Diplomarbeit

EVALUATION OF OXIDTIVE AND NITROSATIVE STRESS IN YOUNG FEMALES OF DIVERSE NUTRIONAL STATUS AND LIFESTYLE BEHAVIOR

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Zusammenfassung

Hintergrund und Ziel: Oxidativer Stress ist ein pathologischer Stoffwechselzustand, der auf ein Ungleichgewicht der pro- und antioxidativen Mechanismen auf zellulärer Basis zurückzuführen ist. Dies kann entzündliche Reaktionen auslösen und führt zu einer destruktiven Progression im Organismus. Ein Überschuss an entstehenden reaktiven Stickstoffspezies verursacht nitrosativen Stress. Beide Phänomene sind an vielen pathologischen Prozessen in unserem Körper beteiligt. Es besteht ein Zusammenhang zwischen oxidativen Stressparametern und dem BMI und nitrosativen Stressparametern und dem Stimmungszustand, so dass der Lebensstil eine wichtige Rolle im Regulationsprozess spielen könnte. Das Ziel dieser Arbeit war es, den oxidativen und nitrosativen Stress bei jungen Frauen mit unterschiedlichem Ernährungsstatus retrospektiv zu bewerten und die Ergebnisse in einen möglichen Kontext von Nahrungsaufnahme, Aktivitätsniveau und Depressionsstatus zu stellen.

Methoden: Es wurden demographische, anthropometrische und lebensstilbezogene Daten einer Kohorte von 107 jungen Frauen mit fünf verschiedenen Ernährungszuständen (18 Patientinnen mit Anorexia nervosa (AN), 27 normalgewichtige Frauen, 22 übergewichtige Frauen, 20 Frauen mit Adipositas und 20 Athletinnen) gesammelt. Die totale antioxidative Kapazität (TAC), die totale oxidative Kapazität (TOC), die endogene Peroxidase-Aktivität (EPA), die Autoantikörper gegen oxidiertes Low-Density-Lipoprotein (oLAb) und Malondialdehyd-modifiziertes Low-Density-Lipoprotein (MDA-LDL-IgM), asymmetrisches Dimethylarginin (ADMA) und symmetrisches Dimethylarginin (SDMA) wurden im Serum gemessen.

Ergebnisse: Bei adipösen Frauen wurde ein signifikant höherer TOC Konzentration im Serum im Vergleich zu normalgewichtigen Frauen ($p = 0,001$) und AN-Patientinnen ($p < 0,001$) festgestellt, zusätzlich war die TOC bei den Athletinnen signifikant höher ($p = 0,008$) als bei den AN-Patientinnen. Die Athletengruppe zeigte im Vergleich zur übergewichtigen Gruppe signifikant niedrigere ($p = 0,048$) oLAb-Titer. Die AN-Patientinnen hatten signifikant niedrigere ADMA-Werte als die normalgewichtigen ($p = 0,007$), die übergewichtigen ($p = 0,033$) und die adipösen Frauen ($p = 0,002$). Darüber hinaus wurde eine positive Korrelation zwischen BMI und TOC, EPA und ADMA sowie

eine negative Korrelation zwischen BMI und MDA-LDL IgM und SDMA gefunden. ADMA war ebenfalls negativ mit dem HAMD-Score korreliert.

Schlussfolgerung: Im untersuchten Kollektiv waren fettleibige junge Frauen stärker von oxidativem Stress betroffen als gleichaltrige Frauen, die nicht übergewichtig waren. Junge Athletinnen zeigten niedrigere oLAb-Titer als Adipöse. AN-Patientinnen zeigten deutlichere Anzeichen von nitrosativem Stress als gesunde junge Frauen. Die vorliegenden Daten ergänzen die bisherigen Erkenntnisse zum Zusammenhang von Lebensstil und oxidativem/nitrosativem Stress mit besonderer Beachtung des Ernährungsstatus.

Abstract

Background and aim: Oxidative stress is determined by an imbalance of pro- and anti-oxidative mechanisms including inflammatory conditions and leads to destructive processes in the organism. It is one of the major risk factors for the development of non-communicable diseases. An excess of emerging reactive nitrogen species induces nitrosative stress. It has been shown previously that oxidative and nitrosative stress are associated with the BMI and the lifestyle of humans. The aim of this thesis was to retrospectively evaluate oxidative and nitrosative stress in young females of different nutritional status and put the results into a possible context of nutritional intake, activity level and depression status.

Methods: Demographic, anthropometric and lifestyle associated data of a cohort of 107 young females of five different nutritional status (18 anorexia nervosa (AN) patients, 27 normal weight women, 22 overweight women, 20 adiposity subjects and 20 athletics) were collected. Total antioxidant capacity (TAC), total oxidant capacity (TOC), endogenous peroxidase activity (EPA), autoantibodies against oxidized low-density lipoprotein (oLAb) and malondialdehyde-modified low-density lipoprotein (MDA-LDL-IgM), asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) were measured in serum.

Results: Significant higher serum TOC was found in obese compared to normal weight women ($p = 0.001$) and AN-patients ($p < 0.001$), additionally TOC was significant higher ($p = 0.008$) in athletes than in AN-patients. Athlete group showed significantly lower ($p = 0.048$) oLAb titres compared to overweight. AN-patients had significant lower ADMA levels than normal weight ($p = 0.007$), overweight ($p = 0.033$) and obese ($p = 0.002$). Furthermore a positive correlation between BMI and TOC, EPA and ADMA as well as a negative correlation between BMI and MDA-LDL IgM and SDMA were found. ADMA was also negative correlated with HAMD score.

Conclusion: In the investigated collective, obese young women were more affected by oxidative stress than women of the same age who were not overweight. Young female athletes showed lower oLAb titres than obese women. AN-patients showed stronger signs of nitrosative stress than healthy young women. The available data complement the

previous findings on the relationship between lifestyle and oxidative/nitrosative stress with special attention to nutritional status.

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Glossar und Abkürzungen

8-epi-PGF2α	8-epi-prostaglandin-F2α
8-OH-2dG	8-hydroxy-2-deoxyguanosine
ADMA	asymmetric dimethylarginine
AN	anorexia nervosa
ApoB-100	apolipoprotein B-100
ATP	adenosine triphosphate
AU	arbitrary unit
BAI	body adiposity index
BDI	Becks depression inventory
BMI	body mass index
Ca^{2+}	calcium
cGMP	cyclic guanosine monophosphate
CK-MB	creatine kinase muscle-brain type
COPD	chronic obstructive pulmonary disease
CRP	C-reactive protein
DNA	desoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
EPA	endogenous peroxidase activity
Fadd	Fas-associated protein with dead domain
Fc	fragment crystallisable
FFA	free fatty acids
GSH	glutathione synthetisase
GTP	guanosine-5´-triphosphate
H_2O_2	hydrogen peroxide
HAMD	Hamilton depression scale
HbA1c	haemoglobin A1c
HDL	high-density lipoprotein
HNE	4-hydroxynonenal/4-hydroxy-2-nonenal
HO_2	perhydroxy radical
HRP	horseradish peroxidase
IgG	immunoglobulin G

IgM	immunoglobulin M
IL-6	interleukin 6
iNOS	inducible nitrogen monoxide synthesase
IPAQ	international physical activity questionaire
L•	lipid radical
LDL	low-density lipoprotein
LH	lipid hydroperoxide
LOO•	lipid peroxy radical
LPS	lipopolysaccharide
MDA	malondialdehyde
MDA-LDL IgM	autoantibodies against malondialdehyde-modified low-density lipoprotein
MET	metabolic equivalent
MPO	myeloperoxidase
MRI	magnetic resonance imaging
NADPH	nicotinamidadinindinukleotidphosphat
NCBI	national center for biotechnology information
NF-kB	nuclear factor ´kappa-light-chain-enhancer´of activated b-cells
NO	nitrogen monoxide
NOS	nitrogen monoxide synthetases
Nox	nicotinamidadinindinukleotidphosphat oxidases
O_2	oxygen
O_2-	superoxide anion
OD	optical density
OH°	hydroxyl radical
oLAb	autoantibodies against oxidized low-density lipoprotein
ONOO-	peroxynitrite
ox LDL	oxidized low-density lipoprotein
P53	protein 53
PI3	phosphoinositide 3
PTEN	phosphatase and tensin homolog
RNA	ribonucleic acid
RNS	reactive nitrogen species

RO•	alkoxy radical
ROO•	peroxy radical
ROS	reactive oxygen species
SDMA	symmetric dimethyarginine
SOD	superoxide dismutase
TAC	total antioxidant capacity
TAS	total antioxidant status
TBARS	thiobarbituric acid reactive substances
TEAC	Trolox equivalent antioxidant capacity
TMB	3,5,3´,5´-tetrametylbenzidine
TMI	transition metal ion
TNFα	tumor necrosis factor alpha
TOC	total oxidant capacity
UQH2	ubiquinol
UV	ultraviolet
VPO1	vascular peroxidase 1
XO	xanthine oxidase

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1 Introduction

Oxygen is essential for many chemical reactions in our body. However, in reactions where oxygen is involved, free oxygen radicals could be generated.[1] A radical is an atom or a molecule which has one or two unpaired electrons. Because it is usually highly reactive and not stable, it could cause damage in a cell by reacting with other molecules.[2] Radicals are thus only a part of the molecules playing a role in oxidative stress. There are also molecules of non radical nature, such as hydrogen peroxide (H_2O_2), causing damaging reactions to cell structures and some of them are even more reactive than radical particles. Summarised they are called reactive oxygen species (ROS).[3]

To control the damage in our body caused by ROS, we need a system to dispose of them. There are molecules which have a detoxifying effect, so-called antioxidants. They are able to remove the ROS respectively to reduce their effect, so they can act as a radical scavenger. [1] Which molecules are included and how antioxidants work is exemplified later. Physiologically a balance between those two processes is obtained. If this equation collapses in favour of the ROS, oxidative stress is formed.[3] The ROS can play an important part in regulation processes in the body; they are for example part of the innate immune system, as explained later.[4] Thus in pathological concentrations they can harm the cell. Because of their responsiveness, oxidation processes of the lipids of the cell membrane and the mitochondrion membrane, breaking of deoxyribonucleic acid (DNA) strands, changes of the activity or the structure of enzymes, ion channels and transport proteins and many other damaging procedures can occur.[1]

A special type of the oxidative stress is the nitrosative stress. In nitrosative stress, reactive nitrogen species (RNS), including nitrogen monoxide (NO), are harming the cell.[5] NO is an important signal molecule, which leads via activating a signal cascade to vasodilatation, thereby it is involved in the regulation process of the width of the coronary vessels as well as the penis erection. Besides it plays a part in the innate immune system in interplay with the ROS.[4] It is generated by different nitrogen monoxide synthetases (NOS). During nitrosative stress a large amount of NO is formed, thus also more reactive types of nitrogen oxides could develop. NO and higher nitrogen oxide types can disturb the signal transduction pathway and the expressions of genes and are associated with autoimmune

diseases, neurodegenerative disorders and many other disease patterns[5], which will be described later.

Oxidative and nitrosative stress are participating in physiological but mainly pathological phenomena in our body[3] and are in the focus of medical research. It is therefore of great interest to investigate the potential influence of nutrition and lifestyle on these processes.

1.1 Reactive Species

There are many different compounds contributing to oxidative stress, of radical and non-radical nature, as mentioned. The most famous is the superoxide anion (O_2-), a one-electron reduction state of oxygen. It is generated in many autoxidation processes.[3] In the respiratory chain, a small amount of oxygen (O_2) reacts to O_2- under the impact of the Nicotinamidadenindinukleotidphosphat (NADPH)-oxidase and the xanthine oxidase (XO).[1] Other two familiar reactive oxygen species are hydroxyl radical (OH°), which is formed by the Fenton and the metal-catalysed Haber-Weiss-reaction, and the hydrogen peroxide.[1,3] Not so well known is the perhydroxy radical (HO_2°), a protonated form of O_2-. Especially the alkoxy (RO°) and peroxy radicals (ROO°), which are formed by oxidation processes of organic molecules such as lipids, play an important role in many diseases, which will be discussed later.[3]

At the centre of nitrosative stress is nitrogen monoxide (NO). As stated previously, there are many different types of NO-synthesases (NOS), with the inducible isoform (iNOS) playing the most important role for nitrosative stress. Expressed on an inflammatory stimulus, it forms NO and L-Citrulin from the catalysis of L-Arginine and molecular oxygen. NO itself is a very long-lived and non reactive radical. However, if NO molecules accumulate, higher and more reactive nitrogen oxides may be generated, especially in the presence of ROS.[5] In table 1 a summary of all important reactive species with their origin is presented.

	Name	Origin
O_2-	superoxide anion	autoxidation processes; respiratory chain
HO_2•	perhydroxyl radical	prodonated form of the superoxide anion
H_2O_2	hydrogen peroxide	formed from superoxide anion (via the hydroxyl radical) or direct from O_2
OH•	hydroxyl radical	formed by the Fenton and the metal-catalysed Haber-Weiss-reaction
RO•, ROO•	alkoxy, peroxy radical	formed by oxidation of organic molecules

NO	nitrogen monoxide	formed by NO-synthesases

Table 1: Reactive species contributing to oxidative and nitrosative stress

1.2 Antioxidants

In order to render the reactive species harmless or to reduce their damage, various systems in the human body are in place. These systems take over prevention and interception as well as repair functions and are summarized as antioxidants. The antioxidant compounds include an enzymatic component with enzymes and ancillary enzymes and non-enzymatic low-molecular substances.[3]

Examples of antioxidant enzymes are superoxide dismutase (SOD) and catalase, which degrade O_2- and subsequently H_2O_2.[1] In addition, various peroxidases are involved in the depletion of reactive species. All of them have a characteristic high cell activity, specific organ and subcellular localizations as well as a metal ion in the active centre such as zinc, iron (heme), selenium, etc. The ancillary enzyme system has a kind of regeneration function by catalysing reduction reactions. The most prominent representative of ancillary enzymes is glutathione.[3]

Some antioxidants cannot be produced by the body, but must be supplied through nutrition. Table 2 shows the 4 most important nutritive antioxidants.

	daily requirement	food source
Tocopherol (Vitamin E)	14-15 mg	sunflower oil, palm oil, nuts, cereals, seeds
Ascorbic Acid (Vitamin C)	♀ 95 mg ♂ 110 mg	fruits, vegetables
Flavonoids	50-100 mg	green plants, onions, red grape juice, red wine, green or black tea, cocoa, chocolate
ß-carotene (Vitamin A)	1 mg	red, yellow and green vegetables

Table 2: Elective nutritive antioxidants, daily requirement and food source[6]

Tocopherol, a member of the vitamin E family, is a lipophilic radical scavenger. The two most important isoforms are α-Tocopherol, which is the dominant form in Vitamin E in humans, and γ-Tocopherol, which we ingest via the diet.[7] Located in the membranes, it offers protection against peroxide formation of unsaturated fatty acids or lipoproteins like the low-density lipoprotein (LDL).[2,8] Free radicals can oxidize unsaturated fatty acids to lipid radicals (RO•), which, by binding with oxygen, can form highly reactive lipid

peroxide radicals (ROO•). Tocopherol stabilizes these radicals by the transfer of a hydrogen atom; the newly formed vitamin E radical is relatively inert and gets regenerated under the influence of vitamin C and glutathione.[8] Ascorbic acid, better known as Vitamin C, is another essential micronutrient with a high antioxidant effect. In many enzyme reactions, such as the procollagen, carnitine and catecholamine biosynthesis, it serves as an electron-donating co-factor. In the same way, it is able to scavenge ROS and RNS as well as antioxidant derived radicals, like the α-tocopheroxyl radical. There is growing evidence that α-tocopherol may even have a prooxidative effect in the absence of ascorbic acid. Ascorbate itself and its radical have low reduction potentials and therefore do not tend to react spontaneously with, for example, O_2 to dangerous peroxyl radicals. Instead, vitamin C can be rapidly enzymatically or spontaneously chemically regenerated from its oxidized forms.[7]

