Review

The atherosclerotic heart disease and protecting properties of garlic: contemporary data

Shela Gorinstein[1], Zenon Jastrzebski[2], Jacek Namiesnik[3], Hanna Leontowicz[4], Maria Leontowicz[4] and Simon Trakhtenberg[5]

[1] Department of Medicinal Chemistry and Natural Products, School of Pharmacy, The Hebrew University-Hadassah Medical School, Jerusalem, Israel
[2] Department of Pharmacology, Institute of Public Health, Warsaw, Poland
[3] Chemical Faculty, Gdansk University of Technology, Gdansk, Poland
[4] Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, Warsaw, Poland
[5] Kaplan University Medical Center, Rehovot, Israel

This article reviews the contemporary data concerning atherosclerosis and protecting properties of garlic. Recent advances in basic science have established a fundamental role for inflammation in mediating all stages of this disease from initiation through progression and, ultimately, the thrombotic complications of atherosclerosis. These new findings provide important links between risk factors and the mechanisms of atherogenesis and garlic properties. Numerous *in vitro* studies have confirmed the ability of garlic to reduce the parameters of the risk of atherosclerosis: total cholesterol, LDL, triglycerides, oxidized LDL. Bioactive compounds and antioxidant potentials in fresh, cooked, boiled and commercial garlic from different regions are presented, using β-carotene, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), nitric oxide (NO), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) with $K_2S_2O_8$ or MnO_2, ferric-reducing/antioxidant power (FRAP), cupric reducing antioxidant capacity (CUPRAC) and others assays for antioxidant status. *In vivo* studies were reviewed on with garlic and cholesterol supplemented diets. The positive influences of garlic on plasma lipids, proteins, antioxidant activity, and some indices of blood coagulation are dose dependent. Garlic could be a valuable component of atherosclerosis-preventing diets only in optimal doses. Many recently published reports show that garlic possesses plasma lipid-lowering and plasma anticoagulant and antioxidant properties and improves impaired endothelial function.

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1 Disease definition

Atherosclerosis, the principal cause of heart attack, stroke and gangrene of the extremities, is responsible for 50% of all mortality in the USA, Europe and Japan. The lesions result from an excessive, inflammatory-fibroproliferative response to various forms of insult to the endothelium and smooth muscle of the artery wall. A large number of growth factors, cytokines and vasoregulatory molecules participate in this process [1, 2].

Nowadays most of the scientists agree that atherosclerosis is a complex process, characterized by an inflammatory, fibro-fatty, proliferative response to damage of the artery wall involving smooth muscle cells, monocyte-derived macrophages, T-lymphocyte and platelets and that hyperlipidemia constitutes a major etiopathological factor for this disease. It was a long way before most of the scientists agree to this definition.

As reported by Kritchevsky [3], the first purely nutritional investigation into experimental atherosclerosis was carried out by Ignatowski in 1908. Believing that a toxic metabolite of animal protein led to atherosclerosis, he fed meat to adult rabbits and milk and egg yolk to weanling rabbits and caused atherosclerosis. The discovery in 1912 that

Correspondence: Dr. S. Gorinstein, Department of Medicinal Chemistry and Natural Products, The Hebrew University of Jerusalem-Hadassah Medical School, P.O.B. 12065, Jerusalem 91120, Israel
E-mail: gorin@cc.huji.ac.il
Fax: +972-2-675-7076

Abbreviations: AGE, aged garlic extract; **CAD**, coronary artery disease

dietary cholesterol *per se* was atherogenic turned attention to fat and cholesterol, eclipsing work on dietary protein. Since this times many thousand experiments, clinical and epidemiological investigations demonstrated the central role of dietary cholesterol in development of atherosclerotic changes in the arteries of animals and humans alike.

Intensive clinical and epidemiological investigations have shown that not only dietary cholesterol takes part in the atherosclerosis process. More than 200 risk factors for atherosclerosis have been identified [4]. The three most important are (i) abnormal lipids including familial hypercholesterolemia [5], (ii) cigarette smoking and (iii) high blood pressure. In addition, many other factors including homocysteine, haemostatic factors such as fibrinogen, C-reactive protein and other are known as factors increasing the risk of atherosclerosis development.

It was demonstrated that among plasma lipids low-density lipoprotein cholesterol (LDL-C) and low HDL-C level are the most atherogenic [6]. However, only oxidized LDL-C particles are able to penetrate arterial walls and cause their occlusion [7, 8].

Atherosclerosis begins in childhood as deposits of cholesterol and its esters, referred to as fatty streaks, in the intima of large muscular arteries. In some persons and at certain arterial sites, more lipids accumulate and covered by a fibromuscular cap form a fibrous plaque [9]. The focal arterial lesion-prone sites play an important role [6]. Key initial participants in these sites include the focal intimal influx and accumulation of LDL-C and a preferential recruitment of blood monocytes. Both are further enhanced in the presence of hyperlipidemia, when the quantity of intimal LDL-C and the oxidative potential of the intima exceed the capacity of macrophages to remove, via the non-down-regulating scavenger receptor, cytotoxic anionic (Ox-LDL) macromolecules. Foam cells, pathognomonic of the fatty streak, form during the receptor-mediated uptake of Ox-LDL by the macrophages. Interstitial free radicals and the excess of Ox-LDL particles injure and kill cells, including the foam cells, with the formation of the necrotic extracellular lipid core, a key transitional step in lesion progression. Monocyte-macrophage recruitment to the intima is likely to be regulated not only by a multiplicity of endothelial adhesive cytokines, integrins, and selectins, but also by the monocyte-specific chemoattractant, MCP-1, constitutively synthesized and secreted by intimal smooth muscle and endothelial cells. Platelets and mural thrombosis directly contribute to subsequent plaque growth, particularly after plaque rupture or fissure and disruption of the thromboresistant endothelial cells [6].

In the atherosclerotic process macrophages are very important for intracellular lipid accumulation and foam cell formation [10]. Monocytes respond to chemotactic factors, cytokines, and macrophage growth factors produced by vascular endothelial cells, smooth muscle cells, and infiltrated cells, by migrating from peripheral blood into the arterial intima and differentiating into macrophages in atherosclerotic lesions. Although various chemotactic factors are known to induce monocyte migration, monocyte chemoattractant protein-1 is the most important and powerful inducer of migration into atherosclerotic lesions. Macrophage colony-stimulating factor is crucial for monocyte/macrophage differentiation and proliferation, and for the survival of macrophages in these lesions. Macrophages also play multifaceted roles in inducing plaque rupture, blood coagulation, and fibrinolysis via the production of various enzymes, activators, inhibitors, and bioactive mediators. During the development of atherosclerosis, macrophages interact with vascular endothelial cells, medial smooth muscle cells, and infiltrated inflammatory cells, particularly T cells and dendritic cells [10].

An increasing body of evidence determined atherosclerosis as an inflammatory disease [11 – 15]. The above-cited investigations led Libby *et al*. [16] to a conclusion that atherosclerosis, formerly considered a bland lipid storage disease, actually involves an ongoing inflammatory response. Recent advances in basic science have established a fundamental role for inflammation in mediating all stages of this disease from initiation through progression and, ultimately, the thrombotic complications of atherosclerosis. These new findings provide important links between risk factors and the mechanisms of atherogenesis.

Smoking contributes significantly to atherosclerosis morbidity and mortality. It affects all phases of atherosclerosis from endothelial dysfunction to acute clinical events, the latter being largely thrombotic. Both active and passive (environmental) smoke exposure predispose to cardiovascular events [17]. Whether there is a distinct direct dose-dependent correlation between smoke exposure and risk is debatable, as some recent experimental clinical studies have shown a nonlinear relation to cigarette smoke exposure [17]. The exact toxic components of cigarette smoke (CS) and the mechanisms involved in CS-related cardiovascular dysfunction are largely unknown, but CS increases inflammation, thrombosis, and oxidation of LDL-C. Recent experimental and clinical data support the hypothesis that cigarette smoke exposure increases oxidative stress as a potential mechanism for initiating cardiovascular dysfunction [17]. The influence of CS is more substantial in persons suffering from diseases, which are connected to atherosclerosis [18]. This fact was supported by the below cited trial.

The aim of this trial was to examine the impact of active smoking and exposure to environmental tobacco smoke (ETS) on the progression of atherosclerosis. A total of 10 914 participants from the Atherosclerosis Risk in Communities (ARIC) study were enrolled between 1987 and 1989. The changes in atherosclerosis were examined from baseline to the 3-year follow-up as indexed by intimal-medial thickness of the carotid artery, assessed by ultrasound, and adjusted for demographic characteristics, cardiovascular risk factors, and lifestyle variables. The authors

found that exposure to cigarette smoke was associated with progression of atherosclerosis. Relative to never-smokers and after adjustment for demographic characteristics, cardiovascular risk factors, and lifestyle variables, current cigarette smoking was associated with a 50% increase in the progression of atherosclerosis. Smoking is of particular concern for patients with diabetes and hypertension [19].

