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Biochemie účinků polyfenolických látek v léčbě vaskulárních onemocnění.

Biochemistry of polyphenols effects in the treatment of vascular disease

Disertační práce

Ph.D. Thesis

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Souhrn

Tato disertační práce se zabývá analýzou učinků polyfenolických látek ve vztahu k oxidativnímu stresu a kardiovaskulárním onemocněním. Práce obsahuje řadu výsledků studia účinků přírodních biologicky aktivních látek červených vín a brambor, a jejich aplikace na modelech spontánní hypertenzních a normotezních experimentálních zvířat.

Metody a přístroje použité v experimentech nám umožnily formulovat několik nových výroků o univerzálním charakteru vztahů mezi antioxidační kapacitou a obsahem polyfenolických látek bez ohledu na zdroj potravin.

Strava je zdrojem minerálních látek, které rovněž přispívají k celkové antioxidační kapacitě, a proto také mohou mít vliv na endogenní antioxidační enzymatický systém tím, že poskytuje základní kofaktory. Experimentálně bylo zjištěno, že koncentrace hořčíku ve víně a extraktu červeného vína známého pro jeho léčebné účinky jsou srovnatelné s minerálními vodami doporučenými pro léčbu a prevenci kardiovaskulárních a metabolických onemocnění. Kromě toho v řadě našich experimentů byly potvrzeny synergické vztahy mezi vybranými minerály.

Klíčová otázka biologické dostupnosti polyfenolů na podporu myšlenky prospěšnosti stravy bohaté na ovoce a zeleninu v prevenci cévních onemocnění byla ověřena na modelech laboratorních zvířat. Polyfenolické sloučeniny byly detekovány v plazmě v koncentraci několikanásobně vyšší u pokusných zvířat léčených polyfenoly ve srovnání s kontrolními skupinami. To vše nám umožňuje dospět k závěru, že tyto látky cirkulují v krvi pokusných potkanů a mohou způsobit pozitivní účinky. Experimentální výsledky potvrdily, že aktivita jednoho z hlavních antioxidačních enzymů superoxid dismutázy a NO syntázy, klíčového enzymu pro udržení normálního cévního tonu, byly zvýšeny ve skupině pokusných zvířat se spontánní hypertenzí, léčených extraktem s vysokým obsahem polyfenolů.

Klíčová slova: polyfenoly, ateroskleróza, červené víno, brambory, NOS, SOD, obsah polyfenolů, antioxidační aktivita, stopové prvky.

Summary

This Ph.D. thesis deals with the deep analysis of polyphenols effects toward vascular disorders. This work provides a number of experimental results of studying both the effects of natural bioactive compounds in red wines and potatoes, and their application to the experiments which includes experimental animal models with spontaneous hypertension.

Methods and equipment used in experimental studies allowed us to make several new statements regarding the universal nature of the relationships between the antioxidant capacity and the polyphenolic content in examined foodstuffs.

Besides this it is also recognized that food is a source of minerals which also contribute to the total antioxidant capacity and therefore may have influence the endogenous antioxidant enzyme system by providing the essential cofactors. Experimentally we have found that the concentrations of magnesium known for its therapeutic action in wine and red wine extract are comparable to the mineral waters recommended for the treatment and prevention of cardiovascular and metabolic diseases. Moreover, synergistic interactions between selected minerals have been found.

The key issue of bioavailability of polyphenols for supporting the idea of the beneficial effects of diet rich in fruits and vegetables toward vascular disease prevention was verified in laboratory animal models. Polyphenolic compounds were detected in plasma in concentrations of several times higher in experimental animals treated with the polyphenols compared to control groups. This enables us to conclude, that these compounds circulate in the blood of experimental rats and may exert positive effects toward vessels. Moreover, our experimental findings have confirmed that activity of one of the main antioxidant enzyme superoxide dismutase and the nitric oxide synthase, an enzyme crucial for the maintaining of normal vascular tonus were increased in the group of experimental animals with spontaneous hypertension treated with the polyphenols rich extract.

Key words: polyphenols, atherosclerosis, red wine, potato, NOS, SOD, phenolic content, antioxidant activity, minerals.

Abbreviations

NOS	nitric oxide synthase
eNOS	endothelial nitric oxide synthase
SOD	superoxide dismutase
CVD	cardiovascular disorders
SHR	spontaneously hypertensive rats
WKY	Wistar Kyoto rats
NADPH	nicotinamide adenine dinucleotide phosphate
ROS	reactive oxygen spices
RNS	reactive nitrogen species
LDL	low density lipoproteins
VLDL	very low density lipoproteins
HDL	high density lipoproteins
NO	nitric oxide
EDHF	endothelium-derived hyperpolarizing factor
NF-κB	nuclear factor κB
L-NAME	N^{ω}-nitro-L-arginine methyl ester
AD	Alzheimer disease
NMDA receptors	*N*-methyl *D*-aspartate receptors
BP	blood pressure
BH4	(6R)-5,6,7,8-tetrahydro-L-biopterin
MCP-1	monocyte chemotactic protein 1
TAC	total antoxidant capacity
TPC	total phenolic content
ABTS radical	2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
TEAC assay	Trolox equivalent antioxidant capacity
FRAP assay	ferric reducing antioxidant power
DPPH	radical 2,2-diphenyl-1-picrylhydrazyl
HPLC	high performance liquid chromatography
AWE	Alibernet wine extract

Contents

Introduction

Recent advances in medical care and prevention of cardiovascular disorders (CVD) are encouraging however vascular diseases remain the main cause of mortality and morbidity across the globe.

Of late, there have been intensive efforts to search for a new effective treatment strategies in treatment of CVD.

Epidemiological and experimental studies support the hypothesis that diets with high contents of plant foods are beneficial in the prevention of chronic diseases and cancer in humans, although the benefits for individuals may depend on their genetic profile [1–10].

An advantage of our time is that the state of medical science and technologies allow us a new look on the disease classification as well as their pathogenesis. Nutritional genomics as well metabolomics and functional food development are among new tools which enable to answer many questions and inconsistency in results raised from the large scale epidemiological studies.

Throughout the history of medicine there have been efforts in searching for drugs that can effectively treat the diseases. Most of the drugs used in modern medicine are initially plant origin. Many biologically active substances have been synthesized from the plant sources. The typical example of such drugs is aspirin (acetylsalicylic acid). This drug has more than 100 years of successful use medicine. Initially the compound was found and isolated from the willow bark extract.

During past decades a lot have been done in studying of the factors and mechanisms promoting the development of cardiovascular disease. Nowadays, oxidative stress is recognized as a principal factor underlying in the pathogenesis of a number of diseases such as cardiovascular and metabolic disorders. This thesis deals with the deep analysis of oxidative stress and it's relationships with the vascular disorders. This work provides a number of experimental results of studying both the effects of natural bioactive compounds in red wines and potatoes, and their application to the experiments which includes experimental animal models.

This thesis provides evidence about the effects of plant polyphenols, with the emphasis on grape, red wine and potato polyphenols. This is due to the fact that grapes and potatoes are the richest and commonly consumed sources of dietary polyphenols in European type of diet [11].

The subsequent sections of the theoretical chapter are structured to describe the properties, sources and effects of polyphenols, as well as vitamins and minerals. Next, I followed the

description of the current concepts regarding the role of the oxidative stress in the development of vascular disorders, the functions of the endogenous antioxidant system, and potential benefits related to the natural bioactive compounds and micronutrients.

In the experimental part the results of biochemical analysis of wine and potatoes were provided, including selected mineral and vitamin content, and significant relationships were described. Finally, the effects of red wine phenolics on superoxide dismutase (SOD) activity as well as endothelial nitric oxide synthase (NOS) activity, and blood pressure trends were tested on the experimental animal models, such as Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). The relevant information regarding the vascular parameters and experimental proof of efficacy of bioactive compounds, vitamins and minerals are summarized.

2 Review of the literature.

2.1 Plant derived bioactive compounds.

General background

There is adequate evidence from observational, in vitro, ex vivo, controlled intervention and animal model studies that consumption of certain foods results in the reduction of oxidative stress and cardiovascular disease incidence [12, 13]. These positive effects are related to the plant bioactive compounds (phytochemicals) and nutrients presented in foodstuffs. In the literature all these mentioned compounds are also known as nutraceuticals.

Bioactive compounds found in plant sources are non-nutritive plant compounds that have protective or disease preventive properties. Thousands of them have been identified in fruits, vegetables, and grains. A spate of report evidence suggests that the benefits of phytochemicals in fruits and vegetables may be even greater than is currently understood, because the oxidative stress induced by free radicals is involved in the development of a wide range of chronic diseases.

These compounds vary widely in chemical structure and function and are grouped accordingly. All bioactive compound found in plant sources can be divided into several groups, such as: plant polyphenols, carotenoids, alkaloids and other groups (Attachment 1. Phytochemicals classification). The major group among phytochemicals is polyphenols. Polyphenols occur naturally in a variety of forms from simple phenolic acids to complex polymerized tannins. They are being intensively studied due to their effects toward human health.

Beside phytochemicals essential role also plays nutrients such as vitamins and minerals. They act synergistically with other bioactive compounds and led to exert higher antioxidant effects comparing to the single compounds [14-17].

Despite the diversity of chemical structures among plant bioactive compounds, all of them exert multiply effects that will be further described.

2.1.1 Properties and major sources of polyphenolic compounds in Western populations

Experimental studies strongly support a role of polyphenols in the prevention of cardiovascular disease, diabetes mellitus, cancer and neurodegenerative disease [1, 18, 19].

Moreover, there are enough evidence to support that polyphenols are bioavailable and exert biological functions in animals and humans [20, 21]. In 2005, Arts and Hollman published a review of several recent epidemiological studies on polyphenols intake and risk of coronary disease. Seven of those studies had clearly showed protective effects attributable to flavonoids [10]. In this study however, authors observed no effect against cancer risk. The Zutphen Elderly Study, published in 2001 had earlier reported the reduced mortality due to chronic diseases amongst men between 65 and 84 years of age under higher catechin intake [22]. Moreover, very recent analysis of efficacy of plant bioactive compounds in human trials by Badimon et al. [1] have found an inverse relationships between polyphenol consumption and CVD mortality in several large scale studies. In fact, such beneficial effects may help to explain the protective CVD effects achieved by food and beverages containing polyphenols [23, 24].

Among all natural antioxidants polyphenols are the largest group, about 8000 compounds that includes mainly flavonoids, phenolic acids, lignans, coumarins, tannins, lignans, xantans and chromons have been described [25]. Polyphenols are divided into several classes according to the number of phenol rings that they contain and to the structural elements that bind these rings to one another. Details of classification and major sources are visualized in the Fig. 1.

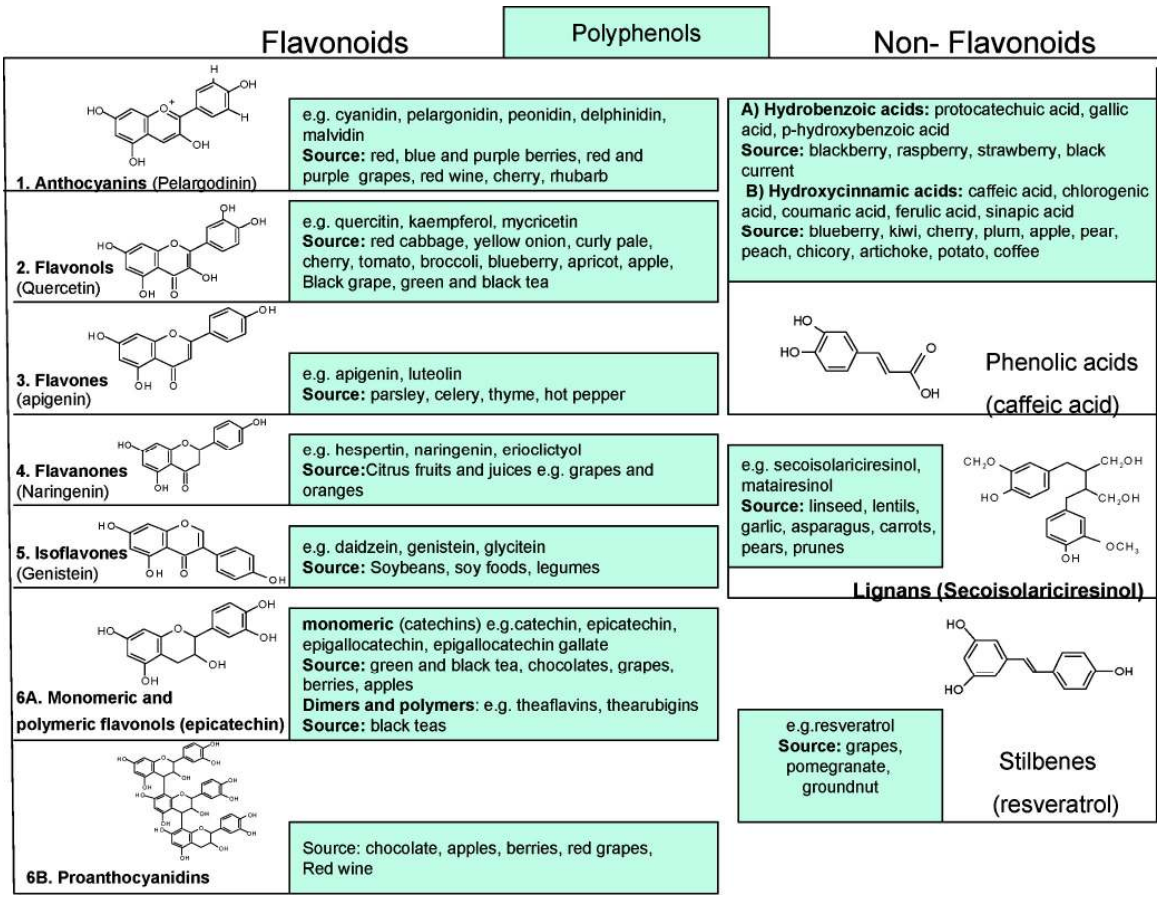


Fig.1 Polyphenols classification with their individual compounds and food sources. Adopted from Singh et al. [26].

Plant polyphenols are non-nutritive, hydrophilic components found in small amounts (micrograms) in all kind of plant-derived food sources such as fruits and vegetables, drinks (wine, coffee, juices) and cereals. In foods and beverages these compounds are associated with sensorial attributes such as colour, bitterness, astringency, which are relevant in products such as wine, tea and grape juice [27-29].

Flavonoids is the biggest and the most widely studied class of polyphenols. Up to date there were more than 4000 single flavonoids identified, and some of them such as catechins and antocyanins, which are presented in red wine are recognized as an important therapeutic agents. [30].

Among the different classes of phenolics flavonoids (anthocyanins, catechins, quercetin), and phenolic acids (gallic, caffeic and chlorogenic acid) have been intensively studied.

Apart from wine or tea, where catechins presented as flavonoid aglicons, dietary flavonoids are glycosides, mainly with D-glucose and in this form are ingested. Glycosylation increases the polarity of flavonoid molecule, which is necessary for storage in plant cell vacuoles [31].

Antocyanins are pigments that provide color for red wines and juices and generally are found skin in grape s and other fruits and vegetables. These compounds at a large extent make up the antioxidant capacities and total phenolic content of fruit and vegetables. Several studies have revealed their cardioprotactive, anti-inflammatory, and other antiviral and antiplatelet aggregation activities. One of the most important sources of these compounds in human diet is red wine that contains up to 350 mg anthocyanins/L [32].

Bioavailability of polyphenols, remains the most essential question to answer in upcoming decade. However, very recent animal study with blueberries has confirmed that anthocyanins are bioavalable in rats. The concentrations of anthocyanins measured in rats were high enough to exert physiological effects ascribed for these compounds [33].

Another class of flavonoids are **catechins**. These compounds are found in high concentration in wine and tea. Catechins have been intensively studied, and positive effects that attribute to the diet rich in fruit and vegetables are mainly related to these substances. These compounds have strong anicancerogenic and anti-inflammatory activities. Studies of bioavalability of catechins detected them in human plasma after consumption of red wine and juices in concentrations, sufficient to exert abovementioned effects [34].

The daily intake of polyphenols could reach 1 g/d. but broadly varies from one region to another and depends highly on dietary patterns of population [35]. Among the major sources of polyphenols in Western populations are found fruits, vegetables (onion, potato), tea, red wine and fruit juices.

The highest concentrations of anthocyanins were found in red wines (up to 350 mg/l), red grapes (3,0-75 mg/kg), chokeberry (20-100 mg/kg), blueberry (2,5-49,7 mg/kg) and oranges (up to 20 mg/l) [26,36].

Catechins are found in high concentrations in beverages such as tea infusions (102−418 mg of total catechins/L) and red wines (27−96 mg/L), and also in grape seeds (Vitis vinifera) (up to 1892 mg/kg), wine grape (red) (800-4000 mg/kg), dark chocolate (up to 610 mg/kg) and in apples (1000-7000 mg/kg of fresh cortex) [37, 26].

Principle sources of quercetin are oninons (284-486 mg/kg fresh eadible part), cherry tomatoes (17-203 mg/kg), apples (21-72 mg/kg), tea infusions (10-25 mg/l) and red wine (4-16 mg/l) [38].

Polyphenols have attracted considerable interest from the scientific community due to the multiple biological effects they exert.

Dietary intake of plant polyphenols is inversely related to the development of cardiovascular disease due to their direct free radical scavenging (antioxidant), antiatherosclerotic, effects on endothelial function (NO production), anti-inflammatory, antithrombogenic, neuroprotective (antiaging), antimicrobial, anticarcinogenic effects and their ability of improving insulin sensitivity [35, 39-43].

These activities of polyphenols are further discussed.

1. Antioxidant effects

Polyphenols have powerful antioxidant activities in vitro, being able to scavenge a wide range of ROS and RNS and chlorine species, such as superoxide, hydroxyl and peroxyl radicals, and hypochlorous and peroxynitrous acid. They can also chelate metal ions [44-47].

Polyphenols such as catechins or quercetin can directly scavenge reactive oxygen species (ROS), such as superoxide ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2) [48], or hypochlorus acid (HOCl) [49], thereby could effectively prevent damage of lipids, proteins and DNA. The phenolic core can act as a buffer and capture electrons from ROS to render them less reactive [50]. Furthermore, polyphenols, quercetin in particular, can chelate metals as iron involved in free radicals formation [51, 52]. Indirectly, polyphenols can interfere with the cellular detoxification systems, such as superoxide dismutases (SOD), catalase or glutathione peroxidases [53, 54]. Moreover, polyphenols can inhibit enzymes generating ROS as xanthine

oxidase and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [52, 55]. Antioxidant potential of polyphenols has been reviewed by Rice- Evans and Miller [56].

Antioxidant properties are related to the both scavenging effect (protection of LDL and VLDL from oxidation) and capacity to suppress inflammation. These protect cell membranes and DNA [57]. Details on this are provided below.

2. Antiatherosclerotic and hypolipemic effects

Another important property of the polyphenolic compounds is their ability to reduce lipid sensitivity to oxidation. In vitro studies with the polyphenolic compounds from red wine, proved their protective effects against oxidized LDL-induced cytotoxicity in endothelial cells [58]. In animals, Vinson et al. [24] reported that polyphenols from red wine or grape juice reduce the plasmatic concentration of lipids in experimental hamsters. This is consistent with studies in humans. Short-term ingestion of purple grape juice has been shown to reduce LDL susceptibility to oxidation in patients with coronary artery disease [59]. This is also related to the study of Frankel et al., where an inhibition of LDL oxidation in humans by resveratrol was observed [60]. These results are also consistent with the study in hypercholesterolemic postmenopausal women with red wine complementation [61].

Study by Hertog et al [62] confirmed that the flavonoids in regularly consumed foods might reduce the risk of death from coronary heart disease in elderly men. Furthermore, a Japanese study reported an inverse correlation between flavonoid intake and total plasma cholesterol concentrations [63].

Recent studies also related the cardiovascular protection by plant polyphenolic compounds to their effects in improving lipid homeostasis. Animal studies and in vitro experiments with human cells demonstrate that polyphenols such as proanthocyanidins (catechins) and naringenin reduce plasma levels of atherogenic apolipoprotein B-triglyceride-rich lipoproteins and LDL-cholesterol but increase antiatherogenic HDL cholesterol [64-66].

3. Effects on endothelial function (NO production)

It is wide recognized that endothelium plays a key role in the control of vascular tone by releasing several vasorelaxing factors, which are nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF). Nitric oxide is produced by the Nitric oxide synthases (NOS). These enzymes due to their key role in the maintaining vessel relaxation have attracted considerable interest for the experiments with the plant bioactive compounds [67].

Recent animal and in vitro studies have established favorable effects of polyphenolic compounds on endothelial function [68]. In cultured endothelial cells, wine, grape juice, grape seed extract polyphenols increased the activity of the endothelial NO synthase and stimulate

NO production [69-71]. In the short term, polyphenols stimulate endothelial NO synthase phosphorylation [72].

Long term exposure to red wine extracts or resveratrol led to increase in enzyme expression and activity [69-70]. The effects of resveratrol on endothelial function may be mediated through an effect on Sirtuin-1, which regulates the expression of genes related to cell survival and the stress response [73, 74]. Furthermore, activation of Sirtuin-1 decreases the activity of p53, a regulator of apoptosis and the cell cycle, and activates AMP-dependent protein kinase, a regulator of cellular energy status [75].

Results of animal studies with the L-NAME and spontaneous hypertension demonstrate that polyphenols from red wine extracts were able to prevent L-NAME-induced hypertension, cardiovascular remodeling and vascular dysfunction via the increase of NO-synthase activity and prevention of oxidative stress. This allows us to hypothesize, the beneficial effects of plant polyphenols in prevention of hypertension may result from their complex influence on the NO balance in the cardiovascular system [76].

Substantial evidence from the human studies supports a benefit of grape beverages on endothelial function [77]. Stein et al. [78] observed that consumption of purple grape juice for 2 or 4 week improved endothelium-dependent brachial artery flow-mediated dilation in patients with coronary artery disease [79]. Dealcoholized wine also improved brachial artery flow-mediated dilation in healthy subjects [80]. Moreover, red wine consumption prevents the acute impairment of endothelial function that occurs following cigarette smoking [81] or consumption of a high-fat meal [82].

In addition to the effects on NO, grapes have important effects on other molecular aspects of vascular function. For instant, flavonoid-containing beverages increase endothelial production of prostacyclin and suppress production of endothelin-1, a potent endothelium-derived vasoconstrictor [83, 84]. In regard to regulation of fibrinolysis, catechins and resveratrol increase protein levels and activity of tissue plasminogen activator, an effect that is likely to be cardioprotective [85]. Finally, there is increasing evidence that polyphenols affect endothelial regulation of inflammation. Red wine constituents reduce adhesion of monocytes to the endothelial surface and block cytokineinduced expression of endothelial adhesion molecules [86]. Thus, polyphenols exert multiple effects in endothelial cells that could reduce cardiovascular risk.

4. Antiinflammatory effects

The importance of inflammation for all stages of atherosclerosis is increasingly recognized and there are data suggesting that dietary polyphenols have antiinflammatory effects.

