universität wien

DIPLOMARBEIT / DIPLOMA THESIS

Titel der Diplomarbeit / Title of the Diploma Thesis

„Fasting and fasting mimetics: Can secondary plant ingredients, such as spermidine, replace Buchinger´s fasting?“

verfasst von / submitted by

Nadja Michlmayer

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

Magistra der Naturwissenschaften (Mag.rer.nat.)

Wien, 2020 / Vienna, 2020

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	UA 190 477 313
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Lehramtsstudium UF Haushaltsökonomie und Ernährung, UF Geschichte und politische Bildung
Betreut von / Supervisor:	Doz. Dr. Alexander Haslberger

Danksagung

An dieser Stelle möchte ich mich bei meinem Partner , meinen Freunden und Freundinnen und meiner Familie bedanken, die mich mein ganzes Studium hindurch unterstützt und ausgehalten haben.

Ich danke der Willenskraft und Stärke, die mich dazu bringen haben lassen, Dinge zu erreichen, von denen ich nie gedacht hätte, dass sie möglich wären.

Ich danke Claire dafür, dass sie meine Arbeit Korrektur gelesen hat und mir damit Sicherheit gegeben hat.

Ich danke meinen Studienkollegen und Studienkolleginnen für die Gegenseitige Unterstützung und den Rückhalt während der gesamten Studienzeit.

Last but not least geht auch ein herzliches Dankeschön an Frau Stephanie Lilja, MSc und Herrn Dr. Alexander Haslberger, die sich meiner Diplomarbeit angenommen haben und mit großer Begeisterung meine Themenauswahl begleitet haben. Vielen Dank für die gute Betreuung und die Unterstützung, die diese Diplomarbeit mit möglich gemacht haben.

Kurzfassung

Diese Diplomarbeit analysiert den Zusammenhang zwischen dem Fasten - dem Prozess des Nahrungsentzuges - der Autophagie und den sekundären Pflanzenstoffen, Spermidin und Resveratrol, und untersucht, ob es möglich ist, durch eine Erhöhung dieser Stoffe den Prozess der Autophagie ohne Fasten einzuleiten. Autophagie ist ein zellulärer Prozess, der geschädigte Organellen oder Zellbestandteile entfernt und unter Hungerbedingungen Energie liefert oder unter Stressbedingungen Schäden repariert.

Diese These gewinnt ihre Bedeutung dadurch, dass wir unseren Körper und unsere Zellen ständig mit Nahrung und damit Energie versorgen, was in der Folge zu einem Zellwachstum führt, das wiederum durch Insulin und den insulinartigen Wachstumsfaktor IGF-1 und mTOR verstärkt wird. Für unsere Zellen bedeutet dies, dass sie kontinuierlich altern. Wenn wir andererseits eine Zeitlang nichts essen, sinken die Insulin- und IGF-1-Spiegel und auch der mTOR kommt zur Ruhe. Die Zellen initiieren dadurch ein "Selbstreinigungsprogramm", das als Autophagie bezeichnet wird. Dies ist ähnlich der Art und Weise, wie sich das Gehirn während des Schlafs reinigt. Während dieses Prozesses können sogar die Proteinaggregate, die im Verdacht stehen, die Alzheimer-Krankheit zu verursachen, aus dem Gehirn gespült werden.

Allerdings kann und darf nicht jeder Fasten. So haben viele Menschen Schwierigkeiten, ihre Essgewohnheiten zu ändern, und andere haben Kontraindikationen, zu denen auch Menschen mit gegenwärtigen Krankheiten und ältere Menschen gehören. Daher könnte es zu einem Nutzen für Millionen von Menschen führen, wenn Resveratrol und Spermidin ähnliche oder gleiche Wirkungen auf den menschlichen Organismus erzielen könnten, ohne dass ein Fasten erforderlich wäre. Ob, welche und wie sekundäre Pflanzenstoffe den menschlichen Organismus beeinflussen, ist Gegenstand umfangreicher Forschung. Diese Arbeit konzentriert sich auf Resveratrol und Spermidin und analysiert, ob diese sekundären Pflanzenstoffe in der Lage sind, Autophagie zu induzieren. Eine abnorme Regulierung der Autophagie wurde im Entwicklungsprozess verschiedener Krankheiten beobachtet, und umgekehrt wurde die stabile Aktivierung der Autophagie mit der Fähigkeit zur Linderung und in einigen Fällen sogar zur Heilung von Krankheitssymptomen in Verbindung gebracht. Diese Arbeit soll einen Überblick über den aktuellen Stand der Forschung zu Spermidin und Resveratrol und deren Auswirkungen auf den menschlichen Organismus geben.

Abstract

This diploma thesis analyses the relationship between fasting — the process of food deprivation — autophagy and the secondary plant substances, spermidine and resveratrol, and investigates whether, through an increase in these substances, it is possible to induce the process of autophagy without the need for fasting. Autophagy is a cellular process that removes damaged organelles or cell components and provides energy under starvation conditions or repairs damage under stress conditions

This thesis gains its importance due to the fact that we constantly supply our body and cells with food, and thereby energy, which subsequently leads to cellular growth which, in turn, is reinforced by insulin and the insulin-like growth factor IGF-1 and mTOR. For our cells this means that they are continuously ageing. On the other hand, if we do not eat for a while, insulin and IGF-1 levels fall and mTOR also comes to a rest. The cells thereby initiate a 'self-cleaning program' called autophagy. This is similar to the way in which the brain cleanses itself during sleep. During this process, even those protein aggregates that are suspected of causing Alzheimer's disease can be flushed out of the brain.

However, not everyone is able to participate in fasting. For example, many people have difficulties changing their eating habits and others have contraindications, which would include people with present diseases and the elderly. Therefore, it could lead to the benefit of millions if resveratrol and spermidine could achieve similar, or the same, effects on the human organism without the need for fasting. Much research has been conducted to identify whether, which and how secondary plant substances influence the human organism. This thesis will focus on resveratrol and spermidine and analyse whether these secondary plant substances are capable of inducing autophagy. Autophagy gains its importance due to its relationship with the development of various diseases, including cancer, neurodegenerative diseases, myopathy and heart disease. Abnormal regulation of autophagy has been observed in the development process of various diseases and, conversely, the stable activation of autophagy has been linked to an ability to alleviate and, in some instances, even cure disease symptoms. This thesis aims to provide an overview of the current state of research on spermidine and resveratrol and their effects on the human organism.

TABLE OF CONTENTS

List of figures

1. Introduction

Prior to exploring the topic and arguments outlined in this diploma thesis, I will explain the concept of, and rational behind choosing, fasting and fasting mimetics. Two years ago, I attended a Ernaehrungswissenschaftliches Seminar (Seminar on Nutritional Science), with Mrs. Professor Petra Rust, wherein a fellow student and I was assigned to write about intermittent fasting. Although the practice was not completely unknown to me, given my environment and personal experience with fasting, there was much that remained to be explored. Throughout the seminar I had the opportunity to further immerse myself in the subject matter. I learned about autophagy, the body's mechanisms to cleaning out damaged cells, and numerous studies and perspectives on the fasting processes. Of particular interest to me was the research of Dr. Frank Madeo. For many years, Madeo studied the fasting processes at the University of Graz and it was through his work that I discovered the interlinkages between the secondary plant ingredient, spermidine, and the process of autophagy. This sparked my interest and laid the foundation for this subsequent work. Coincidentally, a similar connection arose with Prof. Alexander Haslberger and Stephanie Lilja, Mc, who both supervised my diploma thesis. Halsberger and Lilja, from the University of Vienna, are currently working on a study which analyses the supply of secondary plant substances in humans and their positive effects on the human organism, and in which I am also participating.

This work predominantly analyses the 'Buchinger fasting' method and its relationship to the secondary plant substances: spermidine and resveratrol, found in some fruits and vegetables. Furthermore, Halsberger's and Lilja's study also deals with the connection between secondary plant substances and the human metabolism. Research has also been done to discover whether secondary plant substances, such as spermidine or resveratrol, can achieve similar, or even the same effects, as fasting and thereby replace the practical altogether. These substances are said to have a number of positive bodily effects, such as antioxidant and anti-inflammatory effects.

The aim of this diploma thesis is to discover whether secondary plant substances, and in this case spermidine and resveratrol, can replace the fasting process. The fundamental question to this work is whether the secondary plant ingredients spermidine and resveratrol can replace fasting, with similar or even the same positive effects on the human organism?

Therefore, this diploma thesis will begin by introducing the above mentioned substances and subsequently compare and analyse the data from existing studies.

2. Literature Review

2.1. Fasting

Definition

Fasting is the temporary and voluntary renunciation of solid and/or luxury foods. When fasting is carried out correctly, ones body and mind maintain a good performance level without feeling hungry. Fasting affects all dimensions: the body, the mind, the soul and the spirit (SCHUBMANN, 2009, p. 14-16,).

There are a number of different fasting methods and forms, like intermittent fasting or fasting with soups or fasting with the method of Buchinger. Before going into more detail about Buchinger fasting, it is important to clarify some general fasting facts, myths and theories.

In recent years, the topic of fasting has received increasingly more attention. A growing number of people are placing a greater focus is on food, weight and health based on its implications on living a longer and more fulfilling life. Alongside these changing lifestyles, new theories, forms of nutrition, diets and dietary recommendations are constantly being developed. With fasting having one of the oldest histories, if not the oldest, it is therefore interesting to see its ‘comeback’ in modern culture.

The topic of weight has always played an important role in society. Given its positive bodily impact, the renewed interest in the subject matter should be commended. Both men and women are paying increasingly close attention to exercising and training. However, not everyone who has a high bodily weight should be considered ‘overweight’ or a 'medical problem' — given that increased muscle mass needs to be taken into account when analysing weight. The common explanation for overweight is based on the principles related to the energy balance. How does one become overweight? Put simply: by eating more than you burn. These people take in a high number of calories a day and, comparatively, exercise too little. Taken on face value, the energy balance theory sounds logically sound and, on a fundamental level, it is. Energy does not dissolve into thin air and this also applies to the energy we supply to our body. And yet, the principle falls short when we begin to deal with more complex biological organisms, such as a human being or even a mouse (KAST, 2018, p. 271).

Alongside the growing social interest, fasting also became a relevant topic in research. Several studies have demonstrated the effects of fasting on the organism. Prior to conducting studies on humans, hypotheses were tested on mice and impressive and insightful results were secured.

The correlation between fasting and weight control was exhibited by scientists from Japan and the USA by means of an experiment with mice. A test group was fed a high-fat diet on a daily basis. They were allowed to eat as much as they wanted, day and night. The lack of dietary constraints meant they abused the food availability and the mice not only became overweight but also sick. With the increase in fat content, their liver values decreased. The second test group of mice received exactly the same amount of calories. The only difference was that they were fed exclusively at night (i.e. only within a twelve hour time frame). The second group therefore ate twice as much as the first group. Interestingly enough, the second group of mice not only stayed slim, but also healthy. Their livers did not fatten, and their values were stable. This experiment does not mean that everyone who wants to lose weight can now eat as much as they want and gradually slim down, provided they only eat it at night. The experiment should, most certainly, not be understood this way. The main outcome of the study is not to eat compulsively, but rather to allow the body to take breaks in-between. In addition, it is critical to mention that a mouse has a faster metabolism than a human and perceives time differently. Humans, on the other hand, should fast regularly — for at least 20 to 36 hours — to achieve similar effects (STEKOVIC, 2018, p. 63-65).