Flavonoids, like rutin and quercetin, also belong to the group of antioxidant micronutrients. Their structure allows them to capture ROS and complex metal ions. In addition, they are able to prevent oxidative and nitrosative stress by inhibiting certain enzymes, like lipoxygenases, the myeloperoxidase (MPO), the iNOS, etc. MPO can play an important role in the formation of oxidized LDL (ox LDL), which is strongly associated with arteriosclerotic changes of vessels. In addition to the iNOS, the MPO is also involved in the formation of reactive nitrogen species. Epicatechin, a flavonoid found in cocoa, prevents the oxidation of LDL on the one hand and the nitration of tyrosine residues on the other through interfering with the MPO/nitrite and the copper ion systems. In this way flavonoids have a protective effect on the cardiovascular system.[5]

The most important precursor of vitamin A is ß-carotene.[8] It is an oxygen radical scavenger and plays an important part in the prevention of photo-oxidative processes. Due to chronic ultraviolet (UV) exposure or high UV doses, the development of oxidative stress can lead to pathological changes in the skin, such as premature aging or progression of skin tumours. Both the topical and the endogenous application of ß-carotene have a protective effect against these processes.[5]

Other molecules that can intercept oxygen radicals are urate and bilirubin. Heat shock proteins, which are produced in response to particularly stressful conditions, can weaken the effects of ROS.[1] In addition, transport proteins play a role in the detoxification process by exporting reactive species and their harmful products.[3]

1.3 Physiology of reactive species

The two main sources of reactive species in the human body are mitochondria and NADPH oxidases (Nox).[9] In mitochondria, reactive species are produced in the respiratory chain during Adenosine triphosphate (ATP) production. Normally at this process O_2 is reduced to water by complex IV, the cytochrome oxidase.[8] However, 2-4% of the oxygen reacts to ROS, which are neutralized by antioxidant enzymes of the mitochondrion and cytosol.[10] Here the ROS are therefore an undesirable metabolic by-product, which the body has to deal with. In contrast, the seven homologues of the Nox enzymes actively produce ROS. Upon stimulation, the NADPH oxidase is activated and transfers electrons to oxygen, creating the superoxide anion O_2-. It can be converted to other ROS; all of them can perform different tasks.[9]

A subclass of Nox, Nox2, is strongly expressed in certain leukocytes such as granulocytes or macrophages.[9,11] They are activated by signs of invading pathogens, like binding of specific pathogen recognition receptors, microbial products, opsonized particles and integrin-dependent adhesion. The enzyme starts the production of O_2- molecules, which are converted into more disinfectant metabolites.[11] An important step here for defence in neutrophil granulocytes is the formation of hypohalous acids such as hypochlorous acid from H_2O_2.[9,11] The strong antimicrobial activity of these compounds damages both the pathogen and the defence cell itself and possibly even other endogenous cells.[4] However, Nox2 stimulated apoptosis of neutrophil granulocytes may activate a macrophage subtype associated with anti-inflammatory effects and repair of injury via release of neutrophil granular proteins. In this way, Nox2 is involved not only in the immune response itself, but also in the regulation of acute inflammation.[11] In a rare disease called chronic granulomatosis, the granulocytes are not able to produce enough ROS due to a reduction of Nox2 activity as a result of a genetic defect. These patients suffer from immunodeficiency, which is manifested abscesses, pneumonia, lymphadenitis, etc.[12] Inflammatory cytokines and endotoxins also trigger the induction of iNOS, which then produces NO. By forming other RNS and interacting with ROS, it contributes to the same type of immune defence.[8]

The Nox isoforms 1, 2, 4 and 5 are located in the blood vessels and take over an important part of the signal transduction there. The ROS produced promote the action of the phosphoinositide 3 (PI3) kinase pathway, through which, for example, growth factors,

insulin and erythropoietin unfold their effect, by inhibiting phosphatase and tensin homolog (PTEN). In this pathway, the signal is transmitted via enzyme-mediated phosphorylation of amino acids. The task of ROS is to prevent the phosphatase from removing the phosphate group.[1,9]

Nitrogen monoxide plays an even more central role in signal transmission. NO activates the guanylyl cyclase, which catalyses the formation of cyclic guanosine monophosphate (cGMP) from guanosine-5'-triphosphate (GTP). cGMP works as a second messenger and mediates vasodilatation, among other things.[4] In this way, the function of the gastrointestinal tract and the penile erection are regulated. NO donors such as nitroglycerin are also used to treat angina pectoris in order to support blood flow to coronary arteries. In neurons, NO acts as a neurotransmitter and is involved in learning processes.[8]

Furthermore, ROS are required for the synthesis of otoliths, calcium carbonate stones in the equilibrium organ for the measurement of linear acceleration. H_2O_2 is moreover involved in the oxidation of iodide, which is essential for thyroid hormone production.[9]

1.4 Pathophysiology of oxidative stress

Excessive concentrations of reactive species can cause damages at the molecular level, which may have serious consequences for the body. Developed, they are very reactive and are able to oxidize molecules like lipids, proteins, etc. In this way, oxidative stress is involved in many pathological processes, such as vascular damages, nervous disorders, autoimmune diseases, etc.[1]

There are three main ways oxidative stress can damage the cell; through lipid peroxidation, oxidative modification of proteins and DNA damage. These reactions, which partly merge into each other, cause ageing, carcinogenesis and defects in organs.[13]

1.4.1 Lipid peroxidation

Figure 1 shows the mechanism of lipid peroxidation. It may be assumed that the cellular location for lipid peroxidation is the endoplasmic reticulum. Although the exact mechanism is unclear, many reactions might be associated with the initiation of lipid peroxidation, such as the metal induced Haber-Weiss reaction, the Fenton reaction etc. In summary, it can be observed that superoxide radical plays an important role among the reactive species and that peroxidation process is catalysed in the presence of a transition metal ion. In the first step, hydrogen is abstracted from an unsaturated fatty acid, which then reacts with oxygen if present. The resulting lipid peroxy radical can start a chain of radical reactions. By breaking C-C bonds, unsaturated fatty acids aldehydes and alkyl radicals are formed, which again trigger new radical chain reactions. The most important products generated during these processes are malondialdhyde (MDA) and 4-hydroxynonenal/4-hydroxy-2-nonenal (HNE).[3,13] The resulting aldehydes, such as MDA and HNE, are so dangerous because, unlike ROS, they have an uncharged structure. It allows them to move freely through membranes and cytosol, modifying proteins, nucleic acids and membrane lipids.[13]

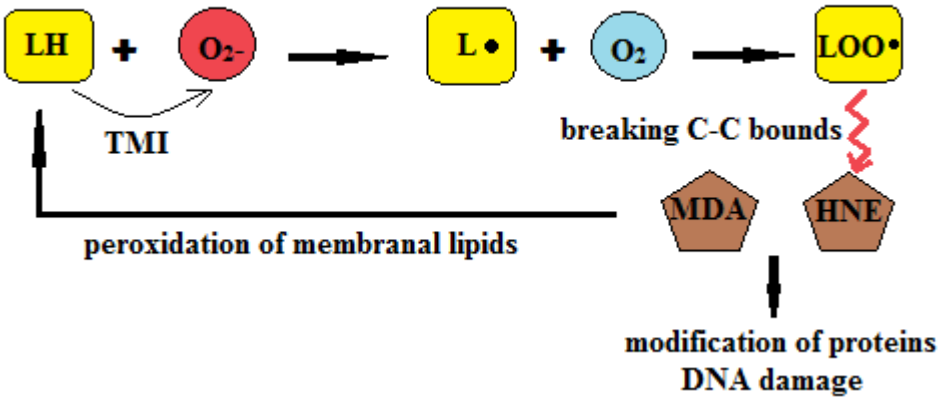

Figure 1: Lipid peroxidation. LH = lipid, O_2- = superoxide anion, TMI = transition metal ion, L• = lipid radical, O_2 = Oxygen, LOO• = lipid peroxy radical, MDA = malondialdhyde, HNE = 4-hydroxynonenal/4-hydroxy-2-nonenal (adapted from [13])

As already mentioned, however, our body has systems to reduce lipid peroxidation. These generally include antioxidant enzymes such as SOD, peroxidases and catalases, which, on the other hand, do not explicitly inhibit the peroxidation of lipids. In contrast, tocopherol reacts specifically with lipid radicals and can thus interrupt the reaction chain.[3]
Failure of these defence mechanisms could, possibly, result in fatal consequences for the cell. The products of these radical chain reactions can damage parts of plasma and intracellular membranes, resulting in changes of the permeability of intra and extracellular compounds. For example, hydrolytic enzymes could migrate from the lysosome and start digestion process. Due to the loss of membrane architecture or direct radical reaction, inactivation of membranal enzymes, protein receptors and, subsequently, the respiratory chain may occur. If the defect of the plasma membrane is too severe, the entire cell is lysed.[3]

1.4.2 DNA damage

Radicals and products of lipid peroxidation react with the heterocyclic bases and sugar residues of the nuclear and mitochondrial DNA, resulting in single and double strand breaks.[13,14] Normally, the body's repair systems are able to reverse the DNA strand damage. However, if the damage is too severe, the introduction of programmed cell death, apoptosis, by the protein 53 (P53) follows.[8] Oxidative stress can also directly induce apoptosis via caspase activation or changes in calcium (Ca^{2+}) permeability of the cell membrane.[1] If, on the other hand, the DNA damage is not detected or if repair is not possible, mutations occur.[8] Double strand breaks are of particular importance here, as the resulting severe genetic mutations can lead to various diseases and carcinogenesis.

Mitochondrial DNA appears to be especially vulnerable to oxidative stress because it is more than twice as often affected by oxidative damage as nuclear DNA is. This could be explained by the "vicious cycle theory of mitochondrial ROS production". Since mitochondrion is one of the main sites of ROS formation, DNA damage occurs simply because of the proximity to reactive species. The resulting mitochondrial dysfunction can lead to an increased formation of ROS, which again endangers the DNA. In addition, mitochondrial DNA has neither protective histones nor sufficient repair mechanisms to reduce oxidative damage. Oxidation products of both nuclear and mitochondrial DNA are associated with the development of various neurodegenerative diseases, aging, tumour progression, etc.[13]

1.4.3 Protein modification

Aldehydic end products like malondialdehyde are also able to affect the apolipoproteins B-100 (ApoB-100) in LDL, modifying them to immunogenic epitopes.[15] Enzymes such as MPO and vascular peroxidase-1 (VPO1), which is strongly expressed in vascular endothelial cells, mediate those processes of oxidation of lipids and proteins in LDL.[16] The resulting oxLDL is not only cytotoxic itself, but also leads to the accumulation of cholesterol in macrophages and foam cell formation. These processes are essential for the development of arteriosclerosis in vessels. In addition, foam cells provide an inflammatory response through secretion of inflammatory mediators.[17] Some of them, such as tumour necrosis factor alpha (TNF-α) and lipopolysaccharide (LPS), may cause an increased expression of VPO 1, implying a higher level of oxLDL.[16] The fact that oxidatively modified LDL is immunogenic could also lead to the formation of autoantibodies against it. These antibodies may not only be independent markers for the progression of arteriosclerosis in the carotid artery; they are also used as established parameters for the measurement of oxidative stress in the body.[18]

However, oxidative stress can not only contribute to development of arteriosclerotic changes in vessels and the associated reduced perfusion, it can also cause damage after therapy in reperfusion phase.[15] After an insufficient supply of oxygen, the re-entry of O_2 can lead to an increased formation of ROS.[1] One of the reasons for this could be the activation of the xanthine oxidase pathway, which is a producer of O_2-. Elicitors of this pathway include heavy muscle contractions, hypoxia and ischemic reperfusion scenarios. Under these conditions, a high calcium release leads to the activation of a protease that

converts the xanthine dehydrogenase, an enzyme involved in purine metabolism, into xanthine oxidase. In the last two steps of purine degradation, xanthine oxidase catalyses the formation of uric acid from hypoxanthine, creating O_2- as a by-product. High ROS levels can lead to a strong activation of ryanodine receptor I (RyRI), inducing aberration in Ca^{2+} release. These abnormalities in calcium homeostasis for example in cardiac cells could result in contractile dysfunction, arrhythmia and myopathy.[19]
Proteins modified by HNE and MDA can alter transcription factors such as the nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-kB) transcription factor, regulating apoptosis, cytokines, cyclins, growth factors, immune regulatory proteins, etc. They can also induce caspase pathways including the Fas/Fas-associated protein with death domain (Fadd) dependent pathway of apoptosis. HNE-modified alpha-enolases or pyruvate kinases, which are both enzymes part of the glycolysis, can lead to altered energy metabolism. All of these processes are involved in the pathogenesis of ageing and cancer.[13]

As already mentioned, part of the oxygen can react to ROS during ATP production. This happens mainly in the presence of NO. NO is able to bind to complex IV and thus inactivates or reduces the activity of the respiratory chain. The result is the production of O_2- due to one electron oxidation of ubiquinol (UQH2) mediated by NO. Then the generated superoxide anion can react with NO and form peroxynitrite (ONOO-), which is a very destructive radical. In this way different reactive nitrosative species can accumulate and lead to nitrosative stress.[19] Target molecules for RNS are primarily molecules with thiol groups and proteins that complex metal ions via sulfur atoms, like transcription factors with zinc fingers. By modifying these compounds, their functions and properties can be altered, which can lead to the development of various diseases.[5,20]

Reactive species arise in the body as an undesirable metabolic by-product, but are also actively formed for physiological reasons. To prevent damage, antioxidants capture the radicals formed. Some of the antioxidant substances are produced by the body itself, while others must be supplied through food. An imbalance between antioxidants and radicals is known as oxidative stress and may result in many pathological processes, such as carcinogenesis, atherosclerotic plaque formation and autoimmune reactions.[5,13] Now it would be interesting to know which factors humans could use to influence oxidative stress.

1.5 Oxidative stress and lifestyle

There are many lifestyle factors that can provoke oxidative stress, such as smoking, alcohol, etc.[21] However, the focus of this thesis is set on the influence of nutrition and physical activity.