Atherosclerotic changes were reported also in young smokers [20]. A multicenter cooperative study, Pathobiological Determinants of Atherosclerosis in Youth (PDAY), was organized to study atherosclerosis in trauma victims 15 – 34 years of age. It was demonstrated that smoking is strongly associated with the prevalence and extent of grossly visible raised lesions in the abdominal aorta. Coronary arteries from 50 smokers and 50 non-smokers were classified microscopically using a system developed by the American Heart Association in order to determine the stage at which smoking affects atherosclerosis. Smokers had over twice as many advanced lesions, as non-smokers (32 *vs*. 14%).

The passive smoking is a risk factor for atherosclerosis. The passive smoking affect the antioxidant defense of human serum, the extent of lipid peroxidation, and the accumulation of LDL-C in cultured human macrophages, the precursors of foam cells in atherosclerotic lesions [21]. The above-cited data provide the pathophysiological background for the recent epidemiological evidence about the increased atherosclerosis risk among passive smokers.

The effects of even brief passive smoking are often nearly as large (averaging 80 to 90%) as chronic active smoking [22].

The importance of the cessation of smoking was underlined by Bolego *et al*. [23]. They found that the incidence of coronary artery and cerebrovascular diseases in ex-smokers consistently decreases after cessation, further underlying the relevance of smoking as a risk factor for these pathological conditions.

There is much evidence that hypertension is one of the major risk factors for atherosclerosis [24, 25]: elevated levels of blood pressure correlate with elevated risk of atherosclerotic complications, although the mechanisms have not been well elucidated. As the cellular and molecular mechanisms of the pathogenesis of atherosclerosis and the effects of hypertension are being more clearly defined, it becomes apparent that the two processes have certain common mechanisms. There is increasing evidence that atherosclerosis should be viewed fundamentally as an inflammatory disease and the endothelium is a likely central focus for the effect of both diseases [26, 27].

In spite of the fact that until now the etiopathogenesis of hypertension is not defined, this illness can be well treated not only by pharmacological means. Weight loss, alcohol reduction, sodium restriction and especially increased physical activity could be helpful in treatment of this disease. Therefore, in a population-based prospective cohort study among men with hypertension, vigorous physical activity was associated with markedly reduced rates of cardiovascular mortality: 6.3 versus 21.0 deaths per 1000 person-years [28]. Reduction of blood pressure greatly reduces the risk of atherosclerotic complications. Decrease of the diastolic blood pressure of 5 to 6 mm Hg reduces the risk of stroke by 40% and the risk of coronary artery disease by 14% [29]. In addition, treatment of systolic blood pressure mostly in the elderly could be effective [30].

The study of lipid metabolism has dominated research into atherosclerosis etiology for decades, although now it is widely recognized that a large number of people with symptomatic atherosclerosis have no detectable evidence of abnormal lipid metabolism [31]. Careful review of epidemiological studies indicates that the classic risk factors, as hypercholesterolemia, cigarette smoking, and hypertension, account for the majority but not the entirety of the etiology and pathogenesis of the clinical complications of atherosclerosis [32]. Evidence has accumulated that factors other than conventional risk factors may contribute to the development of atherosclerosis [33]. These newer risk factors for atherosclerosis include homocysteine, fibrinogen, impaired fibrinolysis, increased platelet reactivity, hypercoagulability, small dense low-density lipoprotein cholesterol, inflammatory-infectious markers and many other [33 – 36].

2 Prevalence

At the beginning of a new millennium atherosclerotic cardiovascular disease remains the leading cause of both death and disability in North America [37]. As far as in 1993 it has been predicted that one in three Americans would eventually die of cardiovascular disease [38]. In following years due to aggressive preventive measures the death rate from atherosclerosis had constantly declined and the largest decline from the previous year was in 1998 – by 9.5% [39]. However, even now, atherosclerosis is the single biggest cause of death in US and Western Europe [37]. In 1999 more than 12 million people in the United States were estimated to suffer from coronary atherosclerosis and approximately 960 000 died from this disease [40]. The economic costs associated with this disease are estimated to be $ 112 billion [41]. The above-mentioned explains why the prevention of atherosclerosis is more than important.

3 Treatment

3.1 Medication

As was mentioned, atherosclerosis is not a bland lipid storage disease and actually involves an ongoing inflammatory response [17]. It did not contradict the claims that plasma lipids are playing a central role in progression and regres-

sion of atherosclerosis. Therefore, the major role in medication of atherosclerosis is plying hypolipidemic agents. During last 40 years many lipid-decreasing drugs were used. At present, the leading agents are statins (lovastatin; pravastatin; simvastatin) [42 – 44]. The below-cited investigations confirm these claims.

In a randomized, double-blind, placebo-controlled, multicenter coronary angiographic trial, 270 patients 37 to 67 years old, with total cholesterol ranging from 190 to 295 mg/dL and angiographically defined coronary artery disease participated. They received a cholesterol-lowering diet and either lovastatin, 80 mg/day, or placebo. As results of such treatment lovastatin lowered total cholesterol level by 32%, LDL-C by 38%, and the apolipoprotein B by 26% and raised the high-density lipoprotein cholesterol by 8.5% ($p < 0.001$). According to angiographic control, for lesions 50% or greater, average percent diameter stenosis increased 0.9% in placebo recipients and decreased 4.1% in lovastatin recipients ($p = 0.005$) [42].

In the next investigation, 447 subjects with serum LDL-C levels > or = 155 mg/dL (> or = 4.0 mmol/L) and total cholesterol (TC) levels <290 mg/dL (<7.5 mmol/L) were randomly assigned to receive either pravastatin 40 mg/day or placebo for 3 years. Atherosclerosis progression was assessed with B-mode ultrasonography. The given treatment reduced the rate of progression by 45% (95% confidence interval [CI]: 16 – 69%, $p = 0.005$) in carotid arteries and by 66% (95% CI: 30 – 90%, $p = 0.002$) in the common carotid arteries. Subjects who received pravastatin had a higher antioxidative capacity of LDL-C, a longer oxidation lag of very low-density lipoprotein (VLDL), and a reduced oxidation rate of LDL-C and VLDL *in vitro* [43].

Eighteen asymptomatic hypercholesterolemic patients with documented aortic and/or carotid atherosclerotic plaques were selected for the next study. Thirty-five aortic and 25 carotid artery plaques were detected. Serial black-blood magnetic resonance imaging (MRI) of the aorta and carotid artery of the patients was performed at baseline and 6 and 12 months after lipid-lowering therapy with simvastatin. Simvastatin induced a significant ($p < 0.01$) reduction in TC and LDL-C levels at 6 weeks that was maintained thereafter. After 12 months of treatment, significant reductions in vessel wall thickness and vessel wall area were observed in both aortic and carotid arteries ($p < 0.001$) [44].

All three cited studies proved that decrease in the plasma lipid levels leads to reduced rate of atherosclerosis progression.

The assessment of the results of measures of primary prevention is very important: it helps to decide if the present treatment is effective.

Primary prevention of coronary heart disease is most appropriate for patients at relatively high risk as shown by Pletcher *et al*. [45]. These authors investigated if measurement of coronary artery calcium could be proposed as a way to improve risk assessment and found that the coronary artery calcium score is an independent predictor of coronary heart disease events. Other authors also examined associations between cardiovascular risk factors and coronary calcification by electron-beam tomography in an unselected population of older subjects (Oei *et al*. [46]). They found that cardiovascular risk factors are associated with coronary calcification.

3.2 Preventive measures

The cessation of smoking is very important: the incidence of coronary artery and cerebrovascular diseases in ex-smokers consistently decreases after cessation, underlying the relevance of smoking as a risk factor for these pathological conditions [24]. In addition, reduction of blood pressure greatly reduces the risk of atherosclerotic complications: as was mentioned, decrease of the diastolic blood pressure of 5 to 6 mm Hg reduces the risk of stroke by 40% and the risk of coronary artery disease by 14% [29].

Therefore, in the prevention of atherosclerosis and its complications the leading measures are decrease of the plasma lipid levels, cessation of smoking and reduction of blood pressure.

An important role in the atherosclerosis prevention is played by proper diets. These diets have to contain a lot of vegetables and fruits and limited quantities of fats [47]. Among these diets the most effective is so-called Mediterranean diet, which contains inter alia oils, vegetables and fruits [48, 49]. Greater adherence to the traditional Mediterranean diet is associated with a significant reduction in total mortality [50, 51].

One of the regularly used vegetable in this diet is garlic (*Allium sativum* L.).