Another key finding that emerged from this research was how strongly the circadian rhythm affects the human organism. Our metabolism works completely differently depending on the time of day. This does not mean that our body can override the foundations of physics but rather that, depending on when or in which rhythm or time window we take in calories, these calories are processed differently. The circadian rhythm of the human body can be traced deep inside our cells, right down to our genes. More than half of our genetic activity is subject to a circadian rhythm. This means that thousands of genes are more or less active depending on the time of day. In a particular organ, such as the liver, this means that in the early morning numerous genes are activated and others are silenced. Depending on the time of day, and based on the gene activity, different cells of our organs produce different proteins. For example: if you give two test subjects the same meal, with one receiving it in the morning and one receiving it in the evening, the body's physical reaction will be entirely different, even if the duration of the fasting period before the meal was the same for both test subjects. For

example, insulin regulates our blood sugar and our insulin sensitivity is highest in the morning. This means that, in the morning our body is able to produce a higher rate of insulin and thereby better regulate our blood sugar after a meal. Nutrients, especially carbohydrates, are therefore best tolerated in the morning. Throughout the course of the day, our blood sugar control systematically decreases. From a blood sugar point of view, when eating in the evening our body perceives it as if we were eating twice as much as in the morning. When we eat, is therefore critical. Due to the increased insulin sensitivity in the morning, our body can process the glucose flood relatively quickly. Towards the evening, the same amount of carbohydrates simply causes our body more trouble. One reason for this is the sleep hormone melatonin, the release of which also largely follows a circadian rhythm. Daylight inhibits the production of melatonin. Conversely, when it is dark, the concentration of melatonin increases and we get tired. The insulin-producing cells of the pancreas are equipped with receptors for melatonin. As soon as melatonin docks to these receptors, the release of insulin is inhibited. The result is a restricted blood sugar regulation in the late evening and at night. One advantage of limiting eating to certain hours during the day is that this allows you to follow the natural day-and-night rhythm of the body, which is determined by light. As already mentioned, all organs of our body, right down to the genetic material, are also affected by the day-and-night rhythm. Put simply, one could say that our organs, such as the intestines, liver, pancreas and many more, are intrinsically established to be more receptive to morning meals due to the gene activity. Throughout the course of the day, patterns of gene activity change. Our body's cells change into a different mode of action. Similar to the constraints on a humans ability to multitask, the body's cells cannot do everything at once. For example, at night time, when our cells are not overly indulged due to food which needs to be processed, their role changes; they perform, what nutritionists like to refer to as, a clean-up job. Clumped or otherwise harmful protein structures and defective cells can be broken down during resting periods. On the other hand, if we eat late in the evening or at night, our cells have to process the incoming food supply, in other words breakdown the nutrients, and are unable to perform their routine clean-up work. Genes of the liver and other organs, which should be in resting mode, are kept active by the unexpected calories (KAST, 2018, p. 272-277).

Fasting is also a medicine for numerous physical ailments, as has been suspected and assumed for many centuries. In recent years, scientific studies have confirmed this ancient wisdom. Perhaps the most important and well-documented example concerns type 2 diabetes. One, if not the core, problem with type 2 diabetes is the obesity of organs, such as the liver and muscles, which then become numb to the insulin signal and are unable to absorb glucose from

the blood. However, positive effects of fasting have also been observed in patients with high blood pressure and rheumatism, disease marked by inflammation and pain in the joints, muscles, or fibrous tissue. The effects are all very impressive in this respect (KAST, 2018, p. 279-280).

If the body needs more heat, the same thing happens as with fasting. Fat is broken down and converted into chemical energy. By breaking up the molecules, energy is produced in the form of chemical compounds that are used either for the function of the cells or for the production of thermal energy, i.e. heat (STEKOVIC, 2018, p. 48).

This process takes place in the brown fatty tissue, an area under the collarbone and around the upper sternum. The difference between white and brown fat is the number of mitochondria. These organelles in our cells are mainly responsible for energy production, but they are also the interface for the metabolism of the cell. In these tiny power stations of the cell, matter is constantly being constructed or broken down; be it fat, proteins, amino acids or sugar. Mitochondria are extremely interesting components of our cells. Their structure is unique and, above all, they have something that no other organelle in our organism has, namely, its own DNA. All other organelles are controlled by DNA in the cell's nucleus, the so-called control room of the cell (STEKOVIC, 2018, p. 49).

It is commonly understood that too much of anything is never good. If the body fasts too frequently, and produces too many ketone bodies, the blood becomes slightly acidic and, in the long run, it overloads our kidneys. In addition, certain fat-soluble, harmful substances that simply accumulate in fat over time suddenly become 'homeless'. In other words, the fat in which they were stored is no longer there. Fat-soluble toxins can cause damage. If they are slowly released from the fat, they cannot cause any real damage, because the kidneys and liver detoxify and dispose of these substances. However, if released too quickly, these two organs will be overloaded and the toxins can do direct damage to the rest of the body (STEKOVIC, 2018, p. 67-66).

Since there are many different methods of fasting, I have decided to go into more detail about one method, namely, the Buchinger fasting method.

2.1.1. Therapeutic fasting according to Buchinger

The term ‘therapeutic fasting’ was coined by the German physician, Otto Buchinger (1878-1966). As a young doctor, Buchinger himself fell ill to the rheumatic fever which led to occupational disabilities. Fasting became his method of healing, to which he later dedicated his life’s work. Today, therapeutic fasting refers to medically supervised, in-patient, fasting which, in the hands of an experienced fasting doctor, can also be done on an out-patient basis. It is a multidisciplinary study, taking into account three dimensions: the somatic, the psychological and the spiritual realm. Traditionally, therapeutic fasting was conducted via the administration of a vegetable broth (1/4 l), fruit or vegetable juices (1/4 l) and honey (30 g) as well as plenty of tea and water. In addition to the physical dimension (medical-therapeutic effects), the psychosocial dimension (psychological changes and group dynamics that arise when people fast together) and a spiritual dimension (natural access to higher states of consciousness, which are reoccurring themes in most world religions) are addressed (DE TOLEDO, 2002, p.189-198).

Therapeutic fasting, according to Buchinger, offers a break or a new beginning. In essence, it is the ability to commence a way of life. Gains can be achieved through renunciation, which trigger a change in eating behaviour on a physical and mental-spiritual level.

Ideally, therapeutic fasting takes place in a clinic for prevention and rehabilitation. It thereby offers a holistic approach. However, well nourished or healthy people can also carry-out this procedure on an outpatient basis, albeit under the guidance of a trained fasting leader.

Procedure
Methodically belongs to fasting according to Buchinger:
• the ample drinking of at least 2 litres of calorie-free liquid (such as mineral water and tea) every day,
• vegetable broth and fruit or vegetable juices (with about 250 kcal per day)
• about 30 g honey and
• regular bowel movements

(SCHUBMANN, 2009, p. 14-17)

It is important to integrate fasting into a holistic, forward thinking, approach which rests on the promotion of health and prevention of diseases and health problems. This includes:
• Physical exercise: It should be individually adapted and should be sufficient every day. Exercise promotes self-esteem and psychosocial integration and has positive metabolic and haemodynamic effects
• Relaxation and rest: e.g. for self-awareness, self-reflection, meditation
• Physiotherapy: e.g. massages, physiotherapy depending on the symptoms, Kneipp treatments
• Nutritional training
• Aftercare programs
• Psychotherapy
• Health information seminars

(SCHUBMANN, 2009, p. 14-17)

There are different effects of fasting with the method of Buchinger. Since human carbohydrate reserves are limited to approximately 1600 kcal, already on the second day of fasting fat begins to break down and metabolise in order to create energy. This leads to a normalization of even strongly increased triglyceride levels in the serum.

Even the blood sugar levels in type 2 diabetics almost always normalise within 5 days. This also explains why it is important to discontinue ones blood sugar-lowering medication prior to beginning the fasting process. The insulin receptor function is significantly improved by weight reduction and also through daily exercise. A low-normal blood sugar level is maintained by using triglycerides for gluconeogenesis.

In addition, there is a low protein catabolism (glucoplastic amino acids), but this is negligible during fasting periods of 10 to 20 days with accompanying physical activity (the muscles, as the largest protein store, are probably largely protected). This effect also occurs during so-called ‘protein-modified fasting’. In addition, with an increasing fasting duration the protein catabolism decreases through the saving mechanisms from approximately 80g per day, to 15g at the end of the fourth week of fasting. During longer periods of fasting, protein is usually supplemented in the form of buttermilk (0.5 l = 17.5 g P.E.).

The serum cholesterol level can also decrease slightly during fasting, firstly due to a lack of food intake and subsequently due to metabolic regulation improvements. In the post-fasting period, a low-fat, and therefore low-cholesterol wholefood, diet should be preferred due to changes in eating habits.

The strong natriuretic-diuretic effect of fasting is well-known. The entire extracellular volume is visibly reduced. Within a few days, the increased blood pressure often sinks due to the reduction of the preload, antihypertensive medicines can be stopped or at least greatly reduced.

At the same time, rheology is improved by reduced fibrinogen and prothrombin reactions (DE TOLEDO, 2002, p. 189-198).

2.2. Sirtuine

Sirtuins, commonly also referred to as silent mating type information regulation 2 homologous (SIRT), can be found in all living organisms, from bacteria and archaebacteria to mammals, and were first discovered in the 1990s in an effort to find yeast mutants with longer life durations. (SINCLAIR, 2006, p. 34-41)

Humans possess a total of seven sirtuins (SIRT1-SIRT7). Just like in yeast, they act as energy sensors in our cells and are activated when there is a lack of energy. Thanks to their properties, sirtuins are therefore multifunctional and regulate many metabolic processes as well as the ageing process (RAUH, 2013).

In fact, an increased activity of a yeast's sirtuin, silent information regulator two (Sir2), can extend its life. It ensures the silencing of certain chromatin regions by deacetylating histones. This attenuation of chromatin activities, such as during replication, recombination and transcription, seems to be essential for prolonging the life of Sirt2. Interestingly, an increase in sirtuin activity also prolongs the life-span of more complex organisms, such as the worm Caenorhabditis elegans or the fruit fly Drosophila melanogaster. Nevertheless, mechanisms that determine the lifespan of these animals fundamentally differ from those of yeast. The lifespan of yeast is expressed in terms of the number of generations; in other words, the number of divisions that a mother cell carries out during its lifetime. In contrast, Caenorhabditis elegans and Drosophila melanogaster consist largely of post-mitotic, non-

dividing, cells and are characterised by a chronological life span. Recent studies suggest that sirtuins also plays an important role in the regulation of the life span of mammals. In more complex species, sirtuins can deacetylate a number of cellular regulatory proteins, in addition to histones, and can influence their activity in a positive or negative manner. However, the life-enhancing effect of increased sirtuin on mice has not yet been documented.

The close link between the sirtuin function and cellular metabolism plays a central role in regulating the lifespan. For example, sirtuins are necessary to activate the life prolonging effect which occurs during the restriction of calories. Studies have shown that in animals, including mammals, a reduced calorie intake leads to a general increase in fitness and prolonged life span. For example, in mice, Sirt1 activity is increased when calorie restrictions are in place. (BOBER, 2007)

Recently, research has focused on how to activate these sirtuins without fasting. To this end, a study is currently underway at the University of Vienna under the direction of Mr. Univ. Doz. Dr. Haslberger and Stephanie Lilja MSc. The aim of the study is to discover whether different sirtuin genes can be activated by SirtFoods and whether these foods are able achieve the same effects as fasting.

