“The role of complex food on atherosclerotic lesion development and composition in genetically modified mice - Studies on fish and chocolate liquor”

Dissertation

to achieve the degree of
Doctor of Nutritional Sciences (Dr. troph.)

of the

Faculty of Natural Sciences III
Institute of Agricultural and Nutritional Sciences,
Martin Luther University Halle-Wittenberg, Germany

presented by

Narges Yazdekhasti-Grau (Master of Science)
born on 18. June 1981 in Isfahan (Iran)

Referee: Prof. Dr. Gabriele Stangl
Prof. Dr. Klaus Eder

Defense on: 15.10.2018

Halle/Saale 2018

Table of content

List of abbreviations

25(OH)D	25-hydroxy vitamin D
ADAM10	a Disintegrin and Metalloprotease 10
ADAM17	a Disintegrin and Metalloprotease 17
AHA	American Heart Association
ALA	Alpha-linolenic Acid
ApoB	Apolipoprotein B
$ApoE^{-/-}$	Apolipoprotein E null
CAD	Coronary Artery Disease
CD	Cluster of Differentiation
CHD	Coronary Heart Disease
CVD	Cardiovascular Disease
DHA	Docosahexaenoic Acid
EPA	Eicosapentaenoic Acid
FA	Fatty Acid
FMO	Flavin Monooxygenase
HDL	High Density Lipoprotein
ICAM-1	Intracellular Adhesion Molecule-1
IL	Interlouekin
JAM-A	Junctional Adhesion Molecule-A
LDL	Low Density Lipoprotein
$Ldlr^{-/-}$	LDL receptor null
LCMUFA	Long Chain Monounsaturated Fatty Acid
MCP-1	Monocyte Chemoattractant Protein-1
mCSF	Macrophage Colony-stimulating Factor
MMP	Matrix Metalloproteinase
mRNA	messenger Ribonucleic Acid
MUFA	Monounsaturated Fatty Acid
NF-κB	Nuclear Factor-kappa B
OxLDL	Oxidized-Low Density Lipoprotein
PUFA	Polyunsaturated Fatty Acid
RCT	Randomized Control Trial
RDA	Recommended Dietary Allowance
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SFA	Saturated Fatty Acid
SMC	Smooth Muscle Cell
SR	Scavenger Receptor

SRA	Scavenger Receptor A
TC	Total cholesterol
TG	Triglyceride
TMA	Trimethylamine
TMAO	Trimethylamine N-oxide
TNF-α	Tumor Necrosis Factor-α
VCAM-1	Vascular Adhesion Molecule-1
$VDR^{-/-}$	vitamin D receptors null
VE-cadherin	Vascular Endothelial-cadherin
VLDL	Very-Low-Density Lipoprotein
VSMC	Vascular Smooth Muscle Cell
WT	wild type

List of figures

1 Introduction

1.1 Atherosclerotic vascular diseases (Atherosclerosis)

Cardiovascular disease (CVD) is a group of diseases that engages both the heart and blood vessels, thereby includes the coronary heart disease (CHD) and coronary artery disease (CAD) among the major conditions (Mendis et al., 2011). CHD and other manifestations of CVDs were not among the main causes of death until the early 20th century, but thereafter a dramatic rise was observed in industrialized countries, peaking around 1960 to 1980 (Levy, 2012). Although CVD is preventable, several factors such as physical inactivity, nicotine abuse and bad nutritional practices (Danaei et al., 2009) are leading to an upsurge of its prevalence in most countries (WHO, 2013). Today, CVDs claim annually more lives than all forms of cancer and chronic lower respiratory disease combined (Benjamin et al., 2017). In 2015, an estimated 17.7 million people died from CVDs, representing 31% of all global deaths and this number is expected to grow over 23.6 million by 2030 (Mendis et al., 2011; WHO, 2017). CVD has been responsible for 47% of all deaths in Europe and 40% of all deaths in the European Union (Nichols et al., 2014). In Germany alone, CVDs accounted for 40 % of all the causes of death in the year 2013 (Statistisches Bundesamt, 2013).

Among the underlying causes of CVDs is Atherosclerosis or “hardening of the arteries”, a chronic inflammatory disease characterized by formation of lipid laden foam cells in the vasculature (Lusis, 2000). In one individual, atherosclerotic plaques may be present at many different locations, in several vessels, most of which may remain asymptomatic (subclinical disease), some become obstructive (stable angina), and others elicit acute thrombosis and lead to obstruction of blood flow to the heart (myocardial infarction), brain (stroke), or lower extremities (critical limb ischemia) (Simionescu and Sima, 2012). The traditional risk factors for atherosclerosis include dyslipidemia, vasoconstrictor hormones incriminated in hypertension, products of glycoxidation associated with hyperglycemia, pro-inflammatory cytokines, hyperhomocysteinemia, infectious microorganisms and smoking (Simionescu and Sima, 2012). Among these risk factors dyslipidemia is a prerequisite for the initiation and progression of about half of arterial lesions, even in the absence of other risk factors (Glass and Witztum, 2001).

All the other traditional risk factors seem to accelerate a disease driven by atherogenic lipoproteins, mainly low-density lipoprotein (LDL), perhaps through either increased atherogenicity of LDL (e.g. particle size, number and composition) or increased susceptibility of the arterial wall (e.g. permeability, inflammation, *etc.*) (Falk, 2006). The plaque initiation and development until the final rupture is orchestrated by a complex array of molecular and cellular events where factors such as mechanical forces (plaque stress, flow shear stress, blood pressure), plaque morphology (thin cap, lipid-rich necrotic core, calcification, hemorrhage, ulcer, *etc.*), cell activities (inflammation, remodeling), blood conditions (cholesterol level, injury-initiated blood changes) play significant roles (McLaren et al., 2011; Tang et al., 2014; Buckley and Ramji, 2015). The mechanism of lesion formation and development is shown in Figure1-1.

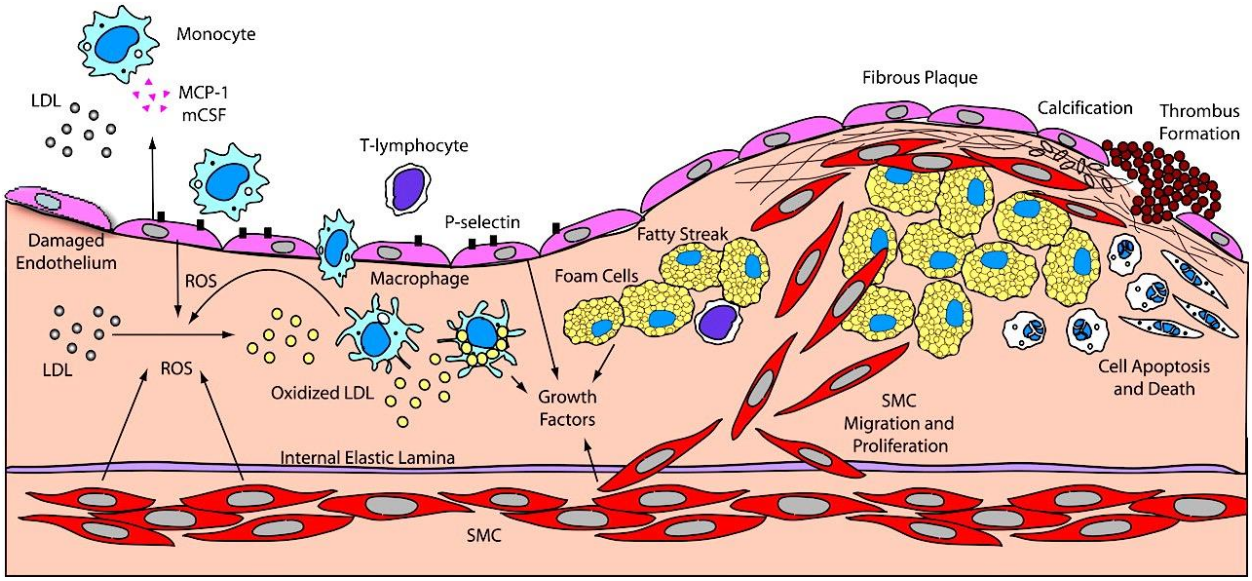

Figure 1-1 ***Development and constituents of an atherosclerotic plaque.*** *Low density lipoprotein (LDL, Monocyte chemoattractant protein-1 (MCP-1), Macrophage colony-stimulating factor (mCSF), Reactive oxygen species (ROS), Smooth muscle cell (SMC). Reproduced from Madamanchi et al. (2005) with permission.*

The initial trigger of atherosclerosis is the trapping and accumulation of the plasma molecules and LDL particles in the intima of medium and large arteries where endothelium is destructed, and blood flow is disturbed (McLaren et al., 2011; Buckley and Ramji, 2015). Destruction of endothelium so-called "endothelial dysfunction" is a condition characterized by excessive generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), reduced

endothelium-mediated vasorelaxation, increased expression of adhesion molecules such as vascular adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1), increased expression of chemotactic molecules such as monocyte chemoattractant protein-1 (MCP-1), enhanced permeability of the cell layer, overproduction of growth factors, and upregulation of several inflammatory genes (Mallika et al., 2007; Sena et al., 2012). Following the retention, LDL particles may undergo oxidative modification due to oxidative excess in the vascular wall, hence turn into oxidized-LDL (OxLDL) and activates the subsequent proinflammatory and proatherogenic process (Karimi et al., 2013). The expression of adhesion molecules allows the adherence of monocytes, T-lymphocytes and platelets to the endothelium (Hansson and Libby, 2006). Among the mediators that regulate the cellular adherence and permeability are "a disintegrin and metalloprotease" 17 (ADAM17) and "a disintegrin and metalloprotease" 10 (ADAM10). These mediators are regarded as transmembrane "molecular scissors" and their task is to proteolytically cleave, or shed, the extracellular regions of transmembrane proteins (Figure 1-2). ADAM17 and ADAM10 act in concert to facilitate the release of soluble forms of proinflammatory cytokines (e.g. tumor necrosis factor-α (TNF-α), *etc*.), adhesion molecules including ICAM-1 and VCAM-1, vascular endothelial-cadherin (VE-cadherin) and junctional adhesion molecule-A (JAM-A); contributing in part to atherogenesis (Schulz et al., 2008; Oksala et al., 2009; Donners et al., 2010; Matthews et al., 2017).

Once entered, monocytes differentiate into macrophages under the influence of macrophage colony-stimulating factor (mCSF) and express several scavenger receptor (SR)s such as cluster of differentiation $(CD)_{36}$. This event is a part of "macrophage trapping", a vicious circle that involves cell retention, oxidation of new LDL and the recruitment of more monocytes which in turn facilitates internalization of more OxLDL (Szondy et al., 2014; Leiva et al., 2015). Internalization of native LDL, occurs at a very low rate besides, LDL receptors may undergo downregulation. In contrast, SRs have a high affinity for OxLDL and they are not down regulated by cellular cholesterol accumulation, leading to massive intracellular lipid deposits (Barbieri et al., 2004). The presence of macrophage foam cells defines the earliest pathological lesion referred as "fatty streak". Most fatty streaks are not occlusive and known to regress (Tabas et al., 2015).

