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Titel | Title

A comparison of the acute effects of pomegranate juice, glucose-water solution and water on exercise induced oxidative stress in women following HIIT

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Abstract

Introduction: Excessive oxidative stress is harmful to the human body and can be caused by many things, one of them is vigorous physical activity. Recently, pomegranate juice has been in the focus of research for its antioxidative properties, which mitigate oxidative stress. This has also been proposed with carbohydrate rich food, like oatmeal, but not with pure glucose solved in water. This could be important as pomegranate juice does also contain sugar in form of glucose and might be benefitting from this combined effect.

Objective: The aim of this master´s thesis was to investigate the acute effects of pomegranate juice (PGJ) and a glucose-water solution (GWS) on exercise-induced oxidative stress following HIIT, as well as the differences between these two beverages.

Methods: Twenty healthy, physically inactive, female subjects, aged 18-40 years, were included in this randomized intervention. They consumed over a series of trials either 250 mL of pomegranate juice (treatment group 1), 250mL of a glucose-water solution which contained 20g of glucose (treatment group 2) or water (control group) two hours prior to engaging in circuit based high intensity resistance training for 20 minutes. Four capillary blood samples on each intervention day, were taken from the participants (fasting, prior, immediately after, 15 minutes after training), and then used to assess the reactive oxygen species (ROS) formation rate with an EPR spectrometer. The total polyphenol content (TPC) and the total antioxidant activity (TAA) of the beverages were assessed by using the Folin-Ciocalteu method and the DPPH antioxidant assay.

Results: The prescribed HIIT training regime was effective in elevating ROS levels in the participants of all groups and thus creating oxidative stress. However, there was no significant difference in effects of the treatment groups (PGJ, GWS) compared to the control.

Conclusion: Based on these result, the effectiveness of HIIT to increase ROS has been demonstrated. Nonetheless, the treatment groups (PGJ, GWS) did not show any significant mitigating effects. This could be due to the TPC and glucose content of the beverages. Therefore, further researched is advised to gain better insight in its application.

Kurzfassung

Einleitung: Zu hoher oxidativer Stress ist schädlich für den menschlichen Körper und kann unter anderem durch höchst intensive physische Aktivität hervorgerufen werden. Granatapfelsaft ist im aktuellen Fokus der Wissenschaft da er Antioxidantien enthält, welche die Auswirkungen des oxidativen Stresses reduzieren können. Dies gilt auch für kohlenhydratreiches Essen, wie Haferflocken. Dieser Effekt wurden jedoch noch nicht für in Wasser gelöste Glukose, ausreichend untersucht. Das könnte von großer Bedeutung sein, da Granatapfelsaft ebenfalls Glukose enthält die dessen Wirkung verstärken könnte.

Ziel: Das Ziel dieser Masterarbeit war einerseits die Analyse der akuten Effekte von Granatapfelsaft (PGJ) und einer Glukose-Wasser Lösung (GWS) auf den durch Bewegung hervorgerufenen oxidativen Stress und andererseits der Vergleich der beiden Getränke untereinander.

Methoden: 20 gesunde, unsportliche, weiblich Teilnehmerinnen im Alter von 18-40 Jahren wurden in dieser randomisierten Interventionsstudie untersucht. Sie konsumierten je 250ml Granatapfelsaft (Untersuchungsgruppe 1), 250ml Glukose-Wasser Lösung mit 20g Glukose (Untersuchungsgruppe 2) und Wasser (Kontrollgruppe) an verschiedenen Trainingstagen, 2 Stunden vor der Durchführung eines Kraftzirkeltrainings, welcher 20 Minuten dauerte. Zu 4 verschiedenen Zeitpunkten wurde den Teilnehmerinnen Blut entnommen (gefastet, vor dem Training, direkt nach dem Training, 15 Minuten nach dem Training), anhand dessen die ROS Bildungsrate mit einem EPR Spektrometer bestimmt wurde. Von den Getränken wurde der Polyphenolgehalt (TPC) und die antioxidative Aktivität (TAA) mittels der Folin-Ciocalteu Methode und des DPPH Assays bestimmt.

Ergebnisse: Das durchgeführte Training führte zu einer signifikanten Steigerung der ROS Bildungsrate in allen Gruppen. Es wurden jedoch keine signifikanten Unterschiede zwischen den untersuchten Getränken (PGJ, GWS) und der Kontrollgruppe festgestellt.

Zusammenfassung: Basierend auf den Resultaten der Studie konnte die Fähigkeit von HIIT in der Steigerung der ROS Bildungsrate gezeigt werden. Jedoch, bewirkten die Interventionsgruppen keinen signifikanten Effekt (PGJ, GWS). Dies könnte durch den TPC und Glukosegehalt der konsumierten Getränke bedingt sein. Daher wird geraten die Anwendung der Substanzen weiterhin zu untersuchen und ihre Wirkungsweisen zu verstehen.

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1 Introduction

The effects that sport, exercise and general energy demands have upon the human body have been thoroughly studied but not exhaustively (Haff & Triplett, 2016). The scope of investigation of these studies range from physical outputs, like strength, speed, or body mass to smallest signaling cells in our body, like m-Tor, pgc1-alpha, acetyl-monophosphate-kinase or the smallest of all, H+ ions (Haff & Triplett, 2016). In this potpourri of scientific disciplines different goals of understanding certain aspects paved the way for greater understanding of the body. While sports scientists seek the merits of a healthy body for various reasons, like performance, health or leisure, which are solely outcome related, it is often overlooked which processes lie underneath. A healthy looking body can be unhealthy if one only judges by appearance. For one to truly state about the healthiness of a person, one would have to look not only at the general visible level but also at the cellular and psychological level. This thesis will focus on the cellular aspects of the body and how exercise and nutrition can impact it. The first thing to know is that the human body seeks homeostasis. The online library of the Encyclopedia Britannica defines homeostasis as "any self-regulating process by which biological systems tend to maintain stability while adjusting to conditions that are optimal for survival. If homeostasis is successful, life continues; if unsuccessful, disaster or death ensues" (Britannica, 2024). Every act of living may destabilize this balance. Even be it something as simple as standing up from a chair or eating an apple. The body has to act according to a stimulus and counterbalance it to return to its natural state. This is on pair with an increase in energy expenditure, which is therefore, on a deep level, associated with death. As a result, the body tries to prepare itself, so that if that incident should occur again it will be better suited to handle it with less energy expenditure. This is called an adaptation process (Peck & Waxman, 2018). One of the most known adaptation processes of the human body today is muscle hypertrophy. Hereby, whether on purpose or by accident, one damages muscle cells by exposing the body to demands which it can barely handle (concentric training) or are even too much to handle (eccentric training). The body then rebuilds the muscle in a stronger bigger format (more contractile elements) so that it is able to cope better with the same stimulus at the next opportunity (Haff & Triplett, 2016). But how does the body actually know how to react? What is it, that elicit these adaptations? It is a cascade of reactions bound to the release of the damaged cells. Depending on which part of the body gets damaged, muscles, bones, or if an energy supply shortage arises the body reacts with molecular signaling. One of those messaging signals that gets

released is oxidative stress, which is caused by an excessive concentration of reactive oxygen species (ROS). The understanding and management of those species will enable, among other things, athletes and common people to better attain their goals of training.

Further, has been shown that nutrition can influence the body's response to free radicals, whether they may be sport induced or otherwise. Tabung et al. (2016) and Kanauchi et al. (2019) demonstrated, based on the Empirical Dietary Inflammatory Index (EDII) that fruit juices can pose anti-inflammatory properties. Furthermore, many studies did investigate different fruit juices and other potential anti-inflammatory beverages, in order to strengthen that claim (Ortega et al., 2021; Wang et al. 2020).

This master thesis is part of a bigger research project which studies the acute impact of different beverages on exercise induced oxidative stress and inflammation. Based on the EDII, among other beverages, the here researched substances were pomegranate juice (PGJ), a glucose-water solution (GWS) and water. Biomarkers for oxidative stress that were tracked included 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 the plasma. It is important to note that the scope of this study only includes the analysis of the level of ROS formation rate in the capillary blood in dependence of the researched substances.

This study is based on a pilot research project conducted by Zeng et al. (2020) where they researched the acute effects of oatmeal consumption on ROS in women following a high intensity interval training (HIIT) session. Their findings indicate a decrease in ROS levels in the capillary blood if oatmeal is consumed before the workout (Zeng et al., 2020). These findings could indicate a ROS protective effect of complex carbohydrates in a solid state.

Additionally, from the literature can also be derived that PGJ could have protective effects against ROS (Ammar et al., 2017; Ammar et al., 2018; Dujaili, Good & Tsang, 2016; Fuster-Muñoz et al., 2016; Machin et al., 2014; Ortega et al., 2020; Wang et al., 2020).

Based on these assumptions, the aim of this master thesis was to investigate the acute effect of pomegranate juice (PGJ) and a glucose-water solution (GWS) on exercise-induced oxidative stress as well as the differences of the effect between these two beverages.

The first hypothesis was, that pomegranate juice and glucose may mitigate the increment of ROS levels after HIIT. The second hypothesis was that pomegranate juice is a more potent suppressor of ROS than glucose.

1.1 Oxidative Stress

Oxidative stress is an interesting phenomenon as it is the base for the oxygen paradox. On the one hand, for the human body, or more general for all aerobic organism that inhabits earth, oxygen is necessary for survival. On the other hand, too much oxygen is toxic and can result in death (Dasgupta & Klein, 2014). As a byproduct of cellular respiration and other biochemical processes, free radicals are being generated in the body. A free radical is defined as "an atom or molecule containing one or more unpaired electrons that is capable of free existence" (Dasgupta & Klein, 2014; de Almeida et al., 2022). The main problem with free radical is, that they do not cause one reaction and then are neutralized, but they are starting a chain reaction. By either donating their electron or taking away from another molecule, they are creating another radical. This process only stops once two radicals interact with each other or antioxidants stabilize the radical (Juan et al., 2021). These radicals can be classified as reactive oxygen species (ROS) or reactive nitrogen species (RNS). Generally speaking, one could say that ROS are primary responsible for oxidative stress. An overview of free radicals is provided in table 1 in accordance to Dasgupta & Klein (2014). These reactions can be homolytic, heterolytic or redox. If the origin of this process is due to bodily functions, these radicals are being called endogenous free radicals. A second source of free radicals can be the environment, for example air pollution, smoking or radiation (Dasgupta & Klein, 2014). If that is the case they are being called exogenous free radicals. An overview of exogenous and endogenous sources of free radicals is given in table 1.

