universität wien

MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

„Acute impact of coffee on exercise-induced oxidative stress "

verfasst von / submitted by

Helia Heidari Dasht Bayaz, BSc

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien, 2023 / Vienna, 2023

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	UA 066 838
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Masterstudium Ernährungswissenschaften Master´s degree programme Nutritional Sciences
Betreut von / Supervisor:	Univ.-Prof. Dr. Daniel König
Mitbetreut von / Co-Supervisor:	

Acknowledgement

I am grateful to my advisor, Univ.-Prof. Dr. Daniel König for their guidance throughout this project. I would also like to express my deepest gratitude to the research project coordinator Markus Gassner, MSc. and the research assistant Dipl.-Ing. Laura Bragagna. Their valuable feedback and assistance played a significant role in developing and shaping my thesis. With their expertise and contribution this thesis attained the quality it now possesses.

I am also thankful to my family for their encouragement and moral support throughout my research process, who have been a source of motivation for me.

I appreciate everyone for their help and support along this way, I am deeply grateful.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

1-RM	*One-Repetition Maximum*
5-RM	*Five-Repetition Maximum*
BMI	*Body Mass Index*
CAT	*Catalase*
CGAs	*Chlorogenic acids*
CMH	*1-hydroxy-4-phosphonooxy-2,2,6,6 tetramethylpiperidine*
DOMS	*Delayed Onset Muscle Soreness*
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDII	*Empirical Dietary Inflammatory Index*
EPR	*Electron Paramagnetic Resonance*
ESR	*Electron Spin Resonance*
FC	*Folin Ciocalteau*
FRAP	*Ferric Reducing Antioxidant Power*
GAE	*Gallic Acid Equivalent*
GPxs	*Glutathione Peroxidase*
GSH	*Glutathione*
HIIT	*High Intensity Interval Training*
HPLC	*High-Performance Liquid Chromatography*
hs-CRP	*high sensitivity C-reactive Protein*
hs-IL-6	*high sensitivity Interleukin 6*
IL-8	*Interleukin 8*
MDA	*Malondialdehyde*
MET	*Metabolic Equivalent of Task*
OJ	*Orange Juice*
ORAC	*Oxygen Radical Absorbance Capacity*
PJ	*Pomegranate Juice*
PREDIMED	*Prevention with Mediterranean Diet*
RMANOVA	*Repeated Measurement Analysis of Variance*
ROS	*Reactive Oxygen Species*
RSA	*Radical Scavenging Activity*
SIT	*Sprint Interval Training*
SOD	*Superoxide Dismutase*
TAA	*Total Antioxidant Activity*

TEAC	*Trolox Equivalent Antioxidant Capacity*
TNFα	*Tumor Necrosis Factor alpha*
TPC	*Total Polyphenol Content*
TRAP	*Total Radical Trapping Antioxidant Power*
Trx	*Thioredoxin*
VO_2max	*Maximum Oxygen Uptake*

Abstract

The aim of this master´s thesis was to investigate the acute effect of consuming coffee on exercise-induced oxidative stress. Coffee is widely consumed worldwide, which is known for its bioactive compounds and antioxidant properties. Existing literature has shown that strenuous and excessive physical activity leads to an increased endogenous production of ROS, which can result in oxidative stress. 20 healthy, physically inactive, female participants, aged 18-40 years, were included in this randomized intervention, who consumed 250 mL filtered coffee (treatment group) and water (control group) two hours prior to engaging in circuit high intensity resistance training for roughly 20 minutes. All subjects submitted four capillary blood samples on each intervention day (fasting, prior, immediately after, 15 minutes after training), which were used to assess the ROS formation rate with an EPR spectrometer. Furthermore, the Folin-Ciocalteu method and the DPPH antioxidant assay were applied to assess the total polyphenol content (TPC) and the total antioxidant activity (TAA) of the beverages. The underlying training protocol effectively elevated the ROS level in all subjects, which is an indicative that HIIT treatment and control group. These results suggest that coffee does not have a notable impact on the ROS formation rate and exercise-induced oxidative stress. However, the optimal dosage and timing of coffee intake in an intervention remains unknown. To gain comprehensive understanding regarding the effect of coffee on the oxidative state further robust and well-controlled interventions are needed.

Ziel dieser Masterarbeit war, den akuten Einfluss des Kaffeekonsums auf die sport-induzierte oxidative Stressreaktion zu überprüfen. Kaffee zählt zu den am häufigsten konsumierten Getränken, welches für seine bioaktiven Substanzen und antioxidativen Eigenschaften bekannt ist. Die vorliegende Fachliteratur belegt, dass intensive und übermäßige physikalische Aktivität einen Anstieg in der endogenen Produktion der radikalen Sauerstoffspezies (ROS) verursacht, was zu oxidativen Stress führen kann. 20 gesunde, inaktive, weibliche Probandinnen im Alter von 18-40 Jahren nahmen an dieser randomisierten Intervention teil, welche 250 mL Filterkaffee (Behandlungsgruppe) und Wasser (Kontrollgruppe) konsumierten und zwei Stunden danach ein 20-minütiges Intervalltraining mit hoher Intensität absolvierten. Insgesamt wurden vier kapillare Blutabnahmen pro Interventionstag durchgeführt (nüchtern, unmittelbar vor, unmittelbar nach und 15 Minuten nach dem Training), welche mithilfe eines EPR Spektrometers zur Bestimmung der ROS-Bildungsrate analysiert wurden. Darüber hinaus wurde anhand der Folin-Ciocalteu Methode und DPPH Antioxidant Methode die Gesamtpolyphenolkonzentration und die antioxidative Kapazität der Getränke bestimmt. Die vorliegende Trainingsintervention erhöhte effektiv die ROS Konzentration, welches als Indikativ für die HIIT-induzierte oxidative Stressreaktion galt. Jedoch konnte kein signifikanter Unterschied zwischen der Behandlungsgruppe und Kontrollgruppe beobachtet werden. Diese Resultate lassen darauf schließen, dass Kaffee keinen positiven Effekt auf die sport-induzierte oxidative Stressreaktion ausübt. Bis zum aktuellen Stand der Forschung wurde die passende Aufnahmemenge und der optimale Zeitpunkt des Kaffeekonsums im Rahmen einer Sportintervention nicht in seiner Gesamtheit erfasst. Für ein umfassendes Verständnis bezüglich der Auswirkung des Kaffeekonsum auf oxidative Parameter sind weitere robuste und kontrollierte Interventionen notwendig.

1 Introduction

In recent years, considerable attention has been drawn to the effect of exercise on the endogenous production of reactive oxygen species (ROS). Thus, the number of studies conducted in this matter has significantly increased. Implementing strategies to counteract the excessive ROS production could be beneficial to reduce the negative consequences on both human health and exercise performance. ROS are natural byproducts of the regular metabolism and play a crucial role in physiological functions in the body. Evidence suggests that moderate-intensity physical activity leads to an elevated ROS production to an extent, where the antioxidant defense system of the body can effectively modulate and neutralize ROS. Consequently, it contributes to an enhanced regenerative capacity and an improved physiological adaption response to exercise. However, strenuous physical activity, such as high intensity interval training (HIIT) or prolonged endurance exercise promote an excessive production of ROS and thus overwhelm the antioxidant defense mechanism, which can lead to oxidative stress. (Simioni et al., 2018), (Lu et al., 2021)

Additionally, it has been reported that nutritional factors such as an adequate intake of antioxidants affect the body´s response to exercise-induced oxidative stress. Based on the „Empirical Dietary Inflammatory Index (EDII) from Tabung et al. and Kanauchi et al. coffee has been categorized as an anti-inflammatory dietary source. The EDII is commonly used to determine the inflammatory potential of foods and beverages. (Kanauchi et al., 2019), (Tabung et al., 2016) Coffee is known for its antioxidative qualities and has been widely studied in association with inflammation and oxidative stress. It is one of the most consumed beverages worldwide. Various epidemiological studies have demonstrated beneficial health effects of consuming coffee due to its great phenolic content. (Paiva et al., 2019)

The present master´s thesis is a constituent component of a larger research project "Acute impact of different beverages on exercise induced oxidative stress and inflammation". The anti-inflammatory beverages used in this project, which were chosen based on the EDII from Tabung et al. and Kanauchi et al. were as followed: matcha, coffee, pomegranate juice (PJ), orange juice (OJ), sugar sweetened water, milk, and water. (Kanauchi et al., 2019), (Tabung et al., 2016) The research project evaluated several biomarkers of oxidative stress, including malondialdehyde (MDA), high sensitivity Interleukin 6 (hs-IL-6), protein carbonyl content, high sensitivity C-

reactive protein (hs-CRP), reactive oxygen species (ROS), and the ferric reducing antioxidant power (FRAP) assay to assess the antioxidant activity of plasma.

The underlying intervention protocol is based on the trial from Zeng et al. They conducted a randomized intervention with 34 young healthy women and aimed to investigate the acute effects of oatmeal consumption prior to a HIIT on the ROS concentration in capillary blood using an EPR spectrometer. They observed a significant decrease in the ROS concentration immediately after the training in the oatmeal group, as opposed to the control group, where the participants completed a HIIT only. (Zeng et al., 2020)

Based on the existing body of research the effect of coffee on oxidative stress remains unclear. Therefore, the aim of this master´s thesis was to investigate the acute effect of consuming coffee on the exercise-induced elevation of reactive oxygen species and oxidative stress.

The underlying hypothesis for this master´s thesis was that coffee, as a dietary antioxidant source, may increase the body´s antioxidant capacity after a HIIT and therefore reduce the exercise-induced oxidative stress by decreasing the endogenous production of reactive oxygen species.

2 Scientific background

2.1 Coffee and its components

Coffee is one of the most common consumed beverages worldwide. There has been significant focus on its effects on human health and decades of research has been conducted in this issue. According to the International Coffee Organization, the estimated world consumption in 2020/21 was 166.35 million 60-kg bags of coffee beans, whereas Finland accounts for the highest consumption amongst European countries with an annual consumption about 12 kg per person. In comparison to that the estimated annual consumption in Austria is 6.8 kg per person. (International Coffee Organization, 2012), (International Coffee Organization, 2021) Comparatively speaking, the United States of America and Brazil are considered as the first and second largest coffee consuming countries in the World, respectively. (International Coffee Organization, 2014)

Due to the rising interest in the consumption of coffee over the last years the development of food based dietary guidelines (FBDGs) has played an increasingly

important role to promote a healthy diet and protect the population from adverse health effects. The effects of consuming coffee are based on a dose-dependent pattern, where both positive and negative effects are possible. (Reyes & Cornelis, 2018) An excessive consumption of coffee can cause cardiovascular complications, anxiety, and insomnia. (Barcelos et al., 2020), (Ciaramelli et al., 2019) Therefore, healthy adults should not exceed a maximum daily intake of four cups of coffee (400 mg caffeine per day), as recommended by the European Food Safety Authority. (EFSA, 2015)

Coffee is primarily divided into two main types based on its species: coffea arabica L. and coffea canephora, commonly known as Arabica and Robusta. These two types represent the majority of the global coffee production. The coffee fruits of the coffee tree are the so called "coffee cherries", from which the green coffee beans are gained. Roasting the green coffee beans and then grinding the roasted beans result in the commercially available coffee. (International Coffee Organization, 2014) According to current research, roasted coffee beans, especially those belonging to the arabica species are known as an important source of bioactive compounds. This is an interesting finding considering that arabica is the most valuable coffee species from a commercial point of view. (Ciaramelli et al., 2019)

The various bioactive compounds in coffee beans are accountable for the potential protective effects of drinking coffee. Based on epidemiological studies it has been hypothesized that coffee may inversely associate with the development of different diseases and inflammatory conditions. The beneficial health effects are based on its antioxidant and anti-inflammatory properties. Some important bioactive compounds are chlorogenic acids (CGAs), trigonelline, diterpenes and caffeine. (Paiva et al., 2019)

Caffeine, a methylxanthine, is the most popular psychoactive compound in the world, which is responsible for the stimulating effect of coffee on the central nervous system (CNS). The latter is the basis for the performance-enhancing effect of caffeine both mentally and physically. Most of the biological effects of caffeine are mediated by the antagonism of the adenosine receptors. Adenosine is described as an inhibitory neurotransmitter in the brain and hence acts as a central nervous system depressant. Adenosine is responsible for regulating sleep, cognitive performance, memory, and

learning. Because of a structural similarity, caffeine can bind to the adenosine receptors and suppress the adenosine receptor functions.
Caffeine leads to an increased production of catecholamines such as dopamine and adrenalin, which are accountable for the induced feeling of wakefulness and alertness after drinking coffee. A common physical consequence of consuming caffeine is an elevated heart rate. (Barcelos et al., 2020)
After oral administration caffeine appears in the bloodstream within minutes, whereas the peak caffeine plasma concentration is reached after 30 to 60 minutes. Caffeine is rapidly absorbed in the stomach and small intestine, and it is metabolized in the liver, where the cytochrome P450 1A2 enzyme (CYP1A2) metabolizes it into its three primary metabolites as followed: paraxanthine, theobromine, and theophylline. After further metabolization only 3 to 5% remain in the caffeine form that can be detected in urine. (Guest et al., 2021)

Caffeine proceeds to be a dietary component of concern in public health, whereas popular caffeine sources are coffee, tea, cocoa, and energy drinks. However, there are some other caffeine containing products which have gained increasing interest over the last years such as chewing gums, energy gels and caffeine-containing capsules. Considerable attention has been drawn to the consumption of caffeine amongst athletes because of its ergogenic effects. (Guest et al., 2021)

The amount of caffeine in each serving depends on the species, the preparation and roasting method (light, medium or dark). Also, the concentration of chlorogenic acids is variable and is affected by the genetics of the species and environmental factors such as temperature and rainfall. (Martini et al., 2016) Chlorogenic acids (CGAs) are the most important polyphenols of coffee beans, accounting for more than 98% of its total phenolic content, with anti-inflammatory and antioxidative properties. CGAs could possess a few additional potential biological effects that are currently being discussed, including antiviral, anticarcinogenic and immunoprotective effects. (Ciaramelli et al., 2019), (Tajik et al., 2017)
The roasting process plays a significant role when it comes to the color and aroma of coffee. The combination of time and temperature determines the degree of roasting. (de Souza et al., 2020) During roasting, the coffee compounds undergo a structural change in a Maillard reaction, which is a non-enzymatic chemical roasting reaction.

