Preventing Cardiovascular Disease. Complementary precision medicine.

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Complementary precision medicine

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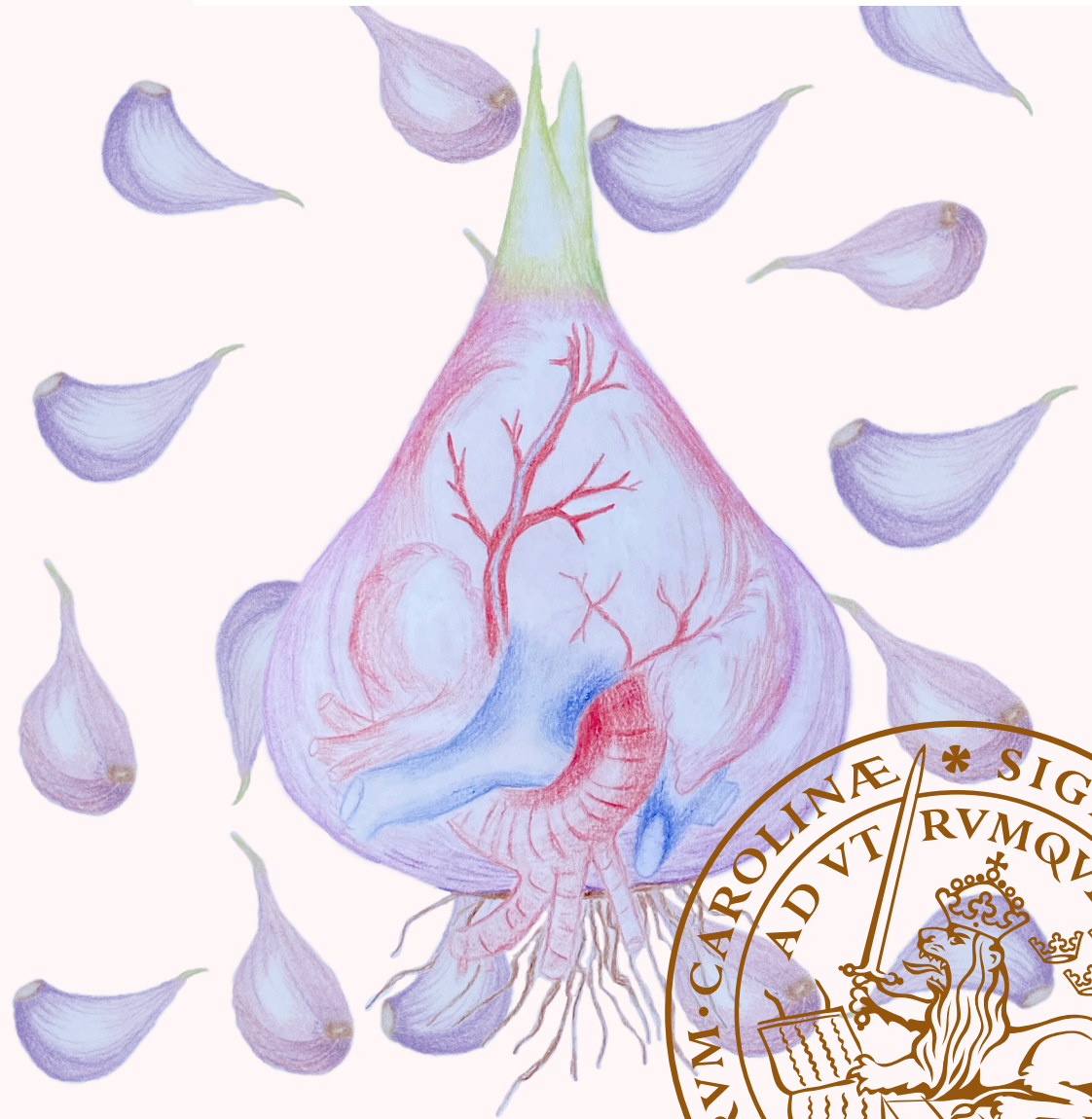

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Complementary precision medicine

Martiné Wlosinska

LUND
UNIVERSITY

DOCTORAL DISSERTATION

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PREVENTING CARDIOVASCULAR DISEASE: COMPLEMENTARY PRECISION MEDICINE

Abstract

Background: Non-communicable diseases are the number one killer worldwide and the leading one, cardiovascular disease (CVD), is responsible for more than 30% of all deaths. CVD is a progressive disease which also makes it economical, easy and effective to prevent. There are many stages of CVD that ultimately can lead to coronary atherosclerosis, measurable as coronary artery calcification (CAC) score by cardiac computed tomography (CT). Before coronary atherosclerosis develops there are many stages of the process: inflammation, reduced endothelial function, hypertension and impaired microcirculation. Precision medicine is a popular novelty in medicine that combines well-established results and medical history with computer science and novel biomedical information.

Aims: The aims of the study were: (I) to evaluate whether aged garlic extract (AGE) can influence CAC and to predict the individual effect of AGE; (II) to assess the effect of long-term treatment with AGE on cutaneous tissue perfusion; (III) to evaluate whether a daily supplement of AGE could reduce inflammation in females with low risk of cardiovascular disease; (IV) to assess the effect of long-term treatment with AGE on peripheral tissue perfusion in patients with confirmed atherosclerosis; and (V) to validate a prediction model to explore whether an individual patient will have a positive effect of AGE on their CAC score and blood pressure.

Methods: Studies I-IV were single-centre parallel randomised controlled studies. Patients were randomised, in a double-blind manner, through a computer-generated randomisation chart to an intake of placebo or AGE (2400 mg daily) for 12 months. In Study I a prediction model was developed using a cross-industry standard process for data mining and in Study V this method for developing prediction models was validated in a new cohort.The cohort used was pooled from previously published studies in the USA.

Results: There was a significant change in CAC progression (OR: 2.95 [1.05–8.27]), in favour of the AGE group. The developed algorithm could predict with 79% precision which patient would have a more favourable effect of AGE on CAC score. Cutaneous microcirculation was measured at 0 and 12 months and the mean post-occlusive reactive hyperaemia (PORH) differed significantly between time points. The mean percent was 102, 64 (174, 15)% change for AGE and 78, 62 (107, 92)% change for the placebo group ($F[1, 120] = 5.\ 95$, $p < 0.016$). Females treated with AGE showed lower levels of inflammatory biomarker interleukin-6 (IL-6) after 12 months of AGE treatment. After 12 months of AGE, an increase of 21.6% (95% CI 3.2%-40.0%, $p < 0.05$) was seen in the relative change of PORH. The same response was seen for CVC and acetylcholine with an increase of 21.4% (95% CI 3.4%-39.4%, $p < 0.05$) in the AGE group. Study V demonstrated that it is possible to develop predictive models.The constructed algorithm was able to predict with 64% precision which patient would have a significant reduction of CAC.

Conclusion: AGE inhibits CAC progression, lowers IL-6, glucose levels and blood pressure and increases the microcirculation in patients at increased risk of cardiovascular events. It is also possible to predict which patient will have a more favourable effect of AGE. AGE lowers IL-6 in females with a low risk of CVD. AGE regenerated peripheral tissue perfusion and increased microcirculation in patients with arteriosclerosis.

For many patients it is essential to know if they will have an effect of a treatment before changing their daily lives. The developed algorithm shows that it is feasible to develop predictive models for answering this question.

Key words
Aged garlic extract, CAC, Placebo-controlled, Double-blinded, Blood pressure, Data science, Predictive models

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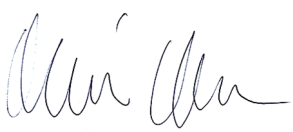
Signature  Date 2021-10-18

Preventing Cardiovascular Disease

Complementary precision medicine

Martiné Wlosinska, MD

LUND
UNIVERSITY

Doctoral Dissertation

Department of Clinical Sciences, Lund

Cardiothoracic Surgery

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“Eat leeks in March, garlic in May,
all the rest of the year the doctors may play”
-Welsh proverb

To Erik, Philip and Oscar,
who kept me motivated

Table of Contents

List of Publications

This thesis is based on the following publications, referred to as Studies I-V:

I. **The effect of aged garlic extract on the atherosclerotic process: a randomized double-blind placebo-controlled trial**.

Wlosinska M, Nilsson AC, Hlebowicz J, Hauggaard A, Kjellin M, Fakhro, M, Lindstedt S. BMC Complement Med Ther. 2020 Apr 29; 20(1):132. doi: 10.1186/s12906-020-02932-5.PMID: 32349742; PMCID: PMC7191741

II. **Aged garlic extract preserves cutaneous microcirculation in patients with increased risk for cardiovascular diseases: A double-blinded placebo-controlled study.**

Wlosinska M, Nilsson AC, Hlebowicz J, Malmsjö M, Fakhro M, Lindstedt S. Int Wound J. 2019 Dec; 16(6): 1487-1493. doi: 10.1111/iwj.13220. Epub 2019 Sep13. PMID31518044.

III. **Aged Garlic Extract Reduces IL-6: A Double-Blind Placebo Controlled Trial in Females with a Low Risk of Cardiovascular Disease**.

Wlosinska M, Nilsson AC, Hlebowicz J, Fakhro M, Malmsjö M, Lindstedt S. Evid Based Complement Alternat Med. 2021 Mar 31; 2021:6636875. doi: 10.1155/2021/6636875. PMID: 33868439; PMCID: PMC8032523.

IV. **Successful improved peripheral tissue perfusion was seen in patients with atherosclerosis after 12 months of treatment with aged garlic.** Lindstedt S, Wlosinska M, Nilsson AC, Hlebowicz J, Fakhro M, Sheik R. Int Wound J. 2021 Feb 16. doi: 10.1111/iwj.13579. Epub ahead of print. PMID: 33590955.

V. **Development and validation of a prediction model for precision aged garlic extract supplement.**

Wlosinska M, Kinninger A, Timglas A, Ollila R, Budoff M, Lindstedt S. Manuscript. 2021

Abbreviations

ACE	Angiotensin converting enzyme
AF	Atrial fibrillation
AGE	Aged garlic extract
AMI	Acute myocardial infarction
ARB	Angiotensin receptor blockers
AV	Atrioventricular
BMI	Body mass index
CAC	Coronary artery calcification
CAD	Coronary artery disease
CCB	Calcium channel blockers
CHD	Coronary heart disease
CONSORT	Consolidate Standards of Reporting Trials
CRISP-DM	Cross industry standard process for data mining
CRP	C-reactive protein
CT	Computer tomography
CVC	Cutaneous vascular conductance
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
eNOS	Endothelial nitric oxidase synthase
ESC	European Society of Cardiology
ESH	European Society of Hypertension
FHS	Framingham Heart Study
HbA1c	Glycated haemoglobin
HDL	High density lipoprotein
HER-2	Human epidermal growth factor receptor
IL-6	Interleukin-6
LDL	Low density lipoprotein

LDV	Laser Doppler Velocimetry
LOOCV	Leave one out cross validation
LSCI	Laser Speckle Contrast Imaging
NCD	Non-Communicable Diseases
NO	Nitric oxidase
NYHA	New York Heart Association
OR	Odds ratio
PORH	Post-occlusive reactive hyperaemia
PU	Perfusion units
PWV	Pulse wave velocimetry
RAS	Renin angiotensin system
RFE	Recursive feature elimination
SAC	S-allylcysteine
SBP	Systolic blood pressure
SD	Standard deviation
SEM	Standard error of the mean
UN	United Nations
VLDL	Very low density lipoprotein
WHO	World Heath Organization

Sammanfattning på Svenska

Den vanligaste dödsorsaken i världen är hjärt- och kärlsjukdom som står för ca 30 % (18 miljoner) dödsfall årligen. Det har resulterat i att FN i samarbete med WHO har beslutat att prioritera prevention och behandling utav hjärt- o kärlsjukdom samt införa behandlingsmål som gemensamt antagits.

Med stigande ålder försämras elasticiteten av blodkärlen, vilket ofta leder till högre blodtryck. Många drabbas även utav att fett och kalk lagras in i kärlväggen så kallad åderförkalkning vilket leder till inflammation och slutligen en förträngning utav kärlen och försämrad blodcirkulation. Drabbas hjärtats blodkärl, som förser hjärtmuskeln med syrerikt blod, kan det leda till hjärtinfarkt som kan vara ett livshotande tillstånd. Åderförkalkning är en progressiv sjukdom som utvecklas under lång tid och därför är det både möjligt, effektivt och hälsoekonomiskt gynnsamt med olika preventiva åtgärder.

Kulturväxten vitlök *(Allium Sativum)* har sedan faraonernas tid nyttjats för olika hälsofrämjande aspekter. I Tutanchamons grav hittades vitlök, och fram till första världskriget användes den i såromläggningar för att minska bakterietillväxt. I modern tid används vitlök främst som en viktig smakförstärkare och krydda i matlagning. Det stigande intresset för bra mat, hälsokost och devisen ”you are what you eat” har medfört att vitlök har hamnat i rampljuset. Fler och fler intresserar sig för hur man kan äta bättre och är nyfikna på vad maten har för effekt på oss och vår hälsa.

Precisionsmedicin kallas med andra ord för ”individuellt anpassad-, personbaserad- eller skräddarsyddmedicin” lika vilket förklarar till stora delar vad det handlar om. Tanken är att anpassa läkemedel, behandling och livsstilsförändringar efter individen eller grupper av individer. Man använder sig utav kombinationer av diagnostiska metoder, genetisk information samt icke-genetiska faktorer så som kön, ålder, levnadsvanor samt även maskininlärning och datorvetenskap för att kartlägga patienter. Genom att dela upp heterogena grupper av patienter som har samma sjukdom i subgrupper beroende på tidigare nämnda faktorer kan man individanpassa läkemedel och behandlingar bättre. Precisionsmedicinen är i nuläget på frammarsch då framstegen inom genetisk forskning, bioinformation så som provtagning samt datorvetenskap och maskininlärning växer för var dag som går och leder ständigt till nya genombrott.

Det är sedan tidigare känt att samma medicin kan ha olika effekt på olika individer. De flesta är även medvetna om att diet, livsstil och genetik skiljer sig mellan individer. Detta medför att patienters, läkares och politikers intresse sammanfaller och precisionsmedicin efterfråga av samtliga.

Syftet med denna avhandling är att kartlägga vilken effekt Aged Garlic Extract (AGE), som är ett populärt hälsokostpreparat baserat på vitlök, har för effekt på

hjärt- och kärlsjukdom i en europeisk population. Vi har även utvecklat en prediktionsmodell, som baserat på individers blodprover och riskfaktorer predikterar vilka som har en bättre effekt av AGE behandling.

2016 till 2018 deltog 135 patienter med förhöjd risk för hjärt- och kärlsjukdom i *Studie I-IV* och samtliga genomfördes som prospektiva randomiserade dubbelblindade placebo-kontrollerade studier. Detta innebär att studierna planerades i förväg och patienterna lottades till aktiv behandling alternativt placebobehandling, utan att varken patienter eller forskare visste vilken behandling som gavs förrän studien avslutades och resultatet analyserades. Upplägget innebär att det yttersta har gjorts för att minimera placeboeffekten. Patienterna undersöktes med datortomografi av hjärtat i början och i vissa fall i slutet av studieperioden och lämnade blodprover och var på kliniska kontroller vid 0-, 4-, 8- och 12-månader. Vid 0- och 12 månader genomgick även patienterna mätningar av mikrocirkulationen i huden på underarmen, dels med Laser Doppler och dels med Laser Speckel. *Studie I* är den största studien på AGE och dess effekt på hjärt-och kärlsjukdom som publicerats. I *Studie I* utvecklade vi en prediktionsmodell, och denna metod valideras på en ny kohort i *Studie V*.

Studie I

Studien fokuserar på AGE och dess effekt på Coronary Artery Calcification (CAC) score, dvs gradering av förkalkningar i kranskärlen på datortomografibilder. Hypotesen var att åderförkalkning i hjärtats kranskärl, som är en progressiv sjukdom och förvärras kontinuerligt, kunde bromsas med AGE.

I studien ingick 104 patienter som hade ett CAC score >1, dvs synliga förkalkningar i kranskärlen. Patienterna lottades sedan till placebo eller AGE-behandling. Efter 12 månaders behandling undersöktes patienterna igen. Vi såg då att oddsen för att en patient skulle tillhöra gruppen med minst CAC-tillväxt var större om AGE-behandling givits jämfört med placebo, både avseende förkalkningar (CAC score), blodsocker (p-glukos), blodtryck (systoliskt blodtryck) samt inflammationsparameter (Il-6). Vi skapade även en prediktionsmodell för att räkna ut om en patient skulle tillhöra gruppen med störst effekt av AGE. Med vår prediktionsmodell kunde vi med 80% träffsäkerhet (accuracy) förutsäga om patienten tillhörde gruppen med bäst effekt av AGE, baserat på patientens mätvärden, riskfaktorer och blodprover vid studiens början.

Studie II

Studien fokuserar på AGE:s effekt på mikrocirkulationen i huden. I studien deltog 122 patienter som genomförde blodflödesmätningar med hjälp av Laser Doppler (LD) i början av studieperioden och efter 12 månaders behandling med AGE/placebo. I denna studie inkluderades samtliga patienter som hade en förhöjd risk för hjärt-och kärlsjukdom beräknat med Framingham risk score. Blodflödet till underarmen blockerades med hjälp utav en blodtrycksmanschett och flödet

uppmättes med LD före, under blockeringen samt efter blodflöded släppts på igen. Resultatet av studien visade att det blev en skillnad på reaktionerna i grupperna, AGE och placebo, där gruppen som behandlats med AGE hade en bibehållen mikrocirkulation efter 12 månader.

Studie III

I studien inkluderades 29 kvinnor med en förhöjd risk för hjärt-och kärlsjukdom, men utan synliga förkalkningar i kranskärlen. Inflammatoriska biomarkörer, blodtryck samt blodfetter analyserades. Blodprover kontrollerades vid studiestart samt efter 12 månaders behandling med AGE/placebo. Patienterna genomgick klinisk undersökning med vägning, mätning, blodtryckskontroll samt kontroll med elektrokardiografi (EKG) vid 0 och 12 månader. Patienterna som behandlats med AGE hade ett lägre IL-6 värde efter 12 månaders behandling.

Studie IV

Mikrocirkulationen i huden studerades på 104 patienter med åderförkalkningar i kranskärlen. Med Laser Speckel maskin mättes mikrocirkulationen före studiens början och efter 12 månaders behandling med AGE/placebo. Laser Speckel Contrast Imaging är en kombinerad kamera och laser som kan mäta rörelser samt förändringar av rörelser i vävnader. Blodflödet till armen blockerades på samma vis som beskrivs i *Studie II*, varpå mikrocirkulationen mättes först vid normal cirkulation till armen, sen vid blockerat flöde och slutligen vid reperfusion (PORH). Förändringen i blodflödet studerades även när acetylcholin, som dilaterar blodkärlen, applicerats lokalt på huden. Studien visar att 12 månaders AGE-behandling gav en förbättrad mikrocirkulation.