Evidence from animal studies confirms that polyphenols are able to exert antiinflammatory effects by inhibition of lipoxygenase, cycloxigenase (COX-1, COX-2) and thereby modulate metabolism of arachidonic acid [87, 88].

The molecular bases of antiinflammatory effects of polyphenols have been linked to inhibition of nuclear factor κB (NF-κB), which governs the regulation of cytokine release and response [86, 89]. Study with incubation of monocytes with catechin has found decrease of their adhesion to endothelial cells [90]. Dietary polyphenols also inhibit activation of nuclear factor-kB in T lymphocyte cell lines [91]. Resveratrol has also been shown to have antiinflammatory effects, including inhibition of adhesion molecule expression [92].

Polyphenols inhibit the production of pro-inflammatory cytokines interleukin -1 (IL-1), and IL-6 by human blood mononuclear cells as well as cytokines from helper T-cells, such as IL-2 and interferon gamma (INFγ) by Th1 cells and IL-4, IL-5 by Th2 cells [93].

Human studies of treatment with lyophilized grape powder for 4 week also confirmed reduction in tissue necrosis factor-α [42].

Therefore, antiinflammatory effects might be a contributing mechanism for the benefits of grape polyphenols against cardiovascular disease.

5. Antitrombogenic effects

Platelet aggregation contributes to both the development of atherosclerosis and acute platelet thrombus formation. Activated platelets adhering to vascular endothelium generate lipid peroxides and oxygen free radicals, which inhibit the endothelial formation of prostacyclin and nitric oxide (NO).

Flavonols are particularly antithrombotic because they directly scavenge free radicals, thereby maintaining proper concentrations of endothelial prostacyclin and nitric oxide [18, 94].

It is well known that arachidonic acid, which is released in inflammatory conditions, is metabolized by platelets to form prostaglandin, endoperoxides, and thromboxane A_2, leading to platelet activation and aggregation [95]. The main antiaggregatory effect of polyphenols is thought to be by inhibition of thromboxane A_2 formation.

Animal studies of selected flavonoids, such as quercetin, kaempferol, and myricetin proof their effects as inhibitors of platelet aggregation. These effects have been shown to depend on NO production [96, 97].

Human studies have also demonstrated antiplatelet effects of grape-derived beverages. Freedman et al. demonstrated that grape juice consumption for 14 days decreased platelet aggregation and superoxide production and increased NO production in healthy volunteers [98].

6. Neuroprotective effects

Aging may be regarded as the major risk factor for all forms of dementi and particularly for Alzheimer disease (AD), affecting up to 18 million people worldwide [99].

Oxidative stress and vascular damage are postulated to play a key role in the development of age related neurodegenerative changes such as dementia and Alzheimer disease. Current drugs used in AD are acetylcholinesterase inhibitors and antagonists to the NMDA receptors. These drugs may only slightly improve cognitive functions but have only very limited impact on the clinical course of the disease. In the past several years, based on in vitro and in vivo studies in laboratory animals, and in humans plant polyphenolic compounds have been proposed as promising therapeutic agents for AD [100-102].

Moreover, several recent studies proved the neuroprotective effects of polyphenols toward individuals with Alzheimer disease [26, 103-105].

One of the promising compounds toward neuronal protection is resveratrol, polyphenol, found in grapes and red wine. Very recent studies show that the beneficial effects of resveratrol are not only limited to its antioxidant and anti-inflammatory action but also include activation of sirtuin 1 (SIRT1) and vitagenes, which can prevent the deleterious effects triggered by oxidative stress. In fact, SIRT1 activation by resveratrol is gaining importance in the development of innovative treatment strategies for stroke and other neurodegenerative disorders [106].

The intake of red wine and other polyphenol rich food and beverages is reported to prevent the development of dementia [107]. The intake of flavonoids was reported to be inversely related to the risk of incident dementia [108].

It is need to mention that polyphenolic rich diet is particularly efficient in early stages of disease development.

7. Antimicrobial effects

The phenolic compounds from various foodstuffs displayed different antimicrobial effects such as antibacterial [109, 110], antifungal [111, 112] and antiviral [113,114].

Chemical structure appears to be critical for these antimicrobial activities attributed to polyphenols. For instant, in the study of Tagurt et al., [115] the core structures with 3,4,5-trihydroxyphenyl groups found in epigallocatechin, epigallocatechin-3-O gallate, castalagin and prodelphinidin showed strong antibacterial activity. This indicated that the number of hydroxyls and the degree of polymerizzation might be pivotal for antimicrobial activity of phenolic compounds.

Recent study by Nikitina et al. [116] has confirmed bacteriostatic activity of polyphenols against gram-positive and gram-negative bacteria. Moreover, it was found a positive correlation between the bacteriostatic activities of plant-derived phenolic compounds with their antioxidant potential.

Rodriguez-Vaquero *et al*. [117] have showed that grape wine inhibited microbial, especially *Escherichia coli* growth, and the inhibition has amplified as the polyphenols concentration increased. The extracts of alcohol-free red and white wine exhibited antimicrobial activity to some pathogens such as *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* [118]. The results suggested that polyphenolic compounds contained in red wines were responsible for the antimicrobial effects. Some studies reported phenolic compounds inhibited other food-borne species such as *Salmonella typhimurium* [119] and *Listerial monocytogenes* [120].

Existing evidence open up possibility for future research in the field of possible use of polyphenols as antimicrobial agents.

8. Anticarcinogenic effects

A growing body of evidence has shown that plant polyphenols have anticancer activity [121, 122].

Some polyphenolic compounds such as fisetin, apigenin, and luteolin are recognised to be potent inhibitors of cell proliferation [123]. A large clinical study has found the presence of an inverse association between flavonoid intake and the subsequent incidence of lung cancer [124]. This effect was mainly ascribed to quercetin, which provided more than 95% of the total flavonoid intake in that particular study.

Researches in anticancer activities of plant phenolic compounds have revealed their dose-dependent character of effects on cells and modulated cell proliferation was notablely [125]. At high concentration, they were attributed to exert direct toxic effects on cancer cells. [126].

9. Improvement of insulin sensitivity

There is increasing evidence that plant polyphenols independently are able to inhibit the alpha-glucosidase and alpha-amylase, enzymes involved in the digestion of carbohydrates. Inhibition of alpha-glucosidase is already an accepted means of controlling post-meal glucose levels in patients suffering from non-insulin-dependent diabetes mellitus (type 2 diabetes) [127, 128].

Different polyphenolic compounds are responsible for the inhibition of alpha-glucosidase and alpha-amylase, which suggests considerable effects on blood glucose levels [57, 129]. Among the all polyphenols, anthocyanins and resveratrol are appeared to be the most effective

inhibitory agents against alpha-glucosidase activity and in inducing the antihyperglicemic effects [130,131].

Experimental studies in animals have proved the intestinal α-glucosidase inhibitory activities and the antihyperglycemic action exerted by the polyphenolic compounds in the same range as synthetic inhibitors (acarbose and voglibose) already being used therapeutically to control non-insulin-dependent diabetes mellitus [132-134]. Moreover, very resent study by Adisakwattana et al, have revealed the synergy between anthocyanins and acarbose [135]. This opens up new possibility of potential clinical application of natural phenolic compounds.

These insulin-like effects in glucose utilization exerted by polyphenols can significantly reduce the post-prandial increase of blood glucose and therefore can be an important strategy in the management of blood glucose level in type 2 diabetic and borderline patients.

10. Estrogen-like effects

Several polyphenolic compounds are also for their hormone –like effects. These compounds belong to the phytoestrogens, and are found in beans, red clover and soya products.

Phytoestrogens (genistein and diadzein) have estrogen-like effects and/or inhibit tyrosine kinases. Phytoestrogens bind to steroid receptors and supposed to have the direct inhibitory effect on activity of human 17β-hydroxysteroid dehydrogenase type 5 (17β-HSD 5) a key enzyme in the metabolism of estrogens and androgenes. This effect contributes to the cancer prevention [136-138].

Besides this, human trials with the dietary supplementation of phytoestrogens (biochanin, genistein, diadzein) have suggested that these compounds have potentially protective effects toward bone density through attenuation of bone loss [139].

2.1.2 Minerals

Minerals as an integral part of every foodstuff play an important role in the body homeostasis. Growing number of clinical evidence proof relationship between the certain level of selected mineral content and the disease progression. Moreover, the pathogenesis of a number of diseases such as, cardiovascular disease, metabolic and neurological disorders has been associated with the changes in balance of certain trace elements [140-142].

Fruit and vegetables, beverages as well as other foodstuffs are the main dietary sources of minerals, such as magnesium, zinc, potassium, copper, iron, calcium, phosphorus, selenium, manganese etc.

Mounting evidence suggest that minerals are able to counteract the development of cardiovascular, metabolic and other diseases [143, 144]. Essential micronutrients and their protective effects will be discussed in this part of the thesis.

Positive effects of minerals in humans are related to their ability to enhance the activity of antioxidant defense system by catalyzing antioxidant enzymes. For instant, several minerals found in foodstuffs, such as copper, zinc and manganese are essential for activity of superoxide dismutase (SOD), a key antioxidant enzyme [145, 146].

Experimental studies with zinc supplementation have revealed an augmentation of catalase, glutathione-s-transferase (GST) and SOD activity after zinc supplementation for 4 months [147].

Zinc is one of the most important and ubiquitous trace elements in the body. Zinc is an important element in preventing free radical formation, in protecting biological structures from damage and in correcting the immune functions, it also possess antiaging effects [146, 148].

Zinc exerts antioxidant effects through catalyzing superoxide dismutase (SOD), acting as a NADPH oxidases inhibitor, inducing the production of metallothionein, an efficient scavenger of $^{\cdot}OH$ radical. Several studies of antioxidant effects of zinc in humans have provide evidence that antioxidant effects of zinc supplementation may lead to downregulation of the inflammatory cytokines through the inhibition of induced NF-κB activation [149, 150].

This antioxidant action of zinc is tightly related to it's preventive action on diabetic complication, since oxidative stress has been considered as the major cause of diabetic cardiomyopathy. Recent study of Song et al. has supported the fact that Zn supplementation is able to prevent heart oxidative damage [151].

Zinc also has an important role in cell-mediated immune functions [152]. It has a pivotal role in the interactions of other trace elements such as copper and iron. Study by McDonald et al. [153] has found that red wines increase absorption of zinc in humans.

Zinc deficiency increases the levels of lipid peroxidation in mitochondrial and microsomal membranes and the osmotic fragility of erythrocyte membranes. Zinc deficiency also produces impaired haemostasis due to defective platelet aggregation, a decrease in T cell number and the response of T-lymphocytes to phytomitogens [154]. Zinc deficit produces growth retardation, anorexia, delayed sexual maturation, iron-deficiency anemia, and alterations of taste [155].

Recent studies also considered zinc deficiency as a risk factor for the atherosclerosis development [140, 156-158].

In addition to zinc, magnesium and potassium play important role in the cardiovascular and metabolic disease prevention as well as contribute to maintenance of bone mineral density, a key indicator of osteoporosis development [159].

Epidemiologic studies have shown that increasing potassium intake lowers blood pressure in the both hypertensive and normotensive people reduce sodium intake, reduces urinary calcium excretion, which reduces the risk of kidney stones formation and helps to prevent bone demineralization [160-161].

Potassium (K) found as a most abundant metal in fruits and wines is also known as a principal intracellular cation in eukaryotes. Potassium is critical for the functions of myocardium and carbohydrate metabolism, it protects from hypertension, insulin resistance and decreases the risk of developing of type 2 diabetes, it also helps to maintain water and electrolyte balance and acid-base status [160, 162].

Potassium could exert it effects in the presence of magnesium that modulate and amplify its metabolic effects [161]. Magnesium protects from insulin resistance (IR) and decreases risk for type 2. diabetes in adults. Intracellular Mg plays a key role in regulating insulin action, insulin-mediated glucose uptake and vascular tone [163, 164].

Another metal, iron (Fe) is essential component of hemoglobin, myoglobin and catalase enzymes at the same time could be pro-oxidant through Fenton reactions. The close relationship between Fe and Cu in human nutrition has been studied for many years. The best-characterized link is provided by caeruloplasmin, a multiCu-binding protein that acts as a serum ferrioxidase and is essential for the mobilisation of Fe from storage tissues. Decreased Cu status has been shown to reduce holo-caeruloplasmin production and impair ferrioxidase activity, leading, in a number of cases, to decreased tissue Fe release and the generation of anaemia [165].

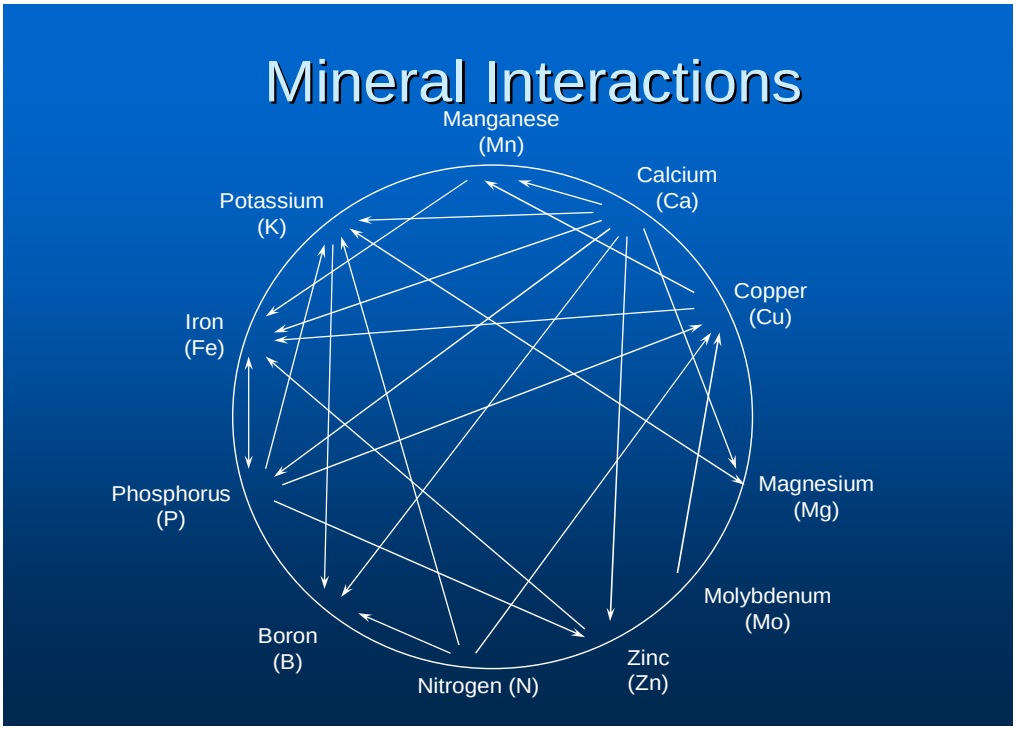
Clinical studies also provided evidence on the role of minerals in the Alzheimer disease development, and a new hypothesis suggests that copper deficiency could lead to onset of Alzheimer disease [166, 167]. It has been also experimentally found that dyshomeostasis of metals such as copper (Cu), iron (Fe) and zinc (Zn) lead induces β-amyloid precipitation and neurotoxicity. Chelating agents offer a potential therapeutic solution to the neurotoxicity induced by copper and iron dyshomeostasis.

A further important element for enzymes of antioxidant defense is selenium (Se). It takes part in the structure of various forms of selenoproteins. Among these selenoproteins are the four forms of glutathione peroxidase (GPx1- intracellular, GPx2- gastrointestinal, GPx3- extracellular (plasma), GPx4- hydroperoxides degrading), an enzyme responsible for lipid hydroperoxides and H_2O_2 removal, Selenium is also incorporated at the active site of

selenoproteins P and W, thioredoxin reductase and the iodothyronine deiodinase enzymes [168].

Regarding the effects of selected minerals to vascular health it is need to mention the key role of calcium (Ca) in the regulation of NOS activity that controls vascular tone. It has been clearly establish that after the enhancement of intracellular Ca^{2+}, eNOS activity markedly increases [169].

Complex characters of interactions among different minerals are visualized in the Fig. 2.



The synergistic relationships between minerals are depicted as arrows.

Fig. 2. Mineral interactions.

2.1.3 Vitamins

Vitamins are organic compounds, required in small quantities for the variety of biochemical functions. Generally they cannot be synthesized by the body and must therefore be supplied in the diet.

Vitamin functions and deficiency disease are well reported in the existing literature. This chapter is focused on the vitamin-vitamin interactions, their action as coenzymes, effects on hormones and possible action toward cardiovascular disease.

Vitamins can be divided into two groups: fat-soluble vitamins and water-soluble vitamins. Vitamins A, D, E and K are the fat-soluble vitamins. They require the presence of fat for their absorption and are stored in the liver. Water-soluble vitamins include vitamin C and the B-complex vitamins. The B vitamins include thiamine (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxine (B_6), folic acid (B_9), cobalamin (B_{12}), and biotin. Water-

soluble vitamins are lost through urination and are not stored in the body. It is wide recognized that vitamins function as enzyme co-factors and they are also essential for the hormones excretion [170].

Several vitamins have possessed antioxidant properties, these vitamins are following, vitamin C, vitamin E, folic acid, vitamins B_6 and B_{12} and vitamin A. They are commonly found in many foods and multivitamins, and their main function is to neutralize the free radicals that are produced in the process of food digestion [7]. Some recent clinical evidence also suggests that B vitamins have the antiinflammatory properties and the ability to reduce the risk of cardiovascular disease development [171-173].

Vitamins being as co-factors of many enzymes play a critical role for their proper function. Here some examples of their action as coenzymes are provided. Vitamin B_3 (niacin) is a functional part of nicotinamide adenine dinucleotide (NAD^+) and NADP. The NAD^+ coenzyme is involved in many types of oxidation and reduction reactions. Riboflavin forms a coenzyme flavin adenine dinucleotide (FAD), which is involved in the oxidation and reduction reactions. Pyridoxine (vitamin B_6) is a coenzyme in transamination and decarboxylation of amino acids and glycogen phosphorylase, and also in modulation of steroid hormone action. Vitamin B_5 (pantothenic acid) is a finctional part of coenzyme A (CoA), participated in the fatty acid synthesis and metabolism [170].

Experimental evidence suggest that supplementation by B-vitamins could increase the estrogen levels in women [174]. Vitamin H (biotin) is also reported to decrease the risk of insulin resistance [175]. In addition, increase in body's ability to process estrogen was observed after vitamin B_6, vitamin B_{12} and choline supplementation.

Very recent animal study also suggests that promising results could be achieved when microelements are applied together with the vitamins. In this study niacin-bound chromium exerts long term metabolic effects, including increase in insulin sensitivity, lowering HbA1C, enhancement of NO activity [176].

According to the oxidative-modification hypothesis in which reactive oxygen species and free radicals play a major role in the pathophysiology of atherosclerosis, supplementation with antioxidants (vitamins A, C, E, folic acid, β-carotene, selenium, zinc) was expected to protect against atherosclerosis [15]. In fact, observational prospective human cohort studies have reported that high dietary intake of foods rich in vitamin E [177], vitamin C [178], and β-carotene [179], have been inversely associated with the incidence of cardiovascular disease. Vitamin C is a potent water-soluble antioxidant that recycles vitamin E, improves endothelial dysfunction (ED) [180]. Vitamin C is also an essential nutrient for the biosynthesis of collagen, L-carnitine, and the conversion of dopamine to norepinephrine [181]. Under

physiological conditions, it functions as a potent reducing agent that efficiently quenches potentially damaging free radicals produced by normal metabolic respiration of the body [182, 183].

Numerous epidemiologic, observational and clinical studies have demonstrated that the dietary intake of vitamin C or plasma ascorbate concentration in humans is inversely correlated to systolic and diastolic blood pressure (BP) and heart rate [180, 184]. Long-term epidemiologic and observational follow-up studies in humans also show a reduced risk of cardiovascular disease [185]. Moreover, the cardioprotective effects of vitamin C may be related to it's ability to protect normal NO synthesis by modulating the redox states of its components, primarily nitric oxide synthase (NOS) cofactor (6R)-5,6,7,8-tetrahydro-L-biopterin (BH_4) [186, 187].

Stabilization and reactivation of the endothelial BH4 by ascorbic acid, and the consequent restoration of the normal biological activities of eNOS [188, 189] and subsequent endothelial NO accumulation, may represent a key mechanism by which vitamin C impacts overall endothelial health. The main effects of vitamin C are summarized in Attachment 2.

Vitamin E, in the form of α-tocopherol, is considered as a key lipophilic antioxidant in human circulation and the vasculature and plays a role in many key processes contributing to the onset and progression of atherosclerosis [15]. Vitamin E supplementation in patients with ischemic heart disease and a moderate hypercholesterolemia resulted in a significantly prolonged lag time and lowered rate of LDL ex vivo oxidation in male patients when compared with postmenopausal women [190].

Major effects of vitamin E toward different molecules are presented in the Attachment 3.

Mounting evidence has suggested that vitamins act synergistically [191, 192]. For instant, vitamin D together with vitamin A and calcium or phosphorus protects the body from colds, diabetes, eye and skin diseases.

Another example of the synergistic interactions is vitamin C and vitamin E. As a lipophilic antioxidant, vitamin E can interact with the lipid components in the vascular systems, especially LDL, and protects them from atherogenic oxidative modification. The lipid-bound a-tocopherols can be oxidized by aqueous-phase radicals and transformed into reactive tocopherol radicals. Oxidized vitamin E can be reduced back to its antioxidant form by vitamin C which reduces the tocopheroxyl radical to tocopherol, thereby acting as a co-antioxidant and inhibiting LDL oxidation [193]. Additionally, vitamin C is capable to sequester aqueous radicals in the plasma before they can oxidize vitamin E in the lipid phase and affords preemptive protection for lipid-bound tocopherols. Study with cigarette smokers,

has provided evidence that the rate of the blood vitamin E oxidation caused by increased oxidative stress is substantially attenuated by vitamin C supplementation [194], indicative of a vitamin E-recycling role for vitamin C and a potential cooperative relationship between vitamins C and E. In fact, this synergy against oxidation of lipoproteins has been shown both in vitro [195] and in vivo [196-198]. Moreover, vitamins C and E can interact synergistically in protection against the development of cardiovascular disorders [199]. For example, when applied in combination, they synergistically attenuate copper-mediated LDL oxidation in vitro [200], downregulate the expression of vascular endothelial growth factor (VEGF) and its receptor VEGF-2 [201], and decrease the activation of NADPH oxidase while increasing that of superoxide dismutase, leading to reduced levels of oxidative stress [202]. These cooperative interactions between these two vitamins have a big clinical significance, because they provide evidence for combined antioxidant therapy in treatment of cardiovascular disorders. Indeed, the therapeutic value of multivitamin treatment has been supported by a number of clinical evidence [203-205].

Research of the synergistic interactions among vitamins appeared to be promising tool to explain their complex effects toward living organisms. Relationships among vitamins are visualized in the Fig.3.