4 Garlic

4.1 History

Garlic was used already at the beginning of the history: it was found in Egyptian pyramids and ancient Greek temples [52]. Some of the earliest references to this medicinal and culinary plant are found on Sumerian clay tablets dating from 2600 – 2100 BC [53]. Garlic was an important medicine to the ancient Egyptians especially for the working class involved in heavy labor and is listed in the medical text *Codex Ebers* (ca. 1550 BC) [53] Ancient medical texts from Egypt, Greece, Rome, China and India each prescribed medical applications for garlic [54]. There is evidence that during the earliest Olympics in Greece, garlic was fed to the athletes as perhaps one of the earliest"performance enhancing" agents [54, 55]. The leading Indian ancient medical text, *Charaka-Samhita* recommends garlic for the treatment of heart disease and arthritis. In another ancient Indian medical textbook *Bower Manuscript*, garlic is recommended for fatigue, parasitic disease, digestive dis-

order and leprosy [53]. In addition, Hippocrates, the well-known physician, has used garlic for treatment of some diseases. It is of interest that cultures, which developed without contact with one another came to similar conclusions about the efficacy of garlic [52]. With the onset of Renaissance, increasing attention was paid in Europe to the medical use of garlic, which continues to the present times.

4.2 Bioactive compounds of garlic and the used garlic preparations

It has been found that the majority of raw garlic is water (65%), and the bulk of the dry weight is composed of fructose-containing carbohydrates, followed by sulfur compounds and protein [54]. Its organoleptic properties are due to sulfuric compounds that are synthesized from gamma-glutamyl-cysteine. As a result of cutting or crushing garlic, the enzyme allinase decomposes alliin to produce allicin, which is responsible for the characteristic flavor [55]. Allicin (diallythiosulfinate), the most active substance of garlic, has been shown to possess a variety of biological activities [56]. In addition, other investigators demonstrated that allicin is the main bioactive component of freshly crushed garlic cloves [57].

Progress in establishing systemic pharmacological effects for fresh, crushed garlic in humans has been hindered by (i) the inability to measure allicin bioavailability, (ii) lack of direct evidence that allicin has significant systemic activity at doses of garlic normally consumed, and (iii) lack of a model for an acute effect [58].

The authors have addressed these problems by quantifying the increases in breath acetone and breath allyl methyl sulfide (AMS). The area under the 48-h curve was measured in humans after consumption of standardized garlic preparations, allicin, and allicin-derived compounds, at the equivalent of 7 g of crushed garlic. Lawson and Wang [58] have shown that the allyl thiosulfinates (mainly allicin) are solely responsible for breath AMS and increased breath acetone. Diallyl trisulfide, diallyl disulfide, ajoene, and S-allylmercaptocysteine, at isomolar dithioallyl, showed the same quantitative effects as allicin. Consumption of AMS at isomolar allyl also gave the same effects as allicin, indicating that AMS is the main metabolite of allicin and is an active metabolite. Lawson and Wang [58] concluded that allicin and allicin-derived compounds are rapidly metabolized to AMS, a compound that stimulates the production of acetone and which can be used to measure the bioavailability of allicin and, hence, the ability of garlic supplements to represent fresh garlic.

The contents of the organosulfur compounds are varying depending on the garlic cultivars [59]. The results of the determination of the organosulfur compounds in two varieties of Iranian garlic [*Allium sativum* var. sativum (I) and *Allium sativum* var. holmense (II)] are cited below. The organosulfur compounds in (I) exhibiting a concentration higher than 1% are diallyl sulfide (1.3%), diallyl disulfide (8%), methyl allyl disulfide (19%), methyl allyl trisulfide (3.2%), diallyl trisulfide (5.5%), diallyl tetrasulfide (2%), 2,3-dimethyl thiophen (1.8%), 5-methyl-1,2,3-thiadiazol (5%), and 1,2-ditiolan-3-carboxilic acid (1.5%). The amount of organosulfur compounds in (II) are diallyl disulfide (2%), methyl allyl trisulfide (6.3%), diallyl tetrasulfide (5%), and cyclopentanthiol (2.5%).

The protein content in garlic is about 3.1%. The electrophoretic patterns of the fresh garlic samples based on the SDS-PAGE system are separated into numerous components (37 bands). The molecular weight range of detected components ranges from 10 to 205 kDa. The majority of the protein bands were in molecular weight range of 24–97 kDa. In all examined patterns two duplicated, more intensive major components (50 and 12 kDa) were detected [60].

The free amino acid content of garlic samples as evaluated in two garlic subspecies *Allium sativum* L. var. opioscorodon (hardneck) and *Allium sativum* L. var. sativum (softneck) ranged from 1121.7 to 3106.1 mg/100 g of fresh weight (mean = 2130.7 ± 681.5 mg/100 g). Hardneck garlic had greater methiin, alliin, and total free amino acids contents compared to softneck garlic. The major free amino acid present in all but one subspecies was glutamine (cv. Mother of Pearl contained aspartic acid as the major free amino acid). Cv. Music Pink garlic (a rocambole hardneck variety) contained the most methiin, alliin, and total free amino acids [61].

The vitamins A, C, E, B-complex, tocopherols and riboflavin were detected in garlic [60]. The concentrations of essential trace elements in raw garlic are 556.1, 446.9, 143.3, 5.5 and 2.5 μg in 100 g fresh weight (FW), for zinc, manganese, copper, selenium and iodine, respectively [60]. The contents of total polyphenols, total and a-tocopherols in raw garlic are high: 120–130, 103.1 and 84.9 mg/100 g FW, respectively [62].

Dietary fibers exercise a plasma cholesterol-lowering effect. The content of total, insoluble and soluble dietary fibers in edible parts of fresh garlic is 23.1 ± 2.1, 14.9 ± 1.2 and 8.2 ± 0.6 g/kg, respectively [60].

The most used garlic preparations are raw garlic *per se*, aged garlic, garlic oil and commercially prepared lyophilized garlic powder, garlic oil, garlic oil macerate and aged garlic extract [63]. Aged garlic is sliced raw garlic stored for 20 months in 15–20% ethanol. This process causes considerable loss of allicin and increased activity of certain newer compounds, like S-allylcysteine (SAC), S-allylmercaptocysteine, allixin and selenium that are stable, highly bioavailable and significantly antioxidant [53].

Nowadays consumers prefer to use commercially prepared garlic. The commercial garlic is prepared by the producers from frozen lyophilized raw garlic samples. The lyophilized garlic is transformed into powder, and in this form is used by the consumers. From 1 kg of raw garlic, 250 g of powdered lyophilized garlic is received [62].

The essential oil content of garlic cloves is 0.2–0.5% and consists of a variety of sulfides, such as DADS and diallyl trisulfide. Eighty percent of the total lipids consists of four fatty acids (FA): linoleic (46–53%), palmitic (20–23%), oleic (4–13%) and α-linolenic acid (3–7%). About 70 other FA were determined, 14 of them above 0.4% and only 4 above 2.5% [64]. Medicinally used garlic oil is mostly prepared by steam distillation process. Steam-distilled garlic oil consists of diallyl (57%), allyl methyl (37%) and dimethyl (6%) mono to hexa sulfides. A typical commercial preparation of garlic oil contains diallyl disulfide (DADS, 26%), diallyl trisulfide (DATS, 19%), allyl methyl trisulfide (15%), allyl methyl disulfide (13%), diallyl tetrasulfide (8%), allyl methyl tetrasulfide (6%), dimethyl trisulfide (3%), penta sulfide (4%) and hexa sulfide (1%) [53].

In pickled garlic, the contents of bioactive compounds are different: salt content ranged from 2.39 to 7.40% and water, protein, and dietary fiber – 86.89%, 3.35%, and 2.1%, respectively. The concentrations (wet weight basis) of other major components are: fat (0.21–0.35%), ash (2.65–8.40%), and sugars (2.21–4.22%). The levels of vitamins: thiamine, 0–0.055 mg/kg, riboflavin, 0.013–0.032 mg/kg, a-tocopherol, 0.36–2.53 mg/kg and ascorbic acid, 0–47.9 mg/100 g [65]. Processing with and without fermentation changes contents of garlic components. On a dry basis, the fermented product had a higher content of riboflavin, α-tocopherol, and most individual amino acids but a lower thiamin level than the unfermented product. Ascorbic acid is totally lost during processing [66].

In order to be an effective remedy for atherosclerosis, garlic has to be plasma cholesterol-lowering, plasma anticoagulant and antioxidant increasing agent and be able to protect the endothelial function of arteries. In the following review of the recently published reports we are trying to find out if garlic could be a cardioprotective agent.