Table 1: List of free radicals, oxidants and possible sources (from Dasgupta & Klein, 2014)

Free Radicals	Oxidants	Endogenous sources	Exogenous sources
Superoxide anion radical	Singlet oxygen	Mitochondrial respiration	Air pollution
Hydroxyl radical	Ozone	Enzymatic reaction	Inorganic particles in air
Hydroperoxyl radical	Hydrogen peroxide	Respiratory burst	Tobacco smoking
Peroxyl radical	Hypochlorite	Strenuous exercise	Some medications
Lipid radical	Nitrous acid	Ischemia/reperfusion	Industrial solvents
Lipid alkoxyl radical	Peroxynitrous acid	Infection	Exposure to radiation
Nitric oxide	Nitrous oxide	Metal ions	
Lipid peroxyl radical		Autoxidation	
Nitrosyl cation			
Thiyl radical			
Protein radical			

The most potent free radical stressors seem to be superoxide and hydrogen peroxide (Dasgupta & Klein, 2014; de Almeida et al., 2022). Superoxide is produced mainly by two sources. One is in the mitochondria as a byproduct of the mitochondrial electronic transport chain (ETC), where leaking electrons bind to oxygen. Second production site would be by cells of the immune system such as macrophages (Dasgupta & Klein, 2014; de Almeida et al., 2022). As they are part of the body's immune defense system they pose a relatively longer half-life than other radicals and are less aggressive. It is important to know, that superoxide is membrane impermeable, thus it cannot leave the cell where it was produced in, but they are able to diffuse within the cell (Dasgupta & Klein, 2014). It can be assumed that if produced on purpose by the immune system, it is necessary in regaining homeostasis in that specific cell it was produced in, and therefore it should stay as long as needed. While contrary to that, if it is produced by accident, through the ETC, it becomes a hindrance that disrupts homeostasis. Hydrogen peroxide on the other hand is membrane permeable, thus it can pass the membrane of a cell. It is also a rather stable substance. To be more specific hydrogen peroxide itself, is a very weak radical. It is a strong oxidant but has very slow reaction kinetics. Its cytotoxicity is given by its ability to produce hydroxyl radicals which can then attack DNA, lipids and proteins (Andrés et al., 2022; Dasgupta & Klein, 2014). It originates either as a non-enzymatic byproduct of the ETC or as an enzymatic byproduct of superoxide and the enzyme superoxide dismutase (SOD 1-3) (Andrés et al., 2022).

1.1.1 Cell damage, cell signaling, cell defense through oxidative stress

Oxidative stress caused by free radicals or oxidants can damage basically every cell in the body, including DNA, lipids and proteins. But destruction is not all they do, as they are vital in upkeeping the balance of homeostasis as was briefly hinted at before in the example of macrophages using them as protection against hostile microbes in the body. Hence, a thorough understanding of its effects may be advantageous if one tries to influence them.

1.1.1.1 Cell damage

ROS can damage nuclear DNA, RNA and mitochondrial DNA. All these attack sites are linked to mutagenesis (mutations), carcinogenesis (cancer) and aging. Mitochondrial DNA is the most effected, as the mitochondria produces ROS during respiration (ETC). Nuclear DNA oxidation can lead to modifications of the nucleotide base (mutagenesis), as single and double strand breaks occur. RNA can be affected but has not been as thoroughly studied as the effects on DNA. At all these sites the manner of attack is similar. A radical, most often hydroxyl radical (HO*), interacts with guanine. The positions C4, C5, C8 of the imidazole ring are being targeted. This results in a mismatch due to the pairing with adenosine and substitutes G with T and C with A in the genome and therefore producing 8-hydroxyguanine (Cheng et al., 1992; Dasgupta & Klein, 2014). Accumulation of 8-hydroxyguanine is linked to the afore mentioned negative effects: mutation, cancer, aging (Cheng et al., 1992).

Furthermore, ROS can also damage lipids. In this light, the structural lipids are of more importance than the energy storage ones. Lipid damage occurs via lipid peroxidation. This is a reaction where cell membranes are being altered through changes of their permeability and fluidity (Dasgupta & Klein, 2014; Juan et al., 2021). The most reactive and hence most aggressive radical for this process is the hydroxyl radical. Other radicals that can be triggers of lipid peroxidation are alkoxyl radicals, peroxyl radicals and peroxynitrite. The initial attack site in lipids are the bis-allylic hydrogens. “Allylic” refers to the carbon molecule to which the hydrogen molecule is attached. That carbon is attached to a carbon, which has a carbon double bond with another carbon molecule that is not the allylic one. “Bis” means that the allylic carbon has two bonds with double bonded carbons (Dasgupta & Klein, 2014; Wagner et al., 1994).

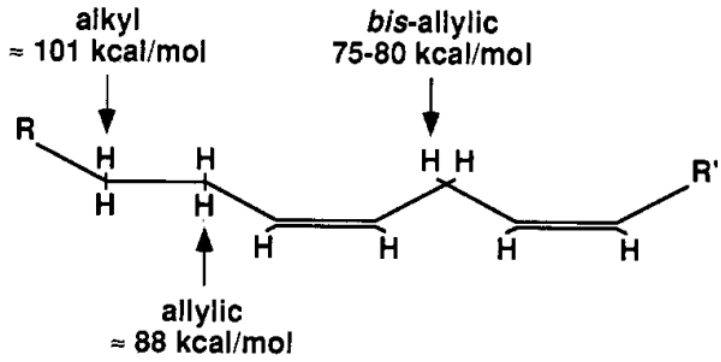

Figure 1: Illustration of allylic and bis-allylic hydrogen positions in unsaturated fatty acids (Wagner et al., 1994)

Consequently, all polyunsaturated fatty acids (PUFAs) can react with free radicals in the manner of lipid peroxidation. The problem with lipid peroxidation is, that it results in harmful byproducts such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE) which lead to cell damage, cell death and are linked to diseases like Alzheimer (Juan et al., 2021). However, not all PUFAs are equally active in this process. The more bis-allylic positions there are, the more susceptible the lipid is for lipid peroxidation (Wagner et al., 1994). The rate at which this lipid peroxidation is taking place varies in the following manner: docosahexaenoic acid (DHA) > eicosapentaenoic acid (EPA) > arachidonic acid > linoleic acid (Dasgupta & Klein, 2014). The first PUFAs to undergo peroxidation are the omega 3 fatty acids, which also have been shown to have anti-inflammatory properties. As they are highly reactive with ROS they can also be seen as antioxidants. However, they lose their antioxidant and anti-inflammatory capabilities fast once they react with the radicals. It is advised to store them without light and oxygen if they are used as supplements. It has to be noted that too much intake of omega 3 might be detrimental to the body, for it again disturbs the oxidative homeostasis (de Almeida et al., 2022).

Since ROS are capable to attack all structural elements within the body, proteins are not excluded as part of their attack sight. Proteins and amino acids are very easily attacked by hydroxyl radicals. To be more precise, methionine and sulfur containing cysteine are very likely to be oxidized by ROS and RNS. This results in a paralysis of the regulation of metabolic pathways (Dasgupta & Klein, 2014; Juan et al., 2021).

1.1.1.2 Cell signaling & cell defense

However, not all radicals are necessarily a bad omen. As shown in table 1 there are different reasons for endogenous radicals. Physiological functions of ROS are to act as messenger substances or immune defense. As cells prefer to operate under redox balance (Dasgupta & Klein, 2014; Schieber & Chandel, 2014), they have to upkeep a certain ROS level. Through small oxidative bursts they can keep their balance to react if they get in contact with other signaling molecules like cytokines, growth factors, various interleukins, tumor necrosis factor α, angiotensin II, platelet-derived growth factors, nerve growth factors, transforming growth factor β1, granulocyte macrophage colony-stimulating factor, and fibroblast growth factor (Dasgupta & Klein, 2014). In small dosages ROS forces the cells to adapt. Redox signaling is therefore involved in cellular differentiation, tissue regeneration and the prevention of aging (Schieber & Chandel, 2014). If the cell is not able to withstand these dosages due to various reasons, for example the inability to regenerate, the cell will die. Hence, ROS regulate autophagy and are responsible for recycling damaged protein, and organelles (Dasgupta & Klein, 2014; de Almeida et al., 2022; Schieber & Chandel, 2014). The molecular switch that is used for that is reactive cysteine from protein breakdown. Correspondingly, this is also where physical activity comes into the picture. Increased activity of the ETC, and cysteine residuals from protein breakdown increase the pro-oxidant part of the redox balance. If the cells are able to cope with the stress, they remain, if not the undergo apoptosis. At the same time the cells that can cope with the stressor have better capabilities to upregulate antioxidant enzymes like catalase, glutathionperoxidase, superoxiddismutase and hence the body as a whole will be better adapted to cope with the stressor as all cells, which were weak in this regard, will have been replaced (Dasgupta & Klein, 2014; de Almeida et al., 2022; Peck & Waxman, 2018).

Although, it has to be considered that permanent disturbances in the redox balance, not only acute oxidative stress, is also a signal for various diseases like tumorigenesis (Schieber & Chandel, 2014). Furthermore, it has to be noted, that there is still a debate about whether autophagy is upregulated to increase cell energetics or to protect from too much ROS from a malfunctioning cell (Dasgupta & Klein, 2014; de Almeida et al., 2022).

Another way in which ROS can act in a positive manner is cellular defense. As ROS attacks the chemical structure of a cell it can also be used against viruses and bacteria. These microorganisms are so small, that if only a little of them is damaged, they can die. Phagocytes as well as enzymes like NADPH oxidases (NOXs), xanthine oxidase, nitric

oxide synthase, and peroxisomal constituents produce oxidative stress, often superoxide, to destroy those pathogens (Dasgupta & Klein, 2014; de Almeida et al., 2022; Juan et al., 2021).

To conclude, the role of ROS in the human cannot be easily defined as good or bad. While, being contributors of basically all known hallmarks of aging including but not limited to telomere attrition, deregulated autophagy, cell senescence (Dasgupta & Klein, 2014; de Almeida et al., 2022), due to their potential in damaging all cells, they are vital in protecting them as well. Although, it would also be a misconception to think, that all ROS has to be downregulated at the same time to protect from damage. The general balance must be upheld for proper cell mechanics. De Almeida et al. (2022) postulates that this could also be one of the main problems with ROS. Since ROS force cells to adapt, they become more resistant to the stimulus of ROS. Therefore, more ROS have to be produced to uphold the redox balance.

1.1.2 History of Free Radicals and Oxidative Stress

Oxidative stress is caused by excessive levels of free radicals (Powers et al., 2016) also known as reactive oxygen species (ROS). The existence of free radicals was first proven to exist in a solution in 1930 by Haber and Weiss. This chemical reaction is now known as the Haber-Weiss-reaction, where hydrogen peroxide interacts with superoxide (Powers et al., 2016). At first the interest in free radicals was mainly fueled because of its role in rubber production. Further interest arose with its possible negative effects on health through radiation poisoning and hyperoxia by damaging cells. As the research continued in the 1950s and 1960s, it was proven that ROS did in fact exists within the human body and are produced by the mitochondria (Dasgupta & Klein, 2014; de Almeida et al., 2022; Powers et al., 2016). In the year 1956 Harman published his theory “free radical theory of ageing”, which proposed that cellular ageing was directly related to the amount of ROS inflicted damage to the cell (de Almeida et al., 2022; Powers et al., 2016). While this theory remained difficult to prove, McCord and Fridovich discovered the enzyme superoxide dismutase (SOD), which was the first convincing prove, that ROS plays an important role in cell biology. Starting from the year 1978 it was shown, that oxidative stress increased through muscular work and caused lipid peroxidation. This reaction was especially high when vitamin E was deficient or suppressed. Quintanilha and Packer’s study in the 1983 showed first that through endurance training, the muscles adapted and increased the level of vitamin E and hence protecting its membrane from radical damage (Powers et al., 2016). As it was now accepted

to be a two component system the definition given for oxidative stress by Sies and Jones in the year 2007 (Powers et al., 2016) is: “an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of the redox signaling and control and/or molecular damage”. In the 1990s it was shown, that oxidative stress was in part responsible for muscular fatigue and maximal force could only be exerted in an optimal redox state. Hence, deviating from this optimal point towards oxidative or antioxidative would decrease muscle force resulting an inverted U-shape for force production (Powers et al., 2016). It was also shown that the rate radicals were produced was proportional to the percentage of maximal force used. Numerous studies proved a dose-response relationship between exercise and the levels of antioxidant enzymes (Powers et al., 2016). In the year 1999 SOD 2 was discovered which is specifically protective for the cardiac muscle to resist ischemia reperfusion injury. Again this adaptation was promoted through endurance training. As the years went by ROS was not only seen as an damaging substance but also as an singling agent for cellular homeostasis. Powers et al. (2016) discuss, that as a signaling agent ROS are critical to promote efficient adaptation to exercise. Powers et al. (2016) refer to the study of Ristow et al. from the year 2009 where it was shown, that a high supplementation with vitamin C (1000mg/day) and vitamin E (400IU/day) prevents the production of proliferator-activated receptor γ coactivator 1α (PGC-1α) which promotes mitochondrial biogenesis. Although not fully proven there is a high probability that ROS might play an important role in epigenetic events.