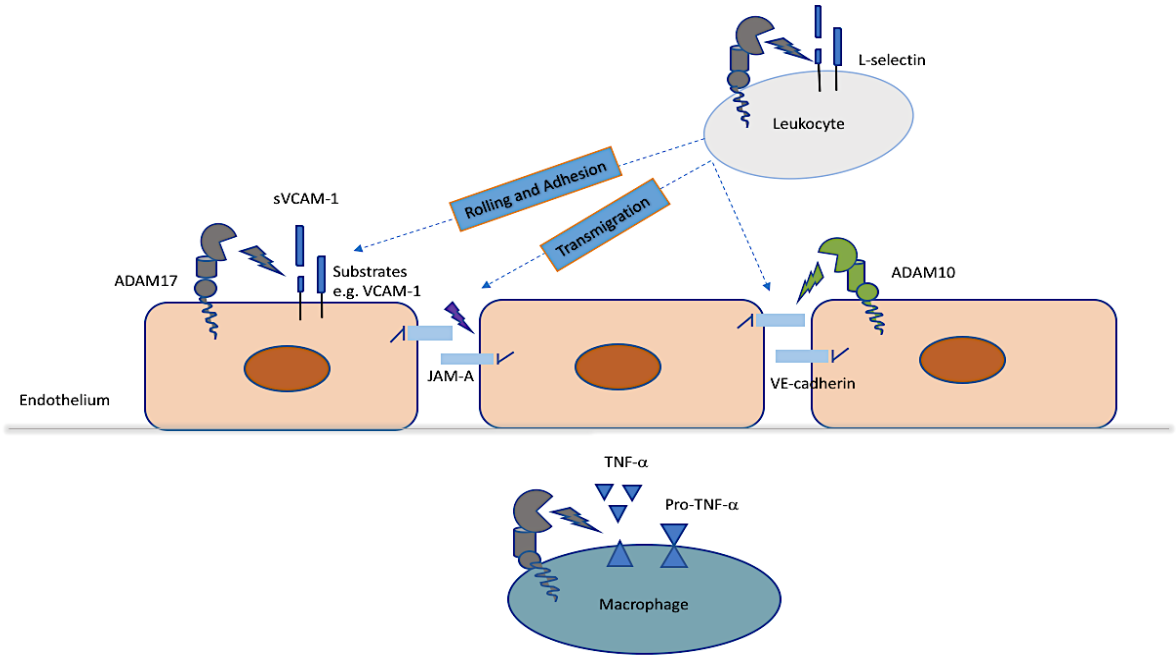

Figure 1-2 ***The role of ADAM10 and ADAM17 shedding activity in atherosclerosis.*** *Schematic summary of ADAMs shedding activities which leads to enhanced vascular adhesion and permibility, pro-inflammatory reactions and atherogenesis. A disintegrin and metalloproteinase 10 (ADAM10), A disintegrin and metalloproteinase 17 (ADAM17), Vascular adhesion molecule-1 (VCAM-1), Tumor Necrosis Factor-α (TNF-α), Junctional adhesion molecule A (JAM-A), VE-cadherin vascular endothelial-cadherin. Adapted from Saftig and Reiss, (2011).*

In disease progression, a fibroproliferative response mediated by vascular smooth muscle cell (VSMC)s leads to plaque growth and enlargement. In fact, smooth muscle cell (SMC)s undergo "phenotypic switch" thereby differentiate from their specialized, contractile state to a secretory phenotype and gain the abilities to migrate into the intima, proliferate and synthesize massive amounts of extra cellular matrix components (Falk, 2006; Badimon and Vilahur, 2014). The extra cellular matrix consists mainly of collagen, but also elastin and proteoglycans (Bentzon et al., 2014). SMCs and collagen-rich matrix play a significant role in plaque stability, hence are considered beneficial in the disease progress (Schwartz et al., 2000). Atherosclerotic plaque growth is also accompanied by a network of microvessels, namely "vasa vasorum", that extends from the adventitia into the base of the plaque and supply oxygen and nutrients to the outer layers of the arterial wall (Tanaka and Sata, 2015). Recent findings have suggested that through vasa vasorum, the inflammatory cytokines and chemotactic factors stimulate VSMCs migration, which in turn result in plaque build-up and expansion (Xu et al., 2015). This might be the reason why atherosclerotic plaques are currently believed to extend not only "inside-out" but also "outside-in" (Tanaka and Sata, 2015; Yagi et al., 2017).

The proliferated VSMCs as well as macrophages located at the center of the plaque may undergo apoptosis due to the lack of oxygen (Lusis, 2000). Depending on the efficiency of efferocytosis, these apoptotic cells may be removed, leading to reduction of the lesion size (Szondy et al., 2014), or they may accumulate and be subjected to secondary necrosis, generating necrotic cores as coherent masses. Apoptosis induces plaque instability through decreases in the number of viable SMCs necessary for collagen production and by compromising the structural integrity of the fibrous cap after release of matrix metalloproteinase (MMP)s from apoptotic bodies (Lusis, 2000). Advanced plaque lesions or so-called "vulnerable" plaques become gradually complex with calcification, new vessel formation, thinning of the fibrous cap and eventually plaque rupture and thrombus formation which lead to cardiovascular and cerebrovascular events (Badimon and Vilahur, 2014).

1.2 Morphologic and histological assessment of atherosclerotic lesion as a tool for risk prediction

Plaque morphology has recently emerged as an important contributory factor to the degree of plaque stenosis (Kanadaşı et al., 2006; Spagnoli et al., 2007; Manoharan et al., 2009). Many scientific studies have analyzed the morphological characteristics of atherosclerotic plaques with the aim of identifying risk markers (Mathiesen et al., 2001; Matter et al., 2009). Histomorphological studies have also led to the understanding that plaque structure can affect the risk of lesion complication and rupture (Damianou and Couppis, 2016). Currently, the routine contribution of histological analysis of plaque in scientific research is almost absent and histological analysis has been mostly used to obtain data for instrumental methods while has little space in clinical use (Ciccone et al., 2011). Histology-based classification of atherosclerotic plaques was first released by the American Heart Association (AHA) categorizing the lesions to types I–VIII (Stary, 2000). Following AHA work, Virmani et al. (2000) introduced an alternative and simpler classification (Figure 1-3), which emphasizes the link between lesion morphology and clinical disease. Both works laid the basis for additional plaque evaluation using other approaches than traditional factors. A series of post-mortem and angiographic studies have identified that the occurrence of cardiovascular events was not reflected by increase in arterial stenosis and the actual plaque size but instead by the stability of the plaque, outlining the plaque composition rather than plaque regression a worthwhile clinical goal to pursue (Braganza and Bennett, 2001; Falk et al.,

1995). The PROSPECT project (Stone et al., 2011), and the joint work of Oxford Plaque Study and AtheroExpress (Howard et al., 2015) have confirmed that the presence of instable plaques is associated with an increased risk of future cardiovascular events.

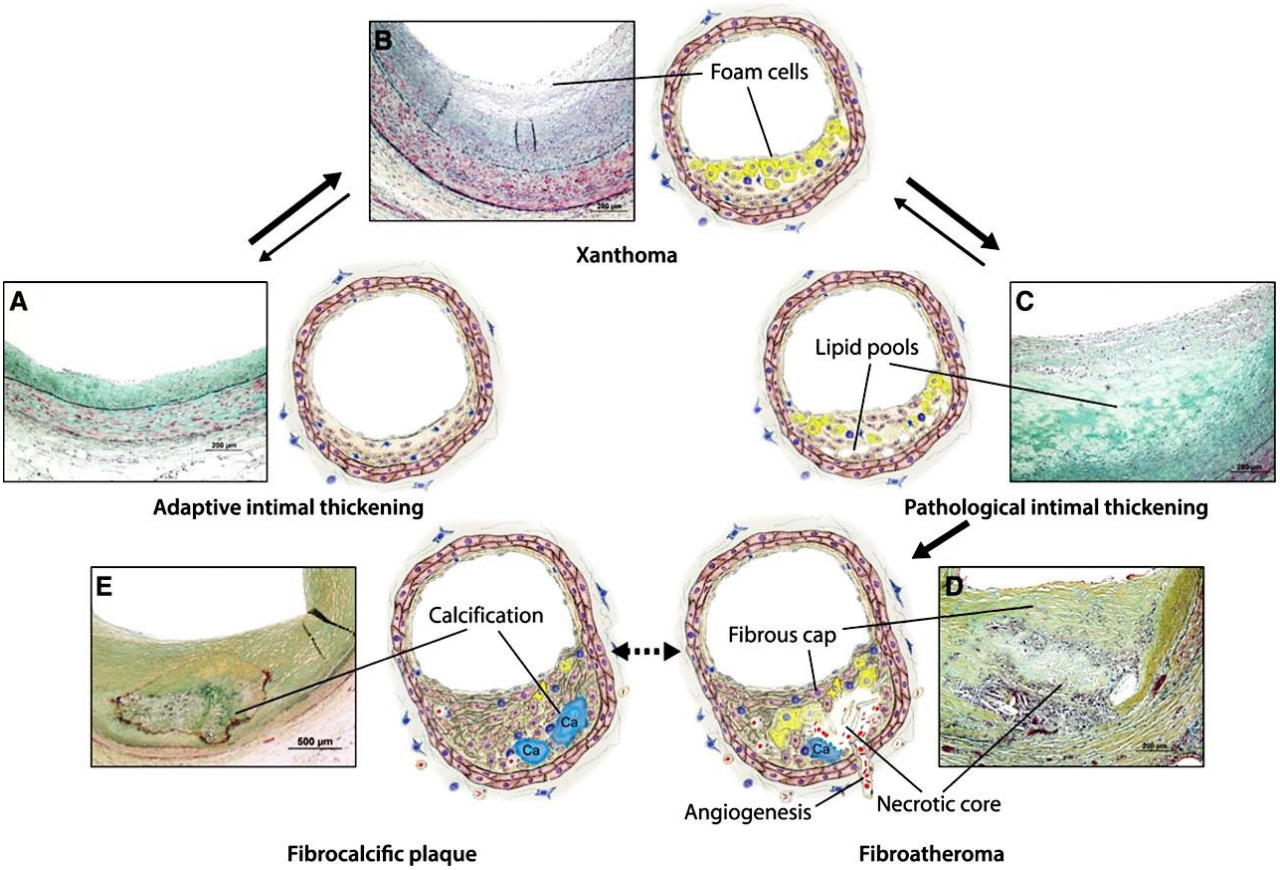

Figure 1-3 ***Lesion types during the atherosclerotic plaque progression.*** ***A****, Adaptive intimal thickening (nonatherosclerotic intimal lesions) characterized by the normal accumulation of smooth muscle cells within the intima in the absence of lipids or macrophages foam cells.* ***B****, Intimal xanthoma (or fatty streak) corresponding to the luminal accumulation of foam cell macrophages without a foam cells or fibrous cap.* ***C,*** *Pathological intimal thickening denotes the presence of smooth muscle cells in a proteoglycan-rich matrix with extracellular lipid pools in the absence of necrosis.* ***D****, Fibroatheroma demonstrating the presence of a necrotic core.* ***E****, Fibrocalcific plaque happens corresponding collagen-rich plaque with significant areas of calcification and few inflammatory cells; a necrotic core might be also present. Reproduced from Bentzon et al. (2014) with permission.*

Two main components which play a significant role in plaque stability are (i) soft, lipid-rich atheromatous mass and (ii) hard, collagen-rich sclerotic tissue (Falk et al., 1995). The sclerotic component, fibrous tissue, is more voluminous and appears to stabilize the plaque and protect against disruption. On the contrary is the less voluminous, less stable and soft atheromatous

component which destabilizes the plaque, and leaves it vulnerable to rupture (Falk et al., 1995; Moreno, 2010). Among the features of plaque instability leading to rupture are large numbers of cells positive for markers such as CD_{68} assumed to be macrophages, a thin or fragmented fibrous cap comprising smooth muscle α-actin–positive cells presumed to be derived from VSMCs and the presence of large necrotic cores with extensive intra- and extra-cellular lipid accumulation (Virmani et al., 2000; Tabas et al., 2015; Bennett et al., 2016).