What are SirtFoods? SirtFoods are plant-based foods composed of polyphenols – secondary plant substances. These substances act on the sirtuins and imitate the ‘lack of energy’ signal which normally results from fasting, dieting and/or exercise. In other words, SirtFoods can activate sirtuins without fasting taking place. In countries such as Japan and India, varying sirtuins are part of the daily diet. Also known as 'blue zones', these countries have the lowest incidences of lifestyle-related diseases, including hypertension, obesity, diabetes, fatty liver and cancer, due to higher situin activating diets. In addition, the polyphenols contained in SirtFoods have been closely linked to positive effects on epigenetic mechanisms and many other genes known for promoting a long and, above all, healthy life (Piwpong, 2018, p. 98-112).

How can you activate sirtuins with SirtFoods? The sirtuin genes, and the resulting sirtuin enzymes, are activated during low energy cycles. Classically, this is achieved by restricting calories, in other words, dieting, fasting and exercising. Alternatively, one can active sirtuins by consuming so-called sirtuin foods which contain so called ‘sirtuin activators’. Active sirtuins can ‘switch on’ genes that, for example, help you lose or maintain weight. They can also activate ‘radical protection’, whereby the anti-oxidative system increases its operations

and thus slows down the ageing process. Lastly, sirtuins are also said to positively influence the regulation of the thyroid gland, the fatty tissue and the metabolisms sugar and fat (Qiu, 2010, p. 1576-1583).

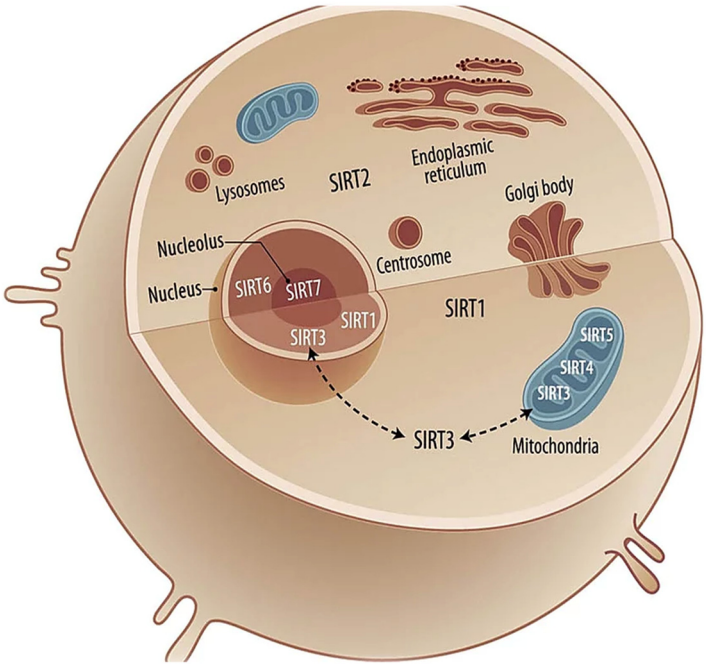

Figure 1: Subcellular localization of sirtuins

SIRT1 is mainly located in the cell nucleus, but it can also be found in the cytosol. SIRT2 is also located in the cytosol, where it has its main site. SIRT3, SIRT4 and SIRT5 are mitochondrial proteins, but SIRT3 can also be located in the cell nucleus and cytosol under different cellular conditions. SIRT6 and SIRT7 are located in the cell nucleus and nucleolus respectively (Alhazzazi, 2011, p. 80-88).

2.3. Secondary plant ingredients

The term ‘secondary plant substances’ covers substances of very different structures. From the roughly 100,000 known secondary plant substances, 5,000 to 10,000 occur in human food (Watzl 2008). As the name suggests, secondary plant substances are formed in small quantities in the secondary plant metabolism. They are a multitude of chemically heterogeneous compounds, which generally have pharmacological effects. Among other reasons, the plant forms these compounds as a type of antibody against pests and diseases, a

growth regulator, as well as for its attractant, fragrance, dye and flavour (SCHEK, 2002, p. 44-52).

Due to their chemical structure and functional properties, secondary plant compounds are divided into different groups, namely: polyphenols, carotenoids, phytoestrogens, glucosinolates, sulphides, monoterpenes, saponins, protease inhibitors, phytosterols and lectins. Although secondary plant compounds are not considered essential human nutrients, they do have an influence on a number of metabolic processes and various health-promoting effects are attributed to them. They are said to protect against various types of cancer and mediate the effects of cardiovascular diseases, such as the dilation of blood vessels and a reduction in blood pressure. Secondary plant compounds also have neurological, anti-inflammatory and antibacterial effects (DGE, 2012).

Knowledge on the importance of secondary plant compounds and their positive health effects has increased considerably. This can largely be explained by the growing focus on large prospective observational studies, called cohort studies, and, above all, intervention studies with isolated secondary plant compounds. Ultimately, only intervention studies provide the necessary causal evidence which demonstrate the relationship between the intake of secondary plant compounds and the resultant disease preventing effects. Since the 2008 Nutrition Report by the German Nutritional Biochemist, Bernhard Watzl (WATZL, 2008), numerous epidemiological studies confirm the effects of secondary plant compounds on human health and their attribution to decreasing the risk of contracting a disease (WATZL, 2008).

Based on the current scientific data, it is possible to evaluate the preventive effect of secondary plant substances. However, recommendations on the daily or average individual intake cannot be concluded at this stage (DGE, 2012).

2.3.1. Spermidine

Spermidine, also referred to as monoaminopropylputrescine, is a secondary plant substance and natural polyamine, and is formed as a byproduct during the formation of spermine from putrescine and decarboxylated S-adenosylmethionine. It is found in all natural organisms, from bacteria to humans, and its highest concentration is most frequently found in the seminal fluid of mammals. Scientists suspect that this phenomenon has an evolutionary background and that its purpose is to protect the genetic material in the sperm. In other words, it helps to preserve the health of the offspring. It is also closely related to cell growth. However, the

exact physiological function of spermidine in the growing cell – in other words in the production of nucleic acids and proteins or membrane stabilisation – has yet to be studied in its entirety (STEINMÜLLER, 2015).

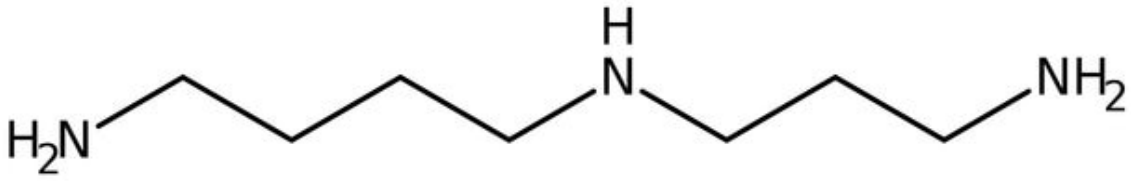

Figure 2: Structural formula of spermidine

Spermidine has several qualities, one of which is autophaghy. Both in unicellular organisms, as well as multicellular organisms such as humans, spermidine activates autophagy. Only under the microscope this little wonder of nature becomes visible. When placing a spermidine on a cell the two immediately interact. Countless small ‘garbage bags’ are formed, in which the cellular waste is collected and digested in a type of ‘cellular stomach’. It activates the same process that is initiated when person has been fasting over multiple days. The only difference is the context (STEKOVIC, 2018, p. 88).

Spermidine prolongs cellular life, and thereby overall life, because it activates autophagy. Several properties are attributed to this substance, including anti-inflammatory and antioxidant functions, and the improvement of proteostasis, respiration and the mitochondrial functions of the metabolism which can have different effects on a singular human organism. These rejuvenation processes are causally associated with spermidine – the polyamine properties of spermidine induce cytoprotective autophagy (MADEO, 2018, p. 1).

In a study in Bruneck, South Tyrol, conducted by Madeo et. al. (2018), the inhabitants of a village were looked at over 20 years and data on nutrition, diseases and causes of death were collected. The results, among others, demonstrated the positive effects of a two and a half milligram daily intake of spermidine. Bruneck reported that the probability of death from heart disease had dropped by more than 25 percent. A more indepth analysis will be provided in the subsequent chapter (MADEO, 2018, p. 4).

As pleasant as a long life may be, it also largely depends on how well the body functions. The most important aspect is to prolong health, and thereby life, and not the other way around. With spermidine this could be achieved. Spermidine not only repairs cellular damage that accumulates with age, but is also able to decrease the changes of developing diseases that are associated with old age. Stekovic's practical experiments on mice have proven this (STEKOVIC, 2018, p. 88-89).

If spermidine slows down the ageing processes, by cleaning up the cellular waste, could it also help against neurodegeneration, in other words, keep the brain young? The assumption is certainly not absurd. The protein waste that autophagy cleans up eventually also accumulates in the brain cells. And this accumulation only increases with age. Although spermidine does not heal cells and thereby the human body, it is one of a few natural substances that influence cell purification. Spermidine can therefore slow down the aging process, neutralize signs of aging or even prevent them from appearing in the first place (STEKOVIC, 2018, p. 90).

The existence of spermidine has been known since the 1970s. However, its traits and functions have only recently been revealed. Science and technology was simply not yet advanced enough to make the necessary discoveries (STEKOVIC, 2018, p. 91).

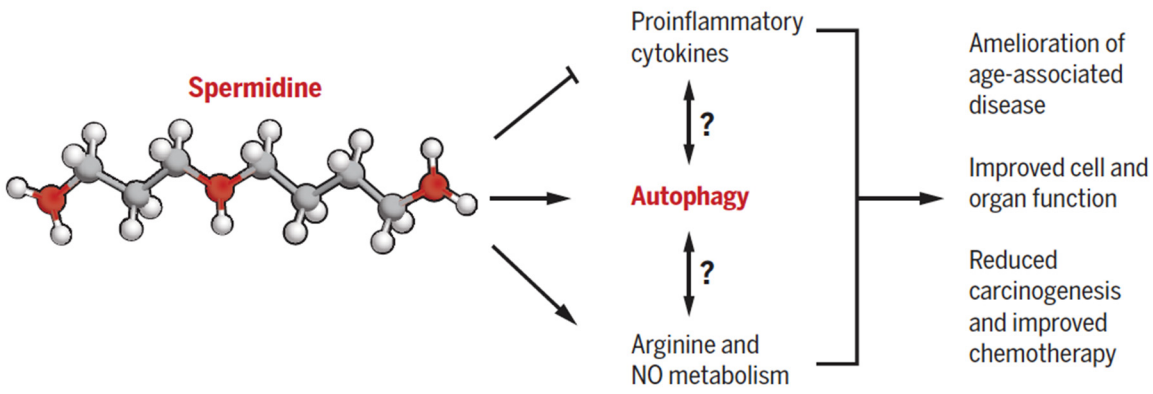

Figure 3: Schematic of health effects that can be mediated or initiated by spermidine

The natural polyamine spermidine has a pronounced cardioprotective and neuroprotective effect. Furthermore, it improves age-related metabolic degradation and stimulates immune surveillance in cancer in animal models. (MADEO, 2018, p. 1)

2.3.2. Resveratrol

Resveratrol is a natural polyphenolic compound synthesized by various plant species, grapes, peanuts and berries.

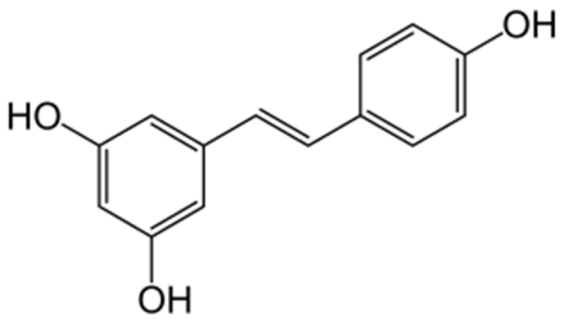

Figure 4: Structural formula of resveratrol

This secondary plant ingredient is said to have properties with a positive effect on the human organism, such as antioxidant, anti-inflammatory, anti-ageing, cardioprotective and neuroprotective effects. For example, Dohyun Park's study from 2016 of mice demonstrated that, similar to fasting, resveratrol can stop inflammatory cells in vitro and prolong life, but without the need for calorie restrictions.