The precise location of ROS production remains somewhat unclear (Powers et al., 2016) and can at least be described as coming from multiple sources (see table 1) (Dasgupta & Klein, 2014; de Almeida et al., 2022). Earlier it was supposed, that the mitochondria would be its primary production source for endogenous ROS, but this is being questioned as the mitochondria produces more ROS in its resting state than in respirations, its adenosine di-phospate (ADP) stimulated state (Powers et al., 2016). This hints to a more sophisticated upkeep of the redox balance than just by the escape of electrons from the ETC.

1.1.3 Methods of Measurements

Oxidative stress can be measured directly and indirectly. Direct measurement can give insight into the specific production of radicals as well as the sight of its origin. But as most ROS molecules have a very short existence (hydrogen peroxide seconds to minutes vs

hydroxyl less than a nanosecond) it becomes very difficult to assess them with high precision and accuracy (Katerji et al., 2019). Therefore, indirect measurements are mostly being used in clinical practice. They are easier to assess, also give insight about the amount of ROS but cannot always pinpoint the origin or single source of the ROS (Katerji et al., 2019).

1.1.3.1 Direct measurements of ROS

One way of directly measuring ROS are fluorogenic probes. Fluorogenic probes can be used to detect the amount of radicals in a solution (Katerji et al., 2019). Hydrogen peroxide, hydroxyl radicals, peroxyl radicals can be measured with 5-(and -6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (DCFDA) (Katerji et al., 2019). DCFDA diffuses into the cell, gets hydrolyzed and then reacts with the radical. In the case of hydrogen peroxide it becomes 2′,7′-dichlorofluorescein (DCF) which is a fluorescent. The resulting intensity of the fluorescent probe can then be measured with flow cytometry or a fluorescence plate reader which in turn will give information about the ROS at cellular level (Katerji et al., 2019). The brighter the fluorescence intensity the more radicals are being produced by the cell. To measure the level of superoxide, one would use dihydroethidium (DHE) which results in a red fluorescence, which emissions are proportional to the amount of superoxide molecules. (Katerji et al., 2019).

Another way of detecting radicals is the electron spin resonance (ESR) or electron paramagnetic resonance (EPR). In this procedure unpaired electrons can be detected. These electrons are paramagnetic, thus this can be measured in a magnetic field. This procedure can also detect poorly reactive radicals by trapping them with certain molecules to form a better measurable species (Rani et al., 2014). These trapping agents can be used in & ex vivo. Ex vivo cannot be used in live humans as the toxicity is too high. Examples therefore would be aromatic traps like salicylate and phenylalanine (Rani et al., 2014). The most common in vivo trap include ascorbic acid (Vitamin C) (Rani et al., 2014).

1.1.3.2 Indirect measurements of ROS

The indirect measures of ROS are also known as fingerprinting. Here the measurement of the site of damage is the primary indicator of the sample. They are yet of little use in human application but can be used in estimating the oxidative harm of nutritional supplements.

One of the most commonly used assessments for lipid damage are thiobarbituric acid-reactive substances, or short TBARS (Dasgupta & Klein, 2014; Rani et al., 2014; Wagner et al., 1994). The first order end products of lipid peroxidation are malondialdehyde (MDA), acrolein and 4-hydroxy-2-nonenal (HNE). But they are not the final product of the lipid peroxidation. Secondary end products are isoprostanes and mono- and serial cyclic peroxides (Dasgupta & Klein, 2014; Rani et al., 2014). Another good marker would be F2-Isoprostane, which derives from arachidonic acid and F4-Isoprostane from eicosapentaenoic and docosahexaenoic acids respectively.

If one wanted to measure DNA damage by ROS, 8-hydroxyguanine is a good indicator (Rani et al., 2014). This will be measured by gas-or liqid chromatography. Here hydroxyl radicals (HO*) are the primary source of ROS that attacks DNA strands and surrounding cellular and mitochondrial DNA (Cheng et al., 1992; Dasgupta & Klein, 2014; Rani et al., 2014).

If ones scope is more focused upon proteins, this can also be separately measured. The result of protein oxidation is protein carbonyl (PC) and derivatives of it (Dasgupta & Klein, 2014). These form from glycation of proteins by sugars, binding of aldehydes, direct oxidation of amino-acid side chains. This will be measured through ELISA (Rani et al., 2014).

1.2 Antioxidants

To counterbalance the oxidative stress the body relies on antioxidants. This is necessary to uphold the redox balance for optimal body functions such as cell signaling (Katerji et al., 2019). Antioxidants work in two ways, either enzymatic or nonenzymic and can be endogenous or diet derived. Furthermore, they can be separated by their inherent way of neutralizing or combating the chain reaction of ROS (see table 2) (Dasgupta & Klein, 2014; de Almeida et al., 2022; Katerji et al., 2019). Dasgupta & Klein (2014) also present a graphic about the effectiveness of some antioxidants (see figure 2). Here it becomes obvious that the neutralizing antioxidants are more effective than the chain breaking ones and that exogenous antioxidants mostly act as chain breakers not in neutralizing way.

Table 1: Excerpt of various antioxidants (Dasgupta & Klein, 2014; de Almeida et al., 2022; Katerji et al., 2019)

Antioxidant	Non-/Enzymatic	Origin	Category
Superoxide dismutase (SOD)	enzymatic	endogenous	neutralizing
Catalase (CAT)	enzymatic	endogenous	neuralizing
Glutathione peroxidase (GPx)	enzymatic	endogenous	neuralizing
Thioredoxin (Trx)	enzymatic	endogenous	neuralizing
Glutathione reductase	enzymatic	endogenous	chain breaking
Vitamin E	non-enzymatic	exogenous	chain breaking
Vitamin C	non-enzymatic	exogenous	chain breaking
ß-carotene	non-enzymatic	exogenous	chain breaking
Uric acid	non-enzymatic	endogenous	chain breaking
Flavonoide	non-enzymatic	exogenous	chain breaking
Bilirubin	non-enzymatic	endogenous	chain breaking

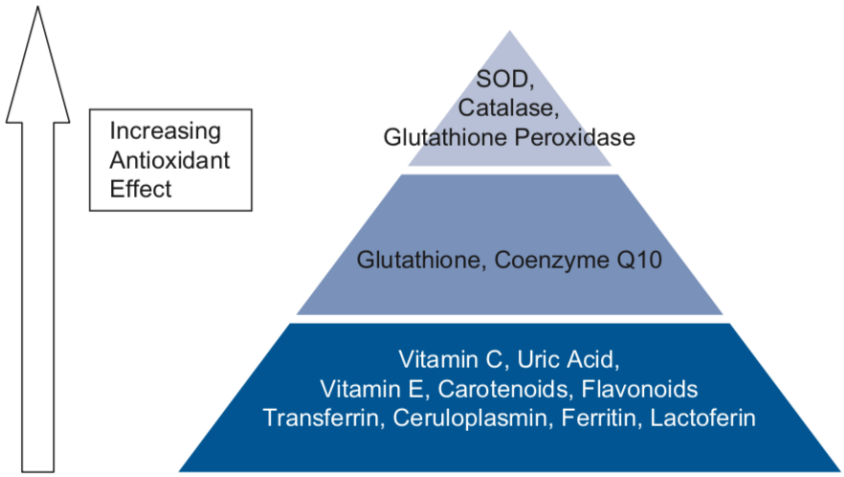

Figure 2: Effectiveness of various antioxidants in the human body (Dasgupta & Klein, 2014, S.12).

The most important antioxidant would be superoxide dismutase (SOD). It turns the highly reactive superoxide anion into hydrogen peroxide and thereby neutralizing it. Hydrogen peroxide is still a ROS but a rather stable one compared to superoxide. SOD is an enzymatic antioxidant and comes in three different forms, that vary in their locations. SOD 1 exist in the mitochondria as well as in the cytosol. SOD 2 is primarily found in the mitochondria and SOD 3 is primarily found outside of the cell. SOD 1 is a dimer (two subunits) and hence

smaller as SOD2 and SOD 3 which are tetramers (four subunits) (Dasgupta & Klein, 2014; de Almeida et al., 2022)

Another enzymatic antioxidant, that works well together with SOD is catalase. It takes hydrogen peroxide, turns it into water and oxygen and thereby neutralizing the radical completely. The same is achieved by glutathione peroxidase (GPx). It can react with hydrogen peroxide as well as noncomplex lipid hydroperoxides, and turns them into water and oxygen. GPx has eight different subgroups GPx 1-8 that vary in their active site in the body (plasma, gut, liver), comes in different isoforms and is also enzymatic. These isoforms can be cytosolic, mitochondrial and sperm nuclear (Dasgupta & Klein, 2014; de Almeida et al., 2022; Katerji et al., 2019). A weaker form of defense against hydrogen peroxide are peroxiredoxins. As they are easier to be deactivated, their purpose it to be more like a sensor of ROS (Dasgupta & Klein, 2014).

All antioxidants until now where neutralizing ones. Up next the chain breaking ones will be discussed. They do not target the ROS directly by changing it, but stop the chain reaction by donation or absorbing an electron and thereby making the ROS stable species (Dasgupta & Klein, 2014). The main distinction between chain breaking antioxidants is whether they are water or lipid soluble. The main water-soluble antioxidants are vitamin C, uric acid and glutathione. The main lipid-soluble ones are vitamin E, carotenoids and polyphenols (Dasgupta & Klein, 2014; de Almeida et al., 2022; Katerji et al., 2019).

The most importance water soluble vitamin is vitamin C. Ascorbic acid protects from superoxide, hydroxyl, hydrogen peroxide and single oxygen (Dasgupta & Klein, 2014). Hence, it helps to prevent damage from lipid peroxidation. Furthermore, it poses the ability to regenerate vitamin E and other antioxidants (de Almeida et al., 2022) and is therefore the most important water-soluble non-enzymatic chain breaking antioxidant (Dasgupta & Klein, 2014, de Almeida et al., 2022). Uric acid is found in the blood and protects from the Fenton reaction as it is reactive with iron (Dasgupta & Klein, 2014). Additionally, it also plays a second role in the redox balance. Should uric acid level get too high, it becomes harmful to the human body. This would results in increased salt sensitivity and lipogenesis. (de Almeida et al., 2022). Glutathione exists in a reduced (GSH) and an oxidized (GSSH) state and the ratio between them (GSH/GSSH) is used as a viable marker for oxidative stress. GPx turns GSH into GSSH. GSSH reacts with H_2O_2 by giving it an electron and thereby converting it to H_20 and O_2. It is also a molecule that is involved in redox signaling (de Almeida et al., 2022).