4.3 Garlic and plasma hyperlipidemia

Hyperlipidemia is the major risk factor of atherosclerosis and the lowering of its level together with increase in plasma anticoagulant and antioxidant activities decreases the morbidity and mortality from this disease [67, 68]. The most studied and reported health-promoting effect of garlic is cardioprotective, which includes the three above-mentioned properties and first of all plasma lipid-decreasing effect [69, 70]. In a comprehensive review, 10 experiments of garlic supplementation in laboratory animals and 11 investigations of humans were reported, most of which confirmed lipid-lowering effect of this vegetable [53]. In the following 4 years after the above-mentioned review the results of new trials were published. We have chosen only four of them, which are connected by use of raw and boiled garlic in experiments on rats [62, 71–73]. In the below-cited animal trials the lipid-lowering effect of diets supplemented with raw or boiled garlic in rats fed cholesterol-containing diets was demonstrated [62, 71, 72]. In all these experiments the same protocol was used: (i) Wistar male rats were randomly divided into control and experimental groups; (ii) the rats of the control group were fed basal diet (BD) that included wheat starch, casein, soybean oil, cellulose, mineral, and vitamin mixtures; (iii) raw or boiled garlic was administered orally; (iv) the duration of the experiments was 28–30 days. To the BD of the experimental groups, 25 mg of lyophilized garlic (equivalent to 500 mg raw garlic/kg body weight) or garlic boiled for 20, 40 and 60 min and 1% of cholesterol was added. After the trial in the groups of rats, whose diets were supplemented with raw or boiled for 20 min garlic a significant hindering in the rise in plasma lipids was registered: total cholesterol (TC), 20.1–26.8%, LDL-C, 39.3–54.2% and triglycerides, 8.6–17.3%, and TC in liver, 27.2–35.7%. No significant changes were found in the level of HDL-C. Addition of garlic boiled for 40 and 60 min did not affect plasma and liver lipid levels.

Based on the results of these experiments the authors concluded that only raw or boiled for 20 min garlic possesses plasma lipid-lowering properties.

In the next experiment, raw and boiled aqueous extracts of garlic were administered daily to normal rats both orally and intraperitoneally for 4 weeks [73]. The serum levels of glucose, cholesterol, and triglycerides were measured. When the rats were treated with a low dose (50 mg/kg) of raw aqueous extract of garlic, no significant changes in the serum glucose levels were observed compared with the control group. However, there was a significant reduction in the cholesterol level of rats receiving a low dose of garlic (11–14%). Rats receiving garlic orally and intraperitoneally also showed a significant reduction in triglyceride levels (38%). When the rats were treated with a high dose (500 mg/kg) of raw garlic, glucose, cholesterol, and triglyceride levels were significantly affected. When boiled garlic extracts were administered at high concentrations (500 mg/kg), there was no effect on the level of serum glucose. However, a relatively small but significant decrease in the concentration of cholesterol and triglycerides was observed in the serum of the rats receiving boiled garlic. The authors concluded that raw garlic had a profound effect in reducing the glucose, cholesterol, and triglyceride levels, whereas boiled garlic had little effect in controlling these parameters.

In the investigations of humans not only raw and aged garlic extract (AGE) but also different medicinal preparations of garlic were used. Therefore, Russian investigators studied effects of allicor (a long-acting garlic drug) on the risk of coronary artery disease (CAD) [74]. They examined 167 patients with hyperlipidemia free of CAD. In men, intake of allicor for 12 months resulted in a 10.7% reduction of a 10-year absolute risk to develop CAD ($p < 0.05$) and decreased a 10-year absolute risk of acute myocardial infarction and sudden death by 22.7% ($p < 0.05$). In women, allicor prevented age-related cardiovascular risk ($p < 0.05$).

Among lipid parameters, the greatest decrease was observed for total and LDL-C cholesterol ($p < 0.05$) by 27.9 and 22.5 and 11.4 and 10.8 mg/dL for men and women, respectively.

A recently published study described the investigation of diabetic patients suffering from hyperlipidemia [75]. A clinical trial was performed in a group of 50 type 2 diabetic patients (39 women, 11 men, cholesterol concentration greater than or equal to 220 mg/dL) to assess the effect of garlic powder tablet on blood glucose, lipid profiles and blood pressure. Each patient received tablets containing 300 mg of garlic powder three times per day for 6 weeks. In the beginning of the study and after 6 weeks supplementation of garlic powder tablets, blood pressure, fasting blood glucose, glycated hemoglobin and lipid profiles were measured. After the trial, the following results were registered: a significant decrease in TC ($p < 0.01$), LDL-C ($p < 0.001$), systolic blood pressure ($p < 0.03$) and a significant increase ($p < 0.02$) in HDL-C. No significant changes in diastolic blood pressure, fasting blood sugar, serum triglycerides and HbA1c were found.

Not only above-cited individual trials confirmed the lipid-lowering effect of garlic and its preparations. Therefore, Stevinson *et al*. [76] search revealed 39 garlic trials in the literature. Thirteen of these trials provided data suitable for statistical pooling. Other were excluded because they were not placebo-controlled, were not randomized, were not double-blind, did not test a monopreparation, did not report total cholesterol level or reported a mean baseline total cholesterol level less than 5.17 mmol/L (200 mg/dL). A total of 796 persons were involved in his research. Baseline values (mean ± SD) in the garlic groups ranged from 5.78 ± 1.06 mmol/L (223 ± 41 mg/dL) to 7.72 ± 3.37 mmol/L (298 ± 130 mg/dL). In the placebo groups, the baseline values ranged from 5.62 ± 0.70 mmoI/L (217 ± 27 mg/dL) to 7.64 ± 1.55 mmol/L (295 ± 60 mg/dL). Ten trials report mean differences that favor garlic over placebo. Only three trials show 95% CI that do not overlap the line of zero effect, indicating significant differences. Meta-analysis of all trials indicated a significant difference ($p < 0.01$) in the reduction of total cholesterol level from baseline in favor of garlic compared with placebo. The weighted mean difference was –0.41 mmol/L (95% CI, −0.66 to −0.15 mmol/L) and (–15.7 mg/dL [CI, −25.6 to −5.7 mg/dL]), respectively. This is equivalent to a 5.8% reduction in total cholesterol levels from baseline due to garlic.

The meta-analysis of the authors focused on the effect of garlic on total cholesterol level. However, because five of the trials also presented other lipid data, they were analyzed to provide an indication of the effect on garlic on HDL and LDL cholesterol levels. The results indicated a nonsignificant difference in the reduction of LDL cholesterol levels between garlic and placebo and a nonsignificant difference in the increase of HDL cholesterol between garlic and placebo. The weighted mean differences were −0.17 mmoI/L (CI, −0.35 to 0.01 mmol/L) (−6.6 mg/L [CI, −13.5 to 0.4 mg/dL]) and 0.07 mmol/L (CI. −0.10 to 0.2 mmol/L) (2.7 mg/dL [CI, −3.9 to 8.9 mg/dL]), respectively [76].

In the very recently published article of Gardner *et al*. [77] the effect of raw garlic *vs*. commercial garlic supplements on plasma lipid concentration in adults with moderate hypercholesterolemia was described.

The cited experiments *in vivo* and investigations of humans show that garlic and its preparations possess plasma lipid-lowering properties.

Despite the reported hypocholesterolemic effect of garlic, the mechanism of this effect is still unclear [78]. In a randomized, double-blind, placebo-controlled intervention study, these authors showed that AGE supplementation was effective in lowering plasma concentration of TC by 7% and LDL-C by 10% in hypercholesterolemic men compared with subjects consuming placebo. Supplementation of AGE to animal diets similarly reduced plasma concentrations of TC and triglycerides by 15 and 30%, respectively. In subsequent experiments using cultured rat hepatocytes, they found 44–87% inhibition of cholesterol synthesis by the water-extractable fraction (WEF), methanol-extractable fraction (MEF) and petroleum ether extractable fraction (PEF) of fresh garlic, and Kyolic (liquid form of AGE). They observed that hydrophilic and hydrophobic compounds of garlic are inhibitory to cholesterol synthesis. Because *S*-allylcysteine (SAC) alone was less potent than Kyolic, which contains SAC and other sulfur compounds, a maximal inhibition appears to require a concerted action of multiple compounds of garlic. In a series of experiments, water-soluble compounds SAC, *S*-ethylcysteine (SEC), and *S*-propylcysteine (SPC) inhibited cholesterol synthesis by 40–60% compared with 20–35% by γ-glutamyl-*S*-allylcysteine (GSAC), γ-glutamyl-*S*-methylcysteine (GSMC) and γ-glutamyl-*S*-propylcysteine (GSPC). Lipid-soluble sulfur compounds (*i. e*. diallyl sulfide, diallyl disulfide, diallyl trisulfide, dipropyl sulfide and dipropyl trisulfide) at low concentrations (0.05–0.5 mol/L) slightly (10–15%) inhibited cholesterol synthesis but became highly cytotoxic at high concentrations (1.0–4.0 mol/L). The authors concluded that the cholesterol lowering effects of garlic extracts, such as AGE, stem in part from inhibition of hepatic cholesterol synthesis by water-soluble sulfur compounds, especially SAC.

