. Neglect of children in exercise oncology research and 2. contradicting effects of catecholamines in exercise and stress studies. The main goal of this thesis is to justify and establish an independent mechanistic research approach in pediatric exercise oncology.

In my literature review, I show that evidence in pediatric exercise oncology is low compared to adult exercise oncology. While effects of exercise on physical outcomes and cancer treatment-related side effects are well-established, direct anti-cancer effects of exercise are less clear. In childhood cancer patients, direct anti-cancer effects of exercise are unknown.

My HIIT intervention with childhood cancer patients shows that safety can be ensured, however such an intervention is feasible only in a small number of physically fit patients that minimally suffer from treatment side effects. HIIT significantly increases heart rate and lactate concentrations but adrenaline levels remain unaltered after the exercise. These results show two important differences between children and adults: 1. Despite the high intensity of the exercise and the significant increase in lactate levels, adrenaline does not increase during the exercise in childhood cancer patients. 2. While HIIT can generally be conducted with adult cancer patients, it is feasible only in a small number of childhood cancer patients and cannot be used as standard care in this cohort.

My cell culture experiments contribute to solving the Cancer Catecholamine Conundrum. While some studies explain the positive effects of exercise on cancer with catecholamines, other studies explain the detrimental effects of stress on cancer with catecholamines. My study provides proof-of-concept that these contradicting findings might be due to differences in adrenergic receptor expression of cancer cells. I cultured two pediatric sarcoma cell lines and analyzed the effect of noradrenaline on cell signaling, proliferation and migration. My work shows that tumor cells, which express adrenergic receptors of all three groups, increase their cAMP signaling and decrease proliferation and migration. Tumor cells, which do not express adrenergic receptors, are not affected by noradrenaline treatment.

5 Future directions

The work of this dissertation shows that it is important to analyze the application of exercise specifically in pediatric oncology. It has to be noted that there is a difference between playful physical activity and physical exercise. While playful physical activity programs are applied safely in some clinical settings for childhood cancer patients, little is known about exercise and its effects in this cohort. My intervention study showed that high-intensity exercise cannot be used as a general adjunct treatment in childhood cancer patients. Additionally, it seems that high-intensity exercise affects childhood cancer patients differently than adults – as adrenaline levels did not increase despite the intensity of the exercise.

Based on the results of this dissertation, the following future research directions arise.

- Increasing sample sizes for pediatric exercise oncology research by multi-center studies.
- Analysis of exercise with different intensities and their physiological effect in childhood cancer patients.
- Metabolomics analyses to search for markers that are affected by exercise specifically in childhood cancer patients. Literature analysis might reveal promising metabolites, which could be analyzed in in vitro experiments for their effects on cancer hallmarks.
- The effect of catecholamines on cancer hallmarks should be tested with more tumor cell lines, as catecholamines might have beneficial, detrimental or no effects. With more cell lines tested, patterns might be detected for the role each adrenergic receptor and its effect on cancer hallmarks.

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Appendix

I Conference Contributions

Conference abstract

Weeber, P., Haferanke, J., Schönfelder, M., Regina, C., von Lüttichau, I., Wackerhage, H. Variable Effekte von Noradrenalin auf zwei Krebszelllinien: Implikationen für die Sporttherapie von Krebspatienten [abstract]. In: 25. dvs-Hochschultag.; 29.-31.03.2022; Kiel, Germany. Hamburg: EDITION CZWALINA FELDHAUS VERLAG GmbH & Co. KG; 2022. p. 199.

Conference poster

Kesting, S., Schönfelder, M., **Weeber, P.**, Pfluger, A., Prehn, C., Kastenmüller, G., Römisch-Margl, W., Wackerhage, H., von Lüttichau, I. Changes in the concentration of metabolites that are known to influence cancer cell signalling and/or behaviour in paediatric cancer patients after exercise [poster]. In SIOP 2020.; 14.-17.10.2020; Virtual Congress. Poster number: 0592 / #848.

Conference talk

Weeber, P. A bout of high-intensity interval training (HIIT) in children and adolescents during acute cancer treatment – A feasibility study [abstract talk]. In Pediatric Exercise Oncology Congress.; 07.-08.04.2022; Virtual Congress.

II Young Investigator Award

1st Pediatric Exercise Oncology Congress

YOUNG INVESTIGATOR AWARD

AT THE 1ST PEDIATRIC EXERCISE ONCOLOGY CONGRESS

This Award is presented to

Peter Weeber

For the talk: A bout of high-intensity interval training (HIIT) in children and adolescents during acute cancer treatment – A feasibility study

This certificate was awarded on 08 April at the 2022.

Dr. Nicole Culos-Reed
Congress president

Dr. Miriam Götte
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