COMENIUS UNIVERSITY IN BRATISLAVA,

FACULTY OF MEDICINE

PROTEINS AND OMEGA-3 FATTY ACIDS: HEALTH BENEFITS DURING AGING

DIPLOMA THESIS

Bratislava 2022 Teresa Ridder

COMENIUS UNIVERSITY IN BRATISLAVA,

FACULTY OF MEDICINE

PROTEINS AND OMEGA-3 FATTY ACIDS: HEALTH BENEFITS DURING AGING

DIPLOMA THESIS

Study Program:	General Medicine
Field of Study:	7.1.1. General Medicine
Training workplace:	Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry
Supervisor:	doc. Ing. Ingrid Žitňanová, PhD.

Bratislava 2022 Teresa Ridder

Comenius University in Bratislava
Faculty of Medicine

THESIS ASSIGNMENT

Name and Surname: Teresa Ridder
Study program: General Medicine (Single degree study, doctor I.II. deg., full time form)
Field of Study: General Medicine
Type of Thesis: Diploma Thesis
Language of Thesis: English
Secondary language: Slovak

Title: Proteins and Omega-3 Fatty Acids: Health Benefits During Aging

Annotation: Aging is associated with various diseases, including sarcopenia, which can lead to disability. Nutrition as a modifiable risk factor may prevent or delay the onset of these geriatric conditions. Of the nutrients, high-quality proteins and omega-3 polyunsaturated fatty acids are of particular interest for their demonstrable effects on the overall health of the body, including skeletal muscle. Many scientific studies point to the important role of omega-3 fatty acids in healthy aging. Omega-3 fatty acids such as EPA and DHA are associated with fetal development, cardiovascular function and Alzheimer's disease.

The aim of this work is to examine the latest results on the associations and role of these nutrients in diseases associated with the aging of the elderly.

Literature: Review of the articles in scientific databases

Supervisor: doc. Ing. Ingrid Žitňanová, PhD.
Institutes: LF. ÚLChBKB - Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry LF UK

Assigned: 05.10.2020

Approved: 12.10.2020

prof. MUDr. Ladislav Turecký, CSc.
Head of the Department/Clinic

……. …….

Student Supervisor

DECLARATION

I hereby declare that this thesis is my original work, and it has been written by me in its entirety.

I have acknowledged all the sources of information which have been used in the thesis.

Bratislava 13.01.2022

…….

Signature

ACKNOWLEDGEMENTS

First, I want to thank my supervisor doc. Ing. Ingrid Žitňanová, PhD. She was always available and very attentively answering my questions. Thank you so much for your help and kindness!

Then I want to thank my parents, without all their support throughout my studies it would have been impossible to accomplish this.

I also wish to thank my friends for their continuous support during our studies and my partner Javier for correcting my English and proofreading.

Thank you.

The work is supported by an EU grant, the CBC program, Interreg V-A-Nutriaging V-0014.

ABSTRACT

RIDDER Teresa: ***Proteins and Omega-3 Fatty Acids: Health Benefits during Aging*** [Diploma Thesis] – Comenius University in Bratislava, Faculty of Medicine; Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry. – Supervisor: doc. Ing. Ingrid Žitňanová, PhD., Bratislava LF UK, 2022, number of pages: 79

Nutrition during old age is of paramount importance in our aging society. The causes of aging have been discussed until today, and most researchers agree that it is a multifactorial process including many different causes. Proteins and omega-3 fatty acids are important dietary components, their increased or decreased intake may have specific effects on the human body. High protein intake can worsen kidney failure but on the other hand prevent sarcopenia. High omega-3 fatty acid intake can lead to a decrease in inflammation, cardiovascular morbidity, and mortality. Effects on other body systems are not researched enough to form a conclusion.

Key words: aging, proteins, omega-3 fatty acids

ABSTRAKT

RIDDER Teresa: ***Proteíny a Omega-3 Mastné Kyseliny: Zdravotné Benefity počas Starnutia*** [Diplomová práca] – Univerzita Komenského v Bratislave, Lekárska fakulta; Ústav lekárskej chémie, biochémie a klinickej biochémie. – Vedúci práce: doc. Ing. Ingrid Žitňanová, PhD., Bratislava, 2022, počet strán: 79

Výživa v starobe má v našej starnúcej spoločnosti dôležitý význam. O príčinách starnutia sa diskutuje dodnes a väčšina výskumníkov sa zhoduje, že ide o multifaktoriálny proces zahŕňajúci mnoho rôznych príčin. Bielkoviny a omega-3 mastné kyseliny sú dôležitou zložkou stravy, ich zvýšený alebo znížený príjem môže mať špecifické účinky na ľudský organizmus. Vysoký príjem bielkovín môže zhoršiť zlyhávanie obličiek, ale na druhej strane zabrániť progresii sarkopénie. Vysoký príjem omega-3 mastných kyselín môže viesť k zníženiu zápalov, kardiovaskulárnej chorobnosti a úmrtnosti. Účinky na iné telesné systémy ešte nie sú dobre preskúmané.

Kľúčové slová: starnutie, proteíny, omega-3 mastné kyseliny

FOREWORD

With the increase of life-expectancy nowadays we can see an increase of age-related diseases all over the world. Nutrition seems to have an effect not only on life-expectancy but also on the number of healthy life years and the overall quality of aging. Some age-related diseases can be positively influenced by altering nutritional factors (Troesch et al., 2020).

Because of the positive health effects of proper nutrition, educating doctors on it can improve their patient's health and quality of life.

This diploma thesis focuses on the effects of omega-3 fatty acids and proteins on age related diseases. It summarizes the information available until today. The information was gathered by searching in PubMed for the terms ""aging"[Title] AND "omega-3"[Title]" (16 results), ""nutrition"[Title] AND "proteins"[Title]" (22 results), ""aging theories"[Title]" (8 results) and ""bioactive substances"[Title]" (49 results) (These findings were last updated on the 29th of November 2021). Other pages used are Web of Science, Jama, New England Journal of Medicine, Scopus, ResearchGate and Science Direct. The alternative spelling "ageing", "ω-3" and "n-3" were considered in the searches. Only articles in English and with full-text availability were used and articles from the last 10 years were preferably selected. Some articles were excluded due to irrelevance. For further clarification purposes additional sources have been selected. To organize the used sources the reference manager Mendeley was used.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

AA	Amino Acid
ADHD	Attention Deficit Hyperactivity Disorder
AGEs	Advanced Glycation End Products
AHA	American Heart Association
ALA	Alpha-Linolenic Acid
BBB	Blood Brain Barrier
CHD	Coronary Heart Disease
CKD	Chronic Kidney Disease
CM	Chylomicron
COPD	Chronic Obstructive Pulmonary Disease
CRP	C-reactive Protein
CVD	Cardiovascular Disease
DHA	Docosahexaenoic Acid
DNA	Deoxyribonucleic Acid
EAA	Essential Amino Acid
ECM	Extracellular Matrix
EPA	Eicosapentaenoic Acid
FDA	Food and Drug Administration

FSH	Follicle Stimulating Hormone
GERD	Gastroesophageal Reflux Disease
GH	Growth Hormone
GHRH	Growth Hormone Releasing Hormone
GIT / GI Tract	Gastrointestinal Tract
HALE	Healthy Aging: A Longitudinal Study in Europe
HDL	High-Density Lipoprotein
hTERT	Human Telomerase Reverse Transcriptase
IGF-1	Insulin-like Growth Factor 1
IL	Interleukin
LDL	Low-Density Lipoprotein
LH	Luteinizing Hormone
mtDNA	Mitochondrial Deoxyribonucleic Acid
NADPH Oxidase	Reduced Nicotinamide Adenine Dinucleotide Phosphate Oxidase
NCD	Non-Communicable Disease
NO	Nitric Oxide
NOX	Types of NADPH Oxidase
PUFAs	Polyunsaturated Fatty Acids
RAAS	Renin-Angiotensin-Aldosterone System

RNA	Ribonucleic Acid
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SA-node	Sinoatrial Node
SCS	Seven Countries Study
SDA	Stearidonic Acid
SENECA	Survey in Europe on Nutrition and the Elderly
SIRS	Systemic Inflammatory Response Syndrome
SOD	Superoxide Dismutase
TGFβ	Transforming Growth Factor β
TNFα	Tumor Necrosis Factor α
UV	Ultraviolet
VLDL	Very-Low-Density Lipoprotein
WHO	World Health Organization
XP	Xeroderma Pigmentosum

INTRODUCTION

According to the World Health Organization (WHO), by 2050 2 billion people will be over 60 years old. It will be twice as many as there were in 2020. In 2050, 22% of people will be over 60 years old. This demographic shift will challenge the healthcare system greatly (World Health Organization, 2018). When looking only at Europe the situation is similar, but the percentages are already a bit higher – in 2019, about one third of people were aged 55 or older, and by 2050 it is projected to reach 40,6% on average (Fig.1) (European Commission, 2021).

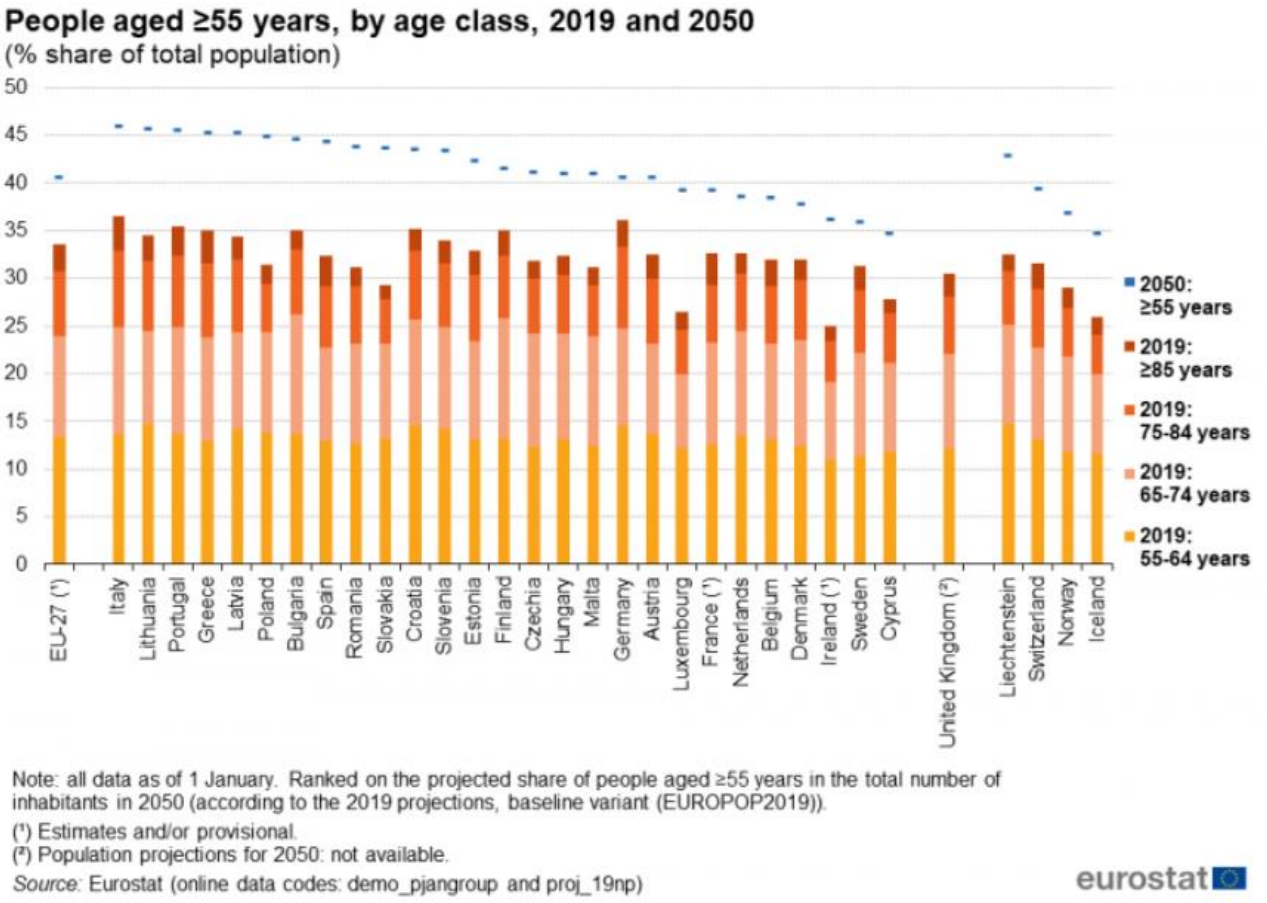

Fig. 1. **People in Europe aged ≥ 55 years, by age class, 2019 and 2050** (European Commission, 2021)

These extra life-years bring great opportunities with it for our society, e.g. more time for education and specialization, but all of this greatly depends on the health of the aging population. Additional years spent with disability and low quality of life do not have such a positive effect. Also, there is great diversity in the abilities of elderly, while some enjoy good functioning and health others may require significant help already at a relatively young age. Chronological age is only loosely associated with such processes. Despite this fact, health at an older age is not random. There are many factors that affect health at an older age, which can be divided into modifiable and non-modifiable. The non-modifiable factors are e.g. genetics, sex, ethnicity, or socioeconomic factors. The modifiable factors mainly include maintaining healthy behaviors such as eating a balanced diet, sufficient physical activity, and the avoidance of harmful substances e.g. tobacco. These factors start to influence the aging processes already at an early age, even fetal factors or how we live as children may have a significant effect (World Health Organization, 2018).

Another important factor is ageism, which is defined as "discrimination against a person on the basis of their age" (World Health Organization, 2018). It is a very prevalent problem in our society today, it may even undermine the healthcare quality of older people due to the lack of acknowledgement of serious health complications. Until today, it is common to assume elderly people to be frail, depending on others to survive, and becoming a burden to our society. These beliefs might further hinder the opportunities of healthy aging (World Health Organization, 2018). The education of doctors and patients is the main key to possibly improve the health of the aging population, especially to optimize the modifiable factors that can lead to a healthier older age.

This thesis focuses on a part of the nutritional factors, the intakes of proteins and omega-3 fatty acids and how they may lead to some health benefits. The thesis is divided into three main parts. The first part summarizes what is known about aging and its manifestations. In the second part the health benefits of an increased or decreased protein intake are discussed. The last part summarizes the information available on omega-3 fatty acids and their health benefits. Both omega-3 fatty acids and proteins are put into context with several age-related manifestations mentioned in the first chapter.

1. AGING

Aging is a complex multifactorial process that until today is not fully understood. It affects the human body on many different levels (molecular, cellular and systemic) and, because of the increasing life-expectancy nowadays, leads to many different diseases, potentially reducing the quality of life and leading to a rise in non-communicable diseases (NCD) (Troesch et al., 2020). Its most definite manifestations are a decreased fertility and increased mortality-risk (da Costa et al., 2016) as well as a higher incidence and prevalence of diseases (Aalami et al., 2003). It is a time-dependent and chronic process – even though often random – it can be seen that some organs, e.g. joints and tendons, exhibit signs of aging earlier if they are used excessively. Other organs such as the brain may even age more slowly if used frequently (Trevisan et al., 2019). Whether aging can be considered a disease or not is still being discussed (Schmeer et al., 2019).

The investigation of the processes behind such phenomena are commonly based on chemistry and biochemistry (da Costa et al., 2016) but, also, scientists of other fields have tried to explain the causes and processes of aging. Many different theories have been formed to elucidate the complex pathological, biological, chemical and psychological aspects of aging. But before getting into these theories and trying to explain why we are aging, a definition of the term "aging" is needed.

Because of the complexity of this phenomenon many different definitions of aging can be found in the literature, some of them already containing a causal theory. It has been described as "[phenotype] = [genotype] + [(diet, lifestyle and environment)]" (Anton et al., 2005). Another definition describes it as a "breakdown of self-organizing systems and reduced ability to adapt to the environment" (Vasto et al., 2010). It is clear that there is not just one single process of aging but multiple processes which are combined and interact with each other on multiple levels (da Costa et al., 2016). Aging does not have a single identifiable cause, but it is influenced by many different factors (Weinert & Timiras, 2003).

The term "senescence" is used for more precision, and is defined as the "progressive deterioration of bodily functions over time". Even though the term "senescence" is more precise, the term "aging" is more common in the literature (Dollemore, 2002).

2. MANIFESTATIONS OF AGING

The manifestations of aging are numerous and affect our body on all levels. All body systems are affected and will be discussed individually in the following chapters (chapter 2.1 – 2.7). These changes are also called NCDs and are often not only age- but also lifestyle-associated (Troesch et al., 2020). Good health at an extremely old age is rare (Khan et al., 2017). Many patients suffer from multiple co-morbidities and need a combination of different drugs (polypharmacy) (Troesch et al., 2020). Additionally, personal adaptation of healthy behavior becomes less likely with age (Tucker et al., 2004).

2.1 Changes in the Cardiovascular System

The cardiovascular system is subject to many changes in the aging patient. A progressive myocyte loss can be observed leading to a reaction which increases the size of the remaining myocytes in the ventricles. The myocardium stiffens as well as large vessels, both arteries and veins. This leads to a poorer expansion function of the heart and a decreased filling during diastole, which results in a lower afterload. Also a cellular loss at the level of the sinoatrial node (SA- node) can be observed, the maximum heart rate decreases as a result (Aalami et al., 2003).

Arteries are also prone to their own age-specific changes. Cardiovascular disease (CVD) has an increased prevalence in the elderly population (da Costa et al., 2016). Coronary heart disease (CHD) is strongly associated with age, it is also the leading cause of death in Europe (Paneni et al., 2017). The vessels become more brittle and the collagen content of the extracellular matrix (ECM) increases on average by 100% (da Costa et al., 2016). In great vessels, such as the aorta, collagen content increases while the amount of elastin decreases (Paneni et al., 2017). Atherosclerotic plaques form and the pulse pressure widens (Khan et al., 2017).