There are also other investigations, which explore the principal mechanism by which garlic decreases cholesterol levels [79]. These authors claim that garlic decreases cholesterol by inhibition of sterol 4a-methyl oxidase. In order to identify the principal site of inhibition in the cholesterolgenic pathway and the active components of garlic, they have treated with aqueous garlic extract or its chemical derivatives cultured hepatoma cells and identified and quantified radiolabeled cholesterol and intermediates. The used extract reduced cholesterol synthesis by up to 75% without evidence of cellular toxicity. Levels of squalene

Table 1. Plasma lipids (mmol/L) and total cholesterol in liver (μmol/g) in rats fed diets supplemented with raw garlic in dose of 500, 750 and 1000 mg/kg body weight and 1% cholesterol[a)]

Diets	TC	LDL-C	HDL-C	TG	TC in liver
Chol	3.69 ± 0.21[a)]	2.08 ± 0.12[a)]	1.61 ± 0.07[a)]	0.88 ± 0.05[a)]	24.1 ± 1.2[a)]
Gar500/Chol	2.81 ± 0.17[b)]	1.19 ± 0.05[b)]	1.62 ± 0.07[a)]	0.75 ± 0.05[b)]	17.7 ± 0.9[b)]
Gar750/Chol	3.45 ± 0.18[a)]	1.81 ± 0.05[a)]	1.64 ± 0.07[a)]	0.83 ± 0.05[a)]	22.9 ± 1.0[a)]
Gar1000/Chol	3.60 ± 0.18[a)]	1.99 ± 0.05[a)]	1.61 ± 0.07[a)]	0.86 ± 0.05[a)]	23.1 ± 1.1[a)]

a) Values are means ± SD, $n = 7$. Means in columns without letters in common differ significantly ($p < 0.05$). Abbreviations used: Chol, nonoxidizedcholesterol; Gar, garlic; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; TC, total cholesterol; TG, triglycerides.

and 2, 3-oxidosqualene were not altered by garlic, indicating that the site of inhibition was downstream of lanosterol synthesis, and identical results were obtained with ^{14}C-acetate and ^{14}C-mevalonate, confirming that 3-hydroxy-3-methylglutaryl-CoA reductase activity was not affected in these short-term studies. Several methylsterols that accumulated in the presence of garlic were identified by coupled GC–MS as 4, 4′-dimethylzymosterol and a possible metabolite of 4-methylzymosterol; both are substrates for sterol 4a-methyl oxidase, pointing to this enzyme as the principal site of inhibition in the cholesterolgenic pathway by garlic. Of nine garlic-derived compounds tested for their ability to inhibit cholesterol synthesis, only diallyl disulfide, diallyl trisulfide, and allyl mercaptan proved inhibitory. The results of this investigation indicate that compounds containing an allyl-disulfide or allyl-sulfhydryl group are most likely responsible for the inhibition of cholesterol synthesis by garlic and that this inhibition is likely mediated at sterol 4a -methyl oxidase.

All above-cited investigations demonstrated the cholesterol-lowering effect of garlic and its preparations. However, also the negative results of garlic treatment, which are described below should be mentioned.

No effect of garlic therapy on major plasma lipoproteins was shown by Superko and Krauss [80]. These two investigators tested the hypothesis that a garlic supplement alters plasma lipids in 50 moderately hypercholesterolemic subjects. A double blind, randomized, placebo-controlled trial in an outpatient lipid research clinic was performed. The results of this investigation showed that garlic therapy has no effect on major plasma lipoproteins and further, that it has no impact on HDL subclasses.

The next cited study also cast doubt on the lipid-lowering effect of garlic [81]. The effect of dried garlic powder on blood lipids, blood pressure and arterial stiffness in a 12-week randomized, double blind, placebo-controlled trial was tested. Seventy-five healthy, normolipidemic volunteers (men and women aged 40–60 years) were assigned to dried garlic powder tablets (10.8 mg alliin (3-(2-propenylsulfinyl)-L-alanine)/d, corresponding to about three garlic cloves) or placebo. The primary outcome measure was serum total cholesterol concentration. Secondary outcome measures were LDL-cholesterol, HDL-cholesterol and triglycerides concentrations, blood pressure and arterial stiffness assessed by pulse wave velocity. No significant differences between the garlic and placebo groups were detected for any of the outcome measures. In conclusion, garlic powder tablets have no clinically relevant lipid- and blood pressure-lowering effects in middle-aged, normolipidemic individuals. The putative anti-atherosclerosis effect of garlic may be linked to risk markers other than blood lipids [81].

In addition, no positive results from garlic consumption on lipid profile in people with mild to moderate hypercholesterolemia were observed by Israeli investigators [82]. Therefore Kerckhoffs *et al*. [83] claim that it is still uncertain whether garlic or garlic preparations can be used as lipid-lowering agents.

It must be underlined that the lipid-lowering effect of garlic is dose dependent (Table 1).

As can be seen only the dose equivalent to 500 mg/kg body weight led to significant decrease in the level of the plasma lipids [71].

Therefore, one of the reasons of the negative results could be a wrong dose of garlic.

4.4 Garlic and plasma antioxidant activity

As was already stated, in order to be effective cardioprotective agent garlic has to possess also antioxidant properties.

It has been shown that raw garlic and AGE contain antioxidant phytochemicals that prevent oxidant damage [84]. Among them unique water-soluble organosulfur compounds, lipid-soluble organosulfur components and flavonoids, notably allixin and selenium. Long-term extraction of garlic (up to 20 months) ages the extract, creating antioxidant properties by modifying unstable molecules with antioxidant activity, such as allicin, and increasing stable and highly bioavailable water-soluble organosulfur compounds, such as *S*-allylcysteine and *S*-allylmercaptocysteine. AGE exerts antioxidant action by scavenging reactive oxygen species (ROS), enhancing the cellular antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase, and increasing glutathione in the cells. AGE inhibits lipid peroxidation, reducing ischemic/reperfusion damage and inhibiting oxidative modification of LDL, thus protecting

endothelial cells from the injury by the oxidized molecules, which contributes to atherosclerosis [84].

Not only AGE possesses antioxidant activity. In addition, the extract of garlic skins (peels) has the same properties [85]. In this study, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of garlic skin extract was evaluated. The active constituents were isolated and subsequently identified by chromatographic techniques. Analysis by HPLC coupled with a photodiode array detector (HPLC-PDA) suggests that these compounds were phenylpropanoids, which had a characteristic absorbance at 300 – 320 nm. LC-MS and NMR analyses allowed the chemical structures of the isolated constituents to be postulated. The proposed compounds were subsequently synthesized and compared with the constituents in the extract using HPLC-PDA and LC-MS. N-trans-coumaroyloctopamine, N-trans-feruloyloctopamine, guaiacylglycerol-beta-ferulic acid ether, and guaiacylglycerol-beta-caffeic acid ether were identified as were trans-coumaric acid and trans-ferulic acid [85].

The antioxidant properties of four main chemical classes of garlic, alliin, allyl cysteine, allyl disulfide, and allicin, prepared by chemical synthesis or purification were investigated [86]. Alliin scavenged superoxide, while allyl cysteine and allyl disulfide did not react with superoxide. Allicin suppressed the formation of superoxide by the xanthine/xanthine oxidase system, probably via a thiol exchange mechanism. Alliin, allyl cysteine, and allyl disulfide all scavenged hydroxyl radicals. Allyl disulfide, alliin, allicin, and allyl cysteine exhibit different patterns of antioxidant activities as protective compounds against free radical damage.

In addition, the next experiment *in vitro* tested whether fresh and commercial shallot and garlic preparations have antioxidant properties [87]. Samples produced by pressing and extraction of bulbs were tested for their ability to decrease free radicals of 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) and the results were compared with commercial preparations of aged garlic and with trolox, butylated hydroxytoluene, N-acetyl cysteine, and gallic acid. The phenolic content of the extracts and the amounts of diallyl sulfides present also were measured. The authors proved that antioxidant activities were directly related to the contents of phenolic compounds, with the fresh freeze-dried extracts significantly more potent than commercial preparations. Hexane-extracted shallot and garlic had the highest antioxidant activity, followed by water extracts, bulb pressings, and commercial products [87].

In order to compare the antioxidant properties of different garlic preparations their antioxidant potential has to be determined [60, 62, 71, 72].

