From the I. medical clinic and policlinic
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The effects of endurance training intervention on circulating cell-free DNA in participants with histologically proven non-alcoholic fatty liver disease.

Die Auswirkungen von Ausdauertrainingsinterventionen auf zirkulierende zellfreie DNA in Probanden mit histologisch nachgewiesener nichtalkoholischer Fettlebererkrankung.

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I. List of abbreviations

AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
BMI	Body-Mass-Index
bp	Base pair
cfDNA	Circulating cell-free DNA
CK	Creatinine kinase
CRP	C-reactive protein
DNA	Deoxyribonucleic acid
DNAse	Deoxyribonucleic acid nuclease
dNTPs	Deoxynucleotide Triphosphates
EDTA	Ethylenediaminetetraacetic acid
g	Gram
HbA1c	Glycated hemoglobin or hemoglobin A1C
HELP Study	Hepatic Inflammation and Physical Performance in Patients With NASH study
HOMA IR	Homeostasis model assessment insulin resistance
HR	Heart rate
IL-6	Interleukin 6
IL-8	Interleukin 8
IR	Insulin resistance
min	Minute
mg	Milligramm

ml	Milliliter
µl	Microliter
MAFLD	Metabolic associated fatty liver disease
mM	Millimolar
mtDNA	Mitochondrial DNA
NAFLD	Nonalcoholic fatty liver disease
NAS	Nonalcoholic fatty liver disease activity score
NASH	Nonalcoholic steatohepatitis
NETs	Neutrophil Extracellular Traps
NETosis	NET activation and release
ng	Nanogram
PCR	Polymerase chain reaction
SD	Standard deviation
SLE	Systemic Lupus Erythematodes
SH	Steatohepatitis
TNF-α	Tumor necrosis factor alpha
U	U

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IV. Zusammenfassung

Das Forschungsgebiet der cfDNA und ihrer Verwendung als potenzieller Biomarker für verschiedene Krankheiten wird größer. Das Ziel dieser Arbeit war: die Auswirkungen von Ausdauertraining auf den cfDNA-Spiegel zu untersuchen, und die Möglichkeit cfDNA als Marker für das Fortschreiten der Krankheit bei Patienten mit NAFLD und NASH zu verwenden.

Weltweit wurde ein Anstieg der Prävalenz bei Patienten mit nichtalkoholischer Fettlebererkrankung und ihrer Subtyp der nichtalkoholischen Steatohepatitis beobachtet. Mit diesem Anstieg besteht für Patienten eine höhere Mortalität aufgrund von bösartigen Erkrankungen und Herz-Kreislauf-Erkrankungen.

Um das Risiko von Komplikationen zu verringern, wurden verschiedene Behandlungen empfohlen. Zu den verfügbaren Optionen gehören Änderungen des Lebensstils und pharmakologische Therapien. Änderungen des Lebensstils, einschließlich Gewichtsverlust und Bewegung, verlangsamen nachweislich sowohl das Fortschreiten der Krankheit als auch die histologischen Befunde bei Patienten mit NAFLD.

Eine Kombination aus der Verwendung nicht-invasiver hepatischer Entzündungsmarker und invasiven Leberbiopsien wurde bislang verwendet, um das Fortschreiten der Krankheit zu verfolgen. Leberbiopsien gelten zwar als Goldstandard, sind aber auch eine teure und invasive Methode, die medizinisches Fachwissen erfordert und mit verschiedenen Risiken verbunden ist. Leberbiomarker ALT und AST sind die beiden am häufigsten als Indikatoren für Leberschäden verwendeten Biomarker. Erhöhte Serumspiegel wurden mit größeren Leberschäden in Verbindung gebracht.

In dieser Arbeit wurden DNA-Fragmenttypen mit 90 bp und 222 bp untersucht. Die Proben wurden auch bei 1600 und 16000 g zentrifugiert. Zum Zeitpunkt „POST“ erreichten die cfDNA-Werte während der Intervention ihren Höhepunkt. Dies wurde sowohl in der Gruppe vor als auch nach der Intervention, sowie in Proben beobachtet, die bei 1600 und 16000 g zentrifugiert wurden. Stunden nach Abschluss des Trainings erreichten die cfDNA-Werte ihren Ausgangswert. Unsere Ergebnisse deckten sich mit vielen anderen Studien, die ebenfalls die Wirkung verschiedener Trainingsformen auf die DNA-Ebene untersuchten.

Nach Abschluss der 8-wöchigen Web-Based Intervention konnten die Teilnehmer ihr Gewicht und ihren BMI senken, ihren Fettleberindex verbessern und die Konzentrationen der Leberbiomarker AST und ALT senken. Im Vergleich dazu waren die cfDNA-Ausgangswerte zum Zeitpunkt „PRE“ nach der Intervention jedoch sowohl bei 90 bp als auch bei 222 bp höher.

Die Methoden zur Bestimmung des Ursprungs von cfDNA entwickeln sich ständig weiter. Neuste Studien, die mit Marker der DNA-Methylierung durchgeführt wurden, haben gezeigt, dass die Gesamt-cfDNA-Spiegel zwischen Patienten mit NAFLD und solchen ohne NAFLD zwar nicht signifikant waren, die methylierten cfDNA-Spiegel jedoch schon.

Wir glauben daher, dass der kombinierte Einsatz unterschiedlicher Methoden der cfDNA-Analyse sowie zusätzlicher nicht-invasiver Verfahren in Verbindung dazu beitragen können, die Genauigkeit der Diagnose und das Fortschreiten von NAFLD und NASH zu verbessern.

1 Introduction

Non-alcoholic fatty liver disease (NAFLD) and its subtype nonalcoholic steatohepatitis have emerged as one of the most common causes of liver disease worldwide. Patients suffering from NAFLD are at risk of increased mortality due to malignancy and cardiovascular disease, as well as liver disease (Rinella et al. 2015). The presence of metabolic syndrome increases the risk of developing NAFLD, whereas physical training and nutrition were both revealed to have a beneficial effect on inflammatory markers, as well as disease progression. Reliable, new approaches for the early detection, diagnosis, and prognosis of NAFLD have been sought after as a replacement for invasive liver biopsies. Liver enzymes and abdominal ultrasounds are just two of the diagnostic tools that are used in the diagnosis and prognosis of the disease, however, their sensitivity is dependent on multiple factors (Rinella et al. 2015). Due to its promising results in other pathologies, cfDNA has been explored as a possible non-invasive substitute.

The treatment of NAFLD is multifaceted. Lifestyle changes have been noted as contributing factors to the improvement of NAFLD (Linden et al. 2016). Additionally, the effect of various forms of exercise on the progression of the disease has also been examined. While some researchers found that a combination of exercise and caloric restriction were most effective and led to liver histological and biochemical improvements (Rodriguez et al. 2012), Hashida et al. determined that significant weight loss was not a requirement for the improvement of hepatic steatosis. It is believed that any quantity of exercise is helpful for enhancing resolution of existing fatty liver or reducing risk of developing new fatty liver (Sung et al. 2016).

cfDNA, or circulating cell-free DNA is DNA which is degraded and found outside of cells within bodily fluids such as cerebrospinal fluid, blood plasma, and urine. It was first discovered by Mendel and Métais in 1948. Since then, research has been conducted to determine its origin. Until recently, it was believed that cfDNA fragments were released into the blood stream upon cell death (Aucamp et al. 2018). Additionally, researchers have hypothesized the origin of cfDNA and believed that it was released from hematopoietic cells (Lui et al. 2002). Recent studies, however, have shown that the largest contributor to the release of cfDNA are neutrophilic granulocyte cells (Fridlich et al. 2023). With the use of several varying approaches,

researchers have been able to identify specific cell tissue markers, thus making advancements in the determination of the origin of cfDNA molecules.

Research into cfDNA is constantly evolving, and with it, so are the methods for its acquirement and analysis. PCR analysis, next generation sequencing and DNA methylation have all been described as prevalent methods for cfDNA quantification and analysis (Bohers et al. 2021).

Various studies have already examined its potential use for the diagnosis, prognosis, and therapy of illnesses, such as breast, gastric, ovary, lung, colon, and prostate cancer, as well as systemic lupus erythematosus (SLE), where higher levels of this nucleic acid have been found (Han et al. 2017, Hendy et al. 2016, Wang et al. 2017, Cherepanova et al. 2008). In addition to the aforementioned pathologies, researchers have also analyzed the effects of diverse training on cfDNA levels. (Atamaniuk et al. 2008, Breitbach et al. 2012, Haller et al. 2017, Tug et al. 2017). However, very few studies have explored the role of cfDNA with regards to non-alcoholic fatty liver disease (NAFLD). A recent study conducted by Chrysavgis et al. examined the relationship between varying levels of cfDNA and the progression of NAFLD. Using total cfDNA and DNA Methylation markers they revealed that cfDNA concentrations were much lower in cirrhotic patients than non-cirrhotic patients, while no significant differences were seen between NAFLD and NASH patients.

Many studies have been conducted with respect to the effect of resistance and endurance training on inflammatory markers of not only fatty liver disease, but also other pathologies. To date, however, only one study examined the possibility of cfDNA as a biomarker for the progression of NAFLD. The effect of exercise interventions has not been studied yet.

The objective of this study was to examine the concentration and kinetics of cfDNA, as well as its fragmentation, before and after an endurance exercise intervention. Moreover, it will be studied if cfDNA could be used as a non-invasive biomarker for monitoring the progression of non-alcoholic fatty liver disease in response to exercise intervention.

2 Literature review

2.1 Non-alcoholic fatty liver disease

An increased prevalence of nonalcoholic fatty liver disease has been seen not only in western countries, but also Asian pacific countries, and is considered to be one of the most common causes for liver disease world-wide (Duseja et al. 2013, see also Rinella et al. 2015). Changes in dietary habits and lifestyle have promoted both an increase in obesity, as well as NAFLD (Duseja et al. 2013). In addition to the male sex, other risk factors have been associated with NAFLD, such as obesity, increasing age, and metabolic syndrome.

NAFLD is defined as the accumulation of fat in the liver (hepatic steatosis), which is determined by imaging or liver histology and caused by factors other than alcohol abuse, medication, or other medical conditions. NAFLD is further divided into two subclasses, nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). The two subclasses can be histologically differentiated from one another. NAFL is defined as the presence of ≥ 5% hepatic steatosis with no evidence of hepatocellular injury in the form of hepatocyte ballooning, while NASH is defined as the presence of ≥ 5% hepatic steatosis with evidence of inflammation with hepatocyte injury (ballooning) with or without fibrosis (Puriet al. 2012).

The pathogenesis of NAFLD is not fully understood. Some hypothesize that insulin resistance is a key component in the development of the disease, while others hypothesize that a second hit in the form of an oxidative injury must take place in order to lead to the full manifestation of the disease.

Although insulin resistance (IR) is commonly found in NAFLD patients, not all patients suffering from NAFLD exhibit IR. By way of the β2-Adrenozeptor, insulin is able to inhibit β-oxidation. This leads to an increased esterification of free fatty acids (FFA), which may serve as a protective measure against the toxic effects of FFAs (Yamaguchi et al. 2007).

Adipokines possess pro- and anti-inflammatory properties and are also believed to play a role in the pathogenesis of NAFLD. Adiponectin, an anti-inflammatory mediator produced by adipose tissue, has been linked with increased glucose uptake and fat oxidation in muscle, as well as improvements in insulin sensitivity (Rabe et al. 2008). Additionally, it has been known to counteract the effects of TNF-α, which is known to have a negative effect on IR. Low serum levels of adiponectin have been observed in patients with NAFLD, whereas higher levels of leptin, a mediator also produced by

adipose tissue, were seen. Increased energy expenditure, stimulation of β-oxidation and enhancement of insulin sensitivity are some of the functions of leptin. In addition, it stimulates the expression of TNF-α, as well as Interleukin-6, which are known to increase IR (Rabe et al. 2008). The increased serum levels have been attributed to leptin resistance.

As the prevalence of NAFLD increases worldwide, researchers have begun searching for a new, cost-effective, and reliable manner for the early detection of liver pathologies. The majority of the patients diagnosed with NAFLD are asymptomatic, however, symptoms may present themselves in the form of fatigue, malaise, and right upper abdominal discomfort. Hepatomegaly is the most common finding during the initial physical examination (Puriet al. 2012). For the diagnosis of NAFLD, four criteria must be met: 1) evidence of hepatic steatosis by imaging or histology, (2) no significant alcohol consumption, (3) absence of other etiologies for hepatic steatosis, and (4) absence of coexisting causes for chronic liver disease (Chalasani et al. 2018).

2.1.1 Biomarkers and inflammatory mediators in Non-alcoholic fatty liver disease

Hepatic biomarkers and inflammatory mediators represent two of the possibilities for tracking disease progression. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the two most common biomarkers used as indicators of hepatic damage. Increases in serum levels have been reported with increased hepatic damage, however asymptomatic increases in serum levels have also been reported. Moreover, various studies have observed normal serum levels in patients suffering from NAFLD. The lack of specificity diminishes the diagnostic value of these hepatic biomarkers (Kim et al. 2008, see also Hadizadeh et al. 2018). Studies have also reported increases in alkaline phosphatase and decreases in serum albumin in NAFLD patients (Hadizadeh et al. 2018).

Inflammatory markers provide another approach for tracking disease progression. Increases in C-reactive protein (CRP), a protein produced in the liver, have been seen in patients with NAFLD. Unfortunately, serum levels are dependent on multiple factors, such as age, sex, smoking, and alcohol consumption (Hadizadeh et al. 2018).

Adiponectin is produced by fat tissue and exhibits anti-inflammatory and anti-lipogenic effects. Studies reported decreased levels of adiponectin with increasing levels of hepatic fat. Additionally, patients suffering from steatohepatitis displayed lower levels

when compared to the control group. Aside from the aforementioned effects, adiponectin also neutralizes TNF-α.

Studies evaluating the role of leptin in the pathogenesis of NAFLD are inconclusive. Various researchers concluded that the serum level coincides with the degree of hepatic steatosis, whereas others were not able to come to this conclusion (Hadizadeh et al. 2018).

Significantly elevated levels of TNF-α and interleukin-6 (IL-6) were seen in patients with severe NAFLD in comparison to control subjects. TNF-α possesses pro-inflammatory attributes and is known to increase insulin resistance, serum triglyceride levels, as well as stimulate the production of hepatic fatty acids. Additionally, it plays a role in the proliferation and apoptosis of hepatocytes and is involved in the development of hepatic fibrosis. Much like TNF-α, IL-6 has been linked with hepatic lipogenesis and impairment of insulin sensitivity. Increased serum levels have been linked with an increase in insulin resistance (Das et al. 2011).

2.1.2 Diagnostics in non-alcoholic fatty liver disease

Ultrasound is an inexpensive, non-invasive, widely available method of abdominal imaging; however, it is user dependent and lacks sensitivity when patients exhibit less than 30% steatosis in the liver biopsy (Ahmed et al. 2015). Findings consistent with an echogenic or bright liver are indicative of a fat accumulation (Puriet al. 2012).

The liver biopsy is considered the gold standard for the histological differentiation of NASH and NAFL, however, it is an expensive and invasive method which requires medical expertise and is accompanied by various risks. It is therefore recommended in situations where patients are found to be at an increased risk for steatohepatitis (SH) and advanced fibrosis, or situations where coexisting chronic liver diseases cannot be excluded without the help of a liver biopsy (Puriet al. 2012, see also Chalasani et al. 2018). Histological characteristics of NAFL include the following alternative criteria: a) steatosis alone, b) steatosis with lobular or portal inflammation, without ballooning, or c) steatosis with ballooning but without inflammation. Evidence of steatosis, ballooning and lobular inflammation are all required for the diagnosis of NASH (Marachesini et al. 2015).

The NAFLD activity score (NAS) is a system used to rank disease activity based on the sum of steatosis (0 to 3), lobular inflammation (0 to 3), hepatocellular ballooning (0 to 2). Fibrosis is not included in the NAS. Activity scores ranging from 0-2 were considered not

characteristic for NASH, scores between 3-4 were evenly distributed amongst the three categories, while scores ≥ 5 were considered indicative of NASH.