1.5.1 Nutrition

The association of oxidative stress and nutrition is in the focus of medical research. Not only because of the antioxidants supplied by food; nutrition itself can cause oxidative stress, even under normal physiological conditions. There are dietary factors that can have inflammatory and pro-oxidant effects.[21]

Diet may trigger epigenetic changes already in perinatal development into adulthood through various pathways, such as the progression of metabolic risk factors and the generation of oxidative stress. An important factor here is over-nutrition, as it could lead to the formation of free radicals and consequently oxidative stress. Inadequate or excessive food supply may create a postprandial imbalance between pro- and antioxidant processes.[21] The supply of glucose stimulates ROS formation in polymorphonuclear leukocytes and mononuclear cells, and a decrease in the antioxidant α-tocopherol.[22] An increase in ROS formation is also observed in the intake of fat and protein, but fat leads to a more significant and prolonged increase in lipid peroxidation compared to protein.[23] Postprandial hyperglycaemia and hyperlipidaemia are conditions whose persistent accumulation could contribute to the development of reproductive and metabolic diseases.[21]

The pathomechanism of how overeating can lead to oxidative stress is demonstrated in figure 2. Over-nutrition and reduced physical activity result in high concentrations of glucose and free fatty acids in the blood. The mitochondria in various cells are thus stimulated to produce more energy, which consequently leads to increased formation of ROS. Certain cells in the organism, such as the muscle adipocytes, are able to resist this condition and become insulin resistant, leading to reduced permeation of glucose and free fatty acids into the cell. Consequently, their plasma concentrations are elevated. Other cells, such as the endothelial cells of the vessels and also the ß cells of the pancreas are

insulin-independent, they are not able to regulate the influx of glucose and free fatty acids. High glucose and free fatty acid concentrations lead here to an increase in energy production, resulting in oxidative stress and associated functional disorders. In the endothelium, this could cause the development of cardiovascular diseases; a ß-cell dysfunction results in changes in insulin secretion. A decrease in insulin secretion in the first postprandial phase could lead to impaired glucose tolerance, which again increases postprandial hyperglycaemia. Permanent conditions such as these may gradually exhaust the ß-cells and trigger the development of diabetes.[21]

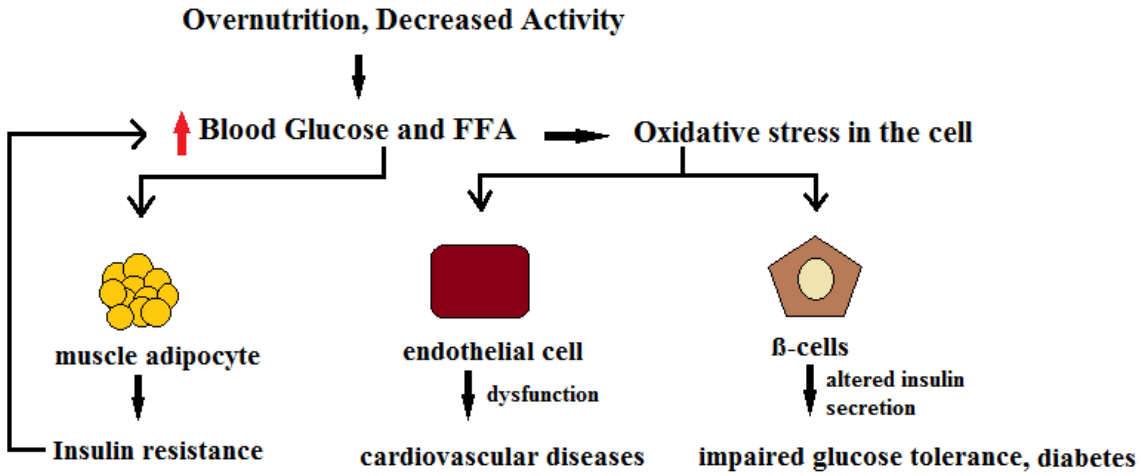

Figure 2: Pathomechanism of overnutrition and oxidative stress. Red arrow represents increase; FFA = free fatty acids (adapted from [21])

Permanently reduced food intake may also have a negative effect on the metabolism. A deficiency of certain nutrients promotes the overproduction of inflammatory mediators, resulting in a chronic inflammatory state in the body.[24] This leads to an increase in catabolism and a reduction in appetite, which in turn increases protein loss. Persistent conditions like these result in malnutrition. Individuals with malnutrition in combination with a lack of antioxidants seem to be more susceptible to oxidative stress. There is a significant parallel association between a decrease in plasma micronutrients and an increase in the intensity of oxidative stress in critically ill children. In addition, malnourished patients show an increase in ROS compared to normal weight patients. This could be due to the chronic inflammatory state with consequent increase in ROS production, on the one hand, and to insufficient intake of carbohydrates, proteins and vitamins associated with the decrease in antioxidant mechanisms, on the other.[25] A lack of antioxidants caused by malnutrition is also related to an increased risk of developing

diseases such as chronic obstructive pulmonary disease (COPD), asthma, neurodegenerative diseases, gastrointestinal diseases, etc. and contributes to poor therapeutic outcomes of these diseases.[26]

1.5.2 Physical activity

There are different results concerning the influence of physical activity on oxidative stress.[21]
Regular physical activity seems to be a way to enhance antioxidant activity and especially to reduce LDL oxidation.[27] According to a trial, a parameter for antioxidant capacity, trolox equivalent antioxidant capacity (TEAC), is significantly higher in trained than in untrained individuals.[28] In addition, regular exercise training is believed to be a valuable physiological tool to prepare the myocardium for sustained oxidative stress. Through exercise, the body's resistance to oxidative stress can be adapted and benefits to the immune system can be achieved.[29]
On the other hand, there is evidence that excessive exercise can increase oxidative stress in the body. The skeletal muscle is considered to be a clear production site for ROS and NO. Intensive training could therefore have the exact opposite effect as described above.[19] For example, increased oxidative stress parameters are found in American football players during competition season.[29] Furthermore, eccentric training and prolonged cycling in untrained individuals appear to increase oxidative stress level throughout the body.[19]

It seems that regular physical activity has a positive effect on the body's resistance and antioxidant capacity, but also that extreme physical activity, especially in untrained subjects, increases the body's oxidative stress.

1.5.3 Anorexia nervosa

Very restrictive food intake, laxative abuse and excessive physical activity could result in underweight and the development of eating disorders such as anorexia nervosa.[1] Anorexia nervosa is characterized by significant and conscious weight loss, distorted body perception and a pathological fear of weight gain.[30] The disease often has a chronic course and is associated with severe vegetative-endocrine disorders and high mortality.[1,30]

A clinical relation between anorexia nervosa and oxidative stress was found in studies with mice, among others. The authors postulated that in anorexic mice there is a deficiency of a complex of the respiratory chain in the mitochondria and therefore the ROS production is increased. The resulting oxidative stress is believed to damage sensitive hypothalamic neurons and thereby induce an impairment of the appetite-regulating neuronal networks.[31] In accordance with these results, a trial with young women suffering from anorexia nervosa also found a disturbance of the activity of this complex, complex I, and increased production of ROS. Malnutrition associated with vitamin and antioxidant deficiencies may also lead to increased oxidative stress.[30] However, it should be noted that there are still some deficiencies in research and it is not clear whether oxidative stress is the cause or consequence of anorexia nervosa.[32]

1.5.4 Overweight and obesity

Persistent over-nutrition and physical inactivity may lead to the development of overweight and even obesity. Due to the way of life in the western world, the incidence rate of overweight and obesity is steadily increasing.[33] This development begins in childhood, and these children are at high risk of becoming obese when they are adults.[34] Obesity is an independent risk factor for many diseases such as arterial hypertension, diabetes mellitus type II, hyperlipidemia, cardiovascular diseases etc. Being overweight by more than 40% is associated with a double probability of premature death.[1]
The definition of overweight and obesity is an abnormal or excessive accumulation of fatty tissue that is associated with a health risk.[33] In obese individuals, the mitochondria of the white adipose tissue appear to be the main origin of reactive species. Adipose tissue releases pro-inflammatory mediators, such as C-reactive protein (CRP) or TNFα, which not only cause damage themselves but also contribute to the formation of radicals. In addition, the expression of antioxidant enzymes is also suggested to be reduced in obese people. This permanent low grade inflammatory status could be associated with a greater increase in oxidative stress.[34]

In fact, there are many studies suggesting that obesity is associated with oxidative stress.[35,36] Obese individuals appear to have higher fasting oxidative stress biomarkers and experience greater levels of exercise-induced oxidative stress.[35] In addition, the acute and adverse oxidative reactions after high-fat food intake seem to be much more severe than in normal weight, well-trained subjects.[21] The connection between oxidative stress and

obesity could be due to the adipose tissue per se or to the conditions associated with obesity, such as hyperglycaemia, hyperlipidaemia or hypertension.[36] However, research suggests that fat is an independent factor in ROS formation.[34] The accumulation of adipose tissue, independent of hyperglycaemic status, may lead to a systemic increase in oxidative stress. This seems to be related to a dysregulated production of adipocytokines.[37]

The Body mass index (BMI), body weight in kg divided by height squared, is an established measure for the classification of body weight. It determines normal weight, underweight, overweight and obesity in accordance with the WHO recommended categories.[38] However, an in-depth analysis of body composition is reasonable for obesity classification since the BMI has known shortcomings in predicting body fat correctly. Especially the excess of body fat has been shown to trigger the processes in the organism that lead to the adverse metabolic states in obesity.[39]
Many studies have already identified a correlation between a high BMI and oxidative stress.[33,40] Either a wide range of subjects[40] or a certain target group, such as children[34], was selected. It is interesting to note that there is a certain research gap in young women and oxidative stress.

1.6 Aims and Hypothesis

The aim of this thesis was to describe 5 established oxidative and 2 nitrosative stress parameters in a cohort of females with different nutritional status and bring the results in a possible context with anthropometric and lifestyle-associated parameters.

Major aims:

(1) To identify to what extent Total antioxidant capacity (TAC), total oxidant capacity (TOC), endogenous peroxidase activity (EPA), autoantibodies against oxidized low-density lipoprotein (oLAb) and malondialdehyde-modified low-density lipoprotein (MDA-LDL-IgM), asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) in serum in AN differ in comparison to other BMI groups and athletes.

(2) To investigate associations of TAC, TOC, EPA, oLAb, MDA-LDL-IgM, ADMA, SDMA and parameters of body composition, depression scales, physical activity, nutritive assessment and laboratory parameters.

We hypothesized that:

(1) TAC, TOC, EPA, oLAb, MDA-LDL-IgM, ADMA, SDMA show significant differences between groups.

(2) TAC, TOC, EPA, oLAb, MDA-LDL-IgM, ADMA, SDMA are associated with the investigated parameters (body composition, depression scales, physical activity, nutritive assessment and laboratory parameters).

2 Material and methods

The data used for this thesis was obtained from the "Energy Sensing in Anorexia nervosa"-study. The study was approved by the local ethical commission of the Medical University of Graz (EK number MUG-26-383ex13/14). All participating patients signed the informed consent and agreed with the blood sampling as well as with the anonymous evaluation of their data. The study was conducted in accordance with the declaration of Helsinki.

2.1 Subjects

The patient population consisted of 107 female subjects aged between 17 and 39 years. The subjects were assigned to 5 groups with different nutritional status, whereas the group assignment was based on BMI categories and lifestyle factors.

2.2 Anthropometric and lifestyle parameters

Anthropometry

For anthropometric information the BMI, body weight in kg per/body height in m squared[41], waist and hip circumference in cm were measured.

Smoking behaviour

Standardised and validated questionnaires and interviews were used to record exercise and nutritional behaviour. The smoking behaviour was assessed by the Fagerström score, a method for determining physical tobacco dependence[42].

Physical activity

The international physical activity questionnaire (IPAQ) was used to measure the activity level of the subjects. The instrument developed by Booth et al. for recording appropriate movement behaviour includes 5 parts: job-related physical activity; transportation physical activity; housework, house maintenance and caring for family; recreation, sport and leisure-time physical activity and time spent sitting. Time reference are the last 7 days. Results are expressed as metabolic equivalent (MET) minutes per week. The IPAQ is internationally encouraged for monitoring and research purposes.[43]

Depression status

Depression status was evaluated using the Beck-Depressions-Inventory (BDI) and Hamilton Depression Scale (HAMD). The BDI is one of the most popular self-assessment instruments worldwide and is intended to reflect the severity of depressive symptoms with a subjective self-assessment of 21 items.[44] The HAMD, invented by Max Hamilton in 1960, encompasses similar categories but is based on external judgment.[45]

Nutritional Intake

Nutritional Intake was assessed by interviewer guided twenty-four hours recalls. The nutritional interviews were performed twice within two weeks. For the evaluation of these records the nutritional software nut.s was used, which is a modular constructed software to calculate nutritional contents and manage allergens. Based on valid and updatable data from various nutritional studies and guidelines, this software is able to perform an automated evaluation of a large number of nutritional anamnesis.[46] In this way, the exact nutritional data of the 107 young females could be analysed. In addition to energy in kcal, proteins, carbohydrates and sugar, fat and its saturated fatty acids and dietary fibres, electrolytes such as sodium and calcium as well as the amount of water and alcohol were recorded. For this thesis, antioxidant parameters were also important, so levels of carotene, vitamin C, vitamin B12, vitamin E, vitamin D, omega-3 fatty acid and omega-6 fatty acid in food intake were determined.

All subjects, with the exception of the majority of anorexia nervosa patients undergoing therapeutic treatment, followed their usual dietary habits. Diets were a criterion for exclusion.

2.3 Laboratory parameters

To find evidence that oxidative stress is related to the nutritional status in young women, established measurement methods and biomarkers of oxidative stress are necessary. Biomarkers are indicators of biological or pathogenic processes that can be objectively measured and evaluated. They should be able to reflect pathways or correlate with the severity of diseases in order to be used as diagnostic tools. Properties such as stability and detectability are of great importance.[47]

Measuring oxidative conditions can be relatively difficult because reactive species are very unstable and have too short half-lives to be measured directly in vivo. Therefore, the determination of molecular products from reactions with ROS and RNS, which are usually much more stable, is usually accessed.[47] A cost-effective, reliable and effortless method to measure the oxidative status is the TOC®/TAC® assay of Labordiagnostik Nord, which will be described in detail later. The measurement is based on the activity of peroxidases, a widespread family of antioxidative enzymes, the most important being glutathione peroxidase. Through these assays endogenous peroxidase activity (EPA), total oxidant capacity (TOC) and total antioxidant status/capacity (TAC) can be quantified. These parameters are frequently used to study oxidative stress and are considered established.[48] To quantify oxidative damage, lipid peroxidation products are often used as biomarkers. One of the most important products is ketoaldehyde MDA, which is produced by peroxidative decomposition of unsaturated lipids. As described above, it can change the Apo B-100 protein in LDL; this product is immunogenetic. The autoantibodies formed against MDA-modified LDL (MDL-LDL IgM) are associated with the extent of arteriosclerosis and coronary heart disease.[47] While oxidized LDL is already an established marker for early signs of atherosclerosis, the role of autoantibodies against oxLDL (oLAb) is discussed controversially.[17] On the one hand, oLAb seems to have a protective effect against oxidative stress, since there is an inverse relation between carotid intima-media thickness and plasma concentration of oxLDL. On the other hand, oLAb is reported to be an indication parameter for lipid peroxidation in vivo. However, research suggests that the immunological effect of oLAbs appears to be anti-atherogenic rather than promoting atherosclerosis. Immuno assays for the measurement of oLAb and MDL-LDL IgM are also considered to be the most reliable method to measure lipid peroxidation in humans.[33]

The endogenous methyl arginines asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) are derivatives of the amino acid L-arginine, a precursor for the formation of nitrogen monoxide. Cardiovascular events and immunological processes are associated with an increase in the concentration of these parameters.[49,50] They can also be used as indicators of nitrosative stress. In Table 3 all described parameters are listed.

	name	significance
TAC	total antioxidant capacity	summary marker for all antioxidative enzymes and scavengers
TOC	total oxidant capacity	summary marker for oxidant status
EPA	endogenous peroxidase activity	summary marker for the activity of all peroxidases
oLAb	autoantibodies against oxidized LDL	marker for lipid peroxidation
MDA-LDL IgM	autoantibodies against MDA modified LDL	marker for lipid peroxidation
AMDA	asymmetric dimethylarginine	marker for nitrosative stress
SDMA	symmetric dimethylarginine	marker for nitrosative stress

Table 3: Established oxidative and nitrosative stress parameters

2.3.1 Total antioxidant capacity (TAC) and total oxidant capacity (TOC)

Serum total peroxide concentrations were determined with a rapid enzymatic in vitro diagnostic assay, TOC® (Labor Diagnostic Nord, Nordhorn, Germany) as previously described by Tatzber et al. The method is based on the enzymatic reaction of peroxides and peroxidases to produce oxygen, which oxidizes the chromogenic substrate 3,5,3´,5´-tetramethylbenzidine (TMB). For the assay protocol, 10µl standards (10µM–1mM H_2O_2) and samples are incubated with the reaction mixture (200µl), consisting of horseradish peroxidase (HRP) (25mU), TMB, and substrate buffer in a proportion of 1:10:100, in uncoated microtiter plates. During oxidation of TMB it changes its colour from colourless to blue. First an absorbance reading is done immediately at a wavelength of 450nm in a plate reader. After an incubation period for 15min the reaction cascade is finished by the addition of sulfuric acid and the colour of the mixture changes to yellow. A second absorbance reading at 450nm is performed. Serum peroxide levels are calculated as the difference of the absorbance readings relating to the hydrogen peroxide standard curve. Results are expressed as arbitrary units (AU).[34,48]