Studie V

I samarbetade med en forskargrupp vid University of California, Los Angeles (UCLA) validerades metoden för utvecklingen av prediktionsmodellen från *Studie I* på en amerikansk kohort som behandlats med AGE i tidigare studier. Patienterna i den amerikanska och vår svenska studiekohort var påfallande olika, varför inklusion- och exklusionskriterier i *Studie 1* tillämpades för att ta fram ett mer jämförbart patientunderlag. Prediktionsmodellen som skapades kunde med 66% träffsäkerhet förutsäga om en patient skulle tillhörda gruppen med bäst effekt av 12 månaders AGE-behandling, baserat på patientens data vid studiernas start.

Sammanfattningsvis analyseras i denna avhandling gynnsamma effekter, av ett hälsokostpreparat som finns tillgängligt i detaljhandeln, på hjärt- och kärlsjukdom. Genom att analysera olika effekter av AGE så som blodtryck, åderförkalkningar i kranskärlen och blodprover, har vi genererat ny kunskap som är lätt mottaglig för gemene man. Algoritmerna som vi har utvecklat i *studie I* och *V* kan användas för att kunna förutse vilken patient som kommer ha mest nytta utav AGE på individnivå.

”Vilken effekt kommer denna behandling ha på mig? ” är en fråga som alla patienter vill ha svar på när de går till doktorn. I *Studie I* och *V* kan vi försiktigt börja besvara den.

Background

Non-communicable Diseases

Non-communicable diseases (NCDs) are the leading cause of death worldwide and responsible for 71% of all deaths. NCDs are chronic diseases that develop over a very long period of time and comprise both genetic- and environmental factors, such as dietary intake, physical activity, smoking and stress. Therefore it is also possible, effective and economical to prevent their development.

Preventing NCDs is such an important mission that back in 2011, the United Nations (UN) adopted a declaration calling on governments, industry and civil society to set up plans by 2013 in order to reduce the risk factors behind the four groups of NCDs: cardiovascular disease (CVD), cancers, chronic pulmonary disease and diabetes. The UN appealed to the World Health Organization (WHO) as the expert agency for health and the frontline of the global effort to prepare recommendations for global targets before the end of 2012. In the 2012 session of the World Health Assembly, member states adopted a resolution known as the 25x25 strategy to reduce premature mortality in NCDs by 25% by the year 2025 [1,2]. The strategy was incorporated into the WHO's Global NCD Action Plan 2013-2020. Later in 2015 the UN General Assembly adopted the resolution "Transforming our world: the 2030 Agenda for sustainable development" where target 3.4 states: "By 2030, reduce by one-third premature mortality from non-communicable diseases through prevention and treatment and promote mental health and well-being [3].

Cardiovascular disease (CVD)

The biggest component of NCDs is CVD. It is estimated that CVD claims the life of 17.9 million people annually (2019) representing 32% of deaths worldwide [4]. CVD is a group of diseases that affect the heart and blood vessels and is divided into six subgroups [4]:

- Coronary heart disease (CHD);
- Cerebrovascular disease;
- Peripheral arterial disease;

- Rheumatic heart disease;
- Congenital heart disease;
- Deep vein thrombosis and pulmonary embolism.

The behavioural risk factors of CVD include unhealthy diet, physical inactivity, tobacco use and harmful alcohol use. The secondary effects of these risk factors may be seen in individuals in the form of obesity, elevated blood lipids, hyperglycaemia and hypertension [4].

In this doctoral thesis we will focus on CHD, and its effects and causes.

Coronary heart disease (CHD)

The most common of the cardiovascular diseases is CHD [5]. The terms CHD, ischaemic heart disease or just heart disease are used interchangeably. CHD results in the loss of blood flow due to obstruction in the arteries providing blood to the heart muscle. The main cause is arteriosclerosis, a build-up of fatty deposits in the vessel wall causing inflammation and a positive feedback loop leading to increased blockage, see Figure 1.

Atherosclerosis

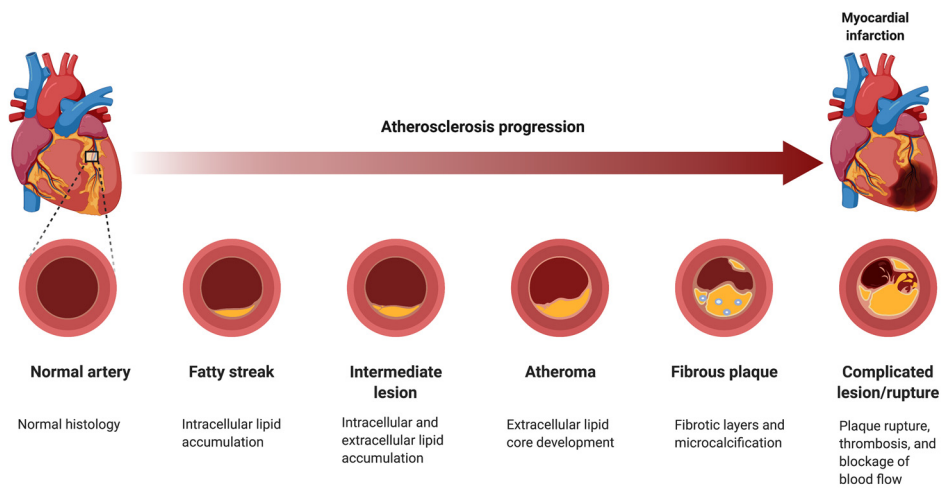

Figure 1. Showing the development of atherosclerosis. Created with BioRender.com

Several pathophysiological mechanisms contribute to atherosclerotic plaque formation. The atherosclerotic process appears to be initiated by inflammation that may proceed to an acute clinical event by the induction of plaque rupture. This can, in turn, cause thrombosis resulting in an occluded vessel leading to oxygen deprivation and consequently cell death. There are multiple parts of the atherosclerotic process that include dysfunctioning endothelium, accumulation of lipoproteins, foam cells and oxidative products in the subendothelial space [6].

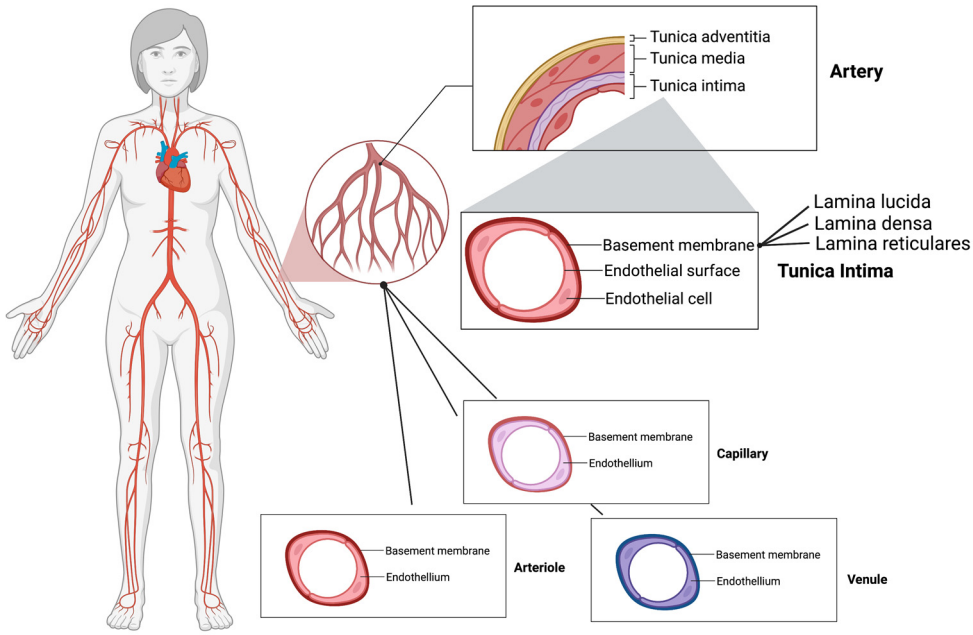

Figure 2. The circulatory system with focus on the microcirculation and the different parts of blood vessels illustrated. Created with BioRender.com

Arteries consist of three separate layers, from the inside out: intima, media and adventitia. The intima is composed of the endothelial surface, the basement membrane and the internal elastic lamina. For many years, the endothelial surface was seen just as a semi-permeable membrane, but this viewpoint has changed. Now it is recognised for its many functions in vascular homeostasis. Its main function is to keep the blood in a liquid form and circulating, and inhibit coagulation. On the other hand, when there is damage to the vessel, the homeostatic process needs to shift the balance and promote coagulation and stop the circulation of blood in that area, see Figure 2 [6].

Shear stress

Shear stress has a role in the formation of atherosclerosis. It is more common with atherosclerotic lesions after bifurcations of the arteries making the haemodynamics of the blood flow part of the process. Shear stress increases with laminar blood flow and can activate endothelial nitric oxidase synthase (eNOS) increasing the amounts of nitric oxidase (NO) available. This leads to a relaxation of the smooth muscle cells and vasodilatation, which promotes endothelial cell survival. Low shear stress or changing shear stress is found after a bifurcation of vessels. This turbulent blood flow reducing the shear stress is therefore a risk factor for plaque formation [7].

Lipoproteins

Hyperlipidaemia is a term describing elevated blood lipids. All dyslipidaemias are either primary, genetic disorders named according to which lipoprotein is raised, or secondary, i.e. a consequence of a different condition. The latter may be diabetes mellitus, alcoholism, nephrotic syndrome, chronic renal failure, hypothyroidism, liver disease, drug intake and others [8]. All lipoproteins are composed of cholesterol, triglycerides and apolipoproteins and can be raised on their own or in combination [9].

Lipids are hydrophobic molecules that need to be bound to proteins, i.e. lipoproteins, in order to be able to be transported in the blood stream. There are four classes of lipoproteins depending on their composition and also size and density: chylomicrons, very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). Lipoproteins are in constant movement and change between the different forms by exchanging molecules with each other and the surrounding tissue [8].

Under certain circumstances lipoproteins pass through the endothelial surface and accumulate in the subendothelial space. They bind to proteoglycans and become trapped inside the vessel wall. The endothelial surface is normally resistant to immune cells, but the presence of lipoproteins makes the surface more permeable and immune cells can enter the space. It is mainly monocytes that enter the space, as a result of which they are transformed to macrophages and start to consume the lipoproteins (specially oxidised LDL). Following this they become foam cells and produce many mediators [10].

Macrophage accumulation and microcalcification

The accumulation of macrophages in the vessel wall can lead to necrotic core formation. This is called plaque necrosis and it can worsen inflammation of the atheroma, attracting even more macrophages, making it a positive feedback loop. In early calcification (i.e. microcalcification), apoptotic bodies and matrix vesicles are released during the death of macrophages and other cells resulting in these structures serving as sites for calcium phosphate crystal formation [11]. The microcalcification

can by itself also promote inflammatory responses from macrophages, stimulating plaque inflammation, additionally creating another positive feedback loop [12]. Macrophages may also stimulate macrocalcification through osteoblastic activation and produce cytokines, such as interleukin-6 (IL-6), interleukin-1β and tumour necrosis factor-α [11].

Chylomicrons

Chylomicrons are produced by the mucosa cells in our intestines mainly from dietary lipids. They carry dietary triacylglycerol, cholesterol and cholesteryl esters to the peripheral tissues [8].

VLDL

VLDL is produced by the liver and is composed of mainly triacylglycerol. Its function is to carry lipids from the liver to the peripheral tissues. When it passes though the circulation its structure changes, it loses triacylglycerol making it denser and it transfers surface components to HDL and receives cholesteryl esters in return. It is thus transformed into LDL. Prior to this, for a short period of time a different particle, intermediate density lipoprotein, is formed before becoming LDL [8].

LDL

LDL has been determined to be the major driving force behind atherosclerosis [13]. Its role is to transport cholesterol in the plasma to the peripheral tissue for the cells to use for synthesis of steroids, bile acid and incorporation into cell membranes. They contain less triacylglycerol than VLDL and have a high concentration of cholesterol and cholesteryl esters. LDL can both deposit the free cholesterol on the surface that it comes into contact with but also by binding to receptors on cell-surface membranes it can be internalised by endocytosis. LDL sometimes becomes chemically modified into ligands by oxidation or acetylation. This modified LDL activates scavenger receptors of macrophages that internalise them. The modified LDL cannot activate the normal pathway for cholesterol regulation inside the cell but, instead, accumulates, thereby transforming the macrophages into foam cells [14].

Hypertension

The pathophysiology behind hypertension has still not been established completely. A small proportion of patients (between 5-8%) have hypertension as a result of renal or adrenal disease. The remainder receive the diagnosis of “Essential hypertension” without a single identifiable cause but instead a combination of different factors due to the impaired function of normal blood pressure regulation [15].

There needs to be a balance between cardiac output and peripheral vascular resistance in order to support normal blood pressure. Usually patients with essential hypertension have normal cardiac output but they have raised peripheral vascular resistance. Small arterioles with walls containing smooth muscle cells regulate peripheral vascular resistance. The continued contraction of these smooth muscle cells is thought to induce structural changes with thickening of the arteriolar vessel wall leading to an irreversible elevation of peripheral vascular resistance [15]. Continuous high blood pressure increases the risk of many cardiovascular events and is one of the leading contributors to premature death in the world. In 2015 it was responsible for nearly 10 million deaths per year and 200 million disability-adjusted life years [16]. The three major cardiovascular events that hypertension can lead to are ischaemic heart disease (4.9 million), haemorrhagic stroke (2.0 million), and ischaemic stroke (1.5 million) deaths per year. Over the last 30 years there have been many advances in diagnostics and treatment for hypertension, but still the disability-adjusted life years attributable to hypertension have increased by 40% since 1990 [17]. Hypertension has a close connection to several cardiovascular events: ischaemic stroke, acute myocardial infarction (AMI), sudden death, haemorrhagic stroke, heart failure, peripheral artery disease and end-stage renal disease. Evidence is accumulating on the connection of hypertension to developing atrial fibrillation (AF) and linking early elevated blood pressure to increased risk of cognitive decline and dementia [18].

In older patients, systolic blood pressure (SBP) tends to be a better predictor for cardiovascular events than diastolic blood pressure (DBP). Elevated DBP is more common in younger patients (<50 years) since DBP often declines from midlife onwards with arterial stiffening. Consequently, pulse pressure (often measured as pulse wave velocimetry [PWV]) increases (i.e. the difference between SBP and DBP) and has a negative prognostic impact [18].

Essential hypertension is a hereditary disease but many environmental factors have a large effect. Dietary- and salt intake, obesity, overweight, psychological stress and smoking all lead to elevation of blood pressure and CVD [19,20]. The definitions of hypertension are listed in Table 1.

Table 1: Definitions of hypertension after repeated measurements

Classification of blood pressure (mmHg)	
Optimal	<120 and <80
Normal	120-129 and/or 80-84
High	130-139 and/or 85-89
Stage 1/Mild hypertension	140-159/90-99
Stage 2/Moderate hypertension	160-179/100-109
Stage 3/Severe hypertension	180/110
Older person > 80 years	160/90
Isolated systolic hypertension	SBP >140 or DBP < 90
Home tested blood pressure	>130/80 (24 h medium)
Patients with diabetes	>140/80

Taken from the European Society of Cardiology (ESC)/European Society of Hypertension (ESH) guidelines from 2018 [18].

Often patients with hypertension also have multiple cardiovascular risk factors, these include:

- Blood lipids outside the normal range;
- Overweight, central obesity;
- Insulin resistance or diabetes;
- Smoking.

These risk factors combined with ageing and organ damage result in the total risk for CVD.

Microcirculation

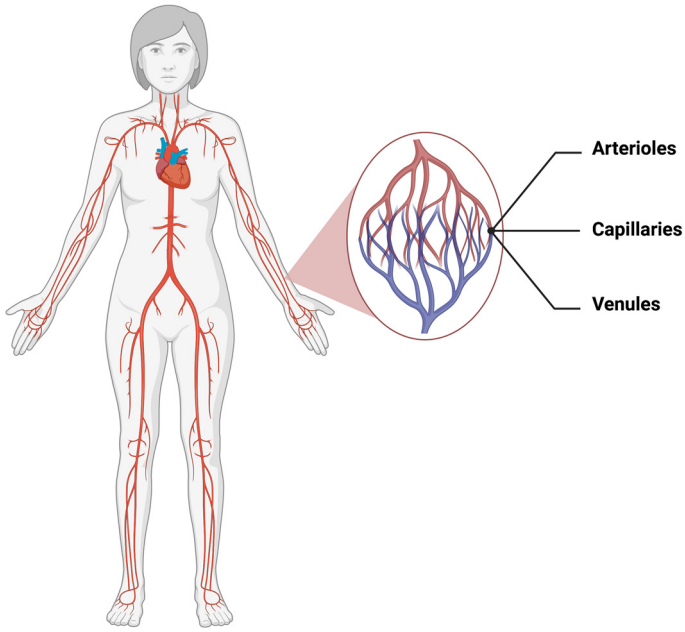

Figure 3. Showing the three major parts of the microcirculation: arterioles, capillaries and venules. Created with BioRender.com

The most important function of the circulatory system is to transport molecules throughout the body. Diffusion of molecules is only efficient over very short distances. For example, the diffusion distance of oxygen in oxygen-consuming tissue is normally less than 100 µm. Transport by a flowing fluid, i.e. convection, does not have such restraints and is used by most organisms. To transport different substances by convection it is easiest done using large vessels, since the resistance to fluid flow is directly related to the diameter of the transportation vessel. The larger the vessel, the lower the resistance. Nevertheless, the transport of substances to all parts of the human organism requires a large amount of very small vessels exceptionally well distributed in all tissues, i.e. the microcirculation [21].

The microcirculation is the final part of the large systemic circulation and consists of microvessels with a diameter smaller than 20 µm. Arterioles, capillaries, venules and their (sub) cellular constituents are the components of the microcirculation, see Figure 3. The most important function of the microcirculation is oxygen transfer from the red blood cells in the capillaries to the parenchymal cells in the surrounding tissue. Oxygen is needed for the parenchymal cells' cellular respiration where it is necessary for the energy requirements needed to sustain their function. All blood-

borne hormones and nutrients to the parenchymal cells, including the functional activity of the immune system and homeostasis, are other functions included in the microcirculation. The regulation of solute exchange between the intravascular and extravascular space and tissue is also part of the microcirculatory functions. Correct functioning of the microcirculation is essential for the viability of the parenchymal cells and following normal organ function [22].

The Framingham Heart Study (FHS)

In 1948 almost nothing was known about the epidemiology of atherosclerosis, hypertension and development of CHD. The US Public Health Service wanted to set up a study answering these questions and Framingham, Massachusetts, a town with a population of approximately 28,000 in close proximity to Boston was chosen for this longitudinal study to take place. The study started out recruiting a first cohort of 5209 individuals with a mean age of 44 years (range 28-74 years) and the first participant entered the Framingham Study research clinic on the 29th September 1948. An executive committee set up in the beginning of the study comprising 15 town members urged that families should not be divided when recruiting for the cohort, which led to very insightful knowledge about the familiar accumulation of CHD development. It also increased the participants' compliance to stay in the study. The original cohort comprised more than 50% women which for that time was visionary. Participants were recruited as termed "normal" by the researchers in the age span of 30-60 years and free from overt CVD because of the age representing the time in life when CVD development occurs. See Box 1 for the original hypotheses and aims from the background paper of the FHS.