2.1.3 Treatment of non-alcoholic fatty liver disease

Successful treatment options for nonalcoholic fatty liver disease are scarce. Among the available options are lifestyle changes and pharmacological therapies. Of the two, lifestyle changes have been proven to both slow disease progression, as well as improve histological findings in patients with NAFLD. Various studies have illustrated the benefits of exercise and its positive effects on inflammatory markers. A combination of resistance and aerobic training led to reductions in both TNF-α levels, as well as circulating leptin levels (Ihalainen et al. 2018). After a 4-week physical training program, patients with an abnormal glucose tolerance showed reduced levels of CRP, a normalization of adiponectin, as well as an increased insulin sensitivity (Oberbach et al. 2006). A reduction in pro-inflammatory mediators such as IL-6 and IL-8 were seen in healthy, young individuals after a 9-week resistance training program (Forti et al. 2017).

Histological improvements have been observed after a 10% reduction in weight is achieved (Linden et al. 2016). Although they are familiar with the benefits of exercise, some NAFLD patients have expressed a lack of confidence due to fear of failure (Zelber-Sagi et al. 2014). While it is recommended that patients with NAFLD undergo weight loss, various studies have also shown that exercise leads to an improvement in hepatic steatosis, even without significant weight loss (Hashida et al. 2017). Exercise intensity and duration are two important variables that directly affect the extent of improvement. Studies have shown that high intensity interval training may provide comparable or better results than continuous moderate-intensity exercise of longer duration (Hallsworth et al. 2015). This provides patients with a less time-consuming alternative for exercise and weight loss.

Researchers have studied various medical alternatives to weight loss strategies in the form of pharmacotherapy. Insulin sensitizers, statins and vitamin E have been explored in various studies. Over the years, pharmacotherapy has proven useful for various patients with NASH, but no single treatment has been found for all patients. Vitamin E yielded the most promising results by showing an improvement in NASH-related inflammation and fibrosis by presumably suppressing lipid peroxidation and oxidative stress (Ahmed et al. 2015).

Insulin sensitizers, such as metformin or pioglitazone, are beneficial for NASH patients with type 2 diabetes. By improving insulin sensitivity in adipose tissue, the liver and muscle, a histological benefit was achieved by reduction of steatosis, lobular inflammation, and hepatocyte ballooning. In spite of the benefits of piogliatozone, it is not without risk, as the food and drug administration warn of a possibility of congestive heart failure in at-risk patients (Ahmed et al. 2015).

Although evidence supports the beneficial effect of pharmacotherapy in the treatment of NASH, a singular strategy has yet to be found. The research appears promising; however, medical therapy has not yet been approved for the treatment of NASH.

An increased emphasis on the early detection of NAFLD may be beneficial for its treatment. While the usefulness of inflammatory mediators and hepatic biomarkers have been explained, researchers are looking for other promising possibilities to further aid in the management of NAFLD.

2.2 Circulating free DNA

2.2.1 Common methods for determination of the origin of cfDNA

Blood samples are collected from participants and are centrifuged to obtain plasma samples. cfDNA is then isolated from the plasma and is quantified and qualified for further analysis. Various forms of analysis have been developed for cancer detection and localization, as well as monitoring. PCR-based methods have been used to analyze point mutations in various forms of cancer. Using next generation sequencing, whole genomes can be analyzed (Bohers et al. 2021). DNA methylation is emerging as a technique to further isolate the origin of cfDNA. It is a biological process which involves the addition of methyl groups to the DNA molecule. It is not only a crucial aspect of cell type-specific gene regulation but is also a fundamental marker of cell identity (Moss et al.). Through analysis of said methylation patterns, researchers have developed a technique to analyze the origin of cfDNA during exercise (Fridlich et al. 2023).

2.2.2 Origin of cfDNA and its release upon cell death

Over the years, cfDNA has received more attention as a potential marker of inflammatory conditions (Frank et al. 2016). The first detection of circulating free nucleic acids was described by Mandel and Métais in 1948 (Ziegler et al. 2002). While DNA is normally found in the nucleus of organisms, cfDNA is a type of DNA found extracellularly within the serum, plasma, and other body fluids (Frank et al. 2016). Up until recently, research regarding the origin of cfDNA was inconclusive, however, advances in the determination of the origin of cfDNA have been made. In the past, it was

hypothesized that cfDNA is released due to cellular breakdown processes, or an active release of DNA (Aucamp et al. 2018).

Necrosis and apoptosis are cellular breakdown mechanisms believed to be responsible for the release of cfDNA. The process of necrosis or premature death of cells occurs more rapidly than apoptosis and results in larger DNA fragments (10000 BP). Apoptosis is the programmed and targeted cause of cellular death. Internucleosomal cleavage of chromatin by way of the DNase yields multiple nucleosomal fragments measuring 160-180 BP in length.

An active release of DNA has been described in the form of NETosis, which is limited to certain hematopoietic cell types. It is characterized as a rapid process involving nuclear disintegration and cell death, which leads to the expulsion of neutrophil extracellular traps (NETs), as well as the snaring and accumulation of defense-related substances and invading microorganisms (Lögters et al., 2009). The molecular weight of the DNA fragments resulting from said process is similar to that of necrosis (Aucamp et al. 2018).

Moss et al. developed an atlas of approximately 25 human tissues from both healthy participants, as well as participants with pathological conditions. Through their research, they found that cfDNA in healthy individuals was also derived from vascular endothelial cells and hepatocytes. Furthermore, they examined the differences in cfDNA based on age. Strikingly, elderly patients exhibited approximately twice the total concentration of cfDNA than that of patients in their 3rd decade of life. They hypothesized that this phenomenon could be attributed to a decrease in the rate of cfDNA clearance in the elderly. Patients suffering from sepsis were revealed to have elevated cfDNA levels, which they attributed to cell death and a strong immune reaction.

Using similar techniques in DNA methylation, Fridlich et al. sought to determine the origin of cfDNA during exercise. In their research model, healthy volunteers were exposed to various running protocols and blood samples were drawn at rest, after completion of a 12-min graded exercise, as well as after a 40 min run. Additionally, blood samples were drawn from volunteers after completion of a half-marathon and full marathon. According to their results, cfDNA levels dramatically increased after completion of exercise and were dependent on effort and duration. In accordance with our findings, cfDNA levels returned to baseline ca. 1 hour after exercise. Using the aforementioned atlas of human tissues, DNA methylation patterns were examined and revealed that approximately half of cfDNA originated from neutrophils, while

approximately a quarter originated from non-leukocyte blood cells, monocytes, lymphocytes, and vascular endothelial cells. These findings correlate with a study undertaken by Neuberger et al., in which blood drawn from patients with hematological malignancies was examined after exercise. Granulocyte derived cfDNA increased from 54,1% to 90.2% after exercise. An increase in leukocyte count, specifically neutrophil count, did not correlate with an increase in neutrophil derived cfDNA (Fridlich et al. 2023).

cfDNA can be derived from serum or plasma. Due to its easy accessibility, it may provide an alternative for the recurrent invasive diagnostic procedures that often plague cancer patients. Though its prognostic or diagnostic benefits may not be limited to cancer, much of the research has been devoted to its application in this field (Ziegler et al. 2002).

2.2.3 cfDNA and its links to various cancers

Increases in cfDNA levels have been observed in patients with breast, gastric, ovary, lung, colon, and prostate cancer (Skrypkina et al. 2016).

Research conducted on patients with lung cancer revealed that higher concentrations of cfDNA were found in patients with early-stage lung cancer in comparison to healthy individuals. The median concentration was highest in patients with stage IV, however, a correlation between advanced tumor stage and increasing cfDNA concentration was not found. In addition, no difference in DNA concentration was seen among the various histological subgroups of lung cancer (Yoon et al. 2009).

Patients with breast cancer stage II-IV exhibited higher concentrations of cfDNA than healthy individuals. In addition, higher concentrations of cfDNA were found to correlate with tumor size, lymph node involvement, histopathological grade, and clinical staging (Tangvarasittichai et al. 2015, see also Wang et al. 2017).

Multiple studies have been conducted on cfDNA concentrations in patients with colorectal cancer before and after surgery. Researchers have concluded that patients with colorectal cancer (CRC) displayed higher concentrations than those in the healthy control group. Intraoperative concentrations of cfDNA were noted to be higher, which is most likely explained by the mechanical and thermal manipulation of the tumor. The high concentrations were still measured 5 days post-operation (Bhangu et al. 2017). Another study concluded that patients with CRC and lymph node positive status or metastases displayed significantly higher cfDNA concentrations than healthy individuals. After the

first surgery, a concentration almost identical to that at the time of diagnosis was noted (Kloten et al. 2017).

Although research involving cfDNA has heavily revolved around cancer, its role in other forms of inflammation has also been studied. Recent studies have found higher concentrations of cfDNA in patients with systemic lupus erythematosus (SLE) than healthy individuals (Zhang et al. 2014, see also Tug et al. 2017). Additionally, researchers found that patients with an active lupus nephritis (ALN) exhibited higher levels than those with an inactive lupus nephritis (Zhang et al. 2014). Multiple studies have deduced that cfDNA may play a role in the pathogenesis of the disease and may be viable as a marker for disease activity and treatment (Hendy et al. 2016, see also Tug et al. 2017).

2.2.4 cfDNA and its role as a marker in sports-related studies

Research involving cfDNA is no longer exclusive to conditions involving tumors and inflammation, as it has also expanded to the realm of sports. Increases in cfDNA concentrations have been recorded after exhaustive exercise (Breitbach et al. 2012). In order to gain a better understanding of its role in sports, researchers have conducted studies on the effect of intensity, duration, and various types of training on cfDNA concentration.

Atamaniuk et al. experimented with the effect of strenuous exercise on cfDNA concentrations in the form of a half marathon and an ultra-marathon. They reported that cfDNA concentrations had significantly increased post-marathon and remained elevated after 24h. Higher levels of cfDNA were found in the 2008 study, which Atamaniuk et al. attributed to the greater physical stress of the ultra-marathon. As a result of the studies, they concluded that cfDNA may prove useful as a tool for the monitoring and quantification of cellular damage (Atamaniuk et al. 2004, see also Atamaniuk et al. 2008).

The effect of duration and relative intensity of cfDNA accumulation was described by Haller et al.. Their results displayed that low-intensity aerobic running led to steady increases of cfDNA throughout the entire exercise, while lactate increases were only noted within the first 10 minutes (Haller et al. 2017).

Tug et al. studied the acute effects during strength exercise and chronic effects of regular strength training on cfDNA concentrations over a period of four weeks. Patients were split into three groups: (i). conservation training (CT) at 60% of the 1 repetition

maximum, (ii). high intensity-low repetition training (HT) at 90% of the 1 repetition maximum, and (iii). differential training (DT) at 60% of the 1 repetition maximum. Significant increases in cfDNA concentrations were noted across all groups. Additionally, Creatinine Kinase (CK) levels were measured, however, no alterations were seen. The authors concluded that short-fragmented cfDNA released during strength training resembles a fast, aseptic inflammatory response, whereas the elevation of longer fragments at baseline demonstrates mild cellular damage due to a new training regimen (Tug et al. 2017).

Even though the majority of the studies have reported increases in cfDNA concentrations post-exercise, one study presented conflicting results. A study conducted by (Shockett et al. 2016) concluded that moderate aerobic treadmill exercise led to a transient decline in cfDNA concentrations after 54 and 90 minutes (Shockett et al. 2016).

Evidence suggests that exercise training aids in the suppression of inflammatory processes (Forti et al. 2017, see also Ihalainen et al. 2018). In addition, training has been linked with an increase in DNase I activity, which promotes transient cfDNA clearance (Velders et al. 2014). This effect, initiated by regular exercise, may provide health benefits through a further suppression of inflammation.

2.2.5 cfDNA and its role as a marker in other medical conditions

As a potential marker for disease activity and progression, cfDNA has showed promising results in various conditions ranging from tumors to autoimmune diseases, such as SLE (Tug et al. 2014, see also Tangvarasittichai et al. 2015, Hendy et al. 2016, and Kloten et al. 2017). Research into this nucleic acid has even extended into the field of sports, where its potential as a marker for cell damage has been uncovered (Atamaniuk et al. 2004, see also Atamaniuk et al. 2008). In contrast, however, very little research can be found linking cfDNA and NAFLD. The evaluation of disease activity and grade of fibrosis is crucial for the diagnosis and treatment of patients with NAFLD. For the assessment of fibrosis, serum markers and ultrasound provide a non-invasive alternative to liver biopsy, however, their specificity is dependent on factors such as percentage of fat or the presence of comorbidities (Kim et al. 2008, see also Ahmed et al. 2015). The evaluation of cfDNA levels in patients with NAFLD could provide a reliable method for the assessment of inflammatory activity. A recent study examined the relationship between cfDNA and liver stiffness, as well as disease activity and severity. 58 NAFLD and 13 healthy controls were included in the study conducted by (Karlas et al. 2017). Of those 58 patients, 26 patients displayed elevated levels of ALT, 18 displayed elevated levels of

AST, and 12 displayed elevated levels of both liver enzymes. According to the controlled attenuation parameter (CAP), 7 patients were classified under mild steatosis, 9 under moderate steatosis, and 42 as advanced steatosis. Karlas et al. found no correlation within the NAFLD between cfDNA concentration, anthropometry, and factors of the metabolic syndrome. In contrast, patients in the NAFLD cohort with increased liver stiffness displayed significantly higher cfDNA concentrations. Based on their findings, Karlas et al. therefore hypothesized that cfDNA concentrations may in fact correlate with established non-invasive markers of NAFLD activity and severity.

2.3 cfDNA degradation

The precise kinetics and clearance of cfDNA remain unclear. DNase I, an enzyme belonging to the phosphodiesterase group, is responsible for the hydrolytic cleavage of DNA within the blood and is produced by the pancreas. Previous studies have outlined the significance of this nuclease and its role in sports, as well as various medical conditions (Velders et al. 2014) concluded that cfDNA increases, brought on by intense exercise, led to increases in DNase concentrations (Velders et al. 2014). In contrast, decreases in DNase activity were accompanied by increases in cfDNA concentration in conditions such as prostate, stomach and colon cancer (Tamkovich et al. 2006, see also Cherepanova et al. 2008). While researchers have made progress regarding the association of nucleases and cfDNA, they have yet to agree on the organ responsible for the uptake and clearance of the nucleic acid. A rapid removal of fetal DNA from the maternal plasma is seen after term delivery, with a mean half-life of approximately 16.3 minutes (Jeong et al. 2007). According to available research, it is believed that the liver is responsible for the clearance of circulating free DNA. Gauthier et al. found that the largest uptake of mononucleosomes was found within the liver, however, small amounts of nucleosomes were found in other organs as well (Gauthier et al. 1996). In addition, some have even attributed a lesser degree of clearance to the kidneys (Saukkonen et al. 2008). Botezatu et al. estimated that approximately 0,5-2.0% of cfDNA found in the blood stream passes through the kidney barrier and is excreted into the urine.

2.4 Pre-analytical phase

The pre-analytical phase comprises of all steps from preparing the patient for specimen collection through processing the specimen. Biological and physiological factors have been shown to influence pre-analytics. This includes, but is not limited to cell contribution, mechanisms of release, cellular movement from proximal space into the extracellular space, structural stability as well as cfDNA half-life. Accidental cell death,

regulated cell death, as well as regulated release from dividing cells all have an effect on the size of cfDNA fragments (Ungerer et al. 2020). Wan et al. has shown for instance, that cfDNA derived from cancer cells with low levels of vascularization can cause hypoxia, further leading to an increased rate of release.

Other factors that can potentially affect pre-analytics are the methods of biospecimen collection. Simple things such as the use of a tourniquet, the types of needles used, or even the types of blood collection tubes used (Ungerer et al. 2020).