In addition, according to Laura Dewald (2019, p. 17) resveratrol has preventive properties against chronic diseases, including diabetes and various metabolic disorders, and can be used as a treatment option.

Resveratrol was first discovered by the Japanese researcher, Michio Takaoka, in 1939. Takaoka isolated the substance from the leaves of the medicinal plant Veratrum grandiflorum, a white lily species. The highest content of resveratrol was found in Japanese knotgrass (*Polygonum cuspidatum* or *Ko-jo-kon* or *Darakchasava*) in 1963 and, until today, it remains the main source of resveratrol extraction (TAKAOKA, 1939, p. 1090-1100).

It was not until 1976 that resveratrol was first detected in red grapes. While the substance is mainly found in the grape skin, small concentrations of resveratrol can also be found in the seeds, stems, vines and roots. Unsurprising, the highest concentration is found in vines whose immune system is under severe strains due to external influences. Conversely, grape vines growing in moderate or stable conditions tend to produce less resveratrol. Therefore, untreated, natural or organic grapes contain significantly more resveratrol than chemically

treated ones. Resveratrol can also be found in, among others, peanuts, blueberries, cranberries, raspberries, mulberries, plums. Resveratrol is sensitive to light and oxygen and survives both the fermentation process and long storage periods. For example, the more time the wine has for natural maceration, the higher the content of resveratrol (STERVBO, 2007, p. 449).

Several studies have demonstrated the anti-inflammatory and growth-inhibiting properties of resveratrol in cancer and primary cells and animal models. The anti-aging effects of resveratrol are due to the activation of SIRT1, the NAD-dependent histone deacetylase. Resveratrol is considered the most powerful SIRT1 activator and there are generally two ways in which resveratrol acts in relation to SIRT1, namely: either by binding directly to SIRT1, and thereby promoting the deacetylation of a number of SIRT1 substrate proteins, or by increasing the NAD+ level (DEWALD, 2019).

In another study conducted by Joseph Baur in 2006, resveratrol improves health and survival of mice on a high-calorie diet and a life-enhancing effect of resveratrol was shown in a mammalian organism fed by a hypercaloric and high-fat diet. The study analysed two mice groups. The control group, was fed a species-appropriate, standard, diet with hardened coconut fat. The second group also received a standard diet enriched with hardened coconut fat but with an additional dose of resveratrol. In both cases, the coconut oil provided approximately 60% of the supplied energy which is unusually high given that the normal (maximum) percentage of energy originating from fat should be no more than 40%. However, coconut oil contains an equally unusual high quantity of saturated fatty acids. In addition, the mice in the second, experimental, group received 22 mg trans-resveratrol per kilogram of body weight per day. This can also be considered high. To achieve a comparable dose, a 70 kg human being would have to drink at least 110 litres of red wine each day for the entirety of their life. Arguably the most important outcome of the study was that the lifespan of the second test group of mice, fed with a fat-rich and high-calorie diet, could be extended to up to 22% merely by administering a daily dosage of resveratrol. However, this diet not only had positive effects on the life span, but also on metabolic processes. While the mice which were fed with a high fat- and high- calorie diet witnessed an increased plasma fasting glucose and insulin concentrations, as well as a reduction in insulin sensitivity, the second, experimental, group — treated with resveratrol — showed no changes in the risk factors associated with the metabolic syndrome. In addition, the resveratrol-treated mice did not develop fattier livers, unlike those fed with a hypercaloric and high-fat diets (BAUR, 2006, p. 337-342).

That being said, and although these results sound very positive and hopeful, studies should always be critically reviewed.

Despite the fact that it has been shown, for the first time, that a substance classified as a ’functional food ingredient’ prolongs the life span of a mammal and, at the same time, prevents the manifestation of the metabolic syndrome resulting from a high-fat super-nutritional diet, these results should be interpreted with caution. The doses of resveratrol administered are in the pharmacological range. Currently, it is still relatively unclear whether and, if so, what type of secondary health risks could occur when consuming more than 1 g of resveratrol as a supplement on a daily basis (corresponding to the amount in approximately 110 L of red wine) (ABRAHAM, 2009, p. 445-453.)

2.4. Autophagy

A human being consists of 100 trillion cells, each with a corresponding role and responsibility Many create waste products and transform it into a necessary component of human existence, including energy or cellular ‘repair’ parts. This cellular cleansing process is called *autophagy*, or *autophagocytosis* to be exact. Molecules that are no longer needed are broken down to produce energy and the system, including each individual cell, regenerate and regain their strength [STEKOVIC, 2018, p. 69].

Autophagy is a recycling process in which damaged or misfolded proteins are broken down to organelles for subsequent reuse. It also ensures general cell homeostasis and proteostasis. Thus, autrophagy removes and recycles cytoplasmic material that accumulates during the aging process (LENZEN-SCHULTE, 2016).

This process can be compared to a built-in "cleaning routine" in the cells. Everything that is no longer needed can be digested by the cell itself and immediately recycled. With age, the balance between the production and breakdown of proteins in human cells is disturbed. As a result, molecular waste, especially protein residues, can accumulate and contribute to diseases such as diabetes, parkinson's and alzheimer's. Intermittent fasting, and fasting in general, activates autophagy and thereby strengthens the immune systems ability to combat the formation of diseases in the cellular system (LENZEN-SCHULTE, 2016).

"It has been clearly documented that periods of food deprivation are able to turn on the autophagy system again. This has two advantages: on the one hand, protein material can be digested and reused. On the other hand, it ensures that defective protein molecules and defective organelles such as mitochondria are broken down from the cell. In this respect, fasting might well be a therapeutically effective means" (Yoshinori Ohsumi, 2016).

Until 2016, when Yoshinori Ohsumi, the Japanese biologist from the Tokyo Institute of Technology received the Nobel Prize for his findings in the field of autophagy, the process was solely studied within the realm of science. In the 1990s, Ohsumi used yeast to explain how the process of autophagy works, which genes are involved, and how to deactivate it. However, only recently did a greater number of scientists find interested in the subject. It was only through Ohsumi's work that the importance of this process for human health could be established (STEKOVIC, 2018, p. 74-75).

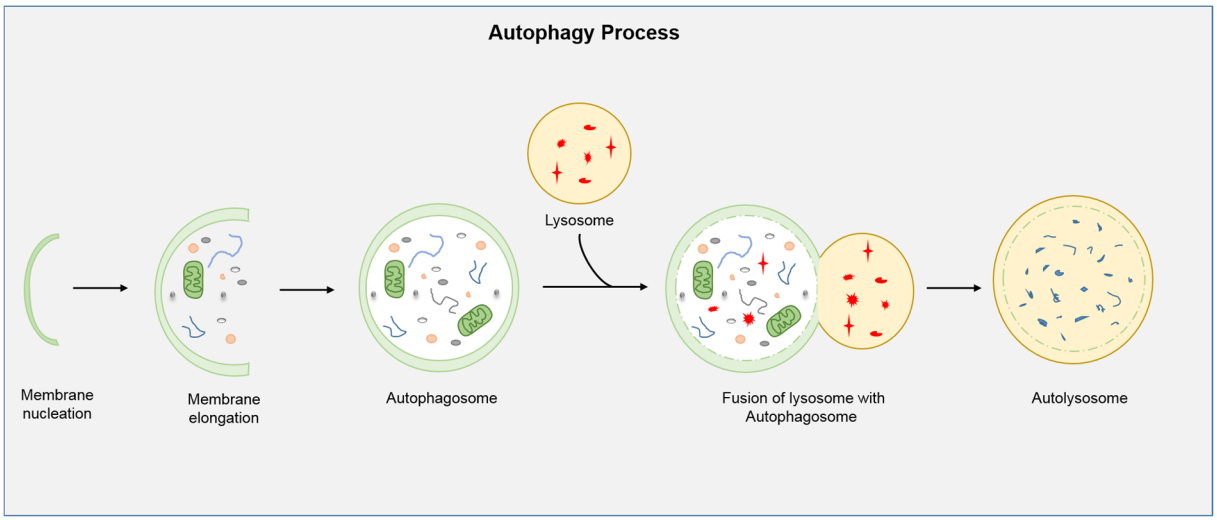

Figure 5: Autophagy Process

In this highly complex process (see Figure 5), cell components which no longer effectively perform their tasks are channeled into the interior of autophagosomes, vesicles with a double membrane that enclose proteins, lipids, membrane components and entire organelles (mitochondria) from the cytoplasm into their interior. The autophagosomes subsequently fuse with lysosomes to form autophagolysosomes, wherein the particles are deconstructed, by acid hydrolases, into their basic building blocks and made available for recycling. Ultimately, this mechanism helps maintain the balance between degradation of old cell and the production of new ones(cellular homeostasis).

Autophagy is continuously active at a basal level, but its activity is heightened when under stress. In extreme situations, for example in cases of severe cell damage, either apoptosis or autophagosomal cell death - a non-apoptotic, programmed cell death - can be induced. Autophagy is thus a mechanism for ensuring the survival of the individual cell, but at the same time also a suicide programme for damaged cells in order to ensure the ultimate goal: the survival of the multicellular organism (LENZEN-SCHULTE, 2016).

Cancer researchers have also studied the process of autophagy and its effects on cancerous cells. This applies first and foremost to tumorigenesis. For a relatively long time, autophagy was regarded a tumour-suppressor because a tumours development was associated with the body's inability to perform autophagy. For example, autophagy-deficient mice have proven to be more prone to developing tumours. Nils-Göran Larsson's et. al. (2016) description of Yoshinori Ohsumi's breakthrough study demonstrated the link between cancer and autophagy, wherein Beclin-1, which is a product of the BECN1 gene, has to potential to mutate into human breast and ovarian cancers. It is important to understand that the BECN1 gene is homologous to the yeast ATG6. This gene regulates the steps in the initiation of autophagy. This finding aroused considerable interest in researchers and scientists trying to understand the role of autophagy in the development of cancer. (LARSSON, 2016, p. 4).

Fasting and autophagy can also help in the detection of advanced stage cancer. In the late stages of cancer, autophagy is activated by the cancer cells themselves in order to eat away at the body. While this is a clever solution, it is also ultimately the cancers' undoing. The destruction of the system also affects cancer itself. When the body no longer exists, the cancer cannot exist either.

Here too, science gives hope. Some studies have suggested that when autophagy activated at the right time it can reduce the side effects of chemotherapy. Although hair loss, nausea, sleeping problems and headaches are not completely eliminated, many patients have reported fewer problems after chemotherapy when simultaneously participating in a special, medically prescribed, fasting cures. (STEKOVIC, 2018, p. 83).

Today we know that autophagy can be activated in every organism and that it most certainly has an influence on the life span, although the exact effects on individual beings may vary. The results of this have been shown in many experiments. No matter in which organism – yeast, worms, flies or mice – autophagy is activated, an increased lifespan can be expected. Even plants are capable of cell purification. The cellular metabolism is like a spider web;

cellular recycling depends on a myriad of mechanisms and explains why this question has not yet been fully clarified scientifically (STEKOVIC, 2018, p. 76-77).