As a result, melanoidins are being produced, which are nitrogen containing brown colored compounds. The level of roasting is negatively correlated with the concentration of chlorogenic acids. (LIczbiński & Bukowska, 2022), (Jung et al., 2017) Studies have shown slight differences regarding the phenolic content between green coffee beans and lightly roasted coffee, however the phenolic loss was doubled in dark roasted coffee. (de Souza et al., 2020) But melanoidins possess antioxidative properties as well, which compensate the loss of CGAs during the roasting process. Even after roasting, coffee is the main dietary source for CGAs. (LIczbiński & Bukowska, 2022), (Jung et al., 2017)

Another bioactive component are diterpenes, such as kahweol and cafestol, which mostly appear in unfiltered coffee. Furthermore, diterpenes are found in greater concentrations in arabica coffee. A high intake of diterpenes, especially cafestol can influence serum lipid levels and liver enzymes. The Tromsø Heart Study by Thelle et al. first published a positive correlation between the consumption of coffee and serum concentration of total cholesterol and triglycerides in 1983. 60% of their study population consumed five or more cups of coffee per day. (Thelle et al., 1983) Both in vivo and in vitro experimental results support this finding. Unfiltered coffee such as French-press coffee, Scandinavian-style boiled coffee and Turkish coffee contain 3-6 mg of each diterpene in one cup. Evidence shows that a long-term consumption of unfiltered coffee increases serum triglycerides and low-density lipoprotein (LDL) in humans. The meta-analysis from Cai et al. included 12 randomized controlled trials with a total of 1182 healthy subjects. The intervention duration varied between three to nine weeks and the amount of coffee intake ranged from two to eight cups (125 mL/cup) per day. This meta-analysis showed a great increase in total cholesterol (TC), total triglycerides (TG), and LDL when consuming ≥ six cups of coffee per day and a significant increase in TC, TG and LDL when consuming boiled coffee compared to filtered coffee. (Du et al., 2020) The mechanism by which diterpenes affect the lipid profile remains unknown. Some studies indicated that kahweol and cafestol exhibit their impact on the serum lipid profile through a reduction in the activity of the LDL-receptor, which causes a decrease in the elimination of LDL from the bloodstream. (Ren et al., 2016)

However, many studies demonstrated strong anti-inflammatory properties for kahweol and cafestol. Thus, more research is needed to clarify the pharmacological and biological effects of diterpenes. (Martini et al., 2016), (Ren et al., 2016)

Because of the beneficial qualities mentioned above it has been hypothesized that coffee consumption is linked to a reduced risk of developing clinical conditions such as cardiovascular diseases, type 2 diabetes mellitus (T2DM), some types of cancer and metabolic syndrome. The anti-inflammatory and antioxidative properties of coffee can lead to oxidative stress-suppressing effects, whereas oxidative stress is considered as a potential basis for developing non-communicable diseases. (Paiva et al., 2019), (Hu et al., 2019) According to Kempf et al. and Yamashita et al. regular coffee consumption can contribute to decrease pro-inflammatory cytokines such as hs-CRP and IL-8 and increase the circulating level of adiponectin. The latter shows beneficial effects in modulating inflammation and hs-CRP is a marker of systemic low-grade inflammation. However, it is important to point out that the study population from Kempf et al. showed an elevated risk for type 2 diabetes mellitus and Yamashita et al. did not exclude subjects with chronic conditions. (Kempf et al., 2010), (Yamashita et al., 2012) Additionally, Lopez-Garcia and colleagues conducted a cross-sectional study with the purpose to evaluate the effect of long-term coffee consumption on oxidative stress markers. They reported no appreciable effect of coffee on the plasma concentration of inflammatory markers in healthy women. However, they reported that a higher coffee intake was associated with a lower CRP plasma concentration in diabetic women. (Lopez-Garcia et al., 2006)

Additionally, the effect of consuming coffee on the plasma antioxidant capacity has been investigated. The study from Moura-Nunes et al. showed that acute consumption of 200 mL instant coffee elevated the plasma antioxidant capacity in healthy adults after 90 minutes, whereas the plasma and serum concentration of endogenous non-enzymatic antioxidants remained unchanged. One serving of coffee, which was used as an intervention beverage, contained 284 mg CGAs and 240 mg caffeine. (Moura-Nunes et al., 2009)

Moreover, the study from Metro et al. demonstrated antioxidative properties of pure caffeine. After a daily oral ingestion of caffeine capsules (5 mg/kg body weight) for seven days an improvement in oxidative stress markers (total antioxidant capacity, malondialdehyde, glutathione, oxidized glutathione) was demonstrated on the eighth day in healthy male participants. (Metro et al., 2017)

However, Shaposhnikov et al. conducted a randomized placebo-controlled intervention trial with 160 healthy male and female participants for eight weeks to investigate the potential antioxidative effects of coffee consumption. They reported that a daily consumption of three or five cups of coffee did not affect the markers of oxidative stress and inflammatory cytokines. No differences were reported in the concentration of CRP, IL-6 and Isoprostane (8-iso-PGF2α). The latter is a biomarker of lipid peroxidation, which reflects the level of oxidative stress in the body.

2.1.1 The effect of coffee on exercise performance

Coffee is a very popular beverage among athletics and in the sports science literature. The beneficial effects of drinking coffee on physical activities are mainly related to the impact of caffeine improving exercise performances. The potential ergogenic and stimulatory effects of caffeine on physical exercises have been studied over the last decades. The primary accepted mechanism to explain the ergogenic effects is its influence on the central nervous system (CNS). Further examinations reported that caffeine can impact the muscle through calcium ion (Ca^{2+}) mobilization, which facilitates muscle contraction and force production. (Guest et al., 2021)

Moreover, other ergogenic effects of caffeine such as delayed muscle fatigue, improved muscular endurance and strength have been discussed in the literature, however the results are not consistent. (Barcelos et al., 2020) The meta-analyses from Grgic et al. reported that an acute caffeine intake, especially in form of capsules, increases muscle strength and power, particularly in the upper body. The most common time used for caffeine administration was 60 minutes prior to the exercise. They only included studies that applied a vertical jump test and a one-repetition maximum test for assessing the muscle power. Nevertheless, improvements in aerobic activities have shown the most benefits from caffeine use by increasing the endurance. (Grgic et al., 2018) Smirmaul and colleagues observed a significant 12% reduction of time until exhaustion due to caffeine intake prior to a high intensity cycling exercise. (Smirmaul et al., 2017)

Considering that caffeine is officially categorized as a stimulant, the International Olympic Committee and National Collegiate Athletic Association have determined a maximum permitted urinary concentration of 15 µg/mL as the threshold for athletes, which is approximately eight cups of coffee or 800 mg of caffeine. However, studies

have demonstrated that caffeine can exert its ergogenic effects at much lower doses. (Barcelos et al.,2020)

The response to caffeine intake can differ between habitual and non-habitual coffee drinkers and the magnitude of the ergogenic effects vary between individuals. According to two meta-analyses several studies reported performance-enhancing effects after moderate caffeine ingestion (3-6 mg/kg body weight). (Shen et al., 2019), (Southward et al., 2018)

The ideal timing of caffeine intake to benefit from its stimulatory impacts depends on the source of caffeine. Some caffeine-containing products such as chew gums may absorb more quickly. (Guest et al., 2021)

2.2 Oxidative stress and free radicals

A free radical is a molecule or an atom with at least one unpaired electron and is characterized as short lived, highly reactive, and unstable due to the odd number of electrons. Thus, it shows a very high affinity to react with the closest stable molecule to take its electron to gain stability. As a result, the targeted molecule can turn to a free radical by losing its electron and initiate a chain reaction with damaging effects to living cells. Reactive oxygen species (ROS) are an example of free radicals, which are formed from a diatomic oxygen (O_2). (Arulselvan et al., 2016) Superoxide ($O_2^{\bullet -}$), hydroxyl- (HO•), peroxyl- (ROO•), and alkoxyl radical (RO•) are other species of ROS.

ROS are divided into free radicals and non-radicals. When two free radicals share their unpaired electrons, non-radicals are created. Additionally, free radicals can be generated from nitrogen, such as nitric oxide/nitrogen monoxide (NO•) and nitrogen dioxide ($NO_2^{\bullet}$). (Gulcin, 2020)

Free radicals are constantly produced from endogenous and exogenous sources. Environmental pollution, alcohol, cigarette smoke, industrial solvents and chemicals, pesticides and medications are exogenous sources that stimulate the formation of free radicals. (Gulcin, 2020)

ROS play an essential role in numerous biological functions in the regular cellular metabolism such as immune responses and cell signaling pathways. They are part of the body’s defense mechanism. Phagocytic cells such as macrophages produce ROS to destroy microbes and foreign organisms.

ROS are mainly produced endogenously in the mitochondrial respiratory chain as byproducts, where oxygen is utilized for energy production in form of adenosine triphosphate (ATP). (Gulcin, 2020) In addition, ROS are important for the regulation of enzymes and gene expression. The impact of free radicals can be described as a dose-dependent-relationship, where low physiological concentrations are necessary for the metabolism and high levels are toxic. (Ozougwu, 2016) This implies that the concentration of ROS determines the transition from their advantageous to harmful effects. However, it remains unclear what concentration triggers this transition. (Di Meo et al., 2016) There is no accurate numeral value that defines their low physiological levels. It is generally accepted that the physiological concentration is in the nanomolar to micromolar range. (Sies & Jones, 2020)
Comparatively speaking, a previous study measured the ROS concentration using the EPR method prior and after an exercise program. The baseline ROS concentrations measured in 34 healthy female participants was about 0.70-0.75 µM/min. (Zeng et al., 2020)

Normally, there is an intracellular balance between reducing and oxidizing reactions, called a redox homeostasis. Excessive stress conditions in the organism can lead to an overwhelming ROS production, which interferes with the redox homeostasis and will eventually lead to oxidative stress. (He et al., 2017) Membrane lipids, proteins and nucleic acids are usual targets for ROS, which can result in damaged cellular structures, modified functions, and consequently induce an inflammatory response. The development of various diseases with accumulating oxidative stress as their physiopathological basis, is a consequence. Also, intracellular damages caused by reactive species can stimulate the process of aging. (Arulselvan et al., 2016)
Inflammation and chronic oxidative stress have been associated with the etiology of many disorders. Oxidative stress as a stimulator of pathologies can be divided in two categories as followed: first, as the primary cause of a pathological condition such as toxicity due to exposure to radiation and second, oxidative stress as the secondary cause of chronic diseases that contributes to the disease progression. (Forman & Zhang, 2021)
Oxidative DNA damages are linked to tumor cell proliferation and tumor progression and therefore can lead to the initiation of cancer. Because of the high-rate

metabolism of cancer cells they can constantly stimulate and preserve a high ROS production and thus maintain their proliferation. (He et al., 2017)

Moreover, studies have shown an association between Alzheimer disease and oxidative stress. Studies were able to demonstrate an increased oxidative stress level in the brain of Alzheimer patients with a high rate of mitochondrial DNA oxidation and protein degeneration. (He et al., 2017)

There is ongoing research to better understand the association between ROS and inflammation. In the literature to date, the specific concentration of ROS that is associated with a higher disease risk is unknown. However, it is generally thought that a persistent imbalance between ROS and antioxidant defenses increases the risk of chronic diseases.

2.2.1 Exercise-induced oxidative stress

It is commonly known that physical activity provokes the production of ROS, due to a high oxygen intake, muscle contraction and muscle injuries while exercising. Especially, strenuous and high intensity exercises are associated with oxidative stress, whereas regular moderate exercises promote health benefits. (Simioni et al., 2018), (Lu et al., 2021) Based on the World Health Organization guidelines, it is recommended that adults engage in moderate-intensity physical activity for at least 150-300 minutes/week, or 75-150 minutes/week of vigorous-intensity exercise, to achieve health benefits. (Bull et al., 2020)

High intensity interval training (HIIT) is a time-efficient form of exercise that stimulates muscles to generate more reactive oxygen radicals. This exercise is characterized by repeated short bouts of intense training, performing with ≥ 90% of maximal oxygen consumption (VO_2max) or >75% of maximal power, with periods of resting or low-intensity exercises.