Various centrifugation protocols have been developed to isolate cfDNA of interest. Cellular breakage as a result of centrifugation speed can cause an increase in unwanted cfDNA (Chiu et al. 2001). Holmberg et al. revealed that while a centrifugation speed of 16000g was sufficient enough to remove maternal DNA, lower speeds such as 1600g led to an elevated level of contamination with maternal DNA. Double centrifugation is now the approach that is most extensively accepted and utilized, however, there is little agreement on the precise speed of each step (Sorber et al. 2019).

Temperature fluctuations or exposure to high temperatures can cause harm to and degrade cfDNA. Research on the appropriate time to keep samples frozen is conflicting. While Kopreski et al. revealed that plasma frozen for 6 years did not have an effect on cfDNA, recent research conducted by Sozzi et al. showed an extensive amount of DNA degradation over the course of 41 months, with an average loss of 30.7% in cfDNA levels per year.

Standard operating procedures between research groups and institutions continue to range significantly. Biological and physiological factors play a significant role in the cfDNA that is to be assessed. In order to accordingly conduct research into cfDNA, the aforementioned factors must be taken into consideration to avoid conflicting results.

3 Materials and methods

3.1 Ethical approval:

All experimental procedures were approved by the Ethics Commission of Rhineland-Palatine (Nr: 837.237.15 (10005))). and were in accordance with the Declaration of Helsinki of the World Medical Association. All study participants gave their written informed consent before beginning the study.

3.2 Test group:

Patients with a histologically proven NAFLD were exposed to an 8-week, personalized Web-based exercise intervention. During this time their progress was monitored by a sports therapist, and their training plans were adjusted accordingly. At various times during the trial, blood was drawn from the participants and inflammatory markers were examined.

46 Individuals (men and women) age 18-70 years took part in the HELP Study, which lasted 8 weeks. They were recruited by physicians at the University Medical Center of the Johannes Gutenberg University of Mainz. The participant's mean age was 41 years (24-61) with a mean BMI of 31.3 kg/m2.

By the end of the study, 3 participants were excluded due to heart arrythmia, poorly controlled insulin-dependent diabetes mellitus, as well as a loss to follow-up. A histologically confirmed case of NASH or fatty liver disease was required for the participants to take part in the study.

In contrast, participants were excluded from the study if any of the following criteria were met: (1) bariatric surgery within the last 5 years, (2) body mass index (BMI) <18.5 kg/m2 or >45 kg/m2, (3) heart attack or stroke within the last 6 months, (4) higher grade coronary artery disease (CADIII-IV), (5) chronic obstructive pulmonary disease (asthma, COPD), (6) renal insufficiency, (7) uncontrolled hypertension or metabolic abnormalities, (8) alcohol consumption >30 g/day (male) and >20 g/day (female), (9) pregnancy, (10) concomitant medication able to cause a secondary NASH (eg, tamoxifen, corticosteroids), (11) concomitant medication able to affect inflammation (eg, tumor necrosis factor antagonists), (12) concomitant anticoagulant medication (eg, phenprocoumon; novel oral anticoagulants, NOAC), (13) other immunological or inflammatory diseases (eg, systemic lupus erythematosus), and (14) musculoskeletal disorders, preventing sport physiological investigations.

3.3 Intervention design:

As described by Pfirmann et al., a gradual cardiopulmonary exercise was undergone by all participants before the start of the study and performed until subjective exhaustion was achieved. The intensity and elevation of the treadmill was increased after completion of each 3-minute stage. Heart rate (HR) and respiratory gas analysis were repeatedly controlled throughout the test, while blood samples were taken from the ear lobe at the end of each stage to assess the lactate concentration. With the help of the Borg scale (6-20), the participant's subjective level of exhaustion was measured 30 seconds before termination of each stage.

The study design and structural content of the website were modeled after various studies (Ritterband et al. 2003, Wantland et al. 2004, van den Berg et al. 2006, as well as Kelders et al. 2012). Akin to Barak et al. (2009), key components of web-based intervention were taken into consideration, such as program content, multimedia aspects, interactive web-based activities, and tailored feedback. Participant registration was completed by an administrator, while a printed instruction manual, as well as an online manual were made available. Upon completion of registration, users were able to change various aspects of their profile, including profile picture, username, and password. Participants' privacy was taken into consideration.

Basic principles of our concept included interaction with a counselor, as well as peer support. A discussion forum and chat room were made available to help improve social support and compliance (Wantland et al. 2004). Additionally, disease-specific questionnaires and informative documents were readily provided by nutritional experts or supervising physicians.

A weekly, secure internal mail supplied participants with their individually tailored exercise plans, which were created by sports scientists to help improve their current condition. In addition to walking or running recommendations, strength and stretching exercises, as well as relaxation exercises were included in the program. Participants were provided with a Polar FT1 HR monitor in order to track their endurance training. In a home-based environment, participants took part in a 45-minute resistance training session using body-weight exercises and elastic resistance bands (of various strengths; Pinofit; Pharmazeutische Präparate GmbH, Hamburg) Illustrative exercise tutorials, as

well as downloadable videos found on the home page were made available to the participants.

Supervisors encourage participants to provide them with training-related information, such as average HR, duration, and subjective level of exertion) at the end of each week. Using this information, trainers adapted the training load for the upcoming weeks. The individual feedback provided by the participants allowed for tailored recommendations based on the needs, problems, and limitations of each participant. The separate examination of strength and endurance training allowed for the adjustment of training volume with respect to duration and or intensity.

Log-in duration and frequency were compiled during the 8-week intervention to assess behavioral usage of the website. An evaluation of training time, exercise interruptions, preference of self-chosen alternative exercises over weekly recommendations, as well as adverse events allowed for the assessment of exercise implementation. User satisfaction concerning the exercise concept and webpage was evaluated using a short questionnaire (8 Likert items). Participants were additionally asked to grade the concept and if they would continue using the website.

3.4 Blood sampling and processing:

Venous blood was drawn from the antecubital vein before, immediately after, as well as 90 minutes after exercise. Capillary blood was drawn from the fingertips before, immediately after, and 5/10/15/30/90 minutes post exercise.

The blood was processed in accordance with the standards set by Breitbach et al. with minor altercations. Venous blood samples were first centrifuged at 4°C, 1,600 g for 10 min (Centrifuge 5424 R, Eppendorf, Hamburg, Germany). The capillary blood taken from the fingertips were centrifuged at 4°C, 1600g for only 2 minutes. To ensure that all cell debris was removed from the plasma, venous samples were also centrifuged at 4°C, 16,000 g for 5 min. Capillary samples were only centrifuged at 1600g. Prior to qPCR analysis, the samples were centrifuged and kept at -20°C for up to 4 months.

3.5 Materials:

Table 1: Materials

Instrument	**Manufacturer**
Piston pipettes 2,5/10/100/1.000 µl Eppendorf Research®/Eppendorf Research® plus/Eppendorf Reference®	Eppendorf AG, Hamburg
Mini laboratory centrifuge	Labnet International, Inc, Edison, NJ, USA
UV Sterilizing PCR workstation	PEQLAB Biotechnologie GmbH, Erlangen
Thermocycler CFX384 Touch™ Real-Time PCR Detection System	Bio-Rad Laboratories GmbH, München
Vortexer Vortex-Genie® 2	Carl Roth GmbH + Co. KG, Karlsruhe
Centrifuge 5810R, Rotor FA-45-24-11	Eppendorf AG, Hamburg
Chemicals	**Manufacturer**
DNA Away®	Carl Roth GmbH + Co. KG, Karlsruhe
dNTPs	Carl Roth GmbH + Co. KG, Karlsruhe
Fluorescein isothiocyanate (FITC)	Sigma-Aldrich Chemie GmbH, Taufkirchen
VELOCITY DNA Polymerase	Bioline GmbH, Luckenwalde
HiFi-Puffer (for the Velocity DNA Polymerase)	Bioline GmbH, Luckenwalde
SYBR® Green I	Sigma-Aldrich Chemie GmbH, Taufkirchen
Water (H2O)	Gibco®, Life Technologies GmbH, Darmstadt
Materials	**Manufacturer**
Butterfly for blood withdrawal: Safety-Multifly®-Set (Nadel: 0,8 x 19 mm)	Sarstedt AG & Co., Nümbrecht
Disinfectant spray: Kodan® Tinktur	Pro Medico Plan GmbH, Mainz
Falcon Tubes 15/50 ml	Greiner Bio-One GmbH, Frickenhausen
Adhesive foil for the qPCR-plates	Greiner Bio-One GmbH, Frickenhausen

Low retention reaction tubes 0,6/1,5 ml	Sigma-Aldrich Chemie GmbH, Taufkirchen
Low retention tips 10/100/1.000 µl	Axon Labortechnik GmbH, Kaiserslautern
Vacutainer for blood samples: S-Monovette® 7,5ml EDTA K3 / 2,7 ml EDTA K3	Sarstedt AG & Co., Nümbrecht
Surgical mask	Carl Roth GmbH + Co. KG, Karlsruhe
Nitrile disposable gloves	Axon Labortechnik GmbH, Kaiserslautern
qPCR plates, 384-Well	Axon Labortechnik GmbH, Kaiserslautern
Reaction tube 0,2/0,5/1,5/2,0 ml	Greiner Bio-One GmbH, Frickenhausen
Software	**Manufacturer /Provider**
Bio-Rad CFX Manager 3.0	Bio-Rad Laboratories GmbH, München
Microsoft Office 2007, 2010	Microsoft Corporation, Redmond, WA, USA
Training materials	**Manufacturer**
Heart rate monitor	Polar, FT1
Resistance bands (various strenghts)	Pinofit; Pharmazeutische Präparate GmbH, Hamburg

3.6 Analysis of the blood samples:

The plasma was diluted in a ratio of 1:10 using sterile H_2O. Diluted plasma (2.2 µl) was then added as a template to 14.3 µl master mix consisting of the following reagents:

Table 2: qPCR reagents

Reagent	Concentration	Preparation 15 µl	Final Concentration
HiFi buffer	5x	3,60	1,2
dNTPs	10mM	0,45	0,30
SYBR Green	10x	0,23	0,15
FITC	0,1x	0,16	0,001

H_2O		7,66	
Polymerase	2 u/µl	0,30	0,04
Primer (for/rev)	3,7 µM	0,61	0,120
Template	1:10 diluted	2	

All reactions included two triplicates of non-template controls: Sterile H_2O, as well as mouse plasma diluted 1:10 in H_2O.

The following primer were used for the L1PA2 90 BP/222 BP fragments:

Table 3: Primer sequences

Sequence	**Description**
TGCCGCAATAAACATACGTG	L1PA2 **forward** primer binding site
GTAATGGGATGGCTGGGTC	L1PA2 **reverse** primer **90bp** binding site
CCTCTCCAGCACCTGTTGTT	L1PA2 **reverse** primer **222bp** binding site

Reactions were carried out in 384-well PCR plates (Axon Labortechnik GmbH, Kaiserslautern) using the Thermocycler CFX384 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories GmbH, München) for qPCR measurement. The cycling conditions were as follows: initial denaturation for 2 min at 98°C, followed by 35 cycles of melting at 95°C for 10 s, annealing at 64°C for 10s and extension at 75°C for 10s.

Table 4: Cycling conditions

Cycling conditions (CFX384)		Protocol:"L1PA2 10S _ET"
Step	**Temperature °C**	**Time**
1	98	02:00
2	95	00:10
3	**64**	0:10 + plate read
5	GOTO 2, 34 more times	
6	Melt Curve 70 - 95 °C, increment 0.5 °C, for 0:10 sec + plate read	

3.7 Statistical analysis:

The qPCR data were captured with the CFX Manager Software version 3.0 (Biorad) and Microsoft Excel 2010 (Microsoft, Redmond, WA). Statistical analysis was performed using SPSS Statistics version 19 (IBM, Chicago, IL), as well as R statistical software R-4.3.1 (R Core Team, 2023). cfDNA values were log transformed before statistical analyses. Normal distribution of data was tested using Shapiro-Wilk test. T-tests or Wilcoxon Rank Sum tests were used to examine group differences for normal on non-normal distributed data, respectively. To study differences between intervention timepoints mixed repeated measures ANOVAs were performed with additional post-hoc T-Tests using rstatix package (version 0.7.2). All figures were created using ggplot2 (version 3.4.2).

4 Results

4.1 Demographic

46 Individuals (men and women) age 18-70 years took part in the HELP Study, which lasted a total of 8 weeks. They were recruited by physicians at the medical university of the Johannes Gutenberg University of Mainz.

By the end of the study, 3 participants were excluded due to heart arrythmia, poorly controlled insulin-dependent diabetes mellitus, as well as a loss to follow-up. A histologically confirmed case of NASH or fatty liver disease was required for the participants to take part in the study.

4.2 Demographic data and metabolic comorbidities of the study cohort

Table 5: Demographic data and metabolic comorbidities of the study cohort.

Variable	N	Mean	Sd	Median	IQR
Age	43	41.953	11.002	39	17
BMI (kg/m2)	43	31.102	4.34	31.5	6.5
Chronic Liver Disease: Quality of Life	40	5.635	0.898	5.77	1.272
Fat Percentage	43	27.921	7.536	27.8	12.7
HbA1c	40	5.65	0.696	5.5	0.5
Height (cm)	43	175.023	10.364	177	14.5
HOMA Insulin Resistance	40	4.345	2.525	3.75	2.453
Weight (kg)	43	95.572	17.458	95.2	27.45
Sex	Male	Female			
	29	14			

Of the 43 patients within the study, the median age was 41 years (range 24-61), with a male-predominance (n=29) (see Table 5). Most patients (n=23, 56.1%) were obese, with a median BMI of 31.3 kg/m2. Within the cohort, there was a high prevalence of NASH (70.7%, n=29), as well as other metabolic comorbidities, such as Diabetes mellitus Typ II (n=11, 26.8%) and arterial hypertension (n=24, 58.5%).

Venous and capillary blood samples were drawn from the patients before, immediately after, as well as 90 minutes after exercise. Additionally, Capillary blood samples were drawn from the fingertips 5/10/15/30 minutes post exercise.

The blood samples were then first centrifuged at 4°C, 1,600 g for 10 min. The capillary blood taken from the fingertips were centrifuged at 4°C, 1600g for 2 minutes. To further

explore the effect of centrifugation, venous blood samples were also centrifuged at 4°C, 16,000 g for 5 min.

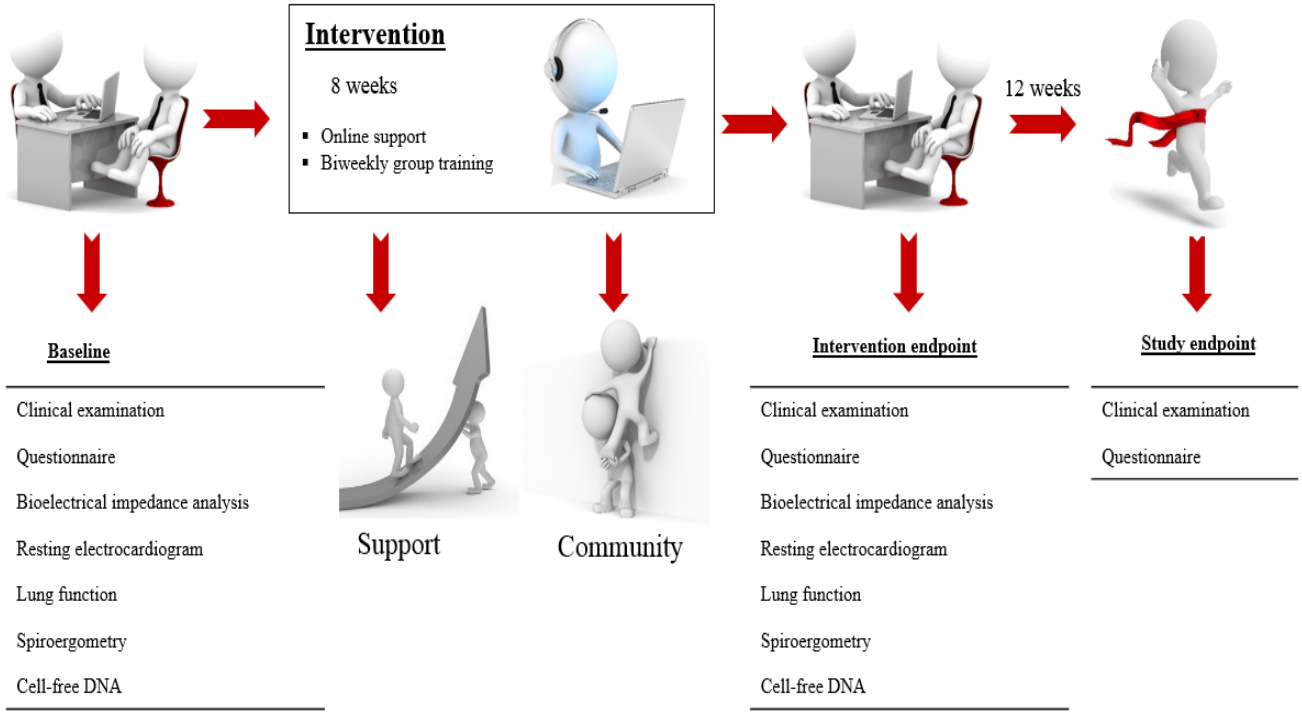

Figure 1: Study design for a web-based intervention program (Pfirmann et al. 2018).