Antioxidants inhibit this reaction and can be detected analogously on the basis of the indirect proportionality of this inhibition reaction with the TAC® assay. TAC is a

summary marker for all antioxidative enzymes and scavengers, which participate in detoxification and neutralization of oxidative stress.[34]

2.3.2 Endogenous peroxidase activity (EPA)

The EPA® assay (Labor Diagnostic Nord, Nordhorn, Germany) for determination of the endogenous peroxidase-activity is based on the similar principle as the TOC® assay described previously. Instead of HRP, however, hydrogen peroxide is added to the reaction mixture. Serum peroxidase activity is calculated in relation to the HRP standard curve. Results are expressed as milliunits per millilitre.[48]

2.3.3 Autoantibodies against oxidized LDL (oLAb) and malondialdehyde-modified LDL (MDA-LDL-IgM)

Titers of oLAb® were measured in serum with a commercial enzyme immunoassay supplied by Biomedica (Vienna, Austria) according to the method of Tatzber and Esterbauer 1995. This enzyme-linked immunosorbent assay (ELISA) is based on the binding reaction of the 1:50 diluted samples to the LDL previously oxidized by cupric ions and bound to the microtiter wells. Detection was done by binding a secondary, peroxidase-coupled anti-IgG antibody, which permitted colorimetric detection of this enzyme with TMB as substrate. Results were expressed as mU/ml.[15]

Malondialdehyde-modified LDL (MDA-LDL IgM®) was measured by ELISA (Omnignostica GmbH, Höflein/Klosterneuburg, Austria) as previously described by Resch, Tatzber et al. MDA-LDL IgM titre were expressed as U/l−1 using external standardization with a standard supplied by the company.[17]

2.3.4 Asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA)

Asymmetric dimethylarginine (AMDA) and symmetric dimethylarginine (SMDA) were measured in Serum or ethylenediaminetetraacetic acid (EDTA) plasma by the AMDA®-ELISA and the SDMA®-ELISA, which are competitive enzyme immunoassays developed by DLD Diagnostika GmbH. First, AMDA is quantitatively converted into N-acyl-AMDA respectively SMDA into N-acyl-SMDA by an acylation reagent. Solid phase bound and free solution antigen compete for a defined number of antibody binding sites. When the

system is in equilibrium, the unbound antigen-antibody complex is removed in one washing step and the corresponding bound complex is detected by a peroxidase conjugate and determined by the conversion of TMB. The TMB/peroxidase reaction is stopped by sulfuric acid and measured at 450 nm. The concentration of the antigen-antibody complex bound to the solid phase is inversely proportional to the concentration of the antigen in the sample. The optical density (OD) values of the standards (linear) are plotted against the corresponding concentrations of the standards (logarithmic). The concentrations of the controls and patient samples can then be read directly from the calibration curve in µmol/l.[51]The endogenous methyl-arginines ADMA and SDMA are derivatives of the amino acid L-arginine. L-arginine, on the other hand, is the precursor to the formation of nitric oxide (NO) in the human body. Several clinical and experimental studies have shown that disease processes associated with NO and nitrosative stress can lead to an increase in the concentration of AMDA and SMDA in plasma or serum.[49,50]

2.4 Statistical analysis

All statistical data analysis was performed using the IBM SPSS version 25 statistic and analysis software. First, data was evaluated for normal distribution using the Shapiro-Wilk test. Results are represented as mean and standard deviation for normal distribution and median and interquartile range for non-normally distributed data.
To identify differences in oxidative and nitrosative stress parameters between the five groups of different nutritional status, the non-parametric Kruskal Wallis Test for more than 2 independent samples was chosen, additionally the Anova was performed for parameters with normal distribution. For the pairwise comparison the Dunn-Bonferoni (non-parametric) and the Tukey (parametric) tests were used as post-hoc tests. Above a p-value of <0.05 the result was considered statistically significant.

2.5 Literature research

A literature search was conducted for background information and to find comparative studies. The research consisted of studying textbooks and screening medical databases. The majority of the studies retrieved were available in the database "Pubmed" of the National Center for Biotechnology Information (NCBI), the others were found using the search engine "Google scholar" of the company Google LCC. Additionally, literature was

provided by the Division of Immunology and Pathophysiology of the Medical University of Graz.

3 Results

3.1 Anthropometric and lifestyle data

The cohort of 107 young females could be assigned to 5 groups with different nutritional status, with group 1 of the anorexia nervosa (AN) patients having a BMI between 13.32 and 17.19 (n=18), group 2 of the normal weight patients between 19.02 and 24.48 (n=27), group 3 of the overweight patients between 25.12 and 29.34 (n=22), group 4 of the obesity patients between 30.44 and 46.89 (n=20) and group 5 of the athletes between 19.09 and 25.88 (n=20).

Significant differences were found between the groups in all anthropometric and lifestyle-associated parameters. The group of athletes had the youngest participants with a significant difference to the overweight and obese subjects. Body weight, BMI, hip and waist circumference increased steadily from group 1 to group 4. Group 5 showed similar values as group 2 of the normal weight. In terms of physical activity, group 5 had on average the highest level of MET-minutes per week, the median value was more than twice as high as the median of anorexia nervosa patients and the overweight. Significant differences were also found between these groups. Furthermore it is interesting to note that the obesity group contained some outliers and therefore had on average a higher IPAQ score than the overweight and anorexia nervosa groups. The BDI as well as the HAMD score were considerably higher in the anorexia nervosa patients than in all other groups. On average, the lowest depression levels were achieved by normal weight and athletes. The characteristics of the different groups are summarized in Table 4.

	AN patients group 1 (n=18)	Normal weight group 2 (n=27)	Overweight group 3 (n=22)	Adiposity group 4 (n=20)	Athletics group 5 (n=20)	p-values
Age (years)	22 (6)	24 (5)	24 (6)	26 (10)	21 (3)	p = 0.002
Weight (kg)	42 (7.2)	59 (10)	74.7 (7.8)	98 (13.1)	64.6 (6.8)	p < 0.001
BMI (kg/m^2)	15.81 (1.74)	21.72 (2.87)	27.08 (1.16)	33.15 (4.52)	21.59 (2.5)	p < 0.001
Waist (cm)	60 (5.5)	70 (6.5)	79 (6.8)	95.5 (18)	71 (6.7)	p < 0.001
Hip (cm)	79 (4.5)	93 (10)	107 (8.3)	122.5 (12)	99 (5.6)	p < 0.001
BDI Score	26 (18)	2 (4)	6 (12)	5 (7)	2.5 (5)	p < 0.001
HAMD Score	18 (11)	2 (5)	5 (6)	4 (5)	2 (4)	p < 0.001
IPAQ Score	2154 (3455)	3792 (6095)	2486.25 (3897)	3352.5 (6384)	6012.25 (4139)	p = 0.018

Table 4: Characteristics of the study population. Data are displayed as median value and interquartile range. p-values of Kruskal Wallis test: significant differences ($p < 0.05$) of all listed parameters could be found.

3.2 Nutritional Intake

Table 5 lists all important nutrient data of the 5 groups. Significant differences were found between the groups in daily intake of saturated fatty acids, dietary fibres, alcohol, carotene, vitamin C, vitamin B12 and vitamin D.

The group of obese women consumed on average the most saturated fatty acids, with a significant difference (Tukey p = 0.005) to the anorexia nervosa patients. The athletes consumed more fibres than the overweight (Dunn Bonferroni p = 0.042). The alcohol intake was highest among normal weight women with a significant difference to the obese group (Dunn Bonferroni p = 0.013), who mostly did not consume any alcohol at all. In addition, obese women consumed significantly less carotene than anorexia nervosa patients (Dunn Bonferroni p = 0.008), normal weight women (Dunn Bonferroni p = 0.005) and athletes (Dunn Bonferroni p = 0.001). The vitamin C intake of the overweight was significantly lower compared to the anorexia nervosa patients (Dunn Bonferroni p = 0.028) and the athletes (Dunn Bonferroni p = 0.043). The normal weight subjects consumed less vitamin B12 (Dunn Bonferroni p = 0.037) and vitamin D (Dunn Bonferroni p = 0.011) than the anorexia nervosa patients.

	AN patients (n=18)	Normal weight (n=27)	Overweight (n=22)	Adiposity (n=20)	Athletics (n=20)	p-values	daily requirement[6]
energy (kcal)	1919 (1472)[a]	1894 (896)[a]	1749 (328)[a]	2065 (616)[a]	2011 (585)[a]	p = 0.187	~2150
proteins (g)	78.6 (51.5)[a]	62.1 (32.9)[a]	60.5 (20)[a]	73.9 (32.3)[a]	73.5 (41.5)[a]	p = 0.072	48
carbohydrates (g)	232.8 (215.5)[a]	200.9 (107)[a]	188.8 (61.4)[a]	209.6 (71.1)[a]	233.4 (104)[a]	p = 0.240	230
sugar (g)	121.7 (118.1)[a]	87.3 (60)[a]	76.9 (46)[a]	110 (68.1)[a]	100.7 (86.3)[a]	p = 0.139	45
fat (g)	79 (39.7)[a]	80.2 (48.8)[a]	66.6 (20.7)[a]	78.9 (33.4)[a]	76 (34.5)[a]	p = 0.46	73
sat. fatty acids (g)	24.6 ± 12.6[b]	32.9 ± 13.4[b]	32 ± 8.2[b]	38.5 ± 13.4[b]	32.6 ± 11.6[b]	p = 0.033*	8
dietary fibres (g)	19.4 (12.8)[a]	20.7 (8.4)[a]	16.6 (6.6)[a]	17.4 (6.6)[a]	23.8 (14.7)[a]	p = 0.05*	30
sodium (g)	2.31 (1.18)[a]	2.41 (1.23)[a]	2.73 (1.23)[a]	3 (1.15)[a]	2.55 (0.99)[a]	p = 0.159	1.5
calcium (mg)	1162 (752)[a]	787 (428)[a]	868 (371)[a]	978 (325)[a]	937 (595)[a]	p = 0.28	1000
water (l)	2.84 (1.29)[a]	3.04 (1.54)[a]	3.5 (1.27)[a]	2.99 (1.24)[a]	3.79 (2.02)[a]	p = 0.055	2.65
alcohol (g)	0.07 (0.57)[a]	0.59 (9.16)[a]	0.19 (7.52)[a]	0 (0.13)[a]	0.05 (0.72)[a]	p = 0.009*	< 10
carotene (Counts)	34500 (20500)[a]	32000(18000)[a]	26000 (13250)[a]	20000(14000)[a]	36500 (19000)[a]	p < 0.001*	
vitamin C (mg)	125.7 (134.7)[a]	80.5 (74.6)[a]	68 (42.1)[a]	87.2 (151)[a]	133.2 (115)[a]	p = 0.013*	95
vitamin B12 (µg)	4.26 (2.7)[a]	2.99 (2.52)[a]	2.97 (1.62)[a]	4.29 (2.58)[a]	3.63 (1.93)[a]	p = 0.01*	4
vitamin E (mg)	13.06 (19.11)[a]	13,52 (5.45)[a]	10.46 (6.52)[a]	12.17 (10.09)[a]	14.03 (11.85)[a]	p = 0.323	14-15
vitamin D (µg)	5.11 (11.44)[a]	1.81 (1.18)[a]	1.78 (2.19)[a]	2.45 (2.7)[a]	2.31 (2.85)[a]	p = 0.026*	20
omega-3 f. a. (g)	2.18 (1.44)[a]	1.25 (1.43)[a]	1.48 (0.61)[a]	1.62 (1.06)[a]	1.36 (2.61)[a]	p = 0.81	

omega-6 f. a. (g)	10.28 (8.02)[a]	13.84 (5.93)[a]	9.01 (6.84)[a]	10.25 (11.19)[a]	14.66 (14.59)[a]	p = 0.165	

Table 5: Nutritional Data. [a] Data are displayed as median value and interquartile range, [b] Data are displayed as mean value and standard deviation. * p-values of Kruskal Wallis Test/Anova < 0.05

3.3 Oxidative Stress Parameters

An overview of all oxidative and nitrosative stress parameters is presented in table 6. The parameters, except SDMA (p=0.164), were overall not normally distributed (p=0.001-0.014). When comparing the median values, differences could be seen between the groups, especially in TOC, oLAb and MDA-LDL IgM concentrations. It is noticeable that the median value of the obesity group and the median value of the athletic group of TOC concentrations were almost twice as high as in all other groups. The athletes also had a much lower median oLAb value than the other groups. The MDA-LDL IgM concentrations were remarkably high in the anorexia patients. The parameters TOC, EPA and oLAb seemed to increase with higher BMI.
At first impression, no major differences in nitrosative stress parameters between the groups seemed to be apparent.

	AN patients group 1 (n=18)	Normal weight group 2 (n=27)	Overweight group 3 (n=22)	Adiposity group 4 (n=20)	Athletics group 5 (n=20)	p-values
TAC (mmol/L)	1 (0.48)[a]	0.98 (0.16)[a]	0.87 (0.14)[a]	0.97 (0.31)[a]	1.01 (0.68)[a]	p = 0.138
TOC (AU)	65.5 (47.4)[a]	67 (67.5)[a]	81 (177.8)[a]	158.2 (169.9)[a]	150.8 (168.9)[a]	p < 0.001*
EPA (mU/mL)	2.9 (2.59)[a]	3.3 (1.7)[a]	3.66 (1.78)[a]	4.18 (3.52)[a]	3.03 (1.46)[a]	p = 0.279
oLAb (mU/mL)	584 (1143)[a]	605 (1365)[a]	685 (992)[a]	668 (1174)[a]	365 (353)[a]	p = 0.035*
MDA-LDL IGM (mU/mL)	137.8 (407.1)[a]	75.5 (77.6)[a]	73.1 (68)[a]	61.4 (49.6)[a]	75.2 (54.7)[a]	p = 0.075
ADMA (µM/L)	0.57 (0.14)[a]	0.67 (0.11)[a]	0.65 (0.15)[a]	0.69 (0.13)[a]	0.58 (0.15)[a]	p = 0.001*
SDMA (µM/L)	0.71 ± 0.1[b]	0.7 ± 0.12[b]	0.71 ± 0.11[b]	0.63 ± 0.09[b]	0.67 ± 0.1[b]	p = 0.077

Table 6: Results of the oxidative and nitrosative stress parameters. a = Data are displayed as median value and interquartile range, b = Data are displayed as mean value and standard deviation. * p-values of Kruskal Wallis Test/Anova < 0.05

3.3.1 Total antioxidant capacity

Group 5 achieved the highest TAC concentration with a maximum of 1.7 mmol/L. The athletes also had the highest interquartile range, as demonstrated in figure 3. All other groups showed outliers in the values, group 2 and 3 even showed extreme outliers decreasing. The highest range was formed by group 4 due to its outliers.

Although the box plots varied widely, the Kruskal Wallis Test (p=0.138) did not find significant differences between the groups in the TAC values. No correlation between BMI and TAC could be identified.