The mechanisms of these arterial effects are based on two main factors: “endothelial dysfunction” and “central arterial stiffness” (Paneni et al., 2017). Endothelial dysfunction leads to an increased thrombotic risk and decreased expansive function of the vessels. It is largely associated with decreased nitric oxide (NO) levels. Increased oxidative stress and chronic

inflammation related to increased reactive oxygen species (ROS) levels are largely the cause of this decrease in NO levels. They are further associated with thrombosis and atherogenesis (Paneni et al., 2017).

Many mechanisms try to compensate for these losses such as increasing the sensitivity to beta-adrenergic stimuli and an increase of the duration of the contraction. Those compensatory mechanisms do not continue without consequences: increased blood pressure and the increase of the size of individual myocytes as well as the increased deposition of collagens lead to left ventricular hypertrophy, further worsening the relaxation abilities of the heart (Aalami et al., 2003). The systolic blood pressure increases while the diastolic blood pressure remains low or even decreases (Khan et al., 2017). A low diastolic pressure also worsens the coronary perfusion, which further worsens the condition of the heart due to ischemia (Paneni et al., 2017).

2.2 Changes in the Pulmonary System

The declines in the function of the pulmonary system over time are markers of aging. The function of the pulmonary system declines consistently with each decade in adults over 70 (Khan et al., 2017). The chest wall becomes more rigid due to calcification in the cartilages and arthritis, which decreases the respiratory muscle's ability to contract. The muscles become atrophic, which puts more of the respiratory effort on the diaphragm and abdominals, which are not able to compensate fully. A ventilation-perfusion mismatch develops leading to a decrease in the efficacy of the respiratory system. Hypercapnia and hypoxia do not stimulate respiration as strongly as they should. This leads to a drop in vital capacity (Aalami et al., 2003).

Chronic obstructive pulmonary disease (COPD) is a typical age-related disease and obstructions of smaller airways become more common with aging (da Costa et al., 2016). Additionally, susceptibility to pneumonia increases (Khan et al., 2017).

2.3 Changes in the Renal System

After the age of 50, the size of the kidneys starts to decrease. Mostly, the cortical tissue is lost, while the medulla does not change significantly. The cause of this tissue loss is due to glomerulosclerosis caused by hypertension, diabetes mellitus and atherosclerosis. Glomerulosclerosis develops and decreases the glomerular filtration rate. Creatinine production decreases as well, which leads to relatively constant creatinine levels in the blood (Khan et al., 2017). Problematically, the reserve for filtration becomes low, which makes it more difficult for the kidneys to compensate e.g. during ischemic events. Also, fibrosis is found in the kidneys, as well as an increase of the tubule length, the renin-angiotensin-aldosterone system (RAAS) is less reactive and renin production decreases. It can lead to acid-base abnormalities, abnormalities with fluids, and electrolyte disbalances (Aalami et al., 2003).

2.4 Changes in the Gastrointestinal System

Changes in the neuromuscular function of the gastrointestinal tract (GIT) are most common in the esophagus and pharynx. Degeneration of the neuromuscular system may lead to symptoms such as reflux or achalasia (Aalami et al., 2003). Gastroesophageal reflux disease (GERD) is a typical age-related disease – not only its incidence but also its severity increase with aging (da Costa et al., 2016). Muscle weakness and failure of muscle coordination can often be secondary to neurological diseases (chapter 2.7). This failure of coordination may lead to secondary structural problems such as atrophy, fibrosis and villi loss (Aalami et al., 2003).

Changes in the structure of the GIT are mostly seen in the colon – diverticulosis is a very common age-related problem. It is caused by a thickening of the muscular layers of the colon: elastin builds up in the *muscularis propria* and *muscularis mucosae*, in a process which may be increased by inflammation and fibrosis (Aalami et al., 2003). Also, the incidence of colon cancer increases with age, as well as the incidences of many other types of cancers (Silva et al., 2015). Abdominal pain, on the other hand, is less frequently recorded in older patients (da Costa et al., 2016).

2.5 Changes in the Hepatobiliary System

After the age of 50, the liver decreases steadily in its size. At the same time, blood flow alterations can be observed. While the size is decreasing, the number of hepatocytes decreases as well. In order to compensate for the loss of hepatocytes, the remaining cells have an increased volume. Despite these processes, the filtration and detoxification function of the liver remain at basal level during aging. The most affected function may be protein synthesis: lower levels of clotting factors and other proteins are produced by the liver in aging patients, still their levels in the blood usually do not significantly decrease (Aalami et al., 2003). Additionally, a mild decrease in albumin levels may be recorded in the elderly (Khan et al., 2017).

2.6 Changes in the Endocrine and Immune System

In women, postmenopausal changes are quite obviously the clearest changes in the endocrine system found during aging. It leads to an estrogen deficiency and a subsequent increase in luteinizing hormone (LH) and follicle stimulating hormone (FSH). The main problem is caused by a rapid bone loss, although later the speed of bone loss decreases. In postmenopausal women, the risk of pathological fractures and osteoporosis is highly increased. Also, the loss of the cardioprotective effects of estrogen cannot go unmentioned. This may be caused by low-density lipoprotein (LDL) increases, while the level of high-density lipoprotein (HDL) decreases. Other unpleasant side effects such as urogenital atrophy, loss of libido and mood swings can happen. Unfortunately, hormonal therapy also has a lot of potentially more severe side effects, which makes it problematic to use (Aalami et al., 2003).

In men, the sex-hormone levels -specifically testosterone- decrease during aging. It can lead to side effects, such as central obesity and muscle loss, which also leads to an increased cardiovascular risk. Testosterone therapy may improve the outcomes (Faulkner & Belin de Chantemèle, 2019).

With age, an impairment of glucose tolerance occurs progressively. The insulin levels decrease as well as the levels of insulin-like growth factor. The reparation of pancreatic beta cells declines with age (Khan et al., 2017).

The thyroid gland function only decreases mildly with age, causing a mild hypothyroidism. In the elderly population, both increased and decreased thyroid stimulating hormone levels are found more frequently (Barbesino, 2019). Meanwhile, the level of parathyroid hormone increases with age, while calcitonin is decreased. This process may also lead to osteoporosis (Aalami et al., 2003).

The levels of cortisol also change in the elderly patient. Patients suffer from sleep problems and waking up early in the mornings. Those changes may be explained by increased levels of cortisol and decreased night production of growth hormone (Kern et al., 1996). The sympathetic body responses may be altered, e.g. thermoregulatory functions may be less functional in elderly, which can cause a higher sensitivity to low temperatures. In relation to the sympathetic dysregulation, there may be a decreased adaptation of blood pressure, heart rate, and pH levels (Aalami et al., 2003).

Inflammatory processes are associated with increased frailty and loss of muscle mass, also known as sarcopenia (Troesch et al., 2020). Overall, the immune function declines with the aging process. The volume of white matter of the bone marrow decreases in older individuals (da Costa et al., 2016). The decreased immunity mediated by T-lymphocytes increases the risk of infections. Responses to interleukin (IL) 2 decrease, and lead to a decreased natural killer cell function. Some mediators of systemic responses increase more in elderly patients rather than in younger patients, which may lead to more complications due to systemic inflammation and higher incidences of systemic inflammatory response syndrome (SIRS) (Aalami et al., 2003). Also, the epithelial barrier function shows signs of impairment, which makes it easier to penetrate for pathological organisms. The hypodermal layer of the skin becomes atrophic (da Costa et al., 2016). The age-related changes in all of these immune functions are collectively called “immunosenescence” (Khan et al., 2017).

2.7 Neurological Changes

The decrease of mitochondrial activity in the neurons is highly associated with the aging process (da Costa et al., 2016). Also, cerebral atrophy, a loss of brain weight (Khan et al., 2017) and a decreased integrity of the blood brain barrier (BBB) (Barnes et al., 2021) are all associated with aging. A diffuse astrocytosis and microglial activation is found in the aging brain (Cutuli, 2017). Alzheimer's disease and Parkinson's disease are related to old age (Trevisan et al., 2019). Incidences of cerebrovascular accidents and delirium increase with age (Aalami et al., 2003). Brain aging is associated with a decreased learning ability and memory problems, as well as the development of neuropsychiatric disorders (Cutuli, 2017). The plasticity of synapses decreases and the building of new neurons becomes impaired (Khan et al., 2017).

Almost all sensory organs are affected by aging: mainly vision, audition, and tactile sensation. In the eye, the rods and cones undergo degenerative changes (Aalami et al., 2003). The lens becomes less flexible due to protein depositions, leading to presbyopia. Age-related cataracts appear more frequently (da Costa et al., 2016). In the ear, several changes occur: the eardrum stiffens, and the cochlea and mechanoreceptors lose sensitivity. Also, reflex time increases as well as the time needed for signal conduction (Aalami et al., 2003).

3. THEORIES OF AGING

Aging has been linked to many different pathophysiological mechanisms and causes. Until this day, over 300 different theories of aging have been formed (da Costa et al., 2016) – most of them remain controversial and unproven (Aalami et al., 2003). Their sheer quantity makes it almost impossible to describe them all. To simplify the theories, they have been grouped according to their similarities (da Costa et al., 2016). There are biological, psychological and sociological theories of aging (Lange & Grossman, 2014). In this paper, the focus lies on the biological theories, which are mostly divided into three groups (Fig.2).

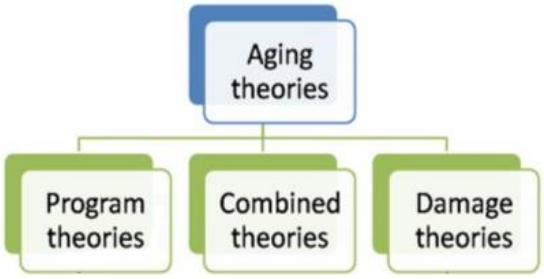

Fig. 2. **Categorization of the main biological theories of aging** (da Costa et al., 2016)

The first group is that of program theories (chapter 4.1). They are also called “adaptive” (Goldsmith, 2016), “intrinsic” or “developmental-genetic” causes of aging (Aalami et al., 2003). The second group are the “error” or “damage” theories (chapter 4.2). They are also called “extrinsic” or “stochastic” causes of aging (Aalami et al., 2003). The third and last group are the combined theories. They contain aspects of both previously mentioned categories (da Costa et al., 2016). Nowadays, most researchers agree that there is not just one singular cause of aging, but that it must be an interplay of many different factors.

Tab. 1. **Biological Theories of Aging**

Name of group of theories	Short description
Program theories	Genetically programmed aging process giving aging species an evolutionary benefit
Theories of programmed senescence	Aging-genes trigger the aging process
Endocrine theories	Decrease of pituitary hormones and increases of insulin growth factor trigger aging
Theories of telomere shortening	Apoptosis caused by reaching the limited number of cell replications is the main aging mechanism
Immunological theories	Decreased immune function causes an increased infection-susceptibility causing aging
Damage theories	Random external events causing accumulative damage to the organism causing aging
Theories of free radicals	Free radicals causing damage to molecules is the main trigger for aging
Mutation accumulation theories	Errors in DNA and its products cause aging
Wear and tear theories	Cells and tissues wear out over time and lose their function and repair mechanisms
Cross-linking theories	Over time molecular cross-links are formed in tissues causing obstruction of important processes
Combined theories	Combination of both groups of theories

Each of the previously mentioned theories (Fig. 2) can be further subdivided into smaller groups (Tab. 1). These subgroups vary widely between different sources and not all of them can be listed here. The main program theories are the theories of programmed senescence (chapter 3.1.1), the endocrine theories (chapter 3.1.2), the theories of telomere shortening (chapter 3.1.3), and the immunological theories (chapter 3.1.4). The main damage theories are the free radical theories (chapter 3.2.1), mutation accumulation theories (chapter 3.2.2), wear and tear theories (chapter 3.2.3), and cross-linking theories (chapter 3.2.4).

Many of the aging theories are based on the previously mentioned pathophysiological changes seen during aging (chapter 2). It often remains unclear whether the changes should be seen as a consequence or a cause of aging.

3.1 Program Theories

Program theories focus on the interplay of environment and genetics (Trevisan et al., 2019). Such theories suggest the existence of an evolutionary benefit in species with a limited lifespan (Goldsmith, 2013). They are also called “active”, “adaptive” (da Costa et al., 2016), “intrinsic”, or “developmental-genetic” theories (Aalami et al., 2003). They suggest the presence of active mechanisms to limit the lifespan of a species (Iouk & Titorenko, 2017).

This would mean that the deterioration of the organism over time leading to death creates space for a new generation with slightly different genes, potential adaptation to the environment, and better chances of survival for the entire species. The altruistic reason would be to lower the risk for overcrowding (Goldsmith, 2016). The individuals of a species compete for limited resources. Thus, to create space for new individuals, it is necessary for the previous ones to disappear (da Costa et al., 2016).

This chapter discusses the genetic influences on aging, genes causing a longer or shorter lifespan, intrinsic mutagenesis, shortening of telomeres, and the immunological changes which may cause aging.

Many of these theories are widely based on simpler animal studies with species which have a much shorter lifespan than humans, as well as a smaller genome (such as yeasts, flies, salmons, or mice). It is difficult to study humans due to their relatively long lifespan and because the genetic information in mammals is very complex. The enormous length of the human genome makes it difficult to identify single genes responsible for aging. Until today, only little is known about the possibilities to transfer this information on the human species (da Costa et al., 2016).

One of the main arguments used against these theories is that, until recently, most humans would die before they had the chance to achieve old age. This would contradict the evolutionary development of an aging mechanism (Kowald & Kirkwood, 2016).

3.1.1 Theories of Programmed Senescence

In some species, certain "age genes" have been identified, which program biochemical pathways leading to death. Salmons die after successful mating, which is probably the best example of a programed death. This can also be observed in other animal species, which hints to the possibility of a programed death in more highly developed species, but not much is known so far. If such a supposition was true, it could be possible to deactivate the responsible genes as to increase the lifespan of individuals of a species. Such deactivating mutations are described as "life-expanding mutations". So far, no mutation has been reported that could entirely stop the process of aging. In some model organisms, a significant increase of the lifespan has been noted after such mutations.

The most employed organism for age prolonging gene research is the nematode *Caenorhabditis elegans* with a lifespan of only a few days to weeks (da Costa et al., 2016). A mutation in the age-1 gene leads to a developmental arrest of the organism in its adult phase. Its lifespan is therefore doubled. This developmental arrest suggests a genetic interaction between development and age. It has been shown that the lifespan of *C. elegans* also depends on other factors e.g. caloric intake and time of onset of caloric restriction (Fontana et al., 2010). Unfortunately, these model organisms are unlikely to reflect insight on the human physiology of aging (da Costa et al., 2016).

3.1.2 Endocrine Theories

According to these theories, the process of aging is controlled by a biological clock regulated by hormones (Jin, 2010). They are also called neuroendocrine theories due to the control that the hypothalamus exerts over hormones (da Costa et al., 2016). Hypothalamic-pituitary-adrenal axis hormones generally decrease during the aging process (Trevisan et al., 2019).

The mainly investigated hormones potentially causing aging are GH, its target IGF-1, and melatonin (Trevisan et al., 2019). Their concentrations generally decrease with age. GH deficiency in humans can cause signs of early aging. Interestingly, these patients live longer than average. Also, in rats with mutations causing a GH deficiency, an increase in life-expectancy and a delayed immune aging is seen (da Costa et al., 2016).

The changes in the biological clock of elderly people lead to the suspicion of melatonin as a cause of aging. Many elderly patients tend to get up early in the morning and go to bed very early in the evening. Melatonin changes may be responsible for these shifts in the biological clock (Trevisan et al., 2019).

3.1.3 Theories of Telomere shortening

The natural chromosome ends are called telomeres. They are non-coding DNA (deoxyribonucleic acid) fragments, which repeat and contain many guanin-bases (Liu et al., 2019). During DNA replication, the 5’ to 3’ direction is the direction of the leading strand. The other strand of DNA is called the lagging strand, and this is where the problem occurs: The enzyme DNA-Polymerase connecting the new bases to the original strand which got separated only moves along the 5’ to 3’ direction. It would have to work against the direction of separation of the DNA in the lagging strand to replicate it successfully. This problem is solved by adding certain RNA (ribonucleic acid) primers to the lagging strand, which are continued by some bases, which are then called Okazaki fragments. These fragments allow the DNA-polymerase to attach and build the lagging strand until the next primer. In the end of the DNA-strand, until the first primer gets attached, the strand cannot be built. This leads to a loss of some part of the telomere with each DNA replication. It is only logical that organisms can only build new cells

effectively for some time without losing a significant part of the telomeres. This is called the "end replication problem" (Fig. 3). Telomere length therefore does play an important role in the aging process and in limiting the maximum life span (Dillin et al., 2008).