Using a web-based program, patients received tailored exercise plans, which were adjusted throughout the 8-week intervention period in accordance with recommendations of the American College of Sports Medicine guidelines.

During the initial phase, three sessions comprised of strength and endurance training were conducted each week. After a 4-week acclimatization period, the exercise intensity and frequency were increased to five sessions per week.

4.3 Pre-analytical aspect: Impact of centrifugation speed on cfDNA concentration

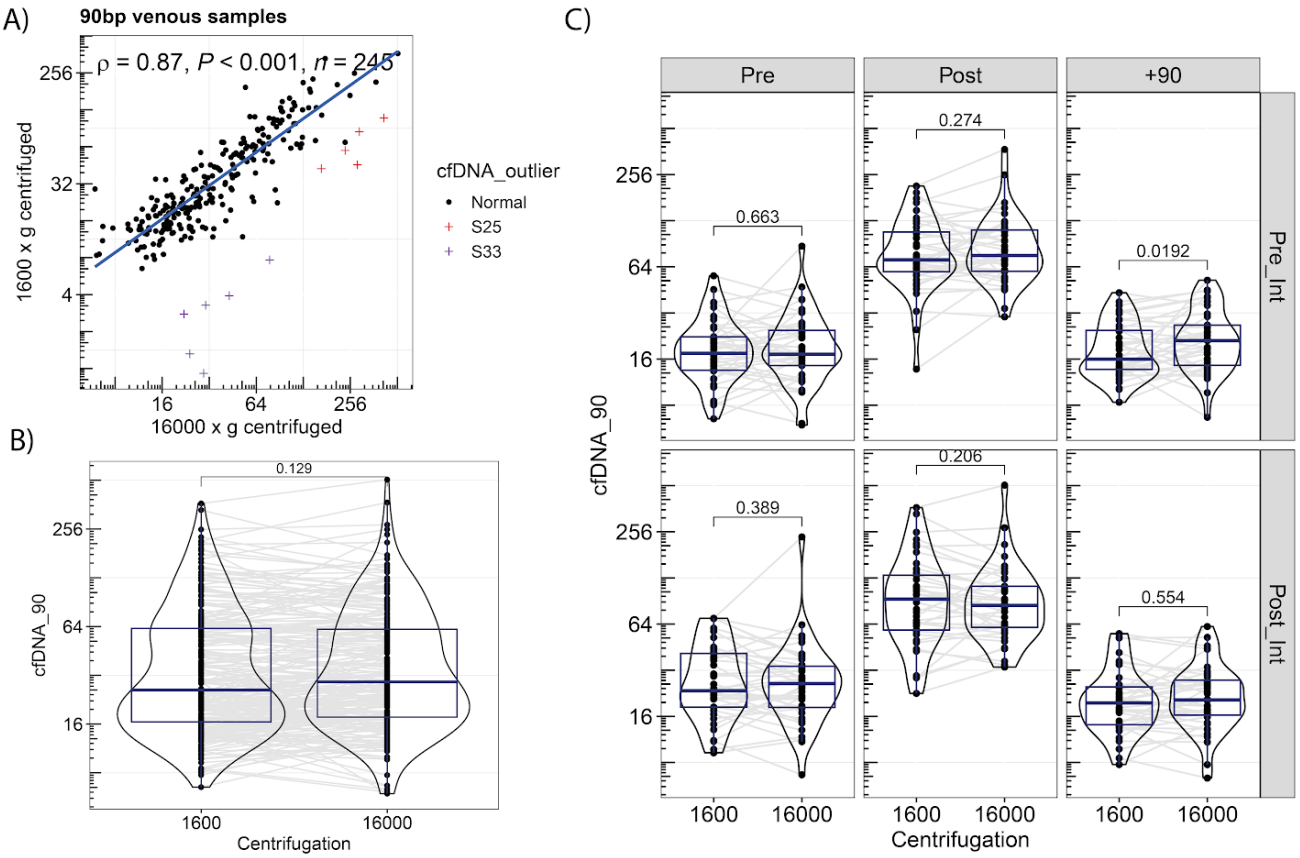

Figure 2: Impact of Centrifugation speed on cfDNA concentration (90bp). A) Spearman rank correlation between 1600 and 16000g centrifuged plasma samples measured with the 90bp assay. S25 and S33 were identified as outlier and were excluded from the analyzes. B) Absolute concentrations of all measured samples . C) Comparison of the centrifugations at the various points in time pre, post and +90 min before and after the intervention.

The median cfDNA levels before the start of the intervention (PRE) were approximately 17.49 (1600g) and 17.23 (16000g). Immediately after exercise completion in the pre-intervention phase (POST) a significant increase in cfDNA was seen, with a median of 71.18 (1600g) and 75.86 (16000g). 90 minutes after completion of the exercise regimen, cfDNA levels that were centrifuged at 1600g reached lower values than initially seen with a median of 15.97. DNA samples that were centrifuged at 16000g, however, revealed an increase in cfDNA with a median of 21.12.

Post-intervention, PRE samples showed a significant increase in the baseline cfDNA levels with a median of 23.52 (1600g) and 26.17 (16000g). Post samples also revealed an increase in the median cfDNA levels in comparison to pre-intervention with a median of 93.15 (1600g) and 84.78 (16000g). Additionally, the median cfDNA levels 90 minutes

after completion of the exercise regimen were comparatively lower than that pre-intervention, with a median of 19.56 (1600g) and 20.49 (16000g).
Comparatively an overall increase in baseline cfDNA levels could be seen post-intervention (PRE), as opposed to pre-intervention (PRE). Incidentally, cfDNA levels showed an overall decrease in baseline values (PRE vs. +90) at 1600g centrifugation in both intervention groups.

In Figure C, a significant difference was seen in p-values in the pre intervention +90 values (p = 0.0192). However, when the p-value was corrected using the Bonferroni correction, no significant differences were seen.

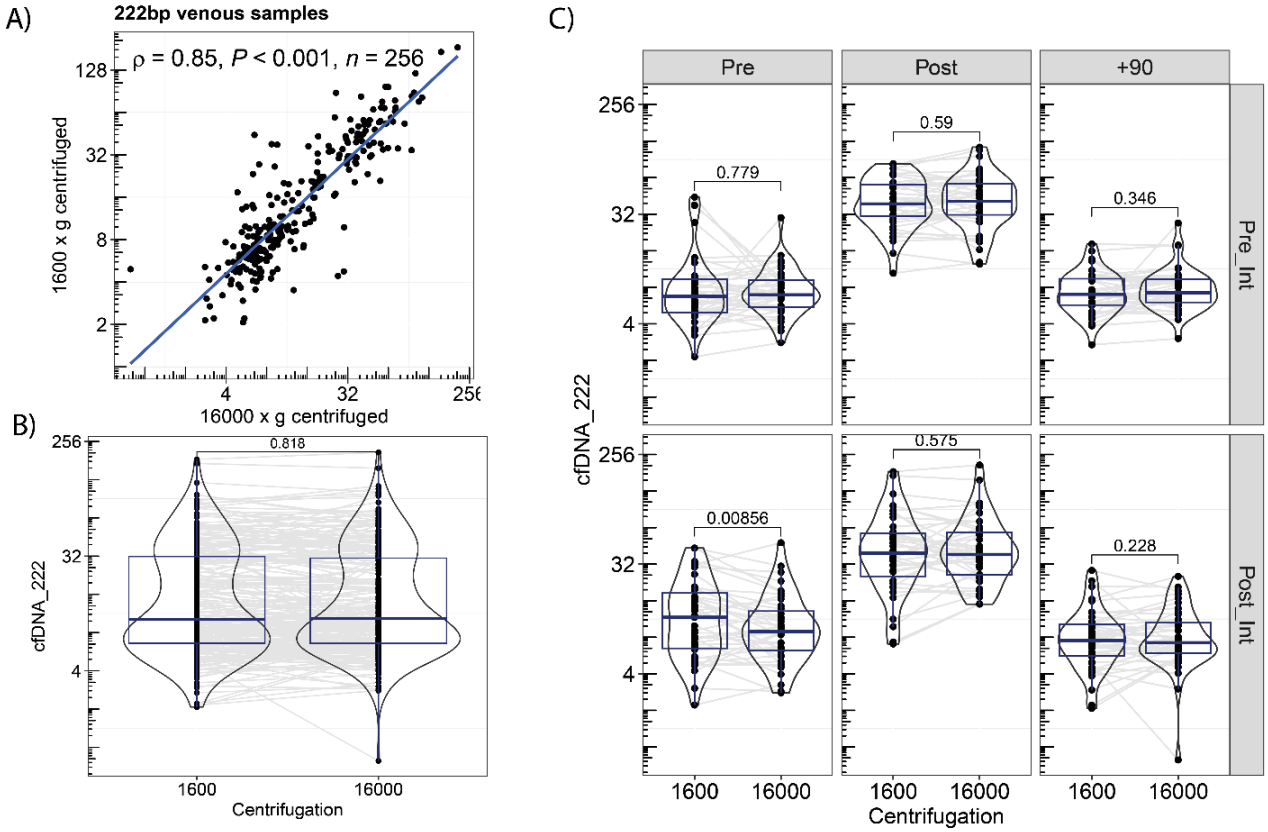

Figure 3: Impact of centrifugation speed on cfDNA concentration (222bp). A) Correlation between 1600 and 16000g centrifuged plasma samples measured with the 222bp assay. B) Absolute concentrations of all measured samples. C) Comparison of the centrifugations at the various points in time pre, post and +90 min before and after the intervention.

The correlation coefficient in Figure A was calculated using the Spearman rank correlation. Normal distribution was tested by means of the Shapiro test and was confirmed for all values except for pre-intervention timepoint PRE, post-intervention

timepoint +90. The aforementioned values were calculated using the Wilcoxon Test.

The median cfDNA levels before the start of the intervention (PRE) were approximately 6.73 (1600g) and 6.94 (16000g). Immediately after exercise completion in the pre-intervention phase (POST) a significant increase in cfDNA was seen, with a median of 38.91 (1600g) and 40.90 (16000g). 90 minutes after completion of the exercise regimen, cfDNA levels at both levels of centrifugation revealed discrete elevations in comparison to their original levels with a median of 7.02 (1600g) and 7.22 (16000g).

Post-intervention, PRE samples showed an increase in the baseline cfDNA levels with a median of 11.67 (1600g) and 8.91 (16000g). Post samples displayed similar median cfDNA values in comparison to pre-intervention with a median of 39.31 (1600g) and 38.43 (16000g). Additionally, the median cfDNA levels 90 minutes after completion of the exercise regimen were similar to the pre-intervention values, with a median of 7.55 (1600g) and 7.21 (16000g).

Comparatively an overall increase in baseline cfDNA levels could be seen post-intervention in the timepoint PRE, as opposed to pre-intervention timepoint PRE. Only slight discrepancies were revealed between the POST median values in the pre- und post intervention groups.

Incidentally, cfDNA levels showed a slight increase in baseline values (PRE vs. +90) in the preintervention group, whereas a decrease was seen in the post-intervention group.

In Figure C, a significant difference was seen in p-values in the postintervention POST values ($p = 0.00856$). However, when the p-value was corrected using the Bonferroni correction ($p_{corrected} = 0.008$), no significant differences were seen.

4.4 Comparison of capillary and venous samples

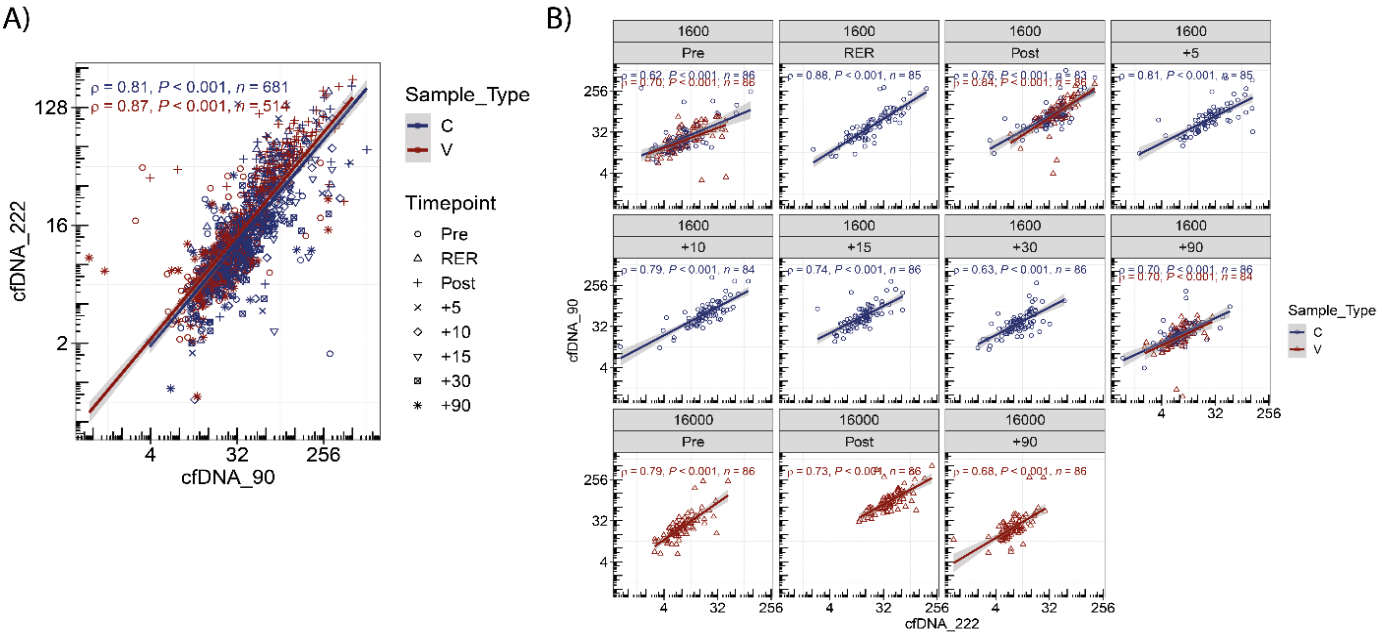

Figure 4: Correlation of 90bp and 222bp measurements. A) Correlation between venous and capillary samples with the 90bp and 222bp assay. B) Correlation of venous and capillary samples at 1600g centrifugation at the various points in time pre, rer, post, +5, +10, +15, +30 and +90 min for 90 bp, as well 16000g centrifugation for timepoints pre, post and +90 in the 222bp assay.

Using the spearman correlation, venous samples, as well as capillary samples were compared with one another during different timepoints in the intervention. As can be seen within the figures, a strong correlation can be noted during various timepoints, especially within the venous samples in the POST timepoint (1600g). Overall venous samples showed a slightly stronger correlation than capillary samples.