The most important fat-soluble nonenzymic chain breaking antioxidant is vitamin E (Dasgupta & Klein, 2014; de Almeida et al., 2022). Vitamin E occurs in eight different states in the body, 4 tocopherols and 4 tocotrienols (Dasgupta & Klein, 2014; de Almeida et al., 2022). The most common form is alpha-tocopherol. It reacts with peroxyl radicals and provides a hydrogen from its phenolic group to the radical to stop the oxidation of polyunsaturated fatty acids (Dasgupta & Klein, 2014; de Almeida et al., 2022). Carotenoids, with its major representative ß-carotene, is also a fat-soluble nonenzymic antioxidant. It can protect from peroxyl radicals and single oxygen (Dasgupta & Klein, 2014; de Almeida et al., 2022).

1.2.1 Polyphenols

Polyphenols have become a recognized substance, that also combat ROS (Dasgupta & Klein, 2014; Pandey & Rizvi, 2009; Pietta, 2000). Polyphenol is an umbrella term for various compounds, which can be separated by their chemical ring structure (see figure 3) (Pandey & Rizvi, 2009). These include phenols, phenolic acid, flavonoids, tannins, stilbenes and lignans (Dasgupta & Klein, 2014; Pandey & Rizvi, 2009; Pietta, 2000). They are largely found in vegetables, fruits and beverages like tea, nuts or dark chocolate (Dasgupta & Klein, 2014; Pandey & Rizvi, 2009; Pietta, 2000). Polyphenols are built by plants to protect them against ultraviolet radiations or other pathogens (Pandey & Rizvi, 2009).

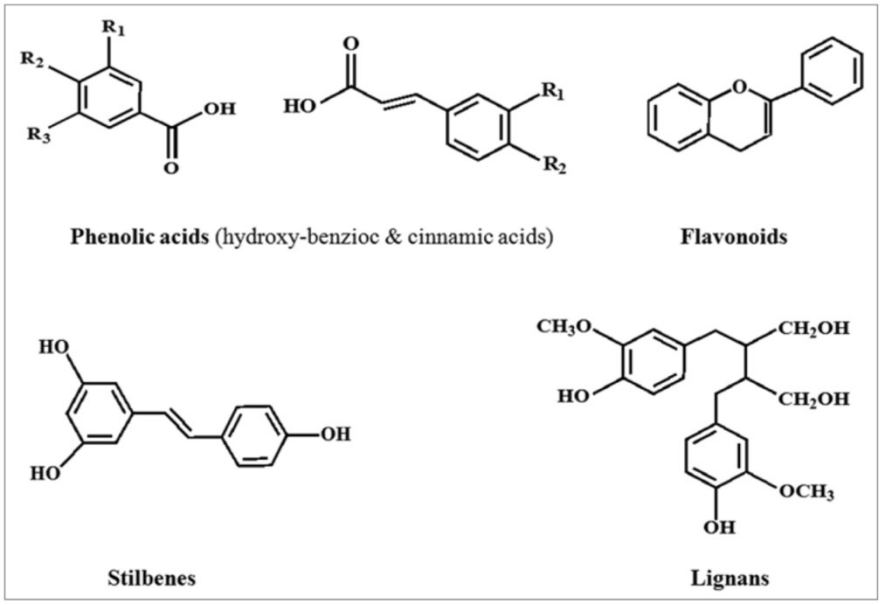

Figure 3: Chemical structure of various polyphenols (Pandey & Rizvi, 2009)

As they protect plants form radiation and other pathogens, by consuming them the human body can also be protected by them from various diseases (Dasgupta & Klein, 2014; Pandey & Rizvi, 2009). Phenols are linked to cardiac protection as they inhibit LDL oxidation, improve endothelial function, increase HDL, act antioxidative and show anti-platelet properties. Studies also support their role as antidiabetic and anti-aging agent (Dasgupta & Klein, 2014; Pandey & Rizvi, 2009). Furthermore, since polyphenols are able to pass through the blood brain barrier there is also a proposed neuroprotective effect (Dasgupta & Klein, 2014; Pandey & Rizvi, 2009).

Important is not only the polyphenol content of a fresh plant, after storage and cooking, but also its bioavailability. In contrast to storage and cooking, which is quite well studied and easy to understand, the bioavailability is very complex. For example, wheat loses 70% of its phenolic acid content during storage, this can be improved by cold storage. As for cooking, older studies promote, that vegetables and fruits should be best consumed raw like onions and tomatoes, for they lose up to 80% after boiling, 65% after microwaving and 30% after frying (Pandey & Rizvi, 2009). Dasgupta & Klein (2014) refer to newer studies proposing that this loss depends on the vegetable. In certain foods, like sweet potatoes, carrots and sweet peppers, cooking or other methods increase the antioxidant content of the food by softening of the food matrix. Therefore, unlike storage cooking cannot by uniformly condemned as bad, but rather has to be judge for each food individually. Another way of affecting polyphenol content in nutrition, especially fruits, is to blend them into juice. Here it is necessary to differentiate between 100% fresh fruit juices and commercial fruit juices. Mahdavi et al. (2011) showed that the polyphenol content of fresh fruit juices is in general significantly higher than in commercial fruit juices. They also suggest that dark juices have a higher polyphenol content than lighter ones.

The bioavailability on the other hand is rather complex and varies between polyphenols. Most polyphenols in foods are in the form of esters, polymers and glycosides (Pandey & Rizvi, 2009), which cannot be absorbed by the small intestine, only aglycones can. This is because neither the liver, intestinal mucosa nor plasma contains esterase. Hence, they need to be hydrolyzed by either enzymes or macrobacteria in the colon and become highly modified. Therefore, it is difficult to differentiate which phenol arrives in what quantity in the system, even if you measure its quantity in the consumed food (Pandey & Rizvi, 2009). As phenolic acid and flavonoids are the most consumed in the human diet (Pandey & Rizvi, 2009) these will be described in more detail.

1.2.1.1 Phenolic acid

Phenolic acids are very common in foods and make up about one third of all polyphenols (Pandey & Rizvi, 2009). They can be classified into two main categories. The hydroxybenzoic, which is mainly found in red and dark fruits and vegetables and is otherwise present in low contents. The other type is hydroxycinnamic, which is highly present in all other food, especially in fruits and vegetables (Oluwole et al., 2022; Pandey & Rizvi, 2009). Phenolic acid is a strong antioxidant against ROS and RNS, by upregulating endogenous antioxidative enzymes, especially superoxide dismutase, and scavenging free radicals itself. For this the phenolic acid uses its hydroxyl group of the aromatic ring (Oluwole et al., 2022). Furthermore, it has protective properties for the gastric tract, diabetes, the cardiac system, neuro-degradation, and inflammation (Oluwole et al., 2022).

1.2.1.2 Flavonoids

Flavonoids are very well studied. In plants, they are often the cause for the colors of flower fruits and leaves (Oluwole et al., 2022; Pandey & Rizvi, 2009). They are water soluble with two benzene rings (C6) and a third C3 ring (Pietta, 2000). They can be separated into six subclasses: flavonols, flavones, flavonones, flavanols, anthocyanins and isoflavones (Oluwole et al., 2022; Pandey & Rizvi, 2009). The differences are made by the number and position of their hydroxyl groups at the C3 ring (Pandey & Rizvi, 2009; Pietta, 2000). For anthocyanins, which have a wide variety of health effect in humans, red fruits are a rich source (Oluwole et al., 2022). They show antiviral, antibacterial, anti-inflammatory, anti-cancerogenic effects, protect the cardiac system, protect the liver, and inhibit lipid peroxidation in animal studies (Oluwole et al., 2022). Other example sources of flavonoids are herbs, seed, spices, nuts, tees, grain. Flavonoids in general cover metals in a chelate like manner and inhibit ROS enhancing enzymes, like NADH-oxidase, lipoxygenase or glutathione S-transferase. Thus prevent the generation of free radicals, especially hydrogen peroxides, as well as facilitating enzymes such as superoxide dismutase (Oluwole et al., 2022). Furthermore, their highly reactive hydroxyl group has radical scavenging properties, which helps to stabilize free radicals itself (Oluwole et al., 2022).

For phenolic acid and flavonoids the gut seems to be their first line of defense against ROS. The most part of ingested flavonoids gets broken down by the microflora which uses them for hydrolysis, cleavage of the heterocyclic oxygen-containing ring, dihydroxylation and decarboxylation (Pietta, 2000). As a byproduct of this usage by the microflora various

phenolic acids are being produced with the antioxidant abilities of its precursors. These new phenolic acid then can be absorbed with the rest of the intact flavonoids they can exert their antioxidant and other abilities in the body (Pietta, 2000). For the intake of flavonoids can be quite high (800mg) through natural sources and their characteristic is to be highly reactive with ROS, they help the body to spare its use of alpha-tocopherol (Vitamnin E) and ß-carotene (Dasgupta & Klein, 2014; Pietta, 2000).

1.2.2 Methods of Measurements of Antioxidants and Polyphenols

In general, the antioxidative capacity of something can be measured in the laboratory by two methods, the total capacity of the compound itself or by the total antioxidative capacity in the blood. The therefore used methods can be divided into further assays. The two most common would be the hydrogen atom transfer (HAT) based assay and the single electron transfer (SET) based assay (Dasgupta & Klein, 2014).

In HAT based assays the compound donates hydrogen atoms to the free radical and thereby neutralizing or reducing the harmfulness of its peroxyl radicals. These reactions are fast, pH independent and solvent independent. The magnitude of the antioxidant capacity is measured via the reduction of the, mostly fluorescence, signal. If the antioxidant can trap the free radicals the signal stays strong. Otherwise the thermal hydrolysis of the peroxyl radicals would interfere with the fluorogenic ability. Hence, the greater the reduction of the signal, the smaller the antioxidative capacity of the compound and vice versa (Dasgupta & Klein, 2014).

SET based assays work by transferring an electron to a compound and thereby reducing its reactivity. This includes free radicals, carbonyls and metal ions. These assays are pH dependent as for in general the ionization potential decreases with an increase in pH. The antioxidant capacity is measured by the change of the color of the fluorescence. This happened due to the reduction of the oxidant by the electron (Dasgupta & Klein, 2014).

For the effects of both methods are based on fluorescence effects they use spectrophotometrically for measurement

A brief overview of examples for HAT and SET assays is given in table 3, which will also be described in the following pages.

Table 2: List of various HAT & SET assays (Dasgupta & Klein, 2014; Katerji et al., 2019; Villaño et al., 2007)

HAT assays	SET assays
Oxygen radical absorbance capacity (ORAC)	2,2-Azino-bis 3-ethylbenzthiazoline-6-sulfonic acid radical scavenging assay (ABTS+)
Total oxyradical scavenging capacity (TOSC)	Cupric reducing antioxidant capacity (CUPRAC)
Total radical trapping antioxidant parameter (TRAP)	Folin-Ciocalteu Reagent (FCR)
2,2-diphenyl-1-picrylhydrazyl (DPPH)	2,2-diphenyl-1-picrylhydrazyl (DPPH)

1.2.2.1 HAT Assays

ORAC (Oxygen radical absorbance capacity) assay is commonly used for investigating chain-breaking antioxidants which are highly present in food. As stated before the loss of fluorescence should be minimal if the food is rich in high quality antioxidants. The result is expressed as Trolox equivalent antioxidant capacity (TEAC, compare table 4) (mmols of Trolox/100g).

TOSC (Total oxyradical scavenging capacity) works similar to the ORAC assay. Furthermore, it can measure both fat- and water-soluble antioxidants, and is able to separate fast and slow acting antioxidants. The result is expressed as TEAC (see table 4).