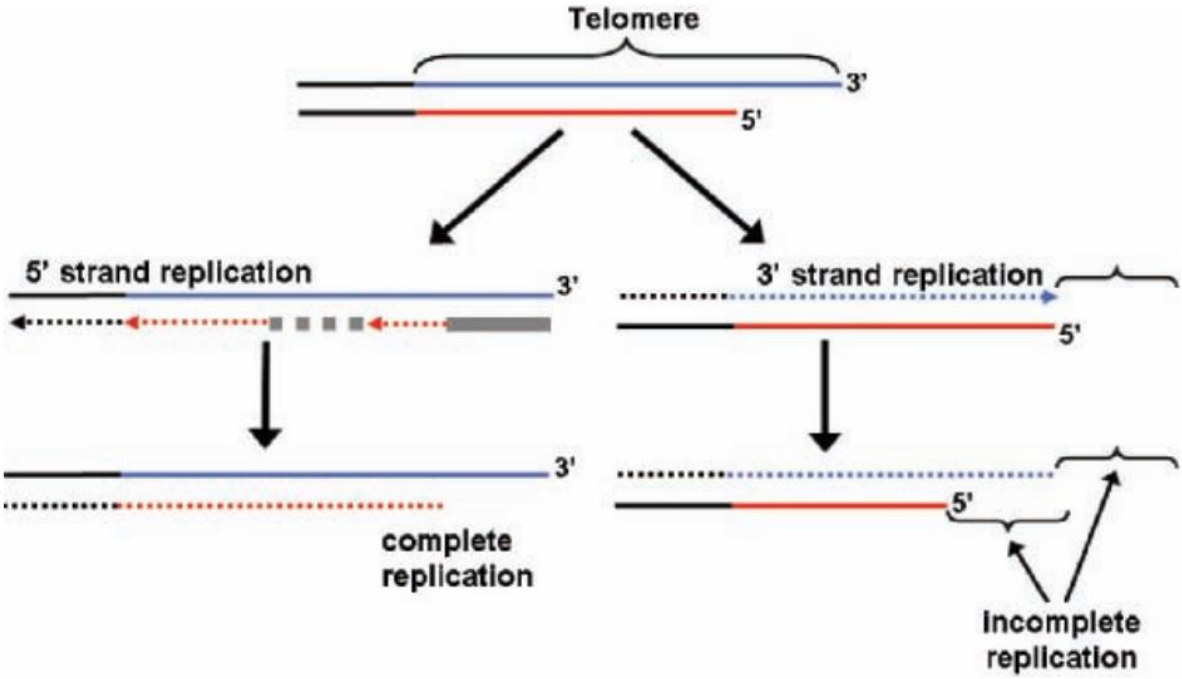

Fig. 3. **The end replication problem** (Dillin et al., 2008)

Telomerase is an enzyme counteracting this mechanism of constant shortening, and it is the only way of lengthening the telomere (Liu et al., 2019). It was discovered in some species, and it can add some G-bases to the end of the DNA strand. Human cells do not contain such an enzyme – except for germ cells, in which hTERT, the human telomerase reverse transcriptase, was identified. In some cancer cells, hTERT becomes activated and leads to immortality of the cell line (Dillin et al., 2008).

Should the length of the telomere become insufficient, the cells protective mechanisms step in. A common consequence is apoptosis, cellular dysfunction, or cell cycle arrest (Dillin et al., 2008). This limit leading to arrest at a specific length of the telomere is called the "Hayflick

limit" (Liu et al., 2019). Cells which have reached a critically shorted length of their telomeres are said to have reached their Hayflick limit (Ndifon & Dushoff, 2016).

Cell senescence becomes visible in cells with short telomeres. Activation of telomerase reduces signs of senescence in cells, and increases their lifespan. Monocellular organisms with large, artificially increased telomeres did, surprisingly, not show an increase in their lifespan. Humans with longer telomers show a decrease in age-related pathology (Liu et al., 2019).

3.1.4 Immunological Theories

Immunological responses become weaker in aging individuals. This process is not only observed in humans but also in other animals. It leads to an increased infection risk and worse outcomes during infection (Trevisan et al., 2019). Innate immune mechanisms cause increased inflammation ("inflamm-aging"), while adaptive immunity involution leads to a decrease of the immune response and its precision ("immuno-senescence") (Schmeer et al., 2019). The immunological theories suggest that this decline in immunity is not accidental, but a planned increase in vulnerability and likelihood of death. Some of the main age-related diseases. Such as cardiovascular diseases, Alzheimer's disease, and cancer are also related to a decline in immune function (Jin, 2010). Senescent cells may escape the elimination by the immune system via a secretion of cytokines. This accumulation of aged cells in aging individuals may be the cause of aging but it may also be caused by it (Schmeer et al., 2019).

3.2 Damage Theories

Damage theories are based on cumulative systemic damage (da Costa et al., 2016). They are also called “extrinsic” or “stochastic” causes of aging (Aalami et al., 2003). According to these theories damage is caused by different external stimuli (da Costa et al., 2016) such as free radicals, radiation, protein cross-linking, or errors in protein synthesis (Aalami et al., 2003). These factors may be modulated to influence aging and may explain the sometimes very different lifespans between individuals of one species, even if they are genetically identical (da Costa et al., 2016). Such theories describe a development of functional loss and accumulation of accidental lesions during aging which are caused by environmental factors via different mechanisms. Those mechanisms make up the different theories found in this category. Researchers nowadays mostly agree that the damage described in these theories is at least partially responsible for the aging process (Trevisan et al., 2019).

3.2.1 Free Radical Theories

ROS are highly reactive molecules causing oxidative damage to the body on a molecular level, especially to lipids, DNA, and proteins. The most important ROS are superoxide, hydrogen peroxide and hydroxyl radical. They are produced in the body by chemical reactions which partially reduce oxygen by adding an electron (da Costa et al., 2016). The main sources of ROS in cells are the mitochondrial transport chain, the nicotinamide adenine dinucleotide phosphate oxidases (NADPH oxidases) and the 5-lipoxygenase (Novo & Parola, 2008) (Fig. 4, Fig. 5, Fig. 6). Their effects are highly significant considering that, over the course of a lifetime, about 2-3% of metabolized oxygen is transformed into ROS. Until today the theory of ROS is one of the most discussed (Liochev, 2013) and most widely accepted (da Costa et al., 2016).

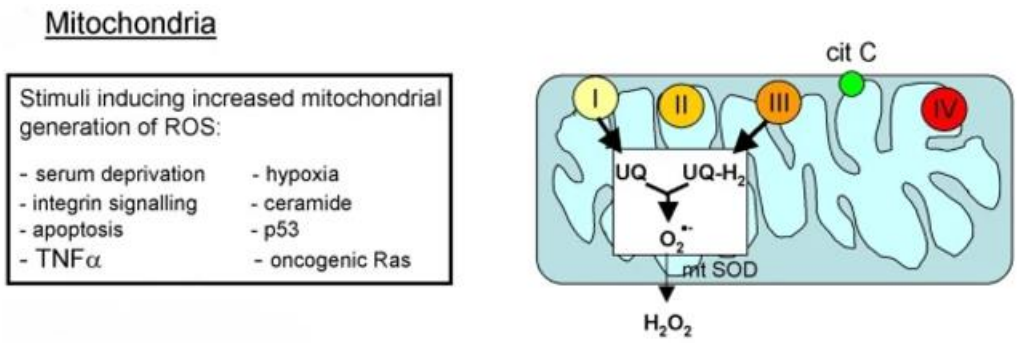

Fig. 4. **Mitochondria: major cellular sources of reactive oxygen species in living cells** (Novo & Parola, 2008)

Mitochondria are one of the main sources of ROS in the human cell. $O_2^{\bullet-}$ radicals are formed at the levels of complex I and III in the mitochondrial electron transport chain. Most of them are converted into H_2O_2 by mitochondrial superoxide dismutase (SOD), H_2O_2 can cross the mitochondrial membrane and therefore it can enter the cytoplasm of the cell (Fig. 4) (Novo & Parola, 2008).

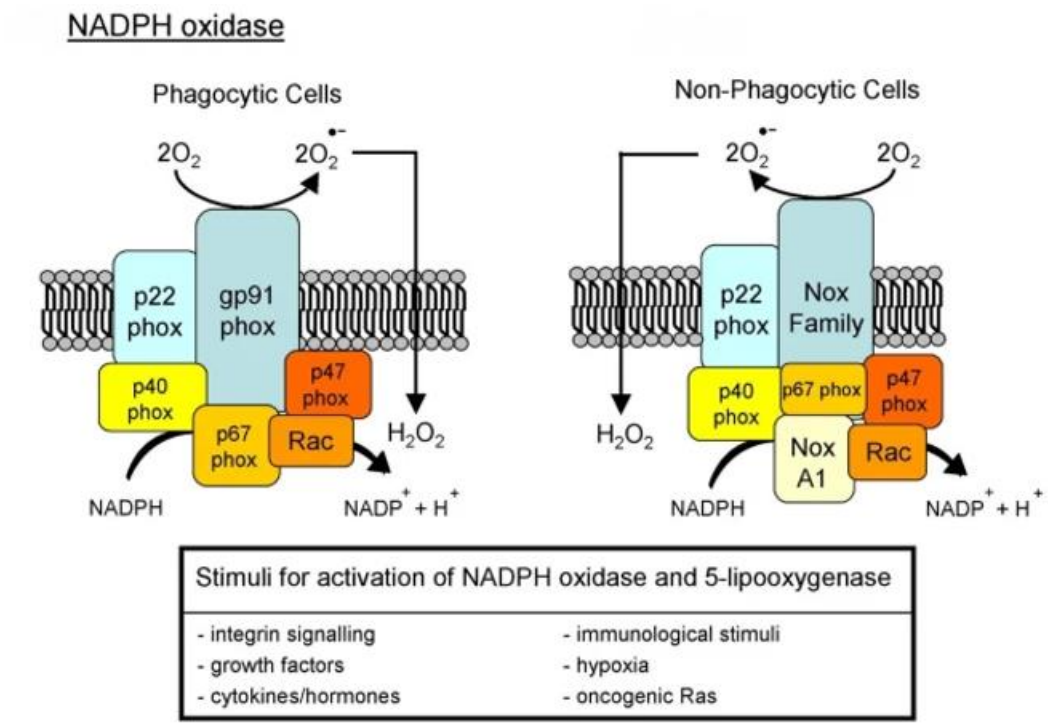

Fig. 5. **Reduced nicotinamide adenine dinucleotide phosphate oxidase: major cellular sources of reactive oxygen species in living cells** (Novo & Parola, 2008)

Two different forms of NADPH oxidase (NOX) can be found in the human body: the classic form in phagocytic cells such as macrophages or neutrophils and the non-classical form in other, non-phagocytic cells (Fig. 5) (Novo & Parola, 2008). The increased production of ROS by these mechanisms after activation has the function to kill invading microorganisms in phagocytic cells. The ROS serve as inflammatory mediators and they are important for innate immune functions (Panday et al., 2015).

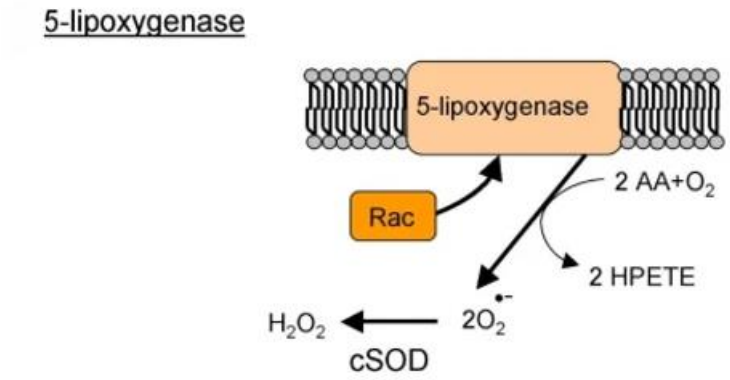

Fig. 6. **5-lipoxygenase: major cellular sources of reactive oxygen species in living cells** (Novo & Parola, 2008)

The enzyme 5-lipoxygenase (Fig. 6) is a part of the synthesis mechanisms of leukotrienes from arachidonic acid (Fig. 16) (chapter 7.4) and one of the mechanisms producing ROS in the human body (Novo & Parola, 2008).

Since some of the reactions producing free oxygen radicals are present in the mitochondria (Fig. 4), mitochondrial maintenance impairment occurs. It is described as one of the main mechanisms inducing aging. Due to the higher exposure of mitochondria to oxygen radicals, mtDNA (mitochondrial DNA) has a higher risk of damage by ROS than nuclear DNA. The organs most vulnerable to ROS damage are heart, brain and skeletal muscle (da Costa et al., 2016).

The theories of free radicals have undergone many changes over the last decades. While it was originally believed that accumulation of damage caused by the radicals would cause aging (Jin, 2010), nowadays researchers discuss the potential anti-aging effects caused by mechanisms counteracting the damage of free radicals. Low levels of free oxygen radicals could, therefore, even possess anti-aging properties, the so called “prosurvival signals” (Liochev, 2013). They act as signaling molecules in the cells. The systems which play a role in reducing oxidative damage activated by increased concentrations of ROS are highly adaptive and induce the expression of antioxidant genes, which have a protective effect (Silva et al., 2015). Researchers argue that the harmful effects of ROS only seem to play a role in defective signaling systems.

Direct damage may not play an important role. This is why some authors regard the traditional theory of ROS as wrong (Lapointe & Hekimi, 2010). Nevertheless, none of these theories are proven and the traditional ROS damage theory is not refuted. The discussion is still ongoing (Liochev, 2013).

It is clear that highly increased levels of ROS are damaging for cells. They may cause chronic inflammation, inducing necrosis or apoptosis (Paneni et al., 2017). Mechanisms counteracting an increased ROS production include caloric restriction, antioxidant rich nutrition, and the avoidance of inflammation (Lange & Grossman, 2014).

Other theories of aging are also based on oxidative damage to cells and their molecules. Similar to ROS also reactive nitrogen species (RNS) may induce aging. They have been associated with some age-related diseases, but the mechanisms remain unknown (da Costa et al., 2016).

Advanced glycation end-products (AGEs) are typically found in tissues of aged individuals and are nowadays highly associated with the aging process. AGEs are also produced in the body under normal circumstances. Additionally, they can enter the body exogenously via food or tobacco. High temperatures, high pH, and a low humidity may increase their formation in the food preparation process. Therefore, highly processed food contains more AGEs than unprocessed food. Decreasing the exposure to these substances may decrease oxidative stress and inflammation in the body and even prolong life (Sharma et al., 2015).

3.2.2 Mutation Accumulation Theories

Mutation accumulation theories are also called “error theories”. They suggest that, over time, errors in DNA and its products -RNA and proteins- accumulate, causing the cells to die. External factors such as radiation, pesticides, organic solvents, or even tobacco smoke are said to predispose these errors to happen more frequently. The theories suggest that a reduction or elimination of these factors could slow down or even stop the process of aging (Lange & Grossman, 2014). The influences of these factors on DNA are the cause of increased instability, which may lead to errors in replication, causing mutations. Several mechanisms have been set in place by cells to detect these errors and remove them. The mutations of those mechanisms are linked to several genetic diseases, called premature aging syndromes. *Xeroderma Pigmentosum* (XP) is probably the best known example of a genetic mutation of DNA repair mechanisms (da Costa et al., 2016). DNA instability is also related to telomere shortening (explained in chapter 3.1.3).

3.2.3 Wear and Tear Theories

Some tissues found in the human body have only limited repair or replacement capabilities. Examples of these tissues are neurons or heart muscle. In other tissues, damage may also not be repaired appropriately if it is too severe. This problem could be exacerbated by aging properties, which cause a decrease of the damage threshold, causing an increased susceptibility for irreparable damage. Studies suggest that aged cells lose their abilities for repair.

According to these theories, the increased use of a tissue, for example, cartilages in a sportsman, can lead to an increase of wear and tear mechanisms. Normal daily activity would also cause damage via wear and tear mechanisms to happen. They may be even more significant than the damage caused by exercise (Lange & Grossman, 2014).

3.2.4 Cross-linking Theories

These theories are also called connective tissue theories. According to them, biochemical processes may create links between structures which are not normally connected (Lange & Grossman, 2014). Protein damage during aging may lead to their cross-linking and an accumulation of toxic substances in cells, called “aggresomes” (Moldogazieva et al., 2019).

3.3 Combined Theories

Gerontologists nowadays mostly agree that there is not just one singular cause of aging. Many different causes are more likely to be at the base of the aging process (Kriete et al., 2006). The combined theories contain aspects of both damage and program theories, in different variations. They are highly complex theories on causes of aging based on many different aspects.

One of the main theorists is Strehler, who tried to combine the existing aging theories and formulated these 4 theses about aging (Strehler, 1977):

> *“1. Aging is universal, and, as such, a phenomenon associated with aging must occur in all individuals of a species, albeit in different degrees”*
>
> *“2. Aging must be intrinsic: the causes must be endogenous and they do not depend on extrinsic factors.”*
>
> *“3. Aging is progressive and must occur incrementally throughout the life-span.”*
>
> *“4. Aging must be deleterious, i.e., a phenomenon associated with aging will only be considered a part of the aging process if it […] holds no advantages for the individual.”*

Since then, many others tried to integrate the aging theories with each other, with some using the previously mentioned criteria. Until today the discussion is not completed.

4. AGING AND BIOACTIVE SUBSTANCES

Bioactive substances are e.g. proteins (Thiyagarasaiyar et al., 2020), fatty acids, vitamins, minerals, and others (Michalak et al., 2021). They are substances found in many foods, e.g. fruits and vegetables (Zielińska et al., 2017), but they can also be synthetically produced and used as supplements (Žmitek et al., 2020). They are also found as additives in many cosmetic products (Thiyagarasaiyar et al., 2020). In the past years, bioactive substances have been attributed many positive health effects, such as immunomodulation or photoprotection. Also anti-aging properties of bioactive substances are studied today, and some effects have already been discovered (Pangestuti et al., 2018).

One of the most discussed sources of bioactive substances is seaweed (Cao et al., 2020; Pangestuti et al., 2018; Su et al., 2020; Thiyagarasaiyar et al., 2020), which interestingly also contains high amounts of omega-3 fatty acids (Cao et al., 2020). The effects of omega-3 fatty acids will be further discussed in chapter 7.

Some effects that are discussed in relation to bioactive substances are their positive influences on skin aging (Michalak et al., 2021), as well as positive effects on age related diseases, such as diabetes or cataracts (Cao et al., 2020).

5. HEALTHY NUTRITION DURING AGING

Nutritional requirements are of fundamental importance throughout the entire life. In old age, some physiological alterations may lead to changes in the body's needs of several nutrients and not only the quality but also the quantity of food must be adapted (Dangour et al., 2007). Both under- and also overnutrition can lead to NCDs (Troesch et al., 2020).

Several problems occur often in elderly populations: oral health declines, swallowing problems occur, the senses of taste and smell change, and the overall mobility is often limited. Combined with a general loss of appetite (Institute of Medicine, 2010), it may lead to undernutrition of the elderly, which is a common problem in old age (Dangour et al., 2007).

Loss of appetite in the elderly may be associated with lower levels of physical activity, decreased energy requirements, and a slower metabolism. Elderly people tend to eat less than they should and, additionally, nutrients which are eaten cannot be as efficiently utilized in elderly patients. To counteract these processes, it is important to supply elderly people with nutrient dense foods (Institute of Medicine, 2010).

Polypharmacy is common in elderly patients (Troesch et al., 2020). Certain drug-nutrient interactions may limit the uptake of some substances, e.g. B-vitamins (Institute of Medicine, 2010).