Similar to cancer researchers, others are investigating whether it is possible to exert a positive influence on the cellular purification process in order to prevent the outbreak of neurodegenerative diseases. Here, too, there are positive results. To date, investigations have mainly been carried out on cell culture model systems and on transgenic mice to which foreign genetic material was transferred or in which autophagy-relevant genes were 'turned off'. Incorrectly folded proteins tend to form insoluble aggregates, which are very toxic. In fly and mouse models that have neurodegenerative diseases, the toxicity of the aggregated proteins are reduced by activating autophagy or through the inhibition of the TOR kinase. Loss of autophagy mechanism in mouse brains, by tissue-specific destruction of Atg5 and Atg7, causes neurodegeneration. Therefore, autophagy prevents the proteins from incorrectly folding in the first place, and therefore has the potential to prevent the rise of neurodegenerative diseases.

Some autosomal recessive human diseases with impaired autophagy are characterized by brain malformations, growth retardation, mental retardation, epilepsy, movement disorders and neurodegeneration (LENZEN-SCHULTE, 2016).

There is still a lot of research being done on the positive influence autophagy can have on dementia and neurodegenerative diseases in general.

3 A Review of existing studies

3.1 Studies

In recent years, increasingly more research institutions and laboratories have taken interest in the topic of fasting mimetics, secondary plant substances and their influence on the human organism. For the purpose of this thesis, I have selected four studies which study the secondary plant substances spermidine and reservatrol and analyse influence on the metabolism. In the following chapter, I will explain the rationale behind the different studies, their respective expertise, results and methodologies.

3.1.1. Spermidine in health and disease

The first study I will explore is the 2018 called 'Spermidins in health and disease' by Frank Madeo,*† Tobias Eisenberg,† Federico Pietrocola, Guido Kroemer.

Prior to exploring the study in more detail, I will briefly introduce the Research Director, Frank Madeo, who was essential for the above mentioned study, the two studies I will subsequently mention and for this current diploma thesis; his work inspired my decision to further investigate spermidine and resveratrol. Professor Dr. Frank Madeo studied biochemistry at the University of Tübingen in Germany and remained there as mentor and leader of research groups until 2004. Since 2004 Madeo has been leading various research groups at the University of Graz. His research interests range from organoprotective effects of a novel natural autophagy inducer and investigations of the role of mitochondrial aspartate transport in cancer to amyloid-beta toxicity in models of Alzheimer's disease. These are just a few examples of his contribution to science and nutrition. In recent years, his main focus has transferred to autophagy, the secondary plant constituent spermidine and how it can activate autophagy. In this context he has chosen to focus his research on yeast, which has already been successfully used as a model for cellular death and ageing research. (University of Graz, Frank Madeo)

The core question of this study was centred around how it is possible to age in health without fasting. Many people find it difficult to adopt healthier eating habits, even when they are aware of the benefits, and many do not manage to start at all. However, those that try, frequently give up after some time and fall back into old habits. The last group of people are, of course, those who are not allowed to fast due to health problems. Given that Univ.-Prof Dr.rer.nat Frank Madeo has already dealt with the fasting process and its effects on the human organism, this experience gave him a new impetus to expand his research and discover whether it would be possible to achieve similar, if not the same, effects as fasting by means of consuming the secondary plant ingredient, spermidine.

Looking at the world population today, chronic diseases such as diabetes, cardiovascular diseases, cancer and neurodegeneration are becoming increasingly more common. For these and other reasons, it is increasingly important to develop strategies to limit human diseases, and particularly those which have a broad socio-economic impact.

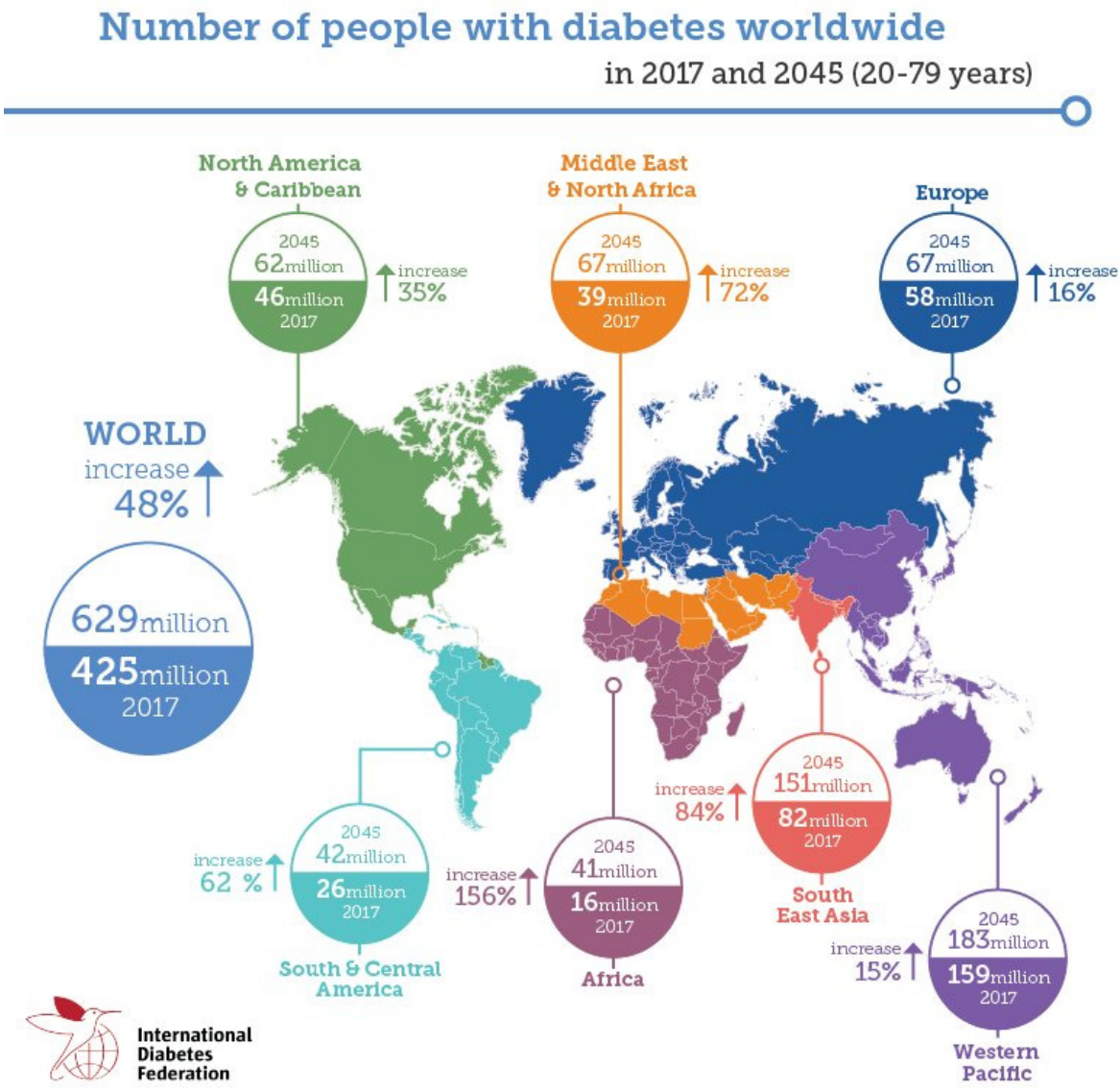

Figure 6: Increase of diabetes worldwide from 2017

Ageing is not just about getting older, but also about ensuring good health while getting older. This requires a delay in the multiple cellular and molecular changes that are responsible for the ageing progress. These changes include a loss of protein degradation capacity, which is strongly associated with dementia, epigenetic changes and genomic instability. This can lead to chronic inflammation and mitochondrial dysfunction. In order to prevent or slow down this process, the substance spermidine gains its importance.

In an autophagy-dependent environment, a consistent additional supply of spermidine can prolong the life span. It can counteract age-related pathologies such as cardiovascular diseases, neuro-degeneration and cancer. Studies conducted on flies and mice can be used as

examples. In flies, the addition of dietary spermidine reduced memory impairment related to age and in mice with multiple sclerosis, it improve the sagittarius autoimmune-guided demyelination of neurons. Spermidine has also been shown to reduce the growth of transplantable tumours, stimulates cancer in combination with chemotherapy, and suppresses tumour development caused by chemical insults in mice. In addition, the increased intake of dietary polyamine correlates with a reduction in the risk of cardiovascular and cancer related mortality rates in epidemiological studies in humans (MADEO, 2018, p.1).

On the one hand, spermidine is counted among the calorie restriction mimetics because it is dependent on autophagy and, on the other hand, it can induce protein deacylation. Since spermidine is part of the daily diet, some clinical studies aim to increase the intake of this polyamine. The use of caloric restriction mimetics (CRM), is interesting because in many countries it has already been established that caloric restriction can have a positive effect on health. That being said, calorie restrictions are not suitable for everyone. It is difficult to establish the optimal calorie restriction level in order to avoid an undersupply. In elderly or sick people, a contraindication for the calorie restriction hypothesis may occur. Therefore, it would be advantageous if the use of CRM would work and could replace a calorie restriction (MADEO, 2018, p. 2).

Since the aim of this study was to determine the effects of polyamines, especially spermidine, on the ageing process and its comorbidities, it was necessary to establish basic factors in advance. In general, polyamines have a positive effect on human health. Positive effects include cardiovascular, anti-tumour and neuroprotective effects, which can be induced via dietary changes or other external factors.

An interesting aspect is that the spermidine concentration in the human organism decreases with age. The synthesis of spermidine and sperm requires the formation of decarboxylated S-adenosyl methionine (dcSAM) from SAM. SAM is an important cofactor in the methylation of proteins, including histones and DNA (MADEO, 2018, p. 3).

In addition to cellular biosynthesis, two other factors are important for the bioavailability of spermidine: oral uptake via food plays and the production by intestinal microorganisms.

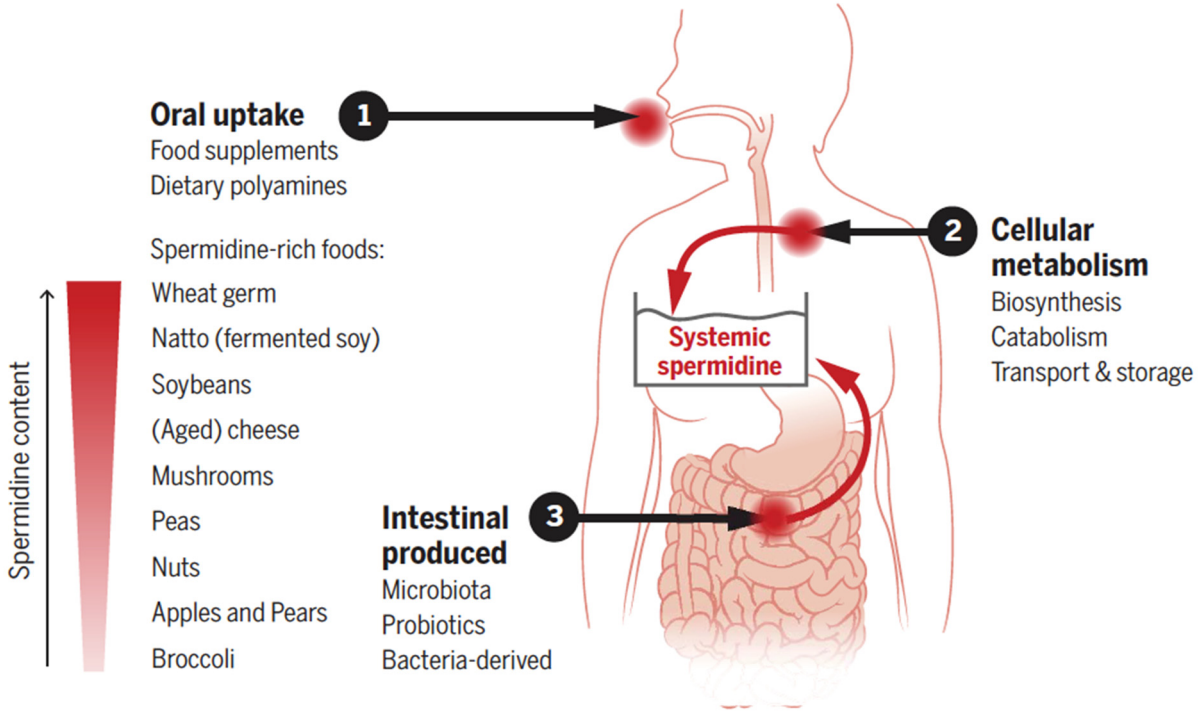

Figure 7: Scheme representing the sources crucial for the bioavailability of spermidine in the whole organism.