The role of VSMC in atherosclerosis is still unclear. The historical view of VSMCs in atherosclerosis is that aberrant VSMC proliferation causes plaque expansion but that VSMCs in advanced lesions are entirely beneficial. This view has been based on the ideas that atherosclerotic plaques consist of homogenous population of VSMCs which can be identified individually from other plaque cells by using standard VSMC immunohistochemical markers (Virmani et al., 2000). However, this historical view, has been challenged by more recent genetic lineage tracing studies which have shown VSMC phenotypic switching results in less-differentiated forms that lack VSMC markers (e.g. macrophage-like cells), and also this switching *per se* promotes atherosclerosis (Gomez et al., 2013; Feil et al., 2014; Shankman et al., 2015). VSMCs are predominantly reparative throughout the atherogenesis, and not the primary driver of plaque formation. Therefore, enhancement and not inhibition of VSMC proliferation in advanced lesions may be beneficial for plaque stability. In contrast, VSMC phenotypic switching to a macrophage-like cell, VSMC death, and senescence may all promote plaque progression, inflammation, monocyte recruitment, and subsequent secretion of VSMC mitogens (Wang et al., 2015a; Bennett et al., 2016). Additionally, the age of VSMCs may influence the inflammatory activities of these cells. A recent study showed that the aged aortic VSMCs from rodents had a higher basal secretion of interlouekin(IL)-6 and also displayed gene upregulation of chemokines and adhesion molecules compared with the young VSMCs. (Song et al., 2012). These properties generate a proinflammatory environment, which promotes migration of more inflammatory cells and eventually leads to plaque enlargement and other features of unstable plaques (Song et al., 2012; Wang et al., 2015a).

Necrotic core contains cellular debris, crystalline cholesterol, lysosomal enzymes, inflammatory cells and macrophage foam cells (Tabas et al., 2015). A larger necrotic core confers a greater risk than a small one (Virmani et al., 2006). The importance of necrotic core size for plaque stability

is clear: the lack of supporting collagen in the necrotic core generates greater tensile stress to the overlying fibrous cap and the enlargement of the core may erode the fibrous cap from below. These factors increase the risk of thrombogenecity of the plaque material and accordingly the risk of clinical events in case of plaque rupture (Fernández-Ortiz et al., 1994). Plaque calcification has also a crucial role in plaque stability. Plaque calcification is an active and controlled intracellular molecular process, occurring in both lipid and connective tissue, and involving the differentiation of macrophages and VSMCs into osteoclast-like cells in a manner resembling the calcification in bone (Pletcher et al., 2004; Karwowski et al., 2012). Although vascular calcification has been recognized to reduce elasticity and increase vascular stiffness, it may also create a barrier to protect plaques against rupture (Wu et al., 2014). Several studies have shown that diffuse, "speckled", or "spotty" deposits have led to local stress and lesion instability whilst large colonies of calcification have generated more stable plaques (Doherty and Detrano, 1994; Abedin et al., 2004; Vengrenyuk et al., 2006). Vascular calcification has been shown to be associated with cardiovascular disease burden (Wu et al., 2014) and also suggested to be an independent risk factor for cardiovascular mortality (Liu et al., 2015). Today, the coronary artery calcium score (total amount of plaque calcification in coronary arteries) is broadly used as an excellent marker for coronary plaque burden, providing prognostic information about the cardiovascular events and fracture beyond that provided by traditional risk factor scoring (Neves et al., 2017).

Altogether, morphological and histological methods that detect the actual plaque size, lipid content and/or the thinnest fibrous cap as well as other critical plaque constituents can be considerably useful in profound understanding of how various interventions (e.g. dietary factors) may affect the plaque structure and help with the prediction of lesion instability and rupture, hence the risk of future CVD events (Butcovan et al., 2016). Histology can provide a platform for exploring morphological features at extremely high resolutions, although this method has some limitations such as its retrospective nature (Phinikaridou et al., 2012). Likewise, there are several invasive and non-invasive imaging modalities which may provide prospective insights into the progression of atherosclerotic plaques although they have much lower resolution compared with the histology. Histological analysis can be performed on vessels from atherosclerotic animals as well as upon excised human vessels collected either postmortem or surgically (Phinikaridou et al., 2012). Over the past 30 years, the vast majority of insights into the natural history of atherosclerosis progress

has come from studies in mouse models which carry genetic mutations, causing them to generate atherosclerotic plaques, identical to those found in humans (Hampton, 2017). Compared with the studies using human subjects, mouse models can be used for invasive interrogation of plaque quantification, plus the privileges of being easier to manage, with controllable nutrition and environmental risk factors (Lee et al., 2017). The most used types of genetically modified mice in atherosclerosis research are LDL receptor null ($Ldlr^{-/-}$) and apolipoprotein E null ($ApoE^{-/-}$) mice that carry mutations which prevent removal of circulating cholesterol by the liver. $ApoE^{-/-}$ mice develop severe dyslipidemia because of diminished clearance of apolipoprotein B (ApoB)-containing lipoproteins, particularly when placed on a high-fat diet, while $Ldlr^{-/-}$ mice develop less of a severe dyslipidemic phenotype which is closer to human dyslipidemia (Zadelaar et al., 2007). Ingesting Western-type diets with additional cholesterol (0.15% - 1.25% w/w) can induce significant atherosclerotic lesions after 12 weeks in $Ldlr^{-/-}$ mice (Lichtman et al., 1999). Plaque development is even more dramatic in $ApoE^{-/-}$ mice fed a Western-type diet as fatty streak lesions can be found as early as 6 weeks (Nakashima et al., 1994). In contrast, the wild type (WT) mouse, is a "high density lipoprotein (HDL)" animal which contributes to its relative atheroresistance.

Mice and humans differ in several parameters that may influence atherogenesis. For example, a critical limitation in $ApoE^{-/-}$ mice is the scarcity of plaque rupture and thrombosis, two major complications of human atherosclerosis. Indeed, murine models do not develop a thick fibrous cap, medial vasa vasorum or even unstable atherosclerotic plaques with overlying thrombosis similar to those observed in humans (Getz and Reardon, 2012). Also, in humans, lesions occur more frequently in the coronary arteries, carotids and peripheral vessels such as the iliac artery while in mice it happens more frequently in the aortic root, aortic arch and innominate artery. The much more rapid heart rate of the mouse and as a result the disturbed blood flow may account for the tendency of atherosclerosis development at these sites (Getz and Reardon, 2012). It must be noted that the regions of disturbed flow or low shear stress are atherosusceptible sites, whereas regions of laminar flow and relatively high shear stress are atheroresistant sites. Although these two regions are subjected to the same systemic risk profiles, yet they react differently to the plaque progress. That is why, more than one site of lesion development in animal models should be examined in order to distinguish between hemodynamics and the cell and cellular factors that influence the atherogenic process (VanderLaan et al., 2004).

1.3 The role of three dietary factors known to influence cardiovascular risks and diseases

1.3.1 Dietary lipids

In the early 1980s, the primary dietary advice for prevention of CVDs emphasized on restrictions in total fats intake, including both saturated and unsaturated fats. Subsequently, the findings from several cohort studies showed that the higher risk for CVD is associated with the increased intake of saturated fatty acids (SFA)s and percentage of calories from fats (Goldbourt et al., 1993; Hu et al., 1999). Paradis and Fodor (1999), further in a systematic review of various dietary fats and their impacts on plasma lipid concentrations, showed that SFAs can significantly increase both LDL and HDL concentrations while unsaturated fats seem to be cardioprotective. Substantial findings then, led the dietary guidelines in the 1990s to stress more on replacing SFAs with unsaturated fats as an approach for risk reduction (Willett, 1998). Successively, several large cohort studies underlined the deleterious effects of *trans* fats and the strong association with higher risks of CHDs (Willett et al., 1993; Hu et al., 1997). Similar to the act of saturated fats, *trans* fats tend to block LDL receptors and prevent their uptake from the bloodstream although they appear to be even worse than SFAs, because in addition to the increased LDL, they tend to lower HDL levels (Mensink and Katan, 1990).

Currently, the role of saturated fats on cardiovascular risks is subjected to many controversial debates (Ramsden et al., 2013; Chowdhury et al., 2014). In 2015, Li et al. reported that replacing SFAs with equivalent energy from polyunsaturated fatty acids (PUFA)s, monounsaturated fatty acids (MUFA)s, or even carbohydrates from whole grains was associated with a significant reduction in CHD risk. Contrarily, a systematic review and meta-analysis of many prospective cohort studies, reported that intake of saturated fats was not associated with all-cause mortality, CVD, CHD, ischemic stroke, or type 2 diabetes, even though *trans* fats, in particular those of industrial origin, were (De Souza et al., 2015). Also, the PURE study, representing data from 18 countries in 5 continents, reported that total fat and types of fat were not associated with CVD, myocardial infarction, or CVD mortality, and compared to total fat and individual types of fat, high carbohydrate intake was associated with higher risk of total mortality (Dehghan et al., 2017). In contrast to SFAs, unsaturated fats and most importantly PUFAs have been repeatedly reported to be cardioprotective (Hu et al., 2002a). Epidemiological (De Caterina et al., 2003) and clinical

trial (Ueshima et al., 2007) studies have reported improvement of lipid profile and reduced risk of myocardial infarction in those who have consumed diet rich in n-3 PUFAs whereas decreased circulating levels of n-3 PUFAs have been associated with higher incidence of CVD events and mortality (Hara et al., 2013). Growing evidence concerning cardiovascular benefits of PUFAs led to the official recommendation by AHA for daily consumption of 500 mg n-3 PUFAs (Krauss et al., 2000; Kris-Etherton et al., 2002) and by the European Food Safety Authority panel that recommends daily consumption of 250 mg n-3 PUFAs (EFSA, 2010a).

Among the essential n-3 fatty acid (FA)s present in human's diet are alpha-linolenic acid (ALA) found in nut and plant oils (Freeman et al., 2006; Bent et al., 2009) as well as docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) found in seafood. The intake of n-3 FAs, either as a supplement or oily fish, can improve n-3 index (defined as the sum of EPA and DHA, expressed as a percentage of the total FA content of red blood cell membranes), and provide several health benefits (Harris and Von Schacky 2004; McCombie et al., 2009). The mechanism linked to the cardioprotective effects of n-3 FAs include their impacts on vascular resistance, blood pressure, inflammation, serum triglyceride levels, platelet aggregation and endothelial function (Paradis and Fodor, 1999; Kris-Etherton et al., 2002; Mozaffarian and Wu, 2011; Chang and Deckelbaum, 2013; Chang et al., 2014). A recent *in vivo* study indicated that dietary PUFAs are able to modify the distribution and bioavailability of other FAs, and particularly to block the absorption of SFAs (Yang et al., 2017). Despite this long list of positive effects, there are other studies including two recent meta-analyses (Kwak et al., 2012; Rizos et al., 2012), and a well conducted randomized control trial (RCT) (Bosch et al., 2012) that have reported ambiguous or even negative results regarding the vascular effects of n-3 FAs, provoking some skeptics to negate their potential health benefits.