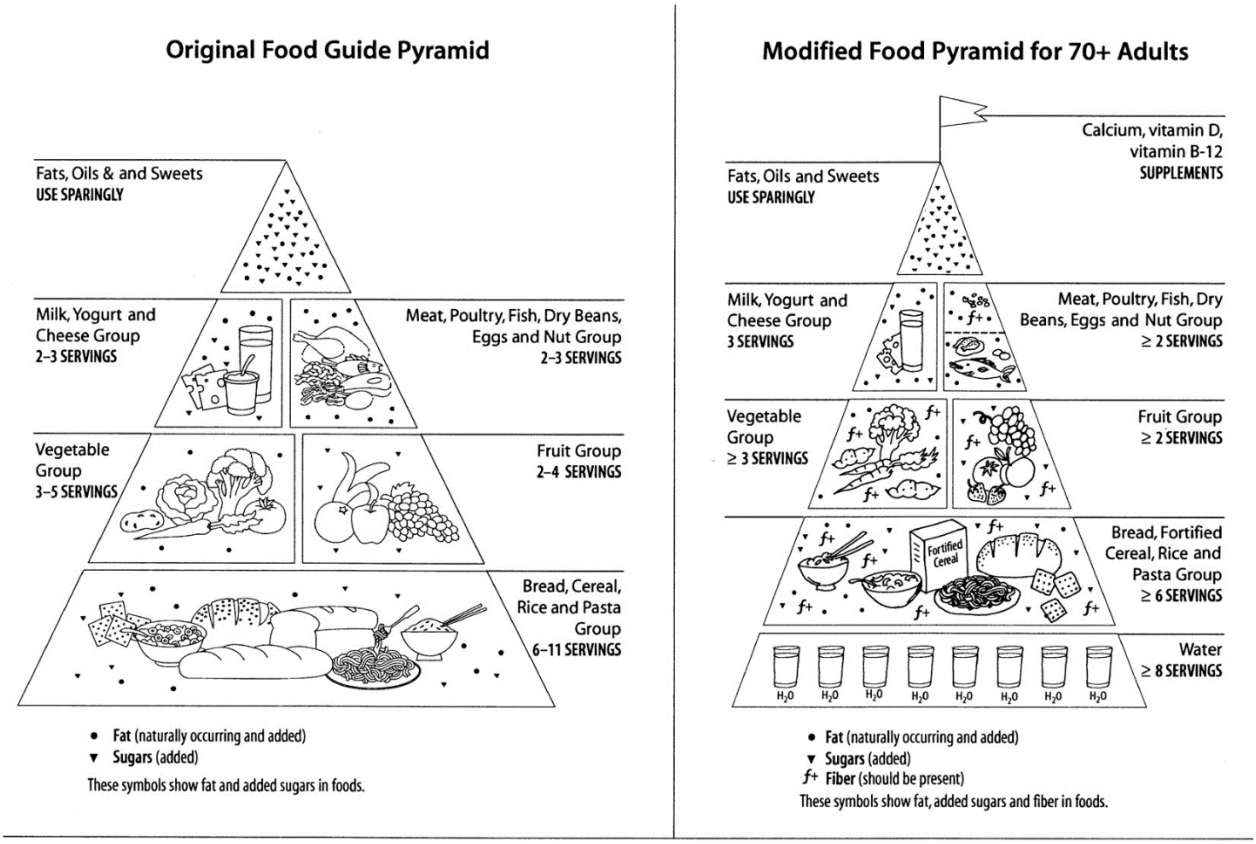

Fig. 7. **Original and modified food pyramid for 70+ adults** (Russell et al., 1999)

Russel published his modified food pyramid for adults over 70 years in 1999 (Fig. 7). The main changes that we see are the importance of controlled water intake because of increased dehydration risks in elderly patients, as well as the reduction of foods containing less nutrients, such as vegetables and fruits. Nutrient supplementation also becomes of greater importance with age, especially of calcium, vitamin D and vitamin B-12 (Russell et al., 1999).

Large studies about nutrition in elderly populations have been carried out in Europe during the last decades. The most known are the Seven Countries Study (SCS) (Keys et al., 1984), the HALE (Healthy Aging: A Longitudinal study in Europe) project (Knoops et al., 2004) and the SENECA (Survey in Europe on Nutrition and the Elderly) study (van Staveren et al., 2002).

The SCS was the first nutritional cohort study performed in elderly populations. It observed over 11,500 men for 25 years (Smit & van Duin, 2020), and concluded that a decreased risk of

coronary artery disease could be found in participants adhering to a Mediterranean diet. Also Japanese participants showed a lower cardiovascular mortality (Keys et al., 1984).

The SENECA study observed the nutrition of over 2,500 elderly people from 12 European countries over the course of 11 years. The study concluded that longer survival rates in elderly are associated with an increased adherence to the Mediterranean diet (van Staveren et al., 2002).

The HALE project started in 2001 and followed up over 7,000 participants from five European countries (Smit & van Duin, 2020). It is based on the SCS and the SENECA study, as well as other studies regarding non-nutritional factors. The study concluded that the adherence to a healthy lifestyle and consuming a Mediterranean diet caused a decline in mortality rates of over 50% (Knoops et al., 2004).

Healthy nutrition and a healthy lifestyle can influence overall health, especially cardiovascular health, and mortality in a positive way (Knoops et al., 2004). Furthermore, the nutritional needs vary between younger and older adults. More attention must be paid to the dosages of nutrients in the elderly since the absorptive capacities are decreased and the metabolism is slowed down. Also possible pharmacological interactions must be considered (Institute of Medicine, 2010).

6. PROTEINS

Proteins are made of amino acids (AA) which are linked together into a chain (Nature Education, 2014). There are 22 different amino acids found in proteins linked together in all possible variations (Ahern, Rajagopal, 2014). This pure amino acid chain is called the primary structure of the protein. The secondary structure of the protein is built by the alpha helices and beta sheet foldings. Both are mostly held together by hydrogen bonds, which makes up a 3-D conformation. This structure becomes even more complex by a series of further foldings called the tertiary structure. The final quaternary structure is a complex made up by several different polypeptide chains (Nature Education, 2014).

Each amino acid contains four groups: a hydrogen, an amine, a carboxyl group and an R part. Three of these groups do not vary in amino acids – the fourth one, the R part, also called "side chain", is unique for each of the 22 amino acids. The four parts are bound together by a central carbon atom (Fig. 8). When two amino acids are bound together, they form a peptide bond between the carboxyl group of one amino acid and the amine group of the other (Fig. 9). If two amino acids are bound together, they are called dipeptide. If more are bound together it is called an oligopeptide or a polypeptide. Proteins are usually the combination of multiple hundreds of amino acids (Ahern, Rajagopal, 2014). The most commonly used method to study these molecular structures is X-ray crystallography (Nature Education, 2014).

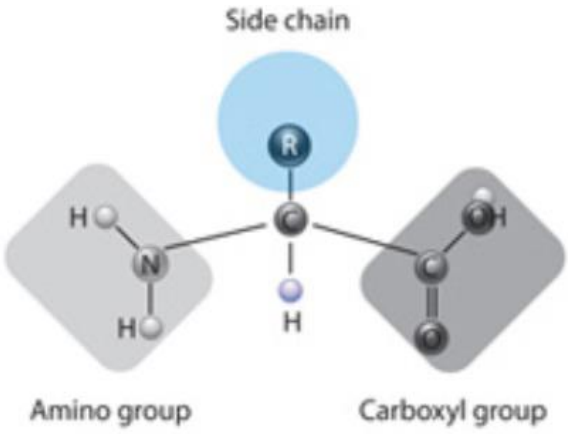

Fig. 8. **General amino acid structure** (Nature Education, 2014)

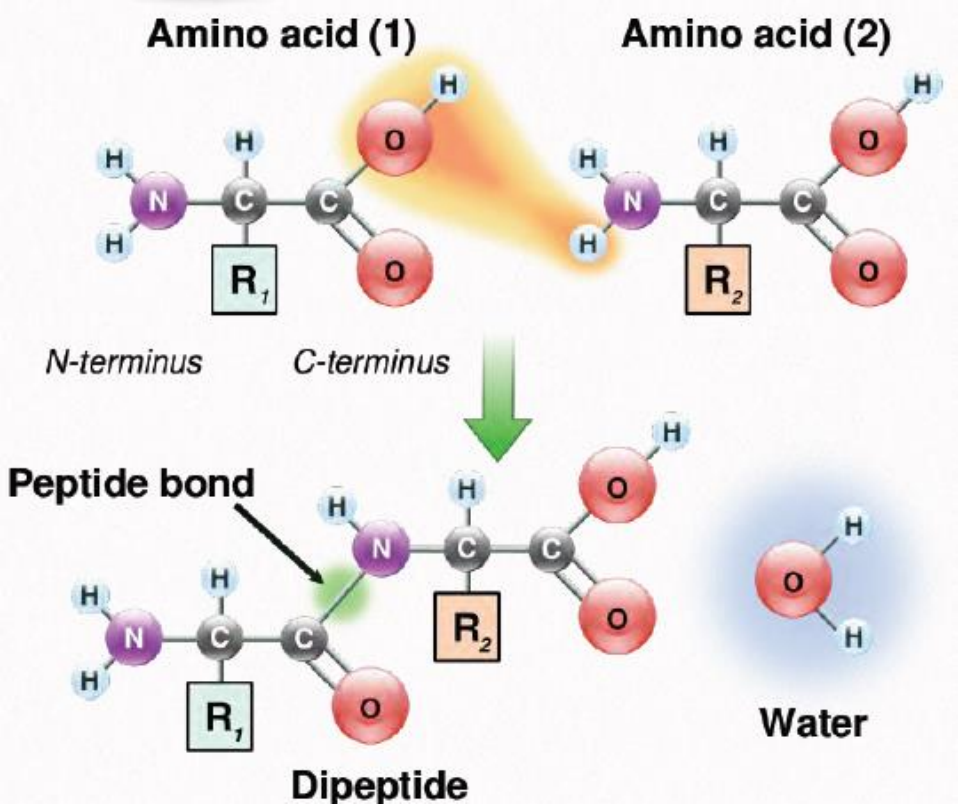

Fig. 9. **Formation of a peptide bond** (Ahern, Rajagopal, 2014)

Proteins are synthesized in a process called protein biosynthesis at the ribosomes, where the process of translation from triplets of messenger RNA to the fitting amino acid takes place. Only 20 out of the 22 existing amino acids have a fitting RNA triplet and play a role in the synthesis of proteins in the human body (Tab. 2). Proteins are further metabolized in the Golgi apparatus, even proteins with the same primary structure can be completely different in their further buildup and function after this process takes place (Nature Education, 2014).

Amino acids are divided into two categories: essential and non-essential (Tab. 2). Essential ones must be included in the diet, while non-essential ones can also be produced in the human body. Under some circumstances, some non-essential amino acids still must be included in the diet, e.g. if their production is not meeting the physiological needs. This may happen for “arginine, cysteine, glutamine, proline, selenocysteine, serine and tyrosine” (Ahern, Rajagopal, 2014).

Tab. 2. **Essential and non-essential amino acids** (Ahern, Rajagopal, 2014)

Essential (9 amino acids)	Non-essential (13 amino acids)
Histidine	Alanine
Isoleucine	Arginine
Leucine	Asparagine
Lysine	Aspartic acid
Methionine	Cysteine
Phenylalanine	Glutamic acid
Threonine	Glutamine
Tryptophan	Glycine
Valine	Proline
	Pyrrolysine (not of relevance in humans)
	Selenocysteine (not of relevance in humans)
	Serine
	Tyrosine

Even though not all amino acids can be produced by the body, all of them can be catabolized (Fig. 10). This happens, for example, in patients consuming a carbohydrate deprived diet or during starvation. They are used to produce energy in these situations by transformation into acetyl-CoA (ketogenic) and products possible to transform into glucose (glucogenic) (Ahern, Rajagopal, 2014). Situations causing stress on the human body, can also cause a shift to increased catabolism of proteins, ultimately leading to a loss of muscle (Weijs et al., 2014).

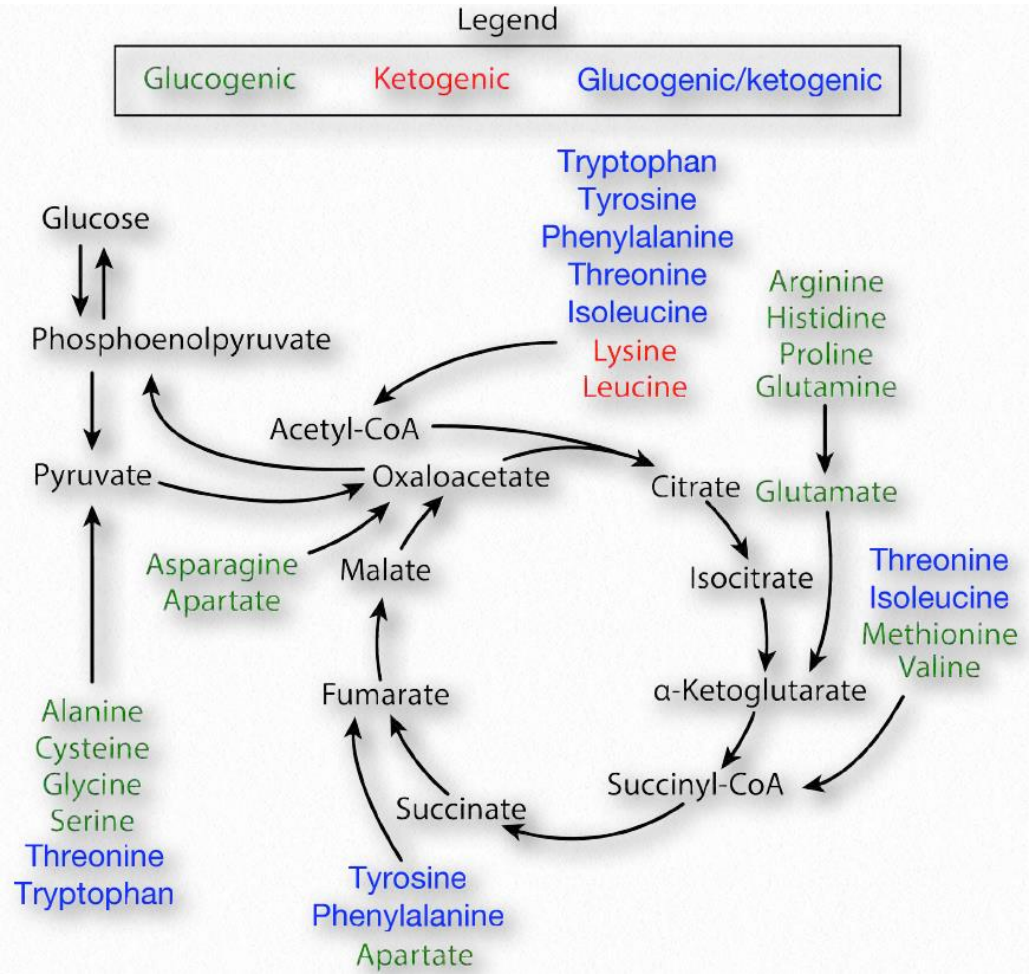

Fig. 10. **Catabolism of amino acids** (Ahern, Rajagopal, 2014)

Proteins are involved in all fundamental processes of the human body. They are the main component of all types of muscle tissue, as well as important structural components of skin and bone. They carry out enzymatic reactions; they are involved in immune functions, oncotic pressure development; they carry lipophilic substances in the blood, and are used for the energy metabolism in gluconeogenesis (Fig. 10) and ketogenesis (Weijs et al., 2014). Severe protein malnutrition leads to a disease called kwashiorkor, which leads to abnormalities in all aforementioned processes and is often fatal if untreated (Benjamin & Lappin, 2021).

Some age related diseases such as Amyloidosis, Alzheimer's disease, and Parkinson's disease, as well as prion-caused spongiform encephalopathies are associated with proteins (Ahern, Rajagopal, 2014) and their misfolding (Pereira & Rodrigues, 2021).

6.1 Sources of Proteins

Most essential amino acids (EAA) are produced by plants (Ahern, Rajagopal, 2014). Nevertheless, many plant sources of protein such as legumes, soy, and grains are not considered high quality sources of proteins. The quality of proteins depends on their bioavailability as well as their composition of AA. If not all essential amino acids are present in high amounts in the source of protein, the human body will eventually run out of these limiting amino acids and will not be able to produce proteins any further. In soy, the limiting amino acids are methionine and cysteine; in legumes, methionine; and in grains, lysine. Plant-derived collagens entirely lack tryptophan. Vegetal sources of protein also generally have lower levels of EAA than animal proteins (Tab. 3) (Joanisse et al., 2021).

Most animal proteins (meat, eggs, and milk products) are considered high quality because they have no limiting EAA. Daily protein recommendations are covered by consumption of one animal-based protein source. To reduce the risk of deficiency of EAA in plant-based diets, a combination of different plant-based protein sources is recommended (Joanisse et al., 2021).

Tab. 3. **Essential amino acids (EAA) and leucine content in different dietary sources**

Essential amino acids (EAA) and leucine content in different dietary protein sources.

Protein source	EAA g/100[a]	EAA g/25 g[a]	Leucine g/100 g[a]	Leucine g/25 g of protein[a]	Protein dose (g) to ingest 3 g of Leucine[a]
Animal-derived proteins					
Egg	16.5	4.1	6.9	1.7	43
Whey	34.1	8.5	10.8	2.7	28
Milk	30.3	7.5	8.7	2.2	34
Caseinate	32.8	8.2	9.0	2.3	33
Casein	24.8	6.2	7.9	2.0	38
Vegetal-derived proteins					
Soy	19.9	4.9	6.8	1.7	44
Wheat	18.0	2.4	6.0	1.5	50
Pea	23.6	5.9	7.1	1.8	42
Potato	29.3	7.3	8.2	2.0	37
Corn	21.0	5.2	13.5	3.4	22
Oat	13.7	3.4	5.7	1.4	52

(Joanisse et al., 2021)

Nowadays, many new and -potentially more sustainable- sources of proteins are emerging. Among them, we can find microorganisms such as microalgae, cyanobacteria or other bacteria, and fungi, as well as insects and dairy byproducts (Pereira & Rodrigues, 2021).