Certain unprocessed foods, made from plant raw materials, are naturally enriched with polyamines. Since it was found that, for example, Natto, durian fruit, fermented soybeans, and Kimchi, fermented Chinese cabbage, contain a higher proportion of polyamines, including spermidine, the food industry was able to respond to this and increase the microbial formation of polyamines by means of bacterial and fungal fermentation. On average, about 7 to 25 mg of spermidine are absorbed daily. The highest values that can be absorbed through food have been linked to the Mediterranean diet. However, these values are still below the amount of spermidine that, according to institute, Swedish Nutrition, would have to be reached in order to achieve the positive effect discussed in this thesis on the human organism.

Studies in mice have shown that oral supplementation of spermidine increases the amount of whole blood, serum and tissue. In mice, the intestinal luminal concentration of spermidine is highly dependent on the intestinal flora, and it is assumed that this is also true for the human organism. The observation of these studies is that food polyamines and polyamine producing bacteria are important for the bioavailability of spermidine.

In fact, the daily intake of 50 to 100 g Natto over a period of 2 months significantly increased the proportion of whole blood sperm in healthy human subjects (MADEO, 2018, p. 5).

Polyamines, especially spermidine, increase the lifespan of multicellular organisms. Cardiovascular diseases and cancer are the most frequent causes of death in humans and mice.

Health and life-stress promoting effects have been documented for dietary or externally supplied spermidine. Spermidine supplements has the potential to extend the lifespan of both invertebrate model organisms and mice. In addition, a polyamine-rich diet reduces the mortality of elderly mice due to external circumstances. As with many other CRMs, spermidine extends the lifespan in a gender-independent manner (MADEO, 2018, p. 6).

Spermidine supplementation reduces the growth of transplantable tumours in mice treated with chemotherapy. This spermidine effect is shared by other CRMs and fasting or hypocaloric diets and is mediated by stimulation of immune surveillance (MADEO, 2018, p. 7).

A spermidine-enhanced autophagy is necessary for several health-promoting effects. Spermidine also suppresses proinflammatory cytokines and improves the bioavailability of arginine, which is required for nitric oxide (NO) biosynthesis and mediates immunomodulatory and antihypertensive effects.

Dietary spermidine protects against cardiac ageing. In elderly mice, it improves the diastolic function, left ventricular elasticity and mitochondrial function. The first results obtained from epidemiological studies confirm the same effects in humans. Uptake of dietary spermidine inversely correlates with the number of cardiovascular disease incidents (MADEO, 2018, p. 8).

By means of autophagy, spermidine is able to suppress tumour formation in healthy cells. This also promotes immune surveillance. The anti-inflammatory effect of spermidine is explained by its action on macrophages. This effect promotes M2 polarization and the suppression of NFkB-dependent proinflammatory cytokines. These macrophages can then suppress the autoimmune reactive T-cells. At the same time, spermidine stimulates the formation of CD8 memory T-cells by inducing autophagy. Spermidine-enhanced autophagy and mitophagy support the elasticity of cardiomyocytes and mitochondrial functions.

Since spermidine has an inhibitory effect on the autoimmune T-cells, demyelination leads to the prevention of neurodegeneration.

Autophagy, in particular mitophagy, plays a fundamental role in maintaining brain function and preventing neurodegeneration.

Furthermore, in flies, it has been found that spermidine supplementation can help to prevent age-related memory disorders and that the flies remain active for longer periods of time. However, these effects are only achievable once autophagy is activated (MADEO, 2018, p. 10).

Overall, these evaluations point to a broad spectrum of neuroprotective effects of externally supplied spermidine, which are relevant for various neurodegenerative movement disorders and types of dementia. In mice, the acute application of spermidine (within a few hours) triggers autophagy in vivo in many tissues such as heart, liver and muscles (4 to 24 hours). Similarly, spermidine induces major changes in the metabolome of the plasma, heart, skeletal muscle and liver similar to those induced by other CRMs or fasting, which is a common phenomena in pharmacological and nutritional interventions. Oral supplementation of spermidine in drinking water induces autophagy in cardiac tissue two to four weeks after intake and reverses the age-related decline in autophagy in the aorta (other tissues still need to be tested) (MADEO, 2018, p. 11).

One aspect which has made current cancer treatment options, include chemotherapy, this successful is the fact that doctors work with an immune-cell-dependent removal of tumor cells. Autophagy enables cancer cells to adapt to internal cell stress (MADEO, 2018, p. 12).

Autophagy facilitates the release of adenosine 5- triphosphate (ATP), which helps to promote the immune systems' recognition of cancer cells. In cells from older donors, it has been observed that when autogaphy is impaired, T-cell function decreases with age.

In elderly mice, CD8+ T-cell responses to influenza vaccination are restored in an autophagy dependent manner by oral spermidine treatment, suggesting an essential role of autophagy and spermidine in memory cell formation.

Dietary spermidine suppresses the age-associated chronic inferior increase in plasma concentration of tumor necrosis factor-a (TNF-a) and other cytokines in mice and inhibits fatal sepsis (MADEO, 2018, p. 13).

In addition, an association between a disturbed polyamine, an arginine metabolism and Alzheimer's disease could be observed in mouse models. Mild cognitive impairments were observed in similar studies with human patients. However, it must also be considered that it is not sufficient to solely measure the level of polyamine spermidine in blood and tissue samples as a biomarker for a general state of health or even for specific diseases (MADEO, 2018, p. 15).

Of course, it is also possible that metabolic pathways are linked by disease-related changes and that this then leads to an increase in polyamine biosynthesis. The complexity of age-associated effects on polyamine metabolism is further enhanced by multi-layered and region-specific changes in the polyamine content of memory-associated brain structures.

Spermidine supplementation prolongs the life- and health- span by protecting against a series of age-related pathologies in differentiated animal models. Although spermidine induces

autophagy and autophagy inhibition shortens the majority of effects of spermidine, additional mechanisms have also been implicated in the health-promoting effects of this polyamine. These potentially autophagy-independent mechanisms include direct antioxidant and metabolic effects on polyamine and its corresponding metabolic pathways, especially the increased bioavailability of arginine and NO production. (MADEO, 2018, p. 16)

3.1.2. Higher spermidine intake is linked to lower mortality a prospective population-based study

The second study I would like to present in this paper, also from 2018, is entitled: 'Higher spermidine intake is linked to lower mortality a prospective population-based study' by Stefan Kiechl, Raimund Pechlaner, Peter Willeit, Marlene Notdurfter, Bernhard Paulweber, Karin Willeit, Philipp Werner, Christoph Ruckenstuhl, Bernhard Iglseder, Siegfried Weger, Barbara Mairhofer, Markus Gartner, Ludmilla Kedenko, Monika Chmelikova, Slaven Stekovic, Hermann Stuppner, Friedrich Oberhollenzer, Guido Kroemer, Manuel Mayr, Tobias Eisenberg, Herbert Tilg, Frank Madeo and Johann Willeit.

The study was conducted based on the knowledge that, in multiple animal models, the administration of spermidine can be associated with a prolonged life span. Given that, to date, the only results stem from animal models, the aim of this study was to investigate the possible relationship between the intake of spermidine in food and the mortality rate and age in humans.

The study analysed 829 participants, aged 45 to 84. It was a prospective community-based cohort study, with 50.1% of participants being women and 49.9% being men. The study was conducted for over 20 years, with routine control checks and questionnaires conducted every 5 years by nutritionists. Food frequencies were validated and a total of 2540 assessments were made in 1995, 2000, 2005 and 2010. From the 829 participants, a total of 341 deaths were recorded between 1995 and 2015 (KIECHL, 2018, p. 371).

In the following paragraph, the method and participants are described in more detail.

The study was conducted in Brunico, a province in Bolzano, Italy, and also the reason for its name: the Bruneck Study. It is a prospective and population-based cohort study in which the population was surveyed in 1990 by an age- and gender-stratified random sample and with re-evaluations planned every five years. The participants were well suited for this study because the annual population mobility was rather low, at 0.2%, and a follow-up rate of more than

90% could be achieved. Another positive aspect was that each participant released their complete medical file for inspection.

The Salzburg Atherosclerosis Prevention Program served as the prospective replication cohort, which included a total of 1770 participants, aged 39 to 67. These studies were conducted between 1999 and 2002 with a follow-up period until September 2013 to confirm the medical status and causes of death. The participants were recruited during a health screening program of large companies. The SAPHIR study included 663 women and 1107 men.

As already mentioned, in the Bruneck study, every 5 years an evaluation was performed by nutritionists. The food intake was assessed using a 118-point food frequency questionnaires (FFQ). For each point in the FFQ a common unit was determined or a portion size was set. Participants were instructed in this system and had to give an average for the previous years in order to determine the nutrient intake (KIECHL, 2018, p. 372 & 373).

In order to accurately estimate the food intake, complex foods were broken down into their individual components and uniform recipes were created. The participants were also given an open section in the FFQ to enter the foods that were not taken into account.

A focal point of the study was, of course, the basal intake of spermidine. The polyamine intake was estimated and calories were adjusted. For this purpose, the residues obtained by regression of the polyamine intake to the total energy intake were used. Furthermore, the ratio of caloric intake to energy expenditure was calculated and called the *caloric ratio*. This was one of the few nutritional characteristics that could be linked to the health and life span of a person.

The Bruneck study collected detailed information on the date, causes and circumstances surrounding the death of all participants. Of these, only one did not survive the follow-up period. (KIECHL, 2018, p. 373)

The following section reflects and summarizes the observations and results. In principle, spermidine intake changed regardless of the season, but the intake was higher in women than in men, and with an increase in age, spermidine intake decreased regardless of gender. The spermidine share was 26% of the total polyamine intake. The different sources of spermidine were identified. The foods with the highest spermidine content were: whole grain with 13.4% of the total amount of spermidine, apples and pears with 13.3%, salad with 9.8%, vegetable sprouts with 7.3% and potatoes with 6.4%.

A total of 341 deaths were recorded during the Bruneck study. Of these, 137 were caused by vascular diseases, 94 by cancer and 110 by other causes. However, the overall rates of death decreased significantly in all groups as spermidine intake increased (KIECHL, 2018, p. 374).

Since the SAPHIR study was used for comparison, the spermidine intake data was also analysed. On the one hand, the intake of spermidine was quantitatively similar to the Bruneck study. On the other hand, the distribution among the type of foods was different. In the SAPHIR study, the largest proportional intake of spermidine was through fruits (24.1%), salad (10.8%) and potatoes (6.6%). The percentage of whole grain cereals was much lower with only 6.5% but the percentage for other vegetables and salad was 30.5%. Eighty-four participants died in total: seven from vascular diseases, 29 from cancer and 12 from other causes.

Both interventions similarly reduced the acetylation of several cellular proteins including histones (and thus the transcription programs) and cytoplasmic enzymes (and thus the metabolic functions). These processes are critical to homeostasis of cells during ageing and starvation, and effectively induced autophagy, a cytoprotective self-digestion process and key to longevity (KIECHL, 2018, p. 376-377).