The above mentioned authors have determined antioxidant potential of raw and boiled garlic by different assays: β-carotene linoleate model system (β-carotene) [88]; radical scavenging activity by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) [88]; scavenging activity against nitric oxide (NO) [89, 90], with 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{+}$) [91], ferric-reducing/antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC).

In addition, other authors found that the antioxidant potential of garlic homogenates blended with distilled water and different amounts of soybean oil was strong and diallyl disulfide was the most stable among the three sulfur compounds of garlic (allicin, diallyl disulfide, and diallyl trisulfide) [92].

Based on combined data of ten published papers [71, 72, 91, 93 – 99] the place of garlic according to antioxidant potential among other popular vegetables is shown in the Table 2. As can be seen, according to antioxidant potential, red pepper ≥ green pepper = garlic = onion ≥ white cabbage.

The results of the determination of the antioxidant potential by different antioxidant assays as shown in the Table 2 are different. In addition, other authors reported the same differences [100]. They found that antioxidant potential of the studied by them vegetables could be put in following order: red pepper > green pepper > white cabbage > white onion and green pepper > red pepper > white onion > white cabbage for FRAP and ORAC assays, respectively.

Siegel *et al*. [101] claim that deposition of lipoproteins and their protein components (apolipoproteins), notably LDL at the surface of endothelial cell membranes and connective tissue matrices in blood vessels constitute one of the initial steps of atherosclerosis. Proteoheparan sulfate can be adsorbed to a methylated silica surface in a monomolecular layer via its transmembrane hydrophobic protein core domain. This binding process was studied by using ellipsometric techniques. It was shown that HDL has a high binding affinity to the receptor and a protective effect on interfacial heparan sulfate proteoglycan layers, with respect to LDL and Ca^{2+} complexation. LDL was found to deposit strongly at the proteoheparan sulfate, particularly in the presence of Ca^{2+}, thus creating the complex formation, proteoglycan-low density lipoprotein-calcium'. This ternary complex build-up may be interpreted as arteriosclerotic nanoplaque formation on the molecular level responsible for the arteriosclerotic primary lesion. On the other hand, HDL bound to heparan sulfate proteoglycan protected against LDL docking and completely suppressed calcification of the proteoglycan-lipoprotein complex. In addition, HDL and aqueous garlic extract were able to reduce the ternary complex deposition and to disintegrate HS-PG/LDL/Ca^{2+} aggregates.

In the next investigation of Siegel *et al*. [102] the studied an *in vitro* biosensor model (PCT/EP 97/05212), the interplay between different lipoproteins in arteriosclerotic nanoplaque formation, as well as aqueous garlic extract (0.2 – 5.0 g/L from LI 111 powder) as a possible candidate drug against arterio/atherosclerosis. These processes were stud-

Table 2. The antioxidant potentials of garlic and some other popular vegetables[a)]

Samples	ORAC (μmol TE/g FW)	FRAP (μmol Fe^{2+}/g FW)	β-carotene (inhibition,%)	DPPH (RSA%)	TEAC (μmol TE/g FW)
Garlic *(Allium sativum)*	8.7 – 8.9	1.9 – 2.7	71.8 – 72.7	62.1 – 66.1	2.5 – 3.3
White onion *(Allium sepa)*	10.2 – 11.2	4.3 – 8.5	67.4 – 68.2	31.1 – 32.9	1.8 – 3.5
Green pepper (*Piper gen.)*	5.5 – 6.3	13.2 – 15.6	70.4 – 71.2	61.1 – 62.1	6.1 – 7.2
Red pepper (*Piper gen.)*	9.01 – 10.3	18.1 – 20.9	73.4 – 78.2	65.1 – 68.1	7.8 – 8.1
White cabbage (*Brassica oleracea capipata)*	11.1 – 13.6	0.7 – 1.6	52.5 – 53.9	49.6 – 51.3	1.7 – 1.9

a) Values are means of five measurements. Abbreviations used: β-carotene, β-carotene linoleate model system; DPPH, radical scavenging activity by 1,1-diphenyl-2-picrylhydrazyl; FRAP, ferric-reducing/antioxidant power; ORAC, oxygen radical absorption capacity; RSA, radical scavenging activity; TEAC, trolox equivalent antioxidant capacity.

ied by ellipsometric techniques quantifying the adsorbed amount (nanoplaque formation) and layer thickness (nanoplaque size). It has been found that proteoheparan sulfate (HS-PG) adsorption to hydrophobic silica was monoexponential and after approximately 30 min constant. The addition of 2.52 mmol/L Ca^{2+} led to a further increase in HS-PG adsorption because Ca^{2+} was bound to the polyanionic glycosaminoglycan (GAG) chains, thus screening their negative fixed charges and turning the whole molecule more hydrophobic. Incubation with 0.2 g/Ll aqueous garlic extract (GE) for 30 min did not change the adsorption of HS-PG. However, the following addition of Ca^{2+} ions reduced the increase in adsorption by 50.8% within 40 min. The adsorption of a second Ca^{2+} step to 10.08 mmol/L was reduced by even 82.1% within the next 40 min. This inhibition of receptor calcification shows that the build-up of the ternary nanoplaque complex is also affected by garlic. Siegel *et al*. [102] concluded that their experiments clearly proved that garlic extract strongly inhibits Ca^{2+} binding to HS-PG. In consequence, the formation of the ternary HS-PG/LDL/Ca^{2+} complex, initially responsible for the ‚nanoplaque' composition and ultimately for the arteriosclerotic plaque generation, is decisively blunted.

Oxidation of LDL has been recognized as playing an important role in the initiation and progression of atherosclerosis [7, 8]. Oxidized LDL, but not native LDL, promotes vascular dysfunction by exerting direct cytotoxicity toward endothelial cells, by increasing chemotactic properties for monocytes, by transforming macrophages to foam cells via scavenger-receptors and by enhancing the proliferation of various cell types, *e.g*. endothelial cells, monocytes and smooth muscle cells; all of these events are recognized as contributing to atherogenesis [103, 104]. These authors found experimental evidence that several garlic compounds can effectively suppress LDL oxidation *in vitro* and that short-term supplementation of garlic to humans increases resistance of LDL to oxidation.

In the next experiment *in vitro*, human LDL was isolated and challenged with a range of oxidants either in the presence or in the absence of AGE or its diethyl ether extract [105].

Oxidative modification of the LDL fraction using $CuSO_4$, 5-lipoxygenase and xanthine/xanthine oxidase was monitored by both the appearance of thiobarbituric-acid substances (TBA-RS) and an increase in electrophoretic mobility. This study indicates that AGE is an effective antioxidant as it scavenged superoxide ions and reduced lipid peroxide formation in cell-free assays. Superoxide production was completely inhibited in the presence of a 10% v/v aqueous preparation of AGE and reduced by 34% in the presence of a 10% v/v diethyl ether extract of AGE. The presence of 10% v/v diethyl ether extract of AGE significantly reduced Cu^{2+} and 15-lipoxygenase-mediated lipid peroxidation of isolated LDL by 81% and 37%, respectively. In addition, it was found that AGE also had the capacity to chelate copper ions. In contrast, the diethyl ether extract of AGE displayed no copper binding capacity, but demonstrated distinct antioxidant properties. These results support the view that AGE inhibits *in vitro* oxidation of isolated LDL by scavenging superoxide and inhibiting the formation of lipid peroxides. AGE was also shown to reduce LDL oxidation by the chelation of Cu^{2+} [105].

The effects of onion and garlic juices on biochemical parameters, enzyme activities and lipid peroxidation were studied also *in vivo* on alloxan-induced diabetic rats [106]. A dose of 1 mL of either onion or garlic juices/100 g body weight was orally administered daily to alloxan-diabetic rats for 4 weeks. It was found that both garlic and onion juices possess antioxidant and anti-hyperglycemic properties.

In most of experiments whole garlic was used. Gonen *et al*. [107], in their study evaluated the antioxidant effects of pure allicin on atherogenesis in experimental mouse models. Daily dietary supplement of allicin, 9 mg/kg body weight, reduced the atherosclerotic plaque area by 68.9 and 56.8% in apolipoprotein E-deficient and LDL receptor knockout mice, respectively, as compared with control mice. LDL isolated from allicin-treated groups was more resistant to $CuSO_4$-induced oxidation *ex vivo* than LDL isolated from control mice. Incubation of mouse plasma with ^{3}H-labeled allicin showed binding of allicin to lipoproteins. By using electron spin resonance, authors demonstrated reduced Cu^{2+} binding to LDL following allicin treatment.

LDL treatment with allicin significantly inhibited both native LDL and oxidized LDL degradation by isolated mouse macrophages. The authors concluded that pure allicin preparation affect atherosclerosis not only by acting as an antioxidant, but also by other mechanisms, such as lipoprotein modification and inhibition of LDL uptake and degradation by macrophages.