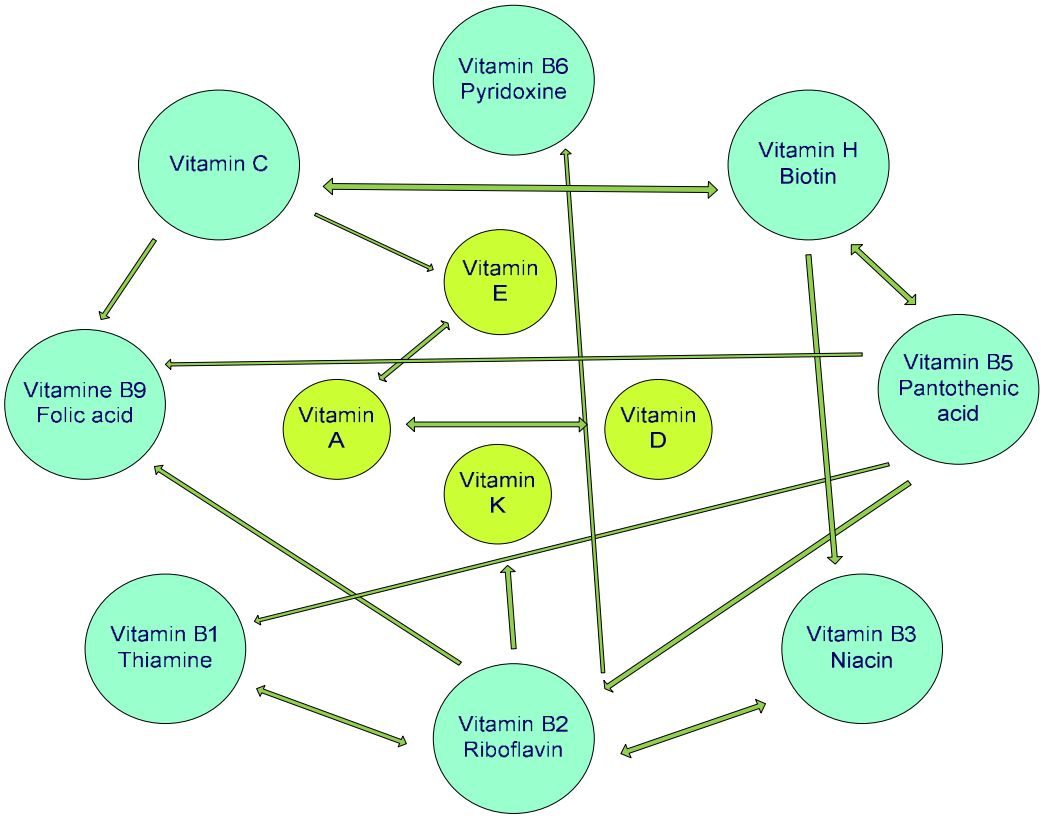


Fig. 3. Vitamin interactions.

2.2 The underlying pathogenesis of vascular disorders. The role of oxidative stress and nitric oxide in atherothrombosis.

World statistics demonstrate that vascular diseases (atherosclerosis) became the major cause of morbidity and mortality among people of all age. According to the World Health Organization, there were nearly 5.5 million strokes – related deaths in 2002 [206].

The question of revealing the main causes in the development of the vascular disease remains relevant up to this date.

Risk factors for cardiovascular disease include hypertension, diabetes mellitus, hypercholesterolemia, and heart failure [207-210].

However, the majority of cardiovascular disease results from complications of atherosclerosis [209].

Oxidative modification hypothesis, in which reactive oxygen species and free radical plays a major role in the pathophysiology of atherosclerosis has been confirmed by the results of experimental and clinical studies [207, 211, 212]. According to this hypothesis increased levels of oxidized low-density lipoprotein (LDL) cholesterol plays central role in the promotion of premature atherosclerosis.

In this regard excess of generation of reactive oxygen species (ROS) represents an important pathological process in atherogenesis. Mounting evidence indicates that chronic and acute overproduction of reactive oxygen species under pathophysiologic conditions plays important role in the development of cardiovascular diseases [213-215].

Experimental studies have also confirmed that each component of the atherosclerotic blood vessel has been demonstrated to increase production of ROS, primarily superoxide anion ($O_2^{-\bullet}$) [216]. Important sources of ROS are vascular smooth muscle cells, endothelial cells, fibroblasts, and infiltrating leukocytes [217]. Production of ROS affects gene transcription, damages DNA, and increases production of inflammatory transcription factors [218].

Although the mechanism of oxidative modification of LDL remains unknown, the importance of oxidation can be seen by the presence of oxidized LDL in atherosclerotic lesions. Experimentally, the amount of oxidized LDL directly related to the atherosclerotic burden [219]. Oxidized LDL induces a series of atherogenic processes, including transcription of proatherogenic genes, production of matrix metalloproteinases and tissue factor, antagonism of endothelial cell production of NO, and promotion of vascular smooth muscle cell apoptosis

[220]. Scavenging of NO leads to the progression of endothelial dysfunction simultaneously increases inflammation, platelet activation, and vasoconstriction.

ROS mediate various signaling pathways that underlie vascular inflammation in atherogenesis: from the initiation of fatty streak development through lesion progress to ultimate plaque rupture. Oxidative stress is appeared to be a universal mechanism for development of many CVD risk factors, which additionally supports its central role in CVD. [169, 209]

The development of atherosclerosis is visualized in the Fig. 4.

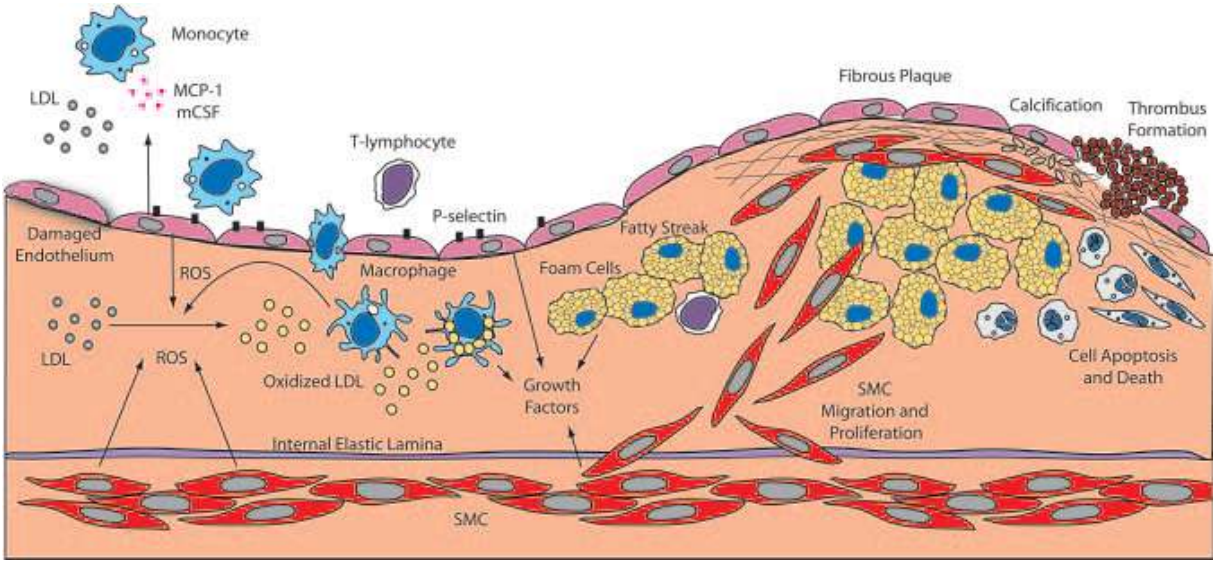


Fig. 4. Development of atherosclerosis.

ROS produced by endothelial cells,SMCs, and macrophages oxidize LDL inthe subendothelial space, at the sites of endothelial damage, initiating events that culminate in the formation of a fibrous plaque. Rupture of fibrous plaque leads to thrombus formation and occlusion of the vessel. Adopted from Madamanchi et al. [211].

Oxidative stress, mechanisms.

The production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is important in both normal physiology and in the pathogenesis of cardiovascular and many other disorders. The ROS include partly reduced forms of molecular oxygen, such as hydroxyl radical (•OH), superoxide anion ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), lipid peroxides, and hypochlorous acid (HClO). Accumulation of ROS is often accompanied by the production of reactive nitrogen species (RNS), such as the highly reactive peroxynitrite anion, a strong oxidant formed by the reaction of $O_2^{-\bullet}$ and nitric oxide. Under physiological conditions, cells protect themselves against ROS damage through endogenous antioxidants that remove free radical intermediates and inhibit oxidation. An imbalance between endogenous oxidants and

antioxidants results in oxidative stress, a condition that contributes to vascular dysfunction and atherogenesis [221].

Sources of ROS

These ROS can be released from nicotinamide adenine dinucleotide (phosphate) (NADPH) oxidase, xanthine oxidase, lipoxygenase, mitochondria, or the uncoupling of nitric oxide synthase (eNOS) in vascular cells [222].

Superoxide anion is produced by the one-electron reduction of oxygen by nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) oxidase, mitochondrial respiration, and other oxidoreductases, such as glucose oxidase and xanthine oxidase [223]. The effects of $O_2-\bullet$ include oxidative damage, the mediation of signal transduction leading to altered gene transcription, posttranslational modification with changes in protein function and enzyme activity ('redox signaling'), and rapid inactivation of NO, leading to endothelial dysfunction. Alterations in both the rate of formation and the extent of scavenging of $O_2-\bullet$ have been implicated in the vascular dysfunction observed in atherosclerosis, hypertension, diabetes mellitus, chronic nitrate tolerance, and postischemic myocardial dysfunction [208, 224].

In the endothelium, NO is synthesized by the Ca^{2+}-calmodulin-dependent nitric oxide synthase (NOS), using L-arginine, O_2, and NADPH as substrates. Nitric oxide is membrane permeable and diffuses throughout the vasculature, promoting smooth muscle cell relaxation by activation of soluble guanylyl cyclase and modulation of cation channels, and, consequently, regulating vascular tone [225]. Additional antiatherogenic actions of NO relate to inhibition of platelet function and inflammatory cell adhesion, promotion of fibrinolysis, and attenuation of smooth muscle cell proliferation [226].

When nitric oxide and O2−• react in a diffusion-controlled process to produce peroxynitrite, which interacts directly with lipids, DNA, and proteins. In the setting of cardiac risk factors and pathological conditions such as atherothrombosis, oxidative stress is associated with impaired NO bioavailability [202, 227,228].

It can be concluded that NO is a key molecule in the maintaining of normal vascular functions. Endothelial dysfunction represents the earliest stage in the atherosclerotic process, and also contributes to the pathogenesis of acute vascular syndromes by predisposing to plaque rupture and intravascular thrombosis.

Molecular links between oxidative stress and atherogenesis

The development of atherosclerosis is accompanied by a chronic inflammatory process of the arterial wall. The most important manifestation of the overproduction of free radical is the

expression of proinflammatory genes that are regulated directly or indirectly by ROS [229]. Such gene products include monocyte chemotactic protein 1 (MCP-1), vascular cell adhesion molecule 1 (V-CAM 1), intercellular adhesion molecule 1 (ICAM-1) and E-selectin. These molecules facilitate endothelial–leukocyte interactions and initiate early stages of atherosclerosis [229]. The increased expression of these inflammatory molecules is largely mediated through redox-sensitive transcription factors such as nuclear factor kappa B (NFκB), activator protein 1 (AP-1), early growth response protein 1 (Egr-1), and hypoxia inducible factor 1β (HIF-1β). These factors are also important for proliferative signals involved in vascular smooth-muscle-cell growth, vascular remodeling and atherogenesis [230].

Additionaly, ROS can activate mitogen-activated protein (MAP) kinases, and both receptor and nonreceptor tyrosine kinases [231].

Protection against ROS

Cell has evolved different mechanisms to protect themselves against ROS. This protection is implemented through the action of endogenous antioxidant system that includes antioxidant enzymes and endogenous antioxidants such as melatonin.

Important antioxidant enzymes presented in arteries include superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) [232].

Superoxide dismutase (SOD)

SOD plays a key antioxidant role by catalyzing the dismutation of $O_2^{-\bullet}$ into oxygen and hydrogen peroxide. For the proper action this enzyme needs cofactors (a metal in the catalytic center). There are three forms of the enzyme in humans. SOD1 c (Cu–Zn–SOD) is located in the cytoplasm, SOD2 (Mn–SOD) in the mitochondria, and SOD3 (Cu– Zn–SOD) is extracellular. In the cardiovascular system, the action of SOD3 lowers supeoxide radical $O_2^{-\bullet}$ and maintains vascular NO level [233, 234]. Extracellular superoxide dismutase may be crucial for the vasodilating activity of extracellular NO by controlling the levels of extracellular $O_2^{-\bullet}$ and preventing the formation of peroxynitrite. The balance between the $O_2^{-\bullet}$ producing oxidases and SOD activities keeps basal $O_2^{-\bullet}$ concentrations below the range in which this species can directly interfere with vascular signaling [235].

Glutathione peroxidase (GPx)

GPx is a selenium-containing antioxidant enzyme. The eight forms of glutathione peroxidase (GPx1- intracellular, GPx2- gastrointestinal, GPx3- extracellular, GPx4- hydroperoxides degrading, selenoproteins P and W, iodotironine deiodinase and mitochondrial selenoprotein) has been described so far. These enzymes responsible for lipid hydroperoxides and H_2O_2

removal [236]. The GPx/glutathione system is appeared to be a major defense in low-level oxidative stress [232].

Catalase (CAT)

The enzyme catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen. CAT is very effective in high-level oxidative stress and protects cells from hydrogen peroxide produced within the cell. The enzyme is especially important in the case of limited glutathione content or reduced glutathione peroxidase activity and plays a significant role in the development of tolerance to oxidative stress in the adaptive response of cells [237]. Catalase needs iron or manganese as a cofactor.

The action of endogenous antioxidant system is visualized in Fig. 5.

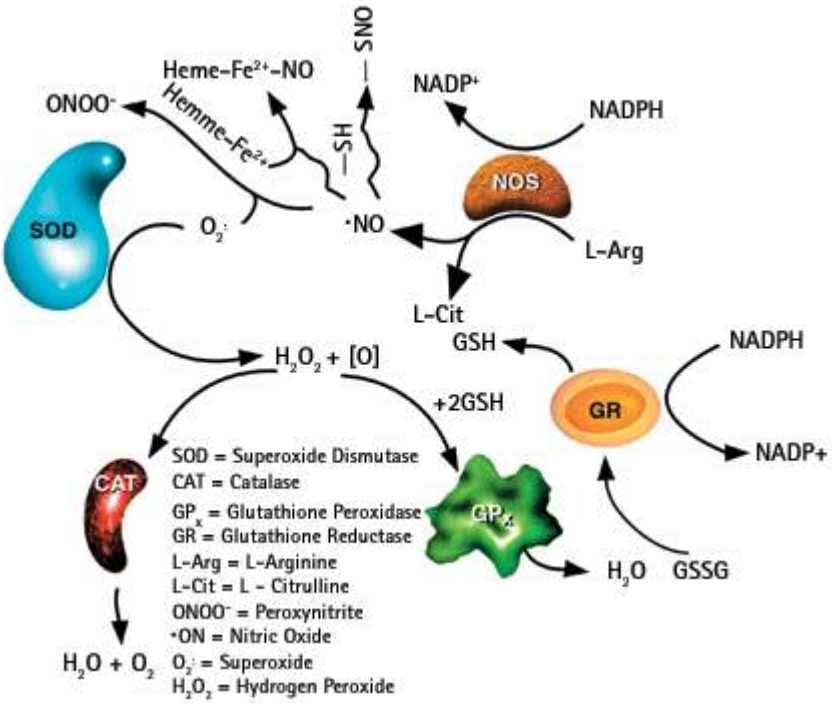


Fig. 5 . Inactivation of reactive oxygen spices by endogenous antioxidant enzyme system

A paradox of metabolism is that while the vast majority of life requires oxygen for its existence, molecular oxygen is a highly reactive molecule that can damage living organisms by conversion to its partially reduced forms, the ROS [238]. Consequently, organisms contain a complex network of low-molecular-weight antioxidant molecules and specific antioxidant enzymes that modulate redox state and prevent oxidative damage of cellular components. In

general, antioxidant systems either prevent ROS from being formed, or remove them before they can damage vital components of the cell [239]. Nonenzymatic antioxidant molecules include uric acid, ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), glutathione (GSH), polyphenolic compounds and mineral serving as cofactors for many enzymes [240].

Nowadays foodstuffs are considered as an important source of essential compounds for living cells that may help them to cope with the excess of ROS.

Modern knowledge of the nature and the bioavailability of dietary antioxidants allow us to assume that food beside phenolics and vitamins also provides minerals that are able to activate endogenous antioxidant enzymes being as their cofactors.

The benefits of natural antioxidant compounds toward endogenous antioxidant system are summarized in the next chapter.

2.3 Endogenous antioxidant system and potential benefits of natural antioxidant compounds.

Many epidemiological studies, including observational studies and randomized controlled trials, have examined the relationship between antioxidants and incidence of CVD [1]. Generally, it was shown that vitamins, minerals and polyphenols intake is associated with the reduction in the incidence of the CVD. However, the results and conclusions of these studies are not consistent. This is due to the fact that in many of these clinical trials there are enormous clinical design problems, methodologic flaws, varied patient population, variable dose and type of vitamin use, improper selection of vitamin used and many other issues that make the studies difficult to interpret [241].

The antioxidant hypothesis

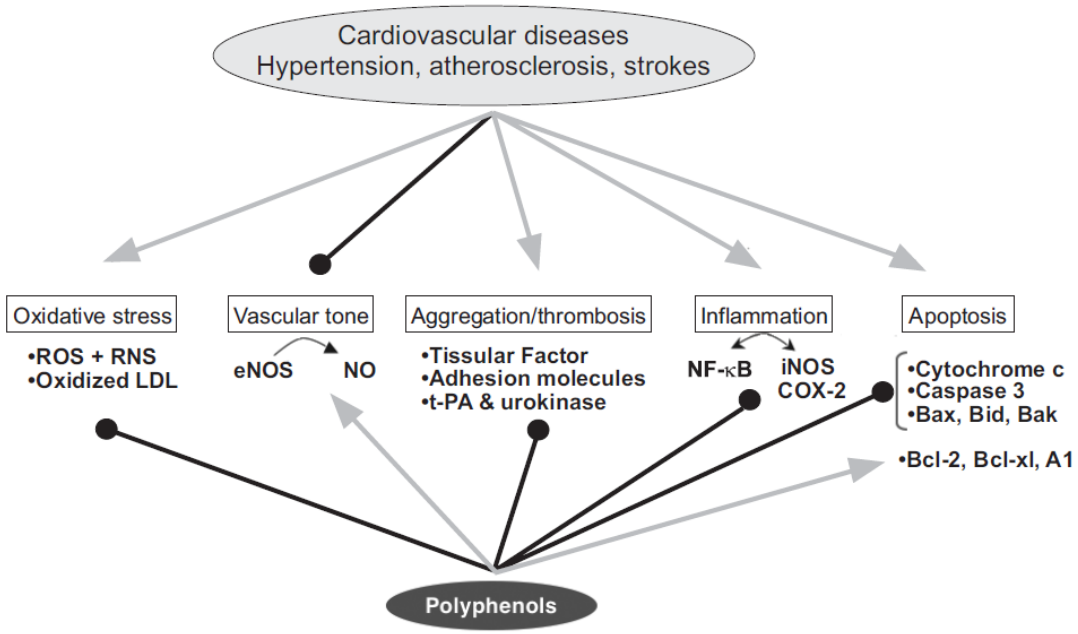
The association between fruit and vegetable intake and reduced risk of chronic disease has been proven by the years of experimental works, large scale clinical trials and epidemiological studies.

Terms such as “French paradox”, and Mediterranean diet have related the reduction of incidence of cardiovascular diseases to the certain type of foodstuffs in the diets.

Previous parts of this thesis provided a great body of evidence about protective effects of plant derived bioactive compounds.

Among all bioactive compounds, polyphenols has attracted great interest from the scientific community due to their abundance in the dietary sources.

Molecular targets of polyphenols that related to their physiological effects are summarized in the Fig.6.



Grey arrows represent a stimulatory effect; black lines represent inhibitory effects.

Fig. 6. Cellular and molecular targets and polyphenols against cardiovascular disease.
ROS- reactive oxygen species, RNS- reactive nitrogen species, LDL- low-density proteins, NOS- endothelial nitric oxide synthase, NO- nitric oxide, t-PA- tissue plasminogen activator, NF-kB- nuclear factor-kB, iNOS- inducible nitric oxide synthase, COX-2- cyclooxygenase-2.

3 Aims of the Ph.D. thesis

This thesis covers two main areas. The first part of experiments is devoted to the isolation, assessment and finding the relationships between the bioactive compounds content and the antioxidant properties in the most consumed foodstuffs in western populations (red wine and potatoes).

The second part was focused on the application of red wine extract on the models of experimental animals with the spontaneous hypertension and their comparison with the normotensive animals.

Here analysis of physiological parameters such as blood pressure development, activity of endogenous antioxidant enzymes (SOD) and nitric oxide release was performed.

The specific aims of this study were following:

A. To find the appropriate methods and techniques that allow to test the hypothesis that antioxidant capacity has a direct relationships with the total polyphenolic content, and to investigate whether this relationship occur in different dietary sources.

B. To evaluate synergistic reactions among bioactive compounds presenting in red wine

C. Using of 2 models of experimental animals to verify hypothesis that polyphenols and other bioactive compounds from the red wine extract are able to affect the physiological parameters as blood pressure, superoxide dismutase activity (SOD) and the nitric oxide synthase activity, the producer of nitric oxide, a factor of vasodilatation.

4 Materials and methods

4.1 Chemicals

Gallic acid, chlorogenic acid, catechin, epicatechin, caffeic acid, gallic acid, quercetin, 4-hydroxybenzoic acid, ABTS radical and Trolox were purchased from Sigma-Aldrich, SOD assay kit (Fluka, Germany), Folin-Ciocalteau reagent, 2,4,6–Tri(2- pyridyl)-s-triazine (TPTZ) was from (Fluka, Germany), commercial set Randox TAS (radical ABTS, Randox Laboratories, Antrim, United Kingdom) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, All the other reagents were of analytical grade.

The examined Cabernet Sauvignon and Merlot wines were purchased in several supermarkets and wine shops in Prague. Samples were selected to be representative of the most consumed foreign wines in the Czech Republic. The selecting criteria for the samples were to find monovarietal wines that are widespread in different parts of the world but not planted in the Czech Republic. Cabernet Sauvignon and Merlot wines fit this criteria and comparative evaluation of their constituents was performed. The alcohol content ranged from 11.5% to 13.5% and 0% in Carl Jung dealcoholised Merlot sample.

The wines examined for the treatment of experimental animals were obtained from the Slovak State Institute of Viniculture (Modra, Slovakia). Samples of Alibernet red wine variety were subjected to the process of dealcoholization, and concentration. Finally, an alcohol free ten times concentrated Alibernet red wine extract was prepared.

Twelve potato samples of different varieties with diverse colored pulps such as there of yellow, three of red and six of blue tuber flesh color were evaluated from two agriculture areas in the Czech Republic.