Furthermore, evidence shows that the lack of sufficient free time is a significant contributing factor to physical inactivity, a global public health concern. As a result, interval training models such as HIIT, are gaining increasing popularity. (Atakan et al., 2021)

Physical activity significantly increases the oxygen demand of the body compared to the resting condition and the oxygen flow through active muscles and other organs rises. Furthermore, muscle damages can often occur during resistance training due

to the involvement of the skeletal muscle including several muscle nerves and fibres. (Lu et al., 2021) Training programs are intended to induce a temporary state of transient fatigue to challenge and improve the body´s regenerative capacity. Both moderate training and HIIT are often applied with the purpose to improve the metabolic adaptions, which has been in fact reported. It has been demonstrated that moderate training provides beneficial effects on the body´s redox status and plays a crucial role in regulating muscle regeneration and HIIT can result in a better muscle mitochondrial capacity, cardiorespiratory fitness, and vascular function. (Moslemi et al., 2023)

However, strenuous physical activity can lead to muscle damages and thus resulting in an increased formation of ROS due to the generated inflammatory response at the site of the muscle injury. As a result, if the recovery phase persists for an extended period, the body might not be able to adjust to the physical workload and it will contribute to a decreased exercise performance. (Magherini et al., 2019), (Canals-Garzón et al., 2022) Any activity that requires six metabolic equivalents per minute or more is considered a strenuous activity. The metabolic equivalent of task (MET) unit is used to express the absolute intensity of an activity. 1 MET is equivalent to the resting metabolic rate or while sitting quietly. (Piercy et al., 2018) Additionally, the inflammatory response due to strenuous exercise can lead to an acute increase of inflammatory biomarkers such as IL-6 and TNFα. (Simioni et al., 2018) The intervention from Richardson et al. demonstrated an exercise-induced inflammatory response in a young and untrained study population. The intervention consisted of a two-week Sprint interval training (SIT), a form of HIIT, which resulted in an elevated concentration of IL-6, IL-8 and TNFα 48 hours after the final training session. (Richardson et al., 2016)

The production of reactive species during physical activity can have both beneficial and harmful effects, which are dependent from the duration, intensity and fitness and nutritional status of the individual. The exercise-induced ROS formation at physiological levels can enhance the antioxidative defense mechanism by increasing the enzymatic antioxidant activity. (Simioni et al., 2018) Moreover, ROS are required for generating the normal muscle force during physical activity, mainly in resistance training. Nevertheless, an overload of ROS during strenuous exercise, which induces oxidative stress can result in contractile dysfunction. In addition, muscle weakness and fatigue can occur. Moreover, research has shown that the production of free

radicals due to intensive physical activity is a common cause of delayed onset muscle soreness (DOMS). The muscle pain experienced hours after an intensive workout is known as DOMS, which usually peaks after 24 to 72 hours. (Torre et al., 2021)

It is important to mention that oxidative stress caused by excessive training only occurs when the intensity of the exercise passes a certain individual threshold. (Magherini et al., 2019) Moreover, the accumulation of ROS depends on the body´s fitness level and capacity to regulate oxidative stress. (Canals-Garzón et al., 2022)
The increased ROS formation during exercise can be demonstrated by determining biomarkers of oxidative stress in both blood and muscle tissue. (Kawamura & Muraoka, 2018). One of the parameters of oxidative stress is malondialdehyde (MDA), which represents the level of free radicals in the body. An increased level of ROS results in an overproduction of MDA, which can be measured in the blood plasma. (Wang et al., 2021)
To prevent exercise-induced oxidative stress and following muscle fatigue it is recommended to avoid prolonged or high intensity exercises in untrained individuals and rather practice a constant, progressive training with sufficient resting and recovery intervals. As a result, the body can adjust its oxidative capacity and detoxify the generated amounts of ROS. (Magherini et al., 2019)

2.3 Antioxidants

An antioxidant is a molecule, which prevents or delays the oxidation of other molecules. The antioxidative defense mechanism maintains a balance between antioxidants and free radicals, which makes them crucial components for the overall well-being of humans. They are characterized by the ability to absorb free electrons and to react with free radicals. They can either directly scavenge ROS, which are considered as primary antioxidants or secondary antioxidants can indirectly stimulate the antioxidant defenses. However, the goal of the antioxidative defense system is not to eliminate free radicals entirely, but to keep them at an appropriate concentration so they can exert their physiological role in the organism. (Gulcin, 2020), (Pisoschi et al., 2021)

Antioxidants are generated endogenously in our body, which are classified as enzymatic and non-enzymatic antioxidants based on their activity. Enzymatic antioxidative defenses include the enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidases (GPxs) and thioredoxin (Trx), while non-enzymatic antioxidants consist of GSH (Glutathione), uric acid, bilirubin, vitamin C, carotenoids, tocopherols and phenolics. Both antioxidative systems are important because they function synergistically, as each can target a specific free radical. (Pisoschi et al., 2021), (Ozougwu, 2016)

Dietary sources such as carotenoids, vitamin C and E are exogenous antioxidant sources, which are mainly found in fruits, vegetables, and high-quality vegetable oils. Both vitamin C and E can counteract lipid peroxidation that can damage the cell membrane and consequently eliminate ROS directly. (Ozougwu, 2016) Antioxidants can occur in both synthetic and natural form. Synthetic antioxidants are added to products to prevent oxidation. (Gulcin, 2020)
Usually, the antioxidative system in the organism is sufficient to prevent any cellular damage caused by free radicals. However, an overload of free radicals accelerates the oxidative state and overwhelms the antioxidative defense mechanism. Furthermore, the ability of the human organism to manage oxidative stress decreases with aging. With advancing age, the concentration of endogenous antioxidants tends to decline. (Ozougwu, 2016)

2.3.1 Polyphenols

Polyphenols are natural compounds found in plants, which are described as the so-called plant secondary metabolites. These phytochemicals are abundant in plant derived foods such as fruits and vegetables, whereas foods with stronger color possess a higher polyphenol content in general. More than 8000 phenolic molecules have been identified, which are structurally characterized by comprising one or more phenolic hydroxyl groups. The number and position of these hydroxyl groups (-OH) represent their biological qualities such as their antioxidant activity. The number of -OH groups is proportional to the number of free radicals a polyphenol can scavenge. The variations among the structure of polyphenols allows a categorization of polyphenols into flavonoids and non-flavonoids. (Di Lorenzo et al., 2021)

Polyphenols are known for their beneficial health effects with evidence supporting their contribution to prevent non-communicable diseases. For instance, the PREDIMED study showed that a Mediterranean diet, which is a diet rich in polyphenols, is associated with a reduced risk for cardiovascular diseases in the elderly. However, this outcome is not solely attributable to the consumption of polyphenols alone, but rather to the consumption of a mixture of essential nutrients. (Ros et al., 2014)

Additionally, phenolic compounds possess the ability to stimulate antioxidative enzymes, which are part of the antioxidant system of the body. (Gulcin, 2020) The required amount of polyphenols needed to achieve their protective effects is a topic of comprehensive debate. The systematic review from Del Bo et al. evaluated the mean total polyphenol intake of the overall population, which was estimated to be around 900 mg/day. The main dietary sources for polyphenols were tea, coffee, red wine, fruit, and vegetables. (Del Bo' et al., 2019)

However, there are inconsistencies in explaining the positive health effects of phenolic compounds. The bioavailability of polyphenols depends on its chemical structure and can be affected by different factors such as thermal treatments and storage conditions. The bioavailability in vivo varies due to their interaction with the food matrix, complex absorption and metabolization. Moreover, due to differences in the gut microbiome among individuals, there is an interindividual variation in the bioavailability of polyphenols. (Kashi et al., 2019) Furthermore, the difficulty in identifying which specific molecule is responsible for a biological action when more than one phenolic compound exist in a sample, creates additional complexity. (Di Lorenzo et al., 2021) Thus, it is less likely that all polyphenols serve as direct antioxidants. Their antioxidant activity appears to be mediated by an endogenous stimulation of the antioxidant capacity via an intracellular signaling pathway. (Bowtell & Kelly, 2019)

Furthermore, polyphenols have become a dietary component of interest among athletes. Due to their antioxidative properties they are frequently used as dietary supplements. In addition to their antioxidant activity, polyphenols are attributed to several biological effects in the human body. A potential role of polyphenols which has been intensively investigated is their impact on the synthesis of mitochondria. The latter can lead to a performance-enhancing effect due to an increased

mitochondria efficiency and thus an increased oxygen supply during physical activity. (Wood dos Santos et al., 2018) Moreover, polyphenols seem to have ergogenic effects due to their enhancing effect on the vascular function, which can lead to an improved muscle perfusion. (Kashi et al., 2019) Trexler and colleagues performed a randomized placebo-controlled trial, and their results showed that an acute ingestion of pomegranate extract, which obtains a high polyphenol content, was associated with an improved blood flow and exercise performance. The blood flow was measured using an ultrasound, which was assessed at baseline, 30 minutes after the administration of pomegranate extract, immediately and 30 minutes after the training. (Trexler et al., 2014)

Current evidence suggests that an acute supplementation of 300 mg polyphenols one or two hours prior to an exercise can lead to an enhanced performance in aerobic and repeated sprint exercise. (Bowtell & Kelly, 2019)

Based on the current literature it appears that the consumption of certain polyphenol rich fruit extracts or juices can positively affect different health biomarkers. (Kashi et al., 2019)

2.4 Electron Paramagnetic Resonance (EPR)

Various measurement methods have been developed to analyze oxidative stress markers in biological systems. Reactive oxygen species are difficult to detect because they possess a short lifetime ranging from nanoseconds to seconds, depending on their reactivity and available cellular antioxidants. (Dikalov et al., 2018) Electron Paramagnetic Resonance (EPR), also described as Electron Spin Resonance (ESR), is a spectroscopic technique that can directly detect free radicals. The unpaired electron in free radicals causes paramagnetic behavior. Every electron has a spin and therefore a magnetic moment. Due to their paramagnetic characteristics, when an external magnetic field is applied, paramagnetic electrons can align either in a parallel or antiparallel direction to the field. These two possible orientations are described as spin states or more accurately as the spin magnetic quantum number (m_s). Therefore, every unpaired electron can exist in two spin states (± ½). When unpaired electrons are exposed to a magnetic field, transitions between the two spin states occur, whereas the parallel direction corresponds to a lower energy level (- ½) and the antiparallel direction to a higher energy level (+ ½).

However, the unpaired electrons mainly align to the parallel direction with the lower energy level. In order to prompt them to align in the higher energy level, radiation from the microwave frequency is used in the magnetic field. An appropriate radiation frequency absorbed by the unpaired electrons will induce the transition between the two spin states. The EPR spectrometer can monitor the radiation absorbed in the sample and convert it into a spectrum. For quantitative interpretations, the area under the signal is proportional to the concentration of free radicals in the sample. Most EPR measurements are done using microwave radiations in the gigahertz range. This is the conventional EPR measurement described as the continuous wave EPR. Without the presence of a magnetic field both spin states have the same energy, which is described as the degenerate state. (Dikalov et al., 2018), (Hogg, 2010)

Due to the short lifetime of reactive radicals, it is necessary to first convert them into more stable radicals to make them detectable for the EPR spectrometer. Two techniques, spin trapping and spin probing, are commonly used to generate stable radicals. Spin traps are implemented in the structure of the sample and react with the transient free radical and spin probes possess reactive compounds that react with the radical to produce a persistent radical species. Compared to the spin traps, spin probes provide an advantage due to their higher efficiency, lower required concentrations to react with radicals and higher sensitivity to the biological system. (Dikalov et al., 2018)

2.5 Folin-Ciocalteu method

The Folin-Ciocalteu (FC) method can be used to determine antioxidative compounds in a variety of plants and plant-derived foods and beverages. It is a very commonly applied method for the assessment of phenolic compounds, which is a development of the Folin Denis reagent. The latter was originally used in the early 19th century for the determination of tyrosine in proteins.

The FC reagent is a complex mixture of heteropoly-phosphotungstates/
molybdates and possesses an intensive yellow color. The FC reagent is commercially available, although it can be prepared in the laboratory. Furthermore, the reagent is light-sensitive and should be stored in the dark. (Agbor et al., 2014), (Sánchez-Rangel et al., 2013)

The mechanism behind this colorimetric method is based on a reduction/oxidation reaction, where the phenolic compounds present in a sample are oxidized with the FC reagent. The reaction results in the formation of blue tungsten and molybdenum. The intensity of the blue color is proportional to the concentration of phenolic compounds. (Singleton & Rossi, 1965) The reaction is better and faster in a basic pH, which is why sodium carbonate is added. (Sánchez-Rangel et al., 2013)
Some criteria need to be met to achieve reliable results from this assay, such as an ideal reaction time and temperature for color development, appropriate volume of extract and the use of a reference standard polyphenol is required. (Agbor et al., 2014)

However, there is a disadvantage of the FC method that should be mentioned. The FC reagent does not only react with phenolic compounds, but rather with any reducing compound present in the sample. Other easily oxidized compounds such as ascorbic acids, amino acids or sugars can interfere and influence the determination of the total phenolic content. However, the results from the FC method correlate well with those obtained from other antioxidative assays such as DPPH or ORAC (Oxygen radical absorbance capacity). (Sánchez-Rangel et al., 2013)

2.6 DPPH antioxidant assay

Investigating antioxidative qualities of natural substances is highly important due to their widespread use in foods, medicine, and cosmetics. Antioxidants are used in the food industry to prevent oxidation processes in foods, which could lead to the formation of undesirable chemical compounds.
The DPPH antioxidant assay is known as a standard and easy method to assess antioxidants and their free radical scavenging activity (RSA%) in a variety of samples. The application of the DPPH radical for antioxidant determination was first published by Blois et al. (Blois, 1958) DPPH (2,2-diphenyl-1-picrylhydrazyl) is a nitrogen centered stable radical, which can persist in solution for hours and the solution appears in a deep purple color. Since DPPH is an artificial radical, it cannot reproduce in an in vivo environment. (Mishra et al., 2012)
Antioxidants react with the DPPH• radical by donating an electron or hydrogen atom, thus reducing it to DPPH-H. Hence, there are two potential mechanisms for phenolic

compounds to react with DPPH. First, a direct abstraction of the phenol H-atom and second an electron transfer from the phenol´s hydroxyl group (-OH) to the DPPH• radical. In solvents such as methanol the second reaction is predominant. (Villaño et al., 2007) This reaction is characterized by a discoloration from deep purple to pale yellow, which can be monitored by a spectrophotometer. The color change is associated with a decrease in absorbance at a characteristic wavelength. DPPH shows maximum absorbance at 515nm in methanol. The reducing capacity of a sample is proportional to the discoloration intensity. According to the literature, methanol provides better radical stability than other solvents such as ethanol or acetone. (Mishra et al., 2012)

There are a few different ways to express the results of the DPPH assay. Commonly, the antioxidant activity of a sample is expressed as the percentage of inhibition of the DPPH radical or the radical scavenging activity (RSA%). Moreover, the TEAC index (Trolox Equivalent Antioxidant Capacity) is often applied, where the total antioxidant activity (TAA) of a sample is compared to the standard Trolox. Trolox (6-hydroxy-2,5,7,8 tetramethylchroman-2- carboxylic acid) is a water-soluble α-tocopherol derivative and has been reported to be an efficient scavenger. (Villaño et al., 2007)

3 Study design and methods

3.1 Participants

In total, 51 female volunteers were screened, and 20 participants successfully completed the intervention. All participants, aged from 18 to 40 years, were healthy and non-smokers or had abstained from smoking for at least six months. They were characterized as not trained or not physically active individuals and they were requested to refrain from medication, exercise and alcohol 24 hours before each intervention day. Their fitness level was assessed using a questionnaire. Participants were required not to engage in physical activities more than 120 minutes per week and a maximum of one strength or resistance training session per week was tolerated. Additionally, a body mass index (BMI) from 18.5 to 25 kg/m^2 and a body fat percentage > 20% were further requirements for inclusion, which were determined along with other anthropometric measurements during the screening process.