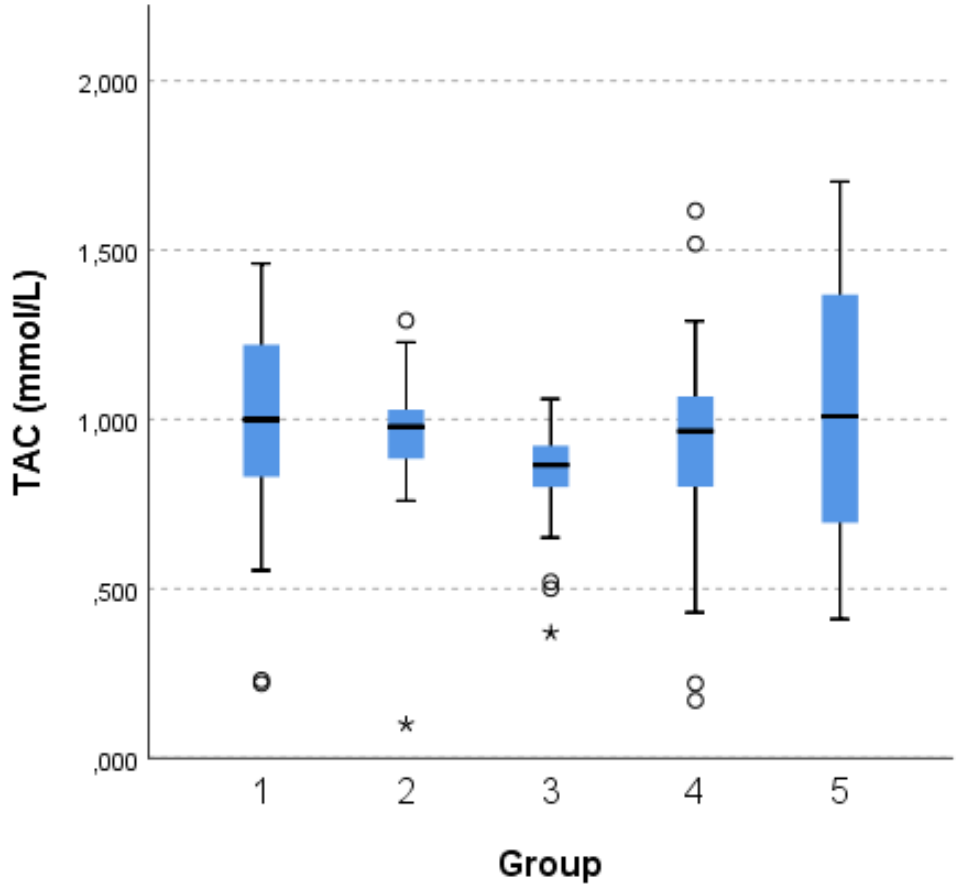

Figure 3: Total antioxidant capacity. O = outliers, * = extreme outliers. Group 1 = AN-patients, group 2 = normal weight, group 3 = overweight, group 4 = obese, group 5 = athletes.

3.3.2 Total oxidant capacity

The TOC values increased steadily from Group 1 to Group 4, with Group 4 showing the highest range and also the highest TOC value with 564 AU, although this value was an outlier. It is also interesting that the median of group 5 was only slightly below the median of group 4.

Significant differences in the groups were found (Kruskal-Wallis $p < 0.001$). Serum TOC was significantly lower in the anorexia nervosa group than in the athlete group (Dunn-Bonferroni $p = 0.008$) and the obesity group (Dunn-Bonferroni $p < 0.001$). In addition, there was a significant difference between the normal weight women and the obese patients (Dunn- Bonferroni p=0.001), obese subjects showing much higher levels. Figure 4

also shows a significant difference between the normal weight and the athletes, but the post-hoc test barely found no statistical significance (Dunn-Bonferroni p = 0.051).
Due to the continuous increase of the TOC value from group 1 to 4, a correlation test between TOC and BMI was performed; a strong association (Spearman R = 0.406, p < 0.001) between the TOC value and the BMI could be identified (Figure 5).

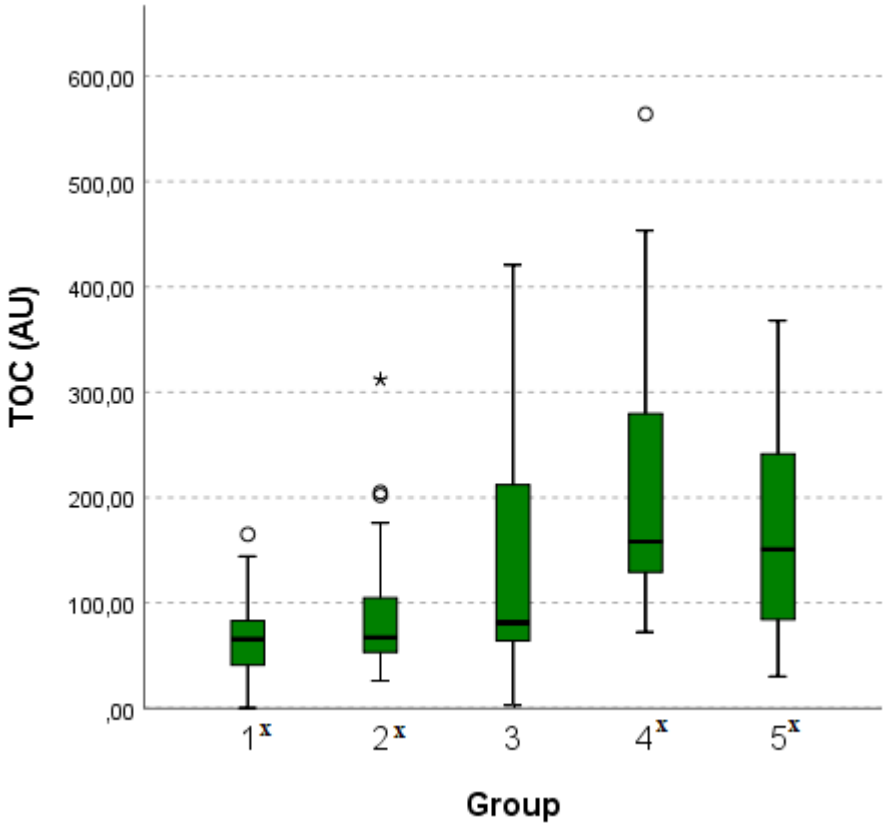

Figure 4: Total oxidant capacity. O = outliers, * = extreme outlier. Group 1 = AN-patients, group 2 = normal weight, group 3 = overweight, group 4 = obese, group 5 = athletes, x: p < 0.001

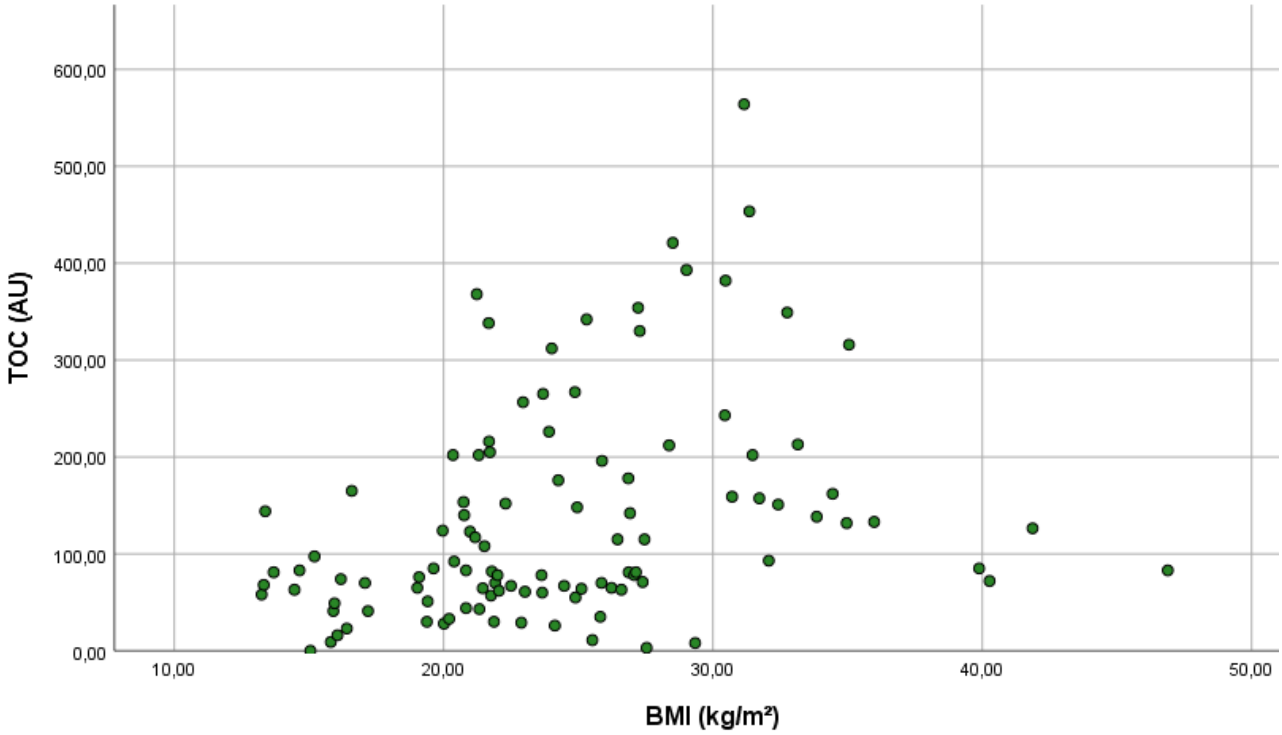

Figure 5: Correlation between BMI and TOC

3.3.3 Endogenous peroxidase activity

Endogenous peroxidase activity (EPA) also seemed to increase from group 1 to group 4. The obesity group had the highest median values, with the maximum of 10.89 mU/mL being set by an extreme value of the normal weight group. In addition, there were a few outliers in the athlete group, otherwise the data were distributed relatively stable. Figure 6 shows the distribution of EPA.

No significant differences in EPA concentration between the groups were found by statistical testing (Kruskal Wallis $p = 0.279$). However, a bilateral correlation was observed between the EPA value and the BMI (Spearman $R = 0.196$, $p = 0.043$), demonstrated in figure 7.

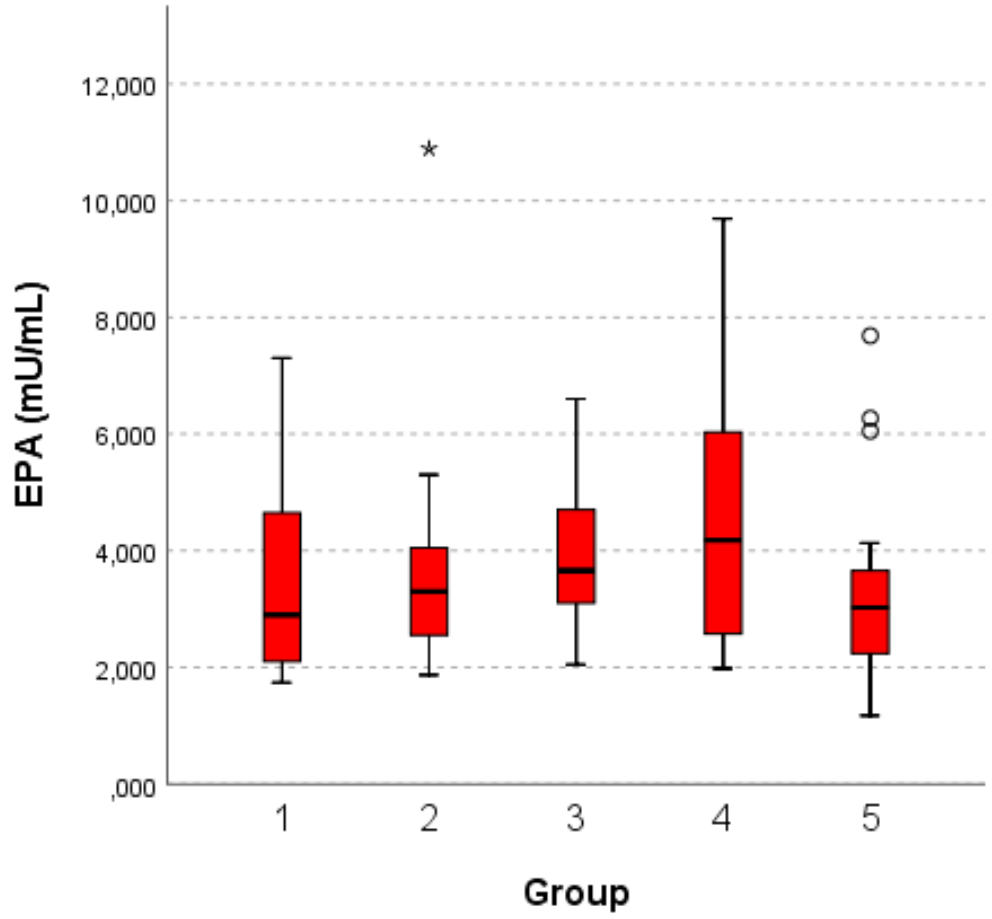

Figure 6: Endogenous peroxidase activity. O = outliers, * = extreme outlier. Group 1 = AN-patients, group 2 = normal weight, group 3 = overweight, group 4 = obese, group 5 = athletes.

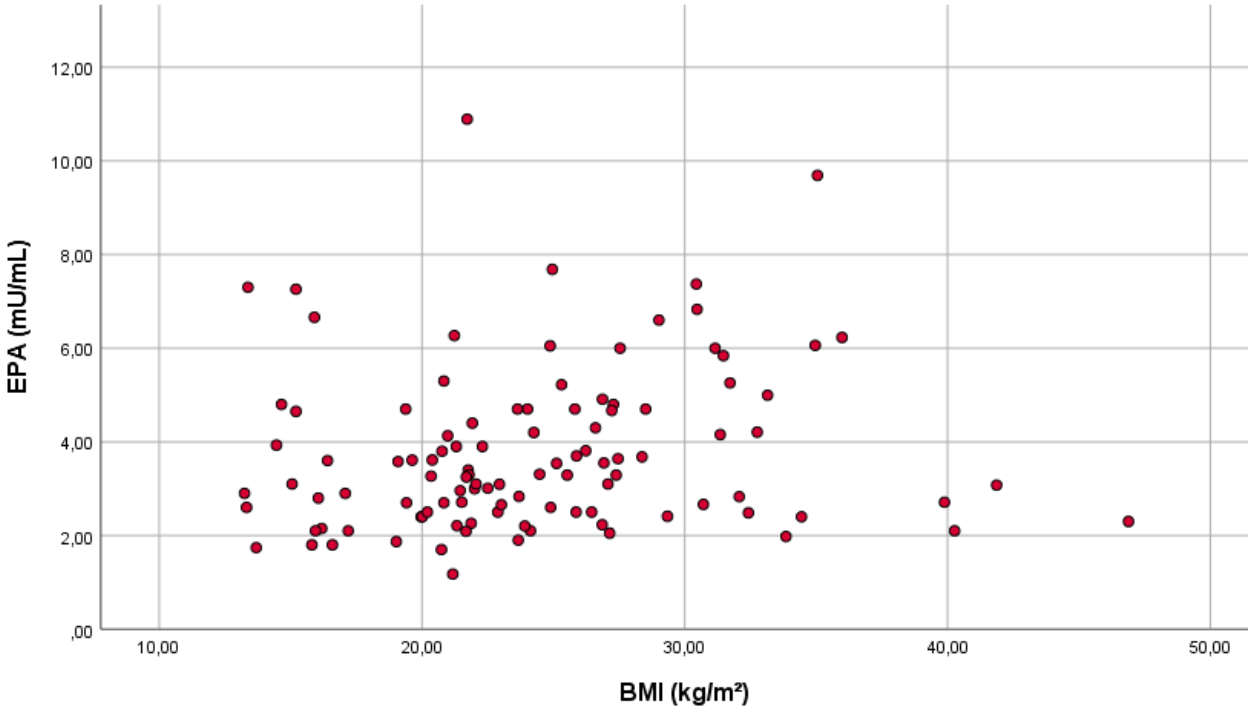

Figure 7: Correlation between BMI and EPA

3.3.4 Autoantibodies against oxidized LDL

In terms of oLAb concentrations, groups 1-4 did not seem to differ much visually (Figure 8). Group 2 achieved the highest value with 4090 mU/mL, the minimum of 37 mU/mL is provided by group 5, which stood out with lower values overall and also had the lowest interquartile distances and span.
The Kruskal Wallis test showed significant differences between the groups with a p value of 0.034. In paired comparison by post hoc tests the athlete group showed significantly lower oLAb values compared to the overweight according to Dunn-Bonferroni (p = 0.048). No correlation between BMI and oLAb could be identified.