Data concerning dietary cholesterol is similarly mixed. Among the considerable number of longitudinal observational studies and meta-analysis of intervention trials, some studies have reported dietary cholesterol increases CVD risk (Howell et al., 1997; Houston et al., 2011), and even independently of plasma cholesterol levels (Shekelle et al., 1981; McGee et al., 1984) whereas others have reported a decreased risk or no change with higher cholesterol intake (Herron et al., 2004; Harman et al., 2008). The link between dietary cholesterol and serum cholesterol appear to be linear up to 600 mg/d of cholesterol intake, but nonlinear for cholesterol intakes more

than 600 mg/d, with negligible effect on serum lipid concentration in most people (McGill, 1979). According to a recent meta-analysis, the rise in serum cholesterol is no longer statistically significant when dietary cholesterol exceeds doses higher than 900 mg/d (Berger et al., 2015). The Dietary Guidelines Advisory Committee has recommended no more than 300 mg/d of cholesterol intake for healthy populations in the United States (Lichtenstein et al., 2006). Similarly, the International Atherosclerosis Society recommended lowering the intake of dietary cholesterol as a strategy for lowering LDL levels (IAS, 2014). Contrary to the guidelines in the United States, other developed and developing countries do not have an upper limit on cholesterol intake but instead they have mainly focused on controlling the intakes of saturated and *trans* fat as major determinants of blood cholesterol levels (Graham et al., 2007).

1.3.2 Dietary proteins

High intakes of dietary protein may also benefit the cardiovascular health by assisting in lipid/lipoprotein profile improvements, weight loss/maintenance, and reducing blood pressure (Hu, 2005; Vasdev and Stuckless, 2010; Leidy et al., 2015). However, the impacts of increased protein consumption must be interpreted with caution because protein is not consumed in isolation but as part of a food matrix. The increased consumption of protein/protein-rich foods certainly results in other changes in the diet such as alterations in the intakes of other nutrients (e.g. saturated fat and refined carbohydrates) and/or foods (e.g. fruits, vegetables, and whole grains), depending on what protein food sources are increased, and for which part of diet they are replaced (Richter et al., 2015). According to the current dietary guidelines, the Recommended Dietary Allowance (RDA) for adults is about 0.8 g protein/kg body weight, although based on individual needs and preferences the range of 10–35% of total calories from protein is acceptable to allow higher flexibility in meal planning (Trumbo et al., 2002; Rodriguez and Miller, 2015). There is also an RDA for each essential amino acid available, which may let individual meet their needs through variety of different protein-providing foods. However, recommendations for the relative contribution of animal- and plant-based sources of protein are still lacking (Trumbo et al., 2002).

For decades, observational studies have suggested that plant and animal protein affect CVD risk differently (Ferdowsian and Barnard, 2009; Altorf-van der Kuil et al., 2010; Zhang et al., 2014). These reports are mixed, depending on characteristics of the population studied and how protein groups are specified (Altorf-van der Kuil et al., 2010; Zhang et al., 2014). For example, the primary findings concerning vascular benefits of dietary plant proteins has come from vegetarian populations, which tend to have a lower blood pressure and plasma cholesterol than their omnivorous counterparts (Sacks et al., 1974 and 1975). Although the Seventh-Day Adventist study discovered that vegetarians also tend to be nonsmokers, consume less alcohol, have a lower body weight, be more physically active, and generally embrace a healthier life-style, all of which prevent the risks for high blood pressure and other cardiovascular risk factors (Armstrong et al., 1977). On the other hand, populations consuming animal-based protein may hugely vary in the type of consumed food (fish compared with red meat), hence contribute differently to CVD risks (Richter et al., 2015). Therefore, the occasionally observed positive contribution of animal proteins in reducing CVD outcomes (e.g. stroke) compared with vegetable protein (Zhang et al., 2014), perhaps is because in those studies fish rather than red meat has been the primary source of animal protein.

A differential effect of plant vs. animal protein on cardiovascular health may additionally pertain to other contributing factors such as non-protein compounds in the food matrix. For instance, several components of red meat, including heme iron, cholesterol, and advanced glycation and lipoxidation end-products, were suggested to be underlying factors responsible for the link between red meat intake and type 2 diabetes as a significant CVD risk factor (Feskens et al., 2013). Whereas, fish as an animal protein provides a different set of nutrients, including n-3 FAs, vitamin D, multiple B vitamins, essential amino acids, and trace elements, which are all known for their contribution to cardiovascular health (Mente et al., 2009; Zheng et al., 2012). A number of cohort studies have reported neither animal protein nor vegetable protein are associated with CHD risk (Hu et al., 2002b; Halton et al., 2006; Haring et al., 2014), while the the nutrients and bioactive components that accompany the protein in the food matrix, such as saturated fat (Hu et al., 1997 and 1999b) polyunsaturated fat (Hu et al., 1997 and 2002a) and vitamins E, B6, and folate (Willett, 1998) are associated with the risk.

The amino acid profile of a protein also plays a significant role. Compared with proteins from animal sources, plant-based proteins provide less amounts of essential amino acids (particularly methionine, lysine, and tryptophan) but are higher in nonessential amino acids (e.g. arginine, glycine, alanine, and serine) (Krajcovicova-Kudlackova et al., 2005). The essential amino acids lysine and methionine have been shown to produce a marked hypercholesterolemic response *in vivo* (Giroux et al., 1999; Krajcovicova-Kudlackova et al., 2005) while nonessential amino acids arginine, as a precursor of the vasodilatory nitric oxide, may be particularly valuable for lowering blood pressure (Appel, 2003; Rodriguez and Miller, 2015). For the most part, the evidence supporting the health effects of plan-based proteins vs. animal-proteins has come from intervention studies in both animals and humans, which have compared soy protein to casein as the animal-based protein (Kritchevsky and Tepper, 1968; Kritchevsky, 1979; Nilausen and Meinertz, 1999). Although, fish protein as an animal protein is characterized with low ratios of methionine-glycine and lysine-arginine which tends to lower cholesterol levels in contrast to the bovine casein. In fact, proteins from variety of fish species have displayed hypocholesterolemic activities *in vivo* when compared with casein (Carroll and Hamilton, 1975; Kritchevsky et al., 1982).

Recent findings have also pointed to the roles of toxic substances as well as gut microbiome in mediating the impacts of animal proteins on CVD. The first findings were from the hypothesis-generating metabolomics studies which revealed that plasma level of trimethylamine N-oxide (TMAO) is correlated with CVD risk (Wang et al., 2011). TMAO is a small organic and odorless compound in the class of amine oxides which is frequently found in the tissues of a variety of marine organisms. TMAO can be formed from trimethylamine (TMA), an odorous gas (fishy smell) which develops as a result of undesirable microbial activities in food products during storage (Lidbury et al., 2014). While these toxic substances can be present in the food matrix, either in the form of TMA or the performed TMAO, they can also be formed through hepatic or microbial metabolism of diet rich in their precursors (e.g. phosphatidylcholine (lecithin), choline, and carnitine), with major food sources including seafood, eggs, liver, beef, and pork (Al-Waiz et al., 1992; Lang et al., 1998; Landfald et al., 2017). As shown in Figure 1-4, following the meal consumption, these precursors are first metabolized to TMA via trimethylamine-lyase enzymes, which are unique to gut microbiota, then further absorbed from gastrointestinal tract and undergoes a subsequent oxidation via a member of hepatic flavin monooxygenase (FMO) family, FMO3, to

generate TMAO (Al-Waiz et al., 1992; Lang et al., 1998). TMAO may in turn contribute to the development of atherosclerosis through upregulation of several SRs such as CD_{36} and scavenger receptor A (SRA), known to promote the uptake of modified lipoproteins, leading to foam cells formation (Wang et al., 2011; Koeth et al., 2013; Wang et al., 2015b).

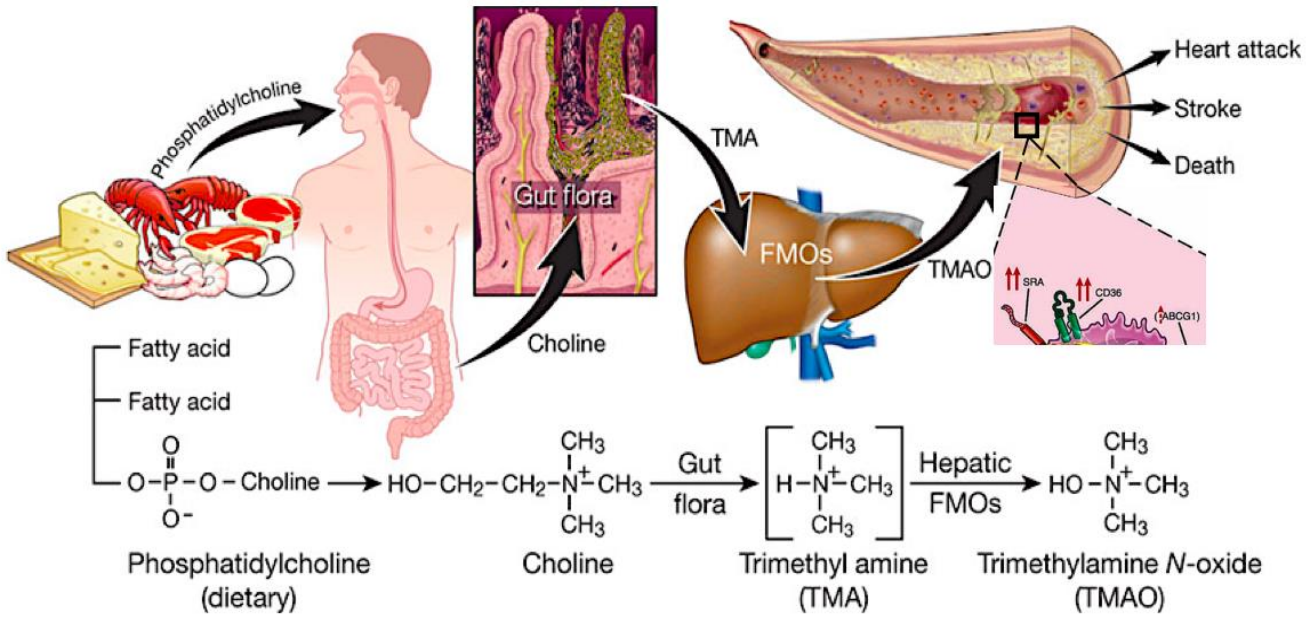

Figure 1-4 ***TMAO generation from dietary phosphatidylcholine/TMA and its contributory role in atherosclerosis.*** *Schematic pathway displays the intestinal-microbiota metabolism of dietary phosphatidylcholine to TMA which is further oxidized to TMAO by a member of FMO. TMAO in turn enhances the expression of scavenger receptors CD_{36} and SRA, hence promoting atherosclerosis development. Hepatic Flavin Monooxygenase (FMO), Cluster of Differentiation 36 (CD_{36}), Scavenger Receptor A (SRA). Reproduced from Wang et al. (2011) with permission.*

The higher concentrations of circulating TMAO has been observed in meat-eaters compared with vegetarian individuals (Koeth et al., 2013). This might be explained in part by the wide-ranging compositional and functional changes that occur in the gut microbiota in response to either a plant-based diet (low-fat and -protein, high- fiber) or an animal-based diet (low-fiber, high-fat and -protein) (David et al., 2014). It is suggested that animal-based diets shift the gut microbial composition to favor bacterial species that prefer TMA containing nutrients (eg, choline and L-carnitine) as a fuel source, hence produce greater amounts of TMA and TMAO following the meal intake (Koeth et al., 2013).

Circulating levels of TMAO has been associated with prevalent CVD in Canadian multiethnic population (Mente et al., 2015) and infarcted coronary artery number in patients undergoing cardiovascular surgery (Mafune et al., 2016) as well as development of type-2 diabetes (Kim et al., 2015). Elevated plasma level of TMAO has been suggested to be a strong predictor of major adverse cardiac events, such as sudden death, nonfatal myocardial infarction and stroke (Tang et al., 2013; Zhu et al., 2016). Putting all together, the evidence may suggest that attempting to assess the cardiovascular impacts of a single protein from either plant or animal sources, may poorly reflect how that protein, within the food matrix, and in presence of other contributing factors may contribute to the risks and diseases.

1.3.3 Secondary plant metabolites

In recent years, secondary plant metabolites such as plant polyphenols have been the subject of increasing scientific interest because of their favorable role in prevention of chronic human diseases (Arts and Hollman, 2005; Scalbert et al., 2005), in particular CVDs (Mink et al., 2007; Cheng et al., 2017). The main health effects derived from polyphenols, with respect to CVD risk preventions have been linked to their strong antioxidant (Heim et al., 2002) and anti-inflammatory (García-Lafuente et al., 2009) activities as well as their effects on endothelial and platelet function (Lamuela-Raventós et al., 2005; Vita, 2005). Cocoa and chocolate products are among the most polyphenol-rich foods (460-610 mg/kg) along with tea and red wine (Spencer et al., 2008; Sudano et al., 2012). The main polyphenol compounds in cocoa are flavonoids and their subfamily flavanols, also called flavan-3-ols (Sudano et al., 2012). European Food Safety Authority has issued two health claims regarding the bioactivity of cocoa flavanols: cocoa flavanols help maintain normal blood pressure (EFSA, 2010b) and endothelium-dependent vasodilation which contributes to normal blood flow (EFSA, 2012). In order to achieve the claimed health benefits, 200 mg/d of cocoa flavanols, provided by 2.5 g of high-flavanol cocoa powder or 10 g of high-flavanol dark chocolate, must be consumed in the context of a balanced diet (Goya et al., 2016).

Flavanols are presented as either monomers (*e.g.* (+)- and (−)-isomers of catechin and epicatechin) or build-up of (epi)catechin subunit polymers, so-called proanthocyanidins (Khan et al., 2014). The molecular structure of flavanols determines the absorption through the gut and the rate of elimination *in vivo*, and subsequently their overall health effects (Habauzit and Morand, 2012).

Monomeric flavanols are absorbed in the small intestine with different rates depending on the type of isomers ((+) or (–) forms) (Donovan et al., 2006). Dimeric and trimeric proanthocyanidins with larger molecular size are degraded by intestinal and colonic microflora followed either by a poor absorption through gastrointestinal tract (about 10-100 times less than monomeric flavanols) or excretion in the feces (Habauzit and Morand, 2012; Field and Newton, 2013). Following the absorption, the monomers and dimers can be methylated, sulfated, or glucuronidated in the liver (Scalbert and Williamson, 2000). In general, these polyphenols have a relatively low bioavailability and a short half-life (Cooper et al., 2008) and also vary greatly in their physiological functions (Park et al., 2000; Kenny et al., 2007).

1.4 Cardiovascular impacts of fish and chocolate liquor as frequently consumed complex foods

Foods are mixtures of more than one active ingredients and the likelihood of their interactions is high. Therefore, recent investigations emphasize on the overall health effects from complete food rather than assessing the role of single nutrients (Hlebowicz et al., 2013; Akesson et al., 2014). Among the complex foods of special interest with respect to cardiovascular health are fish and chocolate liquor which will be discussed further in the following sections.

1.4.1 Fish

Increased consumption of fish and fish oil has been shown to exert beneficial effects to the patients with dyslipidemia, atherosclerosis, hypertension, obesity and inflammatory diseases (Aarsetoey et al., 2012). Several meta-analyses of observational studies have been published evaluating the effect of fish intake on various cardiovascular outcomes (Whelton et al., 2004; Bouzan et al., 2005; König et al., 2005; Xun et al., 2012; Zheng et al., 2012). Fish consumption has been linked to reduced risk of mortality from all causes as well as death from CHD and stroke, using data from 36 countries. According to these findings, an optimum fish intake (approximately 40-60 g/d) has led to an estimated 50% reduction in death from CHD in high-risk populations (Zhang et al., 1999a). Data from a recent meta-analysis showed that there is an inverse association between fish intake and the risk of acute coronary syndrome (Leung Yinko et al., 2014).

Similarly, a recent dose-response meta-analysis of 14 prospective cohort studies suggested that an increase of ~ 20 g/d in fish intake is significantly and inversely associated with the risk of CVD mortality but marginally and inversely associated with the risk of all-cause mortality (Jayedi et al., 2018). Accumulating evidence has led AHA to release a scientific statement, reinforcing its recommendation for intake of a variety of fish, preferably fatty fish, at least twice a week (Kris-Etherton et al., 2002). Similarly, the European Society of Cardiology-European Society of Hypertension 2013 guidelines advised patients with hypertension to eat fish at least twice a week (Mancia et al., 2013). The main health effects of fish consumption are attributed to its n-3 PUFAs which may reach to 40% in fatty fish (He et al., 2004; De Caterina, 2011), but may vary considerably according to season and the district of harvest, with freshwater species containing lower amounts than marine sources (Deckelbaum and Torrejon, 2012; FAO, 2012). The cardioprotective actions of fish oil include anti-arrhythmic activities, reduction of thrombotic and inflammatory processes, endothelial dysfunction and serum triacylglycerol level (Shimokawa, 2001; Kromhout et al., 2012).

Nevertheless, in recent years controversies on the cardiovascular benefits of fish oil have been raised by reports from several recent epidemiological and clinical trials that failed to replicate their health effects on CVD (Burr et al., 2003; Harris, 2013). Several papers have been published (Bjerregaard et al., 2003; Fodor et al., 2014) which have even questioned the reliability of primary evidence through which Bang and Dyerberg (1980) for the first time announced the alleged low occurrence of CAD in Greenland Eskimos due to high consumption of fatty fish (rich in n-3 FAs). Besides, data from the Diet and Reinfarction Trial-2, indicated that oily fish not only had no effects on all-cause mortality or CVD events after 3 to 9 years of follow-up, but also increased sudden cardiac death, largely confined to the subgroup given fish oil capsules (Ness et al., 2002). A systematic review of 11 cohort studies suggested that fish intake was not associated with reduced CHD mortality in low-risk populations whereas, a daily consumption of fish (40-60 g) was associated with lower mortality from CHD in high-risk populations (Marckmann and Gronbaek, 1999). These results may suggest that the discrepancy in the reports might be partly justified with the differences in the populations studied.

In addition to lipids, fish consists of protein, essential amino acids, vitamins and trace elements which their contribution to cardiovascular health cannot be ruled out (Mente et al., 2009; Zheng et al., 2012). For instance, fish is rich in sulfur-containing free amino acid taurine which comprises several cardioprotective properties (Larsen et al., 2013). There are also several other endogenous compounds such as anti-oxidative peptides embedded in fish which protect the n-3 PUFAs in fish from peroxidation (Jensen et al., 2009). The nutrient content of fish differs greatly between the species, and also within the species based on age, sex, environment and season (FAO, 2012). Most of the fish has 15%–20% of total body weight as protein with specific amino acid composition which seem to favor a hypocholesterolemic effects (Shukla et al., 2006; Chiesa et al., 2016). One possible mechanism can be through amplifying the expression of hepatic LDL receptors (Zhang and Beynen, 1993). Fish protein peptides and hydrolysates are also known for their antioxidant activities (Chalamaiah et al., 2012; Najafian and Babji, 2012).

The evidence from epidemiological and clinical studies have been contradictory regarding the role of fish protein on CHD (Bernstein et al., 2010) and its related risk factors such as obesity (Alkerwi et al., 2015). Besides, the findings from human studies comparing the intake of fish protein to other sources of animal protein have indicated that changes in circulating blood lipids have not been consistent, and in some cases were not favorable with respect to CVD risk factors. For example, substitution of lean white fish for other sources of animal protein with comparable or adjusted FAs profile, resulted in higher levels of total cholesterol (TC), HDL and LDL-ApoB in postmenopausal women (Jacques et al., 1992), whereas in premenopausal women, it has led to lower levels of very-low-density lipoprotein (VLDL)-triglyceride (TG) but higher levels of LDL-TG and LDL-ApoB (Gascon et al., 1996). Also, Lacaille et al. (2000) reported that diets high in lean fish protein compared with a combination of beef, pork, veal, eggs, milk, and milk protein in normolipidemic subjects produced lower TG/ApoB and cholesterol/ApoB ratios, but higher total ApoB and LDL-ApoB concentrations.

Additionally, fish seems to have the greatest impact on TMAO circulating levels (Cho et al., 2017) or urinary excursion (Lenz et al., 2004; Dumas et al., 2006) compared with other sources of dietary proteins. In a feeding trial by Cho et al. (2017), fish consumption produced nearly 50 times higher postprandial circulating TMAO concentrations in healthy young men compared to when they consumed eggs (abundant in choline) or beef (abundant in carnitine and choline). These findings,

together with the findings demonstrating the possible exposure to other toxic substances found in fish, such as methylmercury (Chan and Egeland, 2004), have raised the public concern regarding potential harm from fish consumption (Landfald et al., 2017). This might oblige fish consumption advisories to find a balance between the risks and benefits from fish consumption and provide the advice that emphasizes on “valued fish” but warns against “riskier fish" (Ginsberg and Toal, 2009).

1.4.2 Chocolate liquor

For centuries, cocoa-rich chocolate has been known for its good taste along with psychological and other health benefits. Chocolate is a food made by combining cocoa liquor with cocoa butter and sugar (Cooper et al., 2008). Cocoa liquor (chocolate liquor) is the paste made from fully ground, roasted, shelled, and fermented cocoa beans which contains both non-fat cocoa solids and cocoa butter (Hannum and Erdman, 2000). Although chocolate liquor contains relatively high amounts of fats, predominantly MUFAs (33% oleic acid) and SFAs (25% palmitic acid and 33% stearic acid), it is believed they are nonatherogenic and nonhypercholesterolemic for humans (Bracco, 1994) due to its low absorbability and its inhibitory effects on cholesterol absorption (Chen et al., 1989). This is supported by the findings from several human studies which have reported positive effects on blood lipids, through reduced TC, LDL and TG levels and increased HDL levels (Mursu et al. 2004; Baba et al., 2007; Hamed et al. 2008; Mellor et al., 2010; Di Renzo et al., 2013) while others have reported a neutral effect on blood lipids (Kurlandsky and Stote, 2006; Almoosawi et al., 2010).

The consumption of cocoa/chocolate products has been associated with the lower risk of total and cardiovascular mortality, diabetes, myocardial infarction, and/or stroke (Janszky et al., 2009; Buijsse et al., 2010; Oba et al., 2010; Larsson et al., 2011; Petrone et al., 2014). Meta-analyses and systematic reviews of intervention studies have also provided substantial evidence to display that cocoa/chocolate consumption affects multiple cardiovascular risk factors (Shrime et al., 2011) such as blood pressure (Ried et al., 2012) insulin resistance (Hooper et al., 2012), lipid profiles (Jia et al., 2010; Tokede et al., 2011), and flow-mediated vascular dilatation (Hooper et al., 2008).