Box 1: The original hypotheses and aims from the background paper of the Framingham Heart Study.

Foundation of the Framingham Heart Study [23]

Hypothesis given in the background paper for the Framingham Heart Study in 1951

- "It is assumed that these diagnoses [hypertensive and arteriosclerotic disease] do not each have a single cause (as is the case of most infectious diseases), but that they are the result of multiple causes which work slowly within the individual."
- "Clearly, what is required is the epidemiological study of these diseases [hypertensive and arteriosclerotic disease] based on populations of normal composition, including both the sick and the well as they are found in the community."

Original aims of the Framingham Heart Study as presented in 1951

- "Based on as complete a clinical examination as feasible, there are selected out of this initial group those persons who are free of definite signs of these diseases. These persons will be termed the normals, and they will be observed over a period of years until a sizable number are found to have acquired the diseases. At that time a search is made for the factors which influenced the development of disease in the one group and not in the other."
- "As one by-product of this investigation it will also be possible to study the efficiency of various diagnostic procedures in finding heart disease or as indicators of the subsequent development of heart disease. (These findings, of course, have important bearing on the question of including tests for heart disease in mass screening programs.)"
- "A second by-product will be data on prevalence and incidence of cardiovascular diseases."

Taken from the original publication "*70-year legacy of the Framingham Heart Study"*, Box 1 [24].

The cohort was planned to be followed for 20 years and this was achieved in 1968. After 1968, there followed a couple of years of unsure funding but, in 1971, the study was granted federal funds and continued and a second generation of 5125 patients, the G2 cohort, was enrolled. This cohort comprised children of the original cohort and their partners. The ages of the so-called "Offspring G2 cohort" were approximately the same as those of the Original cohort when they were first enrolled. The Original cohort has been followed for approximately 32 cycles every 2 years when the last cycle was finished in 2012-2014, at which time only 40 individuals remained alive. The mean age of the Original cohort at that time was 96 years (range 93-106 years) and they were examined in nursing homes or homes if they were unable to attend the FHS research clinic. In 1995 a small cohort (Omni 1; n = 506) were recruited to accommodate for the changing demographics of Framingham. In 2002 a Third-generation FHS cohort (n = 4095) was recruited comprising the grandchildren of the Original cohort, and an Omni 2 cohort, n = 410, with contemporary minority cohort with similar age distribution was recruited. In 2019 the FHS contained 6477 parent–offspring pairs and 1267 grandparent–grandchildren pairs, plus 5530 sibships and a number of other family line relationships, which are of great value for heritability and genetic studies.

Identifying risk factors for CHD was established by the FHS and one of the most high-impact reports was published in 1961 [25]. Male gender, older age, elevated blood pressure and cholesterol levels and left ventricular (LV) hypertrophy (as assessed by electrocardiogram) were found to be important predictors of the risk of CHD. Following this time, in 1962 and 1964, reports were published on the strong association between smoking and CHD [26,27]. Later, in 1998, a report was published by Wilson et al. with a mathematical algorithm for calculating the 10-year risk of CHD, and in 2008 an algorithm for calculating the general risk of CVD was published and modified [28,29]. These reports form the basis of the Framingham risk score used today.

Treatment of Cardiovascular Disease

Hypertension

Treatment of hypertension can be divided into two categories: lifestyle changes and pharmacological therapies. Hypertensive treatment is well established and high blood pressure is the disease with the most clinical randomised controlled trials published in the world. The relationship between hypertension and coronary artery disease (CAD) is very close and a recent study revealed that hypertension is responsible for 25% of population-attributable risk of a myocardial infarction. Another study concluded that for every 10 mmHg SBP reduction, the incidence of

CAD was reduced by 17%. Multiple meta-analyses have concluded that a reduction of 10 mmHg of SBP or 5 mmHg of DBP reduces the risk of all major cardiovascular events by 20%, all-cause mortality by 10–15%, stroke by 35%, coronary events by 20%, and heart failure by 40% [30]. Even more intensive blood pressure regulations have shown similar results and there is also evidence of a reduction in cardiac events in high-risk groups, such as those with diabetes. The exact optimal level of blood pressure is, however, not defined clearly but evidence supports a target blood pressure of approximately less than 130/80 mmHg in patients with CAD. However, reducing blood pressure to less than 120/80 mmHg is not recommended.

The treatment basis in patients with any stage of hypertension is lifestyle changes. These can prevent or delay the onset of hypertension and reduce the risk of cardiovascular events. The recommendations that have been shown to reduce blood pressure are salt restriction, moderation of alcohol consumption, high consumption of vegetables and fruits, weight reduction and maintaining an ideal body weight, and regular physical activity. Tobacco smoking has an immediate blood pressure-elevating effect that may raise daytime blood pressure; quitting smoking is important for additional reasons, i.e. cancer and CVD prevention. Lifestyle changes have the downside of low persistence over time and lifestyle recommendations should never delay the initiation of drug therapy when a patient has hypertension-mediated organ damage. Patients with high blood pressure often receive these lifestyle recommendations in combination with pharmacological treatment based on the stage of hypertension [18].

Stage 1/Mild hypertension

Younger patients (<65 years) with mild hypertension and established atherosclerotic disease or hypertensive damage should receive pharmacological treatment immediately [31].

Patients with stage 1 hypertension without organ damage or established atherosclerotic disease are recommended to adopt lifestyle changes for 3-6 months and after that a new evaluation should be carried out. If the lifestyle changes have not resulted in reduction of the blood pressure the patient should receive pharmacological treatment.

Stages 2-3/Moderate-severe hypertension

Patients with stages 2-3 hypertension should receive pharmacological treatments immediately. An evaluation and follow-up visit should be planned in a couple of days to weeks depending on the stage. Patients with severe hypertension are often referred immediately to hospital.

Older patients (>65 years)

Older patients (>65 years) should receive pharmacological treatment if their SBP is > 160 mmHg. It is vital that patients with a biological age lower than 65 years should receive advice on lifestyle changes similar to younger patients with hypertension stage 1 [32,33].

Older patients (>80)

The recommendation for older patients is to treat SBP pharmacologically when it is >160 mmHg. It is more common that patients in this age group have comorbidities and many require medication for the treatment of, for example, angina pectoris and heart failure.

See Table 2 for an overview of treatment guidelines and Table 3 for an explanation of the colours connected to the risk level

Table 2. Guidelines for the treatment of hypertension related to risk of cardiovascular disease

Risk assesment Other risk factors, organ damage or disease	**High** normal SBP 130-139 mmHg and/or DBP 85-89 mmHg	**Stage 1** hypertension SBP 140-159 mmHg and/or DBP 90-99 mmHg	**Stage 2** hypertension SBP 160-179 mmHg and/or DBP 100-109 mmHg	**Stage 3** hypertension SBP ≥180 mmHg and/or DBP ≥110 mmHg
No other risk factors	No treatment	Lifestyle changes Consider pharmacological treatment	Lifestyle changes Pharmacological treatment with two substances	Lifestyle changes Innitiate immideately pharmacological treatment with two substances
1-2 risk factors	Lifestyle changes No pharmacological treatment	Lifestyle changes Consider pharmacological treatment	Lifestyle changes Pharmacological treatment with two substances	Lifestyle changes Initiate pharmacological treatment immediately with two substances
≥ 3risk factors	Lifestyle changes No pharmacological treatment	Lifestyle changes Pharmacological treatment with two substances	Lifestyle changes Pharmacological treatment with two substances	Lifestyle changes Initiate pharmacological treatment immediately with two substances
Organ damage due to hypertension, kidney failure stage 3 or diabetes mellitus without organ damage	Lifestyle changes No pharmacological treatment	Lifestyle changes Pharmacological treatment with two substances	Lifestyle changes Pharmacological treatment with two substances	Lifestyle changes Initiate pharmacological treatment immediately with two substances
Atherosclerotic cardiovascular disease, kidney failure stage ≥4 or diabetes mellitus with organ damage	Lifestyle changes Consider pharmacological treatment	Lifestyle changes Pharmacological treatment with two substances substances	Lifestyle changes Pharmacological treatment with two substances	Lifestyle changes Initiate immediately pharmacological treatment with two substances

Table modified according to the Swedish Medical Products Agency's recommendations and modified according to European Society of Cardiology (ESC)/European Society of Hypertension (ESH) guidelines from 2018. Table translated from Swedish, see https://viss.nu/kunskapsstod/vardprogram/hypertoni. Accessed on 14 September 2021 [34].

Table 3. Explanation of the colours used for easier illustration of treatment options in Table 2.

Low risk <1 %	Medium risk 1-4 %	High risk 5-9 %	Very high risk ≥10%

Table modified according to the Swedish Medical Products Agency's recommendations and modified according to European Society of Cardiology (ESC)/European Society of Hypertension (ESH) guidelines from 2018. Table translated from Swedish, see https://viss.nu/kunskapsstod/vardprogram/hypertoni. Accessed on 14 September 2021 [34].

Coronary heart disease

The goal of primary prevention of CVD is to reduce the risk of a future coronary event and development of CHD, i.e. CAD. When patients have diagnosed CAD by experiencing acute coronary symptoms or AMI many pharmacological treatments need to be considered. The following pharmacological treatments are the basis of management of CHD but also have an important role in antihypertensive and lipid-lowering treatments. Several strategies are applied to reduce the risk of cardiovascular events including reducing the risk of blood clots, reducing blood pressure, dilatation of blood vessels and heart stroke modification.

The following pharmacological recommendations are according to the European Society of Cardiology (ESC)/European Society of Hypertension (ESH) guidelines from 2017 and 2019 in combination with the Swedish guidelines found at Janusinfo/kloka listan [18,35-37].

Acetylsalicylic acid

Thrombosis is the most common complication of hypertension by inducing a prothrombotic state but also predisposition for lower extremity artery disease, heart failure and AF. Neither antiplatelet therapy nor anticoagulant therapy is recommended for primary prevention of hypertension alone but in combination with CVD, i.e. secondary prevention with antiplatelet therapy has been shown to have benefits. As secondary prevention, antiplatelet therapy showed an absolute reduction in vascular events of 4.1% compared with placebo. Anticoagulant therapy alone or in combination with antiplatelet therapy (acetylsalicylic acid [aspirin]) is not recommended for patients with only hypertension without any other conditions requiring anticoagulatory treatment such as AF or venous thromboembolism. Thus aspirin is not recommended for primary prevention of hypertension in patients without CVD. In patients with CVD, i.e. for secondary prevention in hypertensive patients, there is a benefit and aspirin is recommended hence most patients are prescribed it (75 mg 1x1) as a lifelong treatment after a coronary event such as AMI. Patients with stable angina pectoris are also prescribed aspirin.

Other antiplatelet therapies, such as ticlopidine and clopidogrel, and newer antiplatelet drugs, such as prasugrel and ticagrelor, have not yet been studied sufficiently to be recommended to be used in hypertensive patients for primary prevention of CVD.

Beta-blockers

Most patients should receive beta-blockers (metoprolol) after an AMI. Metoprolol is given to reduce the size of the infarction and improve the function of the left ventricle.

Cardiac arrhythmias, including ventricular arrhythmias, are predisposed by hypertension and the most common arrhythmia is AF. AF can be considered to be a manifestation of hypertensive heart disease. Patients suffering from AF very often have hypertension and even high-normal blood pressure is associated with AF. The risk of stroke and heart failure increases significantly with an AF diagnosis. Patients suffering from AF require consideration of stroke prevention by anticoagulation treatment. Often patients suffering from AF have a high ventricular rate and beta-blockers or non-dihydropyridine calcium channel blockers (CCBs, e.g. diltiazem and verapamil) are recommended as antihypertensive agents. Often these patients are prescribed beta-blockers but if additional rate control needs to be achieved, a combination with digoxin is recommended.

Blockers of the renin-angiotensin system (RAS)

The primary target with renin-angiotensin system (RAS) treatment is a reduction of blood pressure. With rising age, arterial stiffening increases leading to an elevation in SBP and fall of DBP usually measured by PWV. The loss of arterial elasticity is caused by structural changes of large arterial vessel walls due to arteriosclerosis and the force of blood pressure applied on the arterial wall. Hence, all hypertensive treatments reduce arterial stiffening. This is because reduced blood pressure reduces the force on the arterial wall causing a secondary reduction of PWV. Previously published studies implied that angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) could reduce the PWV on a long-term basis more than expected by the antihypertensive treatment alone. This effect of RAS blockers compared to other antihypertensive drugs has, however, not been demonstrated. A long-term reducing effect on aortic stiffness, consequently a cardiovascular event-reducing effect exceeding the impact of only reducing blood pressure, has also not been shown.

ACE inhibitors should be considered for all patients after an AMI. They are recommended for patients with systolic left ventricle dysfunction, heart failure, diabetes, hypertension and microalbuminuria or kidney failure. If ACE inhibitors are not tolerated well, ARBs can be considered instead. ARB and ACE inhibitors should not be combined since the risk of adverse events arise with particular respect to kidney function and there are no trials that have been able to show a benefit of such a combination. ACE inhibitors and ARBs reduce albuminuria and delay the progress of diabetic and non-diabetic chronic kidney disease.

Lipid-lowering therapy (statins)

Statins are recommended for patients with hypertension who also have an elevated risk of CVD. Such patients often display atherogenic dyslipidaemia characterised by elevated triglycerides and LDL and low HDL, being even more common in patients also suffering from type 2 diabetes or metabolic syndrome. Lipid-lowering statin therapy has been well established as showing a benefit in such patients.

Hypertensive patients without previous cardiovascular events but with LDL >3.4 mmol/L should receive statin therapy since studies have demonstrated that lowering the LDL to <3.0 mmol/L reduced the incidence of cardiovascular events by between 44 and 24%.[18]

All patients should receive statins at the hospital immediately after an AMI. Studies have shown unequivocal benefits of early and intensive statin therapy in acute coronary syndrome. Depending on the programme, 40-80 mg/daily of atorvastatin is given and the goal is an LDL level of <1.8 mmol/L. In the newest guidelines from the ESC/ESH from 2019, a reduction of at least 50% if the baseline LDL is between 1.8 and 3.5 mmol/L is recommended for patients at high risk of CVD.

Calcium channel blockers

Calcium channel blockers (CCB) are prescribed frequently for patients with hypertension. They have an effect on calcium channels in smooth muscle cells in heart and vessels. The mechanism behind the antihypertensive effect is a dilatation of peripheral arterioles reducing the resistance and hence reducing afterload. The heart rate frequency is not affected leading to a reduced oxygen requirement by the heart muscle and an anti-ischaemic effect. The CCB amlodipine also has a suspected direct relaxing effect on the heart's coronary arteries and arterioles causing vasodilatation, leading to an increase in blood flow and oxygen supply to the heart muscle. Some studies report that CCBs have a better effect on slowing the progression of carotid atherosclerosis and left ventricle hypertrophy than beta-blockers. A CCB can be prescribed if the patient is intolerant to beta-blockers or as a combination treatment with beta-blockers and nitrates. Amlodipine is the most studied and is the drug recommended for combination treatment with beta-blockers.

Non-dihydropyridine CCBs (e.g. diltiazem and verapamil) reduce the revenue of energy-rich phosphates and hence the oxygen need. The inhibitory effect on heart contractions with reduced oxygen need is believed to have a beneficial therapeutic effect on angina. Verapamil is thought to have a small effect on frequency reduction and a similar effect as CCBs on coronary vasodilatation and after-load reduction. Non-dihydropyridine CCBs also have an effect on the atrioventicular (AV)-node prolonging the conduction time. This leads to a reduction of the ventricular frequency in AF and flutter. Apart from an effect on the sinus and AV-node, the conduction system of the heart is not affected. A prolonged PQ-time can be observed when the drug is given in high doses or in combination with beta-blockers, which is not recommended due to the risk of heart block.

Nitroglycerin

Nitroglycerin and it derivatives are anti-ischaemic drugs prescribed for reducing the symptoms of chest pain due to oxygen insufficiency in the cardiac muscle in patients with chronic coronary syndrome. In most of these patients anti-ischaemic drugs do not prevent cardiovascular events but often have a good effect on the acute

symptoms. Nitroglycerin induces relaxation of smooth muscle cells, more on the venous side than on the arterial side of the circulatory system. The effect is dependent on the given dose and the patient's individual sensitivity to the drug.

Short-acting nitrates are often prescribed to be taken if the patient feels acute chest pain; they are given as a sublingual spray or tablets that are absorbed quickly and can therefore relieve an attack of angina rapidly. The spray is absorbed more quickly compared to the tablets, which are not to be swallowed but are to be dissolved sublingually and absorbed. Before administration of short-acting nitrates the patient should be seated since standing promotes syncope and a supine position increases venous return and preload. The dilatation of veins leads to a reduced pressure in the left ventricle (reduced preload) because of a reduction in the venous return to the right side of the heart and the reduced vasoconstriction on the arterial side, i.e. reduced blood flow resistance (reduced afterload) lessens the need for oxygen in the heart muscle. Nitrates might also have a small direct dilatory effect on the coronary arteries. This effect has a short duration and passes quickly. The tablets/spray usually comprise 0.3-0.6 mg nitroglycerin and the treatment should be repeated every 5 minutes until the pain disappears. A maximum of 1.2 mg/15 minutes should not be exceeded. If symptoms of angina do not disappear, immediate medical attention is required.

Nitroglycerin can be administered as prophylaxis before any physical exercise that is known to the patient to lead to symptoms of pain. There is a large risk of provoking tolerance with loss of efficacy when nitroglycerin is taken over a prolonged period of time. This is particularly common with long-acting nitrates. A nitrate-free period of approximately 10-14 hours might be necessary in such circumstances. Long-acting nitrates should be prescribed as a second-line therapy for angina relief when initial therapy with a beta-blocker or non-dihydropyridine CCB is contraindicated, poorly tolerated, or proves insufficient to control symptoms. Termination of nitrate therapy should be gradual and not abrupt to avoid a rebound effect. Most common side-effects are headache, hypotension and flushing. Contraindications include hypertrophic obstructive cardiomyopathy, severe aortic valvular stenosis, and co-administration of phosphodiesterase inhibitors (e.g. sildenafil, tadalafil, or vardenafil) or riociguat.

Precision medicine

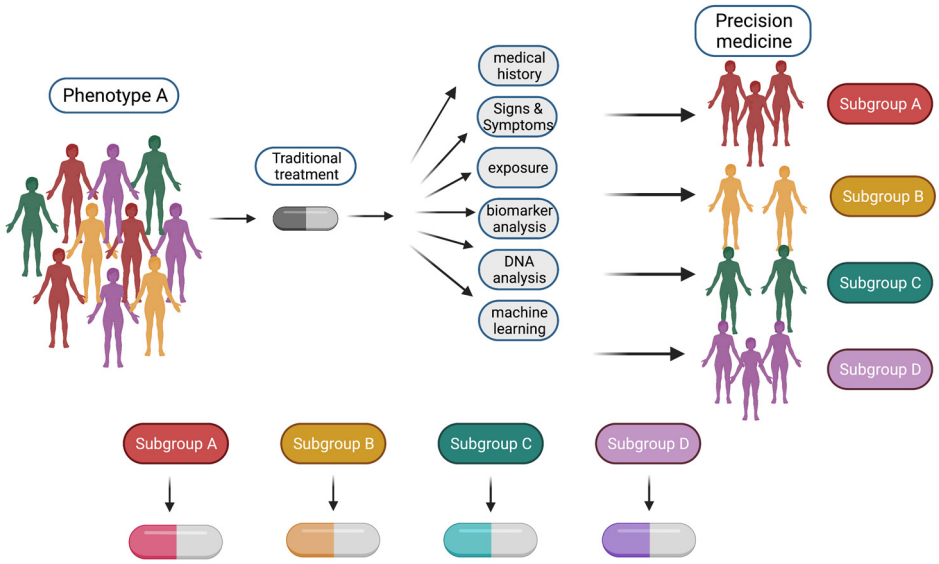

Figure 4. Showing an illustration of the concept precision medicine. Created with BioRender.com

In recent years the term "precision medicine" has become increasingly popular. It has superseded the previously used terms "personalised medicine" and "stratified medicine" which were used interchangeably. The reason behind the change was that physicians have "always treated patients on a personalised level" [38]. Precision medicine incorporates a wide range of individual data, including clinical, genetic, lifestyle and biomarker information apart from the signs and symptoms that were observed previously. The idea is to customise and tailor medical treatments, drugs and recommendations to each individual patient depending on the predicted response or risk of disease, see Figure 4 for an illustration of the concept. An example that is often quoted is when precision medicine is discussed in the analysis of human epidermal growth factor receptor (HER-2) in patients with breast cancer [39]. HER-2 was discovered in patients having a more aggressive form of breast cancer and was found to be a prognostic factor for a more aggressive course of disease. The monoclonal antibody trastuzumab was discovered and it showed beneficial results in clinical studies reducing proliferation of cancer cells expressing HER-2 [38]. Currently trastuzumab is only given to HER-2 positive patients in whom gene expression data are available to guide the treatment decision of that patient, i.e. precision medicine [38].

Lifestyle and daily diet vary greatly between individuals and have been shown to impact on the development of different diseases [40-45]. Drugs have different

effects between different patients and this also needs to be taken into consideration before prescribing medication [46,47].

There are different definitions of precision medicine but they all encompass the multiple sources of information used for decision-making regarding patients. Jameson and Longo defined personalised medicine as "treatments targeted to the needs of individual patients on the basis of genetic, biomarker, phenotypic or psychosocial characteristics that distinguish a given patient from other patients with similar clinical presentations" [48]. The President's Council of Advisors on Science and Technology used this similar definition: "[…] the tailoring of medical treatment to the individual characteristics of each patient. It […] [means] the ability to classify individuals into subpopulations that differ in their susceptibility to a particular disease or their response to a specific treatment" [49]. The purpose is to minimise side-effects for those less likely to respond to a particular treatment and to improve clinical outcomes for others who have a response. This is not a novel concept, but the way in which physicians have practised for centuries, the novelty is the data that are available today for physicians to use if they choose to. Newly discovered biomarkers, DNA analysis and machine learning are tools that are more and more available and research progresses quickly in these areas with novelties discovered frequently.

Patients who are aware of this want to know when consulting a doctor what effect they will have from a certain medicine, treatment or lifestyle change. The medical professionals want to know if the treatment or lifestyle change that they recommend is going to be effective for the individual patient. Politicians want to distribute funds more adequately depending on where they are needed the most. Hence precision medicine is now requested by patients, medical professionals and politicians.

Garlic (*Allium sativum*)

Figure 5. Garlic (Allium sativum) illustration from Medical Botany (1836) by John Stephenson and James Morss Churchill downloaded from Rawpixel.com

Garlic (Allium sativum, Figure 5) is one of the best-known and widespread herbs in the world. The history of using garlic goes back thousands of years and all the ancient civilisations such as the Egyptians, Babylonians, Romans and Chinese used the herb. Garlic is used for culinary purposes but also has an important role in traditional medicine. It is the many sulphur-containing compounds in garlic, recognisable by their strong odour, that its medicinal effects are linked to. The origin of the bulb is not quite clear, the suggested area is central Asia's mountain foothills, but since the proposed ancestor *Allium longiscuspis* is sterile, researchers disagree [50].

Wild garlic originates from areas with tough inland climate with very hot summers and cold winters. The plant is a perennial but in modern agriculture it is often cultivated as an annual. Today garlic is often planted in the autumn, grows until the frost comes, lies dormant in winter, then in early spring shoots spring up rapidly and the plant forms bulbs that mature in late spring or the beginning of summer and then

they are harvested. Previously in the hot scorching summer months the garlic bulb lay dormant until the colder, damper autumn when a new growth's cycle would start. Garlic has no fertile seeds and is propagated through the cloves in the bulbs. Today there are many varieties of garlic but they are all thought to originate from random mutations [51].

Garlic contains many sulphur-containing compounds such as ajoenes (E-ajoene, Z-ajoene), thiosulfinates (allicin), vinyldithiins (2-vinyl-[4H]-1,3-dithiin, 3-vinyl-[4H]-1,2-dithiin), sulphides (diallyl disulfide [DADS], diallyl trisulfide [DATS]) and others. Alliin is transformed to allicin by alliinase enzyme when garlic cells are broken after cutting or crushing. Allicin is responsible for the previously mentioned characteristic strong odour and taste of raw crushed garlic and can easily be damaged by cooking. It also has the ability to provoke allergic reactions, gastrointestinal disorders and provoke intolerance [52]. The contents of raw garlic change depending on environmental circumstances, such as the availability of water and nutrients when the bulb is growing. The temperature also has an effect: lower temperature during winter time makes the bulbs more pungent in smell and taste [51].

Aged Garlic Extract

Garlic supplements can be divided into four main categories: garlic oil, garlic powder, garlic oil macerates and aged garlic extract (AGE). AGE is produced by soaking sliced raw garlic in 15–20% aqueous ethanol for up to 20 months at room temperature. This extract is then filtered and concentrated under reduced pressure at low temperatures [53]. This process causes considerable loss of allicin and increases the concentration of newer compounds, many of which are sulphur-based and water-soluble. The major sulphur compound, S-allylcysteine (SAC), is used to standardise AGE [54]. The result is odourless and tasteless and can therefore be used in placebo-controlled studies unlike the other three forms of garlic.

AGE has been investigated thoroughly for its effect on many aspects of CVD and SAC is the most studied part of AGE. SAC prevents lipid and protein oxidation and has antioxidant properties [55-57]. AGE has been suggested to have a favourable effect on vascular elasticity and endothelial function [58]. It has been shown that AGE increases the production of NO metabolites and it has been proposed that it enhances the production of endothelium-derived NO in rodents [59,60]. Increasing the amounts of NO available leads to a relaxation of smooth muscle cells and vasodilatation which promotes endothelial cell survival. Studies have shown that AGE has a favourable effect on blood lipids [61], platelet function [62,63] and on inflammatory biomarkers; it also reduces progression of atherosclerosis measured by coronary artery calcification (CAC) progression [53,64,65]. It has also been shown to have a positive effect on reducing blood pressure [66-69]. There have been no reports so far of toxic symptoms or interactions with medications [70,71].

Aims of the thesis

The overall aim of this thesis was to study what effect AGE has on different aspects of CVD. In Studies I-IV a Swedish cohort of patients was used. In Study I, II and IV, patients with an increased risk of CVD took part. In Study III only females with a low risk of CVD were included. In Study I an algorithm was developed for predicting which patient will have the best effect of AGE depending on that individual's measurements in the beginning of the study. In Study V the method for developing the predictive model was validated on a new cohort of patients from the USA.

In the planning phase of the prospective study, Study I, the aim was to study CAC score, microcirculatory measurements, blood pressure and different biomarkers and the effect on these of 12 months of placebo or AGE intake. Since the study grew (more patients, women with elevated risk but without CAC) and many more measurements, biological information and the precision medicine approach was taken, the study was subdivided into multiple parts. The specific aims and research questions are presented below for the different studies.

Aims and research question in Study I

The aim in this study was to evaluate whether the CAC score and biomarkers would be affected after 12 months of placebo or AGE intake, using a double-blinded placebo-controlled randomised trial design. We also developed an algorithm with the ability to predict which patient would have the best effect of AGE depending on the baseline values. Patients, both male and female with an increased risk of cardiovascular disease, defined by an elevated Framingham risk score and present CAC on cardiac CT were included in the study.

The specific research questions included:

Do 12 months of AGE or placebo treatment generate changes in:

1. CAC score?
2. Blood pressure (diastolic and systolic)?
3. Fasting blood glucose?

4. Blood lipids (total cholesterol, HDL, LDL, apolipoprotein A and B and triglycerides)?
5. Inflammatory biomarkers (C-reactive protein [CRP] and IL-6)?

And,

6. Generate a prediction model for the effect of 12 months of AGE treatment?

Aims and research question in Study II

In this study we examined the effect of AGE on cutaneous microvascular perfusion, measured with Laser Doppler Velocimetry (LDV), using a double-blinded placebo-controlled randomised trial design. Patients, both male and female with an increased risk of cardiovascular disease defined by elevated Framingham risk score were included in the study.

The specific research question was:

1. Do 12 months of AGE or placebo treatment improve the cutaneous microvascular perfusion measured with LDV in combination with PORH in patients with an increased risk of cardiovascular events?

Aims and research question in Study III

In this study we examined the effect of AGE on females with a low risk of cardiovascular disease defined by the lack of CAC on cardiac CT. Inflammatory biomarkers, blood pressure, and fasting blood glucose and blood lipids were studied.

The specific research question included:

Do 12 months of AGE or placebo treatment generate changes in:

1. Blood pressure (diastolic and systolic)?
2. Fasting blood glucose?
3. Blood lipids (total cholesterol, HDL, LDL, apolipoprotein A and B and triglycerides)?
4. Inflammatory biomarkers (CRP and Il-6)?

Aims and research question in Study IV

In this study we examined the effect of AGE on cutaneous microvascular perfusion measured with Laser Speckle Contrast Imaging (LSCI) using a double-blinded placebo-controlled randomised trial design. Patients, both male and female with an increased risk of cardiovascular disease, defined by elevated Framingham risk score and present CAC on CT were included in the study.

The specific research question was:

1. Do 12 months of AGE or placebo treatment improve the cutaneous microvascular perfusion measured with LSCI in combination with PORH on patients with an increased risk of cardiovascular events?
2. Do 12 months of AGE or placebo treatment improve the cutaneous microvascular perfusion measured with LSCI in combination with Cutaneous Vascular Conductance (CVC) in patients with an increased risk of cardiovascular events?

Aims and research question in Study V

In this study we wanted to validate the method developed in Study I for engineering an algorithm with the ability to predict which patient will have the best effect of AGE. To be able to do this we acquired new patient data from the USA.

The specific research question was:

1. Can you validate the method for developing a predictive model with the aim of identifying patients who would have a more favourable effect of AGE on CAC and SBP after 12 months of AGE intake in a new cohort?

Patients and methods

Study population and method

This thesis is based on two different cohorts of patients, one from Sweden (Studies I-IV) and one from the USA (Study V). The Swedish cohort of patients was recruited through advertisement in newspapers, social media, word of mouth and a press release from Skåne University Hospital, Sweden. Patients with an increased risk of CVD and Framingham risk score > 10 were sought and 175 patients were recruited. All patients underwent a cardiac CT scan and were then divided into two subgroups or excluded. See Figure 6 for an overview.

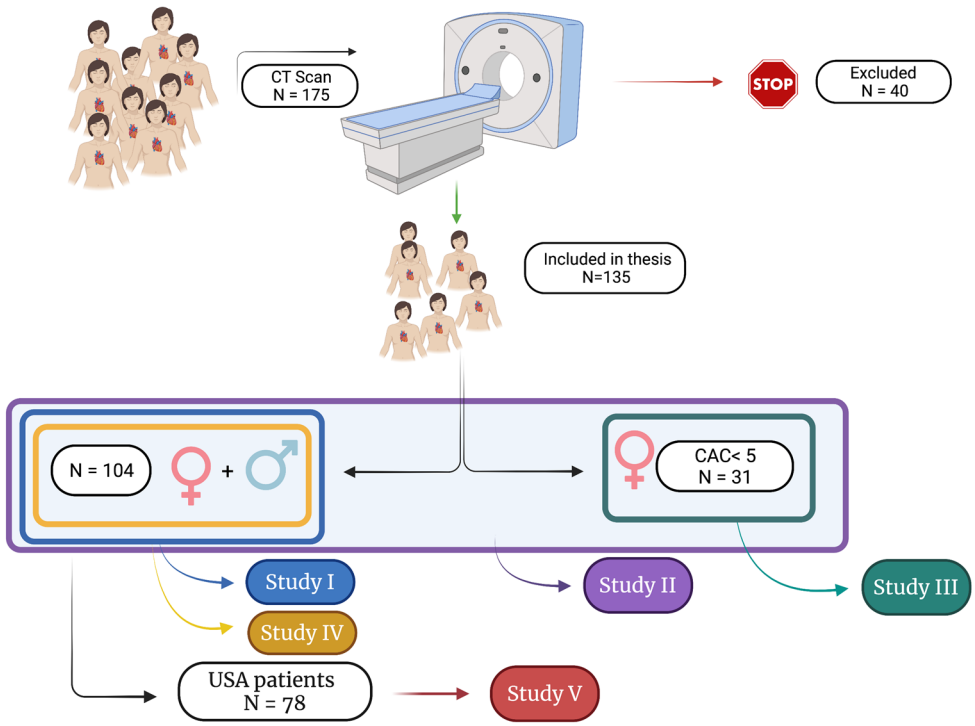

Figure 6. An overview of all patients used in Studies I-V. Created with BioRender.com

Clinical evaluation including medical history, cardiovascular risk factors, prescribed medications, smoking, and alcohol intake was performed at 0, 4, 8, and 12 months for all patients in Studies I-IV. Blood pressure measurements, body mass index (BMI) measurements, electrocardiogram measurements, and assessment of patients' compliance with treatment were carried out at the clinical visits at 0, 4, 8 and 12 months. See Figure 7 for a timeline of the clinical visits of the patients.

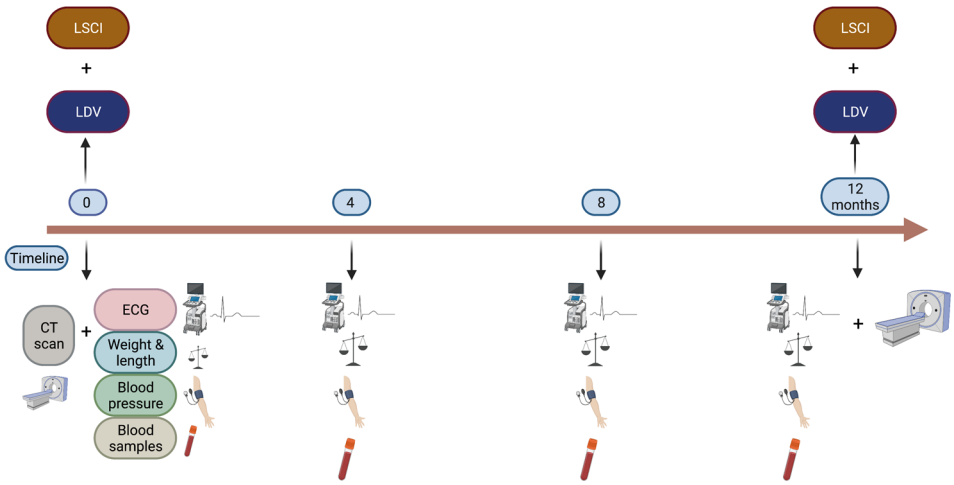

Figure 7. Showing the timeline for Studies I-IV. Created with BioRender.com

Randomised placebo-controlled double-blinded study

The study design that was used in Studies I-IV was a randomised double-blinded and placebo-controlled study. This means that both patient and researchers were blinded to the treatment the patients were receiving until the end of the study and analysis of the results. A randomised placebo-controlled double-blind study is the highest regarded form of study that can be conducted because the placebo effect is minimised as much as possible.

Placebo-controlled clinical trials became widespread after the second World War and randomised placebo-controlled, double-blind trials have become the gold standard for clinical research since then. There was a need to standardise medical treatments and examine what effect they had and the placebo-controlled randomised controlled trial was developed. A study published in the *Journal of the American Medical Association (JAMA)* in 1955 conducted by Henry Beecher defined the "Average significant effectiveness" as being 35.2 (SD 2.2%) [72]. This has later been the number quoted by many as the placebo effect. Before this time medical treatments were justified by the positive effects they had, but a paradigm shift after

the second World War and the above-mentioned published article led to them becoming classified as beneficial only when their effect exceeded that of a placebo. Historically medical practitioners had a more liberal view on the deceiving of patients combined with an assortment of sugar pills, bread pills and coloured water. The view was that placebo pills were used on the disgruntled, unintelligent and hopeless cases for whom no more medical treatments were available and an offered placebo pill then had a calming effect on the patient. The Oxford English Dictionary explaining the meaning of the word uses this historical definition: *"Placebo, n.- A drug, medicine, therapy, etc., prescribed more for the psychological benefit to the patient of being given treatment than for any direct physiological effect; esp. one with no specific therapeutic effect on a patient's condition, but believed by the patient to be therapeutic (and sometimes therefore effective)"* [73]. There is a close connection from an ethics perspective of a shift from "ends justifies the means" to a view whereby instead the virtue of the mean became the standard. At the same time "informed consent" as a concept became more widespread replacing "beneficial for the patient" as the previous ethical standpoint [74].

Placebo-controlled studies are similar to those that scientists called "controlled experiments"; they have clear advantages but they have also been criticised. Patients recruited for studies with a placebo arm often on some level hope for active treatment [75]. Therefore the Declaration of Helsinki, the Nuremberg Code and other guidelines for clinical research stress the importance of the well-being of participants in clinical trials. This is extremely important and emphasised for research carried out on active disease where there is a standard treatment available [76,77].

The patients included in Studies I-IV were mostly under medical supervision and had already used existing treatments according to medical guidelines for CVD. Their regular treatment was not changed in any way and the AGE pills were seen as a dietary complement and therefore the chosen study method of double-blinded placebo randomised controlled trial was possible. The studied substance AGE is odourless and importantly the manufacturer of the AGE product was able to produce placebo pills made from starch looking exactly like the active pill. It would have been possible to choose an open design for the study, but since the patients did not receive any reimbursement for travelling costs and time off work for the clinical visits, blood tests and microvascular measurements, the blinded approach was deemed feasible.

Double blind vs single blind

Single-blind approach is when only the patient is blind to the treatment arm, placebo or active therapy. In our study this approach would have been possible but since we had the ability to perform a double-blinded study and this is more highly regarded, we chose the latter option. A single-blind study can always be criticised for a large

placebo effect and, for studies in which there might be a quite small effect after active intervention, it is important to minimise this outcome.

Computer tomography of the heart and CAC score

CT of the heart (called CT heart or cardiac CT) is performed to evaluate the patient for CAD, defects of the heart valves, tumours of the heart or thrombus inside the heart among other conditions. It is possible when performing a cardiac CT for CAD evaluation to use a predefined scoring system, the Agatston calcium score, to measure the amount of calcium build-up in the cardiac arteries [78].

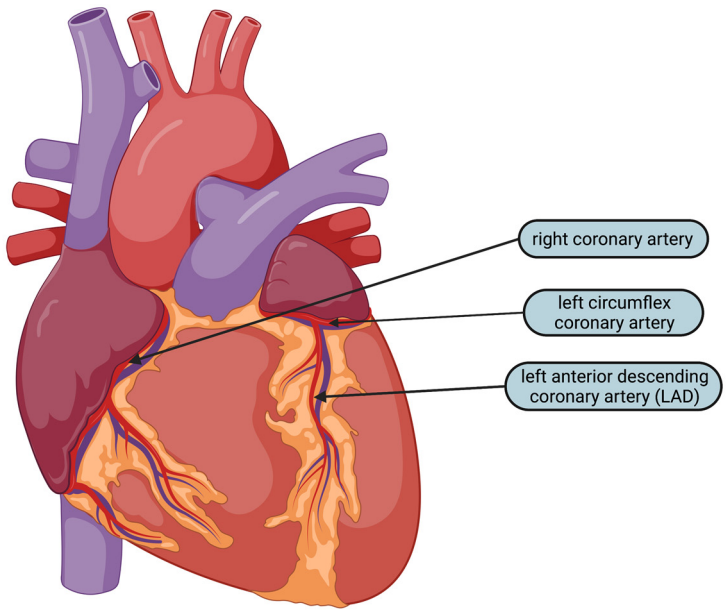

Figure 8. Showing an illustration of the heart with left anterior descending, left circumflex coronary and right coronary arteries outlined. The left main coronary artery is hidden behind the pulmonary artery.

In Studies I-V we used cardiac CT to evaluate CAD with CAC scoring. Continuous 3.0 mm axial slices were taken. Measurement of Agatston calcium score was performed with software, syngo.via, by Siemens. CAC scoring was performed on non-contrast studies by an experienced reader. CAC was defined as a plaque of at least three continuous pixels with a density of > 130 Hounsfield units. The lesion score was calculated by multiplying the lesion area by a density factor derived from the maximal Hounsfield unit within this area, as described by Agatston et al. [78]. The density factor was recorded in the following manner: 1 for lesions with peak

attenuation of 130–199, 2 for lesions with peak attenuation of 200–299, 3 for lesions with peak attenuation of 300–399, and 4 for lesions with peak attenuation of > 400. Total calcium score was determined by totalling individual lesion scores from each of the four main coronary arteries (left main coronary, left anterior descending coronary, left circumflex coronary, and right coronary artery). See Figure 8 for an illustration of the coronary arteries. Cardiac CT was performed at 0 and 12 months.

Laser-Doppler velocimetry (LDV)

Figure 9. Showing the Laser-Doppler velocimetry machine. Reprinted with the permission from Perimed AB.

LDV is a method of non-invasive, continuous measurement of microcirculation in tissue based on the Doppler effect. Most people have experienced the Doppler effect, by hearing an emergency vehicle approaching with the sirens blaring and then the change in the sound when the vehicle has passed. The technique has been known since the mid-19th century and is based on the shift in frequency between an object moving towards a detector or away from it [79]. The LDV machine uses this technique based on the Doppler effect of low-power laser light scattered by static and moving tissue particles, see Figure 9 for the LDV machine. Light bouncing on cells in motion undergoes a change in wavelength (Doppler shift) whilst light bouncing on static objects remains unchanged [80]. The magnitude and frequency distributions of the changes are related directly to the number and velocity of red blood cells. The information is collected by a returning optic fibre, converted into an electronic signal, and analysed. This provides a continuous record of the

microvascular blood flow and tissue perfusion. Therefore changes in the perfusion rate can be observed easily and functional testing of endothelium-dependent microvascular reactivity is possible. Studies have included using LDV for evaluation of cutaneous wound blood flow, microvascular blood flow in the intestinal wall and blood perfusion in a full-thickness eyelid flap amongst other uses [81-89]. A computer analyses and transforms the electronic signal from the LDV machine into arbitrary perfusion units (PU) which can be further compared and analysed. Figure 10A shows the set up of the Doppler machine and 10B shows an illustration of the LDV machine computer output.

In Study II, patients underwent microvascular blood flow measurements using LDV at 0 and 12 months. LDV is sensitive to motion and, therefore, measures have to be put in place to minimise this effect. The measurements were performed in a quiet and temperature-controlled room where the patients acclimatised for 30 minutes before the test. The patients were not allowed to use tobacco, alcohol, caffeine, or take part in exercise 10 hours before the clinical visits. The measurements were made on the forearm with the patient in a comfortable sitting position. A vacuum cushion was used to support the forearm in a perfectly still position.

The measurements were made before partial occlusion, during partial occlusion and during PORH using a manual blood pressure cuff (Boso Varius, AB Henry Eriksson, Bandhagen, Sweden) inflated to 250 mmHg for 3 minutes. Vascular function can be evaluated by observing the response to reactive hyperaemia. Reactive hyperaemia is an increase in blood flow after and because of a temporary occlusion of the blood supply leading to an oxygen deficit in the tissue. In PORH a partial arterial occlusion is performed. After the partial occlusion, the pressure is released, resulting in a large inflow of blood into the previously occluded vessels. The perfusion is measured before the occlusion, during the occlusion and after the occlusion. Parameters related to both occlusion and baseline measurements before occlusion provide information about vascular health. The position of the probe was exactly 10 cm below the elbow joint on the ventral side of the forearm exactly in the middle and chosen so hair, freckles, and broken skin were avoided. The study setup is shown in Figure 10A.

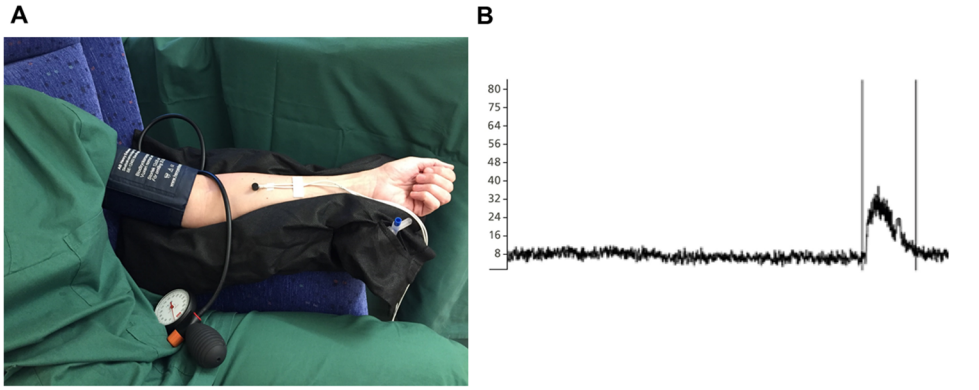

Figure 10. **A**) Clinical set-up of the Laser Doppler velocimetry (LDV) measurements. The patient was in a comfortable sitting position with the forearm on a vacuum cushion. **B**) Example of LDV output in PU units. Post occlusive reactive hyperaemia (PORH) reaction is marked between the vertical lines.

Laser Speckle Contrast Imaging (LSCI)

LSCI is another method of non-invasive, continuous measurement of microcirculation in tissue. LSCI is an instrument that combines a coherent infrared laser light with a photodetection charged coupled device camera. Tissue is illuminated by the laser creating an interference pattern and the backscattered light, with dark and bright areas, creates a speckled pattern. When particles move in the illuminated area, they cause fluctuations in the coherent light that generate motion blurring and changes in the standard deviation of the signal recorded by the camera. Speckle contrast is defined as the ratio ration between the standard deviation and the mean of the intensity in the signal.

In Study IV, LSCI measurements were performed on the skin of the forearm at 0 and 12 months. The LSCI used captures a two-dimensional perfusion map with up to 100 images per second, and at a spatial resolution down to 100 µm/pixel. Perfusion is calculated automatically by the system analysing the variations in the speckle pattern and presented in arbitrary PU. See Figure 11 for the LSCI machine.

Figure 11. A Laser Speckle Contrast Imaging (LSCI) machine. Reprinted with the permission from Perimed AB.

The measurements were made on the forearm before PORH, during partial occlusion and during PORH using a manual blood pressure cuff (Boso Varius, AB Henry Eriksson, Bandhagen, Sweden) inflated at 250 mmHg for 3 minutes. These measurements were complemented with measurements of CVC using acetylcholine in combination with an iontophoresis system (PeriIont Micro Pharmacology System [Perimed AB, Stockholm, Sweden]) to study endothelial function. The system delivers miniscule volumes of drugs non-invasively, in the present study transdermally. Iontophoresis is a technique to transport charged molecules or drugs across a tissue barrier. Combined with the LSCI, iontophoresis is useful for studying endothelial dysfunction. Acetylcholine was the substance used for provoking vasodilatation in the skin. The method is painless and considered safe. Two electrodes are attached to the skin a distance apart from each other and a low intensity electric current is applied between them. One of the electrodes, the active one contains the drug which then follows the current transdermally and the other electrode closes the circuit [90].

The Algorithm

There are many ways of handling large data materials and making them comprehendible. One of the most common ones is called cross industry standard process for data mining (CRISP-DM) [91,92]. It is a method for translating large amounts of information into trends or new patterns and it describes the common approaches used for handling data materials. CRISP-DM encompasses six phases in the data handling process: business understanding, data understanding, data preparation, modelling, evaluation and deployment. It is not a strict system meaning that every step needs to be taken one after the other, but the idea is to jump backwards and forwards between the different phases. Data mining uses the combination of visual effects such as diagrams and equations to extract trends and patterns from the raw data. It is important when using data mining to combine the two approaches of generating data-driven hypotheses with subject-specific information and hypotheses and then using the technique to either reject or confirm the theory. When using only the first approach of data-driven hypotheses it is easy to make mistakes and see trends that do not exist.

In the evaluation phase of CRISP-DM, a validation method is often used. It is a technique for evaluating how well the statistical analysis developed previously will generalise on a new data set. It is mostly used for statistical analyses where the prediction is the goal. Validation methods are used due to the fact that new data for testing a developed statistical analysis are often not available and, therefore, the statistician creates their own testing sub-set. Validation methods are divided into exhaustive and non-exhaustive cross-validation methods. The exhaustive cross-validation methods learn and test all possible ways of dividing the original sample into training and testing subsets. The non-exhaustive method does not do this; instead the method divides the sample in the beginning into sub-sets that are fixed, either as a train- or a test sub-set [93].

Exhaustive cross validation

Leave-one-out-cross-validation (LOOCV) is one of the exhaustive methods. LOOCV is performed by training and testing on the n-1 sample, n representing the total number of samples in the cohort, and a prediction is made for the excluded sample. This process is repeated until all samples have been tested and a mean key performance indicator score from all tests can be obtained. LOOCV can be quite computational burdensome and, therefore, occasionally a k-fold validation approach may be more suitable [94].

Non-exhaustive cross validation

The k-fold cross validation approach includes randomly dividing the data into k-parts, approximately even groups. The k-part is the part that is left out, i.e. the validation test sample. The statistical analysis is tested on the k-1 part of the data

set, which is called the train set. The statistical analysis is then evaluated on the test data set by making a prediction for the data within. A k-fold cross validation approach is less computational burdensome since K<<N. A common approach is 10-fold cross validation. Then 1/10 of a data-set is randomly held out and used as the validation or test set and the remaining 9/10 of the data set is used as a train set. When k = *n* the k-fold cross validation approach is equivalent to LOOCV [95].

In Study I we used CRISP-DM to develop a model for predicting which group out of two, based on their measurements at baseline, the patients would belong to after 12 months of AGE treatment: i.e. one group showing an improved effect of AGE or one group showing less effect. The developed algorithm predicts CAC progression and SBP progression after 12 months of AGE treatment. In Study I we developed the method for generating the prediction model and in Study V we validated the method.

Study I

Table 4. Study I was the first one planned and in the beginning we used these criteria for recruitment of all patients.

Inclusion criteria for study I	Exclusion criteria for study I
Asymptomatic patients	History of myocardial infarction
40-75 years old	Symptoms of ischaemic heart disease
Framingham risk score ≥10	Resting hypotension (systolic <90 mmHg) or hypertension (resting blood pressure >170/110)
CAC > 1	Serum creatinine >140 µmol/L
Stable concomitant medications for at least 4 months prior to randomisation	History of malignancy within the last 5 years or evidence of active cancer
Patients with diabetes (glycated haemoglobin (HbA1c) < 8.0, and stable HbA1c level (variation range within 0.5%) for 6 months.	Conditions interfering with assessment of coronary calcification (metal clips, bypass patients, intracoronary stents) and drug absorption
	Unstable medical disorder
	Heart failure New York Heart Association (NYHA) class III or IV
	Hypersensitivity to AGE
	Diabetic subjects with HbA1c >8.0
	Triglycerides >4.0 mmol/L baseline visit
	Bleeding disorder or stroke or drug abuse
	Prior life-threatening arrhythmia
	Proximal CAC or CAC score >1000 units

Patients and outcomes

Study I was conducted as a double-blinded, placebo-controlled clinical trial. We started to recruit patients in December 2016 and finished in October 2017. A total of 104 patients were recruited according to the inclusion and exclusion criteria shown in Table 4. The patients were randomised to an intake of capsules of 2400 mg AGE daily (two capsules of 600 mg twice daily, Kyolic Reserve formula;

Wakunaga of America Co Ltd., $n = 52$) or two placebo capsules twice daily (starch capsules, $n = 52$) for 12 months. All patients receiving AGE supplement received the same dose. Study investigators, i.e. those assessing outcomes and patients, were blinded to treatment allocation. We aimed for a balanced recruitment of 50% men and women. Patients underwent a cardiac CT scan before entering the study and after 12 months of intervention. Every 4 months the patients met with a study nurse to ensure compliance with the intake of capsules and have a clinical check-up. The primary outcome was changes in CAC score after 12 months of placebo or AGE supplement. Secondary outcome measurements were changes in blood pressure (diastolic and systolic), fasting blood glucose, blood lipids (total cholesterol, HDL, LDL, apolipoprotein A and B and triglycerides) and inflammatory biomarkers (CRP and IL-6). In the study we also developed a prediction model for predicting which patient will have the best effect of AGE depending on that individual's measurements at the beginning of the study. The algorithm was constructed using CRISP-DM; LOOCV was the method used for validating the prediction results.

Statistics

At baseline there were no significant differences in cardiovascular risk factors calculated using the Framingham risk score between the AGE and the placebo group. The majority of the patients in the study were taking medications for hypertension and hypercholesterolaemia when they entered the study. There was, however, a significant difference between the AGE group and the placebo group at baseline measurements at 0 months in BMI where the placebo group had a higher BMI than the AGE group. The AGE group, on the other hand, had a significantly higher CAC score when entering the study. The cholesterol and LDL levels were significantly lower in the placebo group than in the AGE group at baseline. There were more men than women (65% in the AGE group and 66% in the placebo group) in the study despite the quest for gender-balanced groups. These differences were unknown until the study was unblinded at finish.

The placebo and AGE groups differed statistically significantly between each other in many aspects when the study was unblinded after 12 months. Therefore we first needed to perform an adjusted multivariable logistic regression analysis before carrying out the study analyses. This was used for equalising the baseline differences between the groups. Further calculations using multivariable logistic regression analyses adjusted for age, gender, weight, hypertension, hypercholesterolaemia, antiplatelet therapy, heart disease, kidney disease, diabetes, smoking, cancer and alcohol use with median split were used to analyse differences between groups in blood pressure (systolic and diastolic), IL-6 and p-glucose. The data were presented as the probability of belonging to the group with the best result, i.e. lower CAC score, lower blood pressure, lower blood glucose and lower IL-6.

Student's *t*-test and Chi-squared test were used to assess differences between groups. Comparisons of all parameters between the active therapy and placebo were

made by Student's *t*-test. Repeated measures of analysis of variance (ANOVA) were performed to test for differences between groups over time. The associations between changes in the two treatment groups (active therapy and placebo) over 12 months for risk factors, including lipid profile, CAC and CRP were analysed by logistic regression analyses. These analyses were adjusted for demographics, age, gender, and traditional cardiac risk factors. Odds ratios were calculated for median annual change of CAC progression, change in CRP, and other risk factors. All statistical analyses were performed using SPSS V 19.0 (SPSS Institute, Chicago, IL). The level of significance was set to $p < 0.05$. Odds ratio (OR) was presented for belonging to active group or placebo for lower CAC progression, lower blood glucose and lower inflammatory biomarkers.

Study II

Patients and outcomes

Study II was conducted as a double-blinded, placebo-controlled clinical trial. We started to recruit patients in December 2016 and finished in October 2017. A total of 135 patients were recruited according to the inclusion and exclusion criteria, see Table 5. The inclusion and exclusion criteria were the same as those for Study I but with the difference that we included patients without consideration of their CAC score. We only used the Framingham risk score for qualifying patients to this study. The patients were randomised to an intake of capsules of 2400 mg AGE daily (two capsules of 600 mg twice daily, Kyolic Reserve formula; Wakunaga of America Co Ltd., $n = 67$) or two placebo capsules twice daily (starch capsules, $n = 68$) for 12 months. All patients receiving AGE supplement received the same dose. Study investigators, i.e. those assessing outcomes and patients, were blinded to treatment allocation. Every 4 months the patients met with a study nurse to ensure compliance with the intake of capsules and to have a clinical check-up. Patients underwent microvascular measurements at the beginning of the study, 0 months, and after 12 months of intervention. A total of 13 patients were lost to follow up during the study: seven in the AGE group and six in the placebo group. In the AGE group six patients were excluded due to changes in their medications and one due to a withdrawn consent form. In the placebo group two patients withdrew their consent forms, two changed their medication and two were excluded due to one having an acute cerebral event and the second had an acute cardiac event. The primary outcome of this study was to examine whether 12 months of AGE treatment could improve the cutaneous microvascular perfusion measured with LDV in patients with an increased risk of cardiovascular events. The AGE and placebo groups differed only in the aspect of hypercholesterolaemia: this was revealed to be an incidence of 66% in the AGE group and 45% in the placebo group at the baseline, after unblinding of the study.

Table 5. The same inclusion and exclusion criteria were used as for Study I except there was no requirement of CAC >1 in the inclusion criterias for Study II.

Inclusion criteria for Study II	Exclusion criteria for Study II
Asymptomatic patients	History of myocardial infarction
40-75 years old	Symptoms of ischaemic heart disease
Framingham risk score ≥10	Resting hypotension (systolic <90 mmHg) or hypertension (resting blood pressure >170/110)
Stable concomitant medications for at least 4 months prior to randomisation	Serum creatinine >140 µmol/L
Patients with diabetes (HbA1c < 8.0, and stable HbA1c level (variation range within 0.5%) for 6 months	History of malignancy within the last 5 years or evidence of active cancer
	Conditions interfering with assessment of coronary calcification (metal clips, bypass patients, intracoronary stents) and drug absorption
	Unstable medical disorder
	Heart failure NYHA class III or IV
	Hypersensitivity to AGE
	Diabetic subjects with HbA1c >8.0
	Triglycerides >4.0 mmol/L baseline visit
	Bleeding disorder or stroke or drug abuse
	Prior life-threatening arrhythmia
	Proximal CAC or CAC score >1000 units

Statistics

Data were analysed for differences between the two groups, i.e. AGE and placebo, between time points 0 and 12 months. This was performed using ANOVA with a Greenhouse–Geisser correction. This analysis was chosen to minimise for within-subject variability. *Post hoc* tests using the Bonferroni correction were used to make comparisons between time points within the two groups, i.e. AGE supplement and placebo.

All continuous data are presented as a mean value ± standard deviation (SD). The biological zero in LDV measurements was taken into consideration [96]. All statistical analyses were performed using SPSS V 19.0 (SPSS Institute, Chicago, Illinois). The level of significance was set to $p < 0.05$.

Study III

Patients and outcomes

Study III was conducted as a double-blinded, placebo-controlled clinical trial. We started to recruit patients in December 2016 and finished in October 2017. A total of 31 patients were recruited according to the inclusion and exclusion criteria, see Table 6.

Table 6. Study III comprised only women with a CAC ≤5.

Inclusion criteria for Study III	Exclusion criteria for Study III
Asymptomatic patients	History of myocardial infarction
40-75 years old	Symptoms of ischaemic heart disease
Framingham risk score ≥10	Resting hypotension (systolic <90 mmHg) or hypertension (resting blood pressure >170/110)
CAC ≤ 5	Serum creatinine >140 µmol/L
Stable concomitant medications for at least 4 months prior to randomisation	History of malignancy within the last 5 years or evidence of active cancer
Patients with diabetes (HbA1c < 8.0, and stable HbA1c level (variation range within 0.5%) for 6 months	Conditions interfering with assessment of coronary calcification (metal clips, bypass patients, intracoronary stents) and drug absorption
	Unstable medical disorder
	Heart failure NYHA class III or IV
	Hypersensitivity to AGE
	Diabetic subjects with HbA1c >8.0
	Triglycerides >4.0 mmol/L baseline visit
	Bleeding disorder or stroke or drug abuse
	Prior life-threatening arrhythmia
	Proximal CAC or CAC score >1000 units

In this study we included only women with an elevated risk for cardiovascular events according to the Framingham risk score (>10): all women had a CT scan prior to randomisation and all included in the study had a CAC score < 5. CAC score < 5 is considered to be a very low risk for coronary events. The patients were randomised to an intake of capsules of 2400 mg AGE daily (two capsules of 600 mg twice daily, Kyolic Reserve formula; Wakunaga of America Co Ltd., $n = 15$) or two placebo capsules twice daily (starch capsules, $n = 16$) for 12 months. All patients receiving AGE supplement received the same dose. Study investigators, i.e. those assessing outcomes and patients, were blinded to treatment allocation. Every 4 months the patients met with a study nurse to ensure compliance with the intake of capsules and to have a clinical check-up. Patients underwent clinical evaluations including medical history, cardiovascular risk factors, prescribed medications, smoking, and alcohol intake at 0 and 12 months. Blood pressure, BMI and electrocardiogram measurements were also evaluated at 0 and 12 months. Two patients, one in each group, were lost to follow up during the study. One withdrew consent and the second changed her medications. The AGE and placebo group differed only in the aspect of hypercholesterolaemia: more patients in the placebo group had high cholesterol levels. The primary outcome was changes in inflammatory biomarkers (CRP and Il-6) after 12 months of placebo or AGE intake. Secondary outcome measurements were changes in blood pressure (diastolic and systolic), fasting blood glucose, and blood lipids (total cholesterol, HDL, LDL, apolipoprotein A and B, and triglycerides).

Statistics

Data were analysed based on the two groups, i.e. AGE or placebo. Student's *t*-tests and Chi-squared tests were used to assess differences between groups. Comparisons of all parameters between the active therapy and placebo were made with the Student's *t*-test.

All continuous data are presented as a mean value ± SD or ± standard error of the mean (SEM), and all categorical data are reported as percentages or absolute numbers. All statistical analysis was performed using GraphPad Prism (Version 8, GraphPad Software, San Diego, USA). The level of significance was set to $p < 0.05$.

Study IV

Table 7. The same inclusion and exclusion criterias were used for Study IV as for Study I.

Inclusion criteria for Study IV	Exclusion criteria for Study IV
Asymptomatic patients	History of myocardial infarction
40-75 years old	Symptoms of ischaemic heart disease
Framingham risk score ≥10	Resting hypotension (systolic <90 mmHg) or hypertension (resting blood pressure >170/110 mmHg)
CAC > 1	Serum creatinine >140 µmol/L
Stable concomitant medications for at least 4 months prior to randomisation	History of malignancy within the last 5 years or evidence of active cancer
Patients with diabetes (HbA1c < 8.0, and stable HbA1c level (variation range within 0.5%) for 6 months	Conditions interfering with assessment of coronary calcification (metal clips, bypass patients, intracoronary stents) and drug absorption
	Unstable medical disorder
	Heart failure NYHA class III or IV
	Hypersensitivity to AGE
	Diabetic subjects with HbA1c >8.0
	Triglycerides >4.0 mmol/L baseline visit
	Bleeding disorder or stroke or drug abuse
	Prior life-threatening arrhythmia
	Proximal CAC or CAC score >1000 units

Patients and outcomes

Study IV was conducted as a double-blinded, placebo-controlled clinical trial. We started to recruit patients in December 2016 and finished in October 2017. A total of 175 patients were recruited and 104 were admitted to the study according to the inclusion and exclusion criteria, see Table 7.

The patients were randomised to an intake of capsules of 2400 mg AGE daily (two capsules of 600 mg twice daily, Kyolic Reserve formula; Wakunaga of America Co Ltd., $n = 52$) or two placebo capsules twice daily (starch capsules, $n = 52$) for 12 months. All patients receiving AGE supplement received the same dose. Study investigators, i.e. those assessing outcomes and patients, were blinded to treatment

allocation. We aimed for a balanced recruitment of 50% men and 50% women. Patients underwent a cardiac CT scan before entering the study and after 12 months of intervention. Every 4 months the patients met with a study nurse to ensure compliance with the intake of capsules and to have a clinical check-up. Patients underwent microvascular measurements at the beginning of the study, 0 months, and after 12 months of intervention. Eleven patients were lost to follow up: six in the AGE group and five in the placebo group. In the AGE group five patients were excluded due to changes in their medications and one due to withdrawn consent. In the placebo group one patient withdrew consent, two changed their medication and two were excluded due to one having an acute cerebral event and the second had an acute cardiac event. The primary outcome was changes in peripheral tissue perfusion and microcirculation after 12 months of placebo or AGE intake measured using LSCI.

The peripheral tissue perfusion measurements were made on the forearm before partial occlusion, during partial occlusion and during PORH using a manual blood pressure cuff inflated at 250 mmHg for 3 minutes. The LSCI image was framed to the forearm and, when analysed, a region of interest was placed 10 cm below the elbow joint on the ventral side of the forearm. To additionally study the endothelial function, measurements of CVC using LSCI were made. Acetylcholine was the substance used for provoking vasodilatation in the skin.

Statistics

Data were analysed for differences between the two groups, i.e. AGE and placebo, between time points 0 and 12 months. All continuous data were presented as a mean value ± SEM or mean value with 95% confidence interval.

A repeated measures mixed-effect model ANOVA with a Greenhouse–Geisser correction was performed to test for differences between groups between time points 0 and 12 months. *Post hoc* tests using Šídák's multiple comparisons test were used to make correction for multiple measurements. Statistical analyses were performed using GraphPad Prism 9.0 (GraphPad Software Inc., San Diego, CA). Significance was defined as: $p < 0.05$, and $p > 0.05$ (not significant).

Study V

Patients and outcomes

The design of the study is a validation study based on the algorithm developed in Study I. The patients' data in the study were collected from Studies I-IV and also new data were acquired from the USA. All patients in all five studies had an increased risk of cardiovascular disease, defined by the Framingham risk score. All patients underwent a cardiac CT scan at time points 0 and 12 months for definition

of CAC score. Blood pressure was measured and blood samples were collected at the beginning and at the end of the study using standard techniques. All patients received AGE (Kyolic; Wakunaga of America Co Ltd) daily for the duration of the 12 months. We applied the inclusion and exclusion criteria from the Swedish Study I to the USA cohort, hence we excluded patients who received placebo, with CAC score >1000, CAC score <1, CAC score missing and BMI >40. The primary objective was to validate the method for developing a predictive model with the aim of identifying patients who would have a more favourable effect of AGE on CAC and SBP after 12 months of AGE intake.

Statistics

Study V was a validation study of the method developed in Study I. Therefore the statistical method of developing the AGE algorithm used was the same as the one in Study I. The method is based on CRISP-DM. In the first step the target variables are defined. The target variables, i.e. progression of CAC and SBP, were produced using the measured progression of CAC and SBP after 12 months' treatment with AGE. The formula used was:

$$\% - Progression_{12\ months} = \frac{B - F}{B} x100$$

Equation 1. Showing the formula used for creating the target varables CAC progression and SBP progresssion.

B = baseline measurements at 0 months and F = follow up measurements after 12 months of AGE intake. The same formula was used to produce the two target variables.

The patients in the USA cohort were divided by the median into two groups depending on the target variable either CAC or SBP: one group with better effect of AGE after 12 months (i.e. smaller progression of CAC) and one with more progression of CAC (i.e. worse effect of AGE). The same approach was taken with the patients depending on the SBP measurements. The next step included creating predicting variables for the model to use. Baseline measurements such as blood samples, CAC score, blood pressure, BMI and polynomial features[1] of the second-degree and interaction features[2] were used. To reduce the number of features to a reasonable amount, i.e. reduce overfitting and optimising the model, a feature selection function step was carried out. The selected function was recursive feature elimination (RFE). RFE ranks features by importance, discarding the least important features, and re-fitting the model. This step was repeated until a specified number

[1] Polynomials of the second degree are squared variables.

[2] Interaction features are variables that are multiplied with other variables.

of features, 12, remained. Following this was the validation step and as for Study I, LOOCV was chosen for this.

The models were created in Python 3.9.0 (Van Rossum, G. & Drake, F.L., 2009. Python 3 Reference Manual, Scotts Valley, CA: CreateSpace) utilising the following listed libraries: scikit-learn (version 0.23.2), Pandas (1.1.3) and NumPy (1.19.2). All continuous data were presented as a mean value ± SD. Student's *t*-test was used to assess differences between groups. The level of significance was set to $p < 0.05$.

Ethical consideration

All studies were performed according to the principle of the Helsinki Declaration of human rights. All participants signed a written informed consent form before entering the study.

Studies I-IV were approved by the Regional Ethical Review Board in Lund, Sweden, DNR 2016/745. The study protocol was registered at: (https://clinicaltrials.gov/ct2/show/NCT03860350?cond=NCT03860350&draw=2&rank=1, accessed 6 Oct. 21 2021) with ClinicalTrials.gov Identifier: NCT03860350. Studies I-IV were monitored externally by Preventia AB, Sweden, https://www.preventia.se/startsida-2/, accessed 6 Oct. 21 and were conducted according to the CONSORT (Consolidate Standards of Reporting Trials) guidelines and statement [97].

Study V was based on aggregated data from previously published studies in the USA. No individual level data was accessed at any step in the analysis, and no indirect identification of study subjects was possible, hence ethical vetting was not applicable for this study.

Results

Study I

A total of 175 patients with a Framingham risk score ≥ 10 were assessed for the study and underwent a cardiac CT scan. Seventy-one patients were excluded after the initial CT scan: of these 59 did not have a positive CAC score and the remainder had a heavy CAC burden and were sent for further assessment at the cardiology department. A total of 104 patients were included and randomised in the study. During the study period 11 patients were lost to follow-up and were excluded, consequently 93 patients (47 in the AGE group and 46 in the placebo group) were analysed, see Consolidate Standards of Reporting Trials (CONSORT) outlined in Figure 12. No patient in the study had any adverse reaction to the active therapy that required removal from the study.

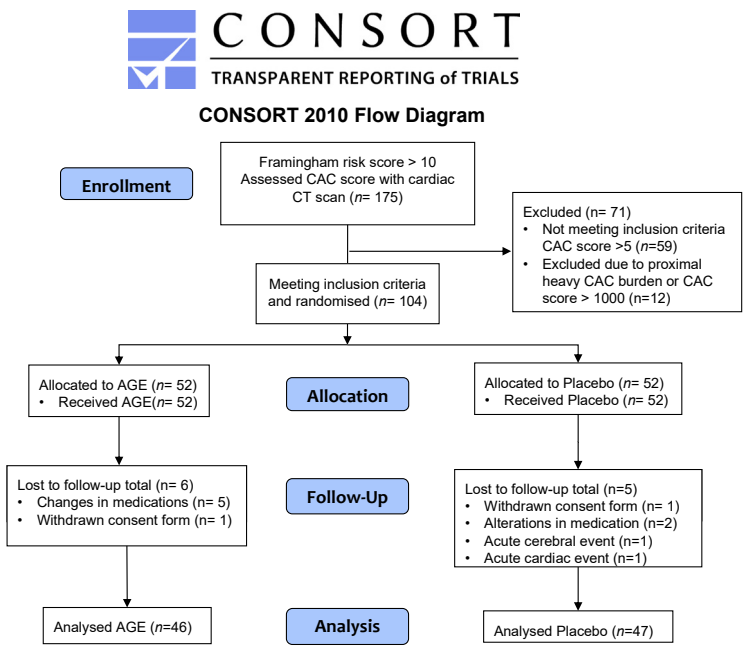

Figure 12 CONSORT statement (Consolidated Standards of Reporting Trials)
Flowchart showing demographics and baseline clinical information of the study cohort.

The effect of AGE on CAC score

The patients underwent a cardiac CT scan at 0 and 12 months and a CAC score was calculated. CAC is considered to be a progressive disease and a progression in CAC score over time is expected. All patients in the study showed an increase in CAC score over the 12-month period. However, the patients in the AGE supplement group had a decreased progression compared to the placebo group. The placebo group had a significantly increased annular CAC progression of 28% compared to the AGE supplement group with 20%. An adjusted multivariable logistic regression analysis was used for equalising the baseline differences between the placebo and the AGE groups. Further calculations using multivariable logistic regression analyses were adjusted for age, gender, weight, hypertension, hypercholesterolaemia, antiplatelet therapy, heart disease, kidney disease, diabetes, smoking, cancer and alcohol use. Median split was used to analyse the data. The adjusted multivariable logistic regression, analysing the CAC score showed that the probability of belonging to the group with the lowest CAC progression was 2.95 (adjusted OR, 95% CI 1.05–8.27, $p = 0.040$) times higher in the AGE group. All results from the median split analysis are shown in Table 8.

Table 8. Results of the logistic regression analysis with median split.

	AGE vs. Placebo		
	Adjusted OR	(95% CI)	*p*-value
CAC	2.95	(1.05-8.27)	0.040
Glucose (mmol/L)	3.1	(1.09-8.85)	0.034
IL-6 (ng/L)	2.56	(1-6.53)	0.049
Cholesterol (mmol/L)	0.57	(0.23-1.43)	0.228
LDL (mmol/L)	0.62	(0.24-1.58)	0.317
Triglyceride (mmol/L)	1.59	(0.6-4.16)	0.349
HDL (mmol/L)	1.43	(0.55-3.76)	0.463
Homocysteine (µmol/L)	1.18	(0.47-2.96)	0.724
ApoB/ApoA	0.9	(0.34-2.39)	0.840
CRP (mg/L)	1.45	(0.56-3.77)	0.447

Coronary artery calcification (CAC), C-reactive protein (CRP). High-density lipoprotein (HDL). Low-density lipoprotein (LDL). Interleukin-6 (IL-6). Apolipoprotein A and B (ApoB/ApoA).

The effect of AGE on glucose

The probability of belonging to the group with the lowest glucose level was 3.10 (adjusted OR, 95% CI 1.09–8.85, $p = 0.034$) times higher in the AGE group compared to the placebo group after 12 months of AGE intake.

The effect of AGE on Interleukin-6 levels (IL-6)

The probability of belonging to the group with the lowest IL-6 was 2.56 (adjusted OR, 95% CI: 1.002–6.53, $p = 0.049$) times higher in the AGE group than in the placebo group after 12 months of AGE intake. There were no significant differences in the lipid profile, BMI or CRP between the AGE and the placebo groups.

Table 9. Systolic blood pressure of the AGE and placebo groups at 0 and 12 months.

	Systolic blood pressure				
	0 months		12 months		
	Mean	(SD)	Mean	(SD)	*p*
AGE (*n*=46)	148	(19)	140	(15)	0.027
Placebo (*n*=47)	142	(29)	142	(14)	0.996

The effect of AGE on blood pressure

Patients' blood pressure was recorded at four time points during the study period 0, 4, 8 and 12 months. A one-way repeated measure ANOVA analysis was performed to examine the blood pressure. The patients in the AGE group showed significantly ($p = 0.027$) lower SBP at the 12 months' follow-up time point: 140 (SD: 15) mmHg compared to baseline 148 (SD: 19 mmHg). No difference in SBP was seen between 0 and 12 months in the placebo group (baseline 142 [SD 29] mmHg and 142 [SD: 14] mmHg at 12 months) ($p > 0.996$), see Table 9. The DBP in the AGE group was 88 (SD: 9) mmHg at 0 months and 85 (SD: 8) mmHg at 12 months ($p \geq 0.06$). The DBP in the placebo group was 87 (SD: 9) mmHg at 0 months and 84 (SD: 10) mmHg at 12 months ($p = 0.049$).

CAC progression and SBP – predicted impact of AGE: CRISP–DM

A logistic regression analysis identified six biomarkers as useful for predicting relative change in CAC progression from baseline to the 12-months' time point. Using these biomarkers, a predictive model was developed to determine the probability of belonging to the group with the lowest annual change in CAC score. The model's accuracy score was 80%, with a precision score of 79% and recall score of 83% using LOOCV.

A logistic regression analysis identified three biomarkers as useful for predicting relative change in SBP progression from baseline to the 12-months' follow up. Using these biomarkers, a predictive model was developed to determine the probability of belonging to the group with the best effect of AGE on SBP, meaning a decrease in the SBP after 12 months of AGE treatment. At a selected probability cut-off value of 0.5, the model's accuracy score was 74%, with precision score of 74% and recall score of 74% using LOOCV.

Study II

In total, 135 patients were enrolled and randomised in the study: 13 participants were excluded during the study and consequently 122 patients, 60 in the AGE group and 62 in the placebo group, were analysed, see CONSORT flow diagram outlined in Figure 13.

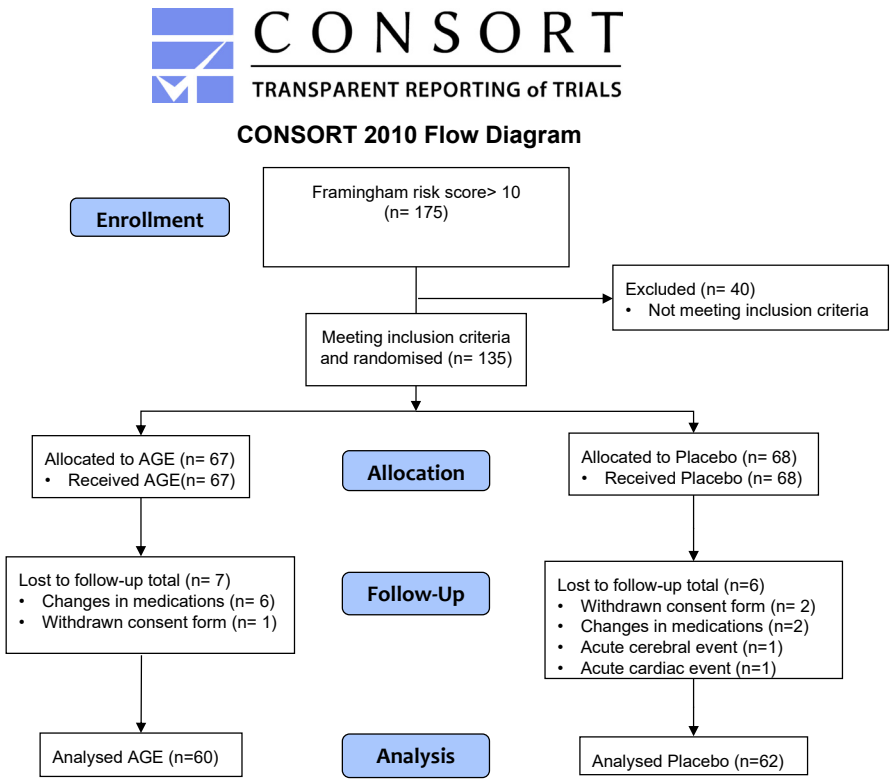

Figure 13 CONSORT statement (Consolidated Standards of Reporting Trials)
Flowchart showing demographics and baseline clinical information of the study cohort.

No patient in the study had any adverse reaction of the active therapy that indicated removal from the study. At baseline, there were no significant differences in cardiovascular risk factors calculated using the Framingham risk score. The measurements were made on the forearm before partial occlusion, during partial occlusion and during PORH using a manual blood pressure cuff inflated to 250 mm Hg for 3 minutes. The position of the probe was exactly 10 cm below the elbow joint on the ventral side of the forearm in the middle and chosen so hair, freckles, and broken skin were avoided.

The effect of AGE on microcirculation

The microvascular blood flow was 9.7 ± (4.9) PU in the AGE group and 9.3 ± (5.0) PU in the placebo group at 0 months before partial occlusion. The microvascular blood flow was 3.5 ± (0.89) PU in the AGE group and 3.8 ± (3.5) PU in the placebo group during the partial occlusion. The microvascular blood flow was 49.9 ± (29.9) PU in the AGE group and 43.9 ± (22.0) PU in the placebo group at 0 months during PORH. The same measurements were made after 12 months of either placebo or AGE treatment. The microvascular blood flow was 16.0 ± (7.4) PU in the AGE group and 13.9 ± (5.8) PU in the placebo group before partial occlusion. The microvascular blood flow was 7.9 ± (3.6) PU in the AGE group and 6.5 ± (3.3) PU in the placebo group during the partial occlusion. The microvascular blood flow was 47.3 ± (22.7) PU in the AGE group and 46.6 ± (23.7) PU in the placebo group at 12 months during PORH. The mean percentage changes between the two time points 0 and 12 months were 102, 64 (174, 15)% change for AGE and 78, 62 (107, 92)% change for the placebo group, respectively, (F [1, 120] = 5. 95, $p < 0.016$) shown in Figure 14.

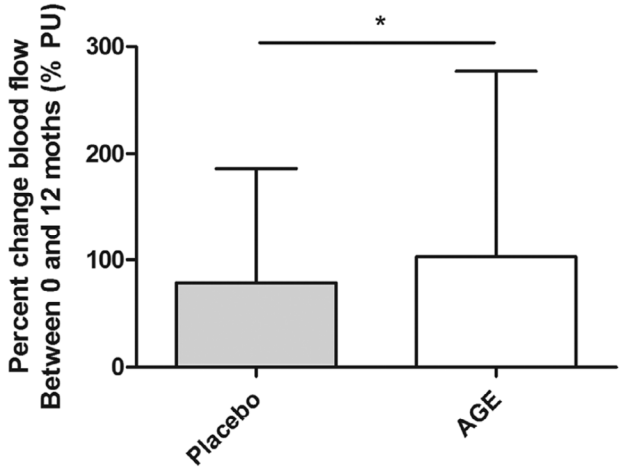

Figure 14 The mean percentage change in blood flow measured using Laser Doppler velocimetry (LDV). Measurements are carried out between 0 and 12 months between the two groups, i.e. AGE and placebo.

Study III

A total of 63 females underwent cardiac CT scan; of these, 31 females had a CAC score ≤ 5 indicating a low risk of coronary disease, meaning that they met the inclusion criteria and were enrolled and randomised in the study. During the study period two patients were lost to follow-up and were excluded, so consequently 29 patients (14 in the AGE group and 15 in the placebo group) were analysed, see

CONSORT outlined in Figure 15. No patient in the study had any adverse reaction to the active therapy that required removal from the study.

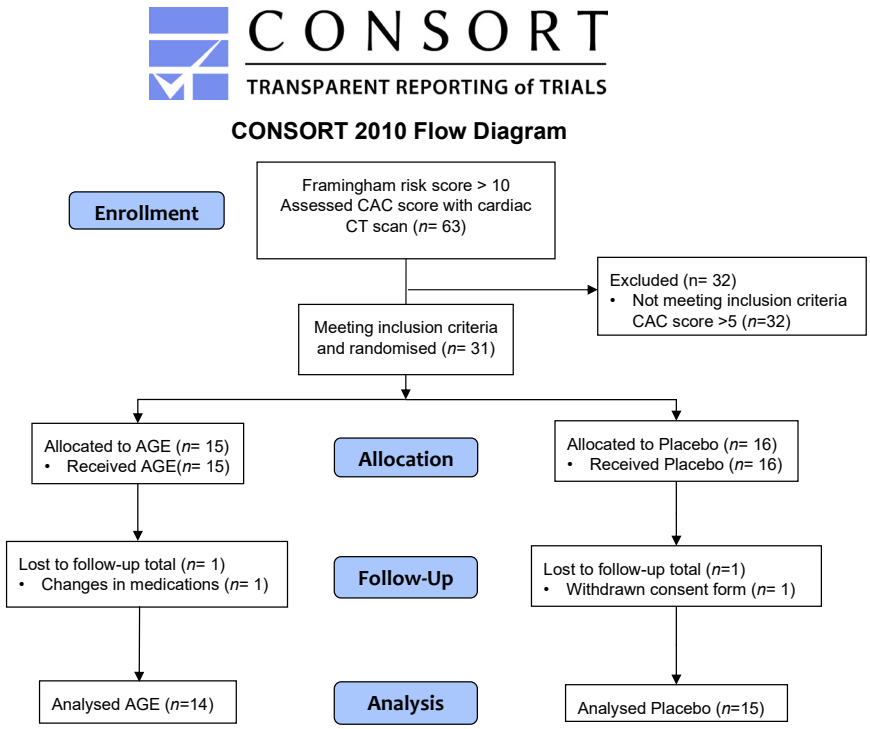

Figure 15 CONSORT statement (Consolidated Standards of Reporting Trials)
Flowchart showing demographics and baseline clinical information of the study cohort.

Table 10: Mean annual changes

	AGE		Placebo		
	n = 14	SD	*n* = 15	SD	*p*
BMI	0.2	(1.7)	0.1	(2.4)	0.88
Systolic BP (mmol/L)	0.9	(12.8)	3.0	(13.9)	0.40
Diastolic BP (mmol/L)	7.9	(34.5)	0.0	(10.1)	0.44
Triglycerides (mmol/L)	-0.3	(39.3)	52.2	(236.7)	0.94
Cholesterol (mmol/L)	-3.9	(12.3)	-3.6	(11.8)	0.94
HDL (mmol/L)	1.1	(13.1)	1.4	(12.8)	0.38
LDL (mmol/L)	-1.6	(15.1)	4.6	(20.7)	0.15
CRP (mg/L)	-27.8	(39.4)	11.3	(65.2)	0.69
ApoB/ApoA1	0.5	(11.6)	2.3	(13.4)	0.08
Homocysteine (µmol/L)	1.1	(15.7)	12.9	(18.3)	0.62
Glucose (mmol/L)	1.9	(12.7)	-0.4	(10.2)	0.36

SD: standard deviation; Apo B/ApoA1: Apolipoprotein B (mmol/L)/Apolipoprotein A1 (mmol/L); BMI: Body mass index (kg/m^2).

Aged Garlic Extract reduces IL-6 and a lipid-lowering trend is noted

At baseline, there were no significant differences in cardiovascular risk factors calculated using the Framingham risk score. There was no significant difference between the AGE group and the placebo group at baseline measurements at 0 months in BMI, blood pressure, blood lipids, or in inflammatory markers. There was a trend towards a lipid-lowering effect in the AGE group; however, this was not significant see Table 10. IL-6 was measured at 0 and 12 months of either AGE or placebo treatment. At baseline, at 0 months, the IL-6 concentration was 4.762 ± 0.701 ng/L in the AGE group and 4.173 ± 0.653 ng/L in the placebo group ($p < 0.05$). After 12 months of treatment, the IL-6 concentration was 3.754 ± 0.493 ng/L in the AGE group and 4.573 ± 0.461 ng/L in the placebo group ($p > 0.05$). (Figure 16). The differences between the two groups were calculated as mean annual percent change ($p < 0.05$).

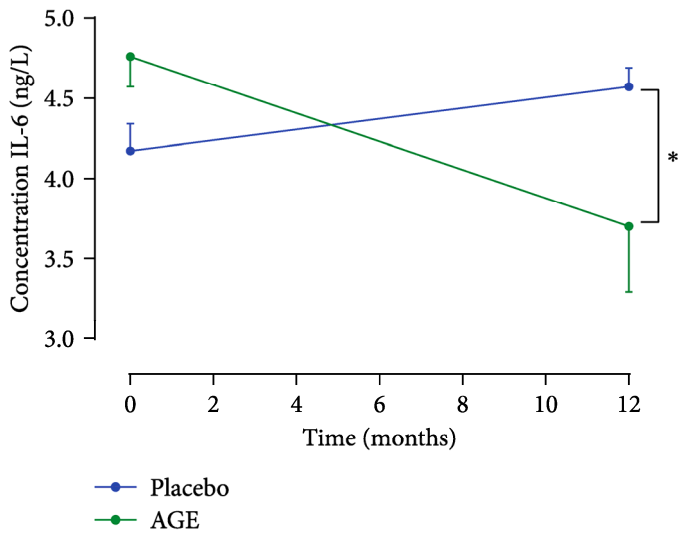

Figure 16. Interleukin-6 concentration at 0 and 12 months of either AGE or placebo treatment. Data are presented as mean ± SEM. The level of significance was set to p <0.05.

Study IV

A total of 175 patients with a Framingham risk score > 10 were assessed for the study and underwent a cardiac CT scan. Seventy-one patients were excluded after the initial CT scan: of these 59 did not have a positive CAC score and the remaining had a heavy CAC burden and were sent for further assessment at the cardiology

department. A total of 104 patients were included and randomised in the study. During the study period 11 patients were excluded, and consequently 93 patients (46 in the AGE group and 47 in the placebo group) were analysed, see CONSORT outlined in Figure 17. No patient in the study had any adverse reaction of the active therapy that indicated removal from the study. At baseline there were no significant differences in cardiovascular risk factors calculated using Framingham risk score. Peripheral tissue perfusion was measured using LSCI at 0 and 12 months. Measurements were taken before, during, and after partial occlusion using a blood pressure cuff creating a PORH response. CVC using acetylcholine iontophoresis was also measured.

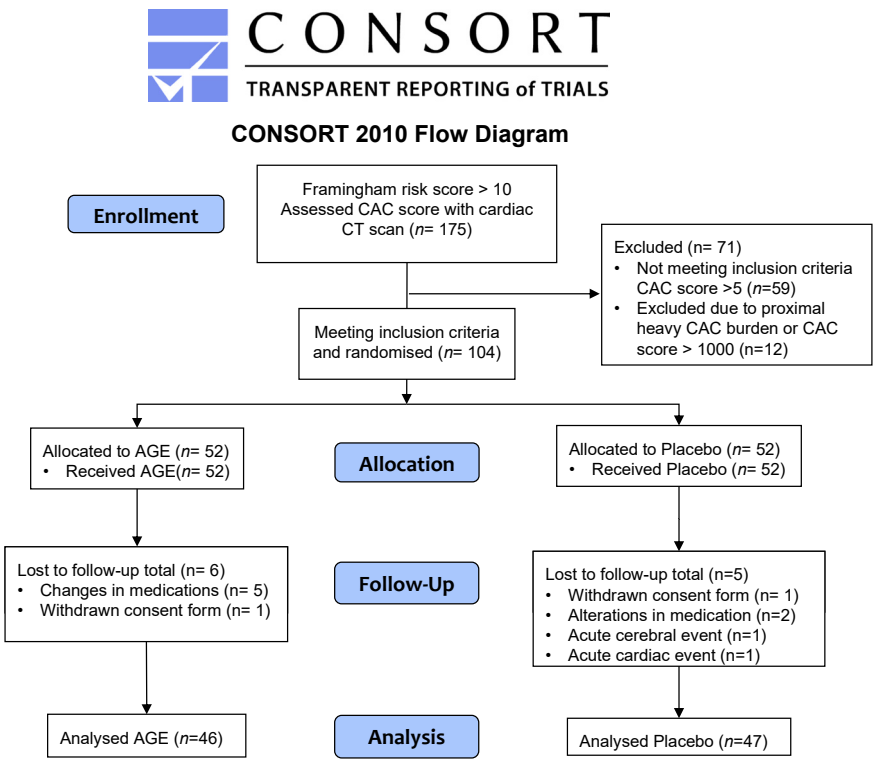

Figure 17 CONSORT (consolidated standards of reporting trials) statement flow chart.
Showing demographics and baseline clinical information of the study cohort

Increased PORH response in patients treated with AGE

The relative change in PORH in the AGE group was 21.3% (95% CI 6.6%-36.0%) compared with -0.3% (95% CI -8.5% to 7.9%) in the placebo group, giving a relative treatment response of 21.6% (95% CI 3.2%-40.0%, $p < 0.05$) after 12 months of AGE intake (Figure 18).

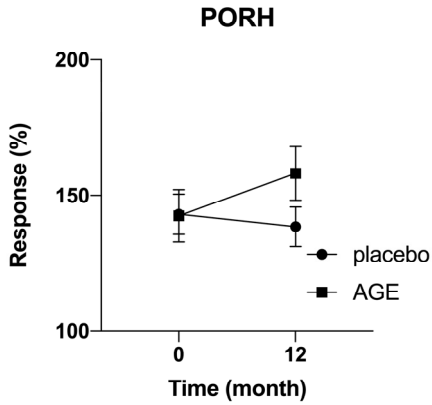

Figure 18 Increased post-occlusive reactive hyperaemia (PORH) response in patients treated with AGE.
Peripheral tissue perfusion was measured at 0 and 12 months. Measurements were taken before, during and after partial occlusion using a blood pressure cuff creating a post-occlusive reactive hyperaemia (PORH) response. A significant increase in relative change in PORH was seen in the AGE group compared with the placebo group.

Improved endothelial function in patients treated with AGE

Endothelial function and vascular response were measured at 0 and 12 months using iontophoresis with acetylcholine provocation. CVC measurements were calculated at rest and at peak vasodilatation in the first 5 minutes following iontophoresis. The relative change in acetylcholine response in the AGE-group was 22.3% (95% CI 7.6%–37.0%) compared with 0.9% (95% CI -7.5% to 9.3%) in the placebo group, giving a relative treatment response of 21.4% (95% CI 3.4% -39.4%, $p < 0.05$) (Figure 19).

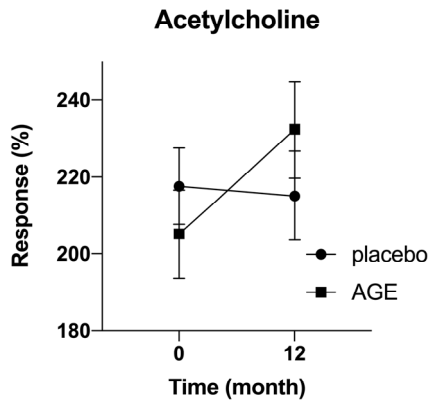

Figure 19 Improved endothelial function in patients treated with AGE.
Endothelial function and vascular response were measured at 0 and 12 months using iontophoresis with acetylcholine provocation. Cutaneous vascular conductance (CVC) measurement was calculated at rest and at peak vasodilatation in the first 5 minutes following acetylcholine iontophoresis. A significant increase in relative change in CVC was seen in the AGE group compared with the placebo group.

Study V

To validate the method, developed in Study I, of developing a prediction model, we received data from four studies, performed in the USA, called the USA cohort. A total of 78 patients who received AGE were included in the present study.

Table 11: Showing the performance metrics

	Accuracy	Precision	Recall
CAC algorithm	65%	64%	69%
SBP algorithm	65%	66%	64%

Performance metrics for each model based on LOOCV showing how well the algorithm performs on coronary artery calcification (CAC) and systolic blood pressure (SBP).

Table 12: Features chosen after elimination

Chosen features after recursive feature elimination (RFE) for the target variable coronary artery calcification (CAC) progression and the mean of the coefficients from the LOOCV. RFE searches for a subset of features by starting with all features in the training set and removing features until the desired number remains.

Coefficients	Predictor variables after RFE for CAC
3.08	LDL
1.69	Gender x Cholesterol
-2.05	Gender x SBP
-1.17	Age x TG
-0.95	BMI x LDL
-1.31	CAC^2
1.73	CAC x Homocysteine
-3.83	Cholesterol x LDL
2.75	Cholesterol x TG
1.93	IL-6 x SBP
-2.09	IL-6 x TG
0.12	LDL^2

Hyperparameters after CAC-model tuning: C-value = 25 penalty = L2-regularisation Solver = Newton-Cg LDL: low density lipoprotein; SBP: systolic blood pressure; TG: triglycerides; BMI: body mass index; CAC: coronary artery calcification; IL-6: interleukin-6.

After the development steps of the prediction model, we validated the model using LOOCV. For each of the 78 subjects in the dataset, we calculated the estimated probability of the i:th subject being in the 50th percentile with the best progression following 12 months of AGE intake. The following mean key performance indicators were acquired: CAC: accuracy 65%, precision 64%, recall 69%, and SBP: accuracy 65%, precision 66%, recall 64%, see Table 11. Twelve features were chosen inclusion in the model. For CAC the 12 features were: LDL, gender x cholesterol, gender x SBP, age x TG, BMI x LDL, CAC2, CAC x homocysteine, cholesterol x LDL, cholesterol x TG, Il-6 x SBP, IL-6 x SBP, IL-6 x TG and LDL2. See Table 12 for an overview and for each feature the associated coefficient. For SBP the 12 features were: gender x DBP, gender x HDL, age x IL-6, age x CRP, CAC x IL-6, CAC x CRP, DBP2, HDL x SBP, HDL x CRP, homocysteine x IL-6,

LDL x CRP and CRP2. See Table 13 for an overview and for each feature the associated coefficient.