TRAP (Total radical trapping antioxidant parameter) is used for measuring the antioxidant capacity of the plasma. It is defined as the number of moles of peroxyl radicals that can be trapped per liter of fluid and it is measured by the amount of time that oxygen uptake is inhibited in the plasma due to the peroxyl radicals (Dasgupta & Klein, 2014). This assays is able to estimate the antioxidant capacity of a compound towards peroxyl radicals, hydroxyl radicals and peroxynitrite. The result is again expressed as TEAC (see table 4). The downside of TRAP is, that it is a complex measurement, which affords long periods of time (Dasgupta & Klein, 2014; Katerji et al., 2019).

1.2.2.2 SET Assays

ABTS+ (2,2-Azino-bis 3-ethylbenzthiazoline-6-sulfonic acid radical scavenging assay) is reduced from dark blue to colorless by the tested antioxidant. It is measured as the reactive relative to 1.0 mmol/L Trolox standard and expressed as TEAC (see table 4). This method

can be easily automated and was originally used to measure the antioxidant capacity of human plasma (Dasgupta & Klein, 2014; Katerji et al., 2019).

CUPRAC (Cupric reducing antioxidant capacity) tests an antioxidant against cupric ion. Here it is reduced to cuprous iron with emits a blue color, which its intensity gives insight of the antioxidant capacity. This assay can measure polyphenols and flavonoids in food and the capacity of human serum. If an ammonium acetate buffer is used it can also measure serum capacity due to ascorbic acid, glutathione and alpha-tocopherol (Dasgupta & Klein, 2014).

FCR (Folin-Ciocalteu reducing capacity) is based on the transfer of an electron of a phenolic substance to the Folin-Ciocalteu agent in an alkaline medium. As the phenolic compound get oxidized the light yellow color changes to blue. Important to know is that this reagent is not soluble in water. The antioxidant capacity is expressed as gallic acid equivalent (µg GAE/mL) (see table 4) (Dasgupta & Klein, 2014).

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a nitrogen based artificial radical. It is rather stable and eluminates a dark purple color. Compared to the upper assays it is special since it is both a HAT- and a SET assay, depending on the environment. In an apolar solvent the assay is primarily HAT based but in a polar environment, like alcohol it is SET based (Villaño et al., 2007). As it is an easy method it is very common to asses total antioxidant activity via the TEAC)or the radical scavenging ability (RSA%) (see table 4) (Villaño et al., 2007).

Table 3: Unit description of antioxidant assays

Units	Explanation
TEAC	Trolox equvialent antioxidant capacity. Trolox is a water soluble derivative of Vitamin E and acts as an antioxidant. Trolox is used as a reference of of the absorbance level of the tested substance (mmol TE/g)
GAE	Gallic acid equivalent. Gallic acid is a stable phenol and is used a a reference for antioxidant ability (µg GAE/mL)
RSA %	Radical scavanging ability % is a calculation: RSA% = (Abs_Blank – Abs_Samp) /Abs_Blank * 100

1.2.3 Pomegranate Juice (PGJ)

PGJ shows one of the highest antioxidant capacities of all beverages (Vučić et al., 2019). This high capacity stems from its abundance of bioactive compounds. Among those phytochemicals are hydrolysable tannins, anthocyanins, polyphenols, flavonoids, lignans, several organic acids, fatty acids, alkaloids, triterpenoids and phytosterols (Vučić et al., 2019). Thus, I can act as an antioxidant by neutralizing and scavenging free radicals as well as metal chelating. Through these antioxidant abilities PGJ is also linked to many potential health benefits like, reducing inflammation by reducing transcription mediators such as tumor necrosis factor alpha, nitric oxide synthases, interleukin 6 and interleukin1-beta. Therefore, it is believed in helping with rheumatoid arthritis and inflammatory bowl disease. It is also researched in reducing the risk of metabolic diseases and various cancer types, but mostly in animal studies (Vučić et al., 2019; Wang et al., 2020). These mediating effects on radicals and various mediators of PGJ have not only been researched for general purposes alone but also in a sport and exercise setting. The systematic review of Ammar et al. (2018) analyzed its effects on physical performance, fatigue and soreness, muscle damage, inflammatory and oxidative stress responses and indicates, that PGJ can have a positive effect on faster post exercise recovery by downregulating inflammatory and oxidative responses to exercise as well as enhance performance. The proposed dosage according to Ammar et al. (2018) would be 750ml/day of polyphenol rich pomegranate supplements within a window of 60min before and up to 48hours after exercise. However, it is also noted that these effects seem more prone to take place if certain parameters are considered:

- PGJ contains more than 0,7g total polyphenols / 0,5 L
- Exercises engage a large muscle mass
- PGJ ingestion more than 60min before exercise

1.2.4 Carbohydrates (CHO)

Carbohydrates (CHO) are one of the most researched macro-nutrients (Chandel, 2021; Reynolds et al., 2019). Dietary CHO are described by their degree of polymerization (DP) and can be put into three major categories (see table 5) (Cummings & Stephen, 2007; Shiv Sanjeevi Sripathi, 2020). First, the smallest form, are sugars with a DP of 1-2, which contain monosaccharides, disaccharides and polyols, than there are oligosaccharides with a DP of 3-9, which can further be divided in to α-glucans (malto-oligosaccharides) and non-α-glucan

(oligosaccharides), and the largest form is named polysaccharides with a DP of 10+, that include starch and non-starch polysaccha- rides (NSPs) (Cummings & Stephen, 2007; Shiv Sanjeevi Sripathi, 2020).

Table 4: Classification of Carbohydrates (Cummings & Stephen, 2007; Shiv Sanjeevi Sripathi, 2020)

Degree of polymerization	Group	Examples
Sugars (1–2)	Monosaccharides	glucose, galactose, fructose
	Disaccharides	maltose, sucrose, lactose
	Polyols (sugar alcohols)	sorbitol, mannitol, maltitol, lactitol, isomalt
Oligosaccharides (3–9)	Maltooligosaccharides (α-glucans)	maltodextrins
	Non-α-glucan oligosaccharides	raffinose, stachyose, fructo and galacto oligosaccharides, inulin polydextrose
Polysaccharides (10+)	Starch	amylose, amylopectin, modified starches
	Non-starch polysaccha- rides (NSPs)	cellulose, hemicellulose, pectin, β-glucan, arabinoxylans,

Some of these carbohydrates are digestible and hence available for energy production and some are not. Non digestible carbohydrates are also known as fiber. Fiber can act as prebiotic element and can be broken down by microbial bacteria throughout a fermentation process. Most unprocessed foods contain a mixture of digestible and indigestible CHOs. The digestible CHO are mostly being absorbed in the small intestine. In the process of digestion, all digestible CHO get broken down into monosaccharides which can be absorbed (Chandel, 2021; Cummings & Stephen, 2007). Although fructose and galactose can as well be absorbed by the body, they later get restructured by the liver to glucagon or fatty acids, for only glucose is being used as energy source of the body (Chandel, 2021). From this it becomes clear that glucose is the most important CHO for the human body. Glucose is stored in the muscle and liver as glycogen, as in a dehydrated state of glucose, or in the blood as active available glucose. Depending on the energetic demands of the body it is either kept at storage

or released into the bloodstream and the working muscle (Chandel, 2021; Shiv Sanjeevi Sripathi, 2020). While on a cellular level only glucose and glycogen can be used, CHO come in a variety of DP sizes which affects is uptake speed, as they need to be broken down first, which requires time. A measurement of the speed of availability is the glycemic index (GI). This is the rate at which the CHO effects blood glucose, hence gets absorbed by the gut and released into the blood stream. A high GI value means a high spike in blood glucose and a low value indicates a rather slow but steady absorption. Regarding exercise and health there are some points that can be taken into consideration. For performance, a CHO with a high GI could be quickly utilized for energy output. For recovery, glycogen storages need to be replenished fast but also over a longer period of time like during sleep, and hormonal responses, insulin, which is an anabolic hormone therefore, not suited for performance or limited in people with diabetes (Chandel, 2021; Cummings & Stephen, 2007; Haff & Triplett, 2016; Shiv Sanjeevi Sripathi, 2020). While these modalities are fairly good understood in humans, potential connections between CHO and ROS lack research. However, there is some evidence that fructans might be able to act as scavengers of ROS in the colon. Fructans cannot be absorbed by the human gut, but they can be used by colonic macrobacteria. In this process they act as a prebiotic and improve gut health, reduce inflammatory responses and benefit intestinal epithelial cells by improving their redox environment (Shiv Sanjeevi Sripathi, 2020). Hence there is less inflammation during the absorption process of the nutrients, less antioxidants might be needed in the gut and would be available elsewhere in the body. A study that hints for this effect to also help with ROS during performance is by Zeng et al. (2020). Zeng et al. (2020) have shown that a portion of oatmeal administered before strenuous exercise did reduce ROS. As oatmeal is a complex structure, with fiber and various micronutrients, compared to plain sugar further research is warranted before definitive answers can be given.

1.3 Exercise and ROS

Physical activity has been deemed essential for a proper life and health span by the World Health Organization (WHO). It has also been shown that physical activity is critical across multiple factors of all ages, for the development and retainment of cognitive functions, weight control, all-cause mortality, cancer, cardiovascular diseases, diabetes and sleep (Bull et al., 2020). As there is a dose response relationship for the beneficial effects, the WHO recommends 150–300min of moderate intensity or 75–150min of vigorous intensity for

adults in the age group 18-64 years (Bull et al., 2020). Further health benefits will arise if two or more days per week a person engages in strength training activities with a moderate to high intensity (Bull et al., 2020). For the strength training benefits it seems that a higher dosage will not result in a higher response. However, for the general activity recommendations it is postulated that, although a certain degree of diminishing returns is immanent with a higher physical fitness, more activity tends to provide more health returns (Bull et al., 2020). Two main factors which are responsible for these positive outcomes are more muscle mass and a higher VO2max (Haff & Triplett, 2016; Klika & Jordan, 2013). As time becomes the limiting factor in the people's daily lives, the most efficient way to spend these active hours becomes the option with 75–150min of vigorous intensity. Therefore, it can be deduced that a higher intensity will result in a better or faster adaptation. This is where the concept of high intensity interval training (HIIT) becomes of great interest. HIIT is a method where intense bouts of exercise alternate with periods of rest. This can be done with endurance classic exercises like running, biking, swimming (Laursen & Buchheit, 2019) or with strength exercises with bodyweight or external resistance (Klika & Jordan, 2013). While there are different modes of implementing this system, that can influence the specific adaptations of the body like strength, power, mitochondrial respiration or cardiac output, there is one common factor. It is a higher energetic requirement which results in a higher oxygen uptake (Klika & Jordan, 2013; Laursen & Buchheit, 2019; Simioni et al., 2018). Higher oxygen uptake implies higher nutrient requirements for the muscle which increases the imposed demands of the electron transport chain. It is also shown that through vigorous exercise the inflammation markers in the blood plasma are increased resulting in higher amounts of tumor necrosis factor (TNF)-α interleukin (IL)-1β, IL-1 receptor antagonist (IL-1ra), TNF-receptors (TNF-R), IL-8 and macrophage inflammatory protein (MIP)-1 (Simioni et al., 2018). This inflammation is due to the bodies reaction to eliminate pathogenic stimuli like bacteria, viruses, damaged or malfunctioning cells in order to regain its homeostasis (Simioni et al., 2018). As stated before in the section about ROS, ETC and the immune response are the biggest endogenous producers of oxidative stress. Therefore, the higher the intensity of the workout or exercise the more ROS will be produced. This ROS pressure fosters the body´s ability to utilize antioxidant defenses and lowers lipid peroxidation levels (Simioni et al., 2018). As an example for the intensity characteristic Çakir-Atabek et al. (2010) shows a reduction of malondialdehyde (MDA) concentration of 21-25% in the lower intensity (70% of maximum) and 35-39% in the higher intensity (85% of maximum) group after exercise and an increase of GSH levels of about 20% in the lower intensity and 22% in