4.2 Methods and equipment

4.2.1 Measurement of Total Antioxidant Capacity

In the wine samples, TAC was measured using spectrophotometric assays on a UV-visible spectrophotometer (PharmaSpec UV- 1700, Shimadzu, Japan). The software parameters were as follows: Shimadzu UV-Probe, Version 2.00-Photometric. TAC was determined using

Trolox equivalent antioxidant capacity (TEAC) assay [242], the ferric reducing ability of plasma (FRAP) assay [243], by DPPH assay [244] and by the commercial kit Randox TAS. All the assays expressed antioxidant power in Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalents (mmol/l).

4.2.2 Measurement of Total Phenolic Content

Total phenolic content was determined using spectrophotometric assay on a UV-visible spectrophotometer (PharmaSpec UV- 1700, Shimadzu, Japan) and was measured using Folin-Ciocalteau reaction [245]. The absorbance was determined at 765 nm using gallic acid as the standard. TPC expressed in gallic acid equivalents (GAE).

4.2.3 Determination of minerals

The red wines elemental composition was determined by AAS method using acetylene/argon flame in Atomic Absorption Spectrometer (Varian Spectra AA 220 FS, Australia) for copper, zinc, selenium and lead, ISE (Ion Selective Electrode) for potassium determination, and Photometry (Roche equipment, Modular E 170, Switzerland) for calcium, magnesium, phosphorus and iron determination.

4.2.4 Measurements of Riboflavin (B_2) and Pyridoxine (B_6) content

Both vitamins determined by HPLC Fluorescent Detection using RECIPE complete set (Recipe Chemicals + Instruments GmbH Munich, Germany). The HPLC system (ECOM ltd., Prague, Czech Republic) was equipped with a pump (ECOM), fluorescence detector (ECOM) and HPLC column included in the kit. The injection volume was 20 μl and the flow rate 1 ml/min. Riboflavin was measured directly with fluorescence detection using excitation and emission wavelengths at 450 nm and 530 nm respectively. Pyridoxine was measured directly with fluorescence detection using excitation and emission wavelengths at 370 nm and 470 nm respectively. Clarity software version 1.5 was used for quantification of the peak areas.

4.2.5 HPLC analysis of single phenolic compounds in red wines and potatoes

The content of gallic acid, chlorogenic acid, catechin, epicatechin,caffeic acid, gallic acid, quercetin and 4-hydroxybenzoic acid was measured with HPLC. HPLC analysis was performed with a Dionex Summit Chromatograph (P680 pump, Ultimate 3000 Photodiode Array Detector, Autosampler ASI-100 driven by Chromeleon) equipped with 150 mm × 4.6 mm Agilent Eclipse XDB-C8 5μm column. Elution was carried out with two solvents: 5 mM KH2PO4 in methanol (A), KH2PO4 in demineralised water (B). The samples were eluted for 20 min at a flow rate of 1 ml per min, with gradient. The column temperature was 20°C and UV detection was ranged from 280 to 325 nm.

4.2.6 HPLC analysis of catechins in animal plasma

Plasma catechin concentration was measured by HPLC Dionex Summit Chromatograph (P680 pump, Ultimate 3000 Photodiode Array Detector, Autosampler ASI-100 driven by Chromeleon) coupled with fluorescence detector, as described by Carando et al. [246]. A LiChrosphere C_{18}, 4 mm × 250 mm, 5 μm particle size analytical column was used as the stationary phase. The mobile phase was heated to 40 °C, delivered at 0.5 mL/min and consisted of two solvents: solvent A = 50 mmol/L ammonium dihydrogen phosphate ($NH_4H_2PO_4$) at pH 2.60, solvent B = 20% solvent A in acetonitrile by Donovan et al. [247]. Briefly, 500 μL of plasma was mixed with 25 μL ascorbic acid–EDTA solution (20% w/v ascorbic acid, 0.1% w/v EDTA), 25 μL of β-glucoronidase and sulphatase (Sigma G-7017) and 250 μL of 0.6 mol/L $CaCl_2$, incubated at 37 °C for 45 min, in order to release conjugated forms of catechin. After incubation, the sample was treated with 700 μL of acetonitrile, and centrifuged at 5000 × *g* for 5 min, in order to precipitate proteins. The supernatant was filtered through 0.45 μm pore size membrane and 25 μL of the sample was injected into HPLC system. Catechin was determined by fluorescence detection at an excitation wavelength of 280 nm and an emission wavelength of 310 nm. All measurements were done in triplicate. Results are expressed as micrograms of catechin per liter.

4.2.7 SOD activity determination

The SOD activity was analyzed using the SOD Assay kit (Fluka). Tissues from the left ventricle and aorta were used as 0.5% and 1% homogenates in the assay. For assay, 200 µl of working solution (WST-1), 20 µl of dilution buffer / standard or sample were mixed in each well. Finally, 20 µl of Enzyme Working Solution was added to each sample and then mix thoroughly. Three blanks were used in this assay: B1 (blank to water, 20 µl of double distilled water), B2 (sample blank – blank to each sample without enzyme) and B3 (enzyme blank – blank without sample). The plate was incubated at 37 °C for 20 minutes and the absorbance was read at 450 nm using a microplate reader (Thermo Scientific Multiscan FC, Finland). SOD activity was calculated using activity of SOD standards and results were expressed in U/ml in plasma and/or U/mg of protein in tissues.

4.2.8 NOS activity determination

NO synthase activity was determined in crude homogenates of the LV, aorta and kidney by measuring the formation of [^{3}H]-L-citrulline from [^{3}H]-L-arginine as previously described by Bredt and Snyder [248] with minor modifications by Pechánová et al. 1997 [249]. Samples were measured in Quanta Smart TriCarb Liquid Scintillation Analyzer (Packard Instrument company, Meriden, CT). NO-synthase activity was expressed as pkat/g of proteins.

4.2.9 Measurement of superoxide level.

The superoxide (O_2^-) was evaluated using Lucigenin Enhanced Chemiluminiscence [250]. Samples of the aorta or left ventricle were stored in Krebs-Henseleit buffer on ice until measurement. Aorta and left ventricle were cut to small pieces up to 15 mg wet weight. Before measurement tissues were equilibrated 20 min at 37 ^{o}C Krebs-Henseleit buffer bubbled with pneumoxid. 50 µmol/l solution of lucigenin in Krebs-Henseleit buffer at 37^{o}C was adapted in dark 10 min and the background chemiluminiscence was measured for 5 min. Sample of aorta or left ventricle was added to lucigenin solution and measured each 30s during 5min in Turner Designs TD-20/20 luminometer and expressed as RLU/mg tissue.

4.3 Animals and treatment

The entire procedures and experimental protocols were approved by the Ethical Committee of the Institute of Normal and Pathological Physiology SAS, and conform to the European Convention on Animal Protection and Guidelines on Research Animal Use. Experimental animals were 6 weeks males old from following groups: normotensive Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). These animals were treated with red wine extract (24,2 mg/kg/day) for 3 weeks. The extract was given in tap water. To ensure that each animal received the complete dose of polyphenolic substances from the Alibernet extract, the calculated amount of liquid extract was given to each cage in the appropriate volume of water and adjusted to the animal's water consumption. Daily water consumption was estimated individually for every animal 1 week before the experiment. During the experiment, water consumption was controlled. All animals were housed as four in one cage at a temperature of 22–24ºC and fed with a regular pellet diet ad libitum.

Systolic blood pressure (SBP) was measured by a noninvasive method of tail-cuff-plethysmography twice a week.

After 3 weeks of treatment, the animals sacrificed and the body weight (BW), heart weight (HW), left ventricle weight (LVW) and right ventricle weight (RVW) were determined. Samples of the heart (LV), aorta and kidney were used to determine NO synthase activity and superoxide dismutase activity (SOD). The plasma samples were immediately frozen and taken for analysis.

4.4 Statistics

In chemical experiments were used the following methods of data processing. Data are presented as mean values ± standard deviation (SD; $n = 3$). The statistical significance was evaluated by Pearson‘s test, which is suitable for small numbers of samples, using GraphPad Prism version 4 software (GraphPad Software, San Diego, California, USA).

Regarding the animal studies ANOVA 6.0 (Duncan test) statistical software was employed.

5 Results

In several studies we examined the biochemistry of foodstuffs such as wines and potatoes, and the experimental trials with the feeding of animals with the red wine extract were done.

5.1 Analysis of antioxidant properties, total phenolics, mineral and selected vitamins content of red wines and red wine extract.

The specific aim of this study was to verify the hypothesis that antioxidant capacity has direct relationships with the total polyphenolic and mineral content, and to investigate that this relationship occurs in different dietary sources.

A number of experiments with red wine and potatoes were done to proof the statement that total antioxidant capacity (TAC) of foodstuff has strong relationship with the total phenolics content (TPC) and in some extent to mineral content. The study also confirmed that this occurs in different dietary sources, e.g. in wines, wine extract and potatoes.

The set of wines comprised of Cabernet Sauvignon and Merlot wines, from different places of origin. Different grape varieties were also chosen to find out the possible influence of grape variety on the TAC in red wines. The samples of wines subjected to the study are visualized in the Table 1.

Table 1. Samples of red wines Cabernet Sauvignon and Merlot subjected to the study.

Wine	Grape variety	Origin	Year	Alcohol content %
1. Finca del Mar	Cabernet Sauvignon	Spain, Valencia	2005	12.5
2. Castel	Cabernet Sauvignon	France, Pays d'Oc	2005	12.5
3. Western Cellary	Cabernet Sauvignon	USA, California	2004	12.5
4. Pinewood Hill	Ruby Cabernet	USA, California	2004	13.5
5. Santa Regina	Cabernet Sauvignon	Chile	2003	13.5
6. Hardy's	Cabernet Sauvignon	South-Eastern Australia	2004	13.0
7. Finca del Mar	Merlot	Spain, Valencia	2005	12.5
8. Castel	Merlot	France, Pays d'Oc	2004	12.5
9. Carl Jung	Merlot	Germany	2005	0
10. Cielo	Merlot	Italy	2005	11.5

For the red wine total polyphenolic analysis a Folin Ciocalteau method were chosen. This method is world-wide recognized for the determination of the total polyphenolic content in food sources. Antioxidant capacities in the set Cabernet Sauvignon and Merlot wines were determined using two different assays of TEAC and FRAP. The results were expressed in trolox equivalents. This approach allows comparing TAC values between each of the wine samples.

Total antioxidant capacity (TAC) and total phenolic content (TPC) of the Cabernet Sauvignon and Merlot red wine samples are presented in Table 2.

Table 2. Total Antioxidant Capacity and Total Phenolic Content of Red Wine samples

Wine	Grape variety	Total Antioxidant Capacity TEAC assay (Trolox mmol/l)	Total Antioxidant Capacity FRAP assay (Trolox mmol/l)	Total Phenolic Content (mg/l GAE)
1	Cabernet Sauvignon	10.5 ± 0.2	9.5 ± 0.2	2414 ± 11
2	Cabernet Sauvignon	16.6 ± 0.4	15.2 ± 0.5	2912 ± 26
3	Cabernet Sauvignon	7.7 ± 0.3	7.0 ± 0.1	1453 ± 16
4	Ruby Cabernet	10.9 ± 0.5	9.6 ± 0.1	2118 ± 19
5	Cabernet Sauvignon	9.9 ± 0.3	9.1 ± 0.1	2365 ± 12
6	Cabernet Sauvignon	11.7 ± 0.6	10.8 ± 0.1	2168 ± 27
7	Merlot	8.9 ± 0.2	8.1 ± 0.1	1737 ± 16
8	Merlot	11.2 ± 0.5	9.7 ± 0.2	2100 ± 25
9	Merlot	9.9 ± 0.2	9.1 ± 0.1	2081 ± 22
10	Merlot	7.5 ± 0.1	6.9 ± 0.1	1447 ± 21
	Intra-assay repeatability (RSD in %, $n = 3$)	5.1	3.2	1.5
	Limits of detection	-	-	3.0

Data are expressed as mean values ± SD ($n = 3$).

Antioxidant activity of red wines measured by TEAC assay ranged between 7.8-16.6 mmol/l in Cabernet Sauvignon samples and 7.5-11.2 mmol/l in Merlot wines. A second method a FRAP assay was also used to assess the antioxidant capacity of red wine samples. According to FRAP assay TAC values ranged between 7.0-15.2 mmol/l in Cabernet Sauvignon wines and 6.9-9.8 mmol/l in Merlot samples. Results from FRAP assay were slightly lower compared to those from TEAC. These small differences in antioxidant values are caused by the TEAC and FRAP methods used for detection. In order to justify and compare the TAC values evaluated by these two different assays a statistical analysis was performed. We have found a strong positive correlation between the TAC values, evaluated by the TEAC and a FRAP assay, with the coefficient of correlation for the 10 pairs of samples ($r = 0.9963$ and $p < 0.0001$).

The total phenolic content of the wine samples determined by using the Folin-Ciocalteau colorimetric method varied from 1447 mg/l in Merlot wines to 2912 mg/l of gallic acid equivalents (GAE) in Cabernet Sauvignon wine samples.

Present research has established that the highest concentrations of polyphenols were detected in French Cabernet Sauvignon 2912 mg/l (wine #2) and Spanish Cabernet Sauvignon 2414 mg/l (wine #1). Lowest concentrations were in Italian Merlot 1447 mg/l (wine #10) and Chilean Cabernet Sauvignon 1453 mg/l (wine #3).

Mean values were used in the comparative analysis of total polyphenol concentrations in Cabernet Sauvignon and Merlot red wine samples. Cabernet Sauvignon and Merlot wines

contain 2238 ± 477 mg/l GAE in average ± SD and 1841 ± 311 mg/l GAE in average ± SD respectively. Total antioxidant capacity showed resembled values higher in Cabernet Sauvignon samples compared to Merlot wines.

Relationship between Antioxidant Capacity and Total Polyphenols in red wines subjected to the experiment.

A significant positive relationship was observed between TAC and TPC values. The total antioxidant capacities based on TEAC and FRAP assays showed strong positive correlation with the Folin-Ciocalteau's total phenolic content ($r = 0.88$ and $p < 0.001$ for TEAC assay) and ($r = 0.89$ and $p<0.001$ for FRAP assay respectively). These finding are visualized in Fig. 7.

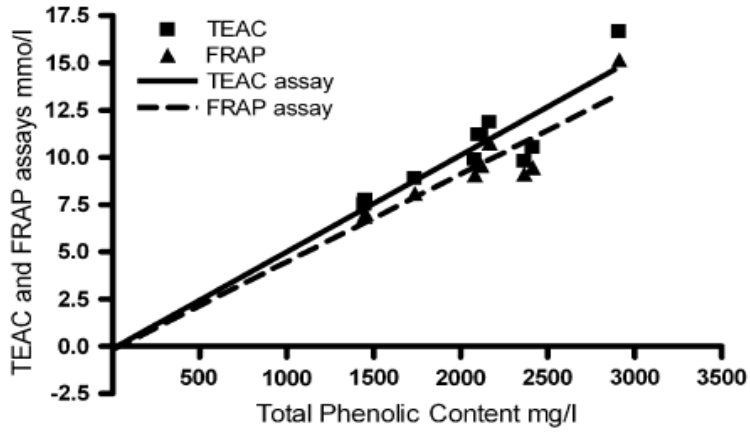


Fig. 7 Correlation between Antioxidant Capacity and Total Polyphenols.

Scatter plot derived from Pearson test, illustrate the statistical correlation between total antioxidant capacity and total phenolic content. TEAC assay: $y = 0.005119 \pm 0.0009553$, $R^2 = 0{,}7821$; FRAP assay: $y = 0.004645 \pm 0.0008290$, $R^2 = 0{,}7970$.

Elemental content in red wines analyzed.

The concentrations of determined mineral elements are different among wines subjected to the study (Table 3).

Table 3. Elemental profile of Cabernet Sauvignon and Merlot wine samples

Wine	Grape variety	K (mg/l)	Mg (mg/l)	P (mg/l)	Ca (mg/l)	Fe (mg/l)	Cu (mg/l)	Se[a] (µg/l)	Pb[a](µg/l)	Zn (mg/l)
1	Cabernet Sauvignon	1231.7	102.6	121.5	57.7	ND	0.2	20.2	14.0	0.4
2	Cabernet Sauvignon	1139.4	89.7	143.2	56.5	ND	0.1	31.5	46.4	0.8
3	Cabernet Sauvignon	1175.4	122.7	245.5	74.5	1.9	0.1	30.0	28.5	0.8
4	Ruby Cabernet	1486.6	106.9	296.7	62.1	1.1	ND	33.6	9.6	0.8
5	Cabernet Sauvignon	1566,0	122.5	251.4	72.1	0.9	0.2	42.0	4.9	0.8
6	Cabernet Sauvignon	1157.4	146.8	266.9	77.0	2.6	0.2	35.0	5.4	1.4
7	Merlot	1092.5	101.8	148.8	64.9	ND	ND	35.8	20.5	0.7
8	Merlot	1054.5	92.6	122.5	68.1	3.1	0.1	23.8	41.1	0.6
9	Merlot	1177.3	117.1	76.9	94.6	0.4	0.1	40.0	9.6	0.3
10	Merlot	1105.0	96.5	131.8	89.4	2.7	0.2	30.7	42.4	0.6
	Intra-assay repeatability (RSD in %, $n = 3$)	1.2	1.4	1.4	1.1	6.3	7.2	4.5	3.5	6.8
	Limits of detection	39.1	0.7	3.1	2.0	0.005	0.02	9.0	3.0	0.06

Data are expressed as mean values ($n = 3$). ND, not detected.

[a] Se and Pb were expressed in µg/l.

The most predominant element detected in all wine samples was Potassium (K). At the same time potassium concentrations were a little higher in Cabernet Sauvignon compared to Merlot. Among other major determined elements were zinc (Zn), phosphorus (P) and magnesium (Mg). Iron (Fe) and copper (Cu) were in several cases below the detectable level. The analysis of the mean values were used in order to find differences in mineral content between red wines of two grape varieties investigated in the current study. This analysis showed that Cabernet Sauvignon wines contain higher levels of potassium (K), magnesium (Mg), phosphorus (P), copper (Cu) and zinc (Zn) compared to Merlot wines.

Vitamins content in examined red wines

Red wine is a source of water-soluble vitamins. Current study determined Riboflavin (B_2) and Pyridoxine (B_6) in all red wine samples. Cabernet Sauvignon had a higher concentration of Pyridoxine (mean value of 23 µg/l $\pm$ 18.8 SD) than Merlot. At the same time, Merlot wines contained higher levels of Riboflavin (mean value of 68 µg/l $\pm$ 16.0 SD) in comparison to Cabernet Sauvignon.

The study of red wine and red wine extract from Alibernet grape variety

Total antioxidant capacity and phenolic content of Alibernet extract.

In this study, samples of Alibernet red wine and extract were examined on total antioxidant capacity (TAC) and total phenolic content (TPC). The results are visualized in Table 4.

Table 4. Antioxidant capacity and phenolic content in red wine and red wine extract.

Sample	TAC	Total phenols
	mmol/l	GAE mg/l
Alibernet wine	35,82	2039± 0,55
Alibernet extract	376,38	24172± 1,26

Antioxidant activity of Alibernet red wine was slightly above 35 mmol/l whereas in Alibernet extract it reaches 376 mmol/l. The results of TAC of red wine and the red wine extract confirm that ten times higher concentration was achieved at the extract. It has been also proven that the process of extraction has not affect the antioxidant properties of the red wine extract.

The total phenolic content of the wine samples determined by using the Folin-Ciocalteau colorimetric method was 2039 mg/l in Alibernet wines and above 24172 mg/l of gallic acid equivalents (GAE) in Alibernet red wine extract samples.

Present research has established that dealcoholised Alibernet red wine extract has more than 11 fold higher Total antioxidant capacity and Total phenolic content compared to Alibernet red wines. Moreover, TAC and TPC values are higher in red wine extract at the same extent. This confirms that extract has preserved all essential properties of wine. This also reveals a significant positive relationship between total antioxidant capacity and the total phenolic content. For determination of the principal phenolic compounds in the Alibernet red wine extract the HPLC analysis was employed. Among the main phenolic compounds were epichatechin and catechin, gallic and caffeic acids. The results are visualized in the Table …

Table 5. HPLC determination of single polyphenolic compounds in Alibernet red wine and extract

Sample	Catechin	Epicatechin	Caffeic acid	Gallic acid	Quercetin	4Hydroxybenzoic acid	Chlorogenic acid
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
Alibernet wine	35,5	17,7	31,0	38,4	29,6	6,3	26,0
Alibernet extract	997,0	1083,7	300,7	452,1	36,2	224,3	177,3

As far as mineral elements are essential for the proper functions of many enzymes and tissues the comprehensive analysis of micro and macro elements were performed.

Potassium (K) was found as a major element detected in Alibernet wine and extract. Interestingly, magnesium concentration in the Alibernet extract is ten times higher compared to red wines, that opens up possibility to consider its concentration relevant to exert physiological effects in experimental animals. Zinc concentration in extract was more than 5,4 g/l, considering the fact that Zn is a co-factor of superoxide dismutase, we could expect it's possible effects toward SOD activity. Other determined elements as phosphorus (P), copper (Cu) and selenium (Se) were in several cases below the detectable level.

The analysis of the mineral profile of red wine extract led us to the conclusion wines, that it was contain approximately ten times higher levels of potassium (K), magnesium (Mg), phosphorus (P), calcium (Ca) and zinc (Zn) compared to Alibernet red wine.

The elemental profile of the Alibernet red wine and wine extract is provided in the Table 6.

Table 6. Elemental content in the Alibernet red wine an d the wine extract.

Sample	K mg/l	Mg mg/l	P mg/l	Ca mg/l	Fe mg/l	Cu mg/l	Se µg/l	Pb mg/l	Zn mg/l
Alibernet red wine	1387,01	131,14	108,97	69,54	1,54	<0,12	<711	<6,22	354,30
Alibernet extract	9069,51	1334,07	<6,2	657,31	24,60	0,26	<711	7,83	5463,92

5.2 Determination of antioxidant capacities and phenolic content in potatoes.

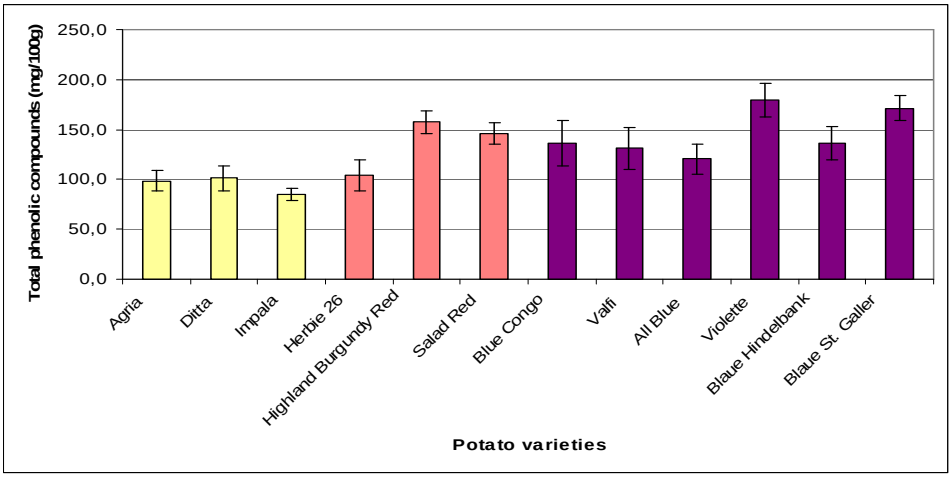
Being a most consumed vegetable of human daily food consumption, potato has attracted considerable interest as a potential source of dietary antioxidants. Current study includes a comparative analysis of antioxidant properties of yellow, red and blue potatoes typical for the Czech Republic with the relation to their phenolic content in fresh, cooked, cooked in steam, microwave heated and fried potato samples. The objective of this experiment was to find the relationship between antioxidant capacity, total phenolic content and the potato variety. In addition, the impact of cooking procedures on antioxidant capacity of potatoes was studied.