Studies in both humans and animals, have mainly addressed the polyphenol contents of cocoa/chocolate for their health effects (Fraga et al., 2005; Grassi et al., 2005; Cienfuegos-Jovellanos et al., 2009; Yamazaki et al., 2010). Yet, chocolate liquor and all cocoa-based products contain several other components which are less accounted for their health impacts. For example, they are rich in methylxanthine compounds, predominantly caffeine and theobromine, both of which are known for their antioxidant (Shively and Tarka, 1984) and neuroprotective (Weisburger, 2001; Smit et al., 2004) activities. Also, dietary fiber extracted from cocoa has been shown to improve the lipidemic profile and serum antioxidant capacity in hypercholesterolemic rats, suggesting its role in cardiovascular risk reduction (Lecumberri et al., 2006 and 2007). Chocolate liquor and all cocoa-based products also contain several minerals necessary for vascular function including dietary magnesium, copper, potassium, and calcium, all known to reduce the risk of cardiovascular diseases (Katz et al., 2011).

Contrary to the evidence supporting the cardioprotective effects of chocolate consumption, several studies failed to show any significant health effects. For example, the EPIC-Norfolk cohort study found no association between chocolate intake and the risk of heart failure after controlling for comorbidities (Kwok et al., 2016). Two cohort studies found that individual who consumed chocolate were more likely to gain weight, which is an independent risk factor for the development of cardiovascular diseases (Greenberg and Buijsse, 2013; Greenberg et al., 2015). Also, data from a RCT evaluating the effects of dark chocolate rich in flavonoid revealed no significant alterations in microcirculatory parameters and the vascular function, in patients with symptomatic peripheral artery disease (Hammer et al., 2015). A meta-analysis of six cohorts and one cross sectional study, showed that only highest levels of chocolate consumption have been associated with lower risk for CVD and 29% reduced risk of stroke and yet it is not possible to establish a clear dose-response relation of chocolate consumption (Buitrago-Lopez et al., 2011). The use of various chocolate products with different polyphenol composition in diverse human sub-population groups (healthy, hypercholesterolemic, obese, dyslipidemic or diabetic) accounts for some of these contradictory findings and certainly influences potential conclusions.

2 Scope of the thesis

To date, most of the studies examining vascular impacts of fish or cocoa/chocolate have assessed their actions in isolation, for example, fish protein hydrolysate or cocoa extracts. However, the inclusion of single agents in the study without considering the potential impact of all nutrients and bioactive compounds in foods which work together in complex mechanisms may cause the occasionally imparted undesirable or contradictory results. Besides, most of the studies assessing the cardiovascular impacts of dietary food and components have been restricted to the analysis of traditional cardiovascular risk factors such as serum lipids, blood pressure, *etc*., while the contribution of morphological and histological analyses has been rare. This work was designed to investigate the role of frequently consumed complex foods (fish and chocolate liquor) on vascular lesion development and composition in genetically modified mice, using several morphological analyses.

Fish is a widely consumed food with various cardiovascular health effects. Studies addressing the vascular effects of fish have mainly focused on fish oil, while data on fish protein is particularly scarce. Therefore, the current work assessed the role of untreated fish protein on atherosclerosis development and composition in $ApoE^{-/-}$ mice using several morphological and histochemical analysis (**Paper I**). Also, as a part of the project investigating the possible involvements of ADAMs in vascular influences of fish oil (University of Kiel), several histochemical analyses were performed on aortic roots of $Ldl^{-/-}$ and WT mice (Martin Luther Univesity) to confirm the atheroprotective effects of fish oil on the vasculature (**Paper II**). Chocolate is another largely consumed complex food, with well-known cardioprotective effects. Nevertheless, most of the studies evaluating the vascular effects of chocolate have applied either cocoa/chocolate extracts or chocolates enriched with high dosages of polyphenols. Accordingly, the contribution of other bioactive components in chocolate for its vascular effects has been often neglected. Thus, in the present study chocolate liquor (**Paper III**) was subjected to investigation for atherosclerotic lesion development and composition using $ApoE^{-/-}$ mice. In the scope of this dissertation the following questions were addressed:

I. **What is the role of fish protein in vascular lesion development and composition? Does it engage the local or systemic risk profile?**

II. **What are the underlying mechanisms for fish protein vascular effects?**

III. **Do morphological assessments of atherosclerotic lesions confirm vascular protective role of fish oil supplementation? What are the underlying mechanisms?**

IV. **What is the role of chocolate liquor in vascular lesion development and composition? Does it engage the local or systemic risk profile?**

V. **What are the underlying mechanisms for chocolate liquor vascular effects?**

3 Experimental studies

3.1 Paper I

Yazdekhasti N, Brandsch C, Schmidt N, Schloesser A, Huebbe P, Rimbach G, Stangl GI: Fish protein increases circulating levels of trimethylamine-N-oxide and accelerates aortic lesion formation in apoE null mice. *Molecular Nutrition & Food Research* 2016; 60:358–36.

Abstract

SCOPE: The protective effect of fish consumption on the cardiovascular system has primarily been ascribed to n-3 fatty acids, but data investigating the vascular effects of fish protein consumption are scarce. This study aimed to investigate the vascular impact of fish protein in a mouse model of atherosclerosis.

METHODS AND RESULTS: Male apoE null mice were fed a Western diet containing 20% fish (turbot) protein, casein, or soy protein, for 16 wk. Morphometric analysis of the aorta revealed that the atherosclerotic plaque area of fish protein fed mice was twofold larger than that in casein- or soy protein-fed mice. The percentage area of calcification deposits in plaques of fish protein fed mice was higher (7.57%) than that in casein-fed (2.86%) or soy protein-fed (3.46%) mice, and fish protein fed mice exhibited higher plaque expression of CD68, CD36, and IL-6 than the other two groups. Fish protein intake was accompanied by increased serum concentrations of trimethylamine-N-oxide (7.03 ± 2.83 μmol/L), as compared with casein (0.92 ± 0.46 μmol/L) and soy protein (1.32 ± 0.54 μmol/L) intake.

CONCLUSION: The present data indicate adverse effects of fish protein on the vascular system, which could be attributable to the high serum trimethylamine-N-oxide concentrations in these mice.

KEYWORDS: Aortic lesion / Fish protein / Inflammation / Mice / Trimethylamine-N-oxide

3.2 Paper II

Speck N, Brandsch C, Schmidt N, **Yazdekhasti N**, Hirche F, Lucius R, Rimbach G, Stangl GI, Reiss K: The Antiatherogenic Effect of Fish Oil in Male Mice Is Associated with a Diminished Release of Endothelial ADAM17 and ADAM10 Substrates. *Journal of Nutrition* 2015; 145(6):1218-26.

Abstract

BACKGROUND: Growing evidence suggests that disintegrin and metalloprotease (ADAM) 17 (ADAM17) and ADAM10 contribute to the pathogenesis of vascular diseases. ADAM17 promotes inflammatory processes by liberating tumor necrosis factor α, interleukin 6 receptor (IL-6R), and tumor necrosis factor receptor 1 (TNFR1). ADAM17 and ADAM10 modulate vascular permeability by cleaving endothelial adhesion molecules such as junctional adhesion molecule A (JAM-A) and vascular endothelial cadherin (VE-cadherin), respectively.

OBJECTIVE: This study was designed to investigate whether a link might exist between the protective effects of fish oil (FO) supplementation against atherosclerosis and ADAM function.

METHODS: Male LDL receptor knockout (LDLR(-/-)) mice and male wild-type (WT) mice were fed a Western diet (200 g/kg fat, 1.5 g/kg cholesterol) containing either 20% lard (LDLR(-/-)-lard and WT-lard groups) or 10% lard combined with 10% FO (LDLR(-/-)-FO and WT-FO groups) for 12 wk. Atherosclerotic lesion development and fatty acid composition of liver microsomes were evaluated. ADAM10 and ADAM17 expression was determined by quantitative real-time polymerase chain reaction and immunoblot analyses. Concentrations of soluble ADAM substrates in plasma and liver extracts were measured by ELISA.

RESULTS: Diets supplemented with FO markedly reduced development of early atherosclerotic lesions in LDLR(-/-) mice (LDLR(-/-)-lard group vs. LDLR(-/-)-FO group mean ± SD: 29.6 ± 6.1% vs. 22.5 ± 4.2%, $P < 0.05$). This was not accompanied by changes in expression of ADAM17

or ADAM10 in the aorta or liver. No dietary effects on circulating TNFR1 (LDLR(-/-)-lard group vs. LDLR(-/-)-FO group mean ± SD: 1.22 ± 0.23 vs. 1.39 ± 0.28, $P > 0.2$) or IL-6R (1.06 ± 0.12 vs. 0.98 ± 0.09 fold of WT-lard group, $P > 0.1$), classical substrates of ADAM17 on macrophages, and neutrophil granulocytes were observed. However, a reduction in atherosclerotic lesions in the LDLR(-/-)-FO group was accompanied by a significant reduction in the circulating endothelial cell adhesion molecules JAM-A (LDLR(-/-)-lard group vs. LDLR(-/-)-FO group mean ± SD: 1.42 ± 0.20 vs. 0.95 ± 0.56 fold of WT-lard group, $P < 0.05$), intercellular adhesion molecule 1 (1.15 ± 0.14 vs. 0.88 ± 0.17 fold of WT-lard group, $P < 0.05$), and VE-cadherin (0.88 ± 0.12 vs. 0.72 ± 0.15 fold of WT-lard group, $P < 0.05$), reflecting reduced ADAM activity in endothelial cells.

CONCLUSION: FO exerted an antiatherogenic effect on male LDLR(-/-) mice that was accompanied by a reduced release of ADAM17 and ADAM10 substrates from endothelial cells. It is suggested that FO-decreased ADAM activity contributes to improved endothelial barrier function and thus counteracts intimal lipoprotein insudation and macrophage accumulation.

KEYWORDS: ADAM; LDLR knockout; atherosclerosis; endothelial permeability; fish oil; shedding

3.3 Paper III

Yazdekhasti N, Brandsch C, Hirche F, Kühn J, Schloesser A, Esatbeyoglu T, Huebbe P, Wolffram S, Rimbach G, Stangl GI: Impact of chocolate liquor on vascular lesions in apoE-knockout mice. *Clinical Science (London)* 2017; 131(20):2549-2560.

Abstract

Cocoa polyphenols are thought to reduce the risk of cardiovascular diseases. Thus, cocoa-containing foods may have significant health benefits. Here, we studied the impact of chocolate liquor on vascular lesion development and plaque composition in a mouse model of atherosclerosis. Apolipoprotein E (apoE)-knockout mice were assigned to two groups and fed a Western diet that contained 250 g/kg of either chocolate liquor or a polyphenol-free isoenergetic control paste for 16 weeks. In addition to fat, protein, and fibers, the chocolate liquor contained 2 g/kg of polyphenols. Compared with the control group, mice fed the chocolate liquor had larger plaque areas in the descending aorta and aortic root, which were attributed to a higher mass of vascular smooth muscle cells (VSMCs) and collagen. Vascular lipid deposits and calcification areas did not differ between the two groups. The aortic tissue level of interleukin-6 (IL-6) mRNA was 5-fold higher in the mice fed chocolate liquor than in the control mice. Chocolate-fed mice exhibited an increased hepatic saturated to polyunsaturated fatty acid ratio than the controls. Although the chocolate liquor contained 14 µg/kg of vitamin D2, the chocolate liquor-fed mice did not have measurable 25-hydroxyvitamin D2 in the serum. These mice even showed a 25% reduction in the level of 25-hydroxyvitamin D3 compared with the control mice. Overall, present data may contribute to our understanding how chocolate constituents can impact vascular lesion development.