Milk contains two types of proteins: whey proteins and casein. Whey proteins are easily digested and quickly absorbed. They contain high amounts of essential amino acids. Additionally, whey proteins stimulate protein anabolism more efficiently than casein. Therefore, whey protein is an excellent supplement for aging individuals. Whey protein can be extracted from byproducts of cheese production (Ticinesi et al., 2016).

6.2 Effects of Proteins on Age Related Diseases

In the modern western diet, protein intake in most individuals is highly increased. This is associated with a slightly increased risk of premature death, as well as worse outcomes in some diseases (Apetrii et al., 2021). In older adults, the situation seems to be a bit different. Studies have shown that about 6% of men over 71 years and 4-6% of women over 50 years fail to consume sufficient amounts of protein (Institute of Medicine, 2010). The general recommendation for adults is that they should consume 0,83 gram of protein per kilo of body weight every day (Dardevet et al., 2021). In older individuals, this level of intake is insufficient to prevent sarcopenia (Ticinesi et al., 2016).

Sarcopenia happens when the skeletal muscle develops anabolic resistance. Amino acids in the blood do not stimulate the synthesis of protein in muscle tissue as much as they do in younger or healthier patients. This leads to a decrease in muscle mass and function, which is called sarcopenia. The process commonly happens in older patients, as well as in critically ill ones, and even in astronauts in space where gravitational forces are absent. Approximately 1,4-2,5% of muscle mass is lost yearly in such patients (Dardevet et al., 2021). This loss of muscle mass increases body fat percentage. This may have an influence on other metabolic functions, and increase the risk of falls in older adults (Institute of Medicine, 2010).

Therefore, a higher daily protein intake of 1 gram per kilo of body weight is recommended for older patients. Patients who are active in a rehabilitation program may benefit from even higher daily levels of 1,2-1,5 gram per kilo of body weight (Ticinesi et al., 2016). A recent study found that stronger, older adults tend to have a protein intake with a daily median of 1,2 gram per kilo of body weight (Wu et al., 2021).

Not only the quantity but also quality of the proteins are important. They should contain sufficient amounts of all EAA, be easily digestible, and the source should not contain other components causing potentially harmful effects. The preparation of the food also plays a role in the uptake of proteins. For example, cooking temperature may play a role (Dardevet et al., 2021). Among individuals with sarcopenia, men and women with the highest protein intakes lost the least amount of muscle mass in three years (Institute of Medicine, 2010). Anabolism of skeletal muscle can also be triggered by increased exercise and insulin administration, which can be recommended in combination with increased protein intake (Weijs et al., 2014).

If the protein intake in an older adult should be increased, it also largely depends on the individual patient's kidney function. Increasing the protein uptake in a patient with kidney disease may increase toxicity and impair renal function even further (Institute of Medicine, 2010). Decreasing protein levels is recommended in such patients, but due to the previously mentioned effects of low protein intake, a minimal daily level of 0,8 gram per kilo of body weight is recommended (Apetrii et al., 2021). Further details on this issue can be read in chapter 6.2.3.

6.2.1 Effects of Proteins on the Cardiovascular System

If protein intake is decreased, muscles essential for survival, such as cardiac muscle tissue, is initially spared from catabolic destruction. Mostly skeletal muscle is degraded for energy production. Ultimately, also, the heart muscle will start to be catabolized, which will lead to heart failure (Weijs et al., 2014).

6.2.2 Effects of Proteins on the Pulmonary System

The pulmonary function can also be decreased if protein malnutrition leads to a catabolism of the respiratory muscle tissue. This may eventually lead to respiratory failure (Weijs et al., 2014).

6.2.3 Effects of Proteins on the Renal System

Chronic kidney disease (CKD) patients are generally advised to reduce their protein consumption. An increase in protein intake may lead to a progression of this preexisting condition. Protein may induce a vasodilation of the afferent arterioles of the glomerulus, causing an increase in glomerular pressure and hyperfiltration. Increased consumption of highly processed sources of protein, such as processed meats, is further associated with an increase in blood pressure, which is the main cause of CKD. End products of the protein catabolism (p-cresyl sulfate, trimethyl aminoxide, and indoxyl sulfate) accumulate excessively in patients with an increased protein intake. This may lead to the onset or an increase of severity of uremic symptoms. Decreased protein intake and an increase in fruit and vegetable intake are even associated with a decreased mortality in patients with CKD (Apetrii et al., 2021).

Nephrologists, on the other hand, do not recommend decreasing daily protein intake below 0,8 gram per kilo of body weight because of the association with loss of muscle mass mentioned previously (Apetrii et al., 2021). Additionally, studies showed an increased loss of bone mass in patients with a decreased protein intake. Previously, it was believed that an increased protein intake would lead to increased renal losses of calcium, leading to a loss of bone mass. This has been refuted (Institute of Medicine, 2010).

6.2.4 Effects of Proteins on the Endocrine and Immune System

The endocrine system largely controls the anabolism and catabolism of muscle proteins. Cortisol leads to an increased catabolism of proteins in muscle, while glucagon promotes gluconeogenesis. Insulin has anabolic effects on the muscle mass (Weijs et al., 2014).

Some proteins may have anti-inflammatory properties. Whey protein stimulates the release of IGF-1, which is known as an anti-inflammatory substance. In some elderly patients, it may even lead to a drop in previously elevated CRP (C-reactive protein) levels. Exercise-induced releases of pro-inflammatory markers CRP and IL-6 were reduced with an increased protein intake in younger patients. Leucine is said to be the main anti-inflammatory amino acid. High levels of it can be found, for example, in red meat (Ticinesi et al., 2016).

Many skin products contain collagen and elastin building blocks. They can potentially improve the cellular metabolism, inhibiting matrix metalloproteinases, and therefore improving the formation of extracellular matrix proteins, which has an overall protective effect on the skin (Žmitek et al., 2020).

6.2.5 Neurological Effects of Proteins

A study showed beneficial effects of a protein-rich extract of the *Musca domestica* larvae over age-related neuronal loss in rats. The substance had a brain-protective and anti-degenerative effect, possibly induced by reducing oxidative stress (Tang et al., 2020).

7. OMEGA-3 FATTY ACIDS

Omega-3 fatty acids are also called n-3 fatty acids, and are also known by their alternative spelling: ω-3 fatty acids (Cholewski et al., 2018). They form, together with omega-6 fatty acids, the group of polyunsaturated fatty acids (PUFAs), meaning they contain more than one double bond (C=C). The first double bond can be found between the 3rd and 4th carbon atom counting from the methyl end in the omega-3 fatty acids, and between the 6th and 7th carbon atom in the omega-6 fatty acids (Fig. 11). Monounsaturated fatty acids have only one double bond (Fig. 12), while saturated fatty acids have no double bonds between their carbon atoms (Fig. 12) (Ahern, Rajagopal, 2014).

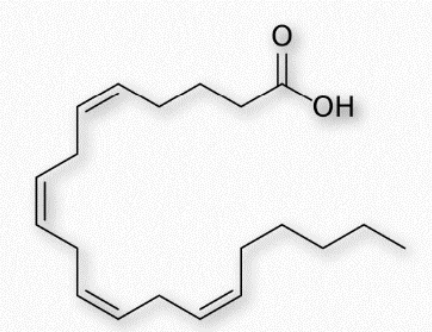

Fig. 11. **Arachidonic acid – a polyunsaturated fatty acid – omega-6 fatty acid** (Ahern, Rajagopal, 2014)

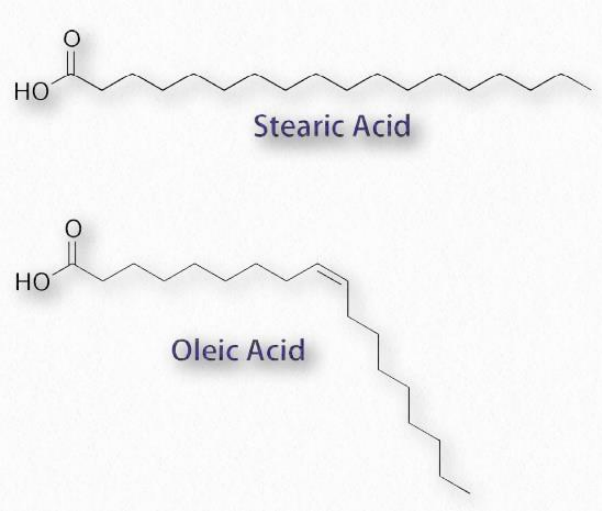

Fig. 12. **Saturated and unsaturated fatty acids** (Ahern, Rajagopal, 2014)

The difference between omega-6 and omega-3 fatty acids is the location of the first double bond. There are two systems to name PUFAs. The omega system starts counting at the methyl (-CH3) end. It is called "omega" because this side of the molecule is considered to be the end part. The delta system starts counting on the opposite side of the molecule, the carboxyl end (-COOH) (Fig. 13). If we used the delta system and started counting from the carboxyl end, the omega-3 fatty acid could also be called a cis-Δ6, because it has a first cis double bond between the 6th and 7th carbon atoms (Ahern, Rajagopal, 2014).

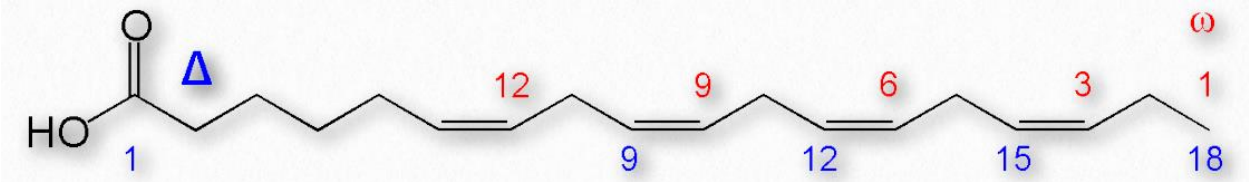

Fig. 13. **Delta (**Δ**) and omega (**ω**) numbering systems for fatty acids** (Ahern, Rajagopal, 2014)

Enzymes producing double bonds are called “desaturases”. Humans, as well as many other animals, have only some types of desaturases and cannot perform all steps of omega-3 fatty acid synthesis in their body (Troesch et al., 2020).

The main omega-3 fatty acid humans require is alpha linolenic acid (ALA) (Calder, 2018). Other types of omega-3 fatty acids can be produced from it by enzymes called desaturases and elongases (Fig. 14) (Calder, 2018). From ALA, eicosapentaenoic acid (EPA, 20 carbon atoms) and docosahexaenoic acid (DHA, 22 carbon atoms) are produced. They are precursor molecules for many physiological mediators, such as prostaglandins, thromboxanes, and leukotrienes. These mediators form the group of eicosanoids (Jain et al., 2015). In humans, this conversion into EPA and DHA is poor. EPA and DHA must also be present in the diet to reach sufficient levels (Troesch et al., 2020). They are therefore considered to be essential as well (Cutuli, 2017).

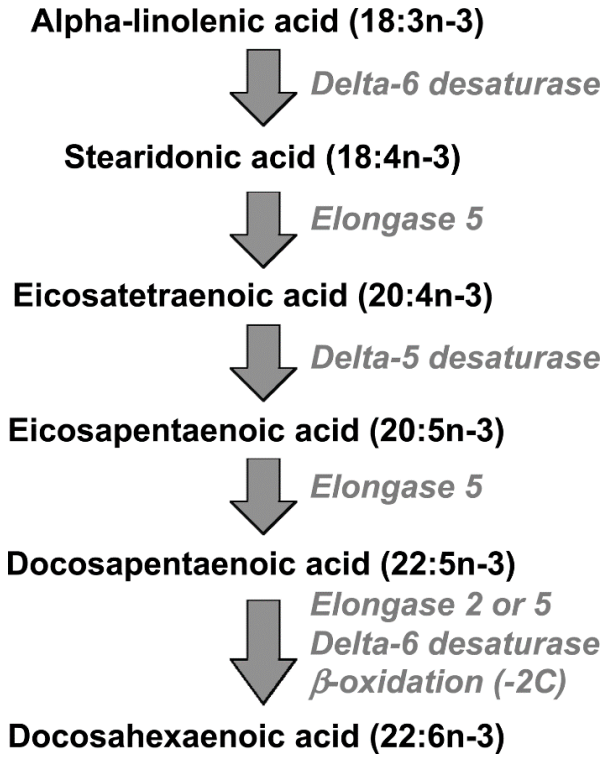

Fig. 14. **The metabolic pathway of biosynthesis of eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid** (Calder, 2018)

Omega-3 fatty acids are considered to have anti-inflammatory effects, while the effects of omega-6 fatty acids are considered to be pro-inflammatory (Cholewski et al., 2018). This may be the cause of the effects of omega-3 fatty acids on the different body systems. The mechanisms are explained in the following chapters (chapter 7.1 and chapter 7.4). Not only is the level of omega-3 fatty acids in the diet important, but also their ratio with omega-6 (Simopoulos, 2002). Interestingly, the anti-inflammatory effect does not increase proportionally with the increase of omega-3 fatty acids in the diet. It is the highest when the intake of both omega-3 and omega-6 fatty acids is high (Ticinesi et al., 2016). The number of daily intakes, timing of doses, and durations of possible effects are still being discussed and may be the cause of ambivalent or even contradicting results of different studies (Troesch et al., 2020).

7.1 Digestion of Omega-3 Fatty Acids and their Transport through Membranes

Fat digestion starts already in the mouth, where the combined forces of chewing, lingual lipase, and some emulsifying phospholipids make lipids more accessible for further digestion steps. Fats are generally hydrophobic and therefore accumulate in groups in the form of droplets, which are separate from hydrophilic food particles. Gastric lipase starts the breakdown of triacylglycerols in the stomach. From there, fat enters the small intestine – the main place of fat absorption. Bile emulsifies the fat due to its ability to attract both water and fat. This drastically increases the surface area of the lipid droplets (Zimmerman, 2020). Long-chain PUFAs are hydrolyzed from triacylglycerols inside the intestinal lumen via the enzyme pancreatic lipase (Cabré et al., 2005). In order to pass through hydrophilic areas, such as the lining of membranes, micelles with their hydrophilic exterior and hydrophobic core are formed. Inside the blood and lymph, long-chain FAs can only be transported within chylomicrons (CM). They are formed inside intestinal cells (Zimmerman, 2020).

Transport of long-chain FAs across the phospholipid membrane had previously been thought to occur via passive diffusion. During the past decade, it was discovered that this is a process regulated by membrane proteins. So called “membrane-associated fatty acid-binding proteins”

or short “fatty acid transporters” regulate and facilitate the uptake of fatty acids. Some of the transporters that have been found are “CD36, plasma membrane-associated fatty acid-binding protein (FABP(pm)), and a family of fatty acid transport proteins (FATP1-6)” (Fig. 15) (Schwenk et al., 2010).

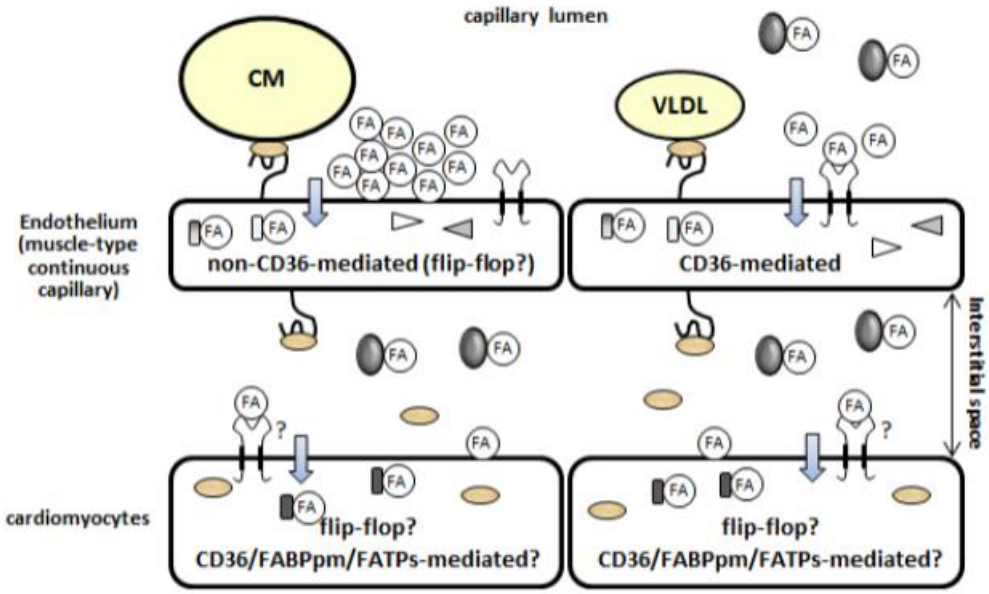

Fig. 15. **Mechanisms of fatty acid uptake by the heart** (Iso & Kurabayashi, 2021)
CM (chylomicron), VLDL (very low density lipoprotein), FA (fatty acid), CD36 (cluster of differentiation 36), FABPpm (plasma membrane-associated fatty acid binding protein), FATPs (fatty acid transport protein)

As seen on figure 15, the exact mechanisms of uptake of fatty acids are still unclear. The image visualizes 4 steps of the process: firstly, the lipolysis before uptake on the endothelial surface; secondly, the uptake of FA from the capillary into the endothelial cell through the plasma membrane; thirdly, the intracellular FA transport through the endothelium and then, fourthly, from there the uptake into the cardiomyocyte. The mechanisms of transport of FA into the interstitial space remain unknown (Iso & Kurabayashi, 2021).

7.2 Sources of Omega-3 Fatty Acids

The main sources of omega-3 fatty acids are almost all types of seafood (Calder, 2018). Fatty fishes have high contents of omega-3 fatty acids, examples of which are salmon, tuna (Covington, 2004) or mackerel (Calder, 2018). Sea mammals, such as whales and seals, also contain increased levels of omega-3 fatty acids in their tissues.