The determination of phenolics in potato resulted in the finding that, among polyphenolic compounds presented in potatoes chlorogenic acid was detected in the highest amounts. Other phenolic substances such as caffeic and protocaffeic acids also occur in small numbers in potatoes. However, the amounts of these compounds are tenfold lower, compared to cholorogenic acid. This may be the reason for the fact that only cholorogenic acid could be determined by the HPLC. Due to this fact the results of total phenolic content in potatoes subjected to the study were expressed in chlorogenic acid equivalents. All potato samples subjected to the study are visualized in the Table 7.

Table 7. List of potato samples.

Sample Number	Cultivar	Tuber flesh colour	Agriculture areas
1	Agria	yellow	Havlíčkův Brod
2	Ditta	yellow	Havlíčkův Brod
3	Impala	yellow	Prague -Suchdol
4	Herbie 26	Red	Havlíčkův Brod
5	Highland Burgundy Red	Red	Prague -Suchdol
6	Salad Red	Red	Havlíčkův Brod
7	Blue Congo	Blue	Prague -Suchdol
8	Valfi	Blue	Havlíčkův Brod
9	All Blue	Blue	Havlíčkův Brod
10	Violette	Blue	Prague -Suchdol
11	Blaue Hindelbank	Blue	Prague -Suchdol
12	Blaue St. Galler	Blue	Havlíčkův Brod

Total phenolic content in 12 potato cultivars were following, the lowest in potatoes with a yellow flesh: from 80 to 100 mg/100 g, compared to 110-150 mg/100 g in red flesh varieties. The highest phenolics content up to 180 mg /100 g was detected in potatoes with blue flash. Greater content of total phenols in the varieties with of colored flesh was associated with a high proportion of oenocyanin, which does not occur in potatoes with white or yellow colored flesh, as visualized in Fig. 8.



Data are expressed as mean ± SD (n = 3).

Fig. 8 The content of total phenolic compounds in different potato varieties.

Total antioxidant capacity in order to eliminate possible discrepancies was determined by both the ABTS assay and the DPPH assay in all potato samples subjected to the study. Statistical analysis provided strong positive correlation (r = 0.93 and p < 0.0001) for the assays.

Strong positive relationships between the total antioxidant capacity (TAC) and the phenolic content were found in all potato cultivars. The significance of these relationships was for DPPH assay (r = 0.87, p < 0.0002) and for ABTS assay (r = 0.71, p < 0.0097).

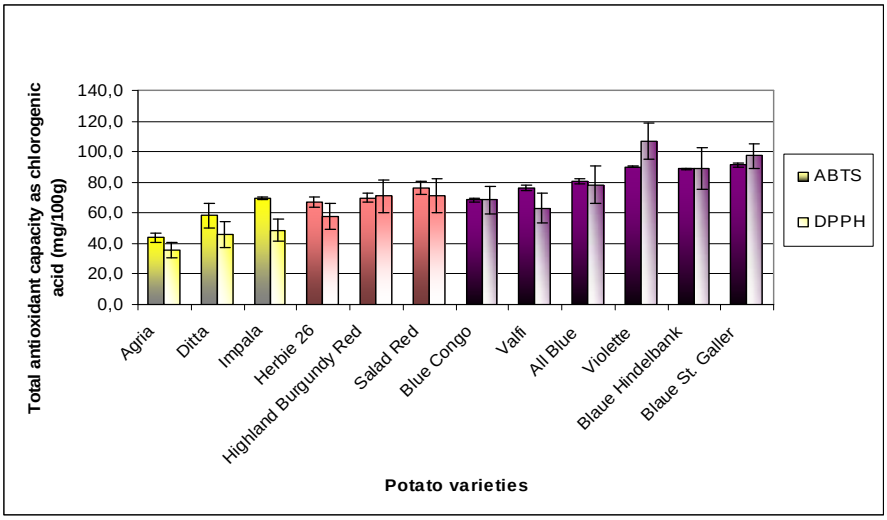
The results of statistical analysis and characteristics of all methods employed in this study are presented in Table 8.

Table 8. The correlation matrix.

r	ABTS	DPPH	TC	HPLC
ABTS	-	0,93	0,71	0,79
DPPH	0,93	-	0,87	0,91
TC	0,71	0,87	-	0,84
HPLC	0,79	0,91	0,84	-

r – linear correlation coefficient, TPC – total phenolic content.

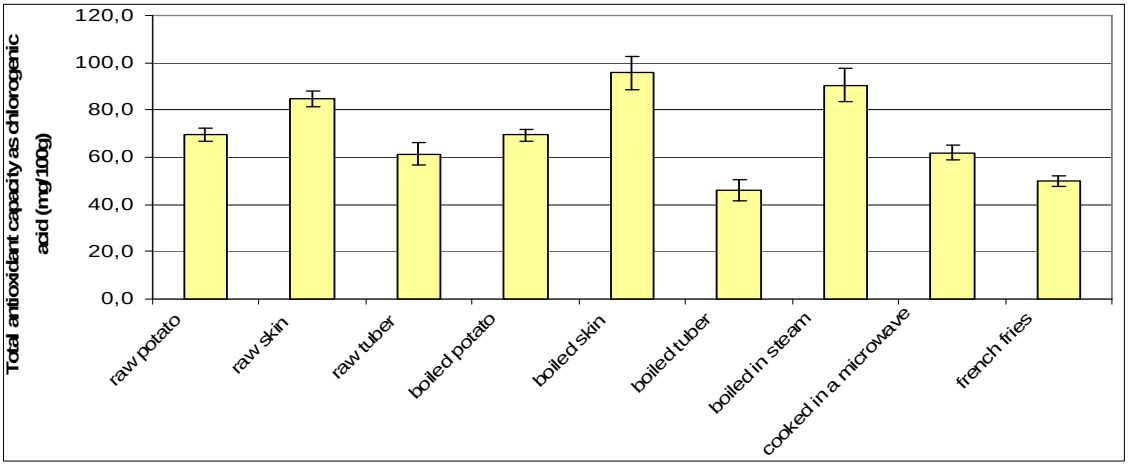
Similarly to phenolic content total antioxidant capacities of potatoes measured by ABTS and DPPH assays were higher in red and blue cultivars compared to the yellow ones. The results of total antioxidant capacities are presented in Fig. 9 and are expressed in chlorogenic acid equivalents.



Data are expressed as mean ± SD (*n* = 3).

Fig. 9. The antioxidant capacity of different potato varieties measured by ABTS and DPPH assays.

Very new results were obtained in the series of experiment evaluating the impact of different cooking procedures on the total antioxidant capacity in potato samples of Impala variety. The potato variety Impala was selected to represent one of the most consumed potatoes in the Czech Republic. It was found that total antioxidant capacity increased by 30% for Impala variety potatoes cooked in steam with the peel, compared to that of the raw tuber. When potatoes were boiled with the peel in water, no change was observed in the antioxidant capacity. All other adjustments lead to a decrease in the antioxidant capacity: when microwaved by 11%, peeled then cooked potatoes by 15%, and when fried in vegetable oil by 29% (**Fig. 10**).



Data are expressed as mean ± SD (*n* = 3).

Fig.10 Antioxidant capacity (ABTS assay) of Impala potato samples after using different cooking procedures expressed in chlorogenic acid equivalents.

5.3 *Bioavailability of catechins in spontaneously hypertensive and Wistar Kyoto animal models.*

Nowadays, bioavailability of plant bioactive compounds is a key question for discovering the mechanisms of their effects. As far as catechins are one of the major polyphenols presented in wine, and other beverages, like tea and juices the goal of this experiment was to measure it's presence in blood circulation of animals using HPLC catechin determination in plasma.

The bioavailability of red wine extract phenolics were studied in WKY normotensive rats and the rats with the spontaneous hypertension. It was found that rats treated with the red wine extract show the concentrations of the principal polyphenols catechin in plasma up to 4,7 mg/l. There were no significant differences in the plasma concentrations of catechins between normotensive (WKY) and spontaneously hypertensive rats (SHR).

For the convenience, the results of catechin concentration in plasma are given in the Table 9. , which contains data on control group and another one received red wine extract.

These results clearly show that animals that did not receive wine extract have in their plasma very low concentrations of catechins or below the detection limit either. On the contrary treated animals have shown significantly higher concentrations of catechins. The catechins concentration varied from the 1,36 mg/l to 4,77 in WKY group, and from 0,6 to 4,57 mg/l in SHR group. In pair 3 of treated WKY animals there was no result due to the lack of the plasma sample.

Table 9. Catechin concentration in plasma of WKY and SHR animals.

№	WKY mg/l of plasma	WKY extract mg/l of plasma	SHR mg/l of plasma	SHR extract mg/l of plasma
1	ND	**1,36**	0,57	**1,85**
2	ND	**4,77**	2,88	**4,33**
3	ND		ND	**4,57**
4	0,52	**1,52**	0,41	**0,9**
5	0,37	**1,51**	0,13	**1,55**
6	0,36	**2,2**	0,24	**0,6**

Listed results allows us to suggest that polyphenols are absorbed and entered into the blood circulation of experimental animals.

5.4 The effects of Alibernet red wine extract on SOD and NOS activities, and superoxide level in tissues of experimental animals with spontaneous hypertension and normotensive.

Physiological parameters of WKY and SHR animals after 3-week treatment with Alibernet red wine extract are summarized in the following table.

Table 10. Effects of Alibernet red wine extract treatment on body weight, heart weight, and blood pressure of WKYand SHRs experimental animals.

	WKY Control (n=6)	WKY red wine extract (n=6)	SHR Control (n=6)	SHR red wine extract (n=6)
Body weight (g)	249 ± 13	253 ± 11	211 ± 12*	216 ± 11*
Heart weight (g)	0,809 ± 0,044	0,841 ± 0,070	0,791 ± 0,046	0,812 ± 0,050
Blood pressure	107 ± 8	108 ± 6	167 ± 12	176 ± 14

*p< 0,05 compared to WKY

The body weight of SHRs was significantly lower compared to WKY. This parameter was not, however, affected by red wine extract treatment in both WKY and SHR.
Similarly, heart weight and blood pressure were not changed by extract treatment in WKY and SHRs.

NO synthase activity

Total NOS activity increased significantly (P<0.05) after the Alibernet wine extract (AWE) treatment of rats with spontaneous hypertension (SHR). This increase of NOS activity was observed in the left ventricle, aorta and kidney of SHRs compared to untreated SHR (Fig.11). The Alibernet wine extract did not change NOS activity in WKY rats.

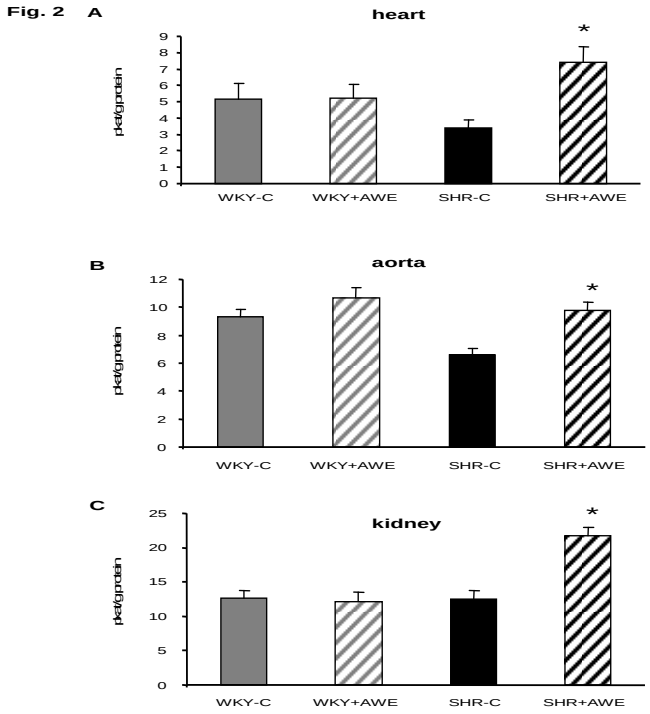


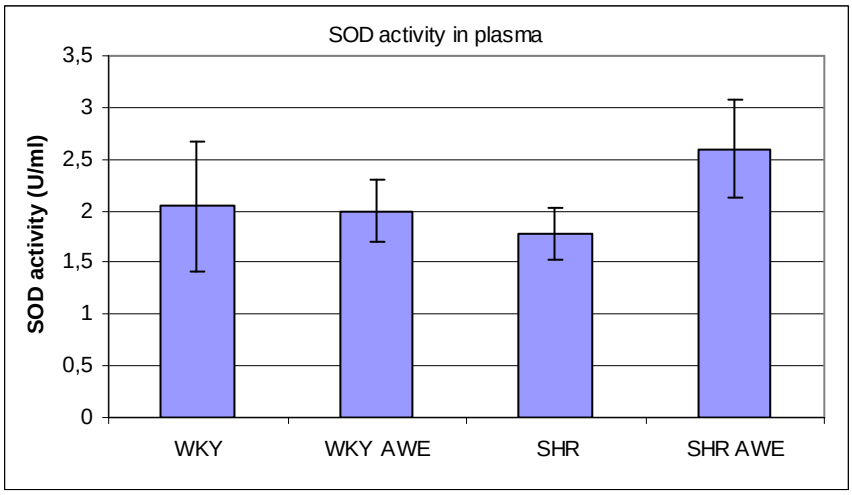
Fig. 11. NOS activity in different organs.

Legend: effect of treatment with Alibernet red wine extract, WKY and SHRs on nitric oxid synthase (NOS) in the left ventricle (LV) (a), aorta (b) and kidney (c). P< 0.05 as compared with control.

Superoxide dismutase (SOD) activity in animals with spontaneous hypertension SHR and WKY.

Changes in SOD activity in plasma.

Significant increase of SOD activity by 45% was observed in plasma of SHR after Alibernet wine extract (AWE) treatment. At the same time, in plasma of control WKY group, superoxide dismutase activity was not significantly changed. These parameters are visualized in Fig. 12. The exact numbers were also provided in Table 11.



Data are expressed as mean ± SD (*n* = 3).

Fig. 12 SOD activity in plasma of WKY and SHR groups.

Table 11. SOD activity in plasma of experimental animals.

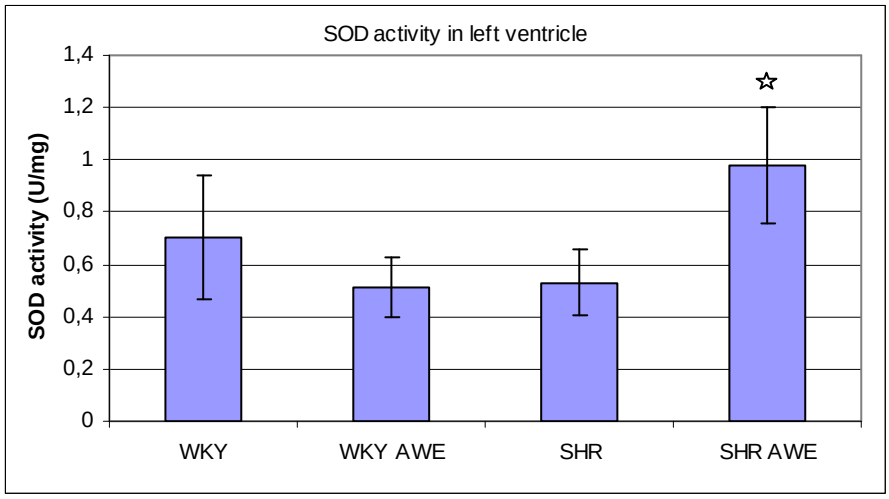
WKY U/ml±SD	**WKY AWE** U/ml±SD	**SHR** U/ml±SD	**SHR AWE** U/ml±SD
2,045±0,632	**1,996**±0,299	**1,788±0,252**	**2,598±0,470***

Changes in SOD activity in left ventricle (LV) and aorta.

Changes in SOD activity were observed in the group of animals with spontaneous hypertension (SHR) where the activity of SOD was significantly increased by 54% in the left ventricle of heart, while SOD activity was not changed in aorta.

At the same time after treatment with the Alibernet red wine extract of WKY group of animals were weren't any significant changes in SOD activity in any of the abovementioned tissues and in plasma either.

These data are visualized in the Fig. 13 and Fig. 14, and in Table 12. and Table 13.



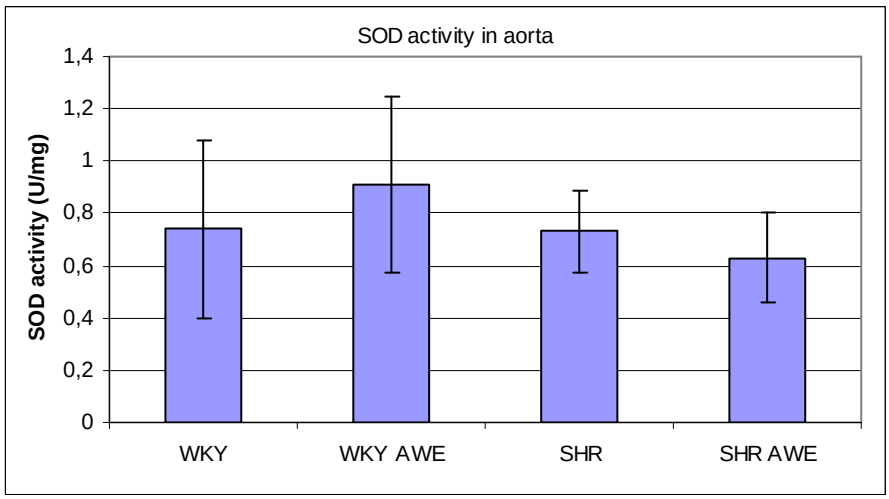
Data are expressed as mean ± SD (*n* = 3).

Fig.13 SOD activity in LV of WKY and SHR groups

Table 12. SOD activity in LV of experimental animals.

WKY U/ml±SD	**WKY AWE** U/ml±SD	**SHR** U/ml±SD	**SHR AWE** U/ml±SD
0,705±0,239	0,510±0,116	**0,529±0,127**	**0,978±0,220***

*p<0.05 SHR vs SHR Alibernet wine extract



Data are expressed as mean ± SD (*n* = 3).

Fig. 14 SOD activity in aorta of WKY and SHR groups

Table 13. SOD activity in aorta of experimental animals.

WKY U/ml±SD	**WKY AWE** U/ml±SD	**SHR** U/ml±SD	**SHR AWE** U/ml±SD
0,739±0,339	0,910±0,339	0,731±0,155	0,630±0,173

Superoxide level in left ventricle and aorta in animals with spontaneous hypertension SHR and WKY.

In this analysis superoxide level was evaluated in left ventricle (LV) and aorta. This is due to the fact that these tissues are pivotal for the imagination the whole picture of oxidative stress in the body. It has been confirmed that rats with spontaneous hypertension as well as control group WKY did not show any significant changes in the level of superoxide after Alibernet wine extract treatment.

Our findings and new results significance and possible application together with the comprehensive analysis of existing published sources on this topic are provided on the subsequent part of this thesis.

6 Discussion

Evidence suggests that a diet high in fruits and vegetables may decrease the risk of chronic disease, such as cardiovascular disease, metabolic disease, and to slowdown aging process. This is due to the plant bioactive compounds such as polyphenols and minerals that may play a key role in the reducing chronic disease risk and development of pathological alterations.

Being one of the most consumed foodstuffs in Western populations red wine and potatoes nowadays have attracted considerable attention from scientific community due to possible protective effects toward cardiovascular system. In several large scale epidemiologic studies, there were proven that positive effects in reducing oxidative stress, prevention and even treatment of cardiovascular and metabolic disorders are attributed to the polyphenols, minerals and vitamins found in dietary sources [1, 18, 19, 37, 241, 251].

Thesis provides numerous data from the experiments on the determination of phenolic and mineral content in red wines and potatoes. The application of red wine extract on two models of experimental animals of WKY and spontaneously hypertensive rats (SHR) allowed us to confirm the positive effects toward NOS activity, a producer of NO, that mediates the vasodilatation, and the activity of the SOD, an enzyme, which plays an important role in the functions of endogenous antioxidant system.

The structure of the discussion part reflects the specific aims of the thesis.

1. The relationships between the antioxidant capacity, phenolic and mineral content in red wines, red wine extract and potatoes.

At the beginning of study there were two questions to test, whether the antioxidant capacity has a direct relationship with the total phenolic content in red wines, and whether this relationship occurs in different food sources.

The results of the study with the red wines of Cabernet-Sauvignon and Merlot grape variety that also included the dealcoholised wine suggested, that polyphenols are crucial for the antioxidant capacity. It was found that Cabernet Sauvignon red wine samples had the highest concentrations of the total polyphenols simultaneously with the highest TAC among the all wines subjected to the study.

Another decisive role also play variety of grapes, this became evident from our experiments, where Cabernet Sauvignon wines possessed higher total antioxidant capacity together with the higher TPC, potassium, magnesium, phosphorus, copper, zinc and vitamin B_6 content compared to Merlot red wines.

Despite considerable variation, the data from the other experimental studies showed that wine and dealcoholized wine enhances absorption of calcium (Ca), phosphorus (P), and magnesium (Mg) and zinc (Zn). This is due to the multiple compounds presented in wine and also due to the natural acidity of wine which may play a role in creating a more favorable intraluminal environment for absorption [252-254].

This enable us to conclude that minerals presented in wine are able to reach blood circulation in required concentrations to exert their effects.

Red wine contains a number of trace elements essential for the proper function of endogenous antioxidant system.

Moreover, copper (Cu), manganese (Mn), selenium (Se) and zinc (Zn) act as co-factors of antioxidant enzymes. Despite the requirement to maintain the balance for the redox trace element such as copper (Cu), which can initiate free radical reactions at the same time it acts as a co-factor for the Cu/Zn- SOD [255].

Despite the fact that the deficiencies are uncommon, the transition to a modern Western diet has led to a substantial decline in potassium (K) intake. A high K intake has the protect effect against insulin resistance, cardiovascular disease and the development of bone pathologies though the maintaining Ca homeostasis [159, 161]. There is close relationship between potassium and magnesium.

This tight connection is illustrated in the study of Humphries et al. [256] and Huerta et al. [163] which focused on a possible protective role of dietary Mg in insulin resistance; their data also demonstrated a high degree of correlation of dietary K with insulin sensitivity. In fact, it was proposed that abnormalities in cellular ion homeostasis may be a major link between cardiovascular and metabolic diseases [257].