The advantages of the Bruneck study are that it is a population-based design which was observed over a long period, 20 years, and has been validated time and again by nutritionists. The causes of death were carefully identified and compared with an independent cohort study (KIECHL, 2018, p. 377).

Ultimately, this study supports the assumption that a spermidine-rich diet could prolong the human life span. This intervention provided the first evidence that there may be a link between spermidine intake and increased survival in humans. The data acquired in the Bruneck study complement the data previously obtained in animal models with worms, flies and mice and other organisms, including yeast and human immune cells. That being said, it is paramount to critically review each and every study and research, particularly when working with people, who may subconsciously be subjective (KIECHL, 2018, p.371 & 378).

3.1.3. Spermidine and resveratrol induce autophagy by distinct pathways converging on the acetylproteome

The third study I would like to present in this paper, from 2011, is entitled: ‘Spermidine and resveratrol induce autophagy by distinct pathways converging on the acetylproteome’ by Eugenia Morselli, Guillermo Mariño, Martin V. Bennetzen, Tobias Eisenberg, Evgenia Megalou, Sabrina Schroeder, Sandra Cabrera, Paule Bénit, Pierre Rustin, Alfredo Criollo,

Oliver Kepp, Lorenzo Galluzzi, Shensi Shen, Shoaib Ahmad Malik, Maria Chiara Maiuri, Yoshiyuki Horio, Carlos López-Otín, Jens S. Andersen, Nektarios Tavernarakis, Frank Madeo and Guido Kroemer.

The study is based on the knowledge that autophagy protects organelles and cells against different stress conditions. The aim was to find out whether it is possible to induce autophagy by feeding the two secondary plant substances, resveratrol and spermidine, as well as by fasting.

If one wants to induce autophagy with resveratrol, the nicotinamide-adenine dinucleotide dependent deacetylase sirtuin 1 (SIRT1) is needed. In this study it was shown that, in contrast to resveratro, the acetylase inhibitor stimulates spermidine autophagy independently of SIRT1 in human and yeast cells as well as in nematodes (MORSELLI, 2011, p.615 & 616).

Sirtuin 1 (SIRT1) is a NAD+-dependent deacetylase. SIRT1 has sufficient transfection expression to stimulate autophagy in human cells. SIRT1 is needed for the hunger-induced autophagy, in other words through the lack of a food supply.

In this research, it was found that spermidine and resveratrol initially undergo different processes, but as the process progresses, the pathways to induce autophagy become unified. This breakthrough has shown that both substances can synergistically stimulate autophagy in vivo in the mouse as well as in vitro in human cells (MORSELLI, 2011, p. 616).

An important fact that could be established by this study was that both substances were comparable in their autophagy stimulating power in relation to human colon cancer HCT 116 cells. Furthermore, it was found that spermidine and resveratrol can trigger autophagy, but through different mechanisms. To investigate the signal transduction pathway of spermidine and resveratrol, the phosphorylation status of several cellular proteins in human colorectal cancer HCT 116 cells was analyzed. Surprisingly, spermidine and resveratrol, alone or in combination, triggered similar changes in the phosphorylation status (MORSELLI, 2011, p. 617).

Since this polyamine and polyphenol does not influence the phosphorylation of the mechanistic target of rapamycin (mTOR), nor that of the ribosomal protein S6 kinase associated with substrate, it is assumed that both substances induce autophagy by AMP-dependent kinase/mTOR-independently. Therefore, a total 100 µM dose of the two substances did not lead to higher autophagy values whereas, if administered alone, they would have.

The next step was to investigate the effects on protein acetylation patterns. Surprisingly, the acetylation status of 170 proteins was modified in response to treatment with resveratrol and

spermidine. Among others, they also form part of the human autophagy protein network.

Both resveratrol and spermidine tended to induce the formation of (de)acetylation of similar proteins, including those relevant to autophagy, such as the substrates ATG5 and LC3 (MORSELLI, 2011, p. 617-620).

It was assumed that even low doses of spermidine and resveratrol, by influencing the balance of acetylation, would be sufficient to induce autophagy. For this reason, HCT 116 cells were tested with different doses and combinations of the two secondary plant compounds to see how this affects the induction of autophagy.

Interestingly enough, in low doses (10 μM) the substances could individually not achieve a high autophagous flux, but in combination they did.

As previously mentioned, resveratrol can only induce autophagy if SIRT1 is also present. Unlike spermidine, which can induce autophagy without SIRT1. Despite the difference in the primary targets of resveratrol and spermidine, both agents activated convergent pathways by stimulating mTOR-independent autophagy and inducing fairly similar changes in the phosphoproteome and, more importantly, the acetylproteome. In tests, resveratrol and spermidine modulated the acetylation of over 100 proteins. Most of these proteins were part of the central network of regulators and executors of autophagy. Thus, it was assumed that the two substances can stimulate autophagy via several different but simultaneous mechanisms involving an enormous number of (de)acetylation reactions. Assuming that resveratrol needs SIRT1 to induce autophagy, and spermidine does so independently of SIRT1, it seems paradoxical that a similar shift in the acetylation pattern stimulated hundreds of proteins, more in the cytosol than in the nucleus, to be deacetylated (MORSELLI, 2011, p. 620 & 621).

In these experiments, it was observed that spermidine, but also resveratrol, stimulated the deacetylation of cytosolic proteins, including ATG5 and LC3, and the acetylation of nuclear proteins, including multiple histones. This data shows that one-tenth of the dose of spermidine or resveratrol can produce an optimally stimulated autophagy. Thus, a higher proautophageal effect can be achieved, which means that the dose-response curve is very steep. It is assumed that this is caused by the compensatory reactions that trigger the homeostasis of the acetylproteome. The partial and somehow simultaneous activation of the deacetylase activity of SIRT1 by resveratrol, and the simultaneous inhibition of acetylases by spermidine, can bring the acetyl proteome out of balance and thus synergistically stimulate autophagy.

One should be aware that it is almost impossible to ingest such high concentrations of spermidine and resveratrol with a normal diet to achieve a pharmacological effect. However, it is a tempting prospect to see that these two substances, together, can induce autophagy in

the human organism and thus help our body to prevent diseases (MORSELLI, 2011, p. 621-628).

3.1.4. Resveratrol induces autophagy by directly inhibiting mTOR through ATP competition

The last study I would like to present in this paper, from 2016, is entitled: 'Resveratrol induces autophagy by directly inhibiting mTOR through ATP competition' by Dohyun Park, Heeyoon Jeong, Mi Nam Lee, Ara Koh, Ohman Kwon, Yong Ryoul Yang, Jungeun Noh, Pann-Ghill Suh, Hwangseo Park and Sung Ho Ryu.

This study focused on the positive health effects of the natural polyphenol, resveratrol. A key process of the positive effects of this polyphenol is the resveratrol induced autophagy, which can lead to a reduction of inflammation or even induction of cancer cell death. Another ambition for this research was to find out which molecule transmits resveratrol induced autophagy. Through this research, it could be determined that the direct inhibition of the mTOR-ULK1 signalling pathway takes place and that this is partly responsible for resveratrol-induced autophagy. It was found that the inhibition of mTOR activity and the presence of ULK1 is indispensable for resveratrol-induced autophagy. Furthermore, it was found that cancer cell suppression by resveratrol is dependent on ULK1. Furthermore, resveratrol was found to inhibit mTOR by docking to the ATP binding pocket of mTOR. This led to the assumption that mTOR is a direct target of resveratrol and that the inhibition of mTOR is necessary for the induction of autophagy.

mTOR functions within two different complexes, namely, mTORC1 (mTOR complex 1) and mTORC2 (mTOR complex 2). These two complexes contain different adaptors and scaffolds for different functions and regulatory mechanisms. Among other things, they also share mTOR as a kinase subunit. mTOR complex 1, mTORC1, is responsible for the control of cell growth and the regulation of different processes of cell proliferation. In addition, mTORC1 also suppresses catabolism by inhibiting autophagy. Inhibition of mTORC1 is sufficient to induce autophagy, and nutrient-insensitive mTOR makes cells insensitive to the autophagy caused by hunger. Active mTOR can inhibit autophagy (PARK, 2016, p.1 & 2).

Resveratrol has been shown in various studies to suppress mTOR activity. SIRT1 is one of the regulators involved in this inhibition mechanism. However, the contradictory results between these reports regarding the requirement of the above-mentioned regulators for resveratrol-

induced mTOR suppression lead to ambiguities. Thus, this study has shown that resveratrol induces autophagy via the mTOR-ULK1 signalling pathway.

This study proved that resveratrol induces autophagy by mTOR inhibition. Furthermore, it was found that mTORC1 activity is inversely correlated with the degree of autophagy. It is therefore assumed that mTORC1 is significantly involved in resveratrol induced autophagy. Another important aspect of this research was the involvement of ULK1 in the induction of autophagy by resveratrol. ULK1 is required for resveratrol induced autophagy. mTOR regulates autophagy by inhibiting phosphorylation of ULK1, and the inhibition of mTOR lowers the inhibitory phosphorylation level of ULK1 and increases autophagy. Therefore, to confirm the involvement of mTOR in resveratrol induced autophagy, and to study the dependence on ULK1, they analyzed the degree of resveratrol induced autophagy in the presence or absence of ULK1 (PARK, 2016, p. 2).

They concluded that resveratrol induces autophagy via the mTOR-ULK1 signaling pathway, which involves the inhibition of mTOR. Resveratrol reduces the viability of mTOR inhibition-sensitive cancer cells in an ULK1-dependent manner. Testing of various cancer cells showed that the resveratrol effect on the viability of cancer cells is largely dependent on the effect of mTOR, which varies according to the type of cancer cell. As a further step, it was investigated whether ULK1 is involved in the suppression of cancer cell viability. Since the investigations showed that ULK1 knockdown leads to a partial restoration of cell viability, and a reduction of resveratrol autophagy induction, it supports the idea that resveratrol induced cellular behavioral changes occur through the mTOR-ULK1 signalling pathway (PARK, 2016, p. 2 & 3).

Another question was whether resveratrol also inhibits mTOR kinase activity in cell-free systems. Investigations showed that this is, in fact, the case. Thus, it is assumed that this inhibition is due to specific binding in the ATP binding site. Resveratrol inhibits mTOR through ATP competition. To verify the simulation results, an in vitro kinase test was performed with different concentrations of ATP (PARK, 2016, p. 4).

Based on these studies, the authors were able to determine that mTOR is a direct target of resveratrol. This assertion is supported by the observation that mTOR activity is inhibited in vitro by the addition of resveratrol. Furthermore, this study provides an explanation for the positive effects of resveratrol. These include the induction of autophagy in response to mTOR and that the viability of cancer cells can be reduced. For the medical sector, it opens up several possibilities to combat diseases associated with highly active mTOR and, of particular interest are neurodegenerative and diabetes related diseases. (PARK, 2016, p. 6)

Resveratrol induces autophagy in various cell lines and in model organisms such as mice. In these reports, the authors showed that the induction of autophagy is necessary for the death of cancer cells and the alleviation of inflammation, and that it may even prolong life. Some studies have shown reduced mTOR activity along with autophagy induction through a resveratrol treatment. However, there is no direct evidence that mTOR is a mediator of autophagy inducted by resveratrol. In this study, using a combinatorial chemical treatment and ULK1 knockdown, it was clearly demonstrated that the mTOR-ULK1 pathway is necessary for autophagy inducted by resveratrol. Further studies are necessary to investigate the involvement of mTOR in autophagy inducted by other natural polyphenols. Inhibition of mTOR have been shown to be a good strategy in the treatment of various diseases such as cancer, neurodegenerative diseases and diabetes. The study conducted by D. Park, will provide a basis for identifying unknown mTOR-based effects of resveratrol (PARK, 2016, p.8).