Exclusion criteria were acute or chronic illnesses, existing iron deficiency or hemoglobin anemia, physical impairment or incompatibleness with the training program, coffee intolerance, and regular resistance training and any recent vaccination. The most recent vaccination was required to have been administered at least seven days prior to the intervention. Furthermore, participants were asked to avoid blood donations during the intervention period.

3.2 Materials and reagents

The required reagents and laboratory equipment to perform the FC-method, DPPH antioxidant assay and the EPR method are listed below.

Table 1.: Reagents for the FC-method

Reagents	Product number	Supplier
Folin-Ciocalteu-reagent	F9252	Sigma-Aldrich, Germany
Sodium carbonate	451614	
Gallic acid	91215	

Table 2.: Reagents for the DPPH antioxidant assay

Reagents	Product number	Supplier
DPPH reagent	D9132	Sigma-Aldrich, Germany
Trolox	93510	
Ethanol (96%)	1.00983.2511	
Methanol (99%, HPLC grade)	1.06035.1000	

Table 3.: Reagents for the EPR method

Reagent	Product number	Supplier
CMH	NOX-02.1-50mg	Noxygen Science Transfer and Diagnostics GmbH, Germany
Krebs-HEPES-buffer	NOX-7.6.1-0.5L	
Oxygen-sensitive label	NOX 15.1–5 µmol/L	

Table 4.: Overview of required laboratory equipment to perform the FC-method, DPPH antioxidant assay and EPR method

Laboratory equipment	**Supplier**	**Description**
Microplate Reader	BMG Labtech, Germany	CLARIOstar® Plus
96-well microplate	BRAND GmbH + Co KG, Germany	clean, flat bottom
Magnetic stirrer equipped with heater	Heidolph, Germany	Product Nr.: MR 3001-K
Rotilabo® Magentic bars	Carl Roth GmbH+Co. KG, Germany	
Vortex mixer	Heidolph, Germany	
Multipette® E3 (1 µl-50 mL)	Eppendorf, Germany	Cat. Nr.: 498000010
Xplorer® Plus (5-100 µL)	Eppendorf, Germany	Cat. Nr.: 4880000783
Combitips® advanced 5mL	Eppendorf, Germany	Cat. Nr.: 0030089456
Combitips® advanced 1.0 mL	Eppendorf, Germany	Cat. Nr.: 0030089430
Manual Finnpipette® (1-10µl, 5-50µl, 50-200µl, 200-1000µl)	Thermo Fisher Scientific, Germany	
Refrigerator		
Parafilm® M	Sigma Aldrich, Germany	Product Nr.: P7793-1EA
Ultrasonic sonicator bath [a]	Bandelin Electronic GmbH & Co.KG, Germany	Product Nr.: RK156H
Wax plates [b]	Noxygen, Germany	NOX-A.3-VPM
Capillary tubes [c]	Hirschmann, Germany	
EPR spectrometer [d]	Bruker, Germany	

[a] This laboratory equipment was only used for the DPPH antioxidant assay, [b, c, d] These laboratory equipment were used for the EPR method.

3.3 Experimental design

This was a randomized controlled crossover intervention study conducted at the University of Vienna, Department of Nutritional Science. All examinations and interventions were done at the Study site, the Nutrition and Training Laboratory (NuTraLab). In the context of this master thesis, a partial aspect of the research project "Acute impact of different beverages on exercise induced oxidative stress and inflammation" was investigated.

The aim of this master´s thesis was to investigate the acute effect of consuming coffee on the exercise-induced elevation of reactive oxygen species and oxidative stress. Participants received clarification about the process of this intervention. All subjects signed a form of consent to participate. The study protocol was approved by the Ethics Committee of University of Vienna with the Reference-ID: 00743. The date of Ethics Committee Approval was: 01.12.2021.

Due to the crossover design, subjects were randomly allocated to both treatment (coffee) and control group (water), with a wash-out period of at least seven days. Initially, as part of the recruitment process, a structured questionnaire was used to collect data about previous diseases, medications, and other lifestyle habits such as smoking. The participants underwent a blood test, a blood pressure measurement, and an electrocardiogram was performed to ensure their overall health. Subjects had to be physically capable to engage in high intensity resistance training for roughly 20 minutes on an empty stomach without experiencing any complications. A week prior to the beginning of the experiment each participant went through a trial run to test the exercise program, during which each training was explained thoroughly by a fitness trainer and the five-repetition maximum (5-RM) per exercise was determined. The 1-RM test is used more commonly, which is defined as the maximum weight an individual can lift while performing the correct technique. The 5-RM is the maximum weight which an individual can lift five times while adhering to the correct technique. This method is used to establish a person´s maximum strength. (Grgic et al., 2020) Comparatively, the advantage of the 5-RM test is a potentially lower risk for muscle injuries during the test phase. The calculations for the maximum weight were done using the formula from Brzycki. (Brzycki, 1998)

Every intervention started in the morning and subjects were asked to follow a 12 hour overnight fast. After arrival at the study site subjects were asked to lie on the medical

examination table and rest for 30 minutes to provide a stable and suitable condition for ROS measurements. During this resting time a 24-hour dietary Recall questionnaire (24h-Recall) was completed with a study staff member. A 24h-Recall was used to assess all foods and beverages consumed on the day before with detailed information about the ingredients, portion size and time of consumption. In case of regular intake of dietary supplements, participants were requested to refrain from any dietary supplements during the intervention period. Along with evaluating the 24h-Recall, alcohol consumption, sleep quality and the use of contraceptives were assessed.

Subsequently, the fasting blood sample was collected, following which subjects received one serving of treatment (1 cup of coffee, 250 mL) or control (1 cup of water, 250 mL) in a randomized sequence.

Afterwards, participants had a two-hour break to rest, which they had to spend at the study site. 30 minutes prior to the second blood collection they were asked to lie down again. The blood sample collections were conducted at fasting, right before, immediately after and 15 minutes after the training. Subjects were asked to remain on the medical examination table between the third and fourth blood sampling. The importance of staying hydrated for the experiment was emphasized at the beginning of every intervention day. The subjects were asked whether they drank at least 500 milliliters of water before their arrival. This procedure was repeated on every intervention day for both the treatment and control group. Every participant completed the experiment within three to four months. Approximately, one or two weeks off from the intervention in case of a minor sickness or a short vacation were granted for each participant. Per intervention day, one to three subjects performed the experiment. The main research project included seven different beverages. Thus, the underlying intervention consisted of a total of seven intervention days per participant.

Furthermore, the total polyphenol content (TPC) and total antioxidant activity (TAA) were assessed according to the FC-method and DPPH antioxidant assay, respectively. These two analytical measurements were done at the laboratory of the Nutritional Science Department of the University of Vienna in the University Center II. As the main research project included further beverages under examination the TPC

and TAA of the following beverages were determined: coffee as the subject of investigation of this master's thesis and matcha, PJ and OJ.

3.4 Statistical analysis

The sample size calculation was performed for the primary outcome of this master´s thesis, the reactive oxygen species formation rate. The sample size was calculated considering preliminary data from Zeng et al., a previous intervention study. (Zeng et al., 2020) A G-Power analysis estimated a total of 20 subjects required for this trial, using a 0.05 significance level and 80% Power, which concluded that 20 subjects needed to successfully complete the intervention.

The statistical analysis required in this master´s thesis was performed using the Software IBM SPSS Statistics 27.0 for Windows. A 2 x 4 repeated measure ANOVA (RMANOVA) was applied to analyse the effect of the training program and the subject of investigation on the level of ROS. The statistical significance was defined as $p\text{-value} < 0.05$.

The evaluation of the TPC and TAA was carried out with Microsoft Excel using a standard curve. The data were reported as the mean ± standard deviation based on triplicate measurements.

3.5 Subject under investigation

An organic arabica filtered coffee (Tchibo Bio Äthiopien) was used in this intervention, which was purchased from a local market named "Tchibo". Each coffee serving was freshly prepared at the study site with 12 g ground coffee in 250 mL hot water using filter paper. Participants were given a cup of coffee after the first blood drawing, which they were asked to consume within 10 minutes. All trial coffee packages were stored at room temperature and in the dark.

3.6 Training intervention

The training started after a two-hour rest at the study site. Participants performed a high intensity circuit resistance training for roughly 20 minutes under the guidance of a trainer. The exercise program was based on the training protocol from Bizheh et al.

with some modifications, which effectively induced an increase in the CRP concentration. (Bizheh & Jaafari, 2011)

50% of the subject´s 5-RM was set as the weight used for each exercise when performing the training on the intervention days. The training program consisted of a ten-minute warmup and three main rounds of five different resistance exercises. (Rowing, lat pull, chest press, leg curl, and leg press). The duration of each exercise was 40 seconds and a ten second break was given between each exercise. There was a one-minute break between the three main rounds. The goal was to reach 20 repetitions per exercise while maintaining the correct exercise technique. The exhaustion level was rated using the Borg scale, with 6 being perceived as not exertive at all and 20 being extremely exertive. Moreover, the Borg scale was used to prevent physical overload and complications during the workout. The goal was to reach an exertion level between 16 to 19 on the Borg scale after an entire exercise session on each intervention day.

3.7 Blood sampling and ROS detection

The total formation rate of the ROS was measured using an e-scan EPR spectrometer, which was equipped with a temperature and gas controller to ensure measurements under in vivo conditions at 37°C. Each participant submitted four blood samples per intervention day at the following times: at fasting, right before, immediately after and 15 minutes after the high intensity circuit resistance training. A qualified person collected the blood samples. The participants were asked to lie down on a medical examination table prior to every blood collection to ensure a suitable condition for the ROS detection. Each time 10 µl capillary blood was drawn directly from the fingertip with a standard Eppendorf pipette. 10 µL oxygen-sensitive label were added to the blood sample to enable monitoring the cellular oxygen consumption. In order to extent the half-life of ROS in the capillary blood CMH (1-hydroxy-4-phosphonooxy-2,2,6,6 tetramethylpiperidine) was used as the spin probe to generate a more stable radical. Thus, 20 µL Krebs-HEPES-buffer diluted spin probe (CMH, 400 µmol/L) were mixed into the blood-label solution. Subsequently, the mixture was vortexed and transferred to a glass capillary tube which was sealed with Noxygen wax. The sealed tube was then put into the cavity of the spectrometer to run the test and acquire data. The following settings were applied for determining the

ROS formation rate: center field: g = 2.011, sweep width: 60 G, sweep time: 5.24s, frequency: 9.76 GHz, power: 20 mW, gain: 1 x 10^3, modulation amplitude: 1.0 G, and number of scans: 10. The Bruker WinEPR Data Processing software was used for the quantitation of EPR spectra.

3.8 Total polyphenol content determination

The total polyphenol content (TPC) was determined using the Folin-Ciocalteu (FC) method according to Singleton et al. with some modifications. (Singleton & Rossi, 1965) The total phenolic content of the following organic beverages was determined: filtered arabica coffee (Tchibo Bio Äthiopien), matcha (dmBio, Matcha Tee Instant, 30 g), pomegranate juice (dmBio, Granatapfel Saft, 330 mL) and orange juice (SPAR Natur*pur, Bio-Orangensaft 100%). All samples were purchased from the local market. The beverages coffee and matcha were freshly prepared on the first measurement day using 12 g filtered arabica coffee and 2 g matcha powder in 250 mL hot water, respectively. After cooling, several dilution sets were prepared for each sample with distilled water. The number of sample dilutions needed for one measurement were used on the first day, and the remaining dilutions were stored in the freezer for further measurements at -20°C. A dilution of 1:2 was chosen for coffee, matcha and OJ, whereas for PJ a dilution of 1:10 was used.

Gallic acid, a known phenolic compound, was used as reference. 10 mg gallic acid was dissolved in 10 mL distilled water in a 10 mL volumetric flask and the sodium carbonate solution was prepared with 35 g sodium carbonate and 100 mL distilled water in a 100 mL volumetric flask. We applied a magnetic stirrer equipped with a heater at 23°C for 15 minutes and 70°C for 30 minutes for optimal dissolution of gallic acid and sodium carbonate, respectively.