Interestingly, magnesium (Mg) in red wines and extracts were presented in the amounts relevant to exert the physiological effects. For instant, Mg in red wines reached concentration up to 146 mg/l, and in Alibernet red wine extract has reached concentrations up to 1337 mg/l.

These findings are in correspondence with the data in the literature published for the mineral content of wines [258-260].

These concentrations of magnesium (Mg) in the red wines and extract are the same level as in mineral waters as Magnesia and Donat Mg respectively, well known for their effects in the treatment of vascular and metabolic disorders [164, 261].

Zinc (Zn) was found as a multipurpose trace element involved besides the prevention of free radicals formation in immune responses, vascular disorders and aging.

Zn is required for structural and functional integrity of more than 2000 transcription factors [262] and 300 enzymes [263]; therefore, almost every signalling and metabolic pathway is in some way dependent on at least one, but often several, Zn-requiring proteins. Recent studies have shown that Zn plays a crucial role in endothelial NO synthase function and in NO signalling. NO synthases are catalytically active only as a dimer of two subunits, the association of which is stabilised by the tetrahedral binding co-ordination of Zn with thiol ligands at the dimer interface [264].

The activity of NO synthase is strongly inhibited by the formation of peroxynitrite, a product of superoxide radical reaction with NO, and is directly related to peroxynitrite-induced release of NO synthase-bound Zn.

Since NO synthase expression is dependent on NF-kB activation, it is also possible that Zn deficiency could influence NO synthase by this mechanism [140].

Furthermore, studies by Reiterer et al. [156] in an atherogenic mouse models confirmed in vivo evidence that zinc deficiency induced proinflammatory events.

This could be an explanation of the effects that trace elements may have influence the NO synthase activity we observed in our experiments.

Magnesium and zinc plays a crucial role in ageing processes. As far as in elderly the deficit of these minerals are the most common case of the progression of insulin resistance, cardiovascular disease and the neurodegenerative alterations in brain function, supplementation of these element seems to a optimal solution in many cases [141, 265-267].

On the base of these examples it could be assumed that minerals in red wines could affect the action of the endogenous antioxidant system, primarily the antioxidant enzyme activities, as well as tissue metabolism and cardiovascular alteration development.

This hypothesis was proved in our experimental treatment of animals with the red wine extract.

Despite the strong antioxidant activities and one of the highest content of polyphenols and other micronutrients in red wines, several studies shows that to achieve the desired concentrations of these compounds it is need to consume the big amounts of wine or grape juices. The feasible solution of this lies in the concentrates that is possible to prepare from the red wines. In our study, to settle this problem, the extract with ten times higher concentration of polyphenols and minerals was used for the treatment of experimental animals of following models: normotensive and spontaneously hypertensive animals.

Further details will be discussed in the part 3 of discussion.

Potatoes

The similar strong relationships between the antioxidant capacity and phenolic content were found in potatoes as for the red wines.

At the same time it is need to mention that the antioxidant capacities as well as total phenolc content of red wines are much higher compared to potatoes. Differences also occur in the principal phenolic compounds. The HPLC analysis has revealed that among the most abundant phenolics in red wines are anthocyanins and catechins whereas a chlorogenic acid in potatoes.

These are consistent with results published for red wines and potatoes by other investigators [268–271].

Similarly, like in red wines results for total antioxidant capacities and total phenolic content in twelve potato samples proved the strong relationships between phenolics and antioxidant properties. It was found that TAC measured by the ABTS and DPPH resembled trends as in total phenolics in all potato samples. These findings related to the relationships between the color of flash in potato and the antioxidant power is consistent with the results published by other investigators.

These results allow us to conclude that total polyphenols contribute in the great extent to the antioxidant capacities of examined foods and beverages. Moreover, this strong positive correlation between TAC and TPC seems to be universal and appears regardless of the type of the foodstuff.

2. Synergy

The synergistic interaction among the polyphenols, minerals and vitamins become the matter of concern in some recent publications. Authors compared the antioxidant activities of the single bioactive compounds with the TAC of foodstuff and beverages and revealed that the total antioxidant capacity measured in the fruits and beverage was higher than the sum of antioxidant activities of single compounds. An assumption was that this occurs due to synergistic interactions among bioactive compounds.

Boyer et al. [272] published relevant information regarding the antioxidant activities of apples. The total antioxidant activity of apples with the peel was approximately 83 μmol vitamin C equivalents, which means that the antioxidant activity of 100 g apples (about one serving of apple) is equivalent to about 1500 mg of vitamin C. However, the amount of vitamin C in 100 g of apples was only about 5.7 mg [273]. Vitamin C is a powerful antioxidant, but these authors show that nearly all of the antioxidant activity from apples

comes from a variety of other compounds. Vitamin C in apples contributed less than 0.4% of total antioxidant activity.

This example was consistent with the findings by the other authors [24, 17]. In our experiments with the red wines and the antioxidant capacities, we found that besides polyphenols certain minerals may contribute to the TAC. The results from the Cabernet Sauvignon and Merlot red wine analysis show that wine with lower phenolic content has higher TAC if it simultaneously has the highest values of Mg, Cu and Zn. However, a sample high in phenolics did not demonstrate the highest TAC when having low mineral content.

Additionally, a positive correlation between the magnesium (Mg) and zinc content (Zn) was found. This correlation was also confirmed in the case of red wine extract.

There are some evidences relating to the synergistic interactions among polyphenols. Mertens-Talcott et al. [274] experimentally verified an enhanced effect of quercetin and the ellagic acid in soft fruits, and particularly in Muscadine grape variety. Other investigators are also considering the possible interactions among polyphenols, and other bioactive compounds. In a very recent study of Peinado et al. [275] it was confirmed that polyphenols and sugars presented in musts act synergistically. It was demonstrated that polyphenols from musts are active in inhibiting radicals, while sugars were highly effective in inhibiting assays mediated by hydroxyl radical formation. So, different molecules as DNA, lipids, and sugars were protected from the oxidation by phenolics. In addition, the deleterious effects of sugars on proteins could be counteracted by phenolics.

Another important aspect of possible synergy are vitamin and minerals interactions. In our study with Cabernet Sauvignon and Merlot wines riboflavin (B_2) and pyridoxine (B_6) were determined. These two vitamins despite the low concentrations of the B vitamins found in red wines, they potentially may affect the metabolism in experimental models by their participation in the reactions of oxidation and reduction in the case of Riboflavin (B_2) and transamination and decarboxylation of amino acids in the case of Pyridoxine (B_6) [170].

These interrelationships between vitamins and minerals could be quite important and became the research topic in several studies.

The most significant example of vitamin action on mineral metabolism is the role played by vitamin D in calcium and phosphorus metabolism. The interrelationship of vitamin C and some minerals is also discussed, with emphasis on its relationship with iron [276, 15]. Vannucci et al. [276] found the possible interactions between pyridoxine and zinc. Our experiments with the red wines also confirmed the positive relationship between pyridoxine and zinc content in Cabernet Sauvignon wines.

Nowadays it is difficult to evaluate all synergistic interactions, however in recent years new analytical technologies will achieve the levels of sophistication. New scientific approaches called metabolomics (the intensive study of very small, previously undetectable metabolites), nutrigenomics and related strategies will allow to elucidate phytochemical complexes and reveal mechanisms by which bioactive compound exert their effects.

3. Animal studies

The question of bioavalability of polyphenols remains to answer in the upcoming decade. In our study catechins were measured in plasma of animals subjected to the experiment (WKY, SHR).

New analytical HPLC techniques and methods enabled us for the first time to determine the concentration of catechins in the plasma of rats with spontaneous hypertension after three weeks of experimental feeding with the red wine extract and to compare these results with the model of normotensive rats. Catechins are one of the major groups of phenolics presented in wine [29, 37].

In our several experiments it was found that catechins were detected in the plasma of rats of both models SHR and WKY treated with Alibernet red wine extract, while in rats of the control groups these substances were found in the concentration of several times lower or even have not been identified.

These data on bioavailability of catechins in our two experimental models allow us to better understand and explain the benefits that are ascribed for polyphenols for the vascular health and the functions of endogenous antioxidant system.

Some increase in catechins concentration was found after the red wine extract treatment. We assume that from polyphenols catechins maybe the main ones which affect both NOS and SOD activities.

It is possible that also another polyphenols like anthocyanins and phytoalexins have similar effects. In the relation to this statement publication of Mazza have described the impact of anthocyanins on stability and total antioxidant capacity of wines [277].

The question of bioavailability of the polyphenolic compounds now is found as a pivotal for the explaining the mechanism of the effects of the bioactive compounds on the vascular health and metabolic disorders. In the results section data on the total and single phenolic content together with the catechins concentration in plasma were given. That allows us to conclude, that polyphenols were circulated in the blood and were able to increase activities of NOS in the examined tissues.

Activities of SOD were also increased in the SHRs treated with Alibernet red wine extract which may indicate that plant bioactive compounds together with the minerals, such as magnesium (Mg) and zinc (Zn) are able to change activities of SOD. This new finding enable us to suggest that the positive effects of fruits and vegetables are related to the increase in activity of the endogenous antioxidant enzymes and enhancing the antioxidant effects. This occurs together with the increase of NO production, important for the vasodilatation and the blood flow improvement. This is particularly important in the case of hypertension, which is known with the elevated free radical and cytokine production, chronic inflammation and the suppression with the NO production.

Alibernet red wine extract increased both NOS and SOD activities only in SHRs, where the abovementioned harmful effects are described. That means that polyphenols may affect only pathological mechanisms while they have no actions in normal conditions. Similar effects of red wine polyphenols were described in L-NAME induced hypertension [76].

This enables us to suggest that polyphenols and micronutrient exert their effects only in models with the developed cardiovascular alterations.

7 Conclusions

The results of the experiments proposed in the Ph.D. thesis, allow us to make a number of important new findings on the content of plant bioactive compounds (polyphenols) in foodstuffs and their role in the antioxidant activity. In this work, a comprehensive biochemical analysis of the most consumed foodstuffs in Western diet was performed. In addition to polyphenols other micronutrients such as minerals were evaluated. Techniques and methods of integrated assessment of wines and red wine extract, enabled us to succeed in experiments with two models of animals WKY and SHR.
The conclusions are:

1. According to our results antioxidant activity is directly related not only to polyphenols but also to micronutrients, such as minerals. This phenomenon appears to be universal, since it was confirmed in wines and potatoes.

2. The content of certain trace elements in wine, for example, magnesium (Mg) is comparable to the mineral waters, known for their therapeutic action. Moreover, contemporary clinical studies found magnesium (Mg) and zinc (Zn) as key micronutrients in the treatment of cardiovascular and metabolic diseases. We found, that Alibernet red wine extract has up to ten folder higher mineral content compared to red wine and to mineral waters as well. These results confirm that wine could be a rich source of essential minerals together with polyphenols.

3. Total antioxidant capacity of fruits, wine, juices and vegetables is higher than the sum of the potentials of individual substances. This occurs due to synergistic interactions among the bioactive substances and micronutrients. This fact was confirmed by our analysis of wine Cabernet Sauvignon, where it was found that in spite of the polyphenols, wine samples with high content of Mg and Zn showed a higher TAC.

4. For the first time catechins were determined in plasma of WKY and SHR after treatment with the red wine extract. Certain concentrations of catechins enable us to conclude, that these compounds circulate in the blood of experimental animals and therefore, the effects that we observed on the activity of NOS and SOD may be attributed to the action of polyphenols.

5. The activity of NOS and SOD were increased in the group of experimental animals with spontaneous hypertension after taking the extract of wine, whereas there were no changes in normotensive rats. It means that Alibernet red wine extract affects only the vasodilatation and antioxidant system of SHR.

6. Our results demonstrate that modification of SOD activity was not due to increases in superoxide. This was proven by direct measurement of superoxide radical in heart (LV) and aorta of the both the SHR and WKY groups, where no significant changes were found. The measurement results show that the increase in SOD activity was not induced by the superoxide radical, but could be caused mainly by the action of substances present in the extract such as minerals and polyphenols.

8 References

1. Badimon L, Vilahur G, Padro T. Nutraceuticals and atherosclerosis: human trials. Cardiovascular Therapeutics. 2010; 28: 202–215.

2. Kris-Etherton PM, Hecker KD, Bonanome MD et al. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. Am. J. Med. 2002; 113(9B): 71-88S.

3. Schröder H. Protective mechanisms of the Mediterranean diet in obesity and type 2 diabetes. J Nutr Biochem. 2007; 18: 149–160.

4. Bazzano LA, He J, Ogden LG, Loria CM, Vupputuri S, Myers L, and Whelton PK. Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first national Health and Nutrition Examination Survey Epidemiologic Follow-up Study. Am. J. Clin. Nutr. 2002; 76: 93-99.

5. Jenkins DJ, Kendall CW, Marchie A, et al. Type 2 diabetes and the vegetarian diet. Am. J. Clin. Nutr. 2003; 78 (suppl): 610-616S.

6. Seifried HE, Anderson DE, Fisher EI, Milner JA. A review of the interaction among dietary antioxidants and reactive oxygen species. J Nutr Biochem. 2007; 18: 567–579.

7. Kaliora, A. and Dedousis, G. Natural antioxidant compounds in risk factors for CVD. Pharmacological Research. 2007; 56: 99-109.

8. He F., Nowson C., MacGregor G. Fruit and vegetable consumption and stroke: meta-analysis of cohort studies. Lancet, 2006; 367: 320-326.

9. Wannamethee, S., Lowe G., Rumley A. et al.: Association of vitamin C status, fruit and vegetable intakes, and markers of inflammation and hemostasis. Am J Clin Nutr. 2006; 83: 567-574.

10. Arts I. and Hollman P. Polyphenols and disease risk in epidemiologic studies. Am J Clin Nutr 2005; 81(suppl), 317S–25S.

11. Brat P., George S., Bellamy A., Du Chaffaut L., Scalbert A., Mennen L., Arnault N., Amiot M. Daily polyphenol intake in France from fruit and vegetables. The Journal of Nutrition. 2006; 136: 2368–2373.

12. Huxley R. and Neil H. The relation between dietary flavonol intake and coronary heart disease mortality: a meta-analysis of prospective cohort studies. European Journal of Clinical Nutrition 2003; 57: 904–908.

13. Dauchet L, Amouyel P, Dallongeville J. Fruit and vegetable consumption and risk of stroke: a meta-analysis of cohort studies. Neurology. 2005 Oct 25; 65(8): 1193-7.

14. Kubena K. and McMurray D. Nutrition and the immune system: A review of nutrient-nutrient interactions. J Am Diet Assoc. 1996; 96: 1156-1164.

15. Kaliora AC, Dedoussis GV, Schmidt H. Dietary antioxidants in preventing atherogenesis. Atherosclerosis. 2006 Jul; 187(1): 1-17.

16. Vannucchi H. Interaction of vitamins and minerals. Arch Latinoam Nutr .1991;41(1):9–18.

17. Liu R. Potential Synergy of Phytochemicals in Cancer Prevention: Mechanism of Action. J. Nutr. 2004; 134: 3479S–3485S.

18. Mulvihill E., and Huff M. Antiatherogenic properties of flavonoids: implications for cardiovascular health. Can J Cardiol 2010: 26: 17A-21A.

19. Scalbert A, Manach C, Morand C, Remesy C, Jimenez L. Dietary polyphenols and the prevention of diseases. Crit Rev Food Sci Nutr 2005; 45: 287-306.

20. Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr 2005; 81(Suppl): 230-42.

21. Williamson G, Manach C. Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. Am J Clin Nutr 2005; 81(Suppl): 243-55.

22. Hertog M., Feskens E., Hollmann P., Katan M, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *The Lancet*, 1993; Vol. 342:1007-1011.

23. Ning P., Clark J., Prasain J., Kim H., White R. and Wyss M. Antihypertensive and cognitive effects of grape polyphenols in estrogen-depleted, female, spontaneously hypertensive rats. Am J Physiol Regul Integr Comp Physiol 2005; 289: R771–R775.

24. Vinson JA, Teufel K, Wu N. Red wine, dealcoholized red wine, and especially grape juice, inhibit atherosclerosis in a hamster model. Atherosclerosis, 2001; 156: 67–72.

25. Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 1998; 56(11): 317–33.

26. Singh M., Arseneault M., Sanderson T., Murthy V., Ramassamy Ch. Challenges for Research on Polyphenols from Foods in Alzheimer's Disease: Bioavailability, Metabolism, and Cellular and Molecular Mechanisms. J. Agric. Food Chem. 2008; 56: 4855–4873.

27. Es-Safi N, Le Guernevé C, Cheynier V, Moutounet M. New polyphenolic compounds formed by evolution of (+)-catechin in hydro-alcoholic solution and their implication in colour changes of grape-derived foods. J Agri Food Chem 2000; 48:4233-40.

28. Shi J., Yu J., Pohorly JE, Kakuda Y. Polyphenolics in grape seeds – biochemistry and functionality. J Med Food 2003; 6(4):291-9.

29. Manach C, Scalbert A, Morand C, Rémésy, C, Jiménez L. Polyphenols: food source and bioavailability. Am J Clin Nutr 2004; 79(5):727-47.

30. Harborne JB, Williams CA. Advances in flavonoid research since. *Phytochemistry* 2000; 55: 481-504.

31. Aherne SA, O'Brien NM. Dietary flavonols: chemistry, food content, and metabolism. Nutrition. 2002 Jan;18(1):75-81.

32. Brouillard R, George F, Fougerousse A. Polyphenols produced during red wine ageing. Biofactors 1997; 6: 403-10.

33. C. Del Bo, S. Ciappellano, D. Klimis-Zacas, D. Martini, C. Gardana, P. Riso, and M. Porrini Anthocyanin absorption, metabolism, and distribution from a wild blueberry-enriched diet (Vaccinium angustifolium) is affected by diet duration in the Sprague-Dawley rat. J. Agric. Food Chem. 2010; 58: 2491–2497.

34. Modun D., Music I., Vukovic J., Brizic I., Katalinic V., Obad A., Palada I., Dujic Z., Boban M. The increase in human plasma antioxidant capacity after red wine consumption is due to both plasma urate and wine polyphenols. Atherosclerosis, 2008; Vol. 197: 250-256.

35. Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. J Nutr 2000; 130: 2073S–85S.

36. Lauro, G.J. and Francis, F. J. (Eds) Natural Food colours, Science and technology. IFT Basic Symposium Series 14, Marcel Dekker, 2000.

37. Arts I., Putte B. and Hollman P. Catechin Contents of Foods Commonly Consumed in The Netherlands. 2. Tea, Wine, Fruit Juices, and Chocolate Milk J. Agric. Food Chem., 2000; 48 (5): 1752–1757.

38. Hertog M., Hollman P., Katan M. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. J Agric food Chem. 1992; 40: 2379-2383.

39. Milner JA. Molecular Targets for Bioactive Food Components. J. Nutr. 2004; 134: 2492S–2498S.

40. Jefremov V, Zilmer M, Zilmer K, Bogdanovic N, Karelson E. Antioxidative effects of plant polyphenols. From protection of G protein signaling to prevention of age-related pathologies. Annals of the New York Academy of Sciences 2007; 1095 (1): 449–457.

41. Fang M, Chen D, and Yang CS. Dietary Polyphenols May Affect DNA Methylation J. Nutr. 2007; 137: 223S–228S.

42. Zern TL, Wood RJ, Greene C, et al. Grape Polyphenols Exert a Cardioprotective Effect in Pre- and Postmenopausal Women by Lowering Plasma Lipids and Reducing Oxidative Stress J. Nutr. 2005; 135: 1911–1917.

43. Manach C, Mazur A and Scalbert A. Polyphenols and prevention of cardiovascular diseases Current Opinion in Lipidology 2005; 16:77–84.

44. Masella R., Di Benedetto R., Varı R., Filesi C., Giovannini C. Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. Journal of Nutritional Biochemistry. 2005; 16: 577–586.

45. Iriti M, Faoro F. Bioactivity of grape chemicals for human health. Nat Prod Commun. 2009; May; 4(5): 611-34.

46. Xia E., Deng G., Guo Y., Li H. Biological Activities of Polyphenols from Grapes Int. J. Mol. Sci. 2010; 1: 622-646.

47. Gollücke AP. Recent applications of grape polyphenols in foods, beverages and supplements. Recent Pat Food Nutr Agric. 2010 Jun; 2(2): 105-9.

48. Pannala AS, Rice-Evans CA, Halliwell B, Singh S: Inhibition of peroxynitrite-mediated tyrosine nitration by catechin polyphenols. Biochem Biophys Res Commun, 1997, 232, 164–168.

49. Binsack R, Boersma BJ, Patel RP, Kirk M, White CR, Darley-Usmar V, Barnes S et al.: Enhanced antioxidant activity after chlorination of quercetin by hypochlorous acid. Alcohol Clin Exp Res, 2001, 25, 434–443.

50. Cotelle N, Bernier JL, Catteau JP, Pommery J, Wallet JC, Gaydou EM: Antioxidant properties of hydroxy-flavones. Free Radic Biol Med, 1996, 20, 35–43.

51. Korkina LG, Afanas'ev IB: Antioxidant and chelating properties of flavonoids. Adv Pharmacol, 1997; 38: 151–163.

52. Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA: Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr, 2001; 74: 418–425.

53. Krinsky NI: Mechanism of action of biological antioxidants. Proc Soc Exp Biol Med, 1992; 200: 248–254.

54. Sies H: Oxidative stress: from basic research to clinical application. Am J Med, 1991; 91: 31S–38S.

55. Orallo F, Alvarez E, Camina M, Leiro JM, Gomez E, Fernandez P: The possible implication of trans-Resveratrol in the cardioprotective effects of long-term moderate wine consumption. Mol Pharmacol, 2002; 61: 294–302.

56. Rice-Evans CA, Miller NJ, Structure-antioxidant activity relationships of flavonoids and isoflavonoids. In: Flavonoids in Health and Disease. Marcell Dekker, Inc., New York, 1998; 199–238.

57. McDougall GJ, Shapiro F, Dobson P, Smith P, Blake A, Stewart D. Different polyphenolic components of soft fruits inhibit alpha-amylase and alpha-glucosidase. J Agric Food Chem. 2005 Apr 6; 53(7): 2760-6.

58. Ou HC, Chou FP, Sheen HM, Lin TM, Yang CH, Huey- Herng Sheu W: Resveratrol, a polyphenolic compound in red wine, protects against oxidized LDL-induced cytotoxicity in endothelial cells. Clin Chim Acta, 2006; Vol 364, Issues 1-2: 196-204.

59. Stein JH, Keevil JG, Wiebe DA, Aeschlimann S, Folts JD: Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. Circulation. 1999; 100: 1050–1055.

60. Frankel EN, Waterhouse AL, Kinsella JE: Inhibition of human LDL oxidation by resveratrol. Lancet. 1993; 341: 1103–1104.

61. Naissides M, Mamo JC, James AP, Pal S: The effect of chronic consumption of red wine on cardiovascular disease risk factors in postmenopausal women. Atherosclerosis. 2006; 185(2): 438-45.