4.5 Interventions effect on cfDNA (90bp and 222bp)

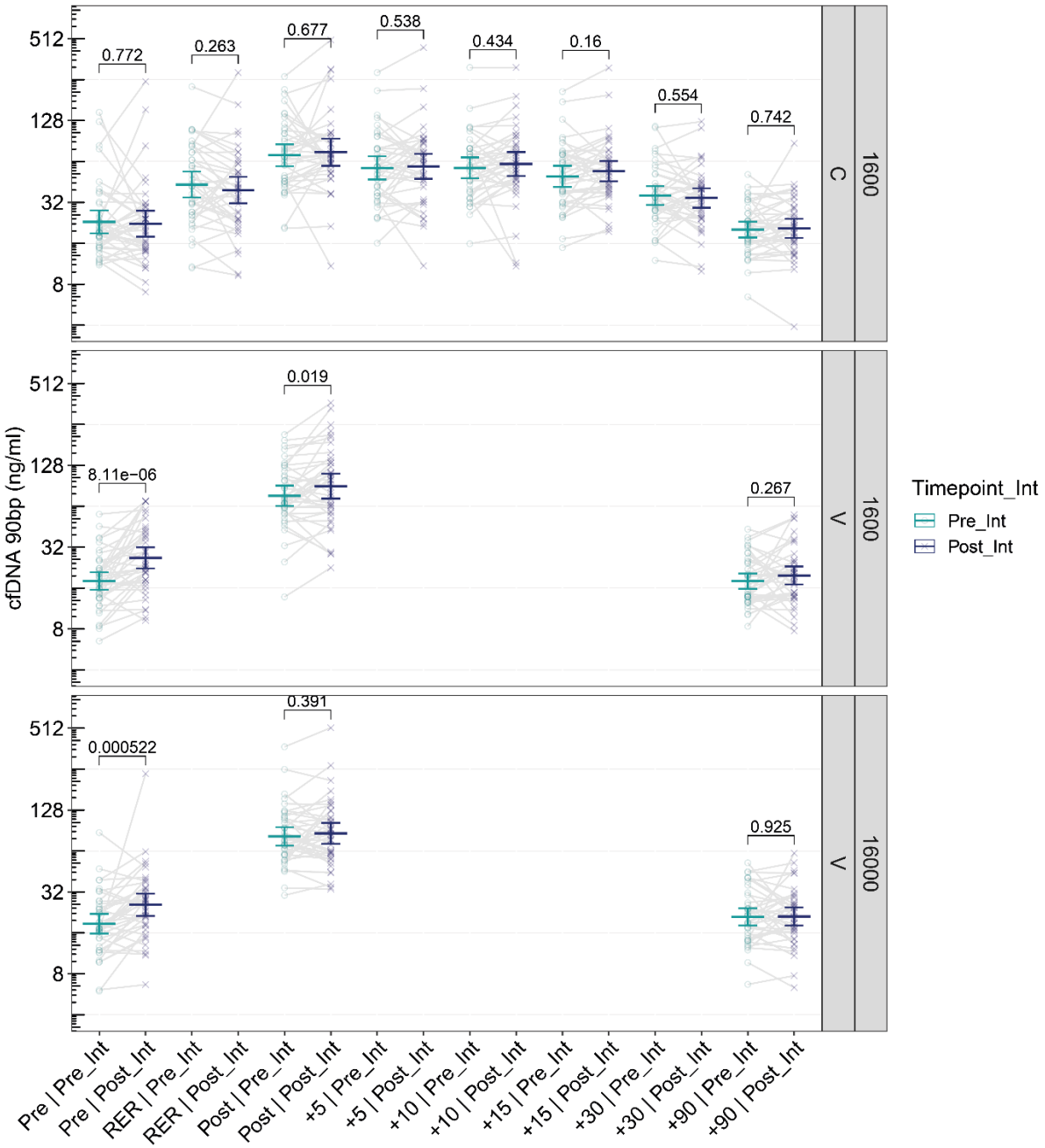

Figure 5: Intervention effect on cfDNA_90bp in , venous and capillary samples.

Figure 5 details the effects of the intervention on cfDNA_90bp. An overview is given with a comparison of capillary samples pre-intervention vs. post-intervention during various timepoints of the study. Additionally, venous samples are compared in a similar manner and are further divided accordingly based on levels of centrifugation. A repeated measures ANOVA was performed to compare the effect of acute exercise and exercise intervention on capillary cfDNA levels. Additionally, a post-hoc T-Test was undertaken to highlight differences in venous samples at the timepoint PRE.

Median capillary cfDNA levels before the start of the intervention (PRE) displayed only slight differences in comparison to post-intervention (19.22 vs 19.93). Immediately after exercise completion in the pre-intervention phase (POST) a significant increase in cfDNA was seen (69.06). In the following 5/10/15/30/90 minutes after completion of the exercise regimen, cfDNA decreased steadily and reached a median than was slightly higher than the initial baseline (20.23).

The baseline of the post-intervention POST samples was discretely higher than that of the pre-intervention (70.76 vs. 69.06). In the post-intervention group, a steady increase in cfDNA was seen after exercise completion, with an incremental decrease over time and an approach towards the baseline after 90 minutes (20.64 vs. 20.23).

Comparatively only a discrete increase in baseline cfDNA levels could be seen in both the post-intervention, as well as pre-intervention groups.

Venous samples centrifuged at 1600g revealed a steady increase throughout the intervention, with an overall decrease in the median 90 minutes after completion of exercise in the pre- und postintervention groups. Samples centrifuged at 16000g also exhibited a steady increase in median cfDNA levels through the intervention, however, cfDNA levels 90 minutes post-exercise were higher than baseline levels in the preintervention group, as opposed to the postintervention group.

A repeated measures ANOVA revealed no significant two-way interaction for the timepoint and the intervention ($F(4.91,167.01) = 1.32$, $p = 0.258$). Acute exercise, however, leads to significantly different cfDNA concentrations at the different timepoints ($F(3.73,126,71) = 68.829$, $p < 0.001$). The results of the post-hoc test can be seen in the upper portion of Figure 5.

The same test was used to test the effect on venous cfDNA levels and revealed a significant two-way interaction for the timepoint and the intervention in both 1600g ($F(2.0,78.0)=6.265$, $p = 0.003$) and 16000g ($F(2.0 , 80.0)= 7.781$, $p < 0,001$). The post hoc T-tests show differences at the timepoint “PRE”.

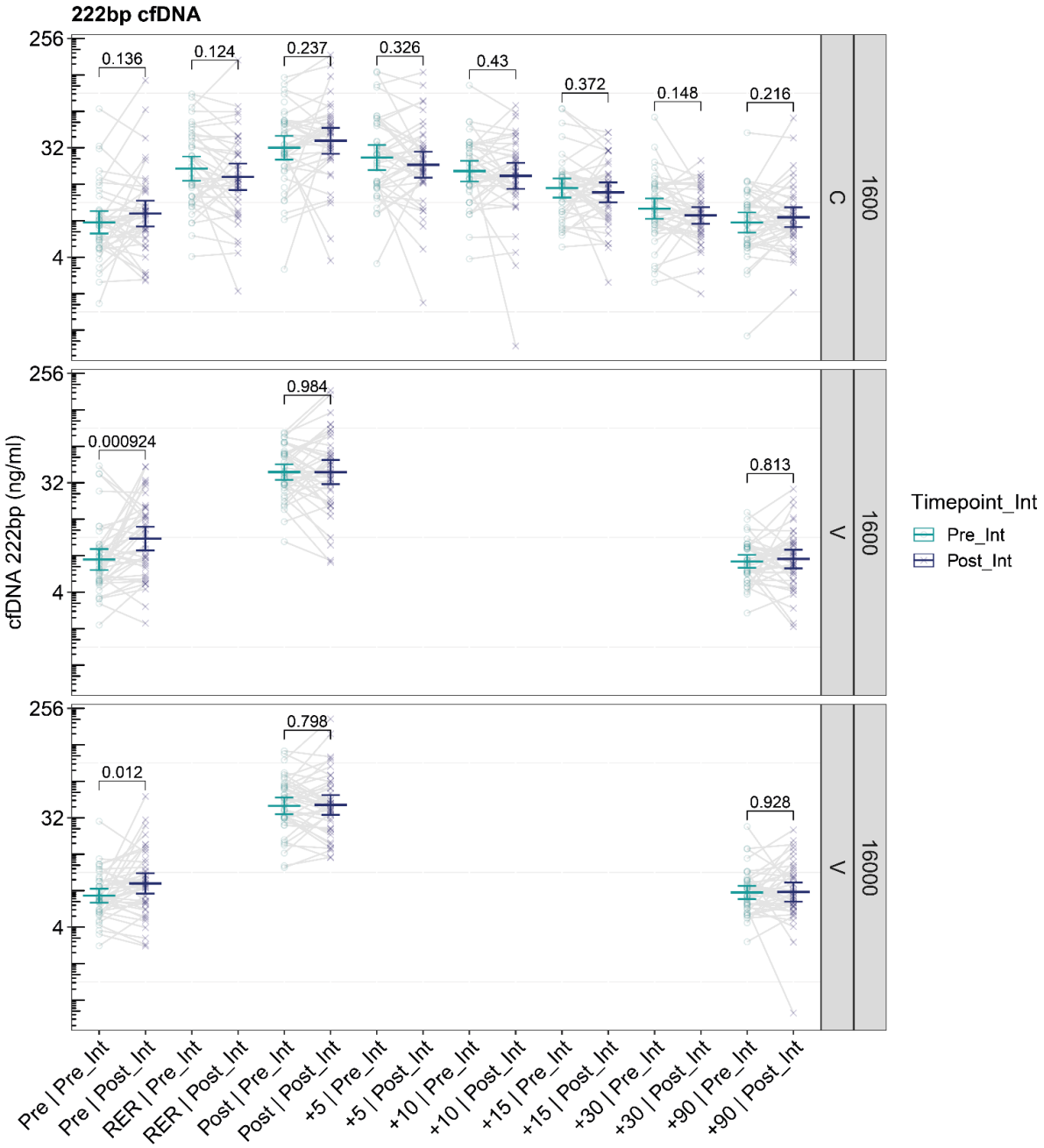

Figure 6: Intervention effect on cfDNA_222bp in venous and capillary samples.

Figure 6 details the effects of the intervention on cfDNA_222bp. An overview is given with a comparison of capillary samples pre-intervention vs. post-intervention during various timepoints of the study. Additionally, venous samples are compared in a similar manner and are further divided accordingly based on levels of centrifugation. A repeated measures ANOVA was performed to compare the effect of acute exercise and exercise intervention on capillary cfDNA levels. Additionally, a post-hoc T-Test was undertaken to highlight differences in venous samples at the timepoint PRE.

A repeated measures ANOVA revealed no significant two-way interaction for the timepoint and the intervention for the timepoint and the intervention ($F(7.0, 245.0) = 1.947$, $p = 0.063$). Acute exercise again, however, leads to significantly different cfDNA concentrations ($F(4.75,166.08) = 67.494$, $p < 0.001$). The results of the post-hoc test can be seen in the upper portion of Figure 6.

The same test was used to test the effect on venous cfDNA levels and revealed a significant two-way interaction for the timepoint and the intervention in both 1600g ($F(2.0,80.0)=6.174$, $p = 0.003$) and 16000g ($F(2.0 , 84.0)= 3.454$, $p < 0,036$). The post hoc T-tests show differences at the timepoint “PRE”.

4.6 Fragmentation index

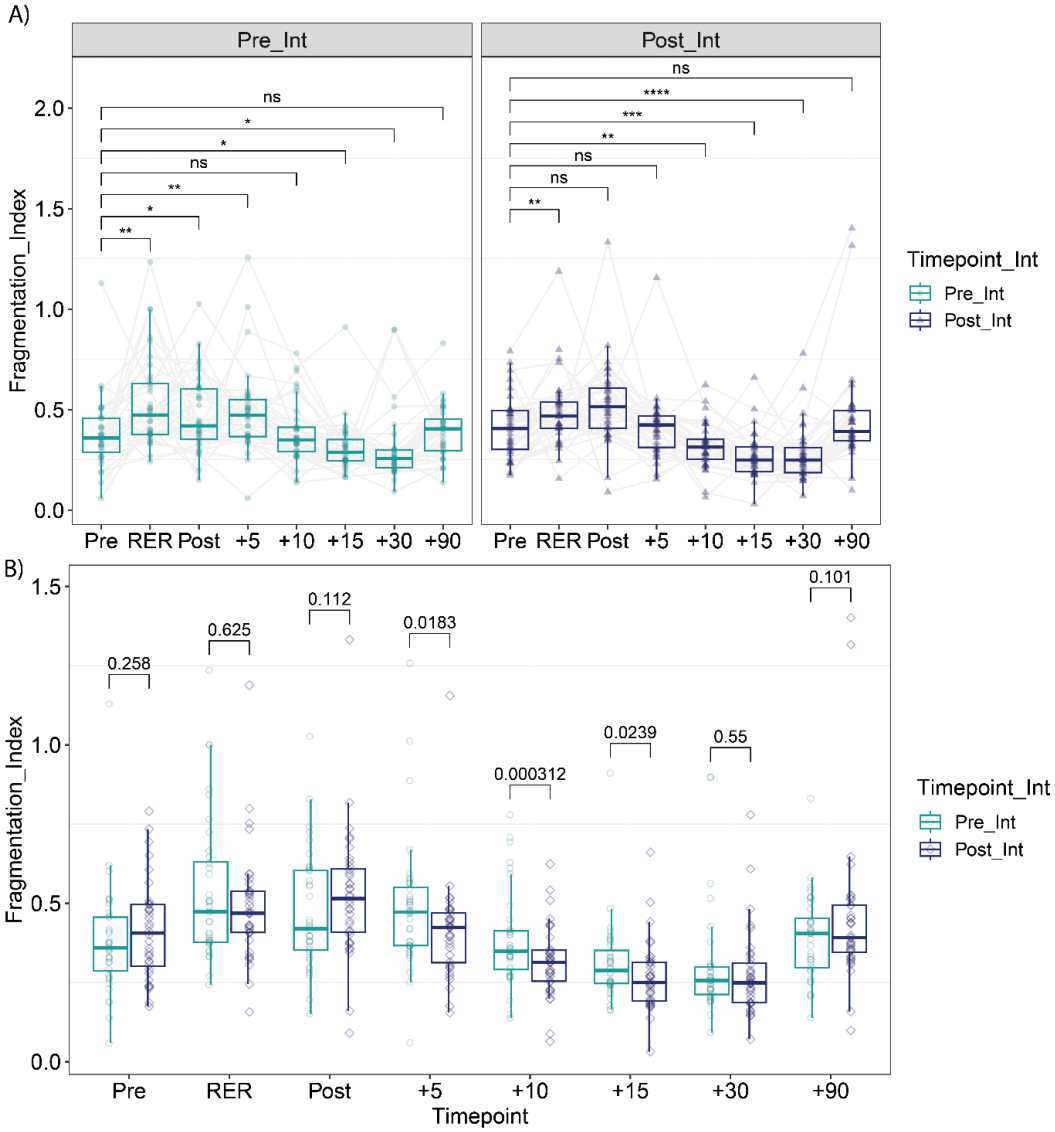

Figure 7: Fragmentation Index for capillary cfDNA_90bp.

Subsection A illustrates the change over time. Using the Wilcoxon test, paired samples were compared with one another. Reductions can be seen after +15 and +30. In subsection B, the undertaken Friedman test did not reveal any significant differences between the intervention timepoints. Paired comparisons were again done using the Wilcoxon test. In order to complete paired comparisons, timepoints from seven different subjects were omitted. These timepoints also included values which were considered outliers.

The diagram above illustrates an increase in median Fragment Index during timepoints “PRE,” “RER” and “POST” in both pre- and post-intervention. Immediately after, a steady decrease is seen in the recovery phases “+5”, “+10,” “+15”, and “+30”. 90 minutes after completion of training, values steady increased to approximately that of the “PRE” values. Timepoints “+5”, “+10,” “+15”, and “+30” also revealed decreases in median Fragmentation Index (0.472 → 0.424, 0.349 → 0.314, 0.288 → 0.250, and 0.256 → 0.249) post-intervention, while timepoints “PRE” and “POST” all showed increases in median Fragmentation Index (0.359 → 0.406, 0.420 → 0.515).