The changes in garlic antioxidant properties subjected to temperature were addressed by some investigators [60, 108]. It was shown that garlic subjected to boiling temperature for more than 20 min losses a significant part of its antioxidant ability [60]. However, not all investigators confirm these findings. Pedraza-Chaverri' *et al*. [108] studied the ability of aqueous garlic extracts to scavenge superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$) in the following aqueous preparations: (a) extracts of boiled garlic cloves (BG), (b) extracts of microwave-treated garlic cloves (MG), and (c) extracts of pickled garlic (PG), and heated extracts of (a) garlic powder (HGP) and (b) raw garlic (HRG). The data were compared with the unheated raw garlic (RG) or with the unheated garlic powder (GP). Extracts of GP and RG scavenged $O_2^{\bullet -}$, H_2O_2, and $OH^{\bullet}$ in a concentration-dependent way. The reactive oxygen species scavenging capacity was not decreased in the aqueous garlic extracts except in MG and HRG (for $O_2^{\bullet -}$) and in HGP and PG (for H_2O_2). The heating before or after garlic cutting was unable to eliminate the capacity of the extracts to scavenge H_2O_2, $O_2^{\bullet -}$, and $OH^{\bullet}$ [108].

As was cited, the oxidation of LDL has been recognized as playing an important role in the initiation and progression of atherosclerosis. All above cited studies *in vitro* and *in vivo* show that garlic possesses strong antioxidant properties and therefore has an important role in atherosclerosis prevention.

4.5 Garlic and plasma anticoagulant activity

Arterial thrombosis is playing a leading role in the death rate from coronary atherosclerosis: in more than in 80% patients with acute transmural infarction of the myocardium thrombotic occlusion of coronary artery was found [109]. Detailed knowledge of the pathophysiology as well as the dynamic nature of coronary thrombus formation provides a valuable tool for correct management and proper adjunctive therapy. Coronary thrombosis is in the majority of cases caused by disruption or fissuring of an atherosclerotic plaque. At the lesion thrombogenic material is exposed to the flowing blood leading to activation of platelets and the formation of a platelet clot. Simultaneously, the coagulation system is activated resulting in increased thrombin formation. Thrombin is a key mediator in arterial thrombosis, due to its effect on both platelets and fibrin generation. Thrombin contributes to the stabilization of an initially loose platelet clot by generating cross-bound fibrin within the thrombus [110]. Therefore, in order to prevent arterial thrombus formation the anticoagulant system must be activated. Recently published papers show the influence of garlic on anticoagulant system.

Antithrombotic effect of garlic consumption is one of the best-investigated [111]. Among the most studied indices of antithrombotic effect is the reduction of the platelet aggregation by garlic and its preparations [112, 113]. A 13-week study was performed in normolipidemic subjects who ingested 5 mL of AGE, Kyolic per day. Aggregation of platelet-rich plasma was induced by ADP. Dietary supplementation with AGE significantly inhibited both the total percentage and initial rate of platelet aggregation at concentrations of ADP up to 10 μmol/L. The K_M for ADP-induced aggregation was approximately doubled after supplementation with AGE, whereas the maximum rate of aggregation was unaffected. Therefore, AGE, when taken as a dietary supplement by normolipidemic subjects, may be beneficial in protecting against cardiovascular disease as a result of inhibiting platelet aggregation [114].

A meta-analytical survey based on 11 electronic databases, references, manufacturers, and experts from January 1966 through February 2000 has been published [112]. The authors included 1798 pertinent records, 45 randomized trials and 73 additional studies. Reports of cardiovascular-related effects were limited to randomized controlled trials lasting at least 4 weeks. Two physicians abstracted outcomes and assessed adequacy of randomization, blinding, and handling of dropouts. Standardized mean differences of lipid outcomes from placebo-controlled trials were adjusted for baseline differences and pooled using random effects methods. It has been found that in comparison with placebo, garlic preparations lead to a significant reduction in platelet aggregation [112].

The underlying mechanism of the platelet aggregation inhibition by AGE was described in the next study [113]. Because calcium mobilization plays an important role in platelet aggregation, the effect of AGE was investigated in this preliminary study. ADP and the calcium ionophore A23187 both stimulated platelet aggregation with a concomitant increase in intracellular calcium ion concentration. When these experiments were repeated in the presence of AGE, both platelet aggregation and calcium mobilization were suppressed. In addition, when platelets were preincubated with AGE, the initial concentration of intracellular calcium was significantly reduced compared with platelets without AGE, confirming the metal-chelating properties of AGE. Platelets loaded with fura-2 acetoxymethyl ester (fura-2 AM) also displayed a reduction in platelet aggregation, and the addition of external calcium did not alter this observation. The data obtained in this study, suggest that AGE probably exerts its inhibitory effect on platelet aggregation either by suppressing the influx of calcium ions by chelating calcium within platelet cytosol or by altering other intracellular second messengers within the platelets [113].

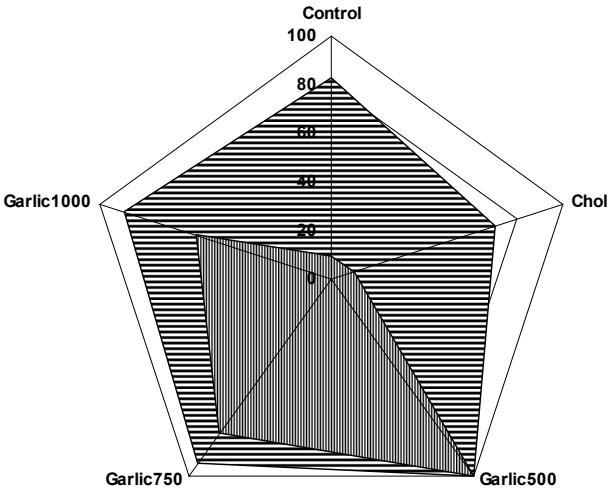

Figure 1. ▤ Total antioxidant capacity (mM TE/L) and ▥ blood clotting time (min) in plasma samples of rats after garlic and cholesterol diets. This figure is adapted from [71].

In addition, other indices of the anticoagulant activity were positively influence by commercial powdered garlic [62]. The authors randomly divided Wistar rats into five diet groups, named Control, Chol, Garlic500, Garlic750, and Garlic1000. Control rats were fed basal diet (BD), which included wheat starch, casein, soybean oil, and vitamin and mineral mixtures. To the BD of the Chol group was added 1% of cholesterol. To the BD of the other three groups (Garlic500, Garlic750, and Garlic1000) were added 1% of cholesterol and commercial garlic equal to 500, 750, and 1000 mg of raw garlic per kilogram of animal weight. After 4 weeks of the experiment a significant decrease in plasma circulating fibrinogen and an increase in the clotting time were found (Fig. 1). The fibrinogenolytic effect of garlic diets was visualized by SDS-PAGE. In the fibrinogen fraction of Garlic500 the 66, 24, and 14- kDa protein bands were detected with weaker protein intensity than in the Garlic750 and Garlic1000.

In a very recently published article, Cavagnaro *et al*. [115] describe the effect of cooked garlic on anti-platelet activity. They examined the *in vitro* anti-aggregatory activity (IVAA) of human blood platelets induced by extracts of garlic samples that were previously heated (in the form of crushed versus uncrushed cloves) using different cooking methods and intensities. These authors also monitored the concentrations of allicin and pyruvate, two predictors of anti-platelet strength. Cavagnaro *et al*. [115] found that oven-heating at 200°C or immersing in boiling water for 3 min or less did not affect the ability of garlic to inhibit platelet aggregation, whereas heating for 6 min completely suppressed IVAA in crushed samples. Prolonged incubation (more than 10 min) at these temperatures completely suppressed IVAA. Microwaved garlic had no effect on platelet aggregation. However, increasing the concentration of garlic juice in the aggregation reaction had a positive IVAA dose response in crushed, but not in uncrushed, microwaved samples. The addition of raw garlic juice to microwaved uncrushed garlic restored a full complement of anti-platelet activity that was completely lost without the garlic addition. Garlic-induced IVAA was always associated with allicin and pyruvate levels. Cavagnaro *et al*. [115] concluded that their results suggest that (i) allicin and thiosulfinates are responsible for the IVAA response, (ii) crushing garlic before moderate cooking can reduce the loss of activity, and (iii) the partial loss of antithrombotic effect in crushed-cooked garlic may be compensated by increasing the amount consumed.

4.6 Garlic and endothelial function of arteries

Endothelial dysfunction of arteries in general and coronary arteries in particular is considered an early phase of atherosclerosis and plays a key role in progression of this decease [116, 117]. There is growing recognition that endothelial dysfunction in addition to its role in early atherosclerosis, also contributes to the later stages of the disease when patients develop clinical symptoms [118]. It was shown in experiments on laboratory animals [119] and investigations of humans [120] that diets supplemented with garlic are able to restore endothelial function.