4 Conclusion

Returning to the hypotheses and questions, I wrote this diploma thesis with the intention to discover whether spermidine or resveratrol, or both secondary plant compounds, are able to replace fasting and induce the process of autophagy.

The Japanese cell biologist, Yoshinori Ohsumi, has already proven that dietary deficiencies can activate and induce autophagy. His research and contribution awarded him the 2016 Nobel Prize in Medicine. It has also been proven that autophagy can be of great advantage for the human organism because it has a preventive effect related to various diseases and particularly to neurodegenerative diseases, including Alzheimer's, in which proteins are deposited in the brain. Autophagy, from the onset, can prevent the deposition of these proteins. In addition, this process — whereby old or damaged cells are recycled and used to build new and healthy cells — can also help with diabetes, cardiovascular diseases and obesity. However, it is important to mention that this is not a panacea, but should rather be taken as a prevention than an acute treatment option. Initially, it may sound as though everyone should start fasting in order to live long and healthy lives. However, a catch remains. Many people find it very difficult to change their eating habits and other groups, such as the elderly or those with contraindications, are not allowed to fast from a medical standpoint. This is one of the main reasons why this topic is so interesting and important to research.

Can secondary plant compounds, such as spermidine and resveratrol, replace fasting with the same or similar effects on the human organism?

This paper summarized different studies with similar aims and explained their main outcomes. Especially in the field of nutrition, and in relation to humans, there may be some pitfalls that should be taken into account when considering the results of these studies. It has been clearly demonstrated that both spermidine and resveratrol can induce autophagy in yeast cells and other organisms such as flies, worms and mice. Yeast has been successfully used as a model for cell death and ageing research. Yeast cells undergo apoptosis in response to ageing, oxidative stress, starvation and expression of pro-apoptotic mammalian proteins. It has been elucidated that autophagy serves as an important cytoprotective pathway in yeast that can induce both apoptotic and necrotic death. In addition, it has also been found that the natural autophagy enhancer, spermidine, prolongs the lifespan of yeasts, flies, worms, and the health of mice. But spermidine not only prolonged the life span; it has also been discovered that the additional intake of dietary spermidine reduced age-related memory impairment in flies and had a positive effect on multiple sclerosis in mice. Additional observations can be made in relation to cancer research. On the one hand, in mice, it was observed that spermidine in combination with chemotherapy can negatively impact the growth of cancer and also manages to reduce the growth of tumours caused by chemical inductions or transplantations in mice. Furthermore, other mice studies showed that, when spermidine is supplemented orally, the amount of whole blood, serum and tissue increases. This suggests that the intestinal luminal concentration of spermidine in mice is strongly dependent on the intestinal flora and it is assumed that this also applies to the human organism. However, not only in mice, but also in epidemiological studies conducted on humans outstanding results have been obtained. For example, an increased dietary intake of spermidine was associated with a reduction in cardiovascular and cancer risk mortality.

With regard to spermidine, the Bruneck study was also conducted over 20 years and included the participation of an entire village in South Tyrol. To date, no other research on the supply of spermidine has been conducted under such a long period of time and with the inclusion of humans. However, studies must always be viewed critically. Data is not always complete or wholly accurate, often because of unintentional human errors.

This leads us to the natural polyphenol, resveratrol. This secondary plant ingredient is said to have many different positive effects on the human organism and it is therefore important to prove these effects. In the two studies, one from Eugenia Morselli from 2011 and the other one from Dohyun Park from 2016, that I have presented, clear evidence was found that

resveratrol can have preventative effects on various diseases. One of the key processes of this natural polyphenol is resveratrol induced autophagy. This process can lead to a reduction in inflammation or even kill cancer cells. While various tests on different cancer cells have shown that the supply of resveratrol via mTOR has a direct effect on the viability of cancer cells, the strength largely depends on the type of cancer cell. This is very relevant for cancer research and could lead to future breakthroughs.

Arguably one of the most relevant findings of resveratrol is that it seems that mTOR is a direct target of resveratrol. This means that resveratrol is able to inhibit the activity of mTOR. This is important because mTOR activity has been linked to many diseases. These include neurodegenerative diseases, such as dementia and Alzheimer's, and diabetes diseases such as diabetes mellitus type 2, which are becoming increasingly more common worldwide and are contributing to rising global medical costs.

Finally, I would like to discuss, in more detail, the results of the combination of spermidine and resveratrol. In the one study that I chose, both spermidine and resveratrol were evaluated for their properties and effects on autophagy and for the way in which they possibly effect the growth and development of different diseases. Interestingly this research demonstrated that both spermidine and resveratrol induce autophagy. However, in order to initiate this induction at the beginning of the pathway, different mechanisms are used and the two substances meet during the process and and autogaphy is initated. This discovery has shown that the two substances can stimulate autophagy in a synergistic way in vivo in mice as well as human cells. It was also exciting to observe that both compounds were able to induce similarly strong autophagy in human colon cancer cells. In order to determine the optimal intensity of autophagy on these colon cancer cells, different doses of spermidine and resveratrol alone, or in combination, were tested. Relatively surprising was the fact that, at low doses, these substances alone did not achieve a high.

Bibliographie

- Abraham, J., & Johnson, R. W. (2009). Consuming a diet supplemented with resveratrol reduced infection-related neuroinflammation and deficits in working memory in aged mice. *Rejuvenation research*, *12*(6), 445-453.

- Alhazzazi, T. Y., Kamarajan, P., Verdin, E., & Kapila, Y. L. (2011). SIRT3 and cancer: tumor promoter or suppressor?. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, *1816*(1), 80-88.BAUR, Joseph A., et al. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature*, 2006, 444. Jg., Nr. 7117, S. 337-342.

- Bober, E., Can Sirtuins combat aging?, Max-Planck-Institut für Herz- und Lungenforschung, Bad Nauheim. Forschungsbericht (2007). (to be found: https://www.mpg.de/319215/forschungsSchwerpunkt; requested on 29.7.2019 at 14:23).

- de Toledo, F. W., Buchinger, A., Burggrabe, H., Gaisbauer, M., Hölz, G., Kronsteiner, W., ... & May, W. (2002). Leitlinien zur Fastentherapie. *Complementary Medicine Research*, *9*(3), 189-198.

- Dewald, L. (2019), Effekte von sekundären Pflanzeninhaltsstoffen auf die zelluläre Seneszenz und den Entzündungsmarker IL-6. Vienna.

- Deutsche Gesellschaft für Ernährung (DGE) (2012): Sekundäre Pflanzenstoffe und ihre Wirkung auf die Gesundheit. (to be found: https://www.dge.de/wissenschaft/weitere-publikationen/fachinformationen/sekundaere-pflanzenstoffe-und-ihre-wirkung/; requested on 2.2.2020 at 17:37).

- Kast, B. (2018). Der Ernährungskompass: das Fazit aller wissenschaftlichen Studien zum Thema Ernährung. C. Bertelsmann Verlag.

- Kiechl, S., Pechlaner, R., Willeit, P., Notdurfter, M., Paulweber, B., Willeit, K., ... & Mairhofer, B. (2018). Higher spermidine intake is linked to lower mortality: a prospective population-based study. *The American journal of clinical nutrition*, *108*(2), 371-380.

- Larsson, N. G., & Masucci, M. G. (2016). Scientific background discoveries of mechanisms for autophagy. *Nobel Prize*. (to be found: http://www.nobelprizemedicine.org/wp-content/uploads/2016/10/Scientific-Background-2016.pdf; requetsted on 20.05.2018 at 20:34).

- Lenzen-Schulte, M., & Zylka-Menhorn, V. (2016). Autophagie: „Selbstverstümmelung" als Überlebensstrategie, in: Deutsches Ärzteblatt, Jg. 113, Heft 40, 7.10.2016, S. A 1740.

- Lilja, S., Effects of fasting, fasting mimetics and metabolites of microbiota on SIRT pathway/epigenetic mechanisms in metabolic syndrome. Vienna (2019).

- Madeo, F., Eisenberg, T., Pietrocola, F., & Kroemer, G. (2018). Spermidine in health and disease. *Science*, *359*(6374), eaan2788.

- Morselli, E., Mariño, G., Bennetzen, M. V., Eisenberg, T., Megalou, E., Schroeder, S., ... & Kepp, O. (2011). Spermidine and resveratrol induce autophagy by distinct pathways converging on the acetylproteome. *Journal of Cell Biology*, *192*(4), 615-629.

- Park, D., Jeong, H., Lee, M. N., Koh, A., Kwon, O., Yang, Y. R., ... & Ryu, S. H. (2016). Resveratrol induces autophagy by directly inhibiting mTOR through ATP competition. *Scientific reports*, *6*(1), 1-11.

- Piwpong, R. (2018). Systematic Review on Anti-Aging Health Care. *Naresuan University Journal: Science and Technology (NUJST)*, *26*(3), 98-112.

- Qiu, X., Brown, K. V., Moran, Y., & Chen, D. (2010). Sirtuin regulation in calorie restriction. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, *1804*(8), 1576-1583.

- Rauh, D., Fischer, F., Gertz, M., Lakshminarasimhan, M., Bergbrede, T., Aladini, F., ... & Steegborn, C. (2013). An acetylome peptide microarray reveals specificities and deacetylation substrates for all human sirtuin isoforms. *Nature communications*, *4*(1), 1-10.

- Schubmann, R. (2009). Therapeutisches Fasten bei Adipositas und metabolischem Syndrom. *Zeitschrift für Komplementärmedizin*, *1*(03), 14-17.

- Schek, A. (2002). Sekundäre Pflanzenstoffe. *Sporternährung*, *5*, 44-52. (tob e found: https://www.researchgate.net/profile/Alexandra_Schek/publication/301328409_Sekundare_Pflanzenstoffe/links/5712242108aeff315ba0cf8c.pdf; requested on 3.2.2020 at 8:14).

- Sinclair, D. A. & Guarente, L. (2006): *Schlüssel zur Langlebigkeit.* In: *Spektrum der Wissenschaft.* 34–41.

- Steinmüller, R. (2015). Biogene Amine in Lebensmitteln. (to be found: https://d-nb.info/1081094796/34; requested on 4.2.2020 at 12:17)

- Stekovic, S. (2018) Der Jungzelleneffekt. Wie wir die Regenerationskraft unseres Organismus aktivieren. Vienna.

- Stervbo, U., Vang, O., & Bonnesen, C. (2007). A review of the content of the putative chemopreventive phytoalexin resveratrol in red wine. *Food Chemistry*, *101*(2), 449-457.(to be found: doi:10.1016/j.foodchem.2006.01.047; requested on 7.2.2020 at 16:03)

- Takaoka, M. (1939). Resveratrol, a new phenolic compound, from Veratrum grandiflorum. *Nippon Kagaku Kaishi*, *60*, 1090-1100.

- Watzl, B., & Rechkemmer, G. (2008). Einfluss sekundärer Pflanzenstoffe auf die Gesundheit. *Deutsche Gesellschaft für Ernährung: Bonn, Germany*, 335-379.

Statement

I hereby declare,

- that I have authored the present master thesis independently, did not use any sources other than those indicated, and did not receive any unauthorized assistance,
- that I have not previously submitted this master thesis topic in any form, in this country or any other, for academic credit,
- and that this work is identical to the one assessed by the registered supervisor.

Wien / Vienna Datum / Date:

Name (Nachname, Vorname) / Name (Last name, First name):

Unterschrift / Signature: