UNIVERSITY OF COPENHAGEN

The effect of Ginkgo biloba and garlic on cardiovascular diseases

19. APRIL 2017

Student: Lea Nymand
KU ID: jsg739
Supervisor: Susanne Bügel

Number of characters: 59,832
ECTS load: 15 point
Does not contain confidential information
Danish titel: *Effekten Ginkgo biloba og hvidløg på hjertekarsygdomme*

ABSTRACT

Aim: The Danish Heart Foundation is updating their website regarding recommendations on dietary supplements in relation to cardiovascular disease. The aim of this report was to describe and discuss the scientific evidence regarding the effect of garlic and Ginkgo biloba in primary and secondary prevention of heart diseases.

Method: A search in Web of Science and SCOPUS was made for randomized, placebo-controlled human-trials investigating either the effect of garlic or G. biloba on heart disease and its risk factors.

Result: Seven RCTs on G. biloba was found. The effect of G. biloba was tested in both patients with increased risk of cardiovascular disease, with cardiovascular disease or healthy subjects. The age ranged wide in these 7 studies with a mean age of 20 to 79 years and the number of subjects ranged from 11-3069. The outcome was measured on blood pressure (BP), triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL) among others. Hard endpoints as mortality and CVD events was the outcome of one of the studies. Sixteen RCT was found on garlic and included both healthy subjects and subjects with symptoms within the metabolic syndrome (MS), diabetes or with coronary heart disease. They had a mean age around 40-70 years and the number of subjects ranged from 12-88. For G. biloba, a significant positive effect was seen in the incident of peripheral vascular disease events, decrease in high-sensitive C-reactive protein (hs-CRP), homeostatic model assessment-insulin resistance (HOMA-IR), interleukin-6 (IL-6), nano plaque formation, prevalence of diabetic nephropathy (NR) and diabetic retinopathy (DR). For garlic, a significant positive effect was seen in TG, TC, LDL, HDL, pulse wave velocity, hs-CRP and digital thermal monitoring (DTM). Only in hypertensive or hyperlipidemia patients a significant positive effect was seen on BP or TC, LDL and HDL respectively.

Conclusion: It is shown that in people with increased risk of plaque formation, inflammation, insulin resistance experienced improvements with the use of G. biloba with a dose of 120-240 mg/day. Likewise, in people with increased level of LDL, TG, BP experienced improvements with the use of garlic supplement with fermented/aged extract of garlic with a dose of 1200-2400 mg/day.

RESUMÈ

Mål: Hjerteforeningen i Danmark vil opdatere deres hjemmesiden mht. anbefalinger af kosttilskud som kan have en effekt på hjertekarsygdomme. Målet med denne rapport var at beskrive og diskutere the videnskabelige grundlag for effekten af Ginkgo biloba og hvidløg i primær og sekundær forebyggelse af hjertekarsygdomme (CVD).

Metode: Der blev foretaget en søgning i "Web of Science" og "SCOPUS". Her blev der søgt efter randomiserede, placebo kontrolleret forsøg (RCT) med mennesker, hvor enten G. biloba eller hvidløg blev indtaget og effekten på CVD og/eller risikofaktorerne herpå.

Resultater: Syv RCT blev fundet vedrørende G. biloba. Effekten af G. biloba blev testet i både patienter med forhøjet risiko for CVD, patienter med CVD og raske personer. Personernes alder i disse 7 studier varierede fra 20-79 år og antallet af personer varierede fra 11-3069. Udfaldet blev målt på blodtryk, triglycerid-niveau i blodet, total-kolesterol, lav-densitet lipoprotein (LDL), høj-densitet lipoprotein (HDL) mf. Der blev også målt på dødeligheden og CVD-hændelser for et af studierne. For hvidløg blev der fundet 16 RCT. Disse inkluderede raske personer og personer med symptomer eller diagnosticeret metabolisk syndrom, diabetes eller med CVD. Antallet af personer varierede fra 12-88 og de have en gennemsnitsalder fra 40 til 70 år. For G. biloba blev der fundet en positiv signifikant effekt på hændelsen af antal perifere karsygdomme. Ligeså fandtes en positiv effekt på høj-sensitiv C-reaktivt protein (hs-CRP), HOMA-IR, IL-6, nano-plak-dannelse, forekomsten af diabetisk øjensygdom og diabetisk nyresygdom. For hvidløg blev der fundet en positiv signifikant effekt på triglycerid-niveauet, total-kolesteroltallet, LDL, HDL, arteriel stivhed, hs-CRP and DTM. Kun i personer med forhøjet blodtryk eller forhøjet niveau af blodlipider fandtes en positiv forbedring på blodtryk, triglycerider, LDL og HDL.

Konklusion: Det har her vist sig at personer med forhøjet risiko for plak dannelse, inflammation og insulin resistens oplevede forbedringer med brug af G. biloba i en dosis på omkring 120-240 mg/dag. Ligeledes oplevede personer med forhøjet blodtryk og niveauer af LDL og triglycerider en forbedring med brug af hvidløg i en dosis på 1200-2400 mg/dag.

PREFACE

This assignment is written during a "Project in practice" course in association with The Danish Heart Foundation.

I have for 8 weeks worked on the association between the risk factors of cardiovascular diseases and the consumption of dietary supplements, specifically G. biloba and garlic.

I want to express my thanks to my supervisor Susanne Bügel for feedback and advise through the course. In addition, I want to say thank you to my colleagues at the Heart Foundation and especially my contact person Lotte Madsen for helping with theoretical questions about cardiovascular diseases as well as mentoring about the whole process and the issues regarding patients counselling.

_____

Lea Nymand
Jsg739
April 19th 2017

Content

ABBREVIATIONS

A2RA: angiotensin II receptor antagonists

ABG: Aged black garlic

ACEI: angiotensin converting enzyme inhibitors

AGE: Aged garlic extract

AGE-S: Aged garlic extract with supplement

AMS: Allyl methyl sulfide

AMSO: allyl methyl sulfoxide

AMSO2: allyl methyl sulfone

ApoB: apolipoprotein B

AUC: area under curve

BB: beta-blockers

BMI: body mass index

BP: blood pressure

CAC: Coronary artery calcium

CAD: Coronary artery disease

CCB: calcium channel blockers

C_{max}: Max concentration

CVD: Cardiovascular disease

CHD: coronary heart disease

coQ10: coenzyme Q10

DBP: Diastolic blood pressure

DC: Dense calcium

DN: diabetic nephropathy

DR: diabetic retinopathy

DTM: Digital thermal monitoring

EAT: Epicardial adipose tissue (b: brown, w: white)

EEG: electroencephalogram

EGb761: Extract of Ginkgo biloba 761

FFM: Free fat mass

FRS: Framingham Risk Scores

GBE: Ginkgo biloba extract

GLT: Ginkgo Leaf Tablets

GP: Garlic powder

HDL: High-density lipoprotein

HRGC-MS: high-resolution gas chromatography-mass spectrometry

HOMA-IR: The homeostatic model assessment-Insulin resistance

HS: Herbal supplements

hs-CRP: high-sensitive C-reactive protein

IL-6: Interleukin 6

IMT: intima-media thickness

LAP: Low-attenuation plaque

LDL: low-density lipoprotein

MGFE: Monascus pilosus garlic fermentation

MS: metabolic syndrome

NCP: Noncalcified plaque

NS: Non-significant

PG: Processed garlic

PWV: Pulse-wave velocity

RCT: Randomized clinical trial

SAC: S-allyl cysteine

SBP: Systolic blood pressure

SD: Standard deviation

SEM: Standard error of mean

SLT: Sailuotong (standardized herbal medicine: Panax ginseng, Ginkgo biloba and Crocus sativus)

TC: Total cholesterol

TG: Triglyceride

TPV: Total plaque volume

Umalb/cr: urinary microalbumin/urinary creatinine

INTRODUCTION

In 2014 in Denmark 466,095 people had a heart disease in sever state. The Danish Heart Foundation advised thousands of heart patients yearly and are focused on al kind of heart diseases and atherosclerosis. They try to have a large amount of their knowledge available on their website to help patients and relatives. Furthermore, other organizations and professional colleagues could make use of the information in their website. The questions being asked, are among others, about food and what to eat and what not to eat in certain circumstances. In the light of new clinical trials and thereby new results in the research area coming up faster than ever, recommendation about supplements should be updated to give advice to the many patients and relatives. Therefore, this report should be the scientific evidence behind the Danish Heart Foundation recommendations on garlic and Ginkgo biloba in primary and secondary prevention of heart diseases.
In many years heart diseases were the main death cause in Denmark, but recently this has changed and now cancer is the most frequent cause of death with 15400 death in 2015. People who died from heart- and other vascular diseases in 2015 was 12504 people.

This report should constitute the grounding for giving advice to those in a higher risk of getting a cardiovascular disease and heart patients in general. With critical eyes and open mind towards how the supplements should be recommended a discussion about the newer studies in this area will shape the best recommendations for garlic and Ginkgo biloba supplement especially in heart patients.

BACKGROUND

Dietary Supplements

It is getting more popular to use herbal remedies and dietary supplements trying to get healthy through food and specific food components. Approximately 5 % (Knudsen, 2014) of the Danish population are using herbal supplements[1] (HS). Regarding the overall supplements use, Denmark has one of the highest consumptions in Europe with 6 out of 10 adults consuming a supplement mostly multivitamins and minerals.

A study from 2013 (Soner et al., 2013) in a Turkish hospital population 48.8 % used HS. Furthermore, it showed that the most used herbals were Green Tea (14.2 %), Rosemary (10.2%), Ginger (9.1%), Fennel (7.5%), Garlic 7.3% and Ginkgo biloba on a 14th place with 1.1%. The use of HS positively correlated with women, government officials, higher education level and higher income. Another study from Turkey (Ipek et al., 2013) a measurement within patients admitted to outpatient cardiology clinic was done in 2013. The patients had diabetes (12%), hypertension (34%), CAD (26%), heart failure (7%), chronic illness (58%), CVD (49%) and history of drug intake (57%). Excluding vitamins and minerals, 12 % of the sample size (n=452) used at least one herbal product and 5 % of them were using more than one. Of those using a least one supplement 32 % were using alternative medicine for the treatment of hypertension and 23 % for treating hyperlipidemia, while 20% used these products to become healthier in general. Of these HS, garlic was the most preferred with 59% using it (Ipek et al., 2013). Also for these heart-patients there was a correlation with the use of HS and education level, furthermore higher frequency of drug intake was also associated with higher HS intake. In another study from South America from 2015 using cardiac patients there was a high prevalence of usage of HS among these patients (56.2%). In contrast to Ipek et al., 2013 a study from 2015 in Turkey herbal users had significantly lower drug consumption than non-users.

With these facts combined with the request from the patients contacting the Danish Heart Foundation (HF), Ginkgo biloba and garlic was decided to be examined.

The above shows that these supplements are used both as a primary and secondary prevention. The primary prevention is intended for healthy people who wish to maintain a healthy lifestyle. These are not the group of people seeking advice in the HF. However, people with slightly increased

[1] A subcategory of dietary supplements only including herbals and no vitamins or minerals

levels of the risk factors contributing to MS are the ones the HF also advise daily. In the secondary prevention group people with a cardiovascular disease (constriction of arteries, arteriosclerosis) belong and people who have survived thrombosis or strokes is in this group also. Therefore, the prevention would have focus on minimizing the risk factors and the mortal outcome.
In the HF people with congenital heart disease or heart failure that is not necessarily in association with lifestyle seek advice as well. For these persons, the prevention will be the same as for healthy persons because they are not necessarily affected by the risk factors.

Ginkgo biloba and garlic

The Latin name, Ginkgo biloba relates to the wood type of Ginkgo trees of the species biloba. The supplement named after this tree consists of an extract of the dried leaves.
G. biloba contains flavonoids (e.g. biflavones, catechin derivatives, flavonol glycosides, proanthocyanidins), organic acids (e.g. 6-hydroxykynurenic-, protocatechuic-, p-hydroxybenzoic-, vanillic-, caffeic-, p-coumaric-, ferulic- and chlorogenic acid) and terpenoids (e.g. bilobalide, ginkgolide A, ginkgolide B, ginkgolide C, ginkgolide J) and ginkgolic acids. EGb761 is a specific standardized extract used in many clinical studies (manufactured by Dr. Willmar Schwabe GmbH & Co. KG, Karlsruhe, Germany), which can be considered as the ‘‘gold standard’’ among G. biloba extracts. The extract of G. biloba leaves have been standardized to contain 24% flavonoid glycosides (containing quercetin, kaempferol, isorhamnetin etc.), 6% terpenoids (in which 3.1% are ginkgolides A, B, C, and J and 2.9% is bilobalide), and 5–10% organic acids (Shi et al., 2010). The potential pharmacological activity of EGb761 involves improved brain energy supply by stabilizing mitochondrial energy production (Unger, 2013). Clinical studies on the efficacy and safety of EGb761 led to the dose recommendations of 120–240 mg/day in the monographs of the WHO (WHO, 1999a).
In 2004, Gaudineau et al. investigated the inhibition of human drug-metabolizing CYP enzymes by EGb761 using a commercially available microtiter-plate-based fluorometric assay. The whole extract inhibited the CYP enzymes 1A2, 2C9, 2D6, 2E1 and 3A4 indicating that G. biloba could have an influence on the effect of heart medicine.

Garlic belongs to the bulbous plants and should be included in a varied diet like a vegetable. Like all other kinds of fruit and vegetables there is a connection between intake and health status. Garlic contains many different vitamins and minerals (DTU The Institute of food, 2017). The specific

mechanisms and biocomponents are making it difficult to know what to extract when making an extraction as supplements. Besides eating it in its fresh condition pills, tablets, capsules and drops are available of different forms of garlic.

The major sulphur containing component of intact garlic is γ-glutamyl-S-allyl-L-cysteines and S-allyl-L-cysteine sulfoxides (alliin). Alliin is a precursor of allicin, methiin, (1)-S-(trans-1-propenyl)-L-cysteine sulfoxide, and cycloalliin. γ-Glutamyl-S-allyl-L-cysteines are converted into S-allyl-cysteines (SAC) through an enzymatic transformation with γ-glutamyltranspeptidase when garlic is extracted with an aqueous solution according to Matsuura et al. 1997 in Amagase 2006. This is verified as both biologically active and bioavailable. Determining the contents of these key precursor compounds is important to evaluate raw garlic. Garlic in unprepared condition contain alliin (0.25-1.15%) in the relation of 1:0.0024, which is converted to the potential bioactive component allicin in the relation 1:0.45, meaning that the allicin content in garlic is 1:0.001. This process is activated by the enzyme allinase which is also contained in the garlic but in different cell components. When the enzyme allinase and alliin get in contact by crushing the garlic, allicin is made. This is a well-known component of garlic but how and where it works is a discussed area.

Allicin may have CVD protective potential through several metabolic pathways. The blood pressure (BP) could be moderated by the induction of vasorelaxation, due to vasodilation (seen in rats (Kim-Park & Ku, 2000)) or stimulation of nitrogen oxide (NO). Prevention of cardiac hypertrophy should be due to the inhibition of reactive-oxygen-species dependent pathways. The inhibition of angiogenesis could be modified by thiol proteins. Suppression of platelet aggregation is combined with the antithrombotic activities, interfering calcium movements that causes depletion of calcium and platelet aggregation to cease at an early stage (Mayeux et al.,1988 in Chan et al., 2013). The prevention of hyperlipidemia is due to the biosynthesis of cholesterol is inhibited via several mechanisms, including reducing incorporation of oleate, inhibiting the activities of acyl-CoA cholesterol acyltransferase and lowering the uptake of cholesteryl esters + inhibits HMG-CoA reductase (Chan et al., 2013). In vitro allicin function as an oxidant by oxidizing all the protein of red blood cells (Amagase, 2006). Opposite Seong et al. (2016) showed that garlic seasoning had lipid oxidation lowering effect in sausages. The anti-microorganism effect seen in petri dishes was a great discovery but the use of this discovery was difficult because of the instability and toxicity of allicin (Amagase, 2006). Animal and in vitro studies of both human and animal cells have

suggested some of these mechanism, but mostly the above is shown in randomized clinical trials (RCT) only resulting in effects in human. Although the fact that allicin is found to cause irritation of skin and GI tract is determined in human studies (Siegers 1992 in Amagase 2006).

The extract is typically made of garlic in a water/alcohol solution. This provides the extract to contain both water soluble and fat soluble components and thereby all the active bio components. The product Kyolic was among others used in Matsumoto et al. 2014. That is an extract made of sliced garlic extracted in aqueous ethanol for 20 month and then filtered and concentrated at low temperature.
For the G. biloba, the dry leaves were put in an acetone/water solution and filtered. Following subsequent purification steps involving liquid–liquid partitioning in order to remove unwanted or harmful compounds (e.g. biflavones, ginkgolic acids) and to concentrate beneficial substances such as the flavonol glycosides and terpene lactone (Unger, 2013)

Overall the lipid-lowering effect is connected to the oil-soluble contents and the cholesterol lowering effect is mostly connected to the water-soluble components. To include all potential beneficial substances from the fresh herbal, a mix of water and acetone or alcohol is often used.

Legislation

In Denmark, all kinds of HS can be sold as a dietary supplement and documentation of the working parts are not necessary, but it cannot be labelled with prevention, relieve and treatment claims. However physiologic or nutritional claims can be suggested, but this must be documented. These dietary supplements are registered as food and therefore maintained under the Danish Food Legislation regulated by the Danish Veterinary and Food Administration. Regarding herbal medicinal products (herbal remedies), these are maintained under the Danish Medicines Act regulated by the Danish medicines Agency. Herbal remedies in no higher concentrations than find in nature are allowed and must be approved by the Danish Medicines Agency before it is put on market. Documentation on the clinical effect of the herbal is demanded. These herbal remedies must contain information about side effects and interactions and these products should be more safe regarding pureness and effect.
G. biloba is both registered as dietary supplement (=7) and as an herbal remedy (=4). Dosage in the dietary supplements ranges from 100 mg to 5000 mg G. biloba extract. The range in herbal

remedies is from 51.7 mg to 100mg. All the herbal remedies mention what the extract correspond to in flavonol glycosides and in terpene lactones. This ranges from 15-24 mg and 3.75-6 mg respectively. This is only mentioned for one of the dietary supplements.

Garlic is registered as dietary supplement (=17) and as an herbal remedy (=1). Garlic are either sold as tablets made of dried garlic powder or as capsules or drops of fermented extract. For garlic powder the dosage vary from 5 mg to 840 mg (corresponding to 0.05-8.4 mg alliin) and for the extract the range is from 0.15 mg to 30 mg, but here it is not certain how concentrated this extract are. For the one herbal remedy the product is made in two dosage form. One where you get 7 mg alliin per day and one where you get 8.4 mg/day.

Cardiovascular diseases

The cardiovascular diseases related to lifestyle include arteriosclerosis, arteriolosclerosis (calcification of large and small arteries respectively), Claudicatio intermittens (peripheral artery disease), angina pectoris (chest pain), cardiac fibrillation, constriction of arteries and when this constriction is in the coronary artery it is called ischemic heart disease. Thrombosis, both cerebral and coronary, apoplexy (stroke) and heart attack is the severe and in worst case the mortal outcome of a CVD.

There is a lot of factors contributing to the development of heart diseases including life style. Most of all the metabolic syndrome risk factors which is:

- Blood pressure: SBP > 140 / DBP > 90
- Triglyceride > 1.7 mmol/l
- Total cholesterol > 5.0 mmol/l
- HDL < men: 1.0 mmol/l, women: 1.3 mmol/l (LDL >3mmol/l)
- Fasting glucose level: >6mmol/l
- Abdominal obesity: men > 94 cm, women > 80 cm

Around 20 % of the Danish population (~950,000) in the range from 20-89 years suffers from increased blood pressure. Around 30 % of those with an increased blood pressure are not aware of it. Around 2 million Danish people between 20-70 years have a LDL> 3 mmol/l and therefore has an increased risk of CVD. 220.000 Danes between 20-100 years have LDL level above 5mmol/l. More than 159.500 suffered from ischemic heart disease. Several kinds of diseases are related to the

heart and/or the arteries. And this indeed affects the whole body as the blood carry oxygen to all tissues throughout the body. However, not all heart diseases can be equally affected by lifestyle and diet. It is possible to be born with an organic heart disease or a cardiac valve disorder. Dependent of the specific disease these people could be in a higher risk of having heart attack or a stroke if they meet the above-mentioned risk factors.

Possible effects of G. biloba and garlic on risk markers of CVD

Several mechanims affect the process of atherosclerosis; blood platelets, clotting proteins to form blood clots, the monocytes ability to move into the vascular wall, the endothelium cells' robustness, the oxidation process of the lipoprotein, and the tendency of the lipoproteins to be combined. Therefore, a lot of relevant measurement can be found. In the studies below, the following has been measured (not all at once): triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), systolic blood pressure, diastolic blood pressure, intima-media thickness, event of diabetic nephropathy (DN), event of diabetic retinopathy (DR), pulse-wave-velocity (PWV), apolipoprotein B (ApoB), homocysteine, adiponectin, high-sensitive C-reactive protein (hs-CRP), homeostatic model assessment-insulin resistance (HOMA-IR), white and brown epical adipose tissue (wEAT and bEAT), digital thermal monitoring (DTM), weight loss, fat free mass (FFM), low-attenuation plaque (LAP), total plaque volume (TPV), non-calcified plaque (NCP), dense calcium (DC) and coronary artery calcium (CAC). These are not equally relevant, thus only some of them are describe further below.

TG is the largest components of the fat we get from the food. Thus, being very variable and is affected by what is eaten, where 95 % is absorbed. This can be corrected by measuring fasting TG level. Fat in the body is stored as TG.

Cholesterol is essential to maintain the normal body functions. That include preserving the integrity of cell membranes, facilitating cell signaling, maintaining the myelin sheath, synthesizing steroid hormones, vitamin D, and coenzyme Q10. Cholesterol levels in the blood are affected by inhibition of the synthesis or by affecting the absorption from the intestine. After production or ingestion of cholesterol it is converted to bile acid which is an important component in the absorption of fat from the intestine. The enzymes CYP7A1 are important in this process. Human can have low or high activity of CYP7A1 and thereby more or less affected by ingestion of too much cholesterol in the blood. Cholesterol either ingested or synthesized cannot be broken down as we do not have the

right enzymes for this process. The cholesterol is needed in the formation of micelles which is important for the absorption of fat. Only 40-50 % of the cholesterol from the food is absorbed. Cholesterol lowering drugs like statins is HMG-CoA reductase inhibitors. This enzyme is necessary in the production of cholesterol in the liver. Whether garlic inhibit the synthesis or the absorption (or both) is not clear. The production of cholesterol in the liver is regulated by the uptake from the intestine.

LDL is the lipoprotein containing most cholesterol and is a transporter of cholesterol to the tissues. Cells in the blood, macrophages among others, take up oxidized LDL particles and these seems toxic for the tissues and thereby release an inflammation state. In addition, LDL particles take up cholesterol unlimited. This is accounted for being the main mechanism behind atherosclerosis (The Danish Medicines Agency).

HDL contains a smaller amount of cholesterol and provides the transport of cholesterol from the tissues back to the liver. HDL level in the blood only vary slightly and is weakly influenced by changes in diet and lifestyle. The transport and uptake of cholesterol in HDL is the only mechanism for removing cholesterol from the blood. Decreased HDL level is often referred to as risk marker and not as LDL a risk maker.

Even though too much cholesterol and fat can be harmful we cannot live without it. Furthermore, sex differences are also a contributor to the higher prevalence of cardiovascular disease in men. Some of the explanations are that the female sex hormone estrogen makes the artery more resistant to cholesterol accumulation. This may also explain why postmenstrual women are at higher risk of atherosclerosis than menstruating women.

Blood pressure (BP) is affected by vasodilation. A diet with a high intake of fruit and vegetables influence the blood pressure positively but it is inconclusive how the mechanisms work. In the arteries endothelium cells constitute the barrier between the blood and the artery wall. The plaque in an artery disease is accumulated beneath these endothelium cells. Therefore, these cells are attributed to have an important function in the development of arteriosclerosis and arteriolosclerosis. Among a range of substances nitrogen oxide (NO) is released from the endothelium cells and is formed by the process of the amino acid arginine formation to citrulline

with help from the enzyme NO synthase resulting in a NO-molecule. This process is happening continuously and is increased by the rate of the blood flow in the artery and thereby the blood pressure. That means the body itself has a mechanism for lowering the blood pressure. In addition, the NO release is decreased by the same risk factors for atherosclerosis like smoking, hypocholesteremia, high age, hypertension (long term) and diabetes. This stimulation of NO may have a blood thinning effect.

White adipose tissue is associated with increase in metabolic disease, obesity and cardiovascular diseases. In addition, expansion of brown adipose tissue is inversely associated with obesity and metabolic disease. The epicardial adipose tissue (EAT) refers to the fat deposit that exists on the surface of the myocardium, thus surrounding and in direct contact with the major coronary arteries and their branches. In Ahmadi et al. (2013) the increase in brown EAT correlated directly with decreases in homocysteine levels and annual percent CAC increased proportionally with decrease in ratio of bEAT/wEAT ratio.

Pulse-wave-velocity (PWV) is also called arterial stiffness. It is calculated as the ratio of the pulse-wave travel distance from the carotid to the radial artery to its transit time (meters per second). The simultaneously record carotid pulse waves, the recording of the radial pulse wave and the electrocardiogram is used for these measurements.

To understand how biocomponents can influence the body the P450-system should be mentioned. The cytochromes P450 (CYPs) are proteins acting as enzymes and induce metabolism of many different substrates in body. This is the most important system in metabolism foreign substances in the body, but also hormones and fatty acids among others. CYPs are located at the inner membrane of the mitochondria or in the endoplasmic reticulum of cells – primary in the tissue of the liver, but is found in other tissues as well. CYP3A4 has the lowest specificity and the highest expression in the liver and gut. This is potentially how the CYP3A4 is able oxidize a wide range of medical drugs. The metabolism of the drug depends on the blood flow through the liver and some medicines decreases the blood flow through the liver thereby an interaction effect can induce here. Drugs may either increase or decrease the activity of the CYP enzymes either by inducing the biosynthesis of an isozyme (enzyme induction) or by inhibiting the activity of the CYP system (enzyme inhibition).

Regarding G. biloba and garlic the P450-system is important when looking at how these remedies can affect the metabolism of other drugs, e.g. statins and warfarin when ingested at the same time. For instance, a very used drug in some heart patients is statins used for the cholesterol lowering effect. A lot of different statins exist e.g. simvastatin, which is an inhibitor of 3-hydroxy-3-metylglutaryl-coenzyme A reductase (HMG-CoA-RI) and is one of the most widely used statins in the treatment of hypercholesterolemia (Zocor, 2011). Another example is lovastatin which also is found naturally all though in smaller doses. Therefore, when using self-prescribed supplements, the interaction of these drugs must be considered. Furthermore, the Danish Medicines Agency writes that the risk of myopathy is increased with interaction with other drugs. In addition, side effects of these drugs should be considered. This can also be a motivator for using herbal substitutes. The most ordinal side effects of statins are muscle pains (myalgia), muscle cramps, headache, dizziness, rash and gastro intestinal discomfort (Danish Medicines Agency, 2016). These side effects are not life threatening, but to live without them would be preferred.

In 2004 statins was taking by 600.000 Danish (Astrup et al., 2004) and the number has increased with 20 % from 2006 to 2014 (The Heart Foundation, 2016). It inhibits the synthesis of cholesterol in the liver and thereby decreases LDL in the blood. Inhibitors of CYP3A4, inhibits the metabolism of some statins (simvastatin, atorvastatin and lovastatin) and thereby affects the mechanisms of statins and increase the risk of myopathy. Likewise, some statins do not use the CYP3A4 enzymes e.g. pravastatin, fluvastatin and rosuvastatin and they should not be affected by other drugs.

The risk of interaction can be reduced with lowered dose.

Other heart medicine includes blood depressing medicines like betablockers and ACE inhibitors which have side effects like fatigue, dizziness, nauseas, diarrhea, disruptive sleep and for ACE inhibitors also dry cough and altered sense of taste.

Warfarin is a heart medicine which has a blood thinning effect. The Medicines Agency measures INR-values to estimate the blood thinning activity. The dose is estimated by the physician from the patient's blood sample. When changing from one product to another new estimate is needed. Therefore, when taking supplement that could interfere with this INR-values it is important that the physician is informed. Warfarin is a mix of enantiomers. (R)-warfarin is mainly metabolized by CYP1A2 and CYP3A4. (S)-warfarin is mainly metabolized by CYP2C9. Drugs that compete for these cytochromes, will either inhibit or induce the effect of the drug. This may increase or decrease the plasma concentration of warfarin, INR or other substances and possible increase the risk of bleeding which can end up with a stroke.

Research Question

To help giving advice to Danish heart patients and person in risk this report has been made to answering if G. biloba and garlic have effect on cardiovascular risk. The primary patients in focus are the low risk group with increased blood pressure and/or too high total and LDL cholesterol. Thereby also answering the questions:

- What are the active components?
- Is there an interaction with CVD drugs?
- What are the optimal dose?
- Is there an upper limit dose, or a minimum dose?
- Are there patients/people for whom it does not work?
- What kind of advice can be given?

METHODE

In this small review a search in Web of Science and SCOPUS was made for randomized, placebo-controlled human-trials investigating either the effect of garlic or ginkgo biloba on heart disease and its risk factors. Only articles in English was used and the search words was “Garlic” or “Ginkgo Biloba” with either words of “heart disease”, “cardiovascular disease”, and “atherosclerosis”. Only papers from 2010 up until today was included. If the title had “rat”, “mice” or any other animal included the study was excluded immediately. Other studies not distinct in the specific topic, the abstract was read before exclusion or inclusion. When the abstract was approved, the whole article was read. In addition, when papers with the right title content showed up to be a review – or when articles referred to older reviews these were used for comparison and discussion. A few studies were found without a placebo group, but tested a dose-response effect, which was included to gain more knowledge of the effect.

Tables were made to make an overview of the articles and the effect of the intervention. Many studies from other countries than Denmark used the unit mg/dl for plasma levels of triglyceride, cholesterol etc. In Denmark, we use the unit mmol/l. Therefore, when compared to the reference values in Denmark values may be converted by the factor 38.67 for cholesterol and 88.57 for triglyceride. Which means that the reference of a normal total cholesterol <5.0 mmol/l is the same as 193.35 mg/dl. In many studies that is referred to as 200 mg/dl. Likewise, for the triglyceride. A normal level <1.7 mmol/l is the same as <150.6 mg/dl (see appendix for normal levels).

RESULT

Ginkgo biloba

Reference	Design	Subjects	Age (years)	Health status	Duration	Dose Mg/daily	Taking medicine	Outcome
Dai et al. 2013 (China)	RCT two-period, two-treatment, balanced, crossover	14 (all men)	22.9 (±2.1)	Healthy volunteers	2 x 14 days 1-month wash-out period	1x 40mg simvastatin cotreated with GBE 2x120 mg	No	↓ AUC_{0-24} (p=0.000), AUC_{0-1} (p=0.001), C_{max} (p=0.002) of simvastatin LDL (p=0.056) TC, HDL, ApoB (NS)
Guo et al. 2012 (China)	two-period, open-label, fixed-sequence design.	16 (all men)	24.8 (±2.9)	Healthy volunteers	After 5 days' wash-out 14 days' intervention	1x 40 mg atorvastatin before and after 14 days of intervention GBE=3x40mg every third day	No	↓ GBE administration reduced AUC_{0-24}, (p = 0.005), AUC_{0-1} (p = 0.03 and C_{max} (p = 0.002) cholesterol lowering efficacy of atorvastatin (NS)
Steiner et al. 2016 (Australia)	RCT double-blind, crossover pilot trial	60	49.19 (±14.28 *)	healthy adults	3 weeks	Sailoutong supplement containing 2 x 27.27 mg ginkgo flavone-glycosides	No	Peripheral pulse pressure (NS) Aortic pressure (NS) Resting heart rate (NS)
Teik et al. 2016 (Malaysia)	Double-blind, crossover, RCT	24	20.2	Undergra duate	A practice day and three testing days with at least 48 hour wash out period.	GBE = Low dose: 1x120 High dose: 1 x 240 mg	No	↓ DBP and SBP (high dose) after cognitive tasks (p<0.05)
Kuller et al. 2010 (USA)	RCT	3069	79	Communi ty volunteers 25% had a history	Mean follow up 6.1 years (max 7.3 years)	GBE = 2 x 120 mg	No	Coronary heart disease event (NS) Incident of myocardial infarction, angina pectoris, Hemorrhagic stroke or stroke (NS) ↓Peripheral vascular disease events (p=0.04)

				of CVD, 55% reported history of hypertension, and 9% had diabetes. 4.5% currently smoked cigarettes.				
Siegel et al. 2014 (Bulgarien)	A clinical pilot study/ prevention randomized, 3-month study (No placebo)	11	37.7 (±2.5*)	MS patients, all smokers	1 month dietary run in phase 2-month treatment with G. biloba	GBE 2 x 120 mg	No statins, calcium antagonists nor nitrite compounds were given	↓ hs-CRP (p=0.0436) ↓ HOMA-IR (p=0.0120) ↓ IL-6 (p < 0.0407) ↓ Nano plaque formation (p < 0.0077).
Zhao et al. 2016 (China)	Double-blinded RCT	140	62.2±6.4/60.7±6.5	Type 2 diabetes outpatients	36 month	Liuwei Dihuang + GLT Placebo also got Liuwei Dihuang	Manegment of blood sugar and blood pressure ect.	TC, TG, HDL, SBP, DBP, IMT, Cerebrovascular events (NS) ↓ prevalence of DR and DN (p<0.05)

*Table 1: result of the G. biloba studies found. Age is presented with mean +/-SD, unless stated with *. * mean +/- SEM*
ApoB: apolipoprotein B, AUC: area under curve, Cmax: Max concentration, DBP: Diastolic blood pressure, DN: diabetic nephropathy, DR: diabetic retinopathy, GBE: Ginkgo biloba extract, GLT: Ginkgo Leaf Tablets, HDL: High-density lipoprotein, HOMA-IR: The homeostatic model assessment-Insulin resistance, hs-CRP: high-sensitive C-reactive protein, IL-6: Interleukin 6, IMT: intima-media thickness, MS: metabolic syndrome, NS: Non-significant, RCT: Randomized clinical trial, SBP: Systolic blood pressure, SD: standard deviation, SEM: standard error of mean, TC: Total cholesterol, TG: Triglyceride

Garlic

Reference	Type	Subjects	Age (years)	Health status	Duration	Dose Daily	Taking medication	Outcome
Higashikawa et al. 2012 (Japan)	double-blind, RCT	55	50.4±12/51.4±11	Volunteers, slightly hyperlipidemia (TG > 120-200 mg/dl)	12 weeks (tested after 4, 8 and 12 weeks)	MGFE: 1x 900 mg (~2mg monacolin K)	No	↓ TG (p=0.007) (only week 8) ↓ TC p=0.003. ↓ LDL (p=0.001). HDL (p=0.257)
Mahdavi-Roshan et al. 2016b (Iran)	RCT cross-over	12	38.00 (±1.86)	Healthy adult participants	2 x 3-week treatment 2-week washout period.	garlic powder tablet = 2x1200 mg	No	DBP and SBP (NS) TG (NS) ↓ TC (p=0.07) HDL (NS) ↓ LDL (p=0.08)
Larijani et al. (2013) (USA)	double-blinded, RCT	65	54±5/55±6	Los-Angeles county firefighters	1 year follow up + quarterly visits	AGE = 4 x 300mg + coQ10 = 4 x 30 mg	No	↓PWV (p=0.009) ↓ hs-CRP (p= 0.02) ↓ DTM (p= 0.02)
Seo et al. 2012 (Korea)	RCT	30	54.4 (±5.4)	Postmenopausal healthy women	12 weeks	AGE = 80mg Exercise= 3 times/week	No	TG, HDL, BP, homocysteine (NS) ↓ Fat-free mass (p<0.01)
Sukandar et al. 2010 (Indonesia)	Randomized dose-response trial	32	35-70	Type 2 diabetes and dyslipidemia, patients	May 2006 – September 2007	Turmeric ethanolic 200mg Garlic aqueous extract - 3 groups 1.2g or 1.6g or 2.4g	No anti-cholesterol and anti.´-diabetic drug.	In the subgroup analysis (TG>200 mg/dl or LDL>150 mg/dl or HDL<50 mg/dl) ↓ TC (low dosage in week 8) (p=0.037) ↑ HDL (low dose week 10) (p=0.01)
Sobenin et al. 2010 (Russia)	RCT	51	56.7 ±1.8*	CHD patients (TC level >200 mg/dl)	12 month	Garlic power 2 x150 mg	No lipidlowering drugs.	TC, TG, HDL (NS) ↓ LDL (p<0.05) ↓ cardiovascular relative risk by 1.5-fold in men (p < 0.05), and by 1.3-fold in women (NS).
Mahdavi-Roshan et al. 2016 (Iran)	RCT	56	59.37 (± 1.28)	CAD patients	3 months	Garlic Powder = 2x 400mg	Yes (all 56)	↓ SBP (p=0.11) For the subgroup analysis (patients with hypertension) ↓SBP (p=0.001)

								TG (p=0.41) LDL (p=0.61) HDL (p=0.85)
Ried et al. 2010 (Australia)	A double-blind parallel RCT	50	66 (±9)	patients with treated hypertension. (SBP≥140 or DBP≥90mmHg)	12 weeks	AGE 4 x 960mg (2.4mg SAC)	ACEI, A2RA, BB, CCB or diuretics	SBP (p = 0.321) ↓SBP (patients SBP>140mmHg) (p = 0.03) DBP (NS)
Ried et al. 2013 (Australia)	A double-blind RCT	79	70 (±12)	general practice patients with uncontrolled systolic hypertension	12 weeks	240 (0.6 mg SAC) 480 (1.2 mg SAC) 960mg (2.4 mg SAC)	Yes	↓ SBP (480 mg) (p<0.001) DBP (NS)
Ried et al. 2016 (Australia)	A double-blind RCT	88	62.3 (±11)	general practice patients and community members with uncontrolled hypertension	12 weeks	AGE = 1.2 g (containing 1.2 mg SAC)	Yes. ¾ took BP medication and ½ other blood thinning	↓ SBP (p=0.02) DBP(p=0.12) ↑ TG negative (0=0.07) TC, LDL, HDL (NS)
Atkin et al. 2016 (UK)	A pilot double blinded RCT	26	49.8	subjects with T2D	12 week (only 4 week with AGE)	AGE= 1200 mg (kyolic)	No	SBP (p=0.94) DBP (p=0.81) TC (p=0.96) HDL (p= 0.46) ↓ TG (p= 0.04 NS in post hoc testing) ↓ HOMA-IR (p=0.05) Hs-CRP (p=0.90)
Gómez-arbeláez et al., 2013 (Columbia)	double-blind, RCT	43	40.79±11	Diagnosis of MS, attending primary health care clinics	24 week (12 weeks of AGE and 12 weeks of placebo)	AGE = 1.2g (kyolic)	No	TG (p = 0.453) TC (p=0.172) LDL (p=0.869) HDL (p=0.911) SBP (p=0.727) DBP (p=0.670) ↓ adiponectin level (p=0.027)
Jung et al 2014 (Korea)	RCT	55	50.48 (±8.58)	Participants with LDL level >130 mg/dl	12 weeks	ABG = 2 x 3g	No	LDL (p= 0.848) TC (p= 0.382) TG (p= 0.158). ↑ HDL p= 0.029

Matsumoto et al. 2014 (Netherlands)	double-blind RCT	55	58.76 ±6.7 (71%men)	MS patients 10 y FRS of CAD of 6–20%.	354 (±41) days	AGE = 2400 mg	No antidiabetic drug, no tobacco use	↑ % LAP (p=0.0049) ↓ %TPV (p=0.06) ↓ %NCP (p=0.09) ↓ % DC (p=0.92)
Ahmadi et al. 2013 (USA)	RCT	60	40–79 years 60±8/61± 10	asymptomatic participants with FRS scores of 10–20%. CAC>30. Free of clinical CAD	12 months	One capsule of AGE-S: AGE = 250 mg B12=100µg Folate= 300 µg B6 = 12.6 mg L-arginine=100mg	Statin	↓ CAC p=0.03 ↓ Homocysteine p=0.004 ↓ Risk of CAC progression (p=0.03) ↑ brownEAT/whiteEAT (p=0.0001)
Han et al. 2011 (Taiwan)	RCT. Parallel feeding.	44	20-60 years	pre-hypertension subjects aged 20 to 60 years with (SBP, 130 to140 mmHg and DBP, 85 to 90 mmHg)	8 weeks	PG: 2x500 mg	No trace element supplements in the previous three months. No gastric or diuretic treatments	↓ SBP (p<0.01) ↓ DBP (p=0.05) TC, TG, GOT, LDL, HDL (NS)

*Table 2: result of the garlic studies found. Age is presented with mean +/-SD, unless stated. * mean +/- SEM*
A2RA: angiotensin II receptor antagonists, ABG: Aged black garlic, ACEI: angiotensin converting enzyme inhibitors, AGE: Aged garlic extract, AGE-S: Aged garlic extract with supplement, BB: beta-blockers, BP: blood pressure, CAC: Coronary artery calcium , CAD: Coronary artery disease, CCB: calcium channel blockers, CHD: coronary heart disease, DBP: Diastolic blood pressure, DC: Dense calcium, DTM: Digital thermal monitoring EAT: Epicardial adipose tissue, FRS: Framingham Risk Scores, HDL: High-density lipoprotein, hs-CRP: high-sensitive C-reactive protein, LAP: Low-attenuation plaque , LDL: low-density lipoprotein, MGFE: Monascus pilosus garlic fermentation, MS: metabolic syndrome, NCP: Noncalcified plaque, NS: Non-significant, PG: Processed garlic, PWV: Pulse-wave velocity, RCT: Randomized clinical trial, SAC: S-allyl cysteine, SBP: Systolic blood pressure, SD: Standard deviation, SEM: Standard error of mean, TC: Total cholesterol, TG: Triglyceride, TPV: Total plaque volume

Ginkgo biloba

In clinical trials investigations of the effect of G. biloba patients with increased risk of cardiovascular disease, with cardiovascular disease or healthy subjects was used. The mean age in these studies ranged from 20 to 79 years and the number of subjects ranged from 11-3069. The outcome measured was wide and expanded from BP, TC, TG, LDL, HDL, hs-CRP, HOMA-IR and hard endpoints such as mortality and CVD events.

In young healthy volunteers G. biloba intake with the synchronous intake of statins did not have any effect on the cholesterol concentration lowering effect of simvastatin compared to placebo group only taking statin (Dai et al., 2013), (Guo et al., 2012). However, the concentration of simvastatin in the plasma was decreased. All volunteers completed the study with no serious adverse events. Two studies used healthy subjects with the aim of seeing an effect on BP (Steiner et al., 2016), (Teik et al., 2016). A significant difference in BP was observed before and after cognitive task. Here the BP (both SBP and DBP) was lowered in the high dose G. biloba group compared to the placebo group. SBP decreased from 124.2±19.2 mmHg to 123.9± 20.6 mmHg in the G. biloba group and increased from 127.6 ±22.6 mmHg to 129.8 ±23.5 mmHg in placebo. Smaller changes were seen for the DBP.

In patients with MS, history of CVD and diabetes no significant changes in TG, TC, HDL and BP was seen (Kuller et al., 2010). Instead an effect in peripheral vascular events was found. Here 23 events were observed in the placebo group and 12 in the G. biloba which made up a significant difference of 0.7%. In diabetes patients, diabetic nephropathy (DN) and diabetic retinopathy prevalence (DR) were all significantly lower in the G. biloba group than in the control group. The prevalence of DR was 5 in the G. biloba group vs 14 in placebo and the prevalence of DN was 10 vs 17 in the G. biloba and control group respectively (Zhao et al., 2016).

Garlic

In table 2 the clinical trials with garlic are shown. The clinical trials included both healthy subjects and subjects with symptoms within the MS, diabetes or with coronary heart disease. They had a mean age around 40-70 years and the number of subjects ranged from 12-88. The outcome measured was wide and expanded from BP, TC, TG, LDL, HDL in most of the studies and with hs-CRP, pulse-wave-velocity (PWV), digital thermal monitoring (DTM), low-attenuation-plaque

(LAP), total-plaque-volume (TPV), non-calcified-plaque (NCP), dense-calcium (DC), coronary-artery-calcium (CAC), brown/white epicardial adipose tissue ratio in the different studies.

In healthy subjects the intake of garlic showed either no effect (Mahdavi-Roshan, Rismanchi, et al., 2016), (Seo et al., 2012) or an effect on TG, TC and LDL (Higashikawa et al., 2012). There was no effect seen in HDL, SBP and DBP in these studies.
In healthy firefighters, a significant effect in PWV (mean decrease =1.21 m/s), CRP (mean decrease in = 1.3) and in DTM AUC (increase =31.3) was seen (Larijani et al., 2013).
Most of the studies used subjects with a disease or syndrome. A significant effect on BP was only seen in patients with hypertension (Mahdavi-Roshan, Nasrollahzadeh, et al., 2016), (Ried et al., 2010), (Ried et al., 2013), (Han et al., 2011), (Ried et al., 2016) and in a study with subject diagnosed with MS no significant change was seen in BP (Gómez-Arbeláez et al., 2013). These patients all received medicine (statin, aspirin, Plavix, B-blocker, ACE-I). Mahdavi-Roshan, Nasrollahzadeh et al. (2016) showed that there was significant improvement beyond the effect of the medication.

TG did not change significantly in any of the studies. TC only decreased in one study, in the subgroup with increased TG, LDL and decreased HDL levels. This decrease (from 248.0+24.7 to 223.6+43.9 mg/dl) was only seen when measured in week 8 and for the low dosage (1200 mg) (Sukandar et al., 2010). LDL only decreased significantly in one study (Sobenin et al., 2010). In this study the patients had increased TC level with >200 mg/dl.
HDL increased significantly in two of the studies. This was in patient with either type 2 diabetes and dyslipidemia (HDL increased from 40.4+7.1 to 47.2+3.9 mg/dl) or with LDL level > 130 mg/dl (HDL increased from 46.86 ±9.40 to 50.36 ±8.85 mg/dl in the ABG group).
One study found a significant effect on the decrease in cardiovascular relative risk in men with 1.5-fold decrease (Sobenin et al., 2010). Likewise, one study found a significant reduction in adiponectin levels. The AGE group increased their levels of adiponectin with a mean of 313.79 ± 179.44 ng/ml whereas the placebo group decreased their levels with 271.88 ± 187.18 ng/ml (Gómez-Arbeláez et al., 2013)

Significant changes in CAC, homocysteine risk of CAC progression and the ratio of bEAT/wEAT was found in one study (Ahmadi et al., 2013). In this a supplement with B vitamins and L-arginine was ingested too.

HOMA-IR, hs-CRP, TPV, NCP and DC was also measured but found non-significant (Atkin et al., 2016), (Matsumoto et al., 2016).

The different kind of supplements

In the garlic studies, different kind of supplement are used. Nine studies used the aged-garlic-extract (AGE) and three used garlic powder (GP). One study used a bacterium fermented garlic extract (MGFE). One study used an aqueous garlic extract (was not explained further) and one used processed garlic (PG) supplement which is described as made like the aged garlic extract. One used aged black garlic (ABG). The different kind of aged garlic extract was made with different duration of fermentation from 2 week to several years.

The AGE showed significant effect in PWV, CRP, DTM, adiponectin level, LAP and HOMA-IR. It did not show an effect in TG, TC, BP, LDL, HDL, CRP. The GP showed significant effect in LDL, SBP (for the hypertensive subgroup) and cardiovascular relative risk. The MGFE had significant changes on TG, TC, LDL. The PG and ABG only showed an effect in BP and HDL respectively. Furthermore, the results indicated that the ABG extract can decrease ApoB and increase the ratio of LDL to ApoB, which are related to low atherogenicity.

Beside the one study with bacteria fermented garlic none of the studies showed an effect on TG. And only one study beside the MGFE study showed an effect on TC.

DISCUSSION

The most important effect seen of the use of G. biloba supplements was in Kuller et al. 2010 where a significant lower incident of peripheral vascular disease events was seen in the intervention group. Here 12 events were seen in the intervention group and 23 events were observed in the placebo group. This study was the largest with 3069 subjects with the longest duration time of 6.1 year on average.

Two studies by Dai et al. 2013 and Guo et al. 2012 are not comparable to the other studies, as the results are limited to these subjects – healthy young men taking statins. These studies showed that G. biloba did not alter the efficacy of simvastatin significantly. Both groups still decreased their levels during the treatment period. These young men probably did not have the need for lowering these levels and the effect or the absence of this could be different in elderly with higher levels and a bigger need for the lowering effect of statins. No other studies of G. biloba in this report used statins.
One study showed an effect of G. biloba on BP (Teik et al., 2016). This was in a cognitive task setting where G. biloba was given one hour before the task. Here a drop from 138.1±26.5 to 123.9±20.6 mmHg was seen in the G. biloba group at the highest dose of 240mg and an increase from 127.6±22.6 to 129.8±23.5 mmHg was seen in the control group for one task. It should be mentioned that the intervention group had a mean SBP of 124.2±19.2 mmHg before treatment of G. biloba and the placebo group had a mean of 127.6±22.6 mmHg meaning that the G. biloba group started with a large increase of the SBP.

In the garlic studies the most efficient results was seen in Higashikawa et al. 2011 after 8 weeks of garlic consumption. These healthy subjects were characterized by having a TG level at 120-200 mg/dl, meaning some of them were mildly hyperlipidemia. Here the TG level decreased with 14.8 % in the garlic group and increased in the placebo with 7.4 %. The TC level decreased with 8.6% in the garlic group and only decreased with 0.1 % in the placebo group. The LDL did also significantly decrease in the garlic group with 14.2 % and did not change in the placebo group. These reductions could help people with slightly increased level to a lower level and thereby reach a healthier level.

In the last study with healthy subjects (Seo et al., 2012), AGE was combined with exercise. Body weight, body fat, BMI, LDL was reduced when the intervention included exercise regardless consumption of AGE. The fat-free mass significantly decreased in the AGE group as the only group from 37.6 ±2.1 to 36.7 ±2.3 kg. This was the only negative effect seen from AGE.

In the garlic studies 5 of the intervention groups was receiving medicine during the intervention. Two studies used subjects who took statins, in three studies subjects used blood pressure/blood thinning therapy. In these, there was no difference between the treatment group and the placebo group. In Ried et al. 2016 there was not seen an increased risk of bleeding in patients taking blood thinning medication together with AGE. In this study a significant decrease in SBP of 5.0 mmHg was shown. In an older study from Ried et al. (2013), patients receiving the same kind of medicine, no difference was seen in the number of side effects in the AGE group compared to placebo. These side effects were gastro intestinal complaints, which caused 3 out of 79 resigned. Other study participants experienced these problems which was despite using the fermented extract without allicin which is known for having an allergic potential and to give gastro intestinal complaints in some people. This could be due to a placebo effect or it could be due to unknown substances.

In the study with asymptomatic participants receiving statins no negative changes occurred and an increase in the risk of coronary artery calcium (CAC) progression were significantly lower in the garlic group compared to control group. In addition, a positive increase in the bEAT/wEAT ratio was seen in the garlic group.

The differentiation between healthy and unhealthy is divided due to the risk factors for CVD mentioned above. In this discussion, age is an important component. As the body gets older it gets weaker and the accumulation of fat and calcium in the arteries will increase. Some people will never notice this and some will be largely affected. Therefore, people with a certain age are at risk and therefore no one are completely healthy in that perspective. Both the firefighters and the postmenopausal women had a mean age around 54 whereas the healthy adults only had a mean age of 38 years. This could indicate that no improvement is to make in those with the age of 38 years, whereas those with the age 54 years could increase or decrease certain parameters to reduce the risk of CVD.

This leads to the discussion about whether you can make a "healthy person healthier". If you have normal level of TG, cholesterol etc., is it possible to reach a "better" normal level? All the risk factors (TC, abdominal obesity, hypertension and insulin resistance) are of continuous scale and therefore it is possible to be in the high end of normal as well as the low end. As mentioned earlier, cut off levels are defined for these risk factors to divide people into high risk group of getting heart disease. In that perspective, you can make a healthy person healthier by for instance moving a TG level from 1.6 to 1.4 mmol/l (140 to 124 mg/dl). Many of these "normal levels" do not have an official minimum but obviously, there is. These minimum levels are not discussed because when eating – eating anything at all – the body will maintain at least minimum normal levels, at least for a while. Likewise, regarding BP you can have a normal low level and that is positive. Therefore, when people are in the high end of the above-mentioned factors, HS may prevent the risk of getting too high levels. The discussion is not about making a person healthier it is a discussion whether you can conclude that you are healthy enough and who are to do that?

For comparison, dietary change and cholesterol lowering medicine decrease the risk of new myocardial infarcts with 25-50 % in patients with symptomatic atherosclerosis. And the risk of getting a new heart attack is 4 % in a year with prescribed medicine (Astrup et al., 2004).

Limitations

As mentioned earlier the studies differed a lot regarding the subjects' health status. When measuring in healthy people you would assume to get smaller changes than you would in subjects with unnormal levels, for instance too high LDL level. Therefore, the results should be interpreted differently.

Besides this the number of participants differed a lot. For G. biloba, the number ranged from 11-3069 subjects – the last number is due to one large study. Without this study the number ranged from 11-140 subjects. In the garlic studies the number ranged from 12 to 88 subjects – these being the ones that accomplished the intervention. Most studies included much more people but after inclusion criteria was achieved and due to withdrawals, changes in prescribed medicine, gastrointestinal complaints, or other issues making it difficult to be compliant the number of subjects decreased.

This is very important regarding the power of the studies. Power calculations is estimated in a very small amount of the studies. The power is an estimate of the risk of type 2 errors (β) which again is

a risk of not detecting a difference even though there is. The power should be at least 80 % (1- β) and depends on the sample size. In the large study by Kuller et al. 2010 a power calculation was made and found to be at 89% - 99 % dependent on the specific variable and the reduction change. A lot of studies did not mention power estimates, but one small study by Dai et al. 2013 (n=14) got a power of 76%, probably due to the small sample size indicating that this study can miss an effect. The 3069-subject study also lasted for a long period (mean = 6.1 year) and had hard endpoints, such as CVD mortality and CVD events. This also makes it easier to maintain a high number of subjects because death is a part of the outcome. The long duration time can influence the compliance of using the supplement. Kuller et al. 2010 estimated the adherence to see how it changed through the years and found that it varied from 90% at 6 months, 79% at 2 years, 73% at 4 years and at the end of the trial, 60.3% of those receiving active therapy were taking their assigned study medication. In addition, it is mentioned that the adherence did not differ between the intervention group and placebo group. The adherence estimate was done using questionnaires. Although it is a risk that people can cheat in this, it is one way of measuring it. Another way is to recollect the pills not ingested, but a true compliance is hard to achieve. Measurement of flavonoids and terpene in the urine could help the compliance measurement but this was not measured.

The duration of the several studies varied from 14 days to 7 years for the G. biloba studies and from 4 weeks to one year for the garlic studies. It is especially interesting to look at the studies where blood lipids were measured at week 4, week 8 and week 12 to see how the values varied. How the duration time is decided is often due to the specific parameter measured and is decided from earlier experience with in the field. In the garlic studies, mostly the duration time is set at 12 weeks and in G. biloba shorter duration is often used. It is not known yet if there is a prevention effect and if the body through a whole lifetime will adapt to e.g. garlic and the active components.
For G. biloba effects was seen after 2 months and after several years. The effects of garlic are seen after 8 weeks and after one year.

Around half the RCTs used in this report is double-blinded. This term “double-blinded” is often used as a verification but it does provide enough information. When a clinical trial is blinded it means that the subjects do not know whether they are getting the intervention or a placebo. With real food, e.g. whole grain vs. refined grain it can be difficult to blind the subjects. Often they find out what they are receiving. For supplements in the form of capsules or pills it is more difficult to

figure out what they are been giving. In the garlic intervention, the subjects might have guessed what group they are in because of the harsh odor issue. Most of the studies does not comment on this issue. Studies by Ried et al. (2010, 2013, 2016) all estimate the blinding success by questionnaire. Around half of the people in the garlic group guessed that they were in this group but many were uncertain. The "double-blinding" term in Ried (2010, 2013, 2016) meant that every patient, investigator, research assistant/nurse and if relevant the general physician were blinded. In Higashikawa et al. (2012) used questionnaires to assess the blinding success and saw 30% guessed correct, 33% incorrect, and 37% answered that they did not know. Furthermore, they saw no difference in the accuracy rate of the blinding between the two groups.

The big issue in measuring in people having a normal everyday life is that you cannot as a researcher control everything in their life which can affect the outcomes. Being a participant in a study might unconsciously make the participant change habits. They might start living healthier even though instructed not to change anything. Opposite, when instructed to follow some guide lines, e.g. more physical activity or a diet this should be controlled.

Another limitation of the methods is the control with the diet. For instance, in Gomez-Arbeláez et al. 2013 the subjects were advised routinely of lifestyle changes (having a diet lower in fat and sugar and increasing physical activity with 30 minutes/day of moderate walking). This could interfere with the positive outcome of increased adiponectin levels all though no changes in BMI was seen. In Seo et al. 2012, LDL concentration decreased in all interventions regardless of exercise compared to placebo group. This meant that AGE had effects comparable to the effects of exercise. In addition, AGE supplements, might provide independent benefits on reducing CVD risk factors for postmenopausal women without hindering the well-known beneficial effects of exercise.

Statistic

In the garlic studies, only one study mention that there is a power of 85 % with a subject size of 50 and a difference in TG with 17.5 % between the control and intervention group. This study by Higashikawa et al. (2012) had 55 participants in the study. A lot of the studies had more than 55 participants but does not mention a power level.

Another important statistical number is what the p-value is calculated from and the unit measuring lipid levels in the blood. Some studies measured both the difference from baseline in placebo and the intervention group and saw if the difference in each group was significantly increased or

decreased (within group). In addition, some studies also looked at the change from baseline in each group and then compared this difference for placebo group and treatment group (between group). It is important to know what difference you are looking for. For instance, if you see a significant change in the treatment group from baseline to after intervention, but you also see this change in the placebo group than you have no significant difference and that is what you are looking for in a controlled trial. Of course, it is the between group difference that is main results here. In the study by Siegel et al. 2014 a significant decrease in hs-CRP, HOMA-IR, IL-6 and nano plaque formation was seen but these reductions were not compared to a placebo group making the results very limited. Furthermore, when testing for several parameters you could end up with a lot of p-values and this itself increases the risk of type 1 error.

On top of that when using the levels from studies around the world you will see that the units are in mg/dl. In Denmark, we use mmol/l and an apparently high decrease/increase is not that much measured in mmol/l.

Dose and the metabolic mechanism

The dose of G. biloba in the studies varied from 120-240 mg, which is the range for what is recommended (WHO, 1999a).
The dose and type of garlic exposure (garlic oil, AGE, GP) varied a lot more. The supplements giving contained from 240 mg to 6000 mg daily of the fermented extract. For the garlic powder it expanded from 300 mg to 2400 mg daily. WHO recommends 400-1200 mg/daily of the dried powder. These wide dosages could be a reason for the widespread results. An interesting problem is that most trial from above mentioned studies used AGE, despite most reviews refers to the bioactive component of allicin, which is explained as being oxidized to diverse sulphur compounds in the fermentation process (Amagase, 2006).
It is known and shown that the flavonol aglycones in G. biloba and allyl disulfide, alliin, allicin, and allyl cysteine in garlic show anti-oxidative effects in for instance meat and possible outcomes for the mechanisms in sausage was lowered cholesterol and reduced lipid oxidation (Seong et al., 2016) and meatballs also showed reduced lipid oxidation (Kobus-Cisowska et al., 2014). However, this cannot be transferred into the mechanism in the human body. Anti-oxidant in vitro may not act in the same way in vivo. Substances probably due have a redox active effect and modify the enzymes system in the body, but how and in what dose is not completely certain.

When it comes to G. biloba there is not much confusion about the powder and the tablets, because the extract is made from the dried leaves and put in to capsules in the before mentioned standards. However, some differences can be found. For instance, it has been found that the yellow leaves are more rich in flavonol aglycones then the green leaves (Kobus-Cisowska et al., 2014). All though, this should not have an effect considering the standardization criteria set up for the EGb761.
Other potential effects accounts for the inhibition of platelet aggregation and vasodilation and modulation of cholesterol metabolism (Bustanji et al., 2011). The flavonol glycosides are converted to their corresponding aglycones and subsequently glucuronidated, sulfated or methylated in the gut or liver (Unger, 2013). The bioavailability of these is low due to an extensive intestinal first pass and therefore the plasma levels is very small (<1µM). Even though the bioavailability is much higher for the terpene lactones (~80%) only a plasma concentration of 0.2 µM is found and may be due to the low concentration in the relevant doses of G. biloba. In the review by Unger 2013 it is concluded that the inhibition of metabolic enzymes such as CYP enzymes by Ginkgo extracts or constituents has not been proven. Transporters of the drug are also main determinants of drug absorption and clearance (Unger, 2013).
In addition to G. biloba some of the studies used other kind of supplement besides G. biloba in their intervention leading to difficulties in concluding anything about the separate supplement. In Steiner et al. (2016) a standardized herbal supplement formula consisting of Panax ginseng, G. biloba and Crocus sativus (saffron) was used, but here no significant effects were found. In Zhao et al. (2016) a six-flavor tea supplement was used both in the intervention group and the placebo group, meaning that the effects seen could be caused by the G. biloba supplement.

A major controversy is the problem about where and how allicin works. Chan et al. (2013) claimed that allicin was absorbed and detected in blood where as Amagase (2006) explained how allicin could not be absorbed or converted and therefore could not be detected in the blood. The trapping of allicin in the gut is due to the binding of fat and protein, thus being too large to diffuse trough the plasma membrane (Amagase, 2006). Opposite Chan et al. (2013) explained how the hydrophobic condition of allicin allows to diffuse trough the cell membrane and in this way, being absorbed from the intestine to the blood. Horev-Azaria et al. (2009 in Chan et al., 2013) showed that that 6 – 18 mM allicin could be detected in blood when 1-3 cloves of fresh garlic are consumed per day. However, another study stated that allicin and its derivatives could not be detected in blood, urine and feces (Lawson & Wang, 2005).The concentration of allicin in the body is therefore

inconclusive. There is agreement on the rapid metabolism and therefore the quick formation of the metabolites allyl methyl sulfide (AMS) and allyl mercaptan. In vitro studies showed how the stability of allicin vary with varying temperature (Fujisawa et al., 2008) from one year at 4 degrees and one day at 37 degrees. Allyl methyl sulfide (AMS), allyl methyl sulfoxide (AMSO) and allyl methyl sulfone (AMSO2) was detected in human breast milk by HRGC-MS after eating garlic (Scheffler et al., 2016), thus it is concluded that allicin itself is not stable and is degraded to secondary substances such as ajoenes, vinyldithiins and different sulfides. Lawson & Wang 2005, showed that the transformation from allicin to AMS is extremely fast. AMSO and AMSO2 are thus assumed to be oxidation products of AMS. And these products together with S-allyl-cysteine (SAC) seems to have the positive effects seen in the body (Amagase, 2006). In addition, in a paper written in collaboration between the Israel Heart Association and the Israel Dietetic Association dried garlic preparations containing alliin and allinase must be enteric coated to be effective because stomach acid inhibits allinase. Because allinase also is deactivated by heat, cooked garlic is less powerful medicinally.

For the garlic interventions three studies added other components to the garlic supplement. Larijani et al. 2013 added Q10, Sukandar et al. (2010) used turmeric ethanolic and Ahmadi et al. (2013) added a B12, B6, folate and L-arginine. All the studies found significant effects of their intervention but this cannot be associated only to the effect of garlic. For instance, did Ahmadi et al. (2013) find a significant decrease in homocysteine, but homocysteine is well known to be affected by vitamin B12, B6 and folate because all these is responsible for the metabolism of this amino acid (Astrup et al., 2004).

Conclusion

G. biloba was tested in two studies for the interaction with simvastatin and its effect on TC, LDL, HDL, ApoB and different ratios within cholesterol. Furthermore, in five other studies G. biloba was tested for an effect on either peripheral pulse pressure, EEG amplitude, DBP, SBP, death of coronary heart disease, incident of myocardial infarction, angina pectoris, strokes and peripheral vascular disease. Besides that, also the effect on hs-CRP, HOMA-IR, IL-6, nano plaque formation, triglyceride, diabetic nephropathy, diabetic retinopathy, intima-media thickness. A positive effect was found for blood pressure after cognitive tasks, the incident of peripheral vascular disease events, decrease in hs-CRP, HOMA-IR, IL-6, nano plaque formation, prevalence of diabetic nephropathy and retinopathy. No negative effect was found as the significant lowering of simvastatin did not influence the cholesterol lowering effect of simvastatin. It appears there is an improvement of peripheral arterial disease. People with increased risk of plaque formation, inflammation, insulin resistance can experience improvements with the use of G. biloba in the recommend dose of 120-240 mg/day.

Garlic was tested in 16 studies for an effect on TG, TC, LDL, HDL, DBP, SBP, PWV, hs-CRP, DTM, FFM, ApoB, %LAP, % TPV, %NCP, %DC, CAC, homocysteine and bEAT/wEAT. A significant positive effect was seen in TG, TC, LDL, HDL, PWV, hs-CRP and DTM. A significant positive effect was seen on BP in hypertensive patients. Furthermore, there was a positive effect on adiponectin level, %LAP, CAC, homocysteine and bEAT/wEAT. Only one significant negative effect was seen for the use of garlic in addition to placebo and the intervention of physical activity. Here a decrease in FFM was seen only for the garlic group.

Subjects with increased level of LDL, TG and BP can experience improvements with the use of garlic supplement with fermented/aged extract of garlic with a dose of 1200-2400 mg/day. Furthermore, the use of these supplements could be a chance to gain the health effects from garlic without the bad breath and without getting the substrate allicin which some people are sensitive to in fresh garlic.

Perspectives

Advise for patients

An important reason for gaining knowledge about a controversial area could be with the aim of patient counselling. Many associations (working with i.e. heart, diabetic or cancer) have an important job in advising and counseling their patients with any questions they may have in regards to their situation with a disease of varying serious degree. Therefore, it is of major concern to gain knowledge, interpret and translate it to more understandable advice to pass on to patients and interested people. In addition, it is important to mention that in Denmark a universal prevention strategy is used to prevent CVD (Astrup et al., 2004). This is in place since first occurrence of CVD is not seen in the small population having several of the risk factors but instead is seen in the larger part of the population with only one or two of the risk factors.

There is a lot of information about supplements to be found on the internet and it can be overwhelming for patients to seek advice here. Most of these web sites are trying to get you to buy these HS. Due to the weak legislation in this field all kind of dried herbs and vegetables, fruits and berries can be put in a capsule in different doses and sold without any documented effect.
More valid recommendations are made by the different patient organizations like the cancer association, the diabetics association and the WHO have made detailed monographs. If G. biloba is wanted as a supplement, for one among many reasons, a daily dose of 120-240 mg is recommended (WHO, 1999a). In addition, interactions and side effects are mentioned. Likewise, a daily dose of 2-5 g fresh garlic cloves, corresponding to 4-12 mg alliin / 2-5 mg allicin, is recommended (WHO, 1999b). For the AGE 1200-2400 mg seemed to be the dose showing an effect.
If you are a cardiac patient and receive heart medicine, the official recommendation is to involve the doctor in the considerations in taking supplements of G. biloba and garlic because of the interaction risk with the heart medicine. If you have only minor alterations in the normal levels of blood lipids and blood pressure you should consider you diet and lifestyle in general. In addition, you should be aware of the many kinds of herbal supplement-products in the market.

Another question to ask is why we are interested in herbal and natural occurring bioactive components? Since medicine already is developed, why then search for more components to help? If you are ill because you have mistreated yourself, then medicine can be a temporary solution, but due to side effects it can only be of limited use. Herbal remedies are suggested to be used in

prevention and treatment when symptoms and illnesses are manageable by oneself. That could include sleeping problems, cough, constipation, edema, joint pains, increased fat levels in the blood, memory problems. Of course, you should not treat yourself if several of these are present and/or other symptoms appear. Small complications or disturbances can be treated with a small supplement if deficiency occurs, a process in the body has gotten ineffective or a change in lifestyle have disturbed the body processes.
Dietary supplements can be used in the same problems as herbal remedies, but they are not allowed to puff the prevention or treatment of diseases. They may suggest to have a nutrition and physiologic effect. Both herbal remedies and dietary supplements can therefore be used in self-treatment.

If the above result and discussion led to a distinctive conclusion and a G. biloba or garlic supplement should be recommended, then a new problem arise. With the dose and kind of contents in these supplements being so diverse should a specific product name be recommended? A big problem arises in who should take the responsibility of choosing a specific product. An official organization will not risk saying something incorrect and will secure that the advice does not harm anyone. A man with a heart disease discover the effect of an HS and did not get any of this knowledge from the patient association where he seeks help and advice. He would probably feel just as mistreated as a man who get the advice of taking HS and do not see any effect. The man in first example feel he got no advice and could have used more knowledge a lot sooner and the other man may feel cheated and have lost money. Most importantly it did not cause any one's life or health – or maybe the man in the first example felt it did. If there is a risk of increased bleeding in some patients on blood thinning medicine and the advice to this patient is to take an herbal supplement and this patient gets a stroke who is to blame?
Of course, an association want to help as thoroughly as possible so not knowing enough about a supplement is a mistake but on the other hand to change opinion as often as is seen in consumer magazine, are more confusing than a help for the consumer. In Astrup et al. 2004 it is pronounced that it is a mistake to use the professional arrogance you perceive as for instance as a doctor. A *"No, you should not take herbal supplements*" answer from the physician is not enough. Doctors and patients counselling should be careful in just rejecting people who wants to try out an herbal remedy/dietary supplements. First, it should be discussed if the reason for the desire to take HS is to make up for an overall unhealthy diet or lifestyle, if it to make an "extra healthy" initiative or if it is

to avoid any artificial medicament. Second it is important to inform the patients about good and bad sides of these supplements and thereby making the patients choose on a well-informed base.

When concluding if G. biloba or garlic have an effect, the subjects tested is very important. When little is known about the effect in vivo it is very difficult to apply the effects of one group of subjects to another. Only one study with G. biloba and two for the garlic studies used patients with CVD or with a history of CVD. The other studies used either metabolic, diabetic, hypertension or healthy subjects. This mean that we do not know if the supplement can prevent a second event.
In healthy (asymptomatic) subjects, it would be a prevention effect that is interesting to look for. The perfect trial should aim at investigating if the supplement has an effect when eating for instance a normal or slightly unhealthy western diet. When comparing cholesterol and triglyceride levels to a placebo group living in the same way without the supplement of either garlic or G. biloba a true effect could be shown, if the levels increased less in the trial group. In this way, a primary prevention would be found.

References

Ahmadi, N., V. Nabavi, F. Hajsadeghi, I. Zeb, F. Flores et al. (2013): Aged garlic extract with supplement is associated with increase in brown adipose, decrease in white adipose tissue and predict lack of progression in coronary atherosclerosis. *International Journal of Cardiology*, Elsevier B.V., Vol. 168:3, pp. 2310–2314.

Amagase, H. (2006): Clarifying the Real Bioactive Constituents of Garlic. *The Journal of Nutrition*, 136, pp. 716–725.

Astrup, A., S. Bügel, J. Dyerberg & S. Stender (2004): *Menneskets Ernæring*. 4th ed., Munksgaard, Copenhagen.

Atkin, M., D. Laight & M.H. Cummings (2016): The effects of garlic extract upon endothelial function, vascular inflammation, oxidative stress and insulin resistance in adults with type 2 diabetes at high cardiovascular risk. A pilot double blind randomized placebo controlled trial. *Journal of Diabetes and Its Complications*, Elsevier Inc., Vol. 30:4, pp. 723–727.

Bustanji, Y., I.M. Al-Masri, M. Mohammad, M. Hudaib, K. Tawaha et al. (2011): Pancreatic lipase inhibition activity of trilactone terpenes of Ginkgo biloba. *Journal of Enzyme Inhibition and Medicinal Chemistry*, Vol. 26:4, pp. 453–459.

Chan, J.Y.Y., A.C.Y. Yuen, R.Y.K. Chan & S.W. Chan (2013): A review of the cardiovascular benefits and antioxidant properties of allicin. *Phytotherapy Research*, Vol. 27:5, pp. 637–646.

Dai, L.-L., L. Fan, H.-Z. Wu, Z.-R. Tan, Y. Chen et al. (2013): Assessment of a pharmacokinetic and pharmacodynamic interaction between simvastatin and Ginkgo biloba extracts in healthy subjects. *Xenobiotica; the Fate of Foreign Compounds in Biological Systems*, Vol. 43:10, pp. 862–7.

Danish Medicines Agency (2016): *Produkt Resumé for Simvastatin "Aurobindo"*, available at: http://www.produktresume.dk/docushare/dsweb/ApplySimpleSearch/Collection-96.

DTU The Institute of food (2017): Garlic, fresh. *2017-02-20*, available at: http://frida.fooddata.dk/ShowFood.php?foodid=205&1.

Fujisawa, H., K. Suma, K. Origuchi, T. Seki & T. Ariga (2008): Thermostability of allicin determined by chemical and biological assays. *Bioscience, Biotechnology, and Biochemistry*, Vol. 72:11, pp. 2877–2883.

Gómez-Arbeláez, D., V. Lahera, P. Oubiña, M. Valero-muñoz, N. De Heras et al. (2013): Aged Garlic Extract Improves Adiponectin Levels in Subjects with Metabolic Syndrome : A Double-Blind , Placebo-Controlled , Randomized , Crossover Study. *Mediator of Inflammation*, Vol. 2013:.

Guo, C.-X., Q. Pei, J.-Y. Yin, X.-D. Peng, B.-T. Zhou et al. (2012): Effects of Ginkgo biloba extracts on pharmacokinetics and efficacy of atorvastatin based on plasma indices. *Xenobiotica; the Fate of Foreign Compounds in Biological Systems*, Vol. 42:February 2017, pp. 784–90.

Han, C.H., J.C. Liu, K.H. Chen, Y.S. Lin, C.T. Chen et al. (2011): Antihypertensive activities of processed garlic on spontaneously hypertensive rats and hypertensive humans. *Botanical Studies*, Vol. 52:3, pp. 277–283.

Higashikawa, F., M. Noda, T. Awaya, M. Ushijima & M. Sugiyama (2012): Reduction of serum lipids by the intake of the extract of garlic fermented with Monascus pilosus: A randomized, double-blind, placebo-controlled clinical trial. *Clinical Nutrition*, Elsevier Ltd, Vol. 31:2, pp. 261–266.

Ipek, G.E., Y. Guray, B. Demirkan, U. Guray, H. Kafes et al. (2013): [The prevalence of alternative herbal medicine and nutritional complementary product intake in patients admitted to out-patient cardiology departments]. *Turk Kardiyol Dern Ars*, Vol. 41:3, pp. 218–224.

Kim-Park, S. & D.D. Ku (2000): Garlic elicits a nitric oxide-dependent relaxation and inhibits hypoxic pulmonary vasoconstriction in rats. *Clinical and Experimental Pharmacology and Physiology*, Vol. 27:10, pp. 780–786.

Knudsen, V.K. (2014): Danskernes forbrug af kosttilskud. *E-Paper Fra DTU Fødevareinstituttet*, 2.

Kobus-Cisowska, J., E. Flaczyk, M. Rudzińska & D. Kmiecik (2014): Antioxidant properties of extracts from Ginkgo biloba leaves in meatballs. *Meat Science*, Vol. 97:2, pp. 174–180.

Kuller, L.H., D.G. Ives, A.L. Fitzpatrick, M.C. Carlson, C. Mercado et al. (2010): Does ginkgo biloba reduce the risk of cardiovascular events? *Circulation: Cardiovascular Quality and Outcomes*, Vol. 3:1, pp. 41–47.

Larijani, V.N., N. Ahmadi, I. Zeb, F. Khan, F. Flores et al. (2013): Beneficial effects of aged garlic extract and coenzyme Q10 on vascular elasticity and endothelial function: The FAITH randomized clinical trial. *Nutrition*, Elsevier Inc., Vol. 29:1, pp. 71–75.

Lawson, L.D. & Z.J. Wang (2005): Allicin and allicin-derived garlic compounds increase breath acetone through allyl methyl sulfide: Use in measuring allicin bioavailability. *Journal of Agricultural and Food Chemistry*, Vol. 53:6, pp. 1974–1983.

Mahdavi-Roshan, M., J. Nasrollahzadeh, A.M. Zadeh & A. Zahedmehr (2016): Does garlic supplementation control blood pressure in patients with severe coronary artery disease? A clinical trial study. *Iranian Red Crescent Medical Journal*, Vol. 18:11, pp. 1–7.

Mahdavi-Roshan, M., M. Rismanchi & J. Nasrollahzadeh (2016): Garlic tablet supplementation reduces lipopolysaccharide-induced TNF-alpha production by peripheral blood mononuclear cells. *European Journal of Inflammation*, Vol. 14:3, pp. 190–195.

Matsumoto, S., R. Nakanishi, D. Li, A. Alani, P. Rezaeian et al. (2016): Aged Garlic Extract Reduces Low Attenuation Plaque in Coronary Arteries of Patients with Metabolic Syndrome in a Prospective Randomized Double-Blind Study. *Journal of Nutrition*, Vol. 146:2, p. 427S–432S.

Ried, K., O.R. Frank & N.P. Stocks (2010): Aged garlic extract lowers blood pressure in patients with treated but uncontrolled hypertension: A randomised controlled trial. *Maturitas*, Elsevier Ireland Ltd, Vol. 67:2, pp. 144–150.

Ried, K., O.R. Frank & N.P. Stocks (2013): Aged garlic extract reduces blood pressure in hypertensives: a dose-response trial. *European Journal of Clinical Nutrition*, Nature Publishing Group, Vol. 67:1, pp. 64–70.

Ried, K., N. Travica & A. Sali (2016): The effect of aged garlic extract on blood pressure and other cardiovascular risk factors in uncontrolled hypertensives: The AGE at Heart trial. *Integrated Blood Pressure Control*, Vol. 9:, pp. 9–21.

Scheffler, L., Y. Sauermann, G. Zeh, K. Hauf, A. Heinlein et al. (2016): Detection of Volatile Metabolites of Garlic in Human Breast Milk. *Metabolites*, Vol. 6:2, pp. 1–24.

Seo, D.Y., S.R. Lee, H.K. Kim, Y.H. Baek, Y.S. Kwak et al. (2012): Independent beneficial effects of aged garlic extract intake with regular exercise on cardiovascular risk in postmenopausal women. *Nutrition Research and Practice*, Vol. 6:3, pp. 226–231.

Seong, P., H. Seo, G.L.S. Cho, H. Van-Ba, P.N. Seong et al. (2016): Cholesterol-lowering and lipid oxidation reduction potentials of traditional seasonings in Salchichon dry-fermented sausages. *Journal of Food Science and Technology*, Springer India, Vol. 53:8, pp. 3364–3373.

Shi, C., J. Liu, F. Wu & D.T. Yew (2010): Ginkgo biloba extract in Alzheimer's disease: From action mechanisms to medical practice. *International Journal of Molecular Sciences*, Vol. 11:1, pp. 107–123.

Sobenin, I. a, V. V Pryanishnikov, L.M. Kunnova, Y. a Rabinovich, D.M. Martirosyan et al. (2010): The effects of time-released garlic powder tablets on multifunctional cardiovascular risk in patients with coronary artery disease. *Lipids in Health and Disease*, Vol. 9:1, p. 119.

Soner, B.C., A.S. Sahin & T.K. Sahin (2013): A survey of Turkish hospital patients' use of herbal medicine. *European Journal of Integrative Medicine*, Vol. 5:6, pp. 547–552.

Steiner, G.Z., A. Yeung, J.-X. Liu, D.A. Camfield, F.M. Blasio et al. (2016): The effect of Sailuotong (SLT) on neurocognitive and cardiovascular function in healthy adults: A randomised, doubleblind, placebo controlled crossover pilot trial. *BMC Complementary and Alternative Medicine*, BMC Complementary and Alternative Medicine, Vol. 16:1, p. no pagination.

Sukandar, E.Y., H. Permana, I.K. Adnyana, J.I. Sigit, R.A. Ilyas et al. (2010): Clinical study of turmeric (Curcuma longa L.) and Garlic (Allium sativum L.) extracts as antihyperglycemic and antihyperlipidemic agent in type-2 diabetes-dyslipidemia patients. *International Journal of Pharmacology*.

Teik, D.O.L., X.S. Lee, C.J. Lim, C.M. Low, M. Muslima et al. (2016): Ginseng and ginkgo biloba effects on cognition as modulated by cardiovascular reactivity: A randomised trial. *PLoS ONE*, Vol. 11:3, pp. 1–20.

The Heart Foundation (2016): Fakta om hjerte-kar-sygsom i Danmark, available at: https://hjerteforeningen.dk/alt-om-dit-hjerte/noegletal/ (accessed 17 February 2017).

Unger, M. (2013): Pharmacokinetic drug interactions involving Ginkgo biloba. *Drug Metabolism Reviews*, Vol. 45:Figure 1, pp. 353–85.

WHO (1999a): *WHO Monographs on Selected Medicinal Plants - Folium Ginkgo*. *Monograph*, available at: http://apps.who.int/medicinedocs/en/d/Js2200e/18.html (accessed 27 March 2017).

WHO (1999b): *WHO Monographs on Selected Medicinal Plants - Bulbus Allii Sativi*. *Monograph*, available at: http://apps.who.int/medicinedocs/en/d/Js2200e/4.html (accessed 27 March 2017).

Zhao, Y., J. Yu, J. Liu & X. An (2016): The Role of Liuwei Dihuang Pills and Ginkgo Leaf Tablets in Treating Diabetic Complications, Vol. 2016:.

APPENDIX

Conversions:

Blood lipids	Mmol/l	Mg/dl
Total cholesterol	<5.0	<193.35
LDL	<3.0	<116.01
HDL	M: >1.0, F: >1.2	M: >38.67, F: >46.40
Triglyceride	<1.7	>150.6

Blood pressure

Normal	mmHg
SBP	<120
DBP	<80