4.7 Correlation of clinical and physiological parameter of the resting values

Figure 8: Significant correlations between clinical parameters and cfDNA_90bp at the timepoint “PRE”. Non-significant correlations are blank.

The figure above details the spearman correlation during the timepoint “pre.” This allows establishment of a connection between cfDNA centrifuged at 16000g and the clinical parameters examined during the study. The fields colored blue or red are considered statistically significant ($p<0.05$). The white fields are considered not statistically significant. No significant correlation could be seen between cfDNA_90 or cfDNA_222 and clinical parameters for liver disease (ALT, AST). BMI, however, displayed a significant positive correlation with cfDNA_222.

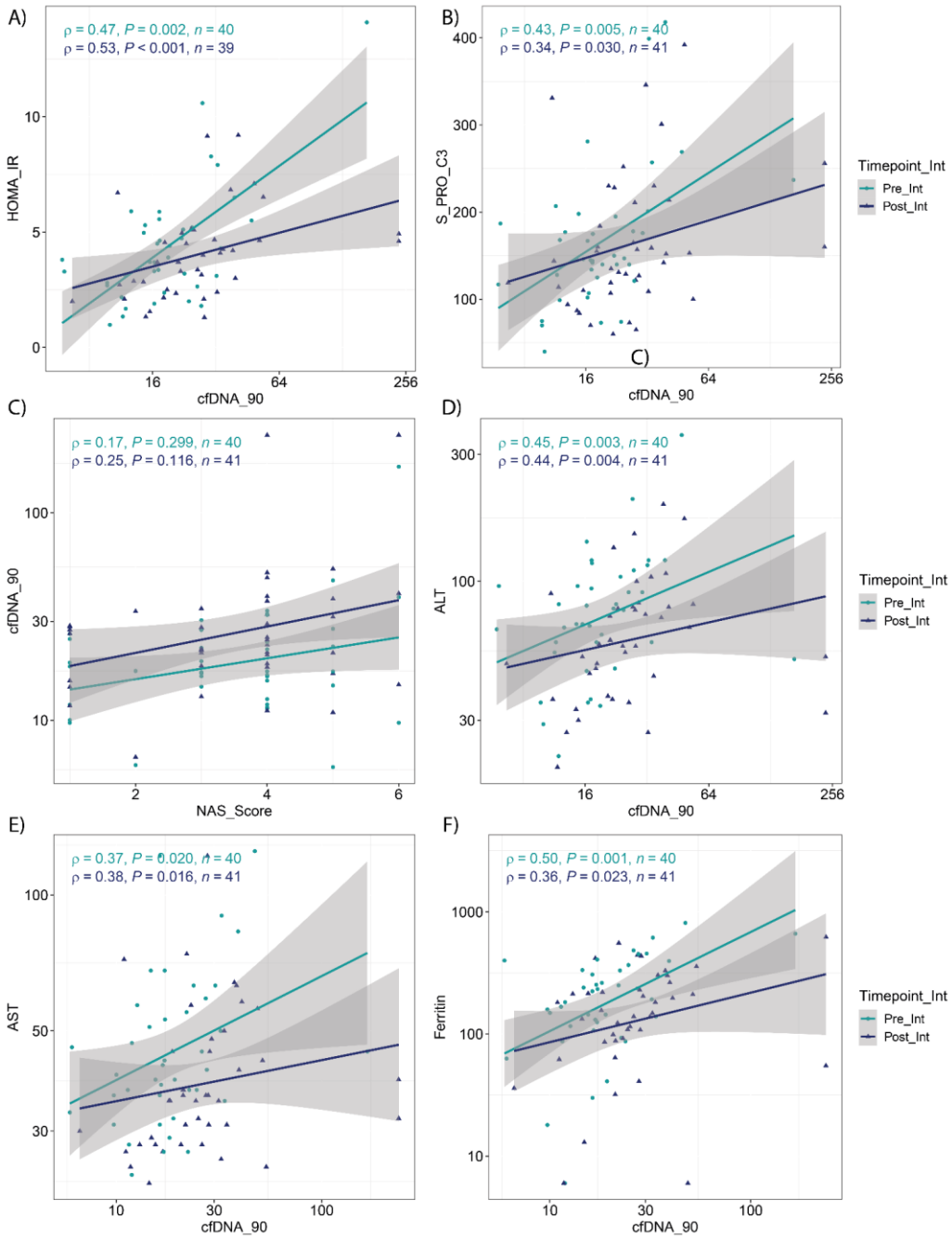

Figure 9: Correlations of individual clinical parameters and cfDNA_90bp at the timepoint “PRE”.

The figure above details the spearman correlation during the timepoint “pre” and displays the positive correlation between cfDNA_90bp and the clinical parameters A)Homeostatic Model Assessment for Insulin Resistance (HOMA_IR), B) Pro C3, C) NAS Score, D) ALT, E) AST, and F) Ferritin.

As can be seen above, all the aforementioned clinical parameters displayed a positive correlation with cfDNA_90bp. Among them, Ferritin, ALT, Pro C3 and HOMA_IR exhibited the strongest positive correlation pre-intervention. Post-intervention, HOMA_IR, ALT and AST exhibited the strongest positive correlation with cfDNA_90bp.

5 Discussion

In this “Discussion” section, the results are summarized and integrated into the context of the literature.

5.1 Summary of the results:

Blood was drawn from all participants in three stages: (i) before the beginning of the intervention and before the beginning of the exercise (PRE), (ii) immediately after its completion (POST), as well as (iii) 90 minutes following completion (+90). Across all pre-intervention groups, increases in median cfDNA levels were detected immediately after completion of exercise. Additionally, median cfDNA levels depreciated rapidly after +90 minutes.

Venous samples and capillary samples were drawn in the same period of time. Capillary samples were additionally drawn 5, 10, 15, 30 minutes after completion of the training exercises. Similar to the venous samples examined, median cfDNA levels in the pre-intervention group followed a similar trend. An increase was seen immediately after completion of the exercise, which then rapidly returned to a slightly elevated baseline in comparison to timepoint PRE.

After completion of the intervention, baseline median cfDNA values (PRE) in capillary and venous samples for both 90bp and 222 bp were elevated in comparison to the baseline before the intervention. Overall, venous samples exhibited a stronger correlation than that of the capillary ($\rho_{venous} = 0.87$, $\rho_{capillary} = 0.81$, $P_{both} < 0.001$)

The effect of acute exercise and exercise intervention on capillary cfDNA levels were tested. No significant two-way interaction was detected for the timepoint and the intervention ($F(4.91,167.01) = 1.32$, $p = 0.258$). Acute exercise, however, was seen to lead to significantly different cfDNA concentrations ($F(3.73,126,71) = 68.829$, $p < 0.001$).

The results of the intervention revealed no significant correlation between cfDNA (90bp and 222bp) and the liver markers for inflammation (ALT/AST). A slightly positive correlation ($\rho=0.34$) was seen between cfDNA_222bp and BMI.

Lastly, positive correlations could be seen at timepoint “PRE” in both intervention groups between cfDNA_90bp and various parameters measured during the study, such as Ferritin, HOMA_IR, S_Pro_C3, NAS Score, AST and ALT. Only correlations with Ferritin, HOMA_IR, AST and S_Pro_C3 were significant.

5.2 Interpretation of the results:

1) Acute endurance training leads to an increase of cfDNA concentrations in both 90bp and 222bp fragment types immediately after training.

The samples drawn from the participants immediately after exercise revealed an increase in cfDNA. Two DNA fragment types were examined in this study, 90bp and 222bp. Additionally, samples were centrifuged at 1600g and 16000g. At the time point “POST,” cfDNA levels reached their maximum during the intervention. This was seen in both pre- und post intervention groups, as well as in both samples centrifuged at 1600g and 16000g.

As previously discussed, various centrifugation protocols have been developed to isolate cfDNA of interest. While a uniform method of centrifugation has not been agreed upon, double centrifugation is now the approach that is most extensively accepted and utilized, however, there is little agreement on the precise speed of each step (Sorber et al. 2019). Chiu et al. 2001 showed that the centrifugation speed can result in an increase in unwanted cfDNA due to cellular breakage. Lower centrifugation speeds of up to 1600g were associated with an increase in contamination with maternal DNA, while higher centrifugation speeds of up to 16000g were sufficient enough to remove said contaminations (Holmberg et al. 2013). For the initial phase of centrifugation, speeds varying from 200 to 2500g are recommended, while speeds ranging from 1600 to 18400g are recommended for the second phase (Ungerer et al. 2020). A three-step processing protocol was tried by El Messaoudi et al. at 16,000g, and they concluded that the plasma cfDNA yield was not different from that after the second centrifugation stage, proving that a two-step processing protocol was enough. In our study, we used a two-step approach centrifugation process. As mentioned earlier in the materials and methods section, venous blood samples were first centrifuged at 4 °C, 1,600 g, for 10 min, while fingertip capillary blood samples were centrifuged at 4 °C, 1600 g, for just two minutes. In a second step, venous samples were additionally centrifuged at 4°C, 16,000 g, for 5 minutes. Using this two-pronged method, we believe that all cell debris was eliminated from the plasma, thus ensuring that only the desired cfDNA was examined. As seen in

Figures 2 and 3, our findings did not reveal a relevant difference in mean cfDNA concentrations when comparing samples that were centrifuged at 1600g and 16000g. Furthermore, our findings also showed that both 90bp and 222bp assays showed only minor differences in mean concentrations, regardless of centrifugation speed. Additionally, with the exception of timepoint PRE, venous and capillary samples did not display relevant differences in mean cfDNA concentrations.

The results of our study regarding an immediate increase in cfDNA concentrations post-intervention correlate with other studies, which, however, examined the effect of resistance training on cfDNA levels (Fatouros et al. 2006, Atamaniuk et al. 2010). Haler et al. found that the duration and intensity of exercise during aerobic running resulted in increases in cfDNA. The study conducted by Breitbach et al. 2012 revealed that varying intensity of exercises also leads to differing elevations in cfDNA. Atamaniuk et al. demonstrated that cfDNA levels are increased by both prolonged aerobic exercise and brief anaerobic exercise, with the highest levels being reached immediately after completion of exercise, with a return to baseline hours later.

Humińska-Lisowska et al. examined the effects of maximal aerobic and anerobic exercise on levels of cfDNA in athletes and a healthy control group. Again, in accordance with our findings, they saw an increase in cfDNA levels immediately after completion of exercise. However, their study revealed a higher level of baseline cfDNA levels in the control group, which they hypothesized may be attributed to psychosocial stress exposure. Furthermore, the findings of their research showed that while the concentration of cfDNA in the control group decreased by approximately 40% 30-60 minutes after exercise, concentrations within the athlete group remained at similar levels. They speculated that in contrary to athletes, the control group may not have fully developed the body’s psychophysical abilities to undertake maximal anerobic effort, thus decreasing the amount of muscle damage and in turn release of cfDNA.

Various mechanisms for the release of cfDNA have been hypothesized, such as apoptosis, necrosis, as well an active release of DNA known as NETosis, which is limited to certain hematopoietic cell types. It is characterized as a rapid process involving nuclear disintegration and cell death, which leads to the expulsion of neutrophil extracellular traps (NETs), as well as the snaring and accumulation of defense-related substances and invading microorganisms (Aucamp et al. 2018). Chevion et al. hypothesized that more strenuous activity led to increase in cfDNA levels due to

leukocyte and muscle cell damage from oxidative stress, as well as acute-phase inflammatory reactions.

DNA methylation patterns were analyzed using an atlas of human tissues, developed by Moss et al., which showed that almost half of cfDNA originated from neutrophils and roughly a quarter from non-leukocyte blood cells, monocytes, lymphocytes, and vascular endothelial cells. These results are consistent with research by Neuberger et al., which analyzed post-exercise blood samples from patients with hematological malignancies. After exercise, granulocyte-derived cfDNA increased from 54.1% to 90.2%. It's interesting to note that a rise in neutrophil count, in particular, did not correspond with an increase in neutrophil generated cfDNA (Fridlich et al. 2023).

Fridlich et al. further examined chromatin structures and gene expression patterns and identified mature neutrophils as the origin of elevated cfDNA after intense exercise.

An increase in the duration and intensity of the exercise, for instance in volunteers who ran a half- or full marathon revealed an increase in cfDNA of neutrophilic origin. Skeletal and cardiac muscle cells were also examined as potential contributors to cfDNA levels during exercise. Fridlich et al. found that intense exercise led to increases in cardiac muscle derived cfDNA, while skeletal muscle cfDNA did not increase.

Further examination of physiological triggers revealed that while physical impact, increases in temperature, as well as hypoxia led to increases in cfDNA levels, alterations in heart rate, ß-adrenergic signaling, glucocorticoids and muscle contraction did not have an effect on cfDNA levels. Lastly, Fridlich et al. discovered that untrained individuals exhibited higher levels of neutrophil derived cfDNA than trained individuals.

2) cfDNA can be used as a non-invasive biomarker for the progression of non-alcoholic fatty liver disease.

In this study participants suffering from non-alcoholic fatty liver disease were chosen to undergo an 8-Week web-based intervention program. cfDNA levels, as well as inflammatory markers and histopathological samples were examined over the course of the study. One of the goals of this study was to examine the possibility of using cfDNA levels as a non-invasive marker for the progression of the disease.

Karlas et al. provided evidence that patients with NAFLD exhibited higher baseline levels of cfDNA in comparison to healthy controls. Additionally, they demonstrated that an increased level stiffness correlated with higher baseline levels. While their study could

not prove a significant association between cfDNA and age, BMI or the presence of type 2 diabetes, our study revealed a significant association between cfDNA 90bp and insulin resistance (Figure 8). An increase in insulin resistance led to an increase in cfDNA levels.

Using DNA methylation of liver tissues, Hardy et al. strived to differentiate the severity of fibrosis levels in patients with NAFLD. The density of methylation was found to be greater than 70% in patients with moderate fibrosis and over 80% in patients with severe fibrosis. Further examination of methylation at the PPARγ gene promoter showed a presence in patients with varying etiologies of fibrosis, suggesting that methylation can be used as a method to track disease progression and development (Hardy et al. 2016).

A further study conducted by Buzova et al. examined levels of median cfDNA and methylated cfDNA in 67 children with biopsy proven NAFLD/MAFLD. They discovered that while median levels of cfDNA were not significantly higher in patients with NAFLD/MAFLD than controls, they determined that plasma levels of methylated cfDNA were significantly higher in patients with NASH than those without. Furthermore, they concluded that varying levels of fibrosis positively correlated with levels of methylated cfDNA. Such findings correlate with a study conducted by Chrysavgis et al., which deduced that serum levels of total cfDNA were not elevated in cirrhotic patients, while higher levels of methylated cfDNA were seen.

After completion of the 8-Week program, participants exhibited a 0.9% decrease in weight (kg) with a median decrease in BMI of 0.4. While the NAFLD fibrosis score remained unchanged, an improvement in the fatty liver index was seen. Additionally, decreases in liver inflammatory markers AST and ALT were noted (Huber et al. 2019). Baseline cfDNA levels, however, were increased in both 90bp and 222bp during the timepoint “PRE” post-intervention when compared to baseline levels before the start of the intervention.

Activity intensity is measured as a percentage of maximum oxygen uptake (Vo2max) or as the 1RM, the maximum weight that can be lifted for one full repetition of the activity. Low-intensity activity produces roughly 60% VO2max, while high-intensity endurance produces >85%. Participants in the study undertaken by Andreatta et al. performed exercises at 80RM and 40RM. They concluded that cfDNA concentrations were significantly higher after performing exercises at 80RM rather than 40RM. They concluded that the concentrations of cfDNA released were therefore dependent on

intensity of the exercise undertaken. The exercise program used in this study included recommendations for walking or running, strength and stretching exercises, as well as relaxation exercises. Resistance training was done at home with bodyweight exercises and resistance bands and lasted approximately 45 minutes. Participants were encouraged to provide relevant training information (e.g. average heart rate, time, subjective perception effort) to the supervisor at the end of each week. Accordingly, the training volume was amended to suit the participants' needs and increases or decreases in training content (duration and intensity) were conducted. Our aim was to evaluate the effect of an appropriate, web-based, 8-week exercise intervention on cardiovascular and respiratory conditions, as indicated by maximal oxygen consumption (maximum oxygen volume [VO2 peak]). Participants in the study exhibited an 8.8% increase in Vo2peak after completion of the intervention (Pfirmann et al. 2019), thus revealing the plausibility of a web-based intervention as a means of improving cardiovascular and respiratory function in patients with NAFLD.