KEYWORDS: IL-6; apoE-knockout mice; atherosclerosis; chocolate liquor; vascular smooth muscle cells

4 Discussion

4.1 The role of fish in atherosclerotic lesion development and composition

The role of fish protein in vascular health and in particular their effects on atherosclerotic plaque progression and composition is not yet clear. The current study assessed the impacts of fish protein on atherosclerotic plaque development and composition in $ApoE^{-/-}$ mice. Animals were fed Western type diet containing 20% fish protein, or casein, or soy protein, for 16 weeks. The main findings were that $ApoE^{-/-}$ mice which consumed fish protein induced a larger vascular lesion area (2-fold larger) with large necrotic cores ($p < 0.001$) compared with mice fed casein or soy protein. Also, extensive calcification was observed on aortic roots of fish protein fed mice (7.57%) compared with the casein (2.86%) and soy protein (3.46%) groups ($p < 0.05$) (**Paper I**). Plaque calcification is a feature of advanced lesions (Otsuka et al., 2014), and often occurs in the presence of apoptotic SMCs and macrophages where they together with other cellular debris form the necrotic cores (Otsuka et al., 2014; Tabas et al., 2015). Necrotic core *per se* is an excellent morphologic marker for lesion instability and predicting rupture-prone plaques (Finn et al., 2010). Therefore, these features suggest adverse effects of fish protein in accelerating atherosclerosis process and shifting the lesion to an advanced-type lesion in an identical time span compared with casein and soy protein (**Paper I**). Similar results were reported by Goulding et al. (1983) where fish protein diet, induced a larger aortic lesion area (c. 70% surface involvement) along by extensive calcification of medial elastic tissue in rabbits, when compared with two isonitrogenous diets containing either soy or milk protein.

On the contrary, several studies in humans and animals have reported atheroprotective role of fish protein. For example, a recent observational study discovered that Portuguese who routinely consumed more fish products (13.4%) compared to Swedes with less fish consumption (7.8%), developed smaller atherosclerotic plaques characterized by less lipids, smaller cores, less apoptosis, and more SMC, reflecting a more stable plaque type in Portuguese (Gonçalves et al., 2015). In this study however, the information about the exact types of seafood dietary sources, the exact amounts of dietary elements, or other contributing factors were not provided, hence cannot be fully comparable with those from the current study (**Paper I**).

Also, two recent *in vivo* studies have suggested antiatherosclrotic properties of lean seafood compared with other sources of animal proteins such as beef (Gabrielsson et al., 2012) or chicken (Jensen et al., 2016). However, in both studies these atheroprotective effects were eventually attributed to the high n-3 PUFA and taurine contents found in the seafood (Gabrielsson et al., 2012; Jensen et al., 2016). Parolini et al. (2014) found that supplementation with % (w/w) salmon protein hydrolysate attenuated atherosclerosis in $ApoE^{-/-}$ mice and reduced several risk factors related to atherosclerotic disorders. Likewise, these results cannot be compared to those from the present study (**Paper I),** because the fish protein used in the study by Parolini et al. (2014) was treated with different proteases and fractionated by micro- and ultrafiltration to obtain peptides <1200 kDa. It has been shown that peptides produced by biotechnical procedures may differ in their biofunctional properties with those emerged from endogenous gastrointestinal proteases (Cam and Mejia, 2012).

Searching for possible roles of amino acids, all proteins were further subjected to the analysis of their amino acid profile. The results showed identical amounts of branched chain amino acids in fish protein and soy protein but higher amounts in casein **(Paper I**). Branched chain amino acids were found by the Insulin Resistance Atherosclerosis Study to be strongly associated with the incidence of diabetes and metabolic abnormalities, both known as underlying causes of atherosclerosis (Lee et al., 2016). The amounts of methionine in fish protein and casein was almost twice the values found in soy protein (**Paper I**). Dietary supplementation with methionine has been shown to promote early atherosclerosis in $ApoE^{-/-}$ mice (Zhou et al., 2001). Of significant importance was the lysine/arginine ratio of the proteins, known to be an important determinant of a protein atherogenic potential (Kritchevsky et al., 1978; Czarnecki and Kritchevsky, 1979). Indeed, the addition of lysine to soy protein has been sufficient to significantly increase its atherogenicity while addition of arginine to casein has lowered its lysine/arginine ratio and reduced its atherogenicity in rabbits (Kritchevsky and Tepper, 1968; Kritchevsky, 1979). In this work (**Paper I**) casein had the highest lysine/arginine ratio (2.56) compared with soy protein (0.92) and fish protein (1.52). Surprisingly the lysin/argenine ratio of fish protein in the present study (1.52) was almost similar to the calculated ratio for fish protein (1.44) in the study by Kritchevsky et al. (1982), where fish protein resulted in smaller lesion size in rabbits. On the other hand, in the study by Goulding et al. (1983) where fish meal diets caused an extensive plaque development (almost

7 times than that of casein), the lysine/arginine ratio of fish protein was only 0.95, similar to the ratio from soy protein in the same study (0.85) as well as the ratio from soy protein used in the present study (0.92). According to these mixed observations, it appears rather complicated to draw a clear pattern between amino acid profile of dietary proteins and their vascular manifestations, hence the amino acid profile of dietary proteins in the current study may not explicitly explain the atherogenic role of fish protein in this study (**Paper I**).

The lipids and fatty acids of all experimental proteins were also evaluated. The result showed strikingly higher amounts of total lipids and total FAs in fish protein, in contrast to casein and soy protein. The fat content in fish protein comprised mainly of SFA (36.55 g/kg) and MUFAs (31.10 g/kg). All the experimental diets were low in PUFA (**Paper I**). Several papers have debated that diet rich in SFA and low in PUFA triggers the atherosclerosis development (Astrup et al., 2011). Also, MUFA-enriched diets and even their replacement with SFA-enriched diets have been associated with an increased risk of atherogenicity in both animals and humans (Brown et al., 2007; Degirolamo et al., 2009). Therefore, it was tempting to speculate that the high levels of SFA and MUFA as well as higher amonts of total fats in fish protein (**Paper I**), have been the main reason for its adverse effects of however, the evidence supporting this idea was not sufficient. For example, the serum lipid parameters were not affected by the total fats and the type of FAs in this study and were comparable across the three groups. Although the reason behind this observation might be that in general $ApoE^{-/-}$ mice seem to have less susceptibility to the changes in dietary FA composition when compared with other animal models of atherosclerosis such as $Ldlr^{-/-}$ mice (Merkel et al., 2001). Even studies addressing the vascular benefits of fish protein and seafood consumption in experimental animals have found no changes in blood lipid concentrations of $ApoE^{-/-}$ mice (Parolini et al., 2014; Jensen et al., 2016), whereas in $Ldlr^{-/-}$ mice the lipid profile has improved (Gabrielsson et al., 2012).

To illustrate the possible involvement of local inflammation in vascular lesion alterations, aortic roots were subjected to immunohistochemical staining of CD_{68} as well as gene expression analyses of CD_{36} and IL-6 (**Paper I**). The results showed a higher expression of CD_{68}, CD_{36} and IL-6 on aortic valves of mice fed fish protein diet ($p < 0.05$) compared with those on casein and soy protein groups, suggesting local atherogenic activity of fish protein. The expression of MMP-9, TNF-α , ICAM-1, and MCP-1 remained comparable among the three groups. Contrarily, Parolini et al.

(2014) detected lower expression of the adhesion molecules ICAM-1 and VCAM-1, and the chemokine MCP-1, in the pooled aortic arch of salmon protein hydrolysate-treated mice (Parolini et al., 2014). Also, mice given cod-scallop exhibited lower expression of VCAM-1 (19%) compared with mice fed chicken (Jensen et al., 2016). Lower circulating levels of proinflammatory proteins such as IL-6, IL-12, TNF-α and MCP-1 have been observed following the treatment of $Ldlr^{-/-}$ /$ApoB^{100/100}$ mice with salmon peptide fractions (Chevrier et al., 2015). The comparative data in human studies is scarce. Two human studies investigating lean fish intake reported no significant effects on inflammatory parameters in patients with CHD (De Mello et al., 2009), or in healthy subjects (Elvevoll et al., 2006). Only, in insulin resistant overweight subjects, C-reactive protein was reduced by 24% following the cod intake while it was increased by 13% in individuals consuming other animal proteins (Ouellet et al., 2008).

Subsequent investigation focused on the role of other contributing factors present in food, such as food toxins. Among the toxic substances available in natural food is TMA, a product of decomposition of plants and animals (Zhang et al., 1999b). TMA is used as an indicator of general fish edibility, spoilage and overall quality. In this study TMA in fish protein was as high as 59.2 mg/100 g tissue which was almost 21-fold higher than the values found in casein or soy protein (**Paper I**). The maximum allowable TMA levels for fish, in international trading, is between 5 to 10 mg/100 g tissue (Johnston et al., 1994). TMA is responsible for the fishy odor of seafood and can be converted to proatherogenic TMAO, an odorless marine compound (Yancey and Siebenaller, 2015). Several *in vivo* studies have shown that the direct provision of TMAO and its dietary precursors (e.g. choline or L-carnitine) within the diet accelerates atherosclerosis development (Wang et al., 2011; Koeth et al., 2013). In contrast, a targeted suppression of microbial TMA/TMAO production has effectively inhibited diet-induced atherosclerosis (Wang et al., 2015b).

Increased consumption of fish and other seafood containing significant quantities of TMA, TMAO or their precursors can increase the TMAO levels in blood circulations (Wang et al., 2011). The circulating concentration of TMAO in the current work (**Paper I**) was nearly 6-fold higher in $ApoE^{-/-}$ mice which consumed fish protein (7.03 ± 2.83 mol/L), as compared with animals fed casein (0.92 ± 0.46 mol/L) and soy protein (1.32 ± 0.54 mol/L). An increased plasma level of TMAO after fish consumption has been reported by several studies including a recent RCT (Cho

et al., 2017). Also, the findings from a pilot study revealed that urinary TMAO may reach to its highest levels in individuals who consumed a seafood diet (> 5000 μmol/l) compared with those who ate other sources of animal proteins such as red meat diet or an egg (139 μmol/l) (Zhang et al., 1999b). Fasting plasma TMAO levels is currently considered as an independent predictor for presence of increased atherosclerotic burden and complexity in patients with CAD (Senthong et al., 2016). Also, Wang et al. (2011) observed that high plasma levels of TMAO in $ApoE^{-/-}$ C57BL/6J mice led to foam cell formation and large aortic atherosclerotic plaque development. This may provide an explanation for the observed athoergenic effects of fish protein in this study (**Paper I**). Surprisingly and in contrast to the others' (Wang et al., 2011; Koeth et al., 2013) and own findings, a recent study in $ApoE^{-/-}$ transgenic mice expressing cholesteryl ester transfer protein indicated that high TMAO levels were inversely, and independently from the changes in plasma lipids associated with aortic lesion size in both aortic root and thoracic aorta; suggesting a protective and not a causative effect of TMAO on atherosclerosis development (Collins et al., 2016). These dissimilar results might be because of the differences in how this study was conducted, particularly the use of male mice that express human cholesteryl ester transfer protein.

TMAO promotes atherosclerosis through arterial endothelial cell activation (Seldin et al., 2016) as well as upregulation of pro-atherogenic SRs and accordingly enhancing the accumulation of cholesterol in foam cells (Koeth et al., 2013; Zhu et al., 2016). Therefore, these results might provide an explanation for the higher expressions of CD_{68} and CD_{36} on mice aorta following the intake of fish protein (**Paper I**). Also, numerous studies in both humans and animals have discovered a potential involvement of TMAO in the expression of inflammatory marker IL-6 through activation of nuclear factor-kappa B (NF-κB) pathway (Seldin et al., 2016; Rohrmann et al., 2016; Ma et al., 2017; Al-Obaide et al., 2017), suggesting a plausible mechanism for the enhanced expression of IL-6 following fish protein intake (**Paper I**). It must be noted, that the amounts of TMA or free TMAO found in the fish highly depend on the fish species (higher amounts in marine- vs. freshwater-species), type of the fish (higher amounts in lean- vs. fatty fish), and the degree of freshness (higher amounts in rotting- vs. fresh fish), hence the finding of the current work cannot be fully associated to other types of fish with different origin or degree of freshness. Nonetheless, the results from this work (**Paper I**) may suggest that fish protein contains various components which may affect the cardiovascular health differently.