The prepared gallic acid solution (1mg/mL) was transferred into a 2 mL Eppendorf tube, which was considered as the stock solution (100µl standard reference + 100 µl distilled water). We used the stock solution to prepare the dilutions of different concentrations (1.95 to 1000 µg/mL) with distilled water, which were used to construct a standard curve.

First, 250 µl of these dilutions were pipetted in a 96-well microplate. Subsequently, 10 µl of each sample dilution plus 240 µl distilled water were transferred to the

microplate with the Eppendorf Xplorer Plus and Eppendorf Multipette, respectively. Afterwards, 5 µl FC reagent and 10 µl sodium carbonate (Na_2CO_3) were added to every well using the Eppendorf Multipette, whereas a three-minute pause between the addition of these two reagents was complied. The total volume in each well was 250 µl. Figure 1 represents the general pipetting scheme of the FC-method. The microplate was covered with parafilm and incubated in the dark for 60 minutes at room temperature. The absorbance at 752nm was determined using the spectrophotometer CLARIOstar® Plus. The absorbance was measured using a blank, which contained distilled water and reagents. Figure 2 shows the gallic acid standard curve (R^2= 0.976) and the equation used to calculate the total phenolic content of the samples. The absorbance of each standard was plotted against their concentration.

The measurement was repeated and the average was taken as the final result and all assays were measured in triplicate. To ensure accurate measurements, a variation coefficient < 5% was determined. The results were expressed as microgram gallic acid equivalent per milliliter (µg GAE/mL).

Figure 1.: General pipetting scheme of the FC-method

PL1												
	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Blank	X1	X1	X1	Blank	Blank	Blank	X9	X9	X9
B	S1	S1	S1	X2	X2	X2	S1	S1	S1	X10	X10	X10
C	S2	S2	S2	X3	X3	X3	S2	S2	S2	X11	X11	X11
D	S3	S3	S3	X4	X4	X4	S3	S3	S3	X12	X12	X12
E	S4	S4	S4	X5	X5	X5	S4	S4	S4	X13	X13	X13
F	S5	S5	S5	X6	X6	X6	S5	S5	S5	X14	X14	X14
G	S6	S6	S6	X7	X7	X7	S6	S6	S6	X15	X15	X15
H	S7	S7	S7	X8	X8	X8	S7	S7	S7	X16	X16	X16

S: Standard, X: Sample

Figure 2.: Standard curve gallic acid

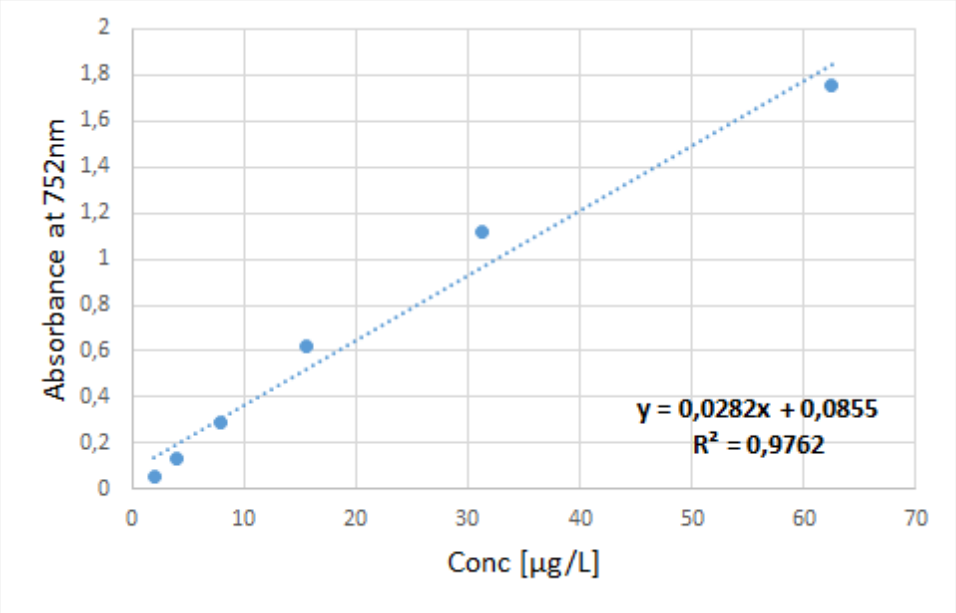


Figure 2 illustrates that the relationship between the absorbance of gallic acid was directly proportional to the polyphenol concentration in terms of gallic acid equivalents. This relationship can be applied to the TPC of the measured samples in this assay.

3.9 Total antioxidant activity determination

In the present study the total antioxidant activity (TAA) of the four beverages mentioned in the previous chapter was assessed according to the DPPH antioxidant

assay. This assay is suitable for a microplate assay format, which allows a simultaneous assessment of multiple samples.

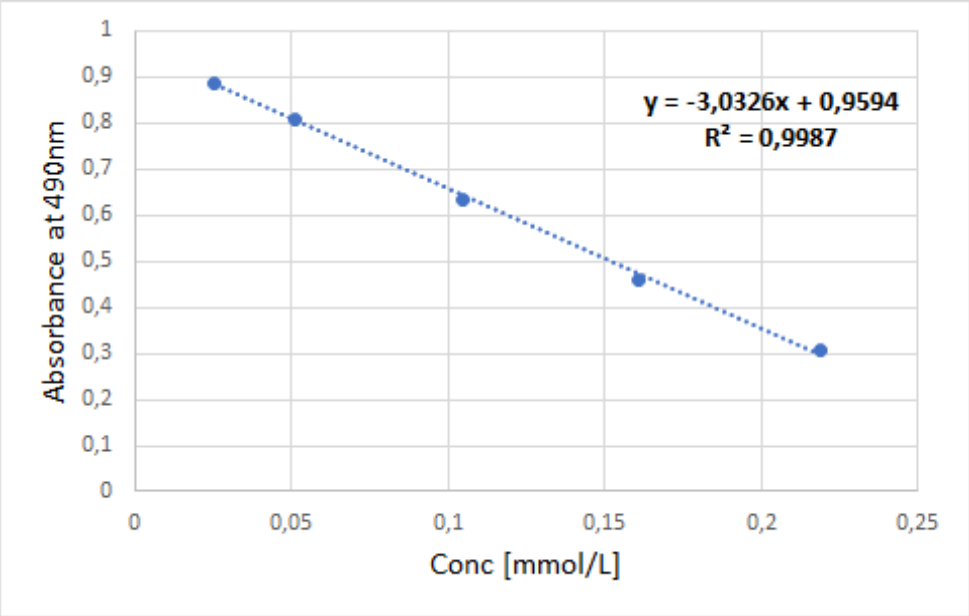
First, the DPPH reagent and Trolox standard had to be dissolved prior to use. The DPPH solution was prepared in a 25 mL volumetric flask with 0.0038 g DPPH in 25 mL methanol (99%, HPLC grade). Subsequently, the glassware was covered with aluminum foil to minimize DPPH degradation. For adequate dissolution we used a magnetic stirrer with stirring bars.

Additionally, a 2.5 mM Trolox standard stock solution was prepared by dissolving 0.0063 g Trolox in 10 mL ethanol (96%) in a 10 mL volumetric flask. The glassware of the Trolox solution was placed in an ultrasonic sonicator bath for three minutes at room temperature for optimal dissolution. If the reagents are not completely dissolved, content may remain on the glassware wall and therefore cause data variation. Both reagent solutions were prepared fresh on each day.

A set of dilutions were made for each sample, and the most appropriate dilution was chosen for the assay. A dilution of 1:10 was chosen for coffee, matcha and OJ, whereas a dilution of 1:50 was used for PJ. 15 µL of each sample dilution was added to the microplate using the Eppendorf Xplorer Plus and following that, 85 µl methanol was pipetted to the sample dilutions in each well using the Eppendorf Multipette.
The Trolox standard stock solution was serially diluted with methanol to generate the Trolox working solution with different concentrations (0.0255, 0.0514, 0.1050, 0.1609, 0.2191,1.260 mM). 100 µl of the Trolox working solution was added to the microplate. Lastly, 100 µl of the DPPH solution were added to each well with the Eppendorf Multipette. The total volume of each well was 200 µl.

The microplate was sealed with parafilm and incubated in dark for 30 minutes. Subsequently, the absorbance was measured against a blank, containing DPPH reagent and methanol at 490nm with the spectrophotometer CLARIOstar® Plus. All measurements were repeated and assessed in triplicate for higher accuracy. To obtain precise measurements, a variation coefficient < 5% was established. Trolox was used as a reference with known concentrations to calculate the antioxidant concentration in the samples with a standard curve (R^2 = 0.99). The sample equivalent of Trolox was calculated with the linear regression y= ax + b and all results were expressed as mmol Trolox equivalent /L.

Figure 3.: Trolox standard curve



According to Figure 3 the relationship between the concentration of Trolox and its absorbance is inversely proportional. With an increasing concentration of Trolox equivalent, the absorbance value decreases, which indicates a higher antioxidant activity. A higher decrease in the absorbance of Trolox means a higher reduction of the oxidizing substances in the sample. The latter principle can be applied to the tested samples within this assay, since Trolox is used as a reference. Hence, the more the absorbance value of the tested sample decreases, the higher is its antioxidant activity.

The RSA% value was calculated as well, which is the percentage of the scavenged DPPH radial and represents the ability of an antioxidant to neutralize a radical. The calculation was done using the following equation, which is consistent with the equation used in previous studies. (Ngamsuk et al., 2019)

RSA% = (Abs_Blank – Abs_Samp) /Abs_Blank * 100

Nevertheless, the DPPH assay has some disadvantages and limitations, which is due to the lack of a standardized description of the assay. Detailed instructions about preparing sample extracts, applying suitable reaction conditions, using appropriate analytical protocols and standard interpretation and expression of data are crucial to enable reliable comparison of assay results. For Instance, the wavelength of

measurement, the solvent, and the pH-level varies between different studies. (Xie & Schaich, 2014)

4 Results

4.1 Baseline characteristics

This intervention included 20 female participants who successfully completed the entire trial. No adverse effects were reported during the intervention period. The baseline characteristics of all subjects were assessed during the screening process, which are summarized in Table 5.

Table 5.: Baseline characteristics of the participants (n=20)

Variable	Mean ± SD	Minimum	Maximum
Age (y)	26.45 ± 4.06	20	37
Body Weight (kg)	58.99 ± 6.82	46.25	70.75
Body Height (m)	1.7 ± 0.06	1.56	1.79
BMI (kg/m^2)	21.0 ± 1.88	18.78	24.95
Fat mass (%)	27,5 ± 3,86	21.8	34.6
WC (m)	0.7 ± 0.07	0.58	0.87
Phase angle (º)	5.1 ± 0.38	4.3	5.9

BMI: Body Mass Index, WC: Waist Circumference

4.2 ROS formation rate

In order to achieve more accurate statistical results, the data were baseline-corrected, which means that the corresponding morning baseline was subtracted from each measurement time point. This adjustment provided an elimination of any confounding factor that might affect the primary outcome, the ROS formation rate.

Table 6 represents the ROS formation rate of both treatment and control group.

Figure 4 shows a reduction of the ROS levels in all subjects from baseline ROS to pre-HIIT ROS, which can be explained by the 2-hour resting period after the consumption of coffee.

However, the represented results show that there was no significant difference observed between the treatment and control group.

Nonetheless, there was a significant difference between the Pre-HIIT and Post-HIIT ROS formation level in general (p=0.007). Thus, the training model can be considered as an effective strategy to induce acute ROS formation.

Table 6.: ROS formation rate in the treatment and control group

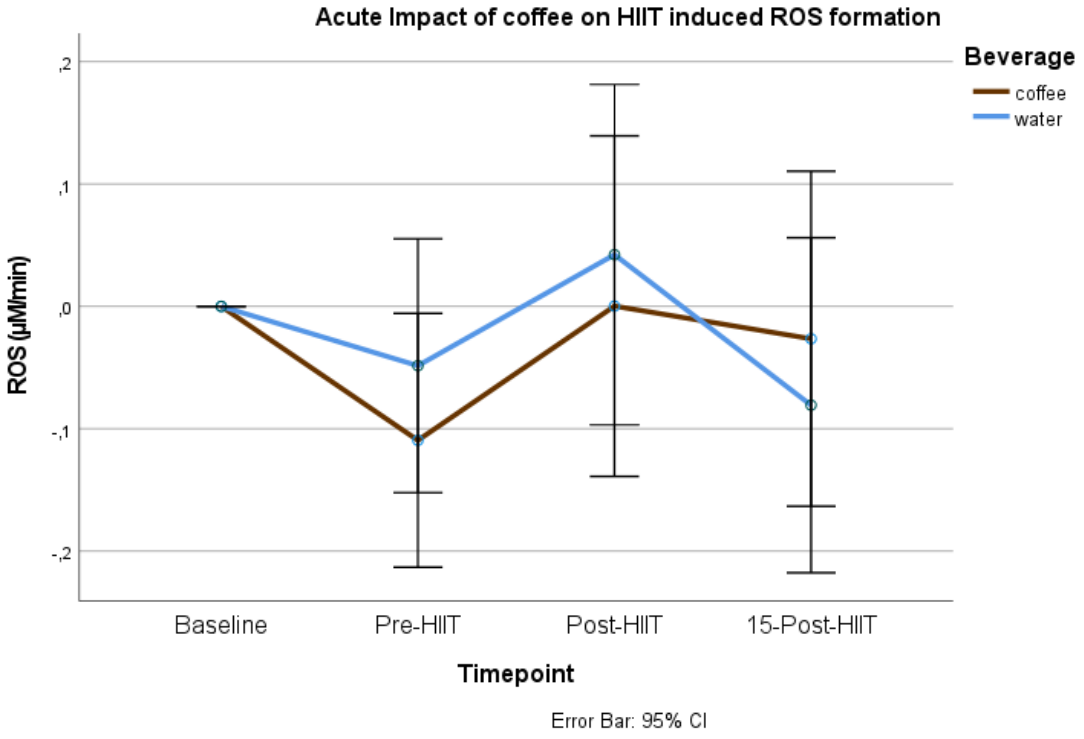
Variable	Time	Treatment [a]	Control [b]	RMANOVA (4 x 2)
ROS µM/min	Baseline	0.00 ± 0.00	0.00 ± 0.00	P > 0.05
	Pre-HIIT	-0.11 ± 0.23	-0.05 ± 0.23	
	Post-HIIT	0.00 ± 0.32	0.04 ± 029	
	15-post-HIIT	-0.03 ± 0.38	-0.08 ± 0.19	

[a] Treatment: Coffee (250 mL)

[b] Control: Water (250 mL)

RMANOVA (4 x 2): four(time) x two (beverages) repeated measures ANOVA.