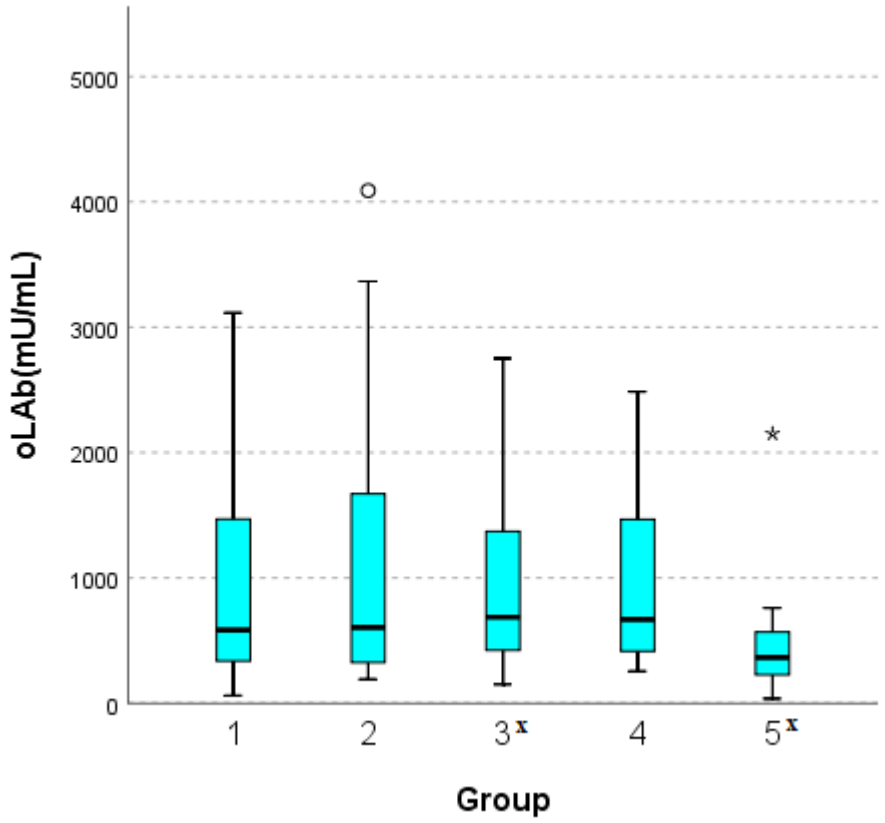

Figure 8: Autoantibodies against oxidized low-density lipoprotein. O = outlier, * = extreme outlier. Group 1 = AN-patients, group 2 = normal weight, group 3 = overweight, group 4 = obese, group 5 = athletes, x: p = 0.034

3.3.5 Autoantibodies against malondialdehyde-modified LDL

The MDA-LDL IgM concentrations were rather low in groups 2-5, while group 1 showed the highest values in average, with a maximum of 1155 mU/mL. The median value was

almost twice as high and the interquartile range was multiple times higher than in the other groups, as also demonstrated in figure 9.

Despite these clear graphical variations, no statistical significance of these differences could be obtained using the Kruskal Wallis test (p = 0.075). However, MDA-LDL IgM were significantly negatively associated with BMI (Spearman R = -0.215, p = 0.026), represented in figure 10.

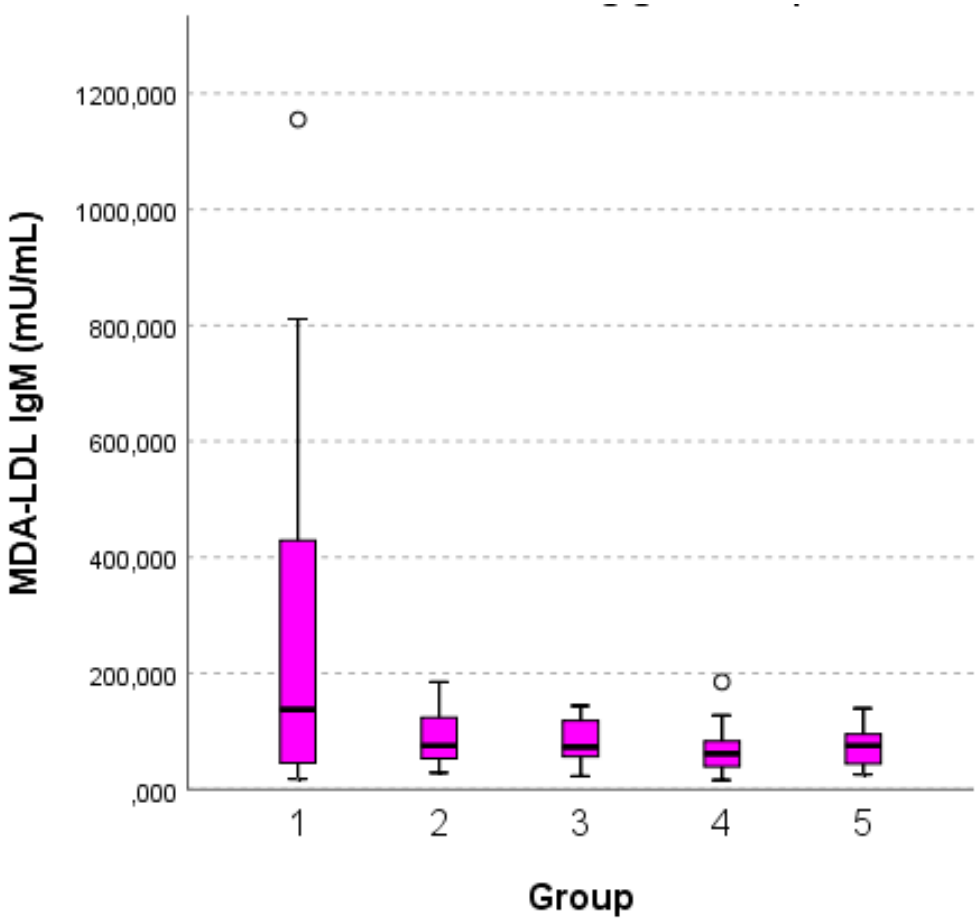

Figure 9: Autoantibodies against malondialdehyde-modified low-density lipoprotein. O = outliers. Group 1 = AN-patients, group 2 = normal weight, group 3 = overweight, group 4 = obese, group 5 = athletes.

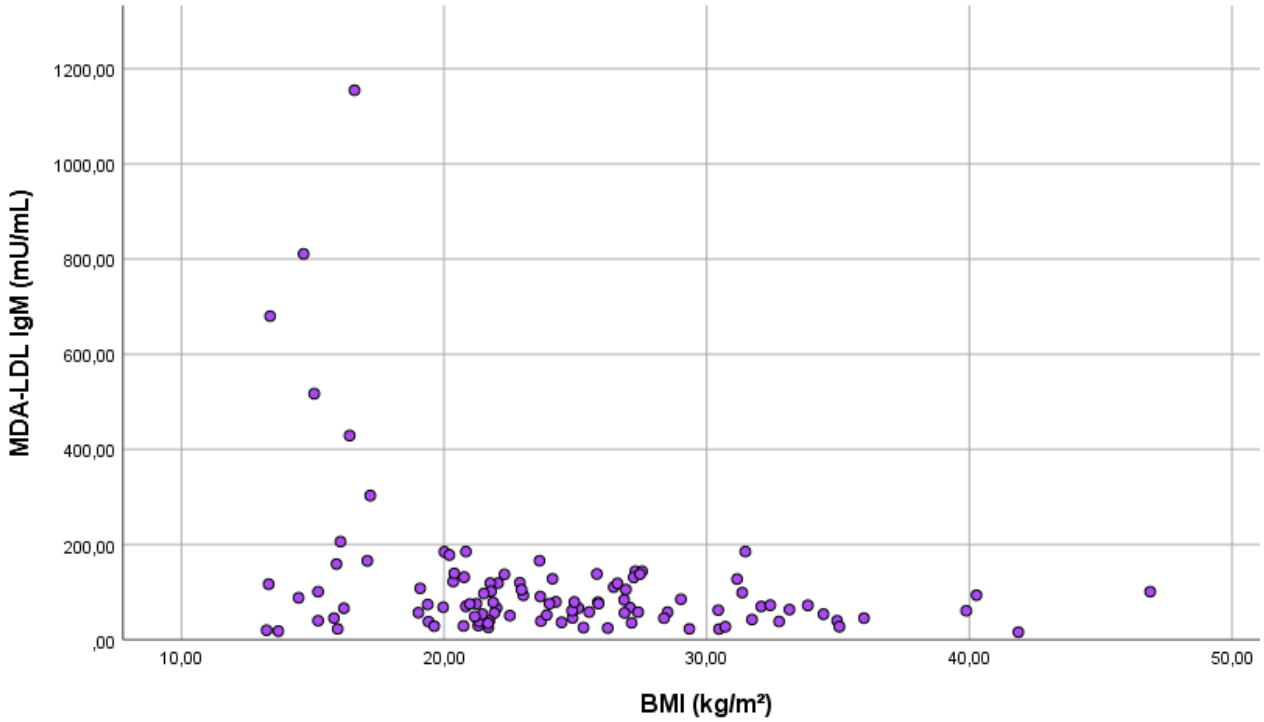

Figure 10: Correlation between BMI and MDA-LDL IgM

3.4 Nitrosative Stress Parameters

3.4.1 Asymmetric dimethylarginine

AMDA concentrations appeared to increase steadily from group 1 to group 4, although group 2 had a higher median value of 0.67 µM/L than the median of group 3 with 0.65 µM/L. There was only one extreme value in group 2, which also represented the maximum of 1.099 µM/L (figure 11).

Statistical group differences were found (Kruskal Wallis p = 0.001) concerning anorexia nervosa patients. Their AMDA levels were significantly lower compared to normal weight (Dunn-Bonferroni p = 0.007), overweight (Dunn-Bonferroni p = 0.033) and obese (Dunn-Bonferroni p = 0.002). As expected, a strong positive correlation was observed between AMDA and BMI (Spearman R = 0.342, p < 0.001), as shown in figure 12. Interestingly, there was also a negative correlation between the HAMD score and AMDA (Spearman R = -0.212, p = 0.029), which is presented graphically in figure 13.

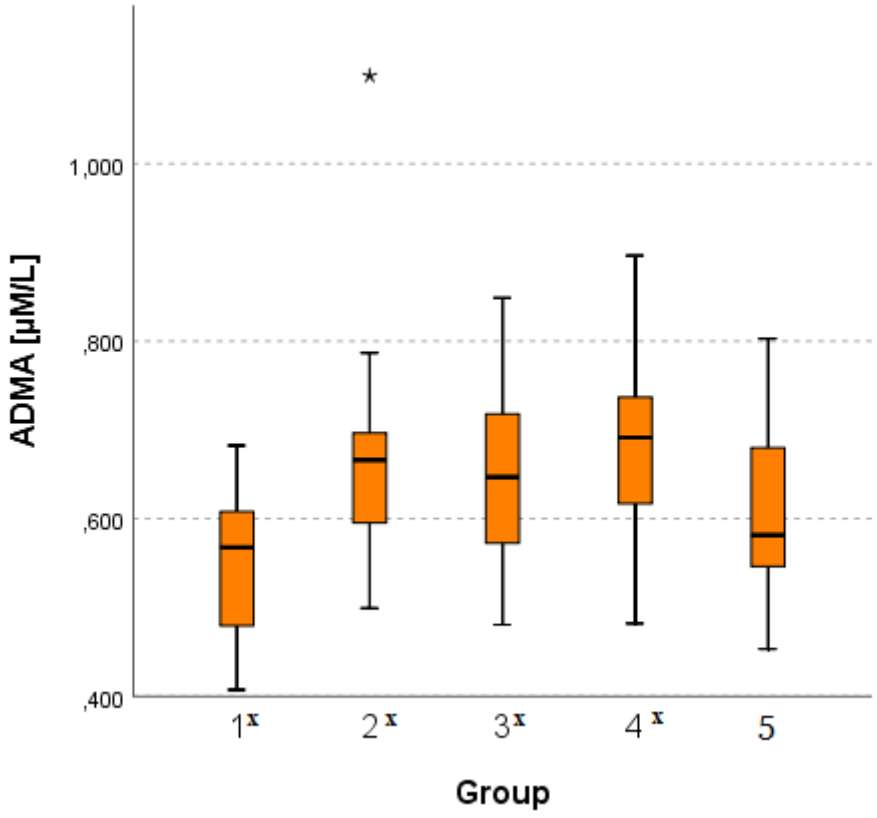

Figure 11: Asymmetric dimethylarginine. * = extreme outlier. Group 1 = AN-patients, group 2 = normal weight, group 3 = overweight, group 4 = obese, group 5 = athletes, x: p = 0.001