As discussed earlier, ALT und AST represent hepatic biomarkers, which are commonly used to determine liver injury, as well as monitor disease development. However, Amarapurkar et al. found a poor connection between blood transaminase levels and histological severity in patients with NAFLD. In spite of having normal ALT levels, they found that 23% of the 81 patients with histologically confirmed NAFLD also had histological evidence of cirrhosis. Such findings were confirmed by Mofrad et al., who stated that individuals with advanced disease frequently have normal levels of liver enzymes, making the identification of at-risk individuals more complex. Scoring systems, such as the NAS have been established as means to measure changes and predict the presence of NASH. However, a study conducted by Brunt et al. concluded that the definitive presence or absence of SH did not always correlate with the NAS.

Karlas et al. demonstrated that patients at risk for advanced fibrosis displayed elevated concentrations of both 90bp, as well as 222bp cfDNA. Furthermore, they showed that while patients with high concentrations of 90bp cfDNA also had increased levels of AST, such findings could not be seen with ALT. According to their study, both ALT und AST did not substantially correlate with 222bp cfDNA. Ferritin and both cfDNA segments showed significant correlations. The findings in our study indicated that increased levels of cfDNA correlated with increased levels of AST, ALT, Ferritin, as well as higher NAFLD scores.

While the determination of cfDNA on its own as a marker for the progression of the disease has yet to be fully established, the use of supplementary non-invasive

techniques in conjunction with cfDNA may aid in the increased accuracy of determining progression of the illness.

5.3 Criticism of chosen methods:

The study began with 46 patients, however, concluded with 43 patients. A comparative study undertaken by Karlas et al. examined 58 NAFLD patients, as well as 13 healthy controls. Due to a lack of healthy control subjects, a direct comparison of cfDNA levels between patients with NASH, NAFL and a healthy cohort could not be achieved. A study containing more participants may have potentially yielded different results.

When implemented correctly, web-based interventions can be beneficial, however, their disadvantages should also be noted.

The participants in our study received illustrated tutorials and videos to help guide them with their exercise regimen. Nonetheless, proper exercise execution cannot be ensured without personal instruction by an expert. In addition, exercise intensity was adjusted weekly based on subjective feedback provided by the patients. Decreases and increases in intensity and duration were both allowed after consultation with a supervisor. Such a decrease could limit the improvement and results observed during the study.

Overall, the intervention was based on the participants' subjective response regarding physical activity during the week and not an objective evaluation. The study relied on the participants' report (Pfirrmann et al. 2018).

Weight management programs are often recommended to patients diagnosed with NAFLD/NASH (Linden et al. 2016). Nutritional experts and supervising physicians provided patients with informative documents and disease-specific questionnaires but did not provide patients with a specific diet plan. A combination of diet and exercise is beneficial for the patient and provides independent results (Linden et al. 2016).

The exercise portion of the intervention was comprised of either resistance training involving body weight exercises and elastic resistance bands or walking and running recommendations. Participants were free to choose one of the two, however, were not supervised during exercise.

Although research regarding the minimum level of intensity or caloric deficit required to see improvements in cfDNA concentrations is inconclusive, studies have shown that both duration and intensity are significantly associated with the accumulation of cfDNA

(Haller et al. 2017). These findings were confirmed by Fridlich et al., which demonstrated that intense exercise led to an increase in cardiac muscle derived cfDNA.

Hashida et al. concluded that low levels of aerobic activity may prove insufficient for the improvement of hepatic steatosis (Rinella et al. 2015). Resistance protocols in studies conducted on the effect of resistance training on hepatic steatosis differ from the model used in our study. The exercises utilized by our study involved elastic resistance bands and body weight, whereas other studies focused on a combination of weighted exercises including biceps curls, calf raises, triceps press, chest press, seated hamstrings curl, shoulder press, leg extensions, and lateral pull down. Participants often completed three sets of 8-12 repetitions and trained three times a week (Hallsworth et al. 2011, see also Jakovljevic et al. 2013, El-Kader et al. 2014, Zelber-Sagi et al. 2014).

Various studies have reported an increase in cfDNA concentration following intense exercise (Atamaniuk et al. 2004). Fatouros et al. concluded that exercise-induced inflammation may also attribute to an increase in cfDNA concentrations. It is therefore feasible that a chronic exercise regimen may have led to an increase in cfDNA levels, thus masking the overall effectiveness of the intervention.

Participants were given a web-based intervention program with explanations and regular check-ups over the course of 8 weeks. Therefore, the findings only examined an acute interval with regards to the effect of acute endurance training on cfDNA levels. An experiment undertaken over a longer period of time could potentially yield different results.

Lastly, an assessment regarding the various training levels of the participants was not undertaken. A participant’s training level before the initiation of the intervention may influence the baseline level of cfDNA concentration, as has been determined by Fridlich et al.

6 Summary (English)

Research into the field of cfDNA and its use as a potential biomarker for various illnesses is growing. The objective of this study was twofold: to research the effects of acute endurance training on cfDNA levels, as well as explore the possibility of using cfDNA as a marker for the progression of disease in patients with NAFLD and NASH.

An increase in prevalence in patients with nonalcoholic fatty liver disease and its subtype nonalcoholic steatohepatitis has been seen worldwide. With said increase, patients are at a higher risk of mortality due to malignancy and cardiovascular disease. In order to decrease the risk of complications, various treatments have been recommended. Among the available options are lifestyle changes and pharmacological therapies. Lifestyle changes including weight loss and exercise have been proven to both slow disease progression, as well as improve histological findings in patients with NAFLD.

A combination of the use non-invasive hepatic inflammatory markers, as well as invasive liver biopsies has been used to track the progression of the disease. While liver biopsies are considered to be the gold standard, they are also an expensive and invasive method which requires medical expertise and is accompanied by various risks. Hepatic biomarkers ALT und AST are the two most common biomarkers used as indicators of hepatic damage. Increases in serum levels have been associated with greater liver damage.

In this work, 90bp and 222bp DNA fragment types were investigated. Samples were also centrifuged at 1600 and 16000 g. At time point "POST," cfDNA levels peaked during the intervention. This was observed in both the pre- and post-intervention groups and in samples centrifuged at 1600 and 16000g. Hours after completion of exercise, cfDNA levels reached their baseline. Our findings coincided with many other studies which also examined the effect of various forms of training on DNA levels.

After completion of the 8-Week web-based intervention, participants were able to decrease their weight and BMI, improve their fatty liver index, as well as decrease concentrations of hepatic biomarkers AST und ALT. In comparison, however, baseline

cfDNA levels were higher in both 90bp and 222bp during the timepoint "PRE" post-intervention.

The methods to determine the origin of cfDNA continue to evolve. Recent studies conducted using DNA methylation markers have shown that while total cfDNA levels did not significantly vary amongst patients with NAFLD and those without, methylated cfDNA levels did.

We therefore believe that the combined use varying methods of cfDNA analysis, as well as additional non-invasive procedures in conjunction may help in the enhanced accuracy of diagnostics and progression of NAFLD and NASH.

7 References

Ahmed, A., Wong, R. J., & Harrison, S. A. (2015). Nonalcoholic fatty liver disease review: diagnosis, treatment, and outcomes. Clinical Gastroenterology and Hepatology, 13(12), 2062-2070.

Amarapurkar, D. N., & Patel, N. D. (2004). Clinical spectrum and natural history of non-alcoholic steatohepatitis with normal alanine aminotransferase values. Tropical gastroenterology: official journal of the Digestive Diseases Foundation, 25(3), 130-134.

Andreatta, M. V., Curty, V. M., Coutinho, J. V. S., Santos, M. Â. A., Vassallo, P. F., de Sousa, N. F., & Barauna, V. G. (2018). Cell-free DNA as an earlier predictor of exercise-induced performance decrement related to muscle damage. International journal of sports physiology and performance, 13(7), 953-956.

Atamaniuk, J., Vidotto, C., Tschan, H., Bachl, N., Stuhlmeier, K. M., & Müller, M. M. (2004). Increased concentrations of cell-free plasma DNA after exhaustive exercise. Clinical chemistry, 50(9), 1668-1670.

Atamaniuk, J., Stuhlmeier, K. M., Vidotto, C., Tschan, H., Dossenbach-Glaninger, A., & Mueller, M. M. (2008). Effects of ultra-marathon on circulating DNA and mRNA expression of pro-and anti-apoptotic genes in mononuclear cells. European journal of applied physiology, 104, 711-717.

Atamaniuk, J., Vidotto, C., Kinzlbauer, M., Bachl, N., Tiran, B., & Tschan, H. (2010). Cell-free plasma DNA and purine nucleotide degradation markers following weightlifting exercise. European journal of applied physiology, 110, 695-701.

Aucamp, J., Bronkhorst, A. J., Badenhorst, C. P., & Pretorius, P. J. (2018). The diverse origins of circulating cell-free DNA in the human body: a critical re-evaluation of the literature. Biological Reviews, 93(3), 1649-1683.

Barak, A., Klein, B., & Proudfoot, J. G. (2009). Defining internet-supported therapeutic interventions. Annals of behavioral medicine, 38(1), 4-17.

Bhangu, J. S., Taghizadeh, H., Braunschmid, T., Bachleitner-Hofmann, T., & Mannhalter, C. (2017). Circulating cell-free DNA in plasma of colorectal cancer patients-A potential biomarker for tumor burden. Surgical Oncology, 26(4), 395-401.

Bohers, E., Viailly, P. J., & Jardin, F. (2021). cfDNA sequencing: technological approaches and bioinformatic issues. Pharmaceuticals, 14(6), 596.

Botezatu, I., Serdyuk, O., Potapova, G., Shelepov, V., Alechina, R., Molyaka, Y., Ananév, V., Bazin, I., Garin, A., Narimanov, M., Knysh, V., Melkonyan, H., Umansky, S., & Lichtenstein, A. (2000). Genetic analysis of DNA excreted in urine: a new approach for detecting specific genomic DNA sequences from cells dying in an organism. Clinical chemistry, 46(8 Pt 1), 1078–1084.

Breitbach, S., Tug, S., & Simon, P. (2012). Circulating cell-free DNA: an up-coming molecular marker in exercise physiology. Sports medicine, 42, 565-586.

Buzova, D., Braghini, M. R., Bianco, S. D., Lo Re, O., Raffaele, M., Frohlich, J., ... & Vinciguerra, M. (2022). Profiling of cell-free DNA methylation and histone signatures in pediatric NAFLD: A pilot study. Hepatology communications, 6(12), 3311-3323.

Chalasani, N., Younossi, Z., Lavine, J. E., Charlton, M., Cusi, K., Rinella, M., Harrison, S. A., Brunt, E. M., & Sanyal, A. J. (2018). The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology (Baltimore, Md.), 67(1), 328–357.

Cherepanova, A. V., Tamkovich, S. N., Bryzgunova, O. E., Vlassov, V. V., & Laktionov, P. P. (2008). Deoxyribonuclease activity and circulating DNA concentration in blood plasma of patients with prostate tumors. Annals of the New York academy of sciences, 1137(1), 218-221.

Chevion, S., Moran, D. S., Heled, Y., Shani, Y., Regev, G., Abbou, B., ... & Epstein, Y. (2003). Plasma antioxidant status and cell injury after severe physical exercise. Proceedings of the National Academy of Sciences, 100(9), 5119-5123.

Chiu, R. W., Poon, L. L., Lau, T. K., Leung, T. N., Wong, E. M., & Lo, Y. D. (2001). Effects of blood-processing protocols on fetal and total DNA quantification in maternal plasma. Clinical chemistry, 47(9), 1607-1613.

Chrysavgis, L., Papatheodoridi, A., Cholongitas, E., Koutsilieris, M., Papatheodoridis, G., & Chatzigeorgiou, A. (2021). Significance of circulating cell-free DNA species in non-alcoholic fatty liver disease. International Journal of Molecular Sciences, 22(16), 8849.

Das, S. K., & Balakrishnan, V. (2011). Role of cytokines in the pathogenesis of non-alcoholic fatty liver disease. Indian Journal of Clinical Biochemistry, 26, 202-209.

Duseja, A., & Chalasani, N. (2013). Epidemiology and risk factors of nonalcoholic fatty liver disease (NAFLD). Hepatology international, 7, 755-764.

El-Kader, S. M. A., Al-Jiffri, O. H., & Al-Shreef, F. M. (2014). Markers of liver function and inflammatory cytokines modulation by aerobic versus resisted exercise training for nonalcoholic steatohepatitis patients. African Health Sciences, 14(3), 551-557.

El Messaoudi, S., Rolet, F., Mouliere, F., & Thierry, A. R. (2013). Circulating cell free DNA: preanalytical considerations. Clinica chimica acta, 424, 222-230.

Fatouros, I. G., Destouni, A., Margonis, K., Jamurtas, A. Z., Vrettou, C., Kouretas, D., ... & Papassotiriou, I. (2006). Cell-free plasma DNA as a novel marker of aseptic inflammation severity related to exercise overtraining. Clinical chemistry, 52(9), 1820-1824.

Forti, L. N., Van Roie, E., Njemini, R., Coudyzer, W., Beyer, I., Delecluse, C., & Bautmans, I. (2017). Effects of resistance training at different loads on inflammatory markers in young adults. European journal of applied physiology, 117, 511-519.

Fridlich, O., Peretz, A., Fox-Fisher, I., Pyanzin, S., Dadon, Z., Shcolnik, E., ... & Dor, Y. (2023). Elevated cfDNA after exercise is derived primarily from mature polymorphonuclear neutrophils, with a minor contribution of cardiomyocytes. Cell Reports Medicine.

Frank, M. O. (2016). Circulating cell-free DNA differentiates severity of inflammation. Biological research for nursing, 18(5), 477-488.

Gauthier, V. J., Tyler, L. N., & Mannik, M. (1996). Blood clearance kinetics and liver uptake of mononucleosomes in mice. Journal of immunology (Baltimore, Md.: 1950), 156(3), 1151-1156.

Hadizadeh, F., Faghihimani, E., & Adibi, P. (2017). Nonalcoholic fatty liver disease: Diagnostic biomarkers. World journal of gastrointestinal pathophysiology, 8(2), 11.
Hallsworth, K., Fattakhova, G., Hollingsworth, K. G., Thoma, C., Moore, S., Taylor, R., ... & Trenell, M. I. (2011). Resistance exercise reduces liver fat and its mediators in non-alcoholic fatty liver disease independent of weight loss. Gut, 60(9), 1278-1283.

Haller, N., Tug, S., Breitbach, S., Jörgensen, A., & Simon, P. (2017). Increases in circulating cell-free DNA during aerobic running depend on intensity and duration. International journal of sports physiology and performance, 12(4), 455-462.

Hallsworth, K., Fattakhova, G., Hollingsworth, K. G., Thoma, C., Moore, S., Taylor, R., ... & Trenell, M. I. (2011). Resistance exercise reduces liver fat and its mediators in non-alcoholic fatty liver disease independent of weight loss. Gut, 60(9), 1278-1283.

Hallsworth, K., Thoma, C., Hollingsworth, K. G., Cassidy, S., Anstee, Q. M., Day, C. P., & Trenell, M. I. (2015). Modified high-intensity interval training reduces liver fat and improves cardiac function in non-alcoholic fatty liver disease: a randomized controlled trial. Clinical science, 129(12), 1097-1105.

Han, X., Wang, J., & Sun, Y. (2017). Circulating tumor DNA as biomarkers for cancer detection. Genomics, proteomics & bioinformatics, 15(2), 59-72.