Figure 4.: Line chart coffee vs. water



4.3 Total polyphenol content and DPPH antioxidant assay

As mentioned previously, this master´s thesis assessed the TPC and the TAA including the RSA% of four different beverages. To obtain information about their TAA, the TEAC index was applied. The results of the assays are summarized in Table 7.

Table 7.: Results of the FC-method and DPPH antioxidant assay

Beverage	TPC (µg GAE/mL)	TEAC (mmol/L)[1]	RSA%	Literature data
Coffee	1126.17 ± 0.03	1.80 ± 0.00	57.10	TPC: - **TEAC**: 5.6-7.0 mmol/L[a] **RSA%**: 56.16 - 82.5[b]
Matcha	1115.17 ± 0.03	1.59 ± 0.02	50.75	**TPC**: 1499.88 - 1736.31 µg GAE/mL [c] TEAC: - **RSA%**: 66.8 - 74.55 [d]
Pomegranate juice	3862.10 ± 0.02	7.00 ± 0.00	44.82	**TPC**: 2502 µg GAE/mL [e] **TEAC**: 24 – 46.9 mmol/L [f] **RSA%**: 34.3 – 75.4 [g]
Orange juice	898.20 ± 0.02	0.23 ± 0.04	8.21	**TPC**: 646.5-910.7 µg GAE/mL [h] **TEAC**: 3.04 mmol/L [i] **RSA%**: 46.7 j

Values are expressed as means ± SD. [1] Results are expressed as Trolox equivalent. [a] (Cruz et al., 2017), [b] (Hudáková et al., 2016), [c] (Jakubczyk et al., 2020), [d] (Trinovani et al., 2022), [e] (Anahita et al., 2015), [f] (Severcan et al., 2020), [g] (Mutahar et al. 2012), [h] (Lee et al., 2016), [i] (Wern et al., 2016), [j] (Klimczak et al., 2007)

5 Discussion

This intervention confirmed that acute strenuous exercise induces oxidative stress by increasing the endogenous ROS production, which is consistent with previous exercise interventions. However, in contrast to the hypothesis of this master´s thesis an acute coffee consumption prior to the training was not capable to attenuate the ROS formation.

The production of free radicals and oxidative stress induced due to intensive exercise has been a topic of discussion for a long time. There exist numerous exercise interventions, but the results are not always consistent. The latter can be explained by the application of different training protocols in each intervention. The duration, intensity and type of the exercise play a crucial role in inducing a physiological response to physical activity, and thus an appropriate training protocol is an important factor in physical activity interventions.

In this intervention a high intensity circuit resistance training was applied, which was tailored to the individual fitness level using the 5-RM test. Oxidative stress induced by exercise has been effectively achieved in other studies performing exercise on treadmills, cycle ergometers or other training programs with a VO_2max > 70% and different training durations. (Lu et al., 2021)

Furthermore, findings suggest that the highest elevation in the ROS production is demonstrated five minutes after a training program in healthy adults, which then recovers gradually within 24 hours. However, different markers of oxidative stress have different recovery rates. (Lu et al., 2021)

Studies have examined the effect of consuming coffee on exercise-induced oxidative stress using both single and multiple training session interventions. The present intervention applied a single bout of HIIT to effectively increase the production of ROS. This approach provided an understanding of the acute effect of coffee on exercise-induced oxidative stress. However, repeated training interventions offer an advantage by minimizing confounding factors including poor sleep or higher stress levels prior to the intervention and hence providing more reliable results. Ferreira et al. conducted an intervention to investigate the effects of acute (one session) and chronic (2 weeks) intensive training (Sprint interval training) on inflammatory markers with or without prior caffeine intake (capsule containing 5 mg/kg caffeine). The acute administration of caffeine one hour prior to the training appeared to mitigate the exercise-induced inflammatory response after a single training session. However, caffeine consumption for two weeks did not result in any advantageous effects on the inflammatory markers. Nonetheless, a reduction in inflammatory markers including the IL-6/IL-10 ratio, was shown after two weeks of SIT regardless of caffeine intake. This finding suggests that there are no beneficial long-term effects of consuming caffeine on the inflammatory profile. (Ferreira et al., 2018) These results are also in

correspondence with the findings from Lopez-Garcia et al. and Shaposhnikov et al., respectively. (Lopez-Garcia et al., 2006), (Shaposhnikov et al., 2018)

The relationship between coffee and exercise has been mainly studied due to the ergogenic effect of caffeine on exercise performance. The number of studies investigating the effect of coffee consumption on the exercise-induced oxidative stress is limited. Most of them used alternative oxidative parameters and methods for their evaluation, such as FRAP assay, TRAP (total radical-trapping antioxidant power) assay, the MDA concentration or the exercise-induced inflammatory response, rather than determining the formation rate of reactive oxygen species in capillary blood. The study from Mrakic-Sposta showed that capillary blood is more suitable to detect ROS due to a significantly higher ROS production level compared to venous blood. (Mrakic-Sposta et al., 2014)

The variation among studies investigating the effect of coffee on oxidative stress can be attributed to the different types of coffee administrated to the study population. The preparation method of the administrated coffee should be considered due to the different caffeine and phenolic content among different coffee brews. The latter is due to the coffee to water ratio, the cup volume, brewing temperature and pressure and the roasting degree. (Olechno et al., 2021)

One of the consequences of the exercise-induced production of free radicals is the delayed-onset muscle soreness (DOMS). The intake of dietary antioxidant supplements to reduce exercise-induced oxidative stress has been widely studied. The systematic review from Torre et al. showed weak evidence for acute or chronic supplementation of vitamin C or E to positively affect DOMS. However, a personalized diet has been acknowledged for its valuable contribution for physically active individuals. (Torre et al., 2021)
Interestingly, it has been reported that interventions with the administration of isolated antioxidants such as vitamin C or E may have adverse effects on the adaptive responses to exercise. However, interventions where subjects consumed antioxidant rich foods or beverages did not support this finding. (Moslemi et al., 2023)

In recent years the effect of polyphenol-rich products on exercise-induced oxidative stress has been a widespread topic of research. The timing of the consumption prior to the training is a critical factor to obtain reliable outcomes, as sufficient time is required for adequate absorption. In this intervention participants consumed the arabica filtered coffee two hours prior to the HIIT, corresponding to a previous study from Zeng et al. (Zeng et al., 2020)
However, considering that the peak concentration of caffeine is typically reached within 30-60 minutes after consumption, a shorter interval between consuming coffee and starting the exercise program could lead to different outcomes. Nonetheless, determining the ideal timing of coffee administration is challenging due to the interindividual variability in metabolism.

In the existing literature, the effect of consuming coffee on inflammatory markers in healthy adults remains inconsistent. According to the current state of knowledge, it appears that coffee does not show an appreciable impact on oxidative stress parameters in healthy adults. (Lopez-Garcia et al., 2006) Nonetheless, the consumption of coffee may offer health benefits to those with underlying disease risk factors. Several experimental and epidemiological studies have investigated the relationship between coffee consumption and T2DM. They observed that in general a habitual consumption of coffee can have protective effects to prevent the development of T2DM or slow down further progression. (Le et al., 2016), (She et al., 2020) Some studies demonstrated reduced circulating levels of pro-inflammatory cytokines including IL-8 and CRP in patients with type 2 diabetes mellitus. (Kempf et al., 2010), (Yamashita et al., 2012), (Shaposhnikov et al., 2018) Coffee contains several bioactive compounds, and the positive effects of consuming coffee cannot be attributed to any single component. Nevertheless, CGAs and caffeine are the primary constituents with antioxidative properties. The number of studies examining the effect of chlorogenic acids on oxidative stress is mainly conducted in animal experiments and in vitro models, while the number of human studies is limited. One of the few human trials that investigated the effect of consuming chlorogenic acid rich coffee is the study from Hoelzl et al. Their findings demonstrated that after five days of consuming four cups of instant coffee containing high levels of chlorogenic acids per day, 26 healthy male and female participants exhibited a decline in diverse markers of oxidative stress including, 8-Isoprostaglandin and reactive oxygen species. The

latter was investigated exposing intact lymphocyte cells to hydrogen peroxide (H_2O_2) and measuring the ROS formation rate after 60 minutes using flow cytometric analysis. (Hoelzl et al., 2010)

Some potential limitations need to be considered in our intervention. The participants were not requested to refrain from coffee in their diet. Thus, an investigation of an acute coffee consumption on the exercise-induced oxidative stress was limited in our study due to the inclusion of both habitual and non-habitual coffee drinkers in the study population. The intervention from Ferreira et al. created a list of all foods and beverages that contained caffeine, which all participants were asked to refrain from 24 hours before and 48 hours after the training session. (Ferreira et al., 2018) Also, following an antioxidant-rich diet during the intervention phase was not an exclusion criterium. In this research project, however, the diet of the previous day was recorded by a 24h-Recall. The analyses of the dietary protocols were considered for the overall conclusions of this study. The advantage of the 24h-Recall was a lower burden on the study participants, which may have a favorable influence on adherence. Ideally, all participants should be placed on an identical diet during the intervention period, which excludes any possible bias regarding nutrition-derived antioxidants. (Kawamura & Muraoka, 2018)

On the other hand, the underlying study protocol ensured that all participants had a similar initial fitness level, which allowed a fair comparison of the outcome. One additional strength of the present study was the homogenous study population, which were healthy females only. The majority of previous exercise interventions included male participants. However, findings suggest that there are no gender-related differences concerning exercise-induced oxidative stress. (Lu et al., 2021) Wiecek et al. reported that there were no differences in the alteration of antioxidant activity after performing a HIIT between male and female subjects. Nonetheless, a higher baseline antioxidant activity in males was reported. (Wiecek et al., 2018)

As previously mentioned, ROS are essential for the regular metabolism at physiological levels. Although there are no specific numeral values to define the physiological ROS levels in the organism, since we assessed the ROS levels of the female subjects in this intervention prior to engaging in any physical activity, the

baseline levels could be an indicative of low physiological levels. The baseline ROS in this intervention were 1.08 ± 0.29 µmol/min. In comparison, the study from Mrakic-Sposta et al. reported ROS levels of 2.01 ± 0.09 and 1.84 ± 0.09 in healthy young male and females with an active lifestyle and sedentary lifestyle, respectively. The mean age of the participants in their study was 19.7 ± 1.1 years, while in this study, it was 26.45 ± 4.06. (Mrakic-Sposta et al., 2014) Furthermore, Zeng and colleagues assessed the ROS levels in an exercise intervention similar to this study protocol. They showed baseline ROS levels of 0.70 ± 0.1 µmol/min in healthy young female adults with a mean age of 25.5 ± 5.1 years. (Zeng et al., 2020)
In regard to the concentration of ROS in the organism, Mrakic-Sposta et al. found a significant difference between the ROS levels in healthy adults and people with health issues. This finding is in correspondence with the existing literature, which suggests that oxidative stress is considered a basis of pathological conditions. They found that subjects suffering from sarcopenia, mild cognitive impairment and sporadic amyotrophic lateral sclerosis showed higher ROS levels of 2.24 ± 0.26, 2.18 ± 0.31 and, 2.49 ± 0.43 µmol/min, respectively. (Mrakic-Sposta et al., 2014)

Based on current understanding, physically active individuals show a better antioxidant defense system and an improved physiological response to exercise-induced stress. Furthermore, active individuals have a higher baseline TAA in the resting state. (Lu et al., 2021)

Based on the FC-method performed in this master´s thesis the TPC of arabica filtered coffee, which was consumed by all participants, was 1126.17 ± 0.03 µg GAE/mL. However, a direct comparison of the results for coffee and matcha with previous studies is unattainable since other studies considered the dry weight of coffee beans or tea leaves in their sample, respectively. (Cruz et al., 2017) Moreover, the preparation method in this study for coffee was drip-brewing, which often leads to an incomplete dissolution of the coffee. The latter is due to the retention of some coffee particles by the paper filter, which can result in polyphenol loss. Additionally, it can be difficult to obtain full dissolution of matcha powder when preparing matcha tea. These are additional contributing factors why a direct comparison of the results of coffee and matcha with previous studies was challenging.