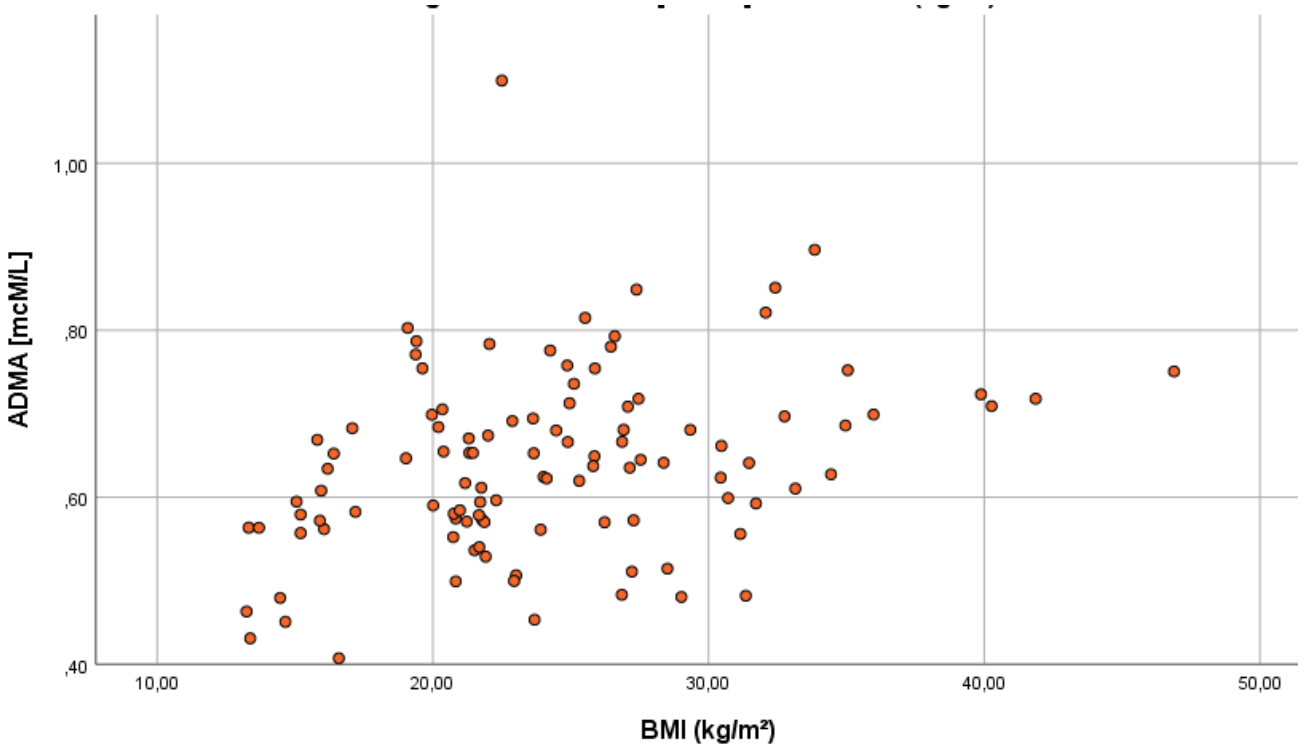

Figure 12: Correlation between BMI and ADMA

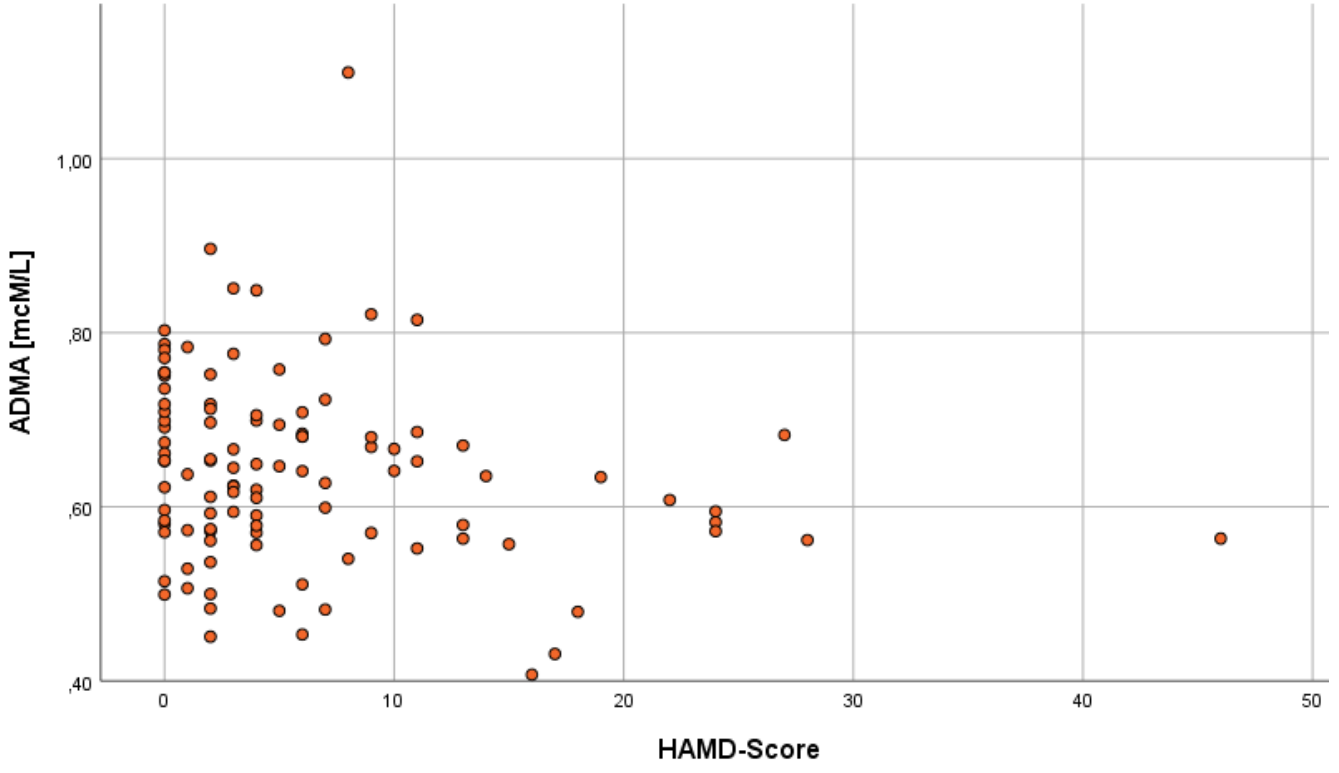

Figure 13: Correlation between HAMD-score and ADMA

3.4.2 Symmetric dimethylarginine

The SDMA value seemed to decrease steadily on average from group 1 to group 4, with a maximum of 0.987 µM/L in group 2. The average concentration of group 5 was 0.67 µM/L, it also had some outliers (figure 14).
Marginally no relevant differences could be found between the different groups in the serum concentrations of SDMA (Anova p = 0.077). However, SMDA was significantly negatively correlated to BMI (Pearson R = -0.237, p = 0.014), which is presented in figure 15.

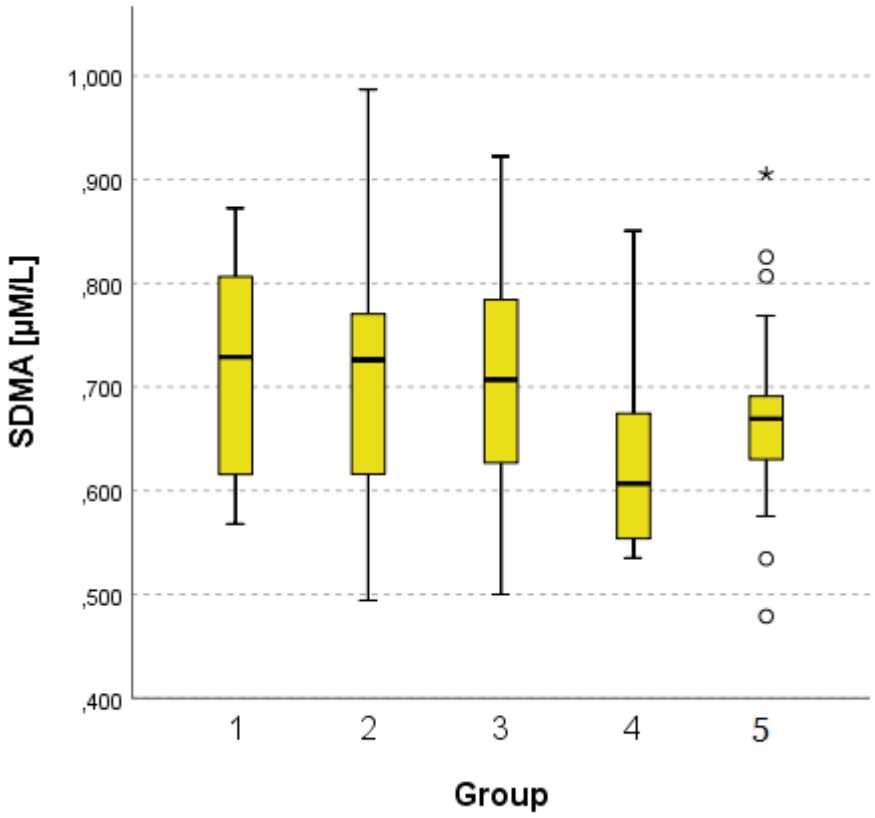

Figure 14: Symmetric dimethylarginine. O = outliers, * = extreme outlier. Group 1 = AN-patients, group 2 = normal weight, group 3 = overweight, group 4 = obese, group 5 = athletes.

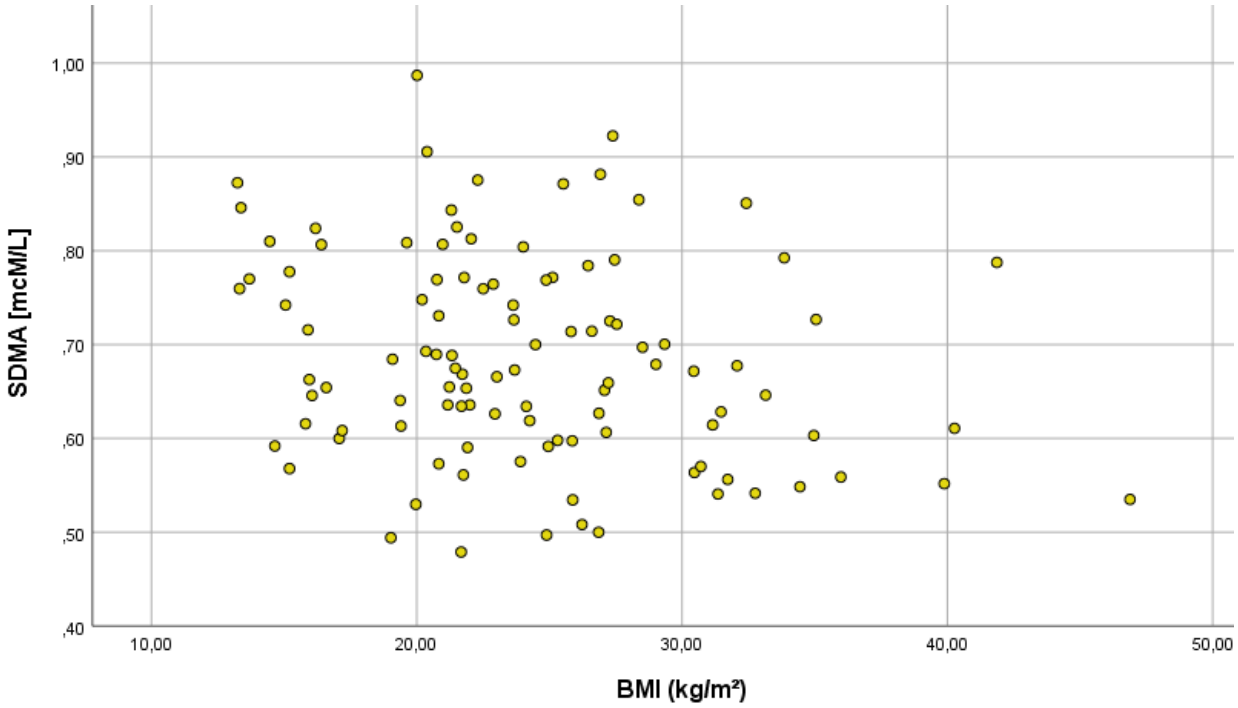

Figure 15: Correlation between BMI and SDMA

4 Discussion

The main objective of this thesis was to put oxidative and nitrosative stress parameters in a possible context with nutritional status and lifestyle factors of young women. Therefore, a cohort of 107 women aged 17-39 years was divided into 5 different groups based on their BMI and physical activity. For each woman, blood samples were used to evaluate 5 laboratory parameters that reflect oxidative stress status (TAC, TOC, EPA, oLAb, MDA-LDL IgM) and 2 laboratory parameters that measure nitrosative stress (ADMA, SDMA).

4.1 Lifestyle and nutritional intake

Significant differences were found between the groups in all anthropometric and lifestyle-associated parameters.
The level of physical activity was highest in group 5 with significant difference to the overweight and anorexia nervosa patients, with the latter reaching the lowest average level. It should be mentioned, however, that the anorexia nervosa patients were in a therapy program where they had to restrain their physical activity level. Interestingly, the group of obese women had higher activity levels than might be expected.
The depression scores BDI and HAMD are both significantly higher in anorexia nervosa patients compared to all other groups. Anorexia nervosa and depression are strongly associated with each other, not only because of their common genetic and environmental risk factors; anorexia nervosa patients also have a higher risk of developing depression. In addition, adolescents suffering from anorexia nervosa seem to be more prone to suicidal thoughts.[52] The lowest values were achieved here by the female athletes. In fact there is evidence that physical exercise could have a positive effect on depression.[53]

Interestingly, despite significant differences in anthropometric and lifestyle-associated parameters, the 5 groups did not differ considerably in their daily energy intake and the intake of the 3 main dietary components fat, carbohydrates and proteins.
However, there were a few differences concerning some dietary antioxidants and vitamins. Thus, the group of obese women consumed significantly less carotene than the anorexia nervosa patients, the normal weight women and the athletes. This could be further linked to their increased oxidative stress parameters. A lack of antioxidants leads to an increased

vulnerability to oxidative stress.[25] However, no differences in TAC concentrations have been found to support this theory.

At first impression, the high dietary intake of anorexia nervosa patients, especially of vitamin C, vitamin B12 and vitamin D, seemed irritating, as a deficiency would actually be expected. Most of the patients were under therapeutic treatment, which included nutritional therapy with high-calorie substitute diet adapted to the level of therapy. This could explain the sufficient intake of nutrients in this group. All other groups, however, followed their traditional eating habits. It is noticeable that for some values, such as vitamin C and especially vitamin D, most of them were unable to reach the recommended daily intake.

4.2 Oxidative Stress

4.2.1 Antioxidant Capacity

No significant differences in TAC concentrations were found between the groups, with athletes showing the highest levels on average. These results are consistent with the results of a study of young Saudi women in which superoxide dismutase (SOD), an antioxidant enzyme, and total antioxidant capacity were measured in serum. No differences were found between the 3 groups, normal weight, overweight and obese.[54] Similarly, Brown et al. found no differences in antioxidant parameters with respect to BMI. However, decreased glutathione synthesisase (GSH) with increased hip circumference, decreased total antioxidant status (TAS) and decreased GSH levels with increased fat levels and a negative correlation between TAS, SOD and GSH with body fat were found.[36] Comparable results were obtained in a Chinese study in which significant lower SOD levels in obese males than in overweight and normal weight males as well as a negative association between intra-abdominal fat measured by magnetic resonance imaging (MRI) and SOD were shown.[55] Rowicka et al. could identify significantly lower TAC concentrations in obese compared to non-obese children.[34]

Even there is no significant difference in the presented groups of different BMI, it is worth to mention that there have been seen differences in bigger cohorts. Wonisch et al. report that the total antioxidant status (TAS) increased continuously from BMI group 1 (BMI < 18.5) to group 4 (BMI 30 - 34.99) and group 5 (BMI >35) again showed lower values than group 3 and 4.[33] It is generally believed that individuals with higher levels of obesity have a greater requirement for antioxidants to prevent free radical damage. Therefore, in the early stages of obesity, the production of antioxidant enzymes may be stimulated, which is associated with an increase of blood antioxidant levels. Chronification of this condition could then lead to depletion of antioxidant sources and thus to a decrease in antioxidant blood levels.[36] Low serum antioxidant levels could also be caused by the redistribution and storage of antioxidants, especially fat-soluble ones, in the fatty tissue.[56]

The highest TAC levels, as mentioned above, were averaged by the athletes, but the difference was not statistically relevant. However, Bloomer et al. found significantly higher Trolox equivalent antioxidant capacity (TEAC), another parameter reflecting the antioxidant status, in trained compared to untrained individuals.[28] Another trial also indicated a direct effect of physical activity on antioxidant status, as the TAS increased

significantly in older subjects with metabolic syndrome after 5 days a week of Tai Chi training for 6 months.[57] In young women, an increase in SOD levels was already measurable after 12 weeks of Tai Chi.[58] This seems to support the hypothesis that moderate physical activity has a positive effect on the body's antioxidant activity. Nevertheless, it should be noted that the same effect as described previously would be possible. Since exercise also stimulates ROS production in the body[19], increased antioxidant levels could be attributed to a reflectively increased production of antioxidants.