In the *in vivo* experiment, rats were fed with 1% raw garlic (RG) supplemented diet. In order to induce pulmonary hypertension monocrotaline (MCT) injections were used. In all cases, RG feeding prevented the exaggerated vasoconstrictory responses of coronary arteries to MCT. However, similar treatments with either boiled garlic (BG) or aged garlic (AG), which do not contain the active allicin metabolite, were ineffective. Further testing of vasoactivity to garlic extracts showed that only RG, but not BG or AG, elicited a potent, dose-dependent dilation on the isolated coronaries. These findings show that positive effect of RG is probably mediated via its active metabolite allicin action on coronary endothelial function and vasoreactivity [121].

Siegel *et al*. [122] have measured the electrophysiological correlation to vasodilatation in human coronary arteries under the influence of garlic extract. They observed that between 0.0002 and 0.2 g powder/L extract concentration, garlic had hyperpolarized the membrane of normal vascular smooth muscle cells of the human coronary artery in a concentration-dependent manner. Correspondingly, the isometric wall tension was decreased. For the garlic constituents allicin and ajoene, a similar course in membrane potential and wall tension for aqueous solutions between 10^{-9} and 10^{-6} mol/L was obtained. These compounds hyperpolarized the cell membrane and relaxed the vascular strips in a concentration-dependent manner. In the author's opinion, the hyperpolarization in vascular smooth muscle supports the concept that garlic extract and its compounds can be classified as phytopharmacological K^+ channel openers.

The endothelial dysfunction caused by increases in vascular oxidant stress decreases bioavailability of nitric oxide (NO) and thus plays a critical role in the vascular pathobiology of hyperhomocysteinemia [123]. AGE contains water- and oil-soluble sulfur compounds that modify the intracellular thiol and redox state, minimize intracellular oxidant stress, and stimulate NO generation in endothelial cells. In a placebo-controlled, blinded, crossover trial in healthy subjects it was examined whether AGE reduces macro- and microvascular endothelial dysfunction during acute hyperhomocysteinemia induced by oral methionine challenge. Acute hyperhomocysteinemia leads to a significant decrease in flow-mediated vasodilation of the brachial artery as determined by vascular ultrasound, indicative of macrovascular endothelial dysfunction. In addition, acute hyperhomocysteinemia leads to a decrease in acetylcholine-stimulated skin perfusion as measured by laser-Doppler flowmetry. Pretreatment with AGE for 6 weeks significantly diminished the adverse effects of acute hyperhomocysteinemia in both vascular territories. Therefore AGE may at least partly prevent a decrease in bioavailability of NO and endothelium-derived hyperpolarizing factor during acute hyperhomocysteinemia [123].

The results of a short-term treatment with AGE also proved that this natural product might improve impaired endothelial function [120]. The aim of the investigators was to test the effect of treatment with AGE on brachial artery flow-mediated endothelium-dependent dilation (FMD) and circulating markers of oxidative stress and systemic inflammation. The trial included 15 men with angiographically proven CAD in randomized, placebo-controlled, crossover design with 2-week treatment and washout periods. During AGE supplementation, FMD increased (44%) significantly ($p = 0.04$) from the baseline and mainly in men with lower baseline FMD. Levels of FMD at the end of AGE treatment were significantly ($p = 0.03$) higher compared with the corresponding levels at the end of placebo treatment when the variation in baseline body weight was taken into account. Markers of oxidant stress (plasma oxidized LDL and peroxides), systemic inflammation (plasma C-reactive protein ad interleukin-6) and endothelial activation (YCAM-1) did not change significantly during the study. These data suggest that even short-term treatment with AGE may improve impaired endothelial function in men with CAD treated with aspirin and a statin.

Therefore, all cited studies indicate that garlic is able to improve an impaired endothelial function.

5 Conclusions

Garlic was used for many millennia as a cure for a wide variety of different conditions. It was shown that garlic contains a wide spectrum of bioactive compounds and high antioxidant activity (Figs. 2 and 3) – the basis of its successful use [124]. This bulbous rooty vegetable became very popular and nowadays some researches claim that garlic is able to cure practically all diseases. However, garlic is not a panacea. Even the most enthusiastic supporters of this

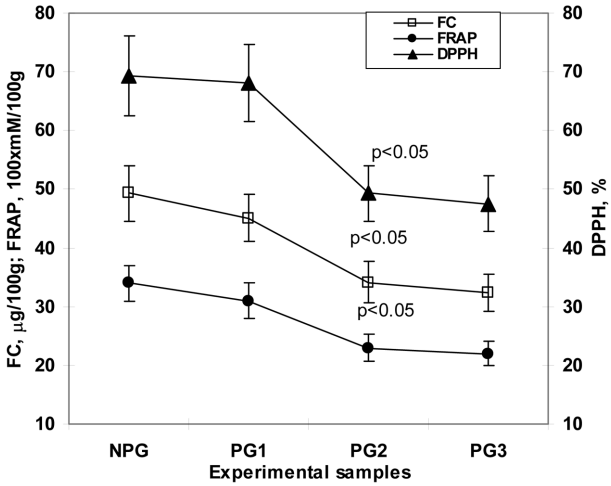

Figure 2. Different radical scavenging assays of garlic (G) samples during processing: NPG, non processed; PG1, PG2, PG3, processed garlic at 100°C for 20, 40 and 60 min. FC, polyphenols, μg/ 100g FW; FRAP, 100 × mM/100 g; DPPH, % inhibition. Values are means ± SD of 5 measurements. Abbreviations: Folin-Ciocalteu (FC); Ferric Reducing/Antioxidant Power (FRAP); 1,1-diphenyl-2-picrylhydrazyl (DPPH). This figure is adapted from [124].

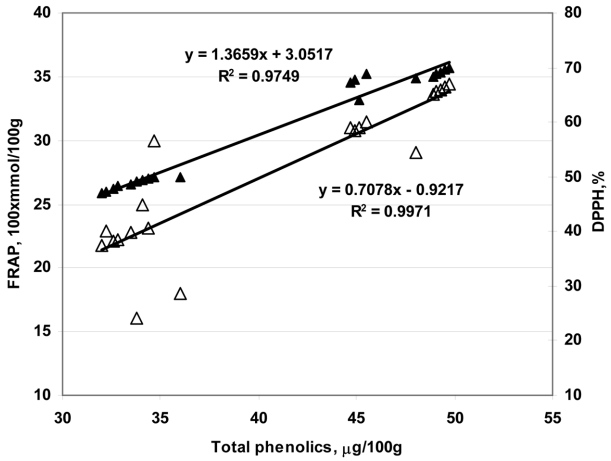

Figure 3. Correlation coefficients for processed and non processed garlic (G) samples and their antioxidant capacities. NPG, non processed; PG1, PG2, PG3, processed garlic at 100°C for 20, 40 and 60 min. (△) FC (μg/100g, X): NPG = 49.3 ± 3.1^a; PG1 = 45.1 ± 3.0^a; PG2 = 34.1 ± 2.7^b 32.4 ± 2.6^b; PG3 = 32.4 ± 2.6^b and FRAP (100 × mmol/100g, Y_1): NPG = 34 ± 2.7^a; PG1 = 31 ± 2.6^a; PG2 = 23 ± 2.1^b; PG3 = 22 ± 2.1^b to (▲) FC (μg/100g, X): NPG = 49.3 ± 3.1^a; PG1 = 45.1± 3.0^a; PG2 = 34.1± 2.7^b 32.4 ± 2.6^b; PG3 = 32.4 ± 2.6^b and DPPH (% inhibition, Y_2): NPG = 69.2 ± 5.1^a; PG1 = 68.1 ± 5.0^a; PG2 = 49.3 ± 3.1^b; PG3 = 47.5 ± 3.1^b. Values are means ± SD of 5 measurements. Values in the same method with different superscript letters are significantly different ($p < 0.05$). This figure is adapted from [124].

natural product agree that first of all garlic is an active cardioprotective agent. Many recently published reports show that garlic possesses plasma lipid-lowering and plasma anticoagulant and antioxidant properties and improves impaired endothelial function. However, some investigators reported that it is still uncertain whether garlic or garlic preparations can be used as lipid-lowering agents. Why after many years of scientific studies there are proponents and opponents of garlic use as lipid-lowering agent? Most authors share the opinion of the below-mentioned investigators [70, 125, 126]. According to them, the negative results obtained in some clinical trials may result from usage of different garlic preparations, unknown active constituents and their bioavailability, inadequate randomization, selection of inappropriate subjects, and short duration of trials. Therefore, they state "although garlic appears to hold promise in reducing parameters associated with cardiovascular disease, more in-depth and appropriate studies are required" [70].

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