The TAA and RSA% of four beverages administrated to all subjects in this intervention were evaluated, which are represented in Table 7. Generally, the higher the RSA% value, the better is the antioxidant activity of a sample. The observed variation among the results of the TAA in PJ and OJ between this and other studies can mainly be attributed to the use of commercially available juices in this intervention, whereas other studies used freshly pressed juices for their analysis. As commercially available juices commonly undergo heating treatments to obtain their long shelf life, it can be concluded that their polyphenol content is often lower than freshly pressed juices. The latter is due to the sensitivity of polyphenols to temperature and storage conditions. (Klimczak et al., 2007) Therefore, the juices used in this intervention possessed a lower antioxidant activity compared to freshly pressed juices, which were used in other studies. Based on the study from Kozik et al. the highest antioxidant activity was observed in fresh cold pressed juice. (Kozik et al., 2015)

Moreover, the vitamin C content in a juice can vary which contributes to different values of TPC and TAA. (Klimczak et al., 2007) Additionally, different origins and cultivars have a considerable impact on the polyphenol content. (Pagliarulo et al., 2016)

The variation of the TAA and RSA% of the filtered arabica coffee consumed in this intervention and other studies is due to the administration of different coffee types and various preparation methods for coffee among studies. (Cruz et al., 2017)

This study determined the TAA of coffee with medium roasting. In this regard a study showed that a higher level of roasting causes a decrease in antioxidant activity due to the reduced phenolic content in coffee. (Asy'ari Hasbullah & Rini Umiyati, 2021) Even though, the TPC of the arabica filtered coffee was determined, the caffeine content, which is an important bioactive constituent, remained unclear. Since various amounts of caffeine in coffee lead to different physiological responses, it would be ideal to quantify the caffeine content for optimal outcome interpretation.

6 Conclusion

Based on the evidence available in the literature, acute high intensity exercise induces oxidative stress. However, regular and long-term intensive training programs appear to reduce oxidative stress. Despite the progress that has been achieved in

this field, further developments in the methodology used to investigate redox signaling pathways are required to identify the specific pathways that contribute to the physiological response to exercise in the human body.

Although some studies have reported positive impacts of consuming coffee on exercise-induces oxidative stress, there are inconsistencies among different studies. Research suggests that coffee may offer potential health benefits and even reduce the risk of some chronic diseases. It is believed that coffee is a dietary antioxidant source with chlorogenic acids being the most abundant bioactive compound of coffee with antioxidative properties. The phenolic compounds found in coffee including CGAs could be responsible for reducing oxidative stress. Phenolic compounds have been associated with decreasing the production of pro-inflammatory cytokines and protecting against oxidative damage. However, the exact mechanism behind the effect of coffee on oxidative stress is not fully understood. Therefore, further well-controlled studies with an optimized study protocol, which include healthy, physically inactive and non-habitual coffee drinkers as their study population are necessary.

According to the current understanding, it can be concluded that engaging in regular physical activity, providing sufficient recovery time between the training sessions, following a balanced diet with essential nutrients including polyphenols can mitigate the harmful effects associated with exercise-induced oxidative stress. (Lu et al., 2021)

7 References

Agbor, G. A., Vinson, J. A., & Donnelly, P. E. (2014). Folin-Ciocalteau reagent for polyphenolic assay. *International Journal of Food Science, Nutrition and Dietetics (IJFS)*, *3*(8), 147-156.

Anahita, A., Asmah, R., & Fauziah, O. (2015). Evaluation of total phenolic content, total antioxidant activity, and antioxidant vitamin composition of pomegranate seed and juice. *International Food Research Journal*, *22*(3). https://doi.org/10.4172/2327-5146.1000164

Arulselvan, P., Fard, M. T., Tan, W. S., Gothai, S., Fakurazi, S., Norhaizan, M. E., & Kumar, S. S. (2016). Role of antioxidants and natural products in inflammation. *Oxidative medicine and cellular longevity*, *2016*. https://doi.org/10.1155/2016/5276130

Asy'ari Hasbullah, U. H., & Rini Umiyati, D. (2021). Antioxidant activity and total phenolic compounds of arabica and robusta coffee at different roasting levels. *Journal of Physics: Conference Series, 1764*. https://doi.org/10.1088/1742-6596/1764/1/012033

Atakan, M. M., Li, Y., Koşar, Ş. N., Turnagöl, H. H., & Yan, X. (2021). Evidence-based effects of high-intensity interval training on exercise capacity and health: A review with historical perspective. *International journal of environmental research and public health*, *18*(13), 7201. https://doi.org/10.3390/ijerph18137201

Barcelos, R. P., Lima, F. D., Carvalho, N. R., Bresciani, G., & Royes, L. F. (2020). Caffeine effects on systemic metabolism, oxidative-inflammatory pathways, and exercise performance. *Nutrition Research*, *80*, 1-17. https://doi.org/10.1016/j.nutres.2020.05.005

Bizheh, N., & Jaafari, M. (2011). The effect of a single bout circuit resistance exercise on homocysteine, hs-CRP and fibrinogen in sedentary middle aged men. *Iranian Journal of Basic Medical Sciences*, *14*(6), 568-573. https://doi.org/10.22038/IJBMS.2011.5057

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181, 1199-1200. https://doi.org/10.1038/1811199a0

Bowtell, J., & Kelly, V. (2019). Fruit-derived polyphenol supplementation for athlete recovery and performance. *Sports Medicine*, *49*(Suppl 1), 3-23. https://doi.org/10.1007/s40279-018-0998-x

Brzycki, M. (1998). *A practical approach to strength training*. McGraw-Hill.

Bull, F. C., Al-Ansari, S. S., Biddle, S., Borodulin, K., Buman, M. P., Cardon, G., ... & Willumsen, J. F. (2020). World Health Organization 2020 guidelines on physical activity and sedentary behaviour. *British journal of sports medicine*, *54*(24), 1451-1462. http://dx.doi.org/10.1136/bjsports-2020-102955

Canals-Garzón, C., Guisado-Barrilao, R., Martínez-García, D., Chirosa-Ríos, I. J., Jerez-Mayorga, D., & Guisado-Requena, I. M. (2022). Effect of antioxidant supplementation on markers of oxidative stress and muscle damage after strength exercise: A systematic review. *International Journal of Environmental Research and Public Health*, *19*(3), 1803. https://doi.org/10.3390/ijerph19031803

Ciaramelli, C., Palmioli, A., & Airoldi, C. (2019). Coffee variety, origin and extraction procedure: Implications for coffee beneficial effects on human health. *Food chemistry*, *278*, 47-55. https://doi.org/10.1016/j.foodchem.2018.11.063

Cruz, R. G. D., Vieira, T. M. F. D. S., & Lira, S. P. D. (2017). Potential antioxidant of brazilian coffee from the region of Cerrado. *Food Science and Technology*, *38*, 447-453. https://doi.org/10.1590/1678-457X.08017

Del Bo', C., Bernardi, S., Marino, M., Porrini, M., Tucci, M., Guglielmetti, S., ... & Riso, P. (2019). Systematic review on polyphenol intake and health outcomes: is there sufficient evidence to define a health-promoting polyphenol-rich dietary pattern?. *Nutrients*, *11*(6), 1355. https://doi.org/10.3390/nu11061355

de Souza, L. D. S., Horta, I. P. C., de Souza Rosa, L., Lima, L. G. B., da Rosa, J. S., Montenegro, J., ... & Teodoro, A. J. (2020). Effect of the roasting levels of Coffea arabica L. extracts on their potential antioxidant capacity and antiproliferative activity in human prostate cancer cells. *RSC advances*, *10*(50), 30115-30126. https://doi.org/10.1039/D0RA01179G

Dikalov, S. I., Polienko, Y. F., & Kirilyuk, I. (2018). Electron paramagnetic resonance measurements of reactive oxygen species by cyclic hydroxylamine spin probes. *Antioxidants & Redox Signaling*, *28*(15), 1433-1443. https://doi.org/10.1089/ars.2017.7396

Di Lorenzo, C., Colombo, F., Biella, S., Stockley, C., & Restani, P. (2021). Polyphenols and human health: The role of bioavailability. *Nutrients*, *13*(1), 273. https://doi.org/10.3390/nu13010273

Di Meo, S., Reed, T. T., Venditti, P., & Victor, V. M. (2016). Role of ROS and RNS sources in physiological and pathological conditions. *Oxidative medicine and cellular longevity*, *2016*. http://dx.doi.org/10.1155/2016/1245049

Du, Y., Lv, Y., Zha, W., Hong, X., & Luo, Q. (2020). Effect of coffee consumption on dyslipidemia: A meta-analysis of randomized controlled trials. *Nutrition, Metabolism and Cardiovascular Diseases*, *30*(12), 2159-2170. https://doi.org/10.1016/j.numecd.2020.08.017

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (2015). Scientific Opinion on the safety of caffeine. *EFSA Journal*, *13*(5), 4102. https://doi.org/10.2903/j.efsa.2015.4102

Ferreira, G. A., Felippe, L. C., Bertuzzi, R., Bishop, D. J., Barreto, E., De-Oliveira, F. R., & Lima-Silva, A. E. (2018). The effects of acute and chronic sprint-interval training on cytokine responses are independent of prior caffeine intake. *Frontiers in physiology*, *9*, 671. https://doi.org/10.3389/fphys.2018.00671

Forman, H. J., & Zhang, H. (2021). Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy. *Nature Reviews Drug Discovery*, *20*(9), 689-709. https://doi.org/10.1038/s41573-021-00233-1

Grgic, J., Lazinica, B., Schoenfeld, B. J., & Pedisic, Z. (2020). Test–retest reliability of the one-repetition maximum (1RM) strength assessment: a systematic review. *Sports Medicine-Open*, *6*(1), 1-16. https://doi.org/10.1186/s40798-020-00260-z

Grgic, J., Trexler, E. T., Lazinica, B., & Pedisic, Z. (2018). Effects of caffeine intake on muscle strength and power: a systematic review and meta-analysis. *Journal of the International Society of Sports Nutrition*, *15*(1), 11. https://doi.org/10.1186/s12970-018-0216-0

Guest, N. S., VanDusseldorp, T. A., Nelson, M. T., Grgic, J., Schoenfeld, B. J., Jenkins, N. D., ... & Campbell, B. I. (2021). International society of sports nutrition position stand: caffeine and exercise performance. *Journal of the International Society of Sports Nutrition*, *18*(1), 1. https://doi.org/10.1186/s12970-020-00383-4

Gulcin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. *Archives of toxicology*, *94*(3), 651-715. https://doi.org/10.1007/s00204-020-02689-3

He, L., He, T., Farrar, S., Ji, L., Liu, T., & Ma, X. (2017). Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. *Cellular Physiology and Biochemistry*, *44*(2), 532-553. https://doi.org/10.1159/000485089

Hoelzl, C., Knasmüller, S., Wagner, K. H., Elbling, L., Huber, W., Kager, N., ... & Cavin, C. (2010). Instant coffee with high chlorogenic acid levels protects humans against oxidative damage of macromolecules. *Molecular nutrition & food research*, *54*(12), 1722-1733. https://doi.org/10.1002/mnfr.201000048

Hogg, N. (2010). Detection of nitric oxide by electron paramagnetic resonance spectroscopy. *Free Radical Biology and Medicine*, *49*(2), 122-129. https://doi.org/10.1016/j.freeradbiomed.2010.03.009

Hudáková, J., Marcinčáková, D., & Legáth, J. (2016). Study of antioxidant effects of selected types of coffee. *Folia Veterinaria*, *60*(3), 34-38. https://doi.org/10.1515/fv-2016-0026

Hu, G. L., Wang, X., Zhang, L., & Qiu, M. H. (2019). The sources and mechanisms of bioactive ingredients in coffee. *Food & Function*, *10*(6), 3113-3126. https://doi.org/10.1039/C9FO00288J

International Coffee Organization. (2012). Trends in coffee consumption in selected importing countries. Retrieved from: https://www.ico.org/documents/icc-109-16e-decisions.pdf (Accessed 04. April.2023)

International Coffee Organization. (2014). Development Strategy for Coffee. Retrieved from: https://www.ico.org/documents/cy2013-14/icc-105-16-r1e-strategy.pdf (Accessed 04. April.2023)

International Coffee Organization. (2021). Statistics - Disappearance (consumption) in selected importing countries. Retrieved from: https://www.ico.org/historical/1990%20onwards/PDF/4b-disappearance.pdf (Accessed 04. April.2023)

Jakubczyk, K., Kochman, J., Kwiatkowska, A., Kałduńska, J., Dec, K., Kawczuga, D., & Janda, K. (2020). Antioxidant properties and nutritional composition of matcha green tea. *Foods*, *9*(4), 483. https://doi.org/10.3390/foods9040483

Jung, S., Kim, M. H., Park, J. H., Jeong, Y., & Ko, K. S. (2017). Cellular antioxidant and anti-inflammatory effects of coffee extracts with different roasting levels. *Journal of Medicinal Food*, *20*(6), 626-635. https://doi.org/10.1089/jmf.2017.3935

Kanauchi, M., Shibata, M., & Iwamura, M. (2019). A novel dietary inflammatory index reflecting for inflammatory ageing. *Annals of Medicine and Surgery, 47,* 44-46. https://doi.org/10.1016/j.amsu.2019.09.012

Kashi, D. S., Shabir, A., Da Boit, M., Bailey, S. J., & Higgins, M. F. (2019). The efficacy of administering fruit-derived polyphenols to improve health biomarkers, exercise performance and related physiological responses. *Nutrients*, *11*(10), 2389. https://doi.org/10.3390/nu11102389

Kawamura, T., & Muraoka, I. (2018). Exercise-induced oxidative stress and the effects of antioxidant intake from a physiological viewpoint. *Antioxidants*, *7*(9), 119. https://doi.org/10.3390/antiox7090119