Hardy, T., Zeybel, M., Day, C. P., Dipper, C., Masson, S., McPherson, S., ... & Mann, J. (2017). Plasma DNA methylation: a potential biomarker for stratification of liver fibrosis in non-alcoholic fatty liver disease. Gut, 66(7), 1321-1328.

Hashida, R., Kawaguchi, T., Bekki, M., Omoto, M., Matsuse, H., Nago, T., ... & Torimura, T. (2017). Aerobic vs. resistance exercise in non-alcoholic fatty liver disease: A systematic review. Journal of hepatology, 66(1), 142-152.

Hendy, O.M., Mona A, E. S., Fatma A, K., Sobhy E, K., Aziza, K., & Salwa R, A. (2016). Circulating cell free DNA as a predictor of systemic lupus erythematosus severity and monitoring of therapy.

Holmberg, R. C., Gindlesperger, A., Stokes, T., Lopez, D., Hyman, L., Freed, M., Belgrader, P., Harvey, J., & Li, Z. (2013). Akonni TruTip(®) and Qiagen(®) methods for extraction of fetal circulating DNA--evaluation by real-time and digital PCR. PloS one, 8(8), e73068.

Huber, Y., Pfirrmann, D., Gebhardt, I., Labenz, C., Gehrke, N., Straub, B. K., ... & Schattenberg, J. M. (2019). Improvement of non-invasive markers of NAFLD from an individualised, web-based exercise program. Alimentary Pharmacology & Therapeutics, 50(8), 930-939.

Humińska-Lisowska, K., Mieszkowski, J., Kochanowicz, A., Stankiewicz, B., Niespodziński, B., Brzezińska, P., ... & Cięszczyk, P. (2021). cfDNA Changes in Maximal Exercises as a Sport Adaptation Predictor. Genes, 12(8), 1238.

Ihalainen, J. K., Schumann, M., Eklund, D., Hämäläinen, M., Moilanen, E., Paulsen, G., ... & Mero, A. A. (2018). Combined aerobic and resistance training decreases inflammation markers in healthy men. Scandinavian journal of medicine & science in sports, 28(1), 40-47.

Jakovljevic, D. G., Hallsworth, K., Zalewski, P., Thoma, C., Klawe, J. J., Day, C. P., ... & Trenell, M. I. (2013). Resistance exercise improves autonomic regulation at rest and haemodynamic response to exercise in non-alcoholic fatty liver disease. Clinical Science, 125(3), 143-149.

Jeong, Y. J., Borgatta, L., Kapp, N., Peter, I., Bianchi, D. W., & Johnson, K. L. (2007). Short-term clearance of cell-free nucleic acids after first-trimester termination of pregnancy. Fertility and sterility, 88(3), 730-733.

Karlas, T., Weise, L., Kuhn, S., Krenzien, F., Mehdorn, M., Petroff, D., ... & Schmelzle, M. (2017). Correlation of cell-free DNA plasma concentration with severity of non-alcoholic fatty liver disease. Journal of translational medicine, 15, 1-9.

Kelders, S. M., Kok, R. N., Ossebaard, H. C., & Van Gemert-Pijnen, J. E. (2012). Persuasive system design does matter: a systematic review of adherence to web-based interventions. Journal of medical Internet research, 14(6), e152.

Kim, W. R., Flamm, S. L., Di Bisceglie, A. M., & Bodenheimer, H. C. (2008). Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology, 47(4), 1363-1370.

Kloten, V., Rüchel, N., Brüchle, N. O., Gasthaus, J., Freudenmacher, N., Steib, F., ... & Dahl, E. (2017). Liquid biopsy in colon cancer: comparison of different circulating DNA extraction systems following absolute quantification of KRAS mutations using Intplex allele-specific PCR. Oncotarget, 8(49), 86253.

Kopreski, M., Benko, F. A., Kwee, C., Leitzel, K. E., Eskander, E., Lipton, A., & Gocke, C. D. (1997). Detection of mutant K-ras DNA in plasma or serum of patients with colorectal cancer. British journal of cancer, 76(10), 1293-1299.
Linden, M. A., Sheldon, R. D., Meers, G. M., Ortinau, L. C., Morris, E. M., Booth, F. W., ... & Rector, R. S. (2016). Aerobic exercise training in the treatment of non-alcoholic fatty liver disease related fibrosis. The Journal of physiology, 594(18), 5271-5284.

Lögters, T., Paunel-Görgülü, A., Zilkens, C., Altrichter, J., Scholz, M., Thelen, S., ... & Jäger, M. (2009). Diagnostic accuracy of neutrophil-derived circulating free DNA (cf-DNA/NETs) for septic arthritis. Journal of Orthopaedic Research, 27(11), 1401-1407.

Lui, Y. Y., Chik, K. W., Chiu, R. W., Ho, C. Y., Lam, C. W., & Lo, Y. D. (2002). Predominant hematopoietic origin of cell-free DNA in plasma and serum after sex-mismatched bone marrow transplantation. Clinical chemistry, 48(3), 421-427.

Marachesini G., Day C. P., Dufour J-F., Canbay A., Nobili V., Ratziu V., ... & Mathus-Vliegen L. European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), & European Association for the Study of Obesity (EASO) (2016). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. Journal of hepatology, 64(6), 1388–1402.

Mofrad, P., Contos, M. J., Haque, M., Sargeant, C., Fisher, R. A., Luketic, V. A., ... & Sanyal, A. J. (2003). Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology, 37(6), 1286-1292.

Moss, J., Magenheim, J., Neiman, D., Zemmour, H., Loyfer, N., Korach, A., ... & Dor, Y. (2018). Comprehensive human cell-type methylation atlas reveals origins of circulating cell-free DNA in health and disease. Nature communications, 9(1), 5068.

Neuberger, E. W., Sontag, S., Brahmer, A., Philippi, K. F., Radsak, M. P., Wagner, W., & Simon, P. (2022). Physical activity specifically evokes release of cell-free DNA from granulocytes thereby affecting liquid biopsy. Clinical epigenetics, 14(1), 1-7.

Oberbach, A., Tönjes, A., Klöting, N., Fasshauer, M., Kratzsch, J., Busse, M. W., ... & Blüher, M. (2006). Effect of a 4 week physical training program on plasma concentrations of inflammatory markers in patients with abnormal glucose tolerance. European journal of endocrinology, 154(4), 577-585.

Pfirrmann, D., Haller, N., Huber, Y., Jung, P., Lieb, K., Gockel, I., ... & Simon, P. (2018). Applicability of a web-based, individualized exercise intervention in patients with liver disease, cystic fibrosis, esophageal cancer, and psychiatric disorders: process evaluation of 4 ongoing clinical trials. JMIR Research Protocols, 7(5), e8607.

Puri, P., & Sanyal, A. J. (2012). Nonalcoholic fatty liver disease: definitions, risk factors, and workup. Clinical Liver Disease, 1(4), 99.

Rabe, K., Lehrke, M., Parhofer, K. G., & Broedl, U. C. (2008). Adipokines and insulin resistance. Molecular medicine, 14(11), 741-751.

Rinella, M. E. (2015). Nonalcoholic fatty liver disease: a systematic review. Jama, 313(22), 2263-2273.

Ritterband, L. M., Gonder-Frederick, L. A., Cox, D. J., Clifton, A. D., West, R. W., & Borowitz, S. M. (2003). Internet interventions: In review, in use, and into the future. Professional Psychology: Research and Practice, 34(5), 527.

Rodriguez, B., Torres, D. M., & Harrison, S. A. (2012). Physical activity: an essential component of lifestyle modification in NAFLD. Nature reviews Gastroenterology & hepatology, 9(12), 726-731.

Saukkonen, K., Lakkisto, P., Pettila, V., Varpula, M., Karlsson, S., Ruokonen, E., ... & Finnsepsis Study Group. (2008). Cell-free plasma DNA as a predictor of outcome in severe sepsis and septic shock. Clinical chemistry, 54(6), 1000-1007.

Shockett, P. E., Khanal, J., Sitaula, A., Oglesby, C., Meachum, W. A., Castracane, V. D., & Kraemer, R. R. (2016). Plasma cell-free mitochondrial DNA declines in response to prolonged moderate aerobic exercise. Physiological reports, 4(1), e12672.

Skrypkina, I., Tsyba, L., Onyshchenko, K., Morderer, D., Kashparova, O., Nikolaienko, O., Panasenko, G., Vozianov, S., Romanenko, A., & Rynditch, A. (2016). Concentration and Methylation of Cell-Free DNA from Blood Plasma as Diagnostic Markers of Renal Cancer. Disease markers, 2016, 3693096.

Sorber, L., Zwaenepoel, K., Jacobs, J., De Winne, K., Goethals, S., Reclusa, P., ... & Pauwels, P. (2019). Circulating cell-free DNA and RNA analysis as liquid biopsy: optimal centrifugation protocol. Cancers, 11(4), 458.

Sozzi, G., Roz, L., Conte, D., Mariani, L., Andriani, F., Verderio, P., & Pastorino, U. (2005). Effects of prolonged storage of whole plasma or isolated plasma DNA on the results of circulating DNA quantification assays. Journal of the National Cancer Institute, 97(24), 1848-1850.

Sung, K. C., Ryu, S., Lee, J. Y., Kim, J. Y., Wild, S. H., & Byrne, C. D. (2016). Effect of exercise on the development of new fatty liver and the resolution of existing fatty liver. Journal of hepatology, 65(4), 791-797.

Tamkovich, S. N., Cherepanova, A. V., Kolesnikova, E. V., Rykova, E. Y., Pyshnyi, D. V., Vlassov, V. V., & Laktionov, P. P. (2006). Circulating DNA and DNase activity in human blood. Annals of the New York Academy of Sciences, 1075(1), 191-196.

Tangvarasittichai, O., Jaiwang, W., & Tangvarasittichai, S. (2015). The plasma DNA concentration as a potential breast cancer screening marker. Indian journal of clinical biochemistry, 30, 55-58.

Tug, S., Helmig, S., Menke, J., Zahn, D., Kubiak, T., Schwarting, A., & Simon, P. (2014). Correlation between cell free DNA levels and medical evaluation of disease progression in systemic lupus erythematosus patients. Cellular immunology, 292(1-2), 32-39.

Tug, S., Tross, A. K., Hegen, P., Neuberger, E. W. I., Helmig, S., Schöllhorn, W., & Simon, P. (2017). Acute effects of strength exercises and effects of regular strength training on cell free DNA concentrations in blood plasma. PloS one, 12(9), e0184668.

Ungerer, V., Bronkhorst, A. J., & Holdenrieder, S. (2020). Preanalytical variables that affect the outcome of cell-free DNA measurements. Critical Reviews in Clinical Laboratory Sciences, 57(7), 484-507.

Van den Berg, M. H., Ronday, H. K., Peeters, A. J., Le Cessie, S., Van Der Giesen, F. J., Breedveld, F. C., & Vliet Vlieland, T. P. M. (2006). Using internet technology to deliver a home-based physical activity intervention for patients with rheumatoid arthritis: A randomized controlled trial. Arthritis Care & Research: Official Journal of the American College of Rheumatology, 55(6), 935-945.

Velders, M., Treff, G., Machus, K., Bosnyák, E., Steinacker, J., & Schumann, U. (2014). Exercise is a potent stimulus for enhancing circulating DNase activity. Clinical biochemistry, 47(6), 471-474.

Wan, J. C., Massie, C., Garcia-Corbacho, J., Mouliere, F., Brenton, J. D., Caldas, C., ... & Rosenfeld, N. (2017). Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nature Reviews Cancer, 17(4), 223-238.

Wang, R., Li, X., Zhang, H., Wang, K., & He, J. (2017). Cell-free circulating tumor DNA analysis for breast cancer and its clinical utilization as a biomarker. Oncotarget, 8(43), 75742.

Wantland, D. J., Portillo, C. J., Holzemer, W. L., Slaughter, R., & McGhee, E. M. (2004). The effectiveness of Web-based vs. non-Web-based interventions: a meta-analysis of behavioral change outcomes. Journal of medical Internet research, 6(4), e40.
Yamaguchi, K., Yang, L., McCall, S., Huang, J., Yu, X. X., Pandey, S. K., ... & Diehl, A. M. (2007). Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. Hepatology, 45(6), 1366-1374.

Yoon, K. A., Park, S., Lee, S. H., Kim, J. H., & Lee, J. S. (2009). Comparison of circulating plasma DNA levels between lung cancer patients and healthy controls. The Journal of Molecular Diagnostics, 11(3), 182-185.

Zelber-Sagi, S., Buch, A., Yeshua, H., Vaisman, N., Webb, M., Harari, G., ... & Shibolet, O. (2014). Effect of resistance training on non-alcoholic fatty-liver disease a randomized-clinical trial. World journal of gastroenterology: WJG, 20(15), 4382.

Zhang, S., Lu, X., Shu, X., Tian, X., Yang, H., Yang, W., ... & Wang, G. (2014). Elevated plasma cfDNA may be associated with active lupus nephritis and partially attributed to abnormal regulation of neutrophil extracellular traps (NETs) in patients with systemic lupus erythematosus. Internal Medicine, 53(24), 2763-2771.

Ziegler, A., Zangemeister-Wittke, U., & Stahel, R. A. (2002). Circulating DNA: a new diagnostic gold mine?. Cancer treatment reviews, 28(5), 255-271

8 Curriculum Vitae

Academic career:

10/2010 – 05/2017	Medical studies at the University of Mainz Overall grade of the medical examination: Gut (2,5)
03/2013	First state examination in medicine
04/2016	Second state examination in medicine, written exam
05/2017	Second state examination in medicine, oral exam

Practical year:

1.Tertial (05/2016 – 09/2016)	Diakonie Hospital, Bad Kreuznach (Internal medicine)
2. Tertial (09/2016 – 12/2016)	Diakonie Hospital, Bad Kreuznach (Orthopedics)
3. Tertial (12/2016 – 04/2017)	Diakonie Hospital, Bad Kreuznach (General surgery)

09/2017 – 11/2024	Dissertation: „ The effects of acute endurance training intervention on circulating cell-free DNA in participants with histologically proven non-alcoholic fatty liver disease” under the supervision of Prof. Dr. med. Schattenberg, as well as Prof. Dr. med. Dr. rer. nat. Perikles Simon, Johannes Gutenberg Universität Mainz
10/2018	Medical license to practice medicine
12/2018	Step 1 of the USMLE (United States Medical Licensing Examination)
04/2018	Step 2 CK of the USMLE (United States Medical Licensing Examination)

Professional experience, clinical experience:

10/2013 – 02/2015	Student assistant (learn and earn) in the coloproctological department of the St. Josefs-Hospital in Wiesbaden
03/2015 – 06/2018	Translation work for the hematology training portal www.hematology.online

07/2019 – 12/2022	Medical resident, St. Marienwörth Hospital, Department for abdominal, visceral and Trauma surgery (Head of the department: Priv.-Doz. Dr. med. Markus Paschold) • Common Trunk in Surgery (24 Months) • Continued medical education Trauma and orthopedics (18 Months)
02/2023 - present	Medical resident, Helios Clinic Munich West, Department for Orthopedic and Trauma surgery (Heads of the department: Prof. Dr. med. Martin Thaler, Alexander Manolopoulos) • Continued medical education: Trauma and orthopedics

Practical training/clinical traineeship:

07/2010 – 10/2010	Practical nurse training in Chippenham Hospital (Internal medicine)
08/2011 – 09/2011	Clinical traineeship in Chippenham Hospital, Richmond, Virginia, USA (Internal medicine)
08/2014 – 09/2014	Clinical traineeship in Chippenham Hospital, Richmond, Virginia, USA (Internal medicine)
07/2015 – 08/2015	Clinical traineeship in Chippenham Hospital, Richmond, Virginia, USA (Internal medicine)
08/2015 – 09/2015	Clinical traineeship in in Dr. Dilly's physician practice, Hargesheim, Rhineland-Palatine (General medicine)

Preparatory College:

09/2009 – 07/2010	Johannes Gutenberg University of Mainz

High School:

08/2004 – 06/2008	Midlothian High School (Midlothian, Virginia, USA)

Foreign language competence:	Englisch (native language) German (fluent) Arabic (conversant)