62. Hertog MG, Kromhout D, Aravanis C, et al. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch Intern Med 1995; 155: 381–6.

63. Arai Y, Watanabe S, Kimira M, Shimoi K, Mochizuki R, Kinae N. Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. J Nutr 2000; 130: 2243–50.

64. Dávalos, C. Fernández-Hernando, F. Cerrato, J. Martínez-Botas, D. Gómez-Coronado, C. Gómez-Cordovés,and M. Asunción. Red grape juice polyphenols alter cholesterol homeostasis and increase LDL-receptor activity in human cells in vitro. J. Nutr. 2006; 136: 1766–1773.

65. Blade C., Arola L. and Salvado M. Hypolipidemic effects of proanthocyanidins and thein underlying biochemical and molecular mechanisms. Mol. Nutr. Food Res. 2010; 54: 37–59.

66. Mulvihill E., Assini J., Sutherland B., DiMattia A., Khami M., Koppes J., Sawyez C., Whitman S. and Huff M. Naringenin Decreases Progression of Atherosclerosis by Improving Dyslipidemia in High-Fat Fed Low-Density Lipoprotein Receptor Null Mice. Arterioscler Thromb Vasc Biol. 2010; 30: 742-748.

67. Schini-Kerth V., Auger C., Kim J., Étienne-Selloum N., Chataigneau T. Nutritional improvement of the endothelial kontrol of vascular tone by polyphenols: role of NO and EDHF. Pflugers Arch - Eur J Physiol, 2010; Vol 459: 853-862.

68. Khoo N., White R., Pozzo-Miller L., Zhou F., Konstance C., Inoue T., Patel R., Parks D.Dietary flavonoid quercetin stimulates vasorelaxation in aortic vessels. Free Radical Biology & Medicine. 2010; Vol. 49: 339–347.

69. Leikert JF, Rathel TR, Wohlfart P, Cheynier V, Vollmar AM, Dirsch VM. Red wine polyphenols enhance endothelial nitric oxide synthase expression and subsequent nitric oxide release from endothelial cells. Circulation. 2002; 106: 1614–7.

70. Wallerath T, Deckert G, Ternes T, Anderson H, Li H, Witte K, Forstermann U. Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase. Circulation. 2002; 106: 1652–8.

71. Duffy S., Vita J. Effects of phenolics on vascular endothelial function. Current Opinion in Lipidology. 2003; Vol.14: 21-27.

72. Lorenz M, Wessler S, Follmann E, Michaelis W, Dusterhoft T, Baumann G, Stangl K, Stangl V. A constituent of green tea, epigallocatechin- 3-gallate, activates endothelial nitric oxide synthase by a phosphatidylinositol- 3-OH-kinase-, cAMP-dependent protein kinase-, and Akt-dependent pathway and leads to endothelial-dependent vasorelaxation. J Biol Chem. 2004; 279: 6190–5.

73. Baur JA, Sinclair DA. Therapeutic potential of resveratrol: the in vivo evidence. Nat Rev Drug Discov. 2006; 5: 493–506.

74. Mattagajasingh I, Kim CS, Naqvi A, Yamamori T, Hoffman TA, Jung SB, DeRicco J, Kasuno K, Irani K. SIRT1 promotes endotheliumdependent vascular relaxation by activating endothelial nitric oxide synthase. Proc Natl Acad Sci USA. 2007; 104: 14855–60.

75. Suchankova G, Nelson LE, Gerhart-Hines Z, Kelly M, Gauthier MS, Saha AK, Ido Y, Puigserver P, Ruderman NB. Concurrent regulation of AMP-activated protein kinase and SIRT1 in mammalian cells. Biochem Biophys Res Commun. 2009; 378: 836–41.

76. Pecháňová O., Bernatova I., Babal P., Martınez C., Kysela S., Stvrtina S., Andriantsitohaina R. Red wine polyphenols prevent cardiovascular alterations in L-NAME induced hypertension Hypertens 2004; 22: 1551-9.

77. Flesch M, Schwarz A, Bohm M. Effects of red and white wine on endothelium-dependent vasorelaxation of rat aorta and human coronary arteries. Am J Physiol. 1998; 275: H1183–90.

78. Stein JH, Keevil JG, Wiebe DA, Aeschlimann S, Folts JD. Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. Circulation. 1999; 100: 1050–5.

79. Lekakis J, Rallidis LS, Andreadou I, Vamvakou G, Kazantzoglou G, Magiatis P, Skaltsounis A-L, Kremastinos D. Polyphenolic compounds from red grapes acutely improve endothelial function in patients with coronary heart disease. European Journal of Cardiovascular Prevention and Rehabilitation, 2005; 12: 596-600.

80. Agewall S,Wright S, Doughty RN, Whalley GA, Duxbury M, Sharpe N. Does a glass of red wine improve endothelial function? Eur Heart J. 2000;21:74–8.

81. Papamichael C, Karatzis E, Karatzi K, Aznaouridis K, Papaioannou T, Protogerou A, Stamatelopoulos K, Zampelas A, Lekakis J, et al. Red wine's antioxidants counteract acute endothelial dysfunction caused by cigarette smoking in healthy nonsmokers. Am Heart J. 2004; 147:5.

82. Cuevas AM, Guasch V, Castillo O, Irribarra V, Mizon C, San Martin A, Strobel P, Perez D, Germain AM, et al. A high-fat diet induces and red wine counteracts endothelial dysfunction in human volunteers. Lipids. 2000; 35: 143–8.

83. Gryglewski RJ, Korbut R, Robak J, Swies J. On the mechanism of antithrombotic action of flavonoids. Biochem Pharmacol. 1987; 36: 317–22.

84. Corder R, Douthwaite JA, Lees DM, Khan NQ, Viseu Dos Santos AC, Wood EG, Carrier MJ. Endothelin-1 synthesis reduced by red wine. Nature. 2001; 414: 863–4.

85. Abou-Agag LH, Aikens ML, Tabengwa EM, Benza RL, Shows SR, Grenett HE, Booyse FM. Polyphyenolics increase t-PA and u-PA gene transcription in cultured human endothelial cells. Alcohol Clin Exp Res. 2001; 25: 155–62.

86. Carluccio MA, Siculella L, Ancora MA, Massaro M, Scoditti E, Storelli C, Visioli F, Distante A, De Caterina R. Olive oil and red wine antioxidant polyphenols inhibit endothelial activation: antiatherogenic properties of Mediterranean diet phytochemicals. Arterioscler Thromb Vasc Biol. 2003; 23: 622–9.

87. Yoon J. and Baek S.Molecular Targets of Dietary Polyphenols with Anti-inflammatory Properties. Yonsei Medical Journal, 2005; Vol. 46: No. 5, 585 – 596.

88. Frei B. and Higdon J. Antioxidant Activity of Tea Polyphenols In Vivo: Evidence from Animal Studies. J. Nutr. 2003; 133: 3275S-3284S.

89. Blanco-Colio LM, Valderrama M, Alvarez-Sala LA, Bustos C, Ortego M, Hernandez-Presa MA, Cancelas P, Gomez-Gerique J, Millan J, et al. Red wine intake prevents nuclear factor-kappaB activation in peripheral blood mononuclear cells of healthy volunteers during postprandial lipemia. Circulation. 2000; 102: 1020–6.

90. Koga T, Meydani M. Effect of plasma metabolites of (+)-catechin and quercetin on monocyte adhesion to human aortic endothelial cells. Am J Clin Nutr. 2001; 73: 941–8.

91. Mackenzie GG, Carrasquedo F, Delfino JM, Keen CL, Fraga CG, Oteiza PI. Epicatechin, catechin, and dimeric procyanidins inhibit PMAinduced NF-kappaB activation at multiple steps in Jurkat T cells. FASEB J. 2004; 18: 167–9.

92. Ferrero ME, Bertelli AE, Fulgenzi A, Pellegatta F, Corsi MM, Bonfrate M, Ferrara F, De Caterina R, Giovannini L, et al. Activity in vitro of resveratrol on granulocyte and monocyte adhesion to endothelium. Am J Clin Nutr. 1998; 68: 1208–14.

93. Tomita M, Irwin K, Xie Z, Santoro T. Tea pigments inhibit the production of type 1 (T_{H1}) and type 2 (T_{H2}) helper T cell cytokines in $CD4^+$ T cells. Phytoter. Res. 2002; 16: 36-42.

94. Oak M., Bedoui J, Schini-Kerth V. Antiangiogenic properties of natural polyphenols from red wine and green tea. Journal of Nutritional Biochemistry 2005; 16: 1 –8.

95. Fraga C. Plant Phenolics and Human Health : Biochemistry, Nutrition and Pharmacology. Wiley, 2010, 593 pp.

96. Osman HE, Maalej N, Shanmuganayagam D, Folts JD. Grape juice but not orange or grapefruit juice inhibits platelet activity in dogs and monkeys. J Nutr 1998; 128: 2307–12.

97. Wollny T, Aiello L, Di Tommaso D, Bellavia V, Rotilio D, Donati MB, de Gaetano G, Iacoviello L. Modulation of haemostatic function and prevention of experimental thrombosis by red wine in rats: a role for increased nitric oxide production. Br J Pharmacol. 1999; 127: 747–55.

98. Freedman JE, Parker C III, Li L, Perlman JA, Frei B, Ivanov V, Deak LR, Iafrati MD, Folts JD. Select flavonoids and whole juice from pyrole grapes inhibit platelet function and enhance nitric oxide release. Circulation. 2001; 103: 2792–8.

99. Mount, C., Downton, C. Alzheimer disease: progress or profit. Nat. Med. 2006, 12, 780–784.

100. Darvesh A, Carroll RT, Bishayee A, Geldenhuys WJ, Van der Schyf CJ. Oxidative stress and Alzheimer's disease: dietary polyphenols as potential therapeutic agents. Expert Rev Neurother. 2010 May;10(5):729-45.

101. Gu Y, Luchsinger JA, Stern Y, Scarmeas N. Mediterranean Diet, Inflammatory and Metabolic Biomarkers, and Risk of Alzheimer's Disease. J Alzheimers Dis. 2010 Aug., in press.

102. Scarmeas N, Luchsinger JA, Mayeux R, Stern Y. . Mediterranean diet and Alzheimer disease mortality. Neurology, 2007 Sep 11; 69(11): 1084-93.

103. Sun A., Wang Q, Simonyi A, and Sun G. Botanical phenolics and brain health Neuromolecular Med. 2008 ; 10(4): 259–274.

104. Mancuso C, Bates TE, Butterfield DA, Calafato S, Cornelius C, De Lorenzo A, Dinkova Kostova AT, Calabrese V. Natural antioxidants in Alzheimer's disease. Expert Opin Investig Drugs. 2007 Dec; 16(12): 1921-31.

105. Ramassamy C. Emerging role of polyphenolic compounds in the treatment of neurodegenerative diseases: a review of their intracellular targets. Eur J Pharmacol. 2006 Sep 1; 545(1): 51-64.

106. Sun A, Wang Q, Simonyi A, Sun G. Resveratrol as a therapeutic agent for neurodegenerative diseases. Mol Neurobiol. 2010 Jun; 41(2-3): 375-83.

107. Orgogozo JM, Dartigues JF, Lafont S, et al. Wine consumption and dementia in the elderly: a prospective community study in the Bordeaux area. Rev Neurol 1997; 153: 185–92.

108. Commenges D, Scotet V, Renaud S, Jacqmin-Gadda H, Barberger-Gateau P, Dartigues JF. Intake of flavonoids and risk of dementia. Eur J Epidemiol 2000; 16: 357–63.

109. Garcia-Alonso, J.; Ros, G.; Vidal-Guevara, M.L.; Periago, M.J. Acute intake of phenolic-rich juice improves antioxidant status in healthy subjects. Nutr. Res. 2006, 26, 330–339.

110. Baydar, N.G.; Sagdic, O.; Ozkan, G.; Cetin, S. Determination of antibacterial effects and total phenolic contents of grape (Vitis vinifera) seed extracts. Int. J. Food Sci. 2006, 41, 799–804.

111. Bruno, G.; Sparapano, L. Effects of three esca-associated fungi on Vitis vinifera L : V. Changes in the chemical and biological profile of xylem sap from diseased cv. Sangiovese vines. Physiol. Mol. Plant Pathol. 2007, 71, 210–229.

112. Jung, H.J.; Hwang, I.A.; Sung, W.S.; Kang, H.; Kang, B.S.; Seu, Y.B.; Lee, D.G. Fungicidal effect of resveratrol on human infectious fungi. Arch. Pharm. Res. 2005, 2, 557–560.

113. de Bruyne, T.; Pieters, L.; Witvrouw, M.; de Clercq, E.; Berghe, D.V.; Vlietinck, A.J. Biological evaluation of proanthocyanidin dimers and related polyphenols. J. Nat. Prod. 1999, 62, 954–958.

114. Chavez, J.H.; Leal, P.C.; Yunes, R.A.; Nunes, R.J.; Barardi, C.R.; Pinto, A.R.; Simoes, C.M.; Zanetti, C.R. Evaluation of antiviral activity of phenolic compounds and derivatives against rabies virus. Vet. Microbiol. 2006, 116, 53–59.

115. Tagurt, T.; Tanaka, T.; Kouno, I. Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease. Biol. Pharm. Bull. 2004, 27, 1965–1969.

116. Nikitina V., Kuzmina L., Melentev A. and Shendel G. Antibacterial activity of polyphenolic compounds isolated from plants of Geraniaceae and Rosaceae families. Applied Biochemistry and Microbiology, 2007, Volume 43, Number 6, 629-634.

117. Rodriguez-Vaquero, M.J.; Alberto, M.R.; Manca-de-Nadra, M.C. Influence of phenolic compounds from wines on the growth of Listeria monocytogenes. Food Control. 2007, 18, 587–593.

118. Papadopoulou, C.; Soulti, K.; Roussis, I.G. Potential antimicrobial activity of red and white wine phenolic extracts against strains of Taphylococcus aureus, Escherichia coli and Candida albicans. Food Technol. Biotechnol. 2005, 43, 41–46.

119. Sivarooban, T., Hettiarachchy, N.S., Johnson, M.G. Physical and antimicrobial properties of grape seed extract, nisin, and EDTA incorporated soy protein edible films. Food Res. Int. 2008, 41, 781–785.

120. Luther, M.; Parry, J.; Moore, J.; Meng, J.H.; Zhang, Y.F.; Cheng, Z.H.; Yu, L. Inhibitory effect of chardonnay and black raspberry seed extracts on lipid oxidation in fish oil and their radical scavenging and antimicrobial properties. Food Chem. 2007, 104, 1065–1073.

121. Coates EM, Popa G, Gill CI, McCann MJ, McDougall GJ, Stewart D, Rowland I. Colon-available raspberry polyphenols exhibit anti-cancer effects on in vitro models of colon cancer. J Carcinog 2007; 6: 4.

122. Hudson, T.S.; Hartle, D.K.; Hursting, S.D.; Nunez, N.P.; Wang, T.T.Y.; Young, H.A.; Arany, P.; Green, J.E. Inhibition of prostate cancer growth by muscadine grape skin extract and resveratrol through distinct mechanisms. Cancer Res. 2007, 67, 8396–8405.

123. Fotsis T, Pepper MS, Aktas E, et al. Flavonoids, dietary-derived inhibitors of cell proliferation and in vitro angiogenesis. Cancer Res 1997; 57: 2916–21.

124. Knekt P, Jarvinen R, Seppanen R, et al. Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. Am J Epidemiol 1997; 146: 223–30.

125. Ramos, S.; Alia, M.; Bravo, L.; Goya, L. Comparative effects of food-derived polyphenols on the viability and apoptosis of a human hepatoma cell line (HepG2). J. Agric. Food Chem. 2005, 53, 1271–1280.

126. Saleem, A.; Husheem, M.; Harkonen, P; Pihlaja, K. Inhibition of cancer cell growth by trude extract and the phenolics of Terminalia chebula retz. fruit. J. Ethnopharmacol. 2002, 81, 327–336.

127. McDougall GJ, Stewart D. The inhibitory effects of berry extracts on digestive enzymes. Biofactors. 2005; 23: 189–195.

128. Anderson R. Chromium and polyphenols from cinnamon improve insulin sensitivity.Proceedings of the Nutrition Society 2008; 67: 48–53.

129. Maurizio Battino, Jules Beekwilder, Beatrice Denoyes-Rothan, Margit Laimer, Gordon J McDougall, and Bruno Mezzetti Bioactive compounds in berries relevant to human health Nutrition Reviews 2009; Vol. 67(Suppl. 1): S145–S150.

130. Matsui T., Ueda T., Oki T., Sugita K., Terahara N., Matsumoto K. α-Glucosidase inhibitory action of natural acetylated anthocyanins. J. Agric. Food. Chem. 2001; 49: b1948-1951.

131. Su H., Hung L., Chen J. Resveratrol, a red wine antioxidant, possesses an insulin-like effect in streptozotocin -induced diabetic rats. Am J Physiol Endocrinol Metab 2006; 290:1339-1346.

132. Toeller M., α-Glucosidase inhibitors in diabetes:efficacy in NIDDM subjects. Eur. J. Clin. Invest. 1994; 24: 31-35.

133. Iwai K., Kim M., Onodera A., and Matsue H. α-Glucosidase Inhibitory and Antihyperglycemic Effects of Polyphenols in the Fruit of Viburnum dilatatum Thunb.*J. Agric. Food Chem.*, 2006; 54 (13): 4588–4592.

134. Tundis R., Loizzo M., Menichini F. Natural products as alpha-amylase and alpha-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update. Mini Rev Med Chem. 2010 Apr; 10(4): 315-31.

135. Adisakwattana S., Charoenlertku P., Yibchok-anun S. α-Glucosidase inhibitory activity of cyanidin-3-galactoside and synergistic effect with acarbose. Journal of Enzyme Inhibition and Medicinal Chemistry. 2009; Vol. 24, No. 1: 65-69.

136. Krazeisen A, Breitling R, Möller G, Adamski J.Human 17beta-hydroxysteroid dehydrogenase type 5 is inhibited by dietary flavonoids. Adv Exp Med Biol. 2002; 505: 151-61.

137. Deluca D, Krazeisen A, Breitling R, Prehn C, Möller G, Adamski J. Inhibition of 17beta-hydroxysteroid dehydrogenases by phytoestrogens: Comparison with other steroid metabolizing enzymes.J Steroid Biochem Mol Biol. 2005; 93(2-5) :285-92.

138. Brozic P, Lanisnik Risner T, Gobec S. Inhibitors of 17beta-hydroxysteroid dehydrogenase type 1. Curr Med Chem. 2008; 15(2): 137-50.

139. Atkinson C., Compston J., Day N., Dowsett M., and Bingham S.The effects of phytoestrogen isoflavones on bone density in women: a double-blind, randomized, placebo-controlled trial.Am J Clin Nutr 2004;79:326 –33.

140. Beattie J. and Kwun I. Is zinc deficiency a risk factor for atherosclerosis? British Journal of Nutrition. 2004; 91: 177–181.

141. Cuajungco M. and Fage K. Zinc takes the center stage: its paradoxical role in Alzheimer's disease. Brain Research Reviews. 2003; 41: 44–56.

142. Kosar F., Sahin I., Taskapan C., Kucukbay Z., Gullu H., Taskapan H., Cehreli S. Trace element status (Se, Zn, Cu) in heart failure. Anadolu Kardiyol Derg 2006; 6: 216-20.

143. Houtman JP. Trace elements and cardiovascular diseases. J Cardiovasc Risk. 1996 Feb; 3(1):18-25.

144. Bao B, Prasad AS, Beck FW, Fitzgerald JT, Snell D, Bao GW, Singh T, Cardozo LJ. Zinc decreases C-reactive protein, lipid peroxidation, and inflammatory cytokines in elderly subjects: a potential implication of zinc as an atheroprotective agent. Am J Clin Nutr. 2010 Jun; 91(6): 1634-41.

145. Frassinetti S, Bronzetti G, Caltavuturo L, Cini M, Croce C. The role of zinc in life: a review. J Environ Pathol Toxicol Oncol 2006; 25(3): 597–610.

146. Stefanidou M, Maravelias C, Dona C , Spiliopoulou C. Zinc: a multipurpose trace element. Arch Toxicol 2006; 80: 1–9.

147. Bhalla P., Chadha V., Dhar R., Dhawan D. Neuroprotective effects of zinc on antioxidant defense system in lithium treated rat brain. Indian Journal of Exp. Biol. 2007; Vol. 45: 954-958.

148. Mocchegiani E, Muzzioli M, Giacconi R. Zinc and immunoresistance to infections in ageing: new biological tools. Trends Pharmacol Sci 2000; 21: 205–208.

149. Prasad AS, Bao B, Beck FW, Kucuk O, and Sarkar FH. Antioxidant effect of zinc in humans. Free Radical Biology & Medicine. 2004; 37(8): 1182–1190.

150. Powell SR. The Antioxidant Properties of Zinc. J. Nutr. 2000; 130: 1447S—1454S.

151. Song Y., Wang J., Li X. and Cai L. Zinc and the diabetic heart. BioMetals. 2005; 18:325–332.

152. Prasad AS. Zinc: Mechanisms of Host Defense. J. Nutr. 2007; 137: 1345–1349.

153. McDonald JT and Margen S. Wine versus ethanol in human nutrition. Zinc balance. Am J. Clin. Nutr. 1980; 33: 1096-1102.

154. Tapiero H, Tew KD. Trace elements in human physiology and pathology: zinc and metallothioneins. Biomed Pharmacother. 2003; 57: 399–411.

155. Barceloux DG. Zinc. Clin Toxicol. 1999; 37:279–292.

156. Reiterer G., MacDonald, Browning J., Morrow J., Matveev S., Daugherty A., Smart E., Toborek M., Hennig B. Zinc Deficiency Increases Plasma Lipids and Atherosclerotic Markers in LDL-Receptor–Deficient Mice. J. Nutr. 2005; 135: 2114–2118,

157. Bruno R., Song Y., Leopard S., Mustacich D.,Tailor A., Traber M., Emily Ho E. Dietary zinc restriction in rats alters antioxidant status and increases plasma F2 isoprostanes. Journal of Nutritional Biochemistry. 2007; 18: 509–518.

158. Munshi A, Babu S, Kaul S, Shafi G, Rajeshwar K, Alladi S, Jyothy A. Depletion of serum zinc in ischemic stroke patients. Methods Find Exp Clin Pharmacol. 2010; 32(6): 433-6.

159. Tucker K., Hannan M., Chen H., Cupples A., Wilson P., and Kiel D. Potassium, magnesium, and fruit and vegetable intakes are associated with greater bone mineral density in elderly men and women. Am J Clin Nutr 1999; 69:727–36.

160. He F. and MacGregor G.Beneficial effects of potassium. BMJ 2001; 323:497–501.

161. Demigne C, Sabboh H, Christian Rémésy C and Meneton P. Protective Effects of High Dietary Potassium: Nutritional and Metabolic Aspects. J. Nutr. 2004; 134: 2903–2906.

162. Young DB and Ma G. Vascular protective effects of potassium. Semin. Nephrol. 1999; 19: 477–486.

163. Huerta MG, Roemmich JN, Kington ML, et al. Magnesium Deficiency Is Associated With Insulin Resistance in Obese Children. Diabetes Care. 2005; 28: 1175–1181.