An excellent plant source of EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) is seaweed (Cao et al., 2020). Fish accumulate omega-3 fatty acids in their body, as seaweed containing high amounts of omega-3 FAs is their main source of food (Shahidi & Ambigaipalan, 2018). Seaweed seems to be the main producer of omega-3 fatty acids. They are the main organisms containing the enzymes needed for *de novo* synthesis of many PUFAs. Also, other organisms have been described to have this ability (de Carvalho & Caramujo, 2018). The type of seaweed described to have most effects is brown seaweed (*Phaeophyta*) (Begum et al., 2021).

Fish also have the ability to transform alpha linolenic acid (ALA) and alpha linoleic acid (LA) into the long-chain PUFAs, DHA, and EPA. Some species of fish have higher levels of conversion than others. Fresh water fish, especially, seem to have increased PUFA production – much higher than marine fish (de Carvalho & Caramujo, 2018). Meat from land animals, on the other hand, contains only negligible amounts of omega-3 fatty acids (Tab. 4) (Calder, 2018), and very low levels of conversion into long-chain PUFAs. These levels are comparable to human conversion levels (Troesch et al., 2020).

Not only is the presence of any omega-3 FA in the food important, but also the ratio between EPA and DHA. This exact ratio is also discussed in the literature (Shahidi & Ambigaipalan, 2018). Between the different species of sea animals, the contents of EPA and DHA vary (Tab. 4) (Calder, 2018).

Tab. 4. **Amount of eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid in gram per 100g in a selection of seafood and meat**

Food	EPA	DPA	DHA	Typical adult portion size (g)	EPA + DPA + DHA (g/portion)
Mackerel	0·71	0·12	1,10	160	3·09
Canned pilchards	1·17	0·23	1·20	110	2·86
Trout	0·23	0·09	0·83	230	2·65
Salmon	0·50	0·40	1·30	100	2·20
Canned sardines	0·89	0·10	0·68	100	1·67
Herring	0·51	0·11	0·69	120	1·56
Crab	0·47	0·08	0·45	85	0·85
Plaice	0·16	0·04	0·10	130	0·39
Cod	0·08	0·01	0·16	120	0·30
Mussels	0·41	0·02	0·16	40	0·24
Haddock	0·05	0·01	0·10	120	0·19
Venison	0·04	0·09	< 0·01	120	0·16
Canned tuna	0·02	0·02	0·14	45	0·08
Lamb	0·03	0·04	0·02	90	0·08
Prawns	0·06	< 0·01	0·04	60	0·06
Chicken	0·01	0·02	0·03	100	0·06
Beef	0·02	0·02	0	90	0·04
Pork	0·01	0·02	0·01	90	0·04

(Calder, 2018)

Since most people in the western world do not consume fish as often as recommended by the WHO, an easy alternative to eating fish is using fish oil. Also, other types of oils, such as vegetable oils, contain omega-3 FAs (Covington, 2004).

Some other foods which do not originate from the sea also contain omega-3 fatty acids to lower degrees or with less optimal DHA:EPA ratios. Examples of such foods are green leafy vegetables, nuts, beans (Novotny et al., 2021), and fungi (Shahidi & Ambigaipalan, 2018).

The ratio between the pro-inflammatory omega-6 fatty acids and the anti-inflammatory omega-3 fatty acids has shifted greatly towards the side of the omega-6 fatty acids in the standard American diet. The average ratio of omega-6 to omega-3 fatty acids used to be 4:1 (or even 1:1), but now it is at about 15 to 1 (Simopoulos, 2002). This is caused by increased dietary levels of omega-6 and decreased omega-3 levels (Covington, 2004). To reduce this ratio, the diet can be adapted. Different oils may be selected for cooking, or fish consumption can be increased. Alternatively, supplements can be used. There are numerous types of omega-3 fatty acid supplements available on the market today – most of which are based on fish oil, some on

algae oils. Supplements significantly increase the amount of omega-3 FAs in the blood and tissues of non-consumers of fatty fish. Attention must be paid to the amounts of EPA and DHA present in those individual supplements and also to their ratio (Tab. 5) (Calder, 2018). Shifting the ratio of omega-6 to omega-3 FA to about 4 to 1 or even lower has shown to be effective (Simopoulos, 2002).

Tab. 5. **Typical eicosapentaenoic acid and docosahexaenoic contents of omega-3 fatty acid supplements**

	EPA (mg/g oil)	DHA (mg/g oil)	EPA + DHA (mg/g/oil)
Cod liver oil	110	90	200
Krill oil	140	65	205
Standard fish oil	180	120	300
Typical 45% fish oil concentrate	270	180	450
Tuna oil	110	350	460
Algal oil used in infant formula	0	>400	>400
Typical 60% fish oil concentrate	360	240	600
Omacor®* (*n*-3 ethyl ester concentrate)	460	380	840
Flaxseed oil	0	0	0

(Calder, 2018)

The exact amounts of EPA and DHA needed are not clear. Most sources recommend an amount of at least 250 mg of combined EPA and DHA per day (Troesch et al., 2020).

7.3 Docosahexaenoic Acid and Eicosapentaenoic Acid

Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are the most important very long-chain omega-3 fatty acids. They are called “very long” in order to be more easily differentiated from the shorter omega-3 fatty acids such as ALA or stearidonic acid (SDA) (Calder, 2018). DHA and EPA can be endogenously produced from alpha-linolenic acid (Fig. 14), but the produced levels are very low (Troesch et al., 2020). DHA is produced from ALA in the liver (de Carvalho & Caramujo, 2018). Their produced levels depend on many individual factors such as diseases, genetics, sex, and age. To maintain adequate blood levels of DHA and EPA, they must be contained in the diet or supplemented. The optimal uptake of DHA and EPA is 250 mg per day, even though -to achieve positive effects in some health conditions- an even higher amount is recommended (Troesch et al., 2020).

Many of the functions of EPA and DHA are synergistic, but some are also distinct (Troesch et al., 2020). The detailed effects which they exert on the different body systems are discussed in the following chapter (chapter 7.4).

7.4 Effects of Omega-3 Fatty Acids

Unlike proteins, omega-3 fatty acids are not usually consumed excessively. Most people have low to very low levels of EPA and DHA in their blood (Troesch et al., 2020). When their concentrations are increased in the blood, they get integrated into the lipophilic cell membranes of all cells, especially in blood and liver cells (Simopoulos, 2002). This leads to changes in the membrane functions, such as changes in fluidity, elasticity, permeability (de Carvalho & Caramujo, 2018), as well as changes in cell signaling, lipid inflammatory mediator production, and gene expression (Troesch et al., 2020).

The mechanism causing the proinflammatory effects of omega-6 fatty acids and anti-inflammatory effects of omega-3 fatty acids can be explained by the competitive inhibition of the same enzymes needed for the conversion of both. Arachidonic acid, which is needed for

pro-inflammatory eicosanoid production, cannot be formed excessively when omega-3 PUFAs are present (Fig. 16) (Davinelli et al., 2018).

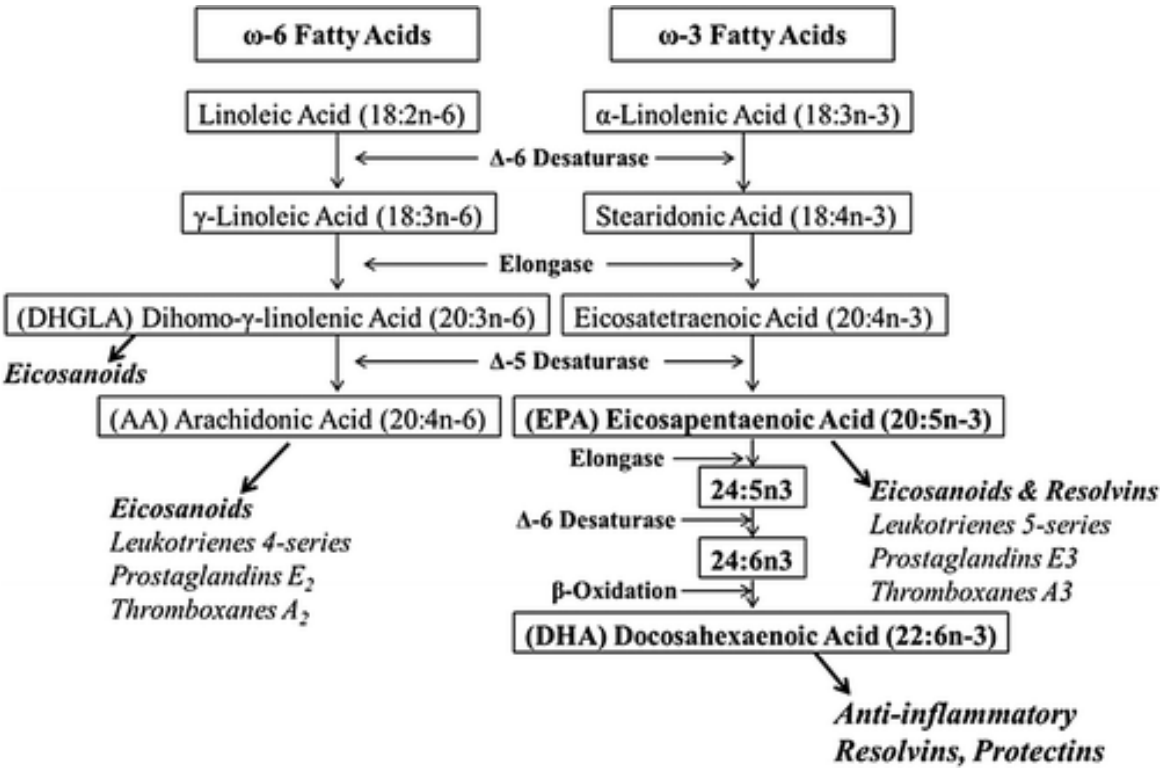

Fig. 16. **Polyunsaturated fatty acids (PUFA) and eicosanoids biosynthesis** (Davinelli et al., 2018)

Proinflammatory eicosanoids, leukotrienes, prostaglandin E_2, and thromboxane A_2 are synthesized from arachidonic acid. If their precursor is mostly replaced by omega-3 fatty acids, these reactions cannot take place and, instead, anti-inflammatory resolvins and protectins are produced in increased amounts (Davinelli et al., 2018).

Many effects of omega-3 fatty acids are still in discussion, and many of their effects are still not fully evaluated and proven in human models (Bischoff-Ferrari et al., 2021). Some recent studies provide evidence that some of the positive effects may have been overestimated in the past (Nicholls et al., 2020; Nissen et al., 2021). Still, the vast majority of studies come to the

conclusion that there are positive effects of omega-3 fatty acids (Novotny et al., 2021). In many cases, the full mechanism of action is hypothesized and not yet understood. Most of the evidence found reflects the positive effects of omega-3 fatty acids on cardiovascular diseases, while other diseases are not yet proven to be significantly positively affected by increased omega-3 fatty acid intake in human models (Bischoff-Ferrari et al., 2021).

Most conditions where the effects of omega-3 fatty acids are being discussed are age related (Troesch et al., 2020). Some effects have also been shown in other conditions, which are not linked to old age, such as asthma in childhood (Miles & Calder, 2017), premenstrual syndrome (Behboudi-Gandevani et al., 2018), maternal depression (Hsu et al., 2018), and attention deficit hyperactivity disorder in children (ADHD) (Chang et al., 2018). The following chapters are focused mostly on the effects on age-related conditions.

Omega-3 fatty acids are officially only used to treat hyperlipidemia (Covington, 2004). Currently, there are four medications available containing omega-3 fatty acids which are approved by the American Food and Drug Administration (FDA) under certain conditions. Such medications are omega-3 ethyl esters A, omega-3-acid ethyl esters and omega-3-carboxylic acid containing both EPA and DHA, and icosapent ethyl, containing only EPA. All of them are prescribed exclusively for adults with highly increased triglyceride levels, even though the mechanism of action is not fully understood (Novotny et al., 2021). No significant side effects or drug interactions are known (Jain et al., 2015; Nissen et al., 2021).

Just as proteins, omega-3 fatty acids also seem to have a beneficial effect on sarcopenia. Studies show that they have anabolic effects on muscle tissue. The mechanisms of such effects remain unclear until today (McGlory et al., 2019).

7.4.1 Effects of Omega-3 Fatty Acids on the Cardiovascular System

Out of all the effects of omega-3 fatty acids, the effects on the cardiovascular system are probably the most researched, with most data available to this day (Novotny et al., 2021). Most of these results are based on the findings of clinical studies but recently also some of the mechanisms could be elucidated (Xie et al., 2021).

The integration of omega-3 fatty acids into the phospholipid membrane of cardiomyocytes has anti-arrhythmic properties, due to changes in the functioning of channels (Endo & Arita, 2016), which decreases the risk of sudden cardiac death (Jain et al., 2015). They also have been associated with an overall decrease in cardiovascular mortality (Davinelli et al., 2018). Omega-3 fatty acids can influence the expression of some genes which are associated with age-related diseases, mostly hyperlipidemia (Xie et al., 2021).

Some of their main effects on the cardiovascular system include: a decrease of atherosclerosis risk (Jain et al., 2015), reduced inflammation in the vessels (Davinelli et al., 2018), and a decrease in blood pressure when given in high doses (Jain et al., 2015). There is a negative association between the incidence of myocardial infarctions and strokes and omega-3 fatty acid consumption (Davinelli et al., 2018).

Therefore, the American Heart Association (AHA) recommends an increased intake of omega-3 fatty acids in the diet of patients with known CHD and hyperlipidemia (Tab. 6).

Tab. 6. **Summary of AHA Recommendations for Omega-3 Fatty Acid Intake**

Summary of AHA Recommendations for Omega-3 Fatty Acid Intake

Patient population	*Recommendation*
No documented history of CHD	Eat a variety of fish (preferably oily) at least twice per week. Include oils and foods rich in alpha-linolenic acid (flaxseed, canola, and soybean oils; flaxseeds and walnuts).
Documented history of CHD	Consume approximately 1 g of EPA plus DHA daily, preferably from oily fish. EPA plus DHA capsule supplements may be used in consultation with a physician.
Needs to lower triglyceride level	Consume 2 to 4 g of EPA plus DHA daily in capsules in consultation with a physician.

(Covington, 2004)

The AHA's recommendation for the overall population is to consume at least 2 portions of fatty fish in a week. Fish should be cooked in ways that do not involve frying (American Heart Association, 2021). For heart protection, about 1g per day of combined EPA and DHA is recommended (Jain et al., 2015), even though higher doses may be needed to reduce triglyceride levels or decrease blood pressure (Covington, 2004).

In the DO-HEALTH aging trial, the effects of omega-3 fatty acids were promising, showing decreased cardiovascular complications in high-risk patients. Until today, only few studies show the effects of their long-term use, or their use in primary disease prevention. Effects, other than the cardiovascular, have only limited data availability until today (Bischoff-Ferrari et al., 2021).

7.4.2 Effects of Omega-3 Fatty Acids on the Pulmonary System

Some conditions regarding the respiratory or pulmonary system may be positively influenced by the increased intake of omega-3 fatty acids. Acute respiratory distress syndrome is a disease caused, for example, by an overactive immune reaction during sepsis. Dimming the inflammatory response in such patients, by giving increased amounts of omega-3 fatty acids in their enteral nutrition, may lead to improvements. The evidence surrounding this topic is sparse, nevertheless. The mortality of patients did not decrease in some studies (Troesch et al., 2020).

It was shown that the proliferation of some lung adenocarcinoma (cell line A549) can be inhibited with omega-3 fatty acid exposure. However, this effect was only seen in an in vitro study and further research is needed (Yao et al., 2014).

7.4.3 Effects of Omega-3 Fatty Acids on the Renal System

A study found no decrease in the levels of kidney dysfunction between a group taking increased levels of omega-3 fatty acids and a placebo group. No positive effects on the renal system could be found (de Boer et al., 2019).

7.4.3 Effects of Omega-3 Fatty Acids on the Gastrointestinal System

Anti-inflammatory properties of omega-3 fatty acids are being researched to reduce the inflammation in patients with inflammatory bowel disease. Inflamed colonic tissue contains lower DHA levels than the healthy or healing tissue (Ungaro et al., 2017). Recent data also suggests changes in the gastrointestinal (GI) flora under the influence of increased levels of omega-3 fatty acids. Organisms subject to research are *Bifidobacteria*, *Lactobacilli*, *Streptococcus* and *Actinobacteria*. They are described to have anti-aging properties due to positive results in animal studies and because they have been found in the gut of very old individuals. A healthy GI flora can decrease inflammation and boost the immune system. Omega-3 fatty acids may change the flora by decreasing blood lipid levels (Xie et al., 2021).

Problems with the digestion of omega-3 fatty acids may occur in patients with a dysfunctional bile system or cirrhosis (see chapter 7.1) (Cabré et al., 2005).

7.4.4 Effects of Omega-3 Fatty Acids on the Immune and Endocrine Systems

The multiple effects of omega-3 fatty acids concerning the immune system are mostly correlated to the previously explained anti-inflammatory effects of omega-3 fatty acids (chapter 7.1). Omega-3 fatty acid intake was shown to decrease levels of CRP, the proinflammatory IL-6, as well as tumor necrosis factor α (TNF- α) in blood. Furthermore, an increase of anti-inflammatory products such as IL-10 and transforming growth factor β (TGFβ) can be observed (Ticinesi et al., 2016). In addition, more serious hyperinflammatory conditions, such as cytokine storm, may be improved by administering omega-3 fatty acids in enteral nutrition. A slight decrease in mortality was recorded in a study where patients were given intravenous omega-3 fatty acids (Troesch et al., 2020).

Immune-mediated inflammatory diseases, such as rheumatoid arthritis, may improve with an increased omega-3 fatty acid intake. Morning stiffness may be reduced, and the number of affected joints may decrease. To see these improvements, high levels of at least 3g of omega-3 fatty acids must be consumed daily (Covington, 2004).

A study showed that the risk of contracting infectious diseases could be lowered by 40% in patients receiving parenteral omega-3 FAs instead of the standard lipid formula (Troesch et al., 2020).