4.2.2 Reactive oxygen species

The TOC and EPA concentrations increased steadily from group 1 the anorexia nervosa patients to group 4 the obese. Both values showed a significant positive correlation to the BMI. In addition, significantly higher TOC levels were shown in the obese compared to the anorexia nervosa patients and normal weight. These results are in agreement with the values of Rowicka et al., who found a significantly higher TOC concentration in obese children compared to non-obese children. Here, a positive association between the duration of obesity and TOC levels could also be established.[34] The results of Wonisch et al. acted slightly different. There the values of the groups BMI 1 (<18.5), BMI 2 (18.5-24.99), BMI 3 (25-29.99) and BMI 4 (30-34.99) were all in a similarly low range. However, group BMI 5 (35-39.99) had twice as high TOC concentrations as all other BMI categories. These results indicate high oxidative stress levels only in extreme obesity.[33] In the study with the young Saudi women a significant difference of 8-hydroxy-2-deoxyguanosine (8-OH-2dG), an indicator for oxidative DNA and ribonucleic acid (RNA) damage, to normal weight persons with a BMI of >27.5 was found. In addition, the value increased proportionally to the BMI, the body adiposity index (BAI), an index to estimate the body fat percentage, the waist circumference and the percentage of body fat. Thus, overweight young women are likely to have increased oxidative DNA damage although they are healthy, i.e. have normal blood glucose levels, normal blood pressure and sufficient exercise.[54] These results are also consistent with the results of this thesis.

There are many different theories about the cause of increased peroxides/oxidative laboratory parameters in obesity. It could be due to the higher proportion of adipose tissue, since the mitochondrion of white adipose tissue is considered to be one of the main sites of ROS.[34] Obese individuals also have an elevated energy expenditure due to weight.[35] Increased metabolism is associated with increased production of ROS.[59] A chronic low

grade inflammatory status in combination with an inadequate antioxidant defence could also lead to high oxidative stress in the body. Another explanation could be the insulin resistance triggered by the fat cells. Adipocytes express pro-inflammatory cytokines such as TNFα and interleukin 6 (IL-6), which interfere with insulin receptor signalling, e.g. by altering key proteins, and have been reported to promote insulin resistance. This leads to stronger hyperglycemic and hyperlipidemic states and increased ROS production in the body. Bloomer et al. gave an indication of this mechanism in their study. They measured xanthine oxidase (XO) activity and hydrogen peroxide levels of obese and non-obese subgroups after a meal. Both groups reacted with an increase in oxidative values on the meal, but the values of the obese were higher overall. In addition, the peak of the values of the non-obese was already reached after 4 hours, whereas the values of the obese still increased after 6 hours, which was the last measurement.[35]

If overweight and obese individuals are more affected by oxidative stress, this could mean that losing weight might reduce oxidative stress. In general, low caloric nutrition increases life expectancy.[1] There is also evidence that maintaining a calorie limit can lead to a discreet reduction in ROS production.[21] Diet and weight loss could already cause a decrease in oxidative stress and its damage after a short time.[60] Similarly, a Roux-en-Y gastric bybass surgery may be able to reduce oxidative parameters by more than 50%.[55]
On the other hand, it would be interesting to know whether the intake of antioxidants could reduce the consequences of oxidative stress in obese people. There are indications that oxidative stress after vascular surgery can be reduced by vitamin substitution.[61,62]
Rosuvastatin is also supposed to decrease endogenous peroxide concentrations and peroxidase activity.[17] It could be therefore possible that an early and correct supplementation of antioxidants in obese patients could prevent or at least reduce the long-term damage caused by oxidative stress.

A higher ROS production in anorexia nervosa patients cannot be confirmed with the TOC and EPA results of this thesis, because the median of the TOC and EPA levels of anorexia nervosa patients was even lower than the median of normal weight.
However, significantly higher TOC levels were found in athletes compared to the women suffering from anorexia nervosa and a trend towards significance was observed compared to the normal weight participants. These results are in accordance with Schippinger et al.

who identified elevated oxidative parameters in American football players and elite alpine ski racers during the competition season.[29,63]

It is also fascinating that the women already showed increased oxidative stress values, although they were young and healthy. This could mean that oxidative conditions might be detected early, even before they can cause irreparable damage. The question therefore arises whether oxidative stress parameters would be good biomarkers for the development of a metabolic syndrome and similar. It would be feasible that by measuring oxidative and nitrosative products, prognostic assumptions could be made about various clinical patterns such as atherosclerosis, coronary heart disease, chronic renal failure, etc. However, it should be noted that the collective studied here is too small to conclude possible derivations to the general population. Nevertheless, the results offer a good approach for planning larger studies.

4.2.3 Lipid peroxidation

One of the most studied areas of oxidative stress in medical research is lipid peroxidation. It is believed to play a major role in the development of metabolic syndrome, atherosclerosis and coronary heart disease.[56] Some parameters that reflect lipid peroxidation have even been proposed as biomarkers for the assessment of atherosclerotic changes in the clinical setting.[33] It may also contribute to the pathogenesis of multiple sclerosis.[64]

There are many studies that investigated a relation of increased lipid peroxidation parameters and obesity. In the paper of Brown et al. lipid hydroperoxides (LH) increased progressively with a higher BMI, with the obese group having a significantly higher value than the normal weight group. Furthermore, LH values were significantly higher in men with a waist circumference of >102cm and in women with a waist circumference of >88cm, respectively. A positive correlation of the LH concentration with the BMI and the waist circumference could be established, indicating the role of elevated body fat and an unfavourable fat patterning within this context.[36] Furukowa et al. could also show a positive relation between BMI and waist circumference and plasma thiobarbituric acid reactive substance (TBARS) and urinary 8-epi-prostaglandin-F2α (8-epi-PGF2α), two other values representing lipid peroxidation.[37]

In this thesis lipid peroxidation was reflected by autoantibodies against oxidized and MDA-modified LDL. However, both oLAb and MDA-LDL IgM values could not be attributed to obesity because the values of group 2, 3 and 4 did not differ. These results are consistent with the study by Latif et al.; there was also no difference in the TBARS values of the examined young women.[54] Similarly, Suzuki et al. could not find any variation in ox LDL or oLAb concentrations between obese and non-obese individuals of both sexes.[56] Rowicka et al. even found lower ox LDL values in obese children, but these results were not statistically significant.[34] However, another study reported significantly higher levels of 8-iso-PGF2a and MDA in obese compared to overweight and normal weight and in overweight compared to normal weight males, and a positive association of these values with BMI and IAF.[55]

Interestingly, the group of athletes was apparent with the lowest oLAb values, with a significant difference to the overweight. These results contradict findings that the oLAb concentration of American football players increased significantly in middle of competition season.[29] In addition, high baseline titre of over 1000 mU/mL in elite skiers were found.[63] This value can only be achieved in the group of young female athletes by an extreme outlier. In order to interpret these results, the significance of oLAb must first be examined more closely. During oxidation of LDL, antigenic epitopes are expressed and thus autoantibodies (oLAb) are formed as an immune response.[29] They are present in the plasma where they can control the increase of oxidized LDL.[56] Therefore, high oLAb titre seems to reflect a strong immune response of the body to radicals and their damage.[15] In this context, elevated oLAb concentrations were found in patients with coronary atherosclerosis, acute myocardial infarction and other vascular diseases.[65] The value was even proposed as a biomarker for in vivo LDL oxidation in high-risk patients with carotid atherosclerosis.[33] However, an inverse relationship was also found between ox LDL and oLAb titre in healthy individuals.[66] In this sense, oLAb would be considered as a protective factor. According to this theory, lower oLAb titres are associated with increased lipid peroxidation because the concentration of free oLAb decreases when it reacts with the antigens of ox LDL and the antibodies are forming epitopes.[62] Tinahones et al. found a higher prevalence of oLAb in young healthy women as well as lower oLAb titres in older persons with cardiovascular risk factors.[65] Schumacher et al. also identified a negative correlation between oLAb and the creatine kinase muscle-brain type (CK-MB) mass in

patients after myocardial infarction. The CK-MB are reflecting the severity of the myocardial infarction. Here low oLAb concentrations were interpreted as formation of free radicals and consecutive lipid peroxidation. The lower peak of the oLAb titre was even 4 hours before the upper peak of the CK-MB concentration, therefore it was speculated whether oLAb could be considered as a prognostic value for myocardial infarction.[18] In another study even lethality was correlated with a continuously decreasing oLAb concentration.[15] On the other hand Steinervoa et al. found extremely high levels of antibodies against ox LDL in 3-month-old children who were not breastfed. In addition, evidence of increased DNA strand breaks was found in these children.[67] The role of oLAb therefore still seems unclear. The low oLAb levels of these young female athletes could on the one hand be interpreted as a sign of increased lipid peroxidation, since athletes are supposedly at higher risk for oxidative stress and lipid peroxidation.[29] This would also be consistent with the elevated TOC values of group 5. On the other hand, the low oLAb concentrations could also be related to a decreased immune response, as less lipid peroxidation takes place in the body and therefore lower levels of antibodies are needed.

The function of autoantibodies against MDA-modified LDL seems similarly unexplained. There is a theory that the nature of the immune response could play different roles. The early immune response with the formation of immunoglobulin M (IgM) antibodies against MDA-LDL is believed to inhibit macrophages from taking up modified LDL molecules and phagocytising apoptotic cells. Additionally, they may prevent macrophages from entering the arterial wall and thus reduce the progression of atherosclerotic changes. Immunoglobulin G (IgG) antibodies of the late immune response are supposed to have exactly the opposite effect because they have a fragment crystallizable (Fc) domain that is recognized by macrophages. This hypothesis is supported by findings of a positive correlation of MDA-LDL-IgG and a negative correlation of MDA-LDL-IgM with the progression of atherosclerotic changes in the carotid artery. There are also different results for MDA-LDL itself. While the value is often associated with lipid peroxidation and its damage, it has also been suggested as a marker for the stability of atherosclerotic plaques.[17] In any case, data on changes in antibody levels in relation to disease progression are currently contradictory. It is therefore very interesting that anorexia nervosa patients have on average a higher MDA-LDL-IgM titre than all other groups with a trend towards significant. Data on lipid peroxidation in individuals suffering from anorexia nervosa are very rare at the moment. Filaire et al. found decreased MDA concentrations in rats after 8

days of food restriction.[68] Another study investigated TBARS levels in anorexia nervosa patients, but could not find significant differences to healthy individuals. However, the patients were in a very early stage of anorexia nervosa.[69] The high antibody levels in these anorexia nervosa patients could indicate an elevated state of lipid peroxidation..

4.3 Nitrosative Stress

As nitrosative stress parameters, asymmetric dimethylarginine and symmetric dimethylarginine were chosen. Significantly low levels of ADMA were found in women suffering from anorexia nervosa compared to normal weight, overweight and obese women. Furthermore, a strong positive correlation of ADMA to BMI was observed. This corresponds to the results of a study by Morelli et al. who also established an association between BMI and nitrosative stress. They measured nitrosative stress through oxidized NO products and thus reflected the bioavailability of NO. In this way, they found not only a reduced bioavailability with increasing BMI but also in relation to hypertension.[70] Furthermore, high ADMA levels are supposed to be related to atherosclerosis and coronary heart disease. Elevated levels of ADMA were found in middle-aged individuals with proven coronary artery calcification compared to individuals without plaque formation. Moreover, ADMA seems to be independently positively associated with the degree of calcification.[71] Krempl et al. observed significantly higher ADMA levels in patients with coronary artery disease compared to healthy controls, and higher levels in patients with unstable angina pectoris than in patients with stable angina. 6 weeks after treatment of these patients with PCI, ADMA levels decreased again in patients without a new cardiovascular event. The patients with persistently elevated ADMA levels had a higher prevalence of new events.[49] In the context of these results, ADMA could serve as a strong risk predictor for cardiovascular events.[72] The reason for the high association with vascular pathologies could be interference with NOS activity.[73] According to another theory, ADMA seems to be crucially involved in the regulation of vascular tone. Following this hypothesis, however, a decrease in ADMA levels is associated with oxidative stress. The consumption of glutathione increases the consumption of homocysteine, which in turn inhibits the catabolism of ADMA in the body. As a result, low ADMA levels would be associated with a high consumption of glutathione and thus with oxidative stress.[74] The increase in ADMA concentration with the BMI in these young women could mean therefore on the one hand elevated levels of nitrosative stress and an increased risk of atherosclerotic changes. On the other hand, the group of anorexia nervosa patients, which had significantly lower concentrations than 3 other groups, could also have an increased consumption of glutathione and thus be more affected by oxidative stress. However, this would mean that the oxidative stress would decrease with higher BMI, which is

contradictory to the TOC results. Another interesting finding concerning ADMA is a negative correlation with the HAMD score. Wigner et al. could establish a connection between depression and oxidative as well as nitrosative stress. Depressed patient exhibited increased NO and nitric peroxide levels. In addition, a high IgM titre against nitrate-modified proteins was found. Reactive substances may be able to degenerate neurons in the hippocampus and thus contribute to neuropathic and psychiatric diseases.[75]

No significant differences between the groups regarding the SDMA value could be found. However, there was a significant negative correlation between SDMA and BMI. In a study by Bode-Böger et al. high ADMA and SDMA levels were observed in patients with coronary artery disease, with SDMA being even a stronger predictor of the degree of stenosis than ADMA. In addition, fat and cholesterol-containing food is considered to raise SDMA levels in animals. SDMA might unfold their pathogenetic effect by indirect inhibition of NO synthesis. In contrast to interact with NOS activity, SDMA could compete with arginine in transport, thus reducing the production and bioavailability of NO. Furthermore, SDMA is not metabolized by an enzyme like ADMA, but excreted via the kidney. In the Henle loop, SDMA can also inhibit the reabsorption of L-arginine.[73] In addition, the SDMA parameter is thought to play a role in renal outcome, as patients with chronic kidney failure have higher levels of SDMA than healthy controls. The SDMA value is suggested to correlate precisely with kidney function and serve as a reference for GFR.[73,76] However, the results of the young women in this study are contradictory to the hypotheses mentioned above. It is known that overweight and obesity are independent risk factors for the development of coronary heart disease, therefore SDMA levels should rise rather than fall with BMI. However, the data on SDMA is far more limited compared to ADMA or oxidative stress parameters, so the role of SDMA is still not fully understood.

4.4 Limitations

Despite new results this thesis had several limitations. First of all, the number of women examined was restricted, especially among the anorexia nervosa patients. It is questionable whether conclusions can be reached about the general population with samples of this size. Further studies should include larger cohorts to verify these findings. Secondly, age can be an important factor for increasing oxidative stress parameters. Although the women were approximately all of similar age, differences could be found between the groups with the athletes being significantly younger than the overweight and the obese women. This may have an additional impact on the results. However, the subjects studied were all of the same sex, which eliminated one possible disruptive factor.

There were also difficulties in the possible interpretation of the results. Although there is plenty of literature on oxidative stress in relation to nutrition, the authors use different parameters to reflect oxidative states. There seems to be a wide range of parameters and measurement methods and the question is whether they can be compared with each other. Apparently, the knowledge about oxidative and nitrosative stress is still so limited that it is not possible to find uniform biomarkers for these conditions. Similarly, there seems to be a lack of knowledge about the biomarkers already available. There are serious discrepancies in the literature on the significance of different parameters, in particular immunological values. Therefore the results obtained are primarily important for planing further studies.

5 Conclusion

In summary the presented data demonstrate that, young obese women were more affected by oxidative and nitrosative stress than women of normal weight and the same age. These results correspond very well with the recent literature in the field of medical research. Young female athletes had lower oLAb titres than women of different nutritional status of the same age. For the first time MDA-LDL-IgM titres are presented of young women suffering from anorexia nervosa pointing out the big variance and very high levels in single individuals. Additionally the reported negative correlation of MDA-LDL-IgM titre to BMI represents important data for future studies in the field.

Oxidative and nitrosative stress and the subsequent damage appear to play a major role in the development of various diseases and in ageing. Further research could provide a better understanding of the biochemical mechanisms and could help to further clarify them. Such conditions may be identified, for example, at an early stage. This knowledge could be used to find early therapeutic strategies against common diseases such as coronary heart disease or serious, sometimes incurable diseases such as many types of cancer.

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