the higher intensity group before exercise, after 6 weeks of different intensity's of strength exercises. But the review of Zhou et al. (2022) showed that intensity alone is not enough. It also takes a certain amount of volume to elicit a high ROS response. Zhou et al. (2022) determines exercise mode from highest ROS production to lowest as follows: high intensity aerobic exercise + resistance training like circuit training from Klika & Jordan (2013) > high intensity aerobic exercise > high intensity intervals with lower volume (strength & endurance) > moderate intensity aerobic exercise. One has to be careful though not to overexpose the body to too much ROS as they are still harmful biological agents. Here the classical quote of Paracelsus applies: "Solely the dose determines that a thing is not a poison". Hence it is important to know when the point of too much ROS, among other stressors of exercise, has been reached and it becomes a limiter of performance. Unfortunately this is highly individual from person to person and their training and health status (Haff & Triplett, 2016). The point where overreaching turns into overtraining cannot be generally described as it can be done with the physical activity guidelines of the WHO. What can be said is that when the frequency, volume, intensity, or duration of the training become excessive and are not interspaced with sufficient recovery, it is likely to happen (Haff & Triplett, 2016). Signs of overtraining and chronic levels of ROS can be hormonal changes, sleep irregularities, loss of appetite, an increase in resting heartrate, depression and decreased immune function (Haff & Triplett, 2016; Simioni et al., 2018). Novices or unhealthy individuals therefore should not train as hard as experienced athletes for they will, in regard to ROS, produce the same relative amount at a much lower intensity, in order to improve their redox balance (Simioni et al., 2018; Zhou et al., 2022). These principle hold true for endurance as well as strength training. Derived from these one can assume that a decrease in ROS during or after exercise might be desirable as it may allow a higher output during the exercise or shorten the needed recovery time.

2 Methods

2.1 Study Design

The study was designed as a randomized controlled crossover trial (RCCT) and was performed by the working group of Professor König at the University of Vienna at the Nutrition and Training Laboratory (NuTraLab). It was a collaboration of the department of sport science with the department of nutritional science and imbedded within an overhead research project which focused on the acute impact of beverages on exercised induced inflammation and oxidative stress in healthy untrained female adults following HIIT.

The scope of this master thesis was to compare the acute effects of pomegranate juice (PGJ), a glucose-water solution (GWS) and water following HIIT induced oxidative stress in inactive women. All subjects gave their consent after being informed about the intervention details. The study protocol was approved by the Ethics Committee of the University of Vienna (Reference – ID: 00743).

At the beginning the subjects underwent a testing process to see if they were eligible as participants, as they needed to perform high intensity interval training (HIIT) with weights for 20 minutes, in a fasted nutritional state, without complications. This processes included:

- A structured questionnaire about personal data, previous diseases, medication lifestyle habits like daily activity, smoking and hormone contraception intake
- A blood test
- A blood pressure measurement
- An electrocardiogram

If subjects were deemed eligible, they were randomly allocated in the treatment groups. The test days had a fixed time line as shown in figure 4. To prevent any interference from the last test day a wash-out period within the RCCT was performed, which lasted at least seven days.

Each subject conducted two familiarization sessions. In the first session the focus was put on learning the exercise techniques. In the second session a 5 repetition maximum (RM) test was performed. Based on the 5-RM, the individuals 1-RM was calculated via a formula introduced by Brzycki (1998) for each exercise. The 1-RM was consequently used in prescribing ones individual workout weight. The workout weight for each subject and each exercise was 50% of the individual´s 1RM in that exercise respectively.

Every Subject had to follow a strict structure on a test day. Before each test day the subjects had to comply to a 12 hour overnight fast. In the morning of the test day the subjects had to drink 500ml of water right after waking up and remain fasted. Upon arriving in the NuTraLab another 500ml of water had to be consumed to ensure sufficient hydration of the subject. First, was a 30-minute resting period to enable a stable environment for the ROS measurements. Here the subjects had to comfortably lay prone on the examination table. During this time a 24-hour dietary/health/physical activity recall questionnaire was completed with a research assistant, to asses all beverages, foods in quality and quantity as well as the subjects weekly physical activity, sleep quality, use of contraceptives, alcohol consumption and health infection within the last week. Furthermore, the day of the female cycle was also noted.

Following this period of rest, fasting blood samples were taken. Afterwards the subjects received their serving of the researched substance. For the treatment group it was either 1cup of pomegranate juicy (250ml) or a glucose-water solution (20g of glucose solved in 250ml of water), and for the control group 1 cup of tap water (250ml). The order of substances was randomized previous to the test days.

Now the second resting period began where the subjects had a two hour time frame, which they had to spend inside the testing facility. Except from exercise, eating and drinking they could spend this time freely. The consummation of tap water was allowed. 30 minutes before the next blood sampling took place, subjects had to lay prone on the examination table again. Afterwards the exercise intervention started. Right after the HIIT training the third blood sample was collected, again on the examination table. Lastly, after continuously laying prone for 15 minutes the fourth and last blood sample was taken.

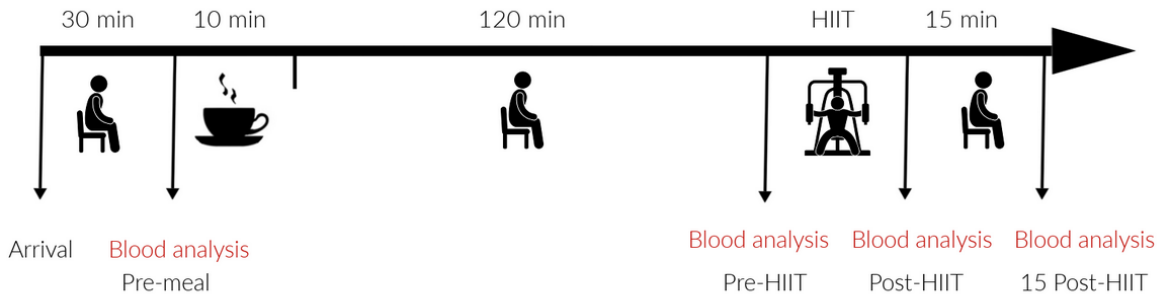

Figure 4: Test day structure (Gassner, 2023)

Each subject completed the experiment within roughly three to four months. This duration was due to the embeddedness of this thesis within a main research project were a total of seven different beverages were tested (coffee, matcha , pomegranate juice, orange juice, low fat cow´s milk, sugar water & tab water). Hence, the whole intervention was at least 7 weeks per subject.

ROS were measured with an EPR spectrometer for each blood sample on the test days. Additionally to the ROS, the total antioxidant activity (TAA) was assessed via DPPH and total polyphenol content (TPC) was assessed with the FCR- method. These measurements were done in a laboratory of the nutrition science department of the University of Vienna.

2.2 Subjects

Out of 51 screened females, 20 were eligible to take part in the study. All subjects (age 26.45 ± 4.06 years; BMI 21.0 ± 1.88 kg/m^2; Bodyfat 27.5 ± 3.86 %) were healthy nonsmokers or at least abstained from smoking since over half a year. Subjects had to be untrained and not physically active (less than 120 minutes of regular physical activity per week) which was assessed using a questionnaire. Other inclusion criteria were a body mass index (BMI) of 18,5 to 25 km/m^2 and a body fat of at least 20% to assure normal hormonal production.

Subjects were instructed to abstain from medication, exercise and alcohol 24 hours prior to each test day and continue to have less than 120 minutes of exercise per week during the study.

Exclusion criteria were:

- Illnesses (acute or chronic)
- Iron deficiency
- Hemoglobin anemia
- Physical impairment which would make the training program impossible
- Intolerances to the various beverages
- Recent vaccination (had to be at least 7 days prior to the intervention)

2.3 Statistical Analysis

In order to ensure a meaningful outcome, the sample size was calculated before the study. Based on the data of Zeng et al. (2020) a G-Power analysis was performed and determined that at least 20 subjects had to complete the study if aimed at a 0.05 level of significance and 80% power. The statistical analysis was performed using IBM SPSS Statistics 27.0 for Windows. The test used was an ANOVA for repeated measures and as aimed at in the power analysis the level of significance was defined as $p\text{-value} < 0.05$.

2.4 Administered substances

The administered substances were 250ml of organic pomegranate juice (PGJ) by DM, 250ml of glucose-water solution (GWS) consisting of 20g of pure glucose, bought by the local pharmacy, solved in 250ml of tap water and plain tap water (W) as a control. Water was the only substance that was allowed to be consumed throughout the whole test day. According to the supplier the PGJ was made out of 100% pomegranates form organic farming and contained 4.8g of sugar per 100ml (see table 6 for nutrition values of PGJ), which adds up to 12.2 g of carbohydrates an 12g of sugar for the 250ml PGJ.

Table 5: Nutritional value of pomegranate juice per 100ml

Nutritional values of pomegranate Juice	per 100ml
Energy	103kJ / 24kcal
Fat	< 0.5 g
of which saturates	<0.1 g
Carbohydrat	4.9g
of which sugars	4.8g
Fiber	<0.5 g
Protein	<0.5 g
Salt	<0.01 g

2.5 Total polyphenol contend (TPC)

TPC was measured with the Folin-Ciocalteu method for the pomegranate juice. Here PGJ was diluted 1:10 with distilled water for the measurement and gallic acid was used as a reference. Gallic acid (10mg) was dissolved with distilled water (10ml) and the sodium carbonate (35g) was dissolved in distilled water (100ml). The stock solution (100µL standard reference + 100L distilled water) consisting of 2 mL of gallic acid solution (1mg/mL), was used to construct the standard curve by measuring different concentrations of it (1,95 to 1000µg/mL). 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 tester. The total volume in each tester was 250 µl. Now the microplate was covered with parafilm and stored in the dark for 60min at room temperature. The absorbance at 752nm was measured with a spectrophotometer CLARIOstar® Plus. The absorbance was measured using a blank, which contained distilled water and reagents. 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) and was 3862,10 µg GAE/ml for the PGJ (see table 9).

2.6 Total antioxidant activity (TAA)

The TAA was measured with DPPH assay. The DPPH reagent was prepared in a 25mL flask with 0,0038g DPPH in 25mL methanol covered with aluminum foil to minimize DPPH degradation. A Trolox standard stock solution was prepared with 0.0063g Trolox in 10mL ethanol in a 10mL volumetric flask. Both reagents were completely dissolved and newly mixed each day. The dilution factor for PGJ was 1:50 and 15µL were put on the microplate with 85µL methanol. From the Trolox standard stock solution different concentrations were derived (0.0255, 0.0514, 0.1050, 0.1609, 0.2191,1.260 mM) and 100µL were added to the microplate. These microplates were incubated for 30minutes and absorbance at 490nm with a spectrophotometer (CLARIOstar Plus), were measured against blanks. Measures were repeated three times to enhance accuracy with a CV < 5%. The Trolox equivalent of the sample was subsequently calculated with a linear regression. And from the absorbance level one could see the antioxidant activity.