Klimczak, I., Małecka, M., Szlachta, M., & Gliszczyńska-Świgło, A. (2007). Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. *Journal of food composition and analysis*, *20*(3-4), 313-322. https://doi.org/10.1016/j.jfca.2006.02.012

Kozik, V., Jarzembek, K., Jędrzejowska, A., Bąk, A., Polak, J., Bartoszek, M., & Pytlakowska, K. (2015). Investigation of antioxidant activity of pomegranate juices by means of electron paramagnetic resonance and UV-Vis spectroscopy. *Journal of AOAC International*, *98*(4), 866-870. https://doi.org/10.5740/jaoacint.SGE4-Kozik

Lee, J. H., Oh, M. K., Lim, J. T., Kim, H. G., & Lee, W. J. (2016). Effect of coffee consumption on the progression of type 2 diabetes mellitus among prediabetic individuals. *Korean journal of family medicine*, *37*(1), 7. https://doi.org/10.4082/kjfm.2016.37.1.7

Lopez-Garcia, E., Van Dam, R. M., Qi, L., & Hu, F. B. (2006). Coffee consumption and markers of inflammation and endothelial dysfunction in healthy and diabetic women. *The American journal of clinical nutrition*, *84*(4), 888-893. https://doi.org/10.1093/ajcn/84.4.888

LIczbiński, P., & Bukowska, B. (2022). Tea and coffee polyphenols and their biological properties based on the latest in vitro investigations. *Industrial Crops and Products*, *175*, 114265. https://doi.org/10.1016/j.indcrop.2021.114265

Lu, Y., Wiltshire, H. D., Baker, J. S., & Wang, Q. (2021). Effects of High Intensity Exercise on Oxidative Stress and Antioxidant Status in Untrained Humans: A Systematic Review. *Biology*, *10*(12), 1272. https://doi.org/10.3390/biology10121272

Magherini, F., Fiaschi, T., Marzocchini, R., Mannelli, M., Gamberi, T., Modesti, P. A., & Modesti, A. (2019). Oxidative stress in exercise training: The involvement of inflammation and peripheral signals. *Free radical research*, *53*(11-12), 1155-1165. https://doi.org/10.1080/10715762.2019.1697438

Martini, D., Del Bo', C., Tassotti, M., Riso, P., Del Rio, D., Brighenti, F., & Porrini, M. (2016). Coffee consumption and oxidative stress: a review of human intervention studies. *Molecules*, *21*(8), 979. https://doi.org/10.3390/molecules21080979

Metro, D., Cernaro, V., Santoro, D., Papa, M., Buemi, M., Benvenga, S., & Manasseri, L. (2017). Beneficial effects of oral pure caffeine on oxidative stress. *Journal of Clinical & Translational Endocrinology*, *10*, 22-27. https://doi.org/10.1016/j.jcte.2017.10.001

Mishra, K., Ojha, H., & Chaudhury, N. K. (2012). Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. *Food chemistry*, *130*(4), 1036-1043. https://doi.org/10.1016/j.foodchem.2011.07.127

Moslemi, E., Dehghan, P., Khani, M., Sarbakhsh, P., & Sarmadi, B. (2023). The effects of date seed (Phoenix dactylifera) supplementation on exercise-induced oxidative stress and aerobic and anaerobic performance following high-intensity interval training sessions: a randomised, double-blind, placebo-controlled trial. *British Journal of Nutrition*, *129*(7), 1151-1162. https://doi.org/10.1017/S0007114522002124

Moura-Nunes, N., Perrone, D., Farah, A., & Donangelo, C. M. (2009). The increase in human plasma antioxidant capacity after acute coffee intake is not associated with endogenous non-enzymatic antioxidant components. *International journal of food sciences and nutrition*, *60*(sup6), 173-181. https://doi.org/10.1080/09637480903158893

Mrakic-Sposta, S., Gussoni, M., Montorsi, M., Porcelli, S., & Vezzoli, A. (2014). A quantitative method to monitor reactive oxygen species production by electron paramagnetic resonance in physiological and pathological conditions. *Oxidative Medicine and Cellular Longevity*, *2014*. https://doi.org/10.1155/2014/306179

Mutahar S, S., Mutlag M, A. O., & Najeeb S, A. Z. (2012). Antioxidant activity of pomegranate (Punica granatum L.) fruit peels. Food and nutrition sciences, 2012. https://doi.org/10.4236/fns.2012.37131

Ngamsuk, S., Huang, T. C., & Hsu, J. L. (2019). Determination of phenolic compounds, procyanidins, and antioxidant activity in processed Coffea arabica L. leaves. *Foods*, *8*(9), 389. https://doi.org/10.3390/foods8090389

Olechno, E., Puścion-Jakubik, A., Zujko, M. E., & Socha, K. (2021). Influence of various factors on caffeine content in coffee brews. Foods, 10(6), 1208. https://doi.org/10.3390/foods10061208

Ozougwu, J. C. (2016). The role of reactive oxygen species and antioxidants in oxidative stress. *International Journal of Research*, *1*(8).

Paiva, C. L. R. S., Beserra, B. T. S., Reis, C. E. G., Dorea, J. G., Da Costa, T. H. M., & Amato, A. A. (2019). Consumption of coffee or caffeine and serum concentration of inflammatory markers: A systematic review. *Critical reviews in food science and nutrition*, *59*(4), 652-663. https://doi.org/10.1080/10408398.2017.1386159

Piercy, K. L., Troiano, R. P., Ballard, R. M., Carlson, S. A., Fulton, J. E., Galuska, D. A., ... & Olson, R. D. (2018). The physical activity guidelines for Americans. *Jama*, *320*(19), 2020-2028. https://doi.org/10.1001/jama.2018.14854

Pisoschi, A. M., Pop, A., Iordache, F., Stanca, L., Predoi, G., & Serban, A. I. (2021). Oxidative stress mitigation by antioxidants-an overview on their chemistry and influences on health status. *European Journal of Medicinal Chemistry*, *209*, 112891. https://doi.org/10.1016/j.ejmech.2020.112891

Ren, Y., Wang, C., Xu, J., & Wang, S. (2019). Cafestol and kahweol: a review on their bioactivities and pharmacological properties. *International journal of molecular sciences*, *20*(17), 4238. https://doi.org/10.3390/ijms20174238

Reyes, C. M., & Cornelis, M. C. (2018). Caffeine in the diet: country-level consumption and guidelines. *Nutrients*, *10*(11), 1772. https://doi.org/10.3390/nu10111772

Richardson, A. J., Relf, R. L., Saunders, A., & Gibson, O. R. (2016). Similar inflammatory responses following sprint interval training performed in hypoxia and normoxia. *Frontiers in Physiology*, *7*, 332. https://doi.org/10.3389/fphys.2016.00332

Ros, E., Martínez-González, M. A., Estruch, R., Salas-Salvadó, J., Fitó, M., Martínez, J. A., & Corella, D. (2014). Mediterranean diet and cardiovascular health: Teachings of the PREDIMED study. *Advances in nutrition*, *5*(3), 330S-336S. https://doi.org/10.3945/an.113.005389

Sánchez-Rangel, J. C., Benavides, J., Heredia, J. B., Cisneros-Zevallos, L., & Jacobo-Velázquez, D. A. (2013). The Folin–Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination. *Analytical Methods*, *5*(21), 5990-5999. https://doi.org/10.1039/C3AY41125G

Severcan, S. S., Uzal, N., & Kahraman, K. (2020). Clarification of pomegranate juice using PSF microfiltration membranes fabricated with nano TiO2 and Al2O3. *Journal of Food Processing and Preservation*, *44*(8), e14559. https://doi.org/10.1111/jfpp.14559

Shaposhnikov, S., Hatzold, T., Yamani, N. E., Stavro, P. M., Lorenzo, Y., Dusinska, M., ... & Collins, A. (2018). Coffee and oxidative stress: a human intervention study. *European journal of nutrition*, *57*, 533-544. https://doi.org/10.1007/s00394-016-1336-4

Shen, J. G., Brooks, M. B., Cincotta, J., & Manjourides, J. D. (2019). Establishing a relationship between the effect of caffeine and duration of endurance athletic time

trial events: A systematic review and meta-analysis. *Journal of science and medicine in sport*, *22*(2), 232-238. https://doi.org/10.1016/j.jsams.2018.07.022

Sies, H., & Jones, D. P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nature reviews Molecular cell biology*, *21*(7), 363-383. https://doi.org/10.1038/s41580-020-0230-3

Simioni, C., Zauli, G., Martelli, A. M., Vitale, M., Sacchetti, G., Gonelli, A., & Neri, L. M. (2018). Oxidative stress: role of physical exercise and antioxidant nutraceuticals in adulthood and aging. *Oncotarget*, *9*(24), 17181. https://doi.org/10.18632/oncotarget.24729

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, *16*(3), 144-158.

Smirmaul, B. P., de Moraes, A. C., Angius, L., & Marcora, S. M. (2017). Effects of caffeine on neuromuscular fatigue and performance during high-intensity cycling exercise in moderate hypoxia. *European Journal of Applied Physiology*, *117*, 27-38. https://doi.org/10.1007/s00421-016-3496-6

Southward, K., Rutherfurd-Markwick, K. J., & Ali, A. (2018). The effect of acute caffeine ingestion on endurance performance: a systematic review and meta–analysis. *Sports Medicine*, *48*, 1913-1928. https://doi.org/10.1007/s40279-018-0939-8

Tabung, F. K., Smith-Warner, S. A., Chavarro, J. E., Wu, K., Fuchs, C. S., Hu, F. B., ... & Giovannucci, E. L. (2016). Development and validation of an empirical dietary inflammatory index. *The Journal of nutrition*, *146*(8), 1560-1570. https://doi.org/10.3945/jn.115.228718

Tajik, N., Tajik, M., Mack, I., & Enck, P. (2017). The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: a comprehensive review of the literature. *European journal of nutrition*, *56*, 2215-2244.

https://doi.org/10.1007/s00394-017-1379-1

Thelle, D. S., Arnesen, E., & Førde, O. H. (1983). The Tromsö heart study: does coffee raise serum cholesterol?. *New England Journal of Medicine*, *308*(24), 1454-1457. https://doi.org/10.1056/NEJM198306163082405

Torre, M. F., Martinez-Ferran, M., Vallecillo, N., Jiménez, S. L., Romero-Morales, C., & Pareja-Galeano, H. (2021). Supplementation with vitamins C and E and exercise-induced delayed-onset muscle soreness: A systematic review. *Antioxidants*, *10*(2), 279. https://doi.org/10.3390/antiox10020279

Trexler, E. T., Smith-Ryan, A. E., Melvin, M. N., Roelofs, E. J., & Wingfield, H. L. (2014). Effects of pomegranate extract on blood flow and running time to exhaustion. *Applied physiology, nutrition, and metabolism*, *39*(9), 1038-1042. https://doi.org/10.1139/apnm-2014-0137

Trinovani, E., Prawira-Atmaja, M. I., Kusmiyati, M., Harianto, S., & Maulana, H. (2022). Total polyphenols and antioxidant activities of green tea powder from GMB 7 and GMB 9 tea clones. *IOP Conference Series: Earth and Environmental Science*, *974*, 012113. https://doi.org/10.1088/1755-1315/974/1/012113

Villaño, D., Fernández-Pachón, M. S., Moyá, M. L., Troncoso, A. M., & García-Parrilla, M. C. (2007). Radical scavenging ability of polyphenolic compounds towards DPPH free radical. *Talanta*, *71*(1), 230-235. https://doi.org/10.1016/j.talanta.2006.03.050

Wang, F., Wang, X., Liu, Y., & Zhang, Z. (2021). Effects of exercise-induced ROS on the pathophysiological functions of skeletal muscle. *Oxidative Medicine and Cellular Longevity*, *2021*, 1-5. https://doi.org/10.1155/2021/3846122

Wern, K. H., Haron, H., & Keng, C. B. (2016). Comparison of total phenolic contents (TPC) and antioxidant activities of fresh fruit juices, commercial 100% fruit juices and fruit drinks. *Sains Malaysiana*, *45*(9), 1319-1327.

Wiecek, M., Szymura, J., Maciejczyk, M., Kantorowicz, M., & Szygula, Z. (2018). Anaerobic exercise-induced activation of antioxidant enzymes in the blood of women and men. *Frontiers in physiology*, *9*, 1006. https://doi.org/10.3389/fphys.2018.01006

Wood dos Santos, T., Cristina Pereira, Q., Teixeira, L., Gambero, A., A. Villena, J., & Lima Ribeiro, M. (2018). Effects of polyphenols on thermogenesis and mitochondrial biogenesis. *International journal of molecular sciences*, *19*(9), 2757. https://doi.org/10.3390/ijms19092757

Xie, J., & Schaich, K. M. (2014). Re-evaluation of the 2, 2-diphenyl-1-picrylhydrazyl free radical (DPPH) assay for antioxidant activity. *Journal of agricultural and food chemistry*, *62*(19), 4251-4260. https://doi.org/10.1021/jf500180u

Yamashita, K., Yatsuya, H., Muramatsu, T., Toyoshima, H., Murohara, T., & Tamakoshi, K. (2012). Association of coffee consumption with serum adiponectin, leptin, inflammation and metabolic markers in Japanese workers: a cross-sectional study. *Nutrition & diabetes*, *2*(4), e33. https://doi.org/10.1038/nutd.2012.6

Zeng, Z., Jendricke, P., Centner, C., Storck, H., Gollhofer, A., & König, D. (2020). Acute Effects of Oatmeal on Exercise-Induced Reactive Oxygen Species Production Following High-Intensity Interval Training in Women: A Randomized Controlled Trial. *Antioxidants*, *10*(1), 3. https://dx.doi.org/10.3390/antiox10010003