Table 13: Features chosen after elimination

Chosen features after recursive feature elimination (RFE) for the target variable decrease of SBP and the mean of the coefficients from the LOOCV. RFE searches for a subset of features by starting with all features in the training set and removing features until the desired number remains. The selected features differ from the CAC progression model.

Coefficients	**Predictor Variables** From RFE for SBP
-1.98	Gender x DBP
2.08	Gender x HDL
-1.69	Age x IL-6
1.55	Age x CRP
1.41	CAC x IL-6
-1.77	CAC x CRP
1.29	DBP^2
-3.30	HDL x SBP
6.93	HDL x CRP
1.44	Homocysteine x IL-6
-2.41	LDL x CRP
-10.25	CRP^2

Hyperparameters after SBP-model tuning: C-value= 100 Penalty = L1-regularisation Solver = Liblinear DBP: diastolic blood pressure; HDL: high density lipoprotein; CRP: C reactive protein; CAC: coronary artery calcification; LDL: low density liprotein; IL-6: interleukin-6.

Discussion

The thesis is based on five studies: four conducted on a Swedish cohort and one conducted on a cohort from the USA. The first four studies were performed methodologically in the same way. They are double-blinded placebo-controlled studies and differ by the measurements made or the subjects studied. The fifth study with a cohort from the USA is based on the method developed in Study I which was further developed, refined and validated in Study V.

AGE and CAC score, blood pressure and the predictive model

Starting with Study I the main findings were a positive effect of 12 months of AGE intake on CAC score and SBP. These findings are in line with previously published studies demonstrating a positive effect of AGE on CAC score and blood pressure in a non-European population with low- to intermediate risk of CVD. [64,65,98-101]. Study I is the first one to be performed on a European population with intermediate- to high risk of CVD. Previous studies have mostly been conducted in the USA. In Study I we also developed a prediction model which could predict which patient will have a significantly reduced CAC progression after 12 months of AGE use.

The study found a significant difference at baseline between the AGE and placebo groups and a multivariable logistic regression adjusted for cardiovascular risk factors was necessary to equalise the baseline differences. The analysis showed that the probability of belonging to the group with the lowest CAC progression was 2.95 (adjusted OR, 95% CI 1.05–8.27, $p = 0.040$) times higher in the AGE group. The results from both the present study and prior studies on AGE and CAC score imply that AGE reduces the progression of CAC in both a European and a USA population.

In Study I there was a positive effect seen on the inflammatory biomarker, IL-6, which is in line with previously published articles [102]. The study found that the probability of belonging to the group with the lowest IL-6 was 2.56 (adjusted OR, 95% CI: 1.002–6.53, $p = 0.049$) times higher in the AGE group than in the placebo group. IL-6 is involved in the inflammatory processes of atherosclerosis [11,103,104]. CAC is the result of the atherosclerotic process and CAC score can predict future cardiovascular events [105]. A positive impact on IL-6 could partly explain the mechanisms behind the lower CAC progression in patients taking AGE

compared to the placebo group. It has been suggested that AGE has a positive impact on vascular endothelial and platelet function [54,63,106-108]. Reduced endothelial function presages a larger risk of CVD. Oxidative stress plays an important role in the pathogenesis and progression of vascular disease. It is believed that AGE has a positive effect on blood pressure by improving vascular endothelial function and reducing the progression of atherosclerotic plaque [108].

The use of data mining and CRISP-DM has increased enormously and it has been used to predict the outcome of various drugs and medical treatments [27, 34, 35]. Individuals respond differently to the same treatments. Environmental and genetic determinants of CVD can vary and these changes could have an impact on the pathogenesis of CVD [36, 37]. Consequently, it is important to tailor diverse types of diets, supplements and treatments depending on each patient. We therefore created an algorithm, the AGE algorithm, to predict the individual results of AGE on CAC progression and SBP. We could predict, with 80% precision, which patient will have a significantly reduced CAC progression after 12 months of AGE supplement. With the same type of prediction model, we could predict with a 74% precision which patients will have a significant blood-pressure lowering effect after 12 months of AGE supplement.

AGE and microcirculation measured by LDV

The main finding in Study II was that 12 months of AGE preserves the microcirculation measured by LDV. The studied patients were the same as the ones included in Study I and additionally women with a Framingham risk score >10 but with a CAC score ≤ 5 were also included. This means that all patients in Study II had an elevated risk for CVD measured with Framingham risk score. These findings are in line with previously published articles. Weiss et al. showed increased microvascular blood flow using acetylcholine-induced vasodilation on the forearm using LDV after AGE use. Ahmadi et al. showed increased microvascular blood flow using digital thermal monitoring after AGE use. Reid et al. showed improved arterial stiffness and increased vascular elasticity measured by PWV after AGE use [58,67,109].

LDV is a method of non-invasive, continuous measurement of microcirculation in tissue. Therefore changes in perfusion can be observed easily and functional testing of endothelium-dependent microvascular reactivity is possible [81,82,84,85]. Changes in microcirculation are associated with reduced endothelial function and low-grade inflammation [103,110,111]. The endothelium lines the interior wall of vessels and has a key function on blood pressure regulation and microvascular blood flow. NO is synthesised by eNOS and causes vasodilation by relaxation of smooth muscle cells of the vascular walls. NO has many functions and plays a vital role in normal endothelial function. Many conditions, including those regularly associated as risk factors for atherosclerosis, such as hypertension, hypercholesterolaemia, and

diabetes mellitus, are associated with reduced release of NO [112-114]. Therefore, endothelial dysfunction has been associated as an early sign of CVD. Experiments that cause vasodilation such as PORH are used to study the endothelial function and tissue perfusion. Reactive hyperaemia is a rise in blood flow as a result of an oxygen deficit in the tissue. A different response is seen in patients with a reduced endothelial function compared to healthy controls.

AGE and IL-6 in women with low risk of CVD

The main finding in Study III was that 12 months of AGE intake lowers levels of the inflammatory biomarker IL-6. This is in line with previously published studies showing a beneficial effect of AGE on inflammation with a lowering effect on IL-6 [102,115]. Study III is the first based on women with a low risk of CVD.

AGE has immunomodulatory effects. S-1-propenylcysteine (S1PC) and SAC are the two most abundantly found amino acids in AGE [116]. S1PC modulates antioxidant gene expression and immune response [117,118]. SAC prevents lipid and protein oxidation and has antioxidant properties [55-57]. IL-6 is involved in the inflammatory processes of atherosclerosis and an elevation of the IL-6 is seen in chronic inflammation [103,104]. IL-6 has been shown to be a predictor of type-2 diabetes mellitus and to decrease insulin sensitivity in hepatocytes. Increased levels of IL-6 are also seen during bacterial and viral infections and physical exercise [119,120]. IL-6 has also been suggested to impact on glucose homeostasis and metabolism both directly and indirectly by its action on skeletal muscle cells, adipocytes and neuroendocrine cells amongst others [121].

The AGE group showed a lowering effect on triglycerides and the placebo group showed a more than 50% increase in triglycerides when studying the mean annular change in lipids after 12 months of AGE intake. The differences were not significant. The same trend was seen when LDL was assessed, but again these changes were not significant. Previous studies have shown that AGE has a significant lipid-lowering effect [53,98,100].

AGE and microcirculation measured by LSCI

The main finding in Study IV was that 12 months of AGE intake increases the microcirculation measured by LSCI. A significant increase of 21.6% (95% CI 3.2%–40.0%, $p < 0.05$) was seen in the relative change of PORH in the AGE group compared with the placebo group. The same response was seen for CVC with an increase of 21.4% (95% CI 3.4%–39.4%, $p < 0.05$) in the AGE group compared with the placebo group.

Atherosclerosis is a progressive disease and involves inflammation and endothelial dysfunction. A secondary manifestation of endothelial dysfunction is reduced tissue

perfusion [110,122]. Chronic conditions may be associated with reduced tissue perfusion and, therefore, improved tissue perfusion is important. In Study IV we selected patients with confirmed atherosclerosis with secondary manifestations visible as CAC. Atherosclerosis is a systemic disease hence patients who have atherosclerosis in the vessels of the heart also have reduced peripheral tissue perfusion. Improving the peripheral tissue perfusion could improve organ function.

In the present study, we used LSCI for visualisation and measurement of tissue perfusion. Compared to LDV measurement, LSCI provides more stable measurements and they are easier to reproduce [123-125]. LSCI is frequently used in studies of endothelial function often combined with a provocation manoeuvre such as iontophoresis and CVC and/or PORH. Both these tests were used in the present study for comparison and to study vascular function. Previous studies have shown that PORH measurements are highly reproducible [126]. In patients with atherosclerosis, the PORH reaction is different to that seen in people without atherosclerosis. The different PORH response to ischaemia in patients with atherosclerosis has been shown to be associated with an increased risk of CVD. [127,128]. Study IV demonstrated that 12 months of AGE treatment improves the PORH reaction significantly, indicating that AGE improves peripheral tissue perfusion and hence lowers the risk of CVD.

Tests of the vascular effect of acetylcholine are often carried out to evaluate the endothelium-dependent microvascular response. Applying acetylcholine to the skin leads to vasodilatation of the tissues [129,130]. A significant increase in CVC in patients receiving AGE compared with patients receiving placebo was seen, again indicating that AGE does improve peripheral tissue perfusion.

The inside wall of blood vessels is covered by endothelium which has major functions in the homeostatic process with respect to blood pressure regulation, microvascular blood flow and peripheral tissue perfusion. NO is synthesised in endothelial cells and causes vasodilatation and is an important modulator of atherosclerosis and peripheral tissue perfusion. Damage to the endothelium by atherosclerosis harms the release of NO. AGE has been shown to have a positive effect on atherosclerosis [63,64,66,98,99,108,131]. AGE exerts its effects through NO-dependent pathways and has therefore been suggested to have a positive effect on endothelial function [58,99,109].

Two different methods, PORH and CVC, were used in Study IV for the evaluation of vascular function. All 93 patients in the study had confirmed atherosclerosis and CAD. Both evaluation methods suggested that 12 months of AGE supplement leads to improved peripheral tissue perfusion. In Study IV, LSCI was used in combination with PORH and CVC, in contrast to a previous study (Study II) where LDV was used instead. LDV is less robust and harder to reproduce compared to LSCI. In Study II, we reported PORH measurements using LDV in 122 patients with an increased risk of developing cardiovascular events [132]. It is hard to compare

results from studies with different methods but our results seem to be in line with each other and with other previous studies [58,67,109,132].

AGE and validation of the method for developing prediction models

The main finding in Study V was that it is feasible to develop prediction models classifying patients into who would have the best results from AGE supplement treatment or not.

Precision medicine is a modern approach to an old problem. For centuries doctors have adapted medicines and treatments to the patient in front of them studying signs, symptoms and questioning them about their medical history and heredity for different diseases and conditions. Precision medicine combines these observations with new-found DNA analyses, biomarker information and computer science. It is a way of customising and tailoring medical- and pharmacological treatment and recommendations to every individual patient depending on the predicted response or risk of disease that they have. Patients with different lifestyles, genes and diets respond to dietary supplements and pharmacological treatments in different ways [40-45,133-136].

Traditionally pharmacological treatment has been prescribed as 'one-size-fits-all', meaning that most patients with a particular condition are prescribed the same drug and in combination with 'trial and error', meaning treatment starts with the most common drug according to local recommendations. The chosen drug is then changed to the second on the list when the patient develops side-effects or the patient does not respond in the expected way. Instead of experimenting on patients in this manner, precision medicine skips the first steps and starts with the drug the individual patient is most likely going to respond to in the prescribed way. On this note we have devised a method for developing algorithms for prediction of the effect an individual patient will have from 12 months of AGE intake. We developed the method in Study I and in Study V validated the method on a new cohort. We could predict with a 64% precision score which patients will have a significantly reduced CAC progression after 12 months of AGE supplement and we could predict with a 66% precision score which patients will have a significant blood-pressure lowering effect after 12 months of AGE supplement.

The machine learning aspect of precision medicine and in our study was applied to the development of the prediction model. To create a model with enough complexity but with good performance, a combination of variables and engineered features was made available for the model to use but we pursued to limit the feature to a minimum. This was to reduce overfitting and increase stability of the model. We tested a model with 10% of the features available, 10 out of 105, but when two additional features were added the model performed better. Consequently, a model with 12 features was chosen. LOOCV was chosen as the validation model after 10-

fold cross validation was deemed to be too sensitive to the random sampling effect. LOOCV is also the same method chosen in Study I. Since the studied sample size is limited in the present study the high computational costs of LOOCV are negligible compared to the benefits.

Limitations

All studies have strengths and limitations. Overall, in all our studies the serum levels of S-allyl cysteine, to ensure intake, was not measured in the AGE supplement group. However, in Studies I-IV the patients were given clinical check-ups every 3 months and every month the study nurse was in contact with the patient to ensure compliance with the intake of the placebo or AGE supplement. All patients were instructed not to change their lifestyle and diet apart from taking the AGE capsules; however, previous studies have shown that patients enrolled in studies are more likely to adopt lifestyle changes. A formal assessment of lifestyle before and after the study was not carried out.

CAC progression was defined as the absolute change in CAC. Analyses of the progression of CAC are statistically challenging because of a high degree of dependence of the CAC score at baseline and because of inter-scan variability. In Studies I-IV the cardiac CT was performed on the same machine by the same staff, and the calculations of the CAC burden were performed by the same two radiologists. In Study V the CT scans were performed in the USA and the data were collected from four different studies.

Both LDV and LSCI are sensitive to movement artefacts and to external factors such as changes in room temperature, stress, caffeine and nicotine intake, and exercise. There is limited knowledge of how differences in skin pigmentation, skin thickness, and proximity to larger vessels and capillary density affect the size of the output. For LDV, this was compensated for to some extent by using standardised measuring techniques according to the study protocol, controlled environment, and meticulous application of the measuring probe, and repeated measures on each patient. LSCI measures on a bigger area and therefore it was deemed that these variations should equalise each other with repeated measures in the same area.

Conclusions

Study I

The study demonstrated that AGE reduces the progression of CAC and that the probability of belonging to the group with the lowest CAC progression was almost three times higher in the AGE group than in the placebo group. Using CRISP-DM a prediction algorithm was created. The algorithm was able to predict with 80% precision which patient would have a significant reduction of CAC progression and with 74% precision which patient would have a significant blood-pressure lowering effect using AGE supplement for 12 months. The study also showed a distinct beneficial effect on inflammation, with a significant lowering effect on IL-6, a blood-pressure lowering effect and a glucose-lowering effect in the AGE group.

Study II

The study demonstrated that 12 months of AGE supplement may preserve and even improve the microcirculation measured by LDV. The effect might be because of an inhibition of the atherosclerotic process, but also by potentially affecting vascular tone and endothelial function.

Study III

The study concluded that AGE lowers IL-6 in females with low risk of CVD development. We were able to conclude that risk prediction with cardiac CT scan in females was superior in estimating the risk of CAD than the Framingham risk score.

Study IV

The study concluded that 12 months of AGE supplement regenerates peripheral tissue perfusion and increases microcirculation in patients with arteriosclerosis and CAD. The effect might be due to an inhibition of the atherosclerotic process, but also by potentially affecting vascular tone and endothelial function.

Study V

The present study demonstrates that it is possible and realistic to develop predictive models classifying patients into who would have the best results from AGE supplement treatment or not.

Future perspective

This thesis has addressed aspects of cardiovascular preventive medicine with a focus on the atherosclerotic process and the role of AGE in it. This was combined with data science and precision medicine.

Double-blinded, placebo-controlled studies are a suitable method for these types of studies but, if I was to conduct a similar study, I would be much more meticulous when randomising patients. The patients in Study I were a very heterogenous group with CAC score ranging from 10-1000 and, unfortunately, they were randomised unevenly. It would have been better to divide them into smaller subgroups and then randomise them for treatment options.

The active substance used in all the studies was AGE but, in the future, it would be very interesting to compare treatment with the different components of AGE: i.e. SAC or S1PC alone, to understand its mechanism of action in clinical applications.

A larger study with more patients would be necessary for clearer results since we could not demonstrate all the lipid-lowering and blood-pressure lowering effects previous studies have shown. I suspect this is due to a too diverse and small study cohort and increasing the size of the cohort would, I suspect, clarify the results. In a large study it would be interesting to include both patients with CAD but also those with an elevated risk of CVD but no visible CAD, representing an early stage of the disease with maybe more room for preventive measurements and treatments. I also suspect that the progressiveness of CAD makes the disease both easy and hard to treat. Patients having passed a certain point in the disease development might be less likely to benefit from AGE and other preventive measures. Comparing patients in two groups with a low CAC score in one group and a high CAC score in the other group would maybe further generate clues to possible preventive measures of CAD development.

Further development of predictive models on larger studies would improve the precision of the model. From a patient's point of view the predictive models are very appealing and patient compliance would probably improve if, in the future, a doctor would be able to recommend a certain treatment with a percentage next to it with the predicted treatment effect. It is alluring to imagine that it might be possible to achieve this one day.

Papers that are not in the thesis

- **A new way of monitoring mechanical ventilation by measurement of particle flow from the airways using Pexa method in vivo and during ex vivo lung perfusion in DCD lung transplantation.**

  Broberg E, Wlosinska M, Algotsson L, Olin AC, Wagner D, Pierre L, Lindstedt S. Intensive Care Med Exp. 2018 Jul 27;6(1):18. doi: 10.1186/s40635-018-0188-z. PMID: 30054767; PMCID: PMC6063805.

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Compared to many other studies "The Garlic study" received a lot of attention from the general public and media and has been easy for patients to understand and embrace which has been very enjoyable. My fellow PhD students at the "kliniska forskarskolan" were a bit more hesitant as well as a few colleagues when telling them that we would study garlic. I hope you are a bit more convinced now and open to this and other alternative treatments.☺

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Preventing Cardiovascular Disease

Martiné Wlosinska graduated from Lund University Medical School, Sweden in January 2015 and underwent her medical internship at Skåne University Hospital in Lund. Simultaneously she began her PhD studies at the Cardiothoracic Department in Lund in December 2016 where she had completed her master thesis and worked before starting the internship. She received her medical licence in February 2018 and continued her work at the Cardiothoracic Department. In February 2019 she began her specialty training in Family Medicine in Lund.

Non-communicable diseases are the number one killer worldwide and the leading one, cardiovascular disease (CVD), is responsible for more than 30% of all deaths. There are many stages of CVD that ultimately can lead to coronary atherosclerosis and, therefore, it is also possible, effective and economical to prevent their development. This thesis is based on four double-blinded placebo-controlled randomised trials studying the effect of a dietary supplement, Aged Garlic Extract (AGE), on CVD development. The popular novelty, precision medicine, was used to develop a prediction model with the ability to predict the benefit of AGE on the individual patient.

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