To research the antioxidants ability to neutralize a radical the radical scavenging ability (RSA%) was calculated as well. Therefore, the equation of Ngamsuk et. al. (2019) (see table 9)

2.7 Training intervention

Subjects performed high intensity resistance training in a circuit training setup. The duration of the training was 20 minutes and was supervised by a sport scientist or trainer. It started with a guided warm up (table 7) and was followed by the main part which consisted of three rounds of 5 exercises (figure 5). The main rounds were interspaced with 1min of passive recovery. This protocol was similar to the protocol of Bizheh et al. (2011) to ensure maximal increase in the ROS concentration.

Table 6: Warm up exercises

Warm Up exercises	Repetions (L & R)
Wrist circles	10x
Forarme circles	10x in- & external rotation
Shoulder circles small	10x in- & external rotation
Shoulder circles big	10x in- & external rotation
1 Leg Hip circles	10x in- & external rotation
Knee circles	10x in- & external rotation
Ankle rotations	10x in- & external rotation
Hip circles	10x
Jumping jacks	20x
High knees	20x
Sideplank + Hipraises with bend knees	10x

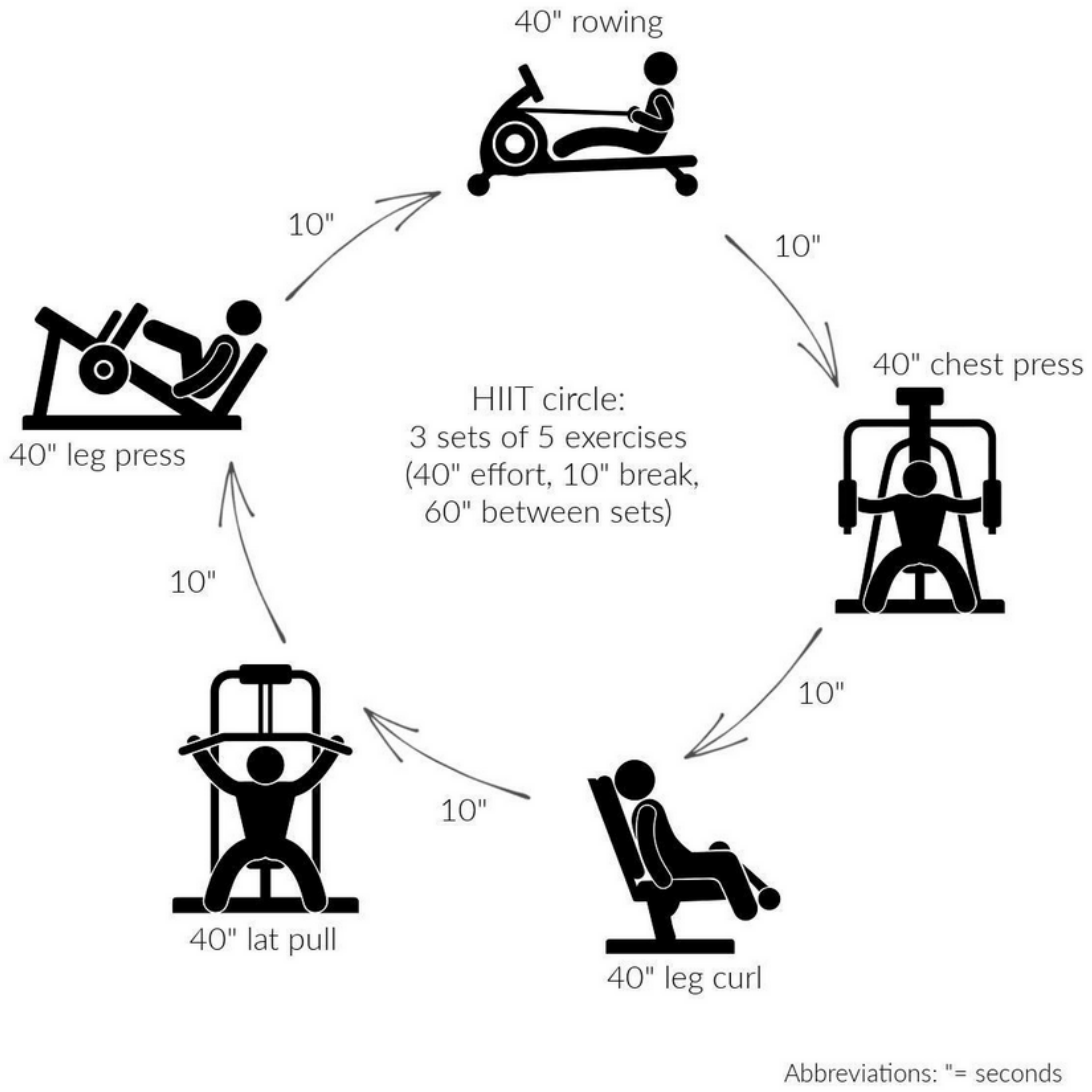

Figure 5: Exercises within the HIIT circle (Gassner, 2023)

2.7.1 Familiarization and 5RM Testing

As the subjects were categorized as novices regarding their training experience, familiarization sessions were needed. This was to provide a certain degree in exercise quality and correctness as well as avoiding initial adaptations through the 1RM test and the first training sessions. Hence, it could be assured that the prescribed % of 1 RM, could be safely handled, was an accurate load and should enable the desired ROS outcome. In the first session, subjects that were eligible had a familiarization session where they were introduced to all the exercises. Trained staff showed them correct positions and key points, to stay effective and safe within each exercise. In the second session a 5-RM Test was performed.

It started with the general intervention warm up and then worked chronologically through the exercises. The test starting with an easy weight for 5 repetitions and then increased each set until only 5 repetitions or less could be performed. This 5-RM was used to calculate the 1 RM with the equation from Brzycki (1998).

$$1RM = kg^* (36 / (37 – reps)) = kg / (1.0278 – 0.0278^*reps)$$

Formula 1: 1RM through 5RM calculation Brzycki (1998)

2.7.2 Training prescription and adaptations

The intervention included a 10 minute warm up (see table 7) and three rounds of HIIT of the different resistant exercises (see figure 5). The workout weight for the HIIT was determined as 50% of the subject´s 1-RM for each exercise. Each exercise was performed for 40 seconds and followed by a 10 second break before starting with the next bout of the next exercise. In those 10 seconds the subjects had to move to the next exercise station which was about 5m away. Between the 3 rounds of this training circuit was a one minute break. Subjects were told to reach at least 20 repetitions per exercise, which could be categorized as a hard goal. If more than 60 total reps over all three sets of one exercise could be performed the workout weight was increased by 5% for the next session, to account for any training adaptions that could decrease ROS output. The training was prescribed as a high volume circuit workout for two reasons. First, previous studies also relied on high volume HIIT to produce sufficient ROS with bodyweight exercises (Klika & Jordan, 2013; Zeng et al. 2020; Zhou et al., 2022). Second, a lower absolute intensity of the 1-RM ensured the subjects safety in handling the weight under fatigue.

The subjects individual exhaustion levels were also measured using the BORG scale (Williams, 2017). This scale classifies physical activities from 6, which is seen as easy, to 20, referring to total exhaustion, and was assesed after every round. The aim was to reach an exhaustion level of 16 to 19 on the Borg scale after the third round of exercise. The BORG scale was a good fit for the high volume HIIT circuit, for this manner of exercise strongly increases heartrate and the BORG scale is highly correlated with heartrate.

2.8 ROS measurements

ROS measures were done by the capillary blood samples which were taken four times during each testing day. The time slots were at fasting upon arrival, right before, direct after and 15 minutes after the HIIT session. Blood samples were all taken in a prone laying position and were collected by the medical staff, with a standard Eppendorf pipette directly from the fingertip. The analysis procedure was based on the pilot study from Zeng et al. (2020). 10 µL of capillary blood were combined with 10µL oxygen sensitive label to make monitoring of the cellular oxygen consumption possible. In order to extend ROS half-life CMH (1-hydrocy-4phosphonooxy-2,2,6,6 tetramethylpiperidine) was used as a spin probe. To measure the ROS in in-vivo conditions an e-scan EPR spectrometer was used, with a temperature and gas controller set to 37°C. For the measuring of the ROS formation rate the following setting were applied: center field: g = 2.011, sweep width: 60 G, sweep time: 5.24s, frequency: 9.76 GHz, power: 20 mW, gain: 1 x 103, modulation amplitude: 1.0 G, and number of scans: 10.Bruker WinEPR Data Processing software was used for the quantitation of EPR spectra.

3 Results

Table 8 provides an overview of the 20 untrained female participants who successfully completed the entry process and took part in this study. No adverse effects were reported during the intervention period.

Table 7: Subject characteristics

Subjects	Mean ± SD	MIN	MAX
Age (years)	26.45 ± 4.06	20	37
Bodyweight (kg)	58.99 ± 6.82	46.25	70.75
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
LBM (%)	58.6 ± 16.44	34.42	77.9
SMM (kg)	19.3 ± 2.32	15.01	22.83
WC (m)	0.7 ± 0.007	0.58	0.87
Phase angle (°)	5.1 ± 0.38	4.3	5.9
BMI: body mass index; LBM: lean body mass; SMM: sceletal muscle mass; WC: waist circumfence			

3.1 Results of TPC and DPPH antioxidant assay

Table 9 provides the results of the total polyphenol content and total antioxidant activity analysis of the pomegranate juice. TPC had a value of 3863.1 µg GAE/mL. Therefore it was 50% higher than the value of a reference study (Anahita et al., 2015). The Trolox equivalent antioxidant capacity (TEAC) had a value of 7, thus it was more than 60% lower than the lower bound of the measured values in the reference study (Severcan et al., 2020). The RSA% was with a score of 44.82 well within the expected boundaries of the reference study (Mutahar et al., 2012).

Table 8: Properties of pomegranate juice

Properties of pomegranate juice	Study sample	Literature data
TPC (µg GAE/mL)	3862.10 ± 0.02	2502 [1]
TEAC (mmol/L)	7.00 ± 0.00	24 – 46.9 [2]
RSA %	44.82	34.3 – 75.4 [3]

[1] Anahita et al. 2015, [2] Severcan et al. 2020, [3] Mutahar et al. 2012

3.2 ROS formation rate through HIIT

First, the data was baseline corrected for accuracy duo to variances in the daily lives of the participants which could be disruptive to the ROS formation rate (subtraction of corresponding morning baseline, hence baseline is always 0). The analysis showed a significant difference for baseline ROS compared to pre-HIIT ROS, 15post-HIIT ROS formation levels (see table 10). It also showed a significant difference between pre-HIIT and post-HIIT ROS levels.

Table 9: ROS formation rate compared to baseline

Variable	Time Point	Mean ± SD
ROS	Baseline vs Pre HIIT	-0.11 ± 0.02 µmol/min *
	Baseline vs. Post HIIT	-0.03 ± 0.02 µmol/min #
	Baseline vs. 15Post HIIT	-0.08 ± 0.02 µmol/min *
p: <0.05; *compared to baseline; # compared to pre HIIT		

Further analysis of the inspected substances (water, pomegranate juice and glucose-water solution), did not show any significant difference between substances in general (table 11) or at the specific points of measurement TP2 & TP3 after HIIT (table 11 & figure 6).