164. Barbagallo M, Dominguez LG. Magnesium metabolism in type 2 diabetes mellitus, metabolic syndrome and insulin resistance. Arch. Biochem. Biophys. 2007; 458(1): 40-7.

165. Sharp P. The molecular basis of copper and iron interactions. Proceedings of the Nutrition Society 2004; 63: 563–569.

166. Maynard C., Bush A., Masters C., Cappai L., Li. Q. Metals and amyloid-β in Alzheimer's disease. Int. J. Exp. Path. 2005; 86: 147–159.

167. Klevay LM. Alzheimer's disease as copper deficiency. Med Hypotheses. 2008; 70(4): 802-7.

168. Brown KM, Arthur JR. Selenium, selenoproteins and human health: a review. Public Health Nutr. 2001; 4(2B): 593-9.

169. Förstermann U. Nitric oxide and oxidative stress in vascular disease. Eur J Physiol. 2010; 459: 923-939.

170. Bender D. Nutritional Biochemistry of the Vitamins, 2nd edition. Cambridge university press, 2003.

171. Shen J, Lai CQ, Mattei J, Ordovas JM, Tucker KL.Association of vitamin B-6 status with inflammation, oxidative stress, and chronic inflammatory conditions: the Boston Puerto Rican Health Study. Am J Clin Nutr. 2010 Feb; 91(2): 337-42.

172. Park Y. Intakes of vegetables and related nutrients such as vitamin B complex, potassium, and calcium, are negatively correlated with risk of stroke in Korea Nutrition Research and Practice (Nutr Res Pract) 2010; 4(4): 303-310.

173. Van Guelpen B, Hultdin J, Johansson I, Stegmayr B, Hallmans G, Nilsson TK, Weinehall L, Witthöft C, Palmqvist R, Winkvist A. Folate, vitamin B12, and risk of ischemic and hemorrhagic stroke: a prospective, nested case-referent study of plasma concentrations and dietary intake. Stroke 2005; 36: 1426-31.

174. Palmas W. Effects of short-term supplementation with ascorbate, folate, and vitamins B6 and B12 on inflammatory factors and estrogen levels in obese postmenopausal women. Int J Vitam Nutr Res. 2006 Jan; 76(1): 34-8.

175. Krajnov A. Vitaminy i mineraly.,Izdatelskij Dom ROZ, 2002.

176. Perricone NV, Bagchi D, Echard B, Preuss HG. Long-term metabolic effects of different doses of niacin-bound chromium on Sprague-Dawley rats. Mol Cell Biochem. 2010 May; 338(1-2): 91-103.

177. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. N Engl J Med 1993; 328:1450–1456.

178. Osganian SK, Stampfer MJ, Rimm E, et al. Vitamin C and risk of coronary heart disease in women. J Am Coll Cardiol 2003; 42: 246–252.

179. Osganian SK, Stampfer MJ, Rimm E, Spiegelman D, Manson JE, Willett WC. Dietary carotenoids and risk of coronary artery disease in women. Am J Clin Nutr 2003; **77**: 1390–1399.

180. Sherman, D.L., Keaney, J.F., Biegelsen, E.S. Pharmacological concentrations of ascorbic acid are required for the beneficial effect on endothelial vasomotor function in hypertension. Hypertension. 2000; 35: 936_941.

181. Li Y. and Schellhorn H. New Developments and Novel Therapeutic Perspectives for Vitamin C J. Nutr. 2007; 137: 2171–2184.

182. Mandl J., Szarka A., and Bánhegyi G. Vitamin C: update on physiology and pharmacology.British Journal of Pharmacology. 2009; 157: 1097–1110.

183. Arrigoni O, De Tullio MC. Ascorbic acid: much more than just an antioxidant. Biochim Biophys Acta. 2002; 1569: 1–9.

184. Duffy, S.J., Bokce, N. and Holbrook. Treatment of hypertension with ascorbic acid. Lancet 1999; 354: 2048-2049.

185. Enstrom, J.E., Kanim, L.E. and Klein, M. Vitamin C intake and mortality among a sample of the United States population. Epidemiology. 1992; 3: 194_202.

186. Baker TA, Milstien S, Katusic ZS. Effect of vitamin C on the availability of tetrahydrobiopterin in human endothelial cells. J Cardiovasc Pharmacol. 2001; 37: 333–8.

187. Huang A, Vita JA, Venema RC, Keaney JF. Ascorbic acid enhances endothelial nitric-oxide synthase activity by increasing intracellular tetrahydrobiopterin. J Biol Chem. 2000; 275: 17399–406.

188. d'Uscio LV, Milstien S, Richardson D, Smith L, Katusic ZS. Long-term vitamin C treatment increases vascular tetrahydrobiopterin levels and nitric oxide synthase activity. Circ Res. 2003; 92: 88–95.

189. Smith AR, Visioli F, Hagen TM. Vitamin C matters: increased oxidative stress in cultured human aortic endothelial cells without supplemental ascorbic acid. FASEB J. 2002; 16: 1102–4.

190. van Tits LJ, de Waart F, Hak-Lemmers HL, de Graaf J, Demacker PN, Stalenhoef AF. Neutrophil superoxide-anion generating capacity in chronic smoking: effect of long-term alpha-tocopherol therapy. J Biosci. 2003 Feb; 28(1): 23-7.

191. Niki R., Noguchi N., Tsuchihashi H., Gotoh N. Interaction among vitamin C, vitamin E, and β-carotene. Am J Clin Nutr 1995; 62: 1322s-1326s.

192. Tesoriere L, Bongiorno A, Pintaudi AM, D'Anna R, D'Arpa D, Livrea MA. Synergistic interactions between vitamin A and vitamin E against lipid peroxidation in phosphatidylcholine liposomes. Arch Biochem Biophys. 1996; 326(1): 57-63.

193. Nagaoka S, Kakiuchi T, Ohara K, Mukai K. Kinetics of the reaction by vitamin E is regenerated which natural by vitamin C. Chem Phys Lipids. 2007; 146: 26–32.

194. Bruno RS, Leonard SW, Atkinson J, Montine TJ, Ramakrishnan R, Bray TM, Traber MG. Faster plasma vitamin E disappearance in smokers is normalized by vitamin C supplementation. Free Radic Biol Med. 2006; 40: 689–97.

195. Murugesan P, Muthusamy T, Balasubramanian K, Arunakaran J. Studies on the protective role of vitamin C and E against polychlorinated biphenyl (Aroclor 1254)-induced oxidative damage in Leydig cells. Free Radic Res. 2005; 39: 1259–72.

196. Krishnamoorthy G, Venkataraman P, Arunkumar A, Vignesh RC, Aruldhas MM, Arunakaran J. Ameliorative effect of vitamins (alphatocopherol and ascorbic acid) on PCB (Aroclor 1254) induced oxidative stress in rat epididymal sperm. Reprod Toxicol. 2007; 23: 239–45.

197. Senthil Kumar J, Banudevi S, Sharmila M, Murugesan P, Srinivasan N, Balasubramanian K, Aruldhas MM, Arunakaran J. Effects of Vitamin C and E on PCB (Aroclor 1254) induced oxidative stress, androgen binding protein and lactate in rat Sertoli cells. Reprod Toxicol. 2004; 19: 201–8.

198. Ramanathan K, Balakumar BS, Panneerselvam C. Effects of ascorbic acid and alpha-tocopherol on arsenic-induced oxidative stress. Hum Exp Toxicol. 2002; 21: 675–80.

199. Carr AC, Zhu BZ, Frei B. Potential antiatherogenic mechanisms of ascorbate (vitamin C) and alpha-tocopherol (vitamin E). Circ Res. 2000; 87: 349–54.

200. Abudu N, Miller JJ, Attaelmannan M, Levinson SS. Vitamins in human arteriosclerosis with emphasis on vitamin C and vitamin E. Clin Chim Acta. 2004; 339: 11–25.

201. Rodriguez JA, Nespereira B, Perez-Ilzarbe M, Eguinoa E, Paramo JA. Vitamins C and E prevent endothelial VEGF and VEGFR-2 overexpression induced by porcine hypercholesterolemic LDL. Cardiovasc Res. 2005; 65:665–73.

202. Chen X, Touyz RM, Park JB, Schiffrin EL. Antioxidant effects of vitamins C and E are associated with altered activation of vascular NADPH oxidase and superoxide dismutase in stroke-prone SHR. Hypertension. 2001; 38: 606–11.

203. Antoniades C, Tousoulis D, Tentolouris C, Toutouza M, Marinou K, Goumas G, Tsioufis C, Toutouzas P, Stefanadis C. Effects of antioxidant vitamins C and E on endothelial function and thrombosis/fibrinolysis system in smokers. Thromb Haemost. 2003; 89: 990–5.

204. Engler MM, Engler MB, Malloy MJ, Chiu EY, Schloetter MC, Paul SM, Stuehlinger M, Lin KY, Cooke JP, et al. Antioxidant vitamins C and E improve endothelial function in children with hyperlipidemia: Endothelial Assessment of Risk from Lipids in Youth (EARLY) Trial. Circulation. 2003; 108: 1059–63.

205. Fang JC, Kinlay S, Beltrame J, Hikiti H,Wainstein M, Behrendt D, Suh J, Frei B, Mudge GH, et al. Effect of vitamins C and E on progression of transplant-associated arteriosclerosis: a randomised trial. Lancet. 2002; 359: 1108–13.

206. Cardiovascular diseases. WHO report, 2003.

207. Briones A. and Touyz R. Oxidative Stress and Hypertension: Current Concepts. Curr Hypertens Rep. 2010; Vol 12: 135-142.

208. Kaneto H., Katakami N., Matsuhisa M. and Matsuoka T. Role of Reactive Oxygen Species in the Progression of Type 2 Diabetes and Atherosclerosis. Mediators Inflamm. 2010.

209. Singh U. and Jialal I. Oxidative stress and atherosclerosis.Pathophysiology 2006; 13: 129–142.

210. Warnholtz A, Nickenig G, Schulz E, Macharzina R, Brasen JH, Skatchkov M, Heitzer T, Stasch JP, Griendling KK, Harrison DG, Bohm M, Meinertz T, Munzel T. Increased NADH-oxidase- mediated superoxide production in the early stages of atherosclerosis: evidence for involvement of the renin–angiotensin system. Circulation 1999; 99: 2027–2033.

211. Madamanchi N, Vendrov A, Rubte M. Oxidative stress and vascular disease, Arterioscler. Thromb. Vasc. Biol. 2005; 25: 29–38.

212. Giustarini D., Dalle-Donne I, Dimitrios Tsikas D. and Rossi R.Oxidative stress and human diseases: Origin, link, measurement, mechanisms, and biomarkers .Critical Reviews in Clinical Laboratory Science, 2009; 46(5-6): 241–281.

213. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease.Clin Chem. 2006 Apr; 52(4): 601-23.

214. Bonomini F, Tengattini S, Fabiano A, Bianchi R and Rezzani R. Atherosclerosis and oxidative stress. Histol Histopathol 2008; 23: 381-390.

215. Förstermann U. Oxidative stress in vascular disease. Nat Clin Pract Cardiovasc Med. 2008; 5(6): 338-49.

216. Maytin M, Leopold J, Loscalzo J. Oxidant stress in the vasculature. Curr Atheroscler Rep. 1999; 1: 156–164.

217. Zalba G, Beaumont J, San Jose G, et al. Vascular oxidant stress: molecular mechanisms and pathophysiological implications. J Physiol Biochem. 2000; 56: 57–64.

218. Griendling KK, Harrison DG. Out, damned dot: studies of the NADPH oxidase in atherosclerosis. J Clin Invest. 2001; 108: 1423–1424.

219. Tsimikas S, Palinski W, Witztum JL. Circulating autoantibodies to oxidized LDL correlate with arterial accumulation and depletion of oxidized LDL in LDL receptor-deficient mice. Arterioscler Thromb Vasc Biol. 2001; 21: 95–100.

220. Kita T, Kume N, Minami M, et al. Role of oxidized LDL in atherosclerosis. Ann NY Acad Sci. 2001; 947: 199–205.

221. Štípek S. a kolektiv. Antioxidanty a volné radikály ve zdraví a nemoci. Praha: Grada, 2000; 314 p.

222. Keaney JF. Oxidative stress and the vascular wall: NADPH oxidases take center stage, Circulation 2005; 112: 2585–2858.

223. Meneshian A, Bulkley GB. The physiology of endothelial xanthine oxidase: from urate catabolism to reperfusion injury to inflammatory signal transduction. Microcirculation 2002; 9: 161–175.

224. White CR, Brock TA, Chang LY, Crapo J, Briscoe P, Ku D, Bradley WA, Gianturco SH, Gore J, Freeman BA, et al. Superoxide and peroxynitrite in atherosclerosis. Proc Natl Acad Sci 1994; 91: 1044–1048.

225. Moncada S, Higgs EA. Nitric oxide and the vascular endothelium Handb Exp Pharmacol. 2006; 176: 213-54.

226. Folts JD Potential health benefits from the flavonoids in grape products on vascular disease. Adv Exp Med Biol 2002; 505:95-111.

227. Vásquez-Vivar J: Tetrahydrobiopterin, superoxide, and vascular dysfunction. Free Radic Biol Med 2009, 47:1108–1119.

228. Touyz RM and Schiffrin EL. Reactive oxygen species in vascular biology: implications in hypertension, Histochem Cell Biol. 2004 Oct; 122(4): 339-52.

229. Kunsch C and Medford RM. Oxidative stress as a regulator of gene expression in the vasculature. Circ Res 1999; 85: 753–766.

230. Stocker R and Keaney JF. New insights on oxidative stress in the artery wall. J Thromb Haemost 2005; 3: 1825–1834.

231. Viedt C. Differential activation of mitogen-activated protein kinases in smooth muscle cells by angiotensin II: involvement of p22phox and reactive oxygen species. Arterioscler Thromb Vasc Biol 2000; 20: 940–9.

232. Wassmann S., Wassmann K. and Nickenig G. Modulation of oxidant and antioxidant enzyme expression and function in vascular cells. Hypertension 2004; 44: 381-386.

233. Jung O, Marklund SL, Xia N, Busse R, Brandes RP . Inactivation of extracellular superoxide dismutase contributes to the development of high-volume hypertension. Arterioscler Thromb Vasc Biol 2007; 27: 470–477.

234. Stralin P, Karlsson K, Johansson BO, Marklund SL. The interstitium of the human arterial wall contains very large amounts of extracellular superoxide dismutase. Arterioscler Thromb Vasc Biol 1995; 15: 2032–2036.

235. Wolin MS. Interactions of oxidants with vascular signaling systems. Arterioscler Thromb Vasc Biol 2000; 20: 1430–1442.

236. Ferrari C. and Torres E. Biochemical pharmacology of functional foods and prevention of chronic diseases of aging. Biomedecine & Pharmacotherapy 2003; Vol57: 251-260.

237. Chelikani P, Fita I, Loewen PC. Diversity of structures and properties among catalases. Cell Mol Life Sci 2004; 61: 192–208.

238. Davies KJ. Oxidative stress: the paradox of aerobic life. Biochem Soc Symp 1995; 61: 1–31.

239. Vertuani S, Angusti A, Manfredini S. The antioxidants and pro-antioxidants network: an overview. Curr Pharm Des 2004; 10: 1677–1694.

240. Sies H. Oxidative stress: oxidants and antioxidants. Exp Physiol 1997; 82: 291–295.

241. Houston M. The role of cellular micronutrient analysis, nutraceuticals, vitamins, antioxidants and minerals in the prevention and treatment of hypertension and cardiovascular disease. Ther Adv Cardiovasc Dis. 2010 Jun; 4(3):165-83.

242. Re R, Pellegrini N, Proteggente A, et al. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine.1999; 26: 1231-1237.

243. Benzie I, Strain J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry. 1996; 239: 70-76

244. Szabo M, Idiţoiu C, Chambre D and Lupea A. Improved DPPH determination for antioxidant activity spectrophotometric assay**,** Chemical Papers, 2007; Vol. 61:, 214-216.

245. Singleton V, Orthofer R, Lamuela-Raventos R. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. Methods of Enzymology. 1999; 299: 152-178.

246. Carando S, Teissedre P, Cabanis J.Comparison of catechin determination in human plasma by high-performance liquid chromatography with two types of detection: fluorescence and ultraviolet. Journal of Chromatography 1998; 707: 195–201.

247. Donovan J, Luthria D, Stremple P, Waterhouse A. Analysis of catechin, epicatechin and their methylated analogs. A comparison of sensitive methods. Journal of Chromatography 1999; 726: 277–283.

248. Bredt, D.S., Snyder, S.H. 1990. Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. Proc Natl Acad Sci U S A., 87(2), 1990, s.682-685.

249. Pecháňová, O., Bernátová, I., Pelouch, V., Šimko, F.: Protein remodelling of the heart in NO-deficient hypertension: the effect of captopril. J Mol Cell Cardiol 1997; 29: 3365-3374.

250. Guzik T.J. & Channon K.M.: Measurement of Vascular Reactive Oxygen Species Production by Chemiluminiscence. In Hypertension Methods and Protocols. Humana Press 2005; 73-89.

251. Davì G, Santilli F, Patrono C. Nutraceuticals in diabetes and metabolic syndrome. Cardiovasc Ther. 2010 Aug; 28(4): 216-26.

252. McDonald JT, Margen S. Wine versus ethanol in human nutrition. II. Fluid, sodium, and potassium balance. Am J Clin Nutr. 1979 Apr; 32(4): 817-22.

253. McDonald JT, Margen S. Wine versus ethanol in human nutrition. IV. Zinc balance. Am J Clin Nutr. 1980 May; 33(5): 1096-102.

254. Caballero B. Nutritional implications of dietary interactions: A review. The United Nations University Press. Food and Nutrition Bulletin *1988;* Vol. 10 .

255. Leung F. Trace elements that act as antioxidants in parenteral micronutrition J. Nutr. Biochem. 1998; 9: 304 –307.

256. Humphries S, Kushner H, and Falkner B. Low Dietary Magnesium Is Associated With Insulin Resistance in a Sample of Young, Nondiabetic Black Americans. Am J Hypertens 1999; 12: 747–756.

257. Resnick, L. M. Cellular ions in hypertension, insulin resistance, obesity and diabetes: an unifying theme. J. Am. Soc. Nephrol. 1992; 3: S78–S85.

258. Frías S, Pérez Trujillo JP, Peña EM, Conde JE. Classification and differentiation of bottled sweet wines of Canary Islands (Spain) by their metallic content. Eur. Food Res. Technol. 2001; 213:145–149.

259. Frías S, Conde JE , Rodríguez MA, Dohnal V, Pérez Trujillo JP. Metallic content of wines from the Canary Islands (Spain). Application of artificial neural networks to the data analysis. Nahrung/Food 2002; 46(5): 370–375.

260. Moreno IM, González-Weller D, Gutierrez V, et al. Differentiation of two Canary DO red wines according to their metal content from inductively coupled plasma optical emission spectrometry and graphite furnace atomic absorption spectrometry by using Probabilistic Neural Networks. Talanta 2007; 72: 263–268.

261. Saris N, Mervaalaa E, Karppanena H, Khawaja J, Lewenstam A. Magnesium: An update on physiological, clinical and analytical aspects. Clinica Chimica Acta 2000; 294: 1–26.

262. Coleman JE. Zinc Proteins: Enzymes, Storage Proteins, Transcription Factors, and Replication Proteins. Annual Review of Biochemistry. 1992; Vol. 61: 897-946.

263. Nomizu T, Falchuk KH, Vallee BL. Zinc, iron, and copper contents of Xenopus laevis oocytes and embryos. Mol Reprod Dev. 1993; 36(4): 419-23.

264. Zou MH, Hou XY, Shi CM, Nagata D, Walsh K, Cohen RA. Modulation by peroxynitrite of Akt- and AMP-activated kinase-dependent Ser1179 phosphorylation of endothelial nitric oxide synthase. J Biol Chem. 2002 Sep 6; 277(36): 32552-7.

265. Barbagallo M, Dominguez LJ. Magnesium and aging. Curr Pharm Des. 2010; 16(7): 832-9.

266. Barbagallo M, Dominguez LJ, Galioto A, Ferlisi A, Cani C, Malfa L, Pineo A, Busardo A, Paolisso G. Role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X. Mol Aspects Med. 2003 Feb-Jun; 24(1-3): 39-52.

267. Song Y, Wang J, Li X, Cai L. Zinc and the diabetic heart BioMetals 2005; 18: 325–332.

268. Han K, Sekikawa M, Shimada K, Hashimoto M, Hashimoto N, Noda T, Tanaka H. Fukushima M. Anthocyanin-rich purple potato flake extract has antioxidant capacity and improves antioxidant potential in rats. British Journal of Nutrition 2006; 96: 1125–1133.

269. Reyes L, Miller J, Cisneros-Zevallos L. Antioxidant capacity, anthocyanins and total phenolics in purple and red-fleshed potato (Solanum tuberosum L.) genotypes. American Journal of Potato Research 2005; 82: 271–277.

270. Lugasi A and Hóvári J. Antioxidant properties of commercial alcoholic and nonalcoholic beverages. Nahrung/Food 2003; 47: 79-86.

271. Burns J, Gardner PT, O'Neil J, et al. Relationship among Antioxidant Activity, Vasodilation Capacity, and Phenolic Content of Red Wines. J Agric. Food Chem. 2000; 48: 220-230.

272. Boyer J and Liu RH. Apple phytochemicals and their health benefits. Nutrition Journal 2004, 3: 5.

273. Eberhardt M, Lee C, Liu RH: Antioxidant activity of fresh apples. Nature 2000; 405: 903-904.

274. Mertens-Talcott SU, Talcott ST, Percival SS. Low concentrations of quercetin and ellagic acid synergistically influence proliferation, cytotoxicity and apoptosis in MOLT-4 human leukemia cells. J Nutr. 2003 Aug; 133(8): 2669-74.

275. Peinado J, Lerma R, Peinado A. Synergistic antioxidant interaction between sugars and phenolics from a sweet wine. Eur Food Res Technol 2010; 231: 363–370.

276. Vannucchi H. Interaction of vitamins and minerals. Arch Latinoam Nutr. 1991; 41(1): 9-18.

277. Mazza G. Anthocyanins in grapes and grape products.Crit. Rev. Food Sci. Nut. 1995; 35: 341-371.

9 Appendices

Attachment 1. Phytochemicals classification.

Attachment 2. The main effects of vitamin C.

Attachment 3. The major effects of vitamin E.

This thesis is based on the following original publications in journals with Impact Factor.

1. Kondrashov A., Ševčík R., Benáková H., Koštířová M., and Štípek S. The key role of grape variety for antioxidant capacity of red wines. The European e-Journal of Clinical Nutrition and Metabolism 2009; Vol.4: e41–e46.

2. Ševčík R., Kondrashov A., Kvasnička F., Vacek J., Hamouz K., Jirušková M., Voldřich M., Čížková H. The impact of cooking procedures on antioxidant capacity of potatoes. Journal of Food and Nutrition Research 2009; Vol. 48 (4): 171–177.