Skin, as an important part of the innate immune system, contains omega-3 fatty acids. They are important both for the function and the structure of the skin. Omega-3 fatty acids have anti-inflammatory, anti-allergic, and hydrating effects on skin. They reduce erythema and, especially, EPA has ultraviolet (UV) light filtering properties. Many skin products available contain omega-3 fatty acids. A deficiency of omega-3 fatty acids causes the skin to dry out, peel off, and become more vulnerable to irritations (Michalak et al., 2021).

Additionally, some effects on the endocrine system have been observed: omega-3 fatty acids may decrease the risk of diabetes (Cao et al., 2020). The osteopenic side effects of the anti-diabetic medication rosiglitazone are prevented when taking fish-oil supplements. They inhibit the formation of osteoclasts, inflammation, and they increase osteogenesis (Cugno et al., 2021). Also, they reduce the levels of the stress hormone cortisol (Madison et al., 2021).

7.4.5 Neurological Effects of Omega-3 Fatty Acids

DHA is one of the main components of gray matter of the brain (Shahidi & Ambigaipalan, 2018), and it is also a component of synaptic membranes (Chen et al., 2020). Therefore, it seems logical that omega-3 fatty acids must have some influences on the neurological system and its functions. Additionally, during the normal aging process, the level of DHA in the brain decreases (Cutuli, 2017) due to a reduced activity of delta 4 desaturase. Increasing the levels of DHA in the brain in aging patients may have effects on neurological age-related diseases (Grodzicki & Dziendzikowska, 2020).

DHA may be important in the prevention of neurodegenerative diseases (Grodzicki & Dziendzikowska, 2020), firstly due to anti-inflammatory, anti-apoptotic, and antioxidant properties; and secondly, due to the positive cardiovascular properties (chapter 7.4.1). By reducing the triglyceride levels, decreasing blood pressure, and decreasing inflammation, the incidence of vascular dementia may be reduced (Ammann et al., 2013). A mediterranean diet

including fish, is also associated with a decreased risk of Alzheimer's disease (Grodzicki & Dziendzikowska, 2020).

Increased levels of omega-3 fatty acids in blood are associated with an increased density of white matter and an increased volume of the hippocampus. They may even protect the brain from age-related damage, as well as improve its maintenance and functioning in old age (Chen et al., 2020). They also increase synaptic plasticity and levels of signaling molecules, which may lead to neurogenesis in the hippocampal area, even in old age. Loss of both gray and white matter can be prevented (Cutuli, 2017).

Furthermore, some other diseases involving the neurological system can potentially be positively influenced by an increase of omega-3 fatty acids. Migraine pain and the frequency of migraines may improve due to a diet rich in omega-3 fatty acids, which proportionally increases the quality of life of patients as well (Slomski, 2021). Also, the influence of toxic substances such as lead, mercury, and organic solvents on the brain may be reduced by PUFAs. Some damage caused by these elements may even be prevented (Chen et al., 2020). Omega-3 fatty acids also improve the integrity of the blood brain barrier (BBB) (Barnes et al., 2021) and may prevent depression (Madison et al., 2021).

On the other hand, one study found no significant difference in cognitive aging between individuals consuming increased amounts of omega-3 fatty acids and individuals not taking increased amounts. This may be attributed to the relatively short time of exposure to omega-3 fatty acids in such study (Ammann et al., 2013).

CONCLUSIONS

As a conclusion I will comment on each of the main parts of this work individually, briefly summarize its main results, and explain my personal view on the topic.

Firstly, the aging theories were summarized. There are three main branches of theories: program theories, damage theories, and combined theories. Overall, there are over 300 different theories of aging, which makes it nearly impossible to explain all of them. Until this day, there is no scientific consensus about how the aging process is caused. Most researchers agree that there must be more than one underlying cause of aging. I personally consider myself a supporter of the combined theories, since there is significant evidence supporting both program theories and damage theories. Furthermore, I am sure the research will continue in this field. It will still take a long time until the complete process of aging and its causes is revealed. Perhaps the results will provide some more information on how to possibly halt or even reverse the process of aging. For now, that can only be a speculation.

In the second part of this thesis, information about proteins and their effects on age related diseases were analyzed. Up to this day, the effects of an increased or decreased protein intake on the human body during aging are not fully understood. Decreased levels of protein intake lead to sarcopenia, an overall catabolic state of muscle tissue. This is associated with an increased mortality and weakness. Therefore, protein intake should generally be increased in older individuals. On the other hand, increased levels of protein intake have shown to be harmful under certain circumstances. High levels of proteins are associated with a progression of chronic kidney disease. A balance must be found considering these facts when determining the optimal intake of proteins for each patient.

Optimally, an increased protein intake should be combined with other factors such as an increase in physical exercise or insulin intake. Either of these factors are used in sarcopenia prevention. The timing of intakes, as well as the type of protein, are both important. The optimal protein supplement seems to be whey protein due to its quick absorption, digestion, and anabolic stimulation. This way, we could potentially reduce sarcopenia risks in patients with chronic kidney disease without increasing the protein intake to harmful levels.

It is important to find the optimal amounts of protein that should be consumed for each patient. Their chronic diseases and personal goals need be considered to ensure they have optimal outcomes. All essential amino acids must be provided in sufficient amounts in the diet. Patients should be educated on which foods to consume when choosing their sources of protein. It is also important to reduce unwanted byproducts, such as fats and salts in processed protein sources. Further research is needed to find the optimal amounts of proteins and to increase alternatives for patients for whom high protein intakes are contraindicated. More specific long-term studies with a control group focusing specifically on protein are so far not available. Such studies would allow us to form more specific conclusions about other positive effects of proteins. It is difficult to design such a study, as it would take a lot of time and possibly have a lot of room for random error due to the different personal lifestyle choices of the participants. Those factors are almost impossible to distinguish from the effects that the proteins may exert. An increased number of participants could help to increase the reliability of the results. Only such a study could further clarify the effects of increased or decreased protein intake on the human body.

In the final part of this thesis, the present information regarding effects of omega-3 fatty acids was summarized. Until today no clear answer can be given, their effects are still being discussed and researched. Some authors can see very clear evidence of the positive effects of omega-3 fatty acids on the human body, while others are not satisfied and request further research. Most authors agree that at least the cardiovascular effects caused by omega-3 fatty acids cannot be negated and their intake is beneficial, especially for risk groups. It is also clear that omega-3 fatty acids have anti-inflammatory properties leading to a regulation of the inflammatory response in the body. Other discussed effects on different body systems still do not have sufficient evidence to support them.

Unlike protein intake, an increased level of omega-3 fatty acids does not seem to have any harmful effects on the body, even in patients with other chronic diseases. There is also a lot of evidence supporting their beneficial effects, especially on the cardiovascular system. Therefore, I would generally recommend an increased intake of DHA and EPA for all patients in their normal diet or via supplements if that is not possible.

The availability of data on the effects of omega-3 fatty acids is not yet optimal. A bigger, more specific long-term study would be helpful, such as I mentioned previously for clarifying the optimal protein intake.

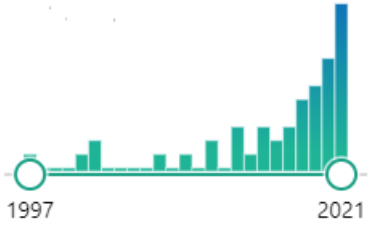

Fig. 17. **Number of studies published to the search “"bioactive substances"[Title]” on PubMed from 1997 to 2021**

The number of studies and papers on bioactive substances such as proteins and omega-3 fatty acids has grown significantly in the recent years, resembling an almost exponential growth (Fig. 17). Overall, there were 49 articles with full-text availability, 14 of them were published in 2021 (the search was last updated on the 29th of November 2021). I have made similar observations when searching for information on omega-3 fatty acids and proteins on PubMed and other online research databases. I suspect that, in the following years, an even further increase can be expected -hopefully- resolving the questions that remain unanswered for now.

Both protein and omega-3 fatty acids are promising nutritional factors, potentially improving outcomes in seniors. Despite this fact, they are currently recommended in clinical guidelines very sparsely. Hopefully, the recent increase in data will lead to more generous inclusions and recommendations in guidelines, making supplements more accessible for patients and physicians.

LIST OF BIBLIOGRAPHIC REFERENCES

Aalami, O. O., Fang, T. D., Song, H. M., & Nacamuli, R. P. (2003). Physiological Features of Aging Persons. *Archives of Surgery*, *138*(10), 1068–1076. https://doi.org/10.1001/archsurg.138.10.1068

Ahern, Rajagopal, T. (2014). Biochemistry Free For All. In *Toxicological Sciences* (Vol. 137, Issue 2). https://academic.oup.com/toxsci/article/1676330/Table_of_Contents

American Heart Association. (2021). *Fish and Omega-3 Fatty Acids*. https://www.heart.org/en/healthy-living/healthy-eating/eat-smart/fats/fish-and-omega-3-fatty-acids

Ammann, E. M., Pottala, J. V, Harris, W. S., Espeland, M. A., Wallace, R., Denburg, N. L., Carnahan, R. M., & Robinson, J. G. (2013). ω-3 fatty acids and domain-specific cognitive aging: secondary analyses of data from WHISCA. *Neurology*, *81*(17), 1484–1491. https://doi.org/10.1212/WNL.0b013e3182a9584c

Anton, B., Vitetta, L., Cortizo, F., & Sali, A. (2005). Can we delay aging? The biology and science of aging. *Annals of the New York Academy of Sciences*, *1057*, 525–535. https://doi.org/10.1196/annals.1356.040

Apetrii, M., Timofte, D., Voroneanu, L., & Covic, A. (2021). Nutrition in Chronic Kidney Disease-The Role of Proteins and Specific Diets. *Nutrients*, *13*(3). https://doi.org/10.3390/nu13030956

Barbesino, G. (2019). Thyroid Function Changes in the Elderly and Their Relationship to Cardiovascular Health: A Mini-Review. *Gerontology*, *65*(1), 1–8. https://doi.org/10.1159/000490911

Barnes, S., Chowdhury, S., Gatto, N. M., Fraser, G. E., & Lee, G. J. (2021). Omega-3 fatty acids are associated with blood-brain barrier integrity in a healthy aging population. *Brain and Behavior*. https://doi.org/10.1002/brb3.2273

Begum, R., Howlader, S., Mamun-Or-Rashid, A. N. M., Rafiquzzaman, S. M., Ashraf, G. M., Albadrani, G. M., Sayed, A. A., Peluso, I., Abdel-Daim, M. M., & Uddin, M. S. (2021). Antioxidant and Signal-Modulating Effects of Brown Seaweed-Derived Compounds against Oxidative Stress-Associated Pathology. *Oxidative Medicine and Cellular Longevity*, *2021*, 9974890. https://doi.org/10.1155/2021/9974890

Behboudi-Gandevani, S., Hariri, F.-Z., & Moghaddam-Banaem, L. (2018). The effect of omega 3 fatty acid supplementation on premenstrual syndrome and health-related quality of life: a randomized clinical trial. *Journal of Psychosomatic Obstetrics and Gynaecology*, *39*(4), 266–272. https://doi.org/10.1080/0167482X.2017.1348496

Benjamin, O., & Lappin, S. L. (2021). *Kwashiorkor*.

Bischoff-Ferrari, H. A., de Godoi Rezende Costa Molino, C., Rival, S., Vellas, B., Rizzoli, R., Kressig, R. W., Kanis, J. A., Manson, J. E., Dawson-Hughes, B., Orav, E. J., da Silva, J. A. P., Blauth, M., Felsenberg, D., Ferrari, S. M., Theiler, R., & Egli, A. (2021). DO-HEALTH: Vitamin D3 - Omega-3 - Home exercise - Healthy aging and longevity trial - Design of a multinational clinical trial on healthy aging among European seniors. *Contemporary Clinical Trials*, *100*, 106124. https://doi.org/10.1016/j.cct.2020.106124

Cabré, E., Hernández-Pérez, J. M., Fluvià, L., Pastor, C., Corominas, A., & Gassull, M. A. (2005). Absorption and transport of dietary long-chain fatty acids in cirrhosis: a stable-isotope-tracing study. *The American Journal of Clinical Nutrition*, *81*(3), 692–701. https://doi.org/10.1093/ajcn/81.3.692

Calder, P. C. (2018). Very long-chain n-3 fatty acids and human health: fact, fiction and the future. *The Proceedings of the Nutrition Society*, *77*(1), 52–72. https://doi.org/10.1017/S0029665117003950

Cao, L., Lee, S. G., Lim, K. T., & Kim, H.-R. (2020). Potential Anti-Aging Substances Derived from Seaweeds. *Marine Drugs*, *18*(11). https://doi.org/10.3390/md18110564

Chang, J. P.-C., Su, K.-P., Mondelli, V., & Pariante, C. M. (2018). Omega-3 Polyunsaturated Fatty Acids in Youths with Attention Deficit Hyperactivity Disorder: a Systematic Review and Meta-Analysis of Clinical Trials and Biological Studies. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *43*(3), 534–545. https://doi.org/10.1038/npp.2017.160

Chen, C., Xun, P., Kaufman, J. D., Hayden, K. M., Espeland, M. A., Whitsel, E. A., Serre, M. L., Vizuete, W., Orchard, T., Harris, W. S., Wang, X., Chui, H. C., Chen, J.-C., & He, K. (2020). Erythrocyte omega-3 index, ambient fine particle exposure, and brain aging. *Neurology*, *95*(8), e995–e1007. https://doi.org/10.1212/WNL.0000000000010074

Cholewski, M., Tomczykowa, M., & Tomczyk, M. (2018). A Comprehensive Review of Chemistry, Sources and Bioavailability of Omega-3 Fatty Acids. *Nutrients*, *10*(11). https://doi.org/10.3390/nu10111662

Covington, M. (2004). Omega-3 Fatty Acids. *American Family Physician*, *70*(1), 133–140. https://www.aafp.org/afp/2004/0701/afp20040701p133.pdf

Cugno, C., Kizhakayil, D., Calzone, R., Rahman, S. M., Halade, G. V, & Rahman, M. M. (2021). Omega-3 fatty acid-rich fish oil supplementation prevents rosiglitazone-induced osteopenia in aging C57BL/6 mice and in vitro studies. *Scientific Reports*, *11*(1), 10364. https://doi.org/10.1038/s41598-021-89827-8

Cutuli, D. (2017). Functional and Structural Benefits Induced by Omega-3 Polyunsaturated Fatty Acids During Aging. *Current Neuropharmacology*, *15*(4), 534–542. https://doi.org/10.2174/1570159X14666160614091311

da Costa, J. P., Vitorino, R., Silva, G. M., Vogel, C., Duarte, A. C., & Rocha-Santos, T. (2016). A synopsis on aging—Theories, mechanisms and future prospects. *Ageing Research Reviews*, *29*, 90–112. https://doi.org/10.1016/j.arr.2016.06.005

Dangour, A. D., Grundy, E. M. D., & Fletcher, A. E. (2007). *Ageing Well - Nutrition, Health, and Social Interventions*. CRC Press.

Dardevet, D., Mosoni, L., Savary-Auzeloux, I., Peyron, M. A., Polakof, S., & Remond, D. (2021). Important determinants to take into account to optimize protein nutrition in the elderly: Solutions to a complex equation. *Proceedings of the Nutrition Society*, *80*(2), 207–220. https://doi.org/10.1017/S0029665120007934

Davinelli, S., Corbi, G., Righetti, S., Sears, B., Olarte, H. H., Grassi, D., & Scapagnini, G. (2018). Cardioprotection by Cocoa Polyphenols and ω-3 Fatty Acids: A Disease-Prevention Perspective on Aging-Associated Cardiovascular Risk. *Journal of Medicinal Food*, *21*(10), 1060–1069. https://doi.org/10.1089/jmf.2018.0002

de Boer, I. H., Zelnick, L. R., Ruzinski, J., Friedenberg, G., Duszlak, J., Bubes, V. Y., Hoofnagle, A. N., Thadhani, R., Glynn, R. J., Buring, J. E., Sesso, H. D., & Manson, J. E. (2019). Effect of Vitamin D and Omega-3 Fatty Acid Supplementation on Kidney Function in Patients With Type 2 Diabetes: A Randomized Clinical Trial. *JAMA*, *322*(19), 1899–1909. https://doi.org/10.1001/jama.2019.17380

de Carvalho, C. C. C. R., & Caramujo, M. J. (2018). The Various Roles of Fatty Acids. *Molecules (Basel, Switzerland)*, *23*(10). https://doi.org/10.3390/molecules23102583

Dillin, A., Karlseder, J., Allsopp, R., Passos, J. F., Nelson, G., & ... (2008). Telomeres and Telomerase in Ageing, Disease, and Cancer Molecular Mechanisms of Adult Stem Cell Ageing. In K. L. Rudolph (Ed.), *Springer*. Springer.

Dollemore, D. (2002). *Aging Under the Microscope: A Biological Quest. National Institutes of Health, National Institute on Aging*.