Table 10:Time point dependent ROS measurements of PGJ, GWS & Control

		Mean ± SD		
Variable	**Time Point**	**PGJ**	**GWS**	**Control**
ROS	Baseline vs Pre HIIT	-0.13 ± 0.40 µmol/min	-0.11 ± 0.22 µmol/min	-0.05 ± 0.23 µmol/min
	Baseline vs. Post HIIT	0.02 ± 0.38 µmol/min	0.03 ± 0.37 µmol/min	0.04 ± 0.30 µmol/min
	Baseline vs. 15Post HIIT	-0.15 ± 0.30 µmol/min	-0.05 ± 0.25 µmol/min	-0.08 ± 0.19 µmol/min

PGJ: pomegranate juice 250ml; GWS: glucose-water solution 250ml (20g Sugar); Controll: Water; p: <0.05

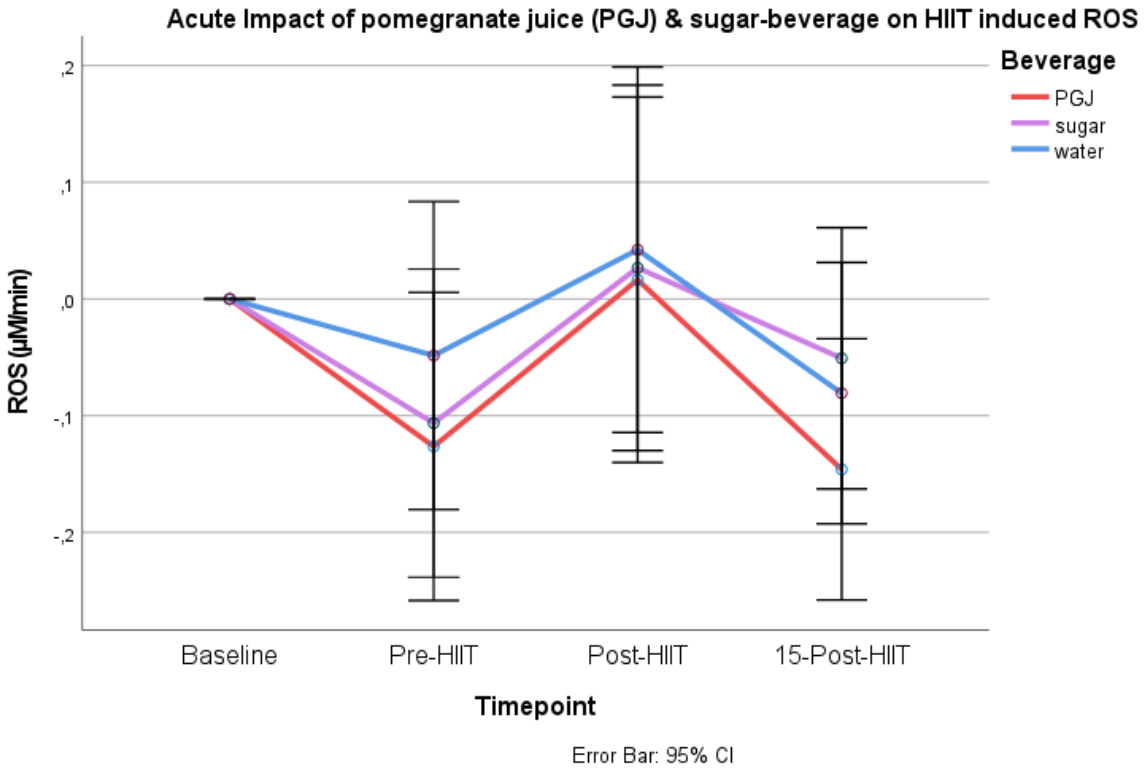

Figure 6: ROS at different blood sampling time points (Gassner, 2023)

4 Discussion

This intervention study continued to validate that acute HIIT is successful in elevating ROS formation in untrained people, as has been shown in the literature (Zhou et al., 2022). The important part seems to be the combination of high intensity with high volume. In this study an intensity was given by the researcher and the volume was, although also given, most of the time, the maximum repetition effort of the subjects with 15+ repetitions. This modality also enabled a high aerobic response. Once understood what properly spikes ROS in a population, researchers can choose from a variety of exercise modalities (running, lifting weights, bodyweight exercises), if they want to research ROS levels. This is helpful as some researchers may be limited in their strength and conditioning equipment.

However, this studies outcomes do not support the claim of acute protective effects of pomegranate juice or a glucose-water solution against increases in ROS at any timepoint in the study. Hence, the first hypothesis of this master thesis could not be proven to be correct. Neither could the second hypothesis be proven, that stated pomegranate juice could reduce ROS more than a glucose-water solution if consumed before the HIIT activity. This could be seen as contrast to other studies which showed an effect (Ammar et al., 2017; Ammar et al., 2018; Dujaili, Good & Tsang, 2016; Fuster-Muñoz et al., 2016; Machin et al., 2014; Ortega et al., 2020; Wang et al., 2020). The differences which could have led to this will be analyzed and discussed in the following paragraphs to be taken into account for further research.

First, the focus will be put on the digested carbohydrates. Zeng et al. (2020), showed that 1g /kg BW of carbohydrates consumed before training in form of oatmeal was able to reduce ROS formation. Carbohydrates in this form are little in pure sugar and more in a complex-chained form, including dietary fiber. This study did use 20g of pure glucose solved in 250ml of water. Therefore, two factors could have influenced the difference in outcomes. The first factor is the quantity of carbohydrates (CHO). For instance, this study did administer 0,34g / kg BW of carbohydrates before HIIT compared to the 1g / kg BW by Zeng et al. (2020). Furthermore, as this study used pure glucose, there could not have been any possible effects of dietary fiber or other phytochemicals. Hence proposing that it was not the CHO intake itself but also the dietary components with it that may be important for reducing ROS.

The second factor is metabolic uptake speed. For oatmeal is a complex meal it can be estimated that the nutrient uptake takes longer than the one of liquid glucose. Although the

2h waiting period should balance this effect, secondary mechanisms like insulin response, blood sugar content or fermentation in the colon could have been influencing the results.

Second, the focus will be put on pomegranate juice and its anti-oxidative properties. Many studies support the protective effects of PGJ against ROS during performance (Ammar et al., 2017; Ammar et al., 2018; Dujaili, Good & Tsang, 2016; Fuster-Muñoz et al., 2016; Machin et al., 2014; Ortega et al., 2020; Wang et al., 2020). It has to be noted that there are also studies which do not support the positive effects of PGJ or are inconclusive (Morvaridzadeh et al., 2020; Trombold et al., 2011). Ammar et al. (2018) collected these findings in his systematic review and postulated that PGJ shows positive effects but three points needed to be taken in to consideration: benefits seems to be more unlikely in unilateral movements, PGJ had to be rich in polyphenols (>1,69 g/l) and ingestion time has to be more than 1h before exercise. Although these points have been taken into consideration the facilitating effects in suppressing ROS could not be measured. However, it could still be a dosage problem within this study. Ammar et al. (2017) used 500ml of PGJ and this study used 250ml of PGJ. In contrast, this study used 965 mg GAE/serving with no effects, whereas Machin et al. (2014) used 650mg GAE/serving and had positive effects. Another influencing factor could also be the duration of supplementation. In the present study it was a single drink of PGJ, whereas Dujaili, Good & Tsang (2016), Lamb et al. (2019) and Fuster-Muñoz et al. (2016) did supplement PGJ for 1 week, 9 days and 21 days respectively. For this reason a permanent supplementation with PGJ could be more advantageous than a single dose. Here the feasibility of permanently drinking PGJ also have to be considered. In the long run the fair amount of sugar contained in PGJ might cancel out the positive effects on health and longevity for some individuals. This might also be the reason for studies researching pomegranate extract supplements instead of its pure juice form (Wang et al., 2020).

A further possible effect that could be researched, would be the combined effect of PGJ and CHO. This study cannot provide any evidence for it, but the reviewed studies do hint at it. It stands to reason that if CHO, especially fiber, pose a dampening effect on ROS, the increase of CHO or fiber in PGJ could prove beneficial. This benefit of mixed sources could have a better anti-oxidative effect than more of one source.

In spite of the results of this study it is important to note that further research into ROS dampening supplements could have important information on trainings strategies. For if one can actively influence the redox balance and the adaptive strategies of the body, these could

be of great interest for professional sports or for medical applications for unhealthy populations. The indications for its effect are already given (Ammar et al., 2017; Ammar et al., 2018; Dujaili, Good & Tsang, 2016; Fuster-Muñoz et al., 2016; Machin et al., 2014; Ortega et al., 2020; Trombold et al., 2011; Wang et al., 2020) for an increase in antioxidants would speed up the recovery, for it reduces soreness, and decrease ROS during exercise or increases voluntary muscle contraction. In a professional sporting context this could result in less fatigue after hard practices or games, which could possible lead to less injuries, enable more hard practices or make it possible to push even harder in the hard practice. Having less fatigued athletes, and hence less injured ones, increases availability what is of utmost important in sports. To be able to have more hard training session will result in more quality training hours what is very important for the development of an athlete if he wants to improve. In the setting health and exercise of the general population these factors hold true nonetheless. Most people overexert themselves once they start to be active. For they want to cramp in everything at once after sometimes years of sedentary lives. Here a reduced ROS response could help them in the initial phase of exercise to keep them injury free and motivated.

On the other hand, the effects of ROS could also be willingly kept high after an intense training session as to force more adaptation from one single training bout. This will not be skill related but purely physical. If fatigue is not the main factor, like shortly before a holiday or when certain intense practices need to be cycled, this could amplify the stimulus and thus the adaptation response. This would increase the antioxidant response as the body would be more resilient for the next training without needing to change the training structure.

Eventually, these training considerations are the reasons why the details of the acute response to antioxidative supplements need to be studied. Once the quantity and also its time effects are understood, one may use it as a potential tool. For if it is possible to have effects for only one trainings session it can be used for everyday monitoring and manipulation. If it is only possible to guarantee effects via long term intake, the time horizon needs to be carefully planned as once antioxidative supplements build up in the body they will have a certain residual within the body.

5 Conclusion

To conclude, the effects of the underlaying cell signaling processes of the body are often not visible from the outside but are linked to measurable performance outputs and physiological adaptations. One of those cell signals are ROS, which can harm the body but also foster beneficial adaptations. This study illustrates that ROS can be provoked through vigorous activity. Consequently, training stimuli have to be carefully managed as its effects on health cannot always superficially be seen. Previous studies have shown nutrition to be able to mitigate the effects of ROS. However, this study did not find any difference between pomegranate juice, a glucose-water solution and the control (water) regarding their influence on ROS following HIIT. Hence the first hypothesis has not proven to be correct. Despite the best efforts to comply to the recommended usage of pomegranate juice and the glucose-water solution, no effect could be shown. This can be due to varying circumstances in quantity, quality and timing. The assumption of the author is that, for glucose the dosage was not sufficient in quantity and rich in fiber and for pomegranate juice a single dosage might not be enough for its impact to show properly. The second hypothesis could not be analyzed as any difference in the effect of those beverages would still make no difference in the ROS in the plasma.

For this reason, the literature is still inconclusive on this topic. There needs to be further research before it can be properly applied in training practices. This author would recommend on the one hand for pomegranate juice, to increase dosages or freshness of the juice to increase TPC or TEAC up to the values of the reference studies in table 9. For glucose on the other hand, it is recommended to increase the absolute amount to match the values of Zeng et al. (2020) and administer more complex carbohydrates in liquid form.

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Eigenständigkeitserklärung

Ich erkläre, dass ich die vorliegende Arbeit selbstständig verfasst habe und nur die ausgewiesenen Hilfsmittel verwendet habe. Diese Arbeit wurde weder an einer anderen Stelle eingereicht (z. B. für andere Lehrveranstaltungen) noch von anderen Personen (z. B. Arbeiten von anderen Personen aus dem Internet) vorgelegt.

Wien, 31.1.25