Endo, J., & Arita, M. (2016). Cardioprotective mechanism of omega-3 polyunsaturated fatty acids. *Journal of Cardiology*, *67*(1), 22–27. https://doi.org/10.1016/j.jjcc.2015.08.002

European Commission. (2021). *Eurostat Statistic Explained*. https://ec.europa.eu/research/foresight/index.cfm

Faulkner, J. L., & Belin de Chantemèle, E. J. (2019). Sex hormones, aging and cardiometabolic syndrome. *Biology of Sex Differences*, *10*(1), 30. https://doi.org/10.1186/s13293-019-0246-6

Fontana, L., Partridge, L., & Longo, V. D. (2010). Extending healthy life span--from yeast to humans. *Science (New York, N.Y.)*, *328*(5976), 321–326. https://doi.org/10.1126/science.1172539

Goldsmith, T C. (2013). Arguments against non-programmed aging theories. *Biochemistry. Biokhimiia*, *78*(9), 971–978. https://doi.org/10.1134/S0006297913090022

Goldsmith, Theodore C. (2016). Evolution of Aging Theories: Why Modern Programmed Aging Concepts Are Transforming Medical Research. *Biochemistry. Biokhimiia*, *81*(12), 1406–1412. https://doi.org/10.1134/S0006297916120026

Grodzicki, W., & Dziendzikowska, K. (2020). The Role of Selected Bioactive Compounds in the Prevention of Alzheimer's Disease. *Antioxidants (Basel, Switzerland)*, *9*(3). https://doi.org/10.3390/antiox9030229

Hsu, M.-C., Tung, C.-Y., & Chen, H.-E. (2018). Omega-3 polyunsaturated fatty acid supplementation in prevention and treatment of maternal depression: Putative mechanism and recommendation. *Journal of Affective Disorders*, *238*, 47–61. https://doi.org/10.1016/j.jad.2018.05.018

Institute of Medicine. (2010). *Providing Healthy and Safe Foods As We Age: Workshop Summary* (L. Pray, C. Boon, E. A. Miller, & L. Pillsbury (eds.)). The National Academies Press. https://doi.org/10.17226/12967

Iouk, T., & Titorenko, V. I. (2017). A laboratory test of evolutionary aging theories. In *Aging* (Vol. 9, Issue 3, pp. 600–601). https://doi.org/10.18632/aging.101215

Iso, T., & Kurabayashi, M. (2021). Cardiac Metabolism and Contractile Function in Mice with Reduced Trans-Endothelial Fatty Acid Transport. *Metabolites*, *11*(12). https://doi.org/10.3390/metabo11120889

Jain, A. P., Aggarwal, K. K., & Zhang, P. Y. (2015). Omega-3 fatty acids and cardiovascular disease. *European Review for Medical and Pharmacological Sciences*, *19*(3), 441–445. https://doi.org/10.5005/jp/books/14130_101

Jin, K. (2010). Modern biological theories of aging. In *Aging and Disease* (Vol. 1, Issue 2, pp. 72–74). https://doi.org/10.1093/jn/119.6.952

Joanisse, S., McKendry, J., Lim, C., Nunes, E. A., Stokes, T., Mcleod, J. C., & Phillips, S. M. (2021). Understanding the effects of nutrition and post-exercise nutrition on skeletal muscle protein turnover: Insights from stable isotope studies. *Clinical Nutrition Open Science, 36*, 56–77. https://doi.org/10.1016/j.nutos.2021.01.005

Kern, W., Dodt, C., Born, J., & Fehm, H. L. (1996). Changes in cortisol and growth hormone secretion during nocturnal sleep in the course of aging. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences, 51*(1), M3-9. https://doi.org/10.1093/gerona/51a.1.m3

Keys, A., Menotti, A., Aravanis, C., Blackburn, H., Djordevic, B. S., Buzina, R., Dontas, A. S., Fidanza, F., Karvonen, M. J., & Kimura, N. (1984). The seven countries study: 2,289 deaths in 15 years. *Preventive Medicine, 13*(2), 141–154. https://doi.org/10.1016/0091-7435(84)90047-1

Khan, S. S., Singer, B. D., & Vaughan, D. E. (2017). Molecular and physiological manifestations and measurement of aging in humans. *Aging Cell, 16*(4), 624–633. https://doi.org/10.1111/acel.12601

Knoops, K. T. B., de Groot, L. C. P. G. M., Kromhout, D., Perrin, A.-E., Moreiras-Varela, O., Menotti, A., & van Staveren, W. A. (2004). Mediterranean diet, lifestyle factors, and 10-year mortality in elderly European men and women: the HALE project. *JAMA, 292*(12), 1433–1439. https://doi.org/10.1001/jama.292.12.1433

Kowald, A., & Kirkwood, T. B. L. (2016). Can aging be programmed? A critical literature review. *Aging Cell*, *15*(6), 986–998. https://doi.org/10.1111/acel.12510

Kriete, A., Sokhansanj, B. A., Coppock, D. L., & West, G. B. (2006). Systems approaches to the networks of aging. *Ageing Research Reviews*, *5*(4), 434–448. https://doi.org/10.1016/j.arr.2006.06.002

Lange, J., & Grossman, S. (2014). *Gerontological Nursing: Competencies for Care* (K. L. Mauk (ed.); 4th ed.). JONES & BARTLETT PUB INC. https://samples.jbpub.com/9781284104479/Chapter_3.pdf

Lapointe, J., & Hekimi, S. (2010). When a theory of aging ages badly. *Cellular and Molecular Life Sciences*, *67*(1), 1–8. https://doi.org/10.1007/s00018-009-0138-8

Liochev, S. I. (2013). Reactive oxygen species and the free radical theory of aging. *Free Radical Biology & Medicine*, *60*, 1–4. https://doi.org/10.1016/j.freeradbiomed.2013.02.011

Liu, J., Wang, L., Wang, Z., & Liu, J.-P. (2019). Roles of Telomere Biology in Cell Senescence, Replicative and Chronological Ageing. *Cells*, 1–10.

Madison, A. A., Belury, M. A., Andridge, R., Renna, M. E., Rosie Shrout, M., Malarkey, W. B., Lin, J., Epel, E. S., & Kiecolt-Glaser, J. K. (2021). Omega-3 supplementation and stress reactivity of cellular aging biomarkers: an ancillary substudy of a randomized, controlled trial in midlife adults. *Molecular Psychiatry*, *26*(7), 3034–3042. https://doi.org/10.1038/s41380-021-01077-2

McGlory, C., Calder, P. C., & Nunes, E. A. (2019). The Influence of Omega-3 Fatty Acids on Skeletal Muscle Protein Turnover in Health, Disuse, and Disease. *Frontiers in Nutrition*, *6*, 144. https://doi.org/10.3389/fnut.2019.00144

Michalak, M., Pierzak, M., Kręcisz, B., & Suliga, E. (2021). Bioactive Compounds for Skin Health: A Review. *Nutrients*, *13*(1). https://doi.org/10.3390/nu13010203

Miles, E. A., & Calder, P. C. (2017). Can early omega-3 fatty acid exposure reduce risk of childhood allergic disease? *Nutrients*, *9*(7), 1–16. https://doi.org/10.3390/nu9070784

Moldogazieva, N. T., Mokhosoev, I. M., Mel'nikova, T. I., Porozov, Y. B., & Terentiev, A. A. (2019). Oxidative Stress and Advanced Lipoxidation and Glycation End Products (ALEs and AGEs) in Aging and Age-Related Diseases. *Oxidative Medicine and Cellular Longevity*, *2019*, 3085756. https://doi.org/10.1155/2019/3085756

Nature Education. (2014). *Essentials of Cell Biology*. https://www.nature.com/scitable/ebooks/essentials-of-cell-biology-14749010/122996920/

Ndifon, W., & Dushoff, J. (2016). The Hayflick Limit May Determine the Effective Clonal Diversity of Naive T Cells. *Journal of Immunology (Baltimore, Md. : 1950)*, *196*(12), 4999–5004. https://doi.org/10.4049/jimmunol.1502343

Nicholls, S. J., Lincoff, A. M., Garcia, M., Bash, D., Ballantyne, C. M., Barter, P. J., Davidson, M. H., Kastelein, J. J. P., Koenig, W., McGuire, D. K., Mozaffarian, D., Ridker, P. M., Ray, K. K., Katona, B. G., Himmelmann, A., Loss, L. E., Rensfeldt, M., Lundström, T., Agrawal, R., … Nissen, S. E. (2020). Effect of High-Dose Omega-3 Fatty Acids vs Corn Oil on Major Adverse Cardiovascular Events in Patients at High Cardiovascular Risk: The STRENGTH Randomized Clinical Trial. *JAMA*, *324*(22), 2268–2280. https://doi.org/10.1001/jama.2020.22258

Nissen, S. E., Lincoff, A. M., Wolski, K., Ballantyne, C. M., Kastelein, J. J. P., Ridker, P. M., Ray, K. K., McGuire, D. K., Mozaffarian, D., Koenig, W., Davidson, M. H., Garcia, M., Katona, B. G., Himmelmann, A., Loss, L. E., Poole, M., Menon, V., & Nicholls, S. J. (2021). Association Between Achieved ω-3 Fatty Acid Levels and Major Adverse Cardiovascular Outcomes in Patients With High Cardiovascular Risk: A Secondary Analysis of the STRENGTH Trial. *JAMA Cardiology*, *6*(8), 910–917. https://doi.org/10.1001/jamacardio.2021.1157

Novo, E., & Parola, M. (2008). Redox mechanisms in hepatic chronic wound healing and fibrogenesis. *Fibrogenesis & Tissue Repair*, *1*(1), 5. https://doi.org/10.1186/1755-1536-1-5

Novotny, K., Fritz, K., & Parmar, M. (2021). *Omega-3 Fatty Acids.*

Panday, A., Sahoo, M. K., Osorio, D., & Batra, S. (2015). NADPH oxidases: an overview from structure to innate immunity-associated pathologies. *Cellular & Molecular Immunology*, *12*(1), 5–23. https://doi.org/10.1038/cmi.2014.89

Paneni, F., Diaz Cañestro, C., Libby, P., Lüscher, T. F., & Camici, G. G. (2017). The Aging Cardiovascular System: Understanding It at the Cellular and Clinical Levels. *Journal of the American College of Cardiology*, *69*(15), 1952–1967. https://doi.org/10.1016/j.jacc.2017.01.064

Pangestuti, R., Siahaan, E. A., & Kim, S.-K. (2018). Photoprotective Substances Derived from Marine Algae. *Marine Drugs*, *16*(11). https://doi.org/10.3390/md16110399

Pereira, R. N., & Rodrigues, R. M. (2021). Emergent Proteins-Based Structures-Prospects towards Sustainable Nutrition and Functionality. *Gels (Basel, Switzerland)*, *7*(4). https://doi.org/10.3390/gels7040161

Russell, R. M., Rasmussen, H., & Lichtenstein, A. H. (1999). Modified Food Guide Pyramid for people over seventy years of age. *The Journal of Nutrition*, *129*(3), 751–753. https://doi.org/10.1093/jn/129.3.751

Schmeer, C., Kretz, A., Wengerodt, D., Stojiljkovic, M., & Witte, O. W. (2019). Dissecting Aging and Senescence-Current Concepts and Open Lessons. *Cells*, *8*(11). https://doi.org/10.3390/cells8111446

Schwenk, R. W., Holloway, G. P., Luiken, J. J. F. P., Bonen, A., & Glatz, J. F. C. (2010). Fatty acid transport across the cell membrane: regulation by fatty acid transporters. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, *82*(4–6), 149–154. https://doi.org/10.1016/j.plefa.2010.02.029

Shahidi, F., & Ambigaipalan, P. (2018). Omega-3 Polyunsaturated Fatty Acids and Their Health Benefits. *Annual Review of Food Science and Technology*, *9*, 345–381. https://doi.org/10.1146/annurev-food-111317-095850

Sharma, C., Kaur, A., Thind, S. S., Singh, B., & Raina, S. (2015). Advanced glycation End-products (AGEs): an emerging concern for processed food industries. *Journal of Food Science and Technology*, *52*(12), 7561–7576. https://doi.org/10.1007/s13197-015-1851-y

Silva, G. M., Finley, D., & Vogel, C. (2015). K63 polyubiquitination is a new modulator of the oxidative stress response. *Nature Structural & Molecular Biology*, *22*(2), 116–123. https://doi.org/10.1038/nsmb.2955

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, *56*(8), 365–379. https://doi.org/10.1016/s0753-3322(02)00253-6

Slomski, A. (2021). Diets High in Omega-3 Fatty Acids Might Ease Migraines. *JAMA*, *326*(8), 691. https://doi.org/10.1001/jama.2021.12645

Smit, L., & van Duin, S. (2020). *Seven Countries Study*. https://www.sevencountriesstudy.com/

Strehler, B. L. (1977). *Times, Cells, and Aging* (2nd ed.). https://www.sciencedirect.com/book/9780126732603/times-cells-and-aging

Su, W., Wang, L., Fu, X., Ni, L., Duan, D., Xu, J., & Gao, X. (2020). Protective Effect of a Fucose-Rich Fucoidan Isolated from Saccharina japonica against Ultraviolet B-Induced Photodamage In Vitro in Human Keratinocytes and In Vivo in Zebrafish. *Marine Drugs*, *18*(6). https://doi.org/10.3390/md18060316

Tang, Y., Feng, P., Gui, S., Jin, X., Zhu, J., & Lu, X. (2020). The Protective Effects of Protein-Enriched Fraction from Housefly (Musca domestica) against Aged-Related Brain Aging. *Journal of Nutritional Science and Vitaminology*, *66*(5), 409–416. https://doi.org/10.3177/jnsv.66.409

Thiyagarasaiyar, K., Goh, B.-H., Jeon, Y.-J., & Yow, Y.-Y. (2020). Algae Metabolites in Cosmeceutical: An Overview of Current Applications and Challenges. *Marine Drugs*, *18*(6). https://doi.org/10.3390/md18060323

Ticinesi, A., Meschi, T., Lauretani, F., Felis, G., Franchi, F., Pedrolli, C., Barichella, M., Benati, G., Di Nuzzo, S., Ceda, G. P., & Maggio, M. (2016). Nutrition and Inflammation in Older Individuals: Focus on Vitamin D, n-3 Polyunsaturated Fatty Acids and Whey Proteins. *Nutrients*, *8*(4), 186. https://doi.org/10.3390/nu8040186

Trevisan, K., Cristina-Pereira, R., Silva-Amaral, D., & Aversi-Ferreira, T. A. (2019). Theories of aging and the prevalence of Alzheimer's disease. *BioMed Research International, 2019*. https://doi.org/10.1155/2019/9171424

Troesch, B., Eggersdorfer, M., Laviano, A., Rolland, Y., Smith, A. D., Warnke, I., Weimann, A., & Calder, P. C. (2020). Expert Opinion on Benefits of Long-Chain Omega-3 Fatty Acids (DHA and EPA) in Aging and Clinical Nutrition. *Nutrients*, *12*(9). https://doi.org/10.3390/nu12092555

Tucker, J. S., Klein, D. J., & Elliott, M. N. (2004). Social control of health behaviors: a comparison of young, middle-aged, and older adults. *The Journals of Gerontology. Series B, Psychological Sciences and Social Sciences*, *59*(4), P147-50. https://doi.org/10.1093/geronb/59.4.p147

Ungaro, F., Tacconi, C., Massimino, L., Corsetto, P. A., Correale, C., Fonteyne, P., Piontini, A., Garzarelli, V., Calcaterra, F., Della Bella, S., Spinelli, A., Carvello, M., Rizzo, A. M., Vetrano, S., Petti, L., Fiorino, G., Furfaro, F., Mavilio, D., Maddipati, K. R., … Danese, S. (2017). MFSD2A Promotes Endothelial Generation of Inflammation-Resolving Lipid Mediators and Reduces Colitis in Mice. *Gastroenterology*, *153*(5), 1363-1377.e6. https://doi.org/10.1053/j.gastro.2017.07.048

van Staveren, W. A., de Groot, L. C. P. M. G., & Haveman-Nies, A. (2002). The SENECA study: potentials and problems in relating diet to survival over 10 years. *Public Health Nutrition*, *5*(6A), 901–905. https://doi.org/10.1079/PHN2002376

Vasto, S., Scapagnini, G., Bulati, M., Candore, G., Castiglia, L., Colonna-Romano, G., Lio, D., Nuzzo, D., Pellicano, M., Rizzo, C., Ferrara, N., & Caruso, C. (2010). Biomarkes of aging. *Frontiers in Bioscience - Scholar*, *2 S*(2), 392–402. https://doi.org/10.2741/s72

Weijs, P. J. M., Cynober, L., DeLegge, M., Kreymann, G., Wernerman, J., & Wolfe, R. R. (2014). Proteins and amino acids are fundamental to optimal nutrition support in critically ill patients. *Critical Care (London, England)*, *18*(6), 591. https://doi.org/10.1186/s13054-014-0591-0

Weinert, B. T., & Timiras, P. S. (2003). Invited review: Theories of aging. *Journal of Applied Physiology*, *95*(4), 1706–1716. https://doi.org/10.1152/japplphysiol.00288.2003

World Health Organization. (2018). *Ageing-and-Health @ Www.Who.Int*. https://www.who.int/news-room/fact-sheets/detail/ageing-and-health

Wu, S.-Y., Yeh, N.-H., Chang, H.-Y., Wang, C.-F., Hung, S.-Y., Wu, S.-J., & Pan, W.-H. (2021). Adequate protein intake in older adults in the context of frailty: cross-sectional results of the Nutrition and Health Survey in Taiwan 2014–2017. *The American Journal of Clinical Nutrition*, *114*(2), 649–660. https://doi.org/10.1093/ajcn/nqab070

Xie, S.-H., Li, H., Jiang, J.-J., Quan, Y., & Zhang, H.-Y. (2021). Multi-Omics Interpretation of Anti-Aging Mechanisms for ω-3 Fatty Acids. *Genes*, *12*(11). https://doi.org/10.3390/genes12111691

Yao, Q. H., Zhang, X. C., Fu, T., Gu, J. Z., Wang, L., Wang, Y., Lai, Y. B., Wang, Y. Q., & Guo, Y. (2014). ω-3 polyunsaturated fatty acids inhibit the proliferation of the lung adenocarcinoma cell line A549 in vitro. *Molecular Medicine Reports*, *9*(2), 401–406. https://doi.org/10.3892/mmr.2013.1829

Zielińska, M. A., Białecka, A., Pietruszka, B., & Hamułka, J. (2017). Vegetables and fruit, as a source of bioactive substances, and impact on memory and cognitive function of elderly. *Postepy Higieny i Medycyny Doswiadczalnej (Online)*, *71*(0), 267–280. https://doi.org/10.5604/01.3001.0010.3812

Zimmerman. (2020). *Digestion and Absorption of Lipids*. https://med.libretexts.org/@go/page/481

Žmitek, K., Žmitek, J., Rogl Butina, M., & Pogačnik, T. (2020). Effects of a Combination of Water-Soluble CoenzymeQ10 and Collagen on Skin Parameters and Condition:Results of a Randomised, Placebo-Controlled,Double-Blind Study. *Nutrients*, *12*(3). https://doi.org/10.3390/nu12030618