One of these components is fish oil, which is widely believed to be atheroprotective. The mechanism governing the cellular and molecular actions of fish oil is not yet clear. Of special interest for the project conducted by the research group at the Kiel University was to investigate the possible involvement of ADAM17 and ADAM10 in atheroprotective effects of fish oil using $Ldlr^{-/-}$ and WT mice (**Paper II**). The use of $Ldlr^{-/-}$ mice in this study was because of their higher susceptibility to dietary lipids compared with the other mice models such as $ApoE^{-/-}$ mice (Asset et al., 2001; Zampolli et al., 2006). The animals were fed a Western diet containing either 20% lard ($Ldlr^{-/-}$ -lard and WT-lard groups), or 10% lard combined with 10% fish oil ($Ldlr^{-/-}$ -FO and WT-FO groups) for 12 weeks. The aortic roots of the experimental animals underwent histochemical staining at the laboratory of Martin Luther University for assessment of total plaque area, lipid deposits and collagen content.

As expected, $Ldlr^{-/-}$ mice developed extensive vascular lesions compared with WT mice, characterized with more lipid deposits and higher collagen content (p *for all* < 0.001). Diet supplemented with fish oil significantly inhibited the lesion development and lipid accumulations in $Ldlr^{-/-}$ mice compared with lard fed counterparts (**Paper II**). Similar reports have been released by other animal studies. An incremental substitution of fish oil for SFs, strongly inhibits the atherosclerosis development in $Ldlr^{-/-}$ mice, exhibiting only small lesions (~100.103 mm^2) after 12 weeks of intervention (Chang et al., 2014). Yang and coworkers (2016) showed that in $Ldlr^{-/-}$ mice a diet enriched with fish oil-derived long chain MUFA (LCMUFA) alone, significantly reduced the atherosclerotic lesions by 50 % and 45% compared with the control (no LCMUFA) and olive oil diets respectively (Yang et al., 2016). Besides, mice fed fish oil-derived LCMUFA displayed a more stable plaque morphology characterized by fewer lipid deposits and less macrophages accumulation. Although $LDL^{-/-}$ mice are suggested to constitute the most appropriate model for studying the vascular effects of n-3 FAs (Zampolli et al., 2006; Asset et al., 2001), several studies have shown that fish oil has similarly inhibited atherosclerotic lesion formation in $ApoE^{-/-}$ mice (Wang et al., 2004; Eilertsen et al., 2011; Sun et al., 2014). Only few human studies have investigated the role of fish oil on atherosclerotic plaque size and composition. A histological study demonstrated that the carotid atherosclerotic lesions from patients who consumed fish oil before carotid endarterectomy were less heavily infiltrated with macrophages compared with those who ate sunflower oil. Besides, these plaques were more likely to be fibrous-cap atheromas and

displayed fewer signs of inflammation, suggesting the role of fish oil in plaque stability (Thies et al., 2003). In the current study plaque collagen content was not affected by the diet (**Paper II**), although in an earlier study, both ApoE$^{-/-}$ and Ldlr$^{-/-}$ mice exhibited a higher plaque collagen content along with increased SMCs, but less macrophages, after consumption of a diet rich in EPA (Matsumoto et al., 2008).

Recent animal studies have suggested various mechanisms for atheroprotective role of fish oil (Yang et al., 2011; Brown et al., 2012; Yang et al., 2013; Sun et al., 2014). It is also suggested that fish oil may exert a direct influence on all components of atherosclerosis at the site of the lesions (Yang et al., 2016). Among the local mediators are ADAMs, which participate in both cellular adhesion and proteolytic cleavage of various cell surface molecules, therefore determine the cellular fate, proliferation, and growth through various mechanisms (Blobel, 2005, Edwards et al., 2008; Holdt et al., 2008; Schulz et al., 2008; Oksala et al., 2009; Donners et al., 2010). Of special interest in the second work (**Paper II**) was to investigate the role of ADAM10 and ADAM17 in antiatherogenic actions of fish oil. Since, studies in human and mice have detected ADAM10 and ADAM17 localizations at the site of atherosclerotic lesions (Canault et al., 2006; Satoh et al., 2008; Donners et al., 2010), their cellular abundance in the aorta was also quantified in this work. The findings showed no significant genotype-specific or nutrition-dependent differences amongst all four groups (data from immunohistochemical analysis) (**Paper II**).

Since fish oil is known to alter the course of inflammatory stimulation during the disease progress (Thies et al., 2003; Weber and Noels, 2011), the extent of inflammation involved in the early atherogenesis in Ldlr$^{-/-}$ mice was studied (**Paper II**). The plasma and liver concentrations of classical macrophage- and leukocyte-derived inflammatory markers (e.g. TNF-α, IL-6, *etc*.) were evaluated. Despite the clear lesion development in knockout mice compared to the WT mice, and also smaller lesion size in knockout mice fed fish oil than those fed with lard, there were no significant difference in the circulating and hepatic levels of cytokines among the groups; only a higher plasma concentration of MCP-1 was detected in knockout mice compared to the WT mice (**Paper II**). According to previous data, fish oil treatment in laboratory mice has resulted in decreased (Sadeghi et al., 1999; Bhattacharya et al., 2007), increased (Petursdottir et al., 2002; Carlson et al., 2015), or unaltered (Wallace et al., 1999) inflammatory responses. The inconsistent findings might point to the application of different mouse models, high/low dosage of fish oil

supplement, and/or the level of fish oil purification (Petursdottir et al., 2002; Carlson et al., 2015). Similar discrepancy has been observed in results from human studies. Several human studies have emphasized that dietary marine n-3 FAs were associated with reduced plasma levels of inflammatory markers (Madsen et al., 2001; Lopez-Garcia et al., 2004), while others have reported no significant changes in circulating proinflammatory cytokines obese hypertriglyceridemic subjects following the treatment with PUFA-rich diet (Skulas-Ray et al., 2011). According to the report from a recent systematic review of intervention studies, in healthy individuals and people with cardiovascular risk and established CVD, the effects of fish oil on inflammatory markers has been mixed with either reduction, or no change, in only one of the inflammatory markers (e.g. IL-6), but the reasons for these controversies are yet unknown (Myhrstad et al., 2011).

The next attempt was to assess the ADAMs activity in aorta and several other organs (**Paper II**). ADAMs shed several cell-signaling molecules and cell adhesion molecules, contributing to successful recruitment of leukocytes to the inflammation site. ADAM17 is the main shaddase of VCAM-1, ICAM-1, and JAM-A (Gooz, 2010; Scheller et al., 2011), while ADAM10 mainly releases VE-cadherin (Schulz et al., 2008). VCAM-1 and ICAM-1 facilitates leukocyte adhesion respectively to the vascular endothelium (Garton et al., 2003; Ponnuchamy and Khalil, 2008) and integrins (Tsakadze et al., 2006), while JAM-A and VE-cadherin participate in leukocyte diapedesis through the endothelial layer (Ponnuchamy and Khalil, 2008; Vestweber, 2008; Koenen et al., 2009). The research group at the University of Kiel further addressed the question whether dietary fish oil supplementation has affected the generation of these substrates in aorta and other organs (**Paper II**). According to their findings, the atheroprotective effects of fish oil supplementation was reflected in a significant decline in the plasma concentration of JAM-A, ICAM-1, and VE-cadherin, as well as a slight reduction in soluble VCAM-1 concentration. Several human studies have reported reduced circulating levels of soluble VCAM-1 in elderly subjects after supplementation with EPA and DHA (Miles et al., 2001) and reduced soluble ICAM-1 and soluble VCAM-1 in the bloodstream of patients with metabolic syndrome after EPA supplementation (Yamada et al., 2008). In a study by Zhao et al. (2004), diet rich in PUFA resulted in significant decrease in circulating concentrations of ICAM-1, VCAM-1 and E-selectin. Comparable data from animals are scarce, since most of the animal studies have focused on the gene expression of these molecules in respect to the fish oil supplementation (Casós et al., 2008).

Also, data from cell culture studies have shown that subsequent to the exposure to marine n-3 PUFAs, substantial reductions have been observed in expression of adhesion molecules on monocytes (Hughes et al., 1996) and endothelial cells (De Caterina et al., 1994; Weber et al., 1995; Collie-Duguid and Wahle, 1996), and also in expression of JAM-A in human endothelium (Massaro et al., 2016). In conclusion, the findings from this work suggest that the reduced ADAMs shedding activity in endothelial cells has directly contributed to endothelial barrier improvement and suppressed atherogenesis following the fish oil supplementation (**Paper II**).

4.2 The role of chocolate liquor in atherosclerotic lesion development and composition

The evidence regarding the effects of cocoa/chocolate on the atherosclerosis progress are not conclusive. The present trial was conducted to investigate the role of chocolate liquor on vascular lesion development and composition in $ApoE^{-/-}$ mice fed a semisynthetic Western diet, containing 250 g/kg of either chocolate liquor, or a polyphenol-free isoenergetic control paste for 16 weeks (**Paper III**). The chocolate liquor contained ~2 g/kg polyphenols, in particular epicatechin (1.07 ± 0.05 g/kg). The results retrieved from *en face* analysis showed a significantly larger lesion area in total aorta of mice fed chocolate liquor compared to the controls ($p < 0.05$). Similarly, the data from histochemical and immunohistochemical assessments of aortic roots displayed that mice fed chocolate liquor had a larger plaque size ($p < 0.05$), characterized with more VSMCs (3-fold higher, $p < 0.01$) and larger collagen area ($p < 0.01$). The rest of plaque components including calcified area, macrophages and lipid content remained identical between the two groups (**Paper III**). Collagen contributes to the plaque structural integrity and mechanical strength therefore, "not enough" collagen leads to plaque weakness and vulnerability, while "too much" collagen could also lead to arterial stenosis (Rekhter, 1999). Most of the collagen in the plaque is produced by SMCs. In fact, migrated and proliferated SMCs can synthesize up to 25 to 46 times more collagen, contributing to the fibrous nature of the plaque (Rekhter, 1999; Doran et al., 2008). In addition to the role of VSMCs in plaque collagen production, collagen also plays a significant role in VSMC proliferation and migration (Rocnik et al., 1998). Therefore, the parallel increase in both collagen and VSMCs observed here (**Paper III**) can be owing to their mutual interactions. Since these two plaque components, VSMCs and collagen, are known to stabilize the atherosclerotic plaques (Rudijanto, 2007; Badimon and Vilahur, 2014), it can be speculated that chocolate liquor has also induced a stable-type of atherosclerotic lesions in $ApoE^{-/-}$ mice.

Comparable to own findings, Yakala et a. (2013) similarly reported unfavorable effects of chocolate consumption on atherosclerosis. According to their results, ApoE*3-Leiden mice fed two different types of chocolates ("chocolate A" with relatively higher polyphenol and lower fiber content compared to "chocolate B") and particularly those fed with "chocolate A", experienced increased plasma-cholesterol levels and extensive atherosclerotic plaque formation (Yakala et al., 2013). On the contrary, Guan et al. (2016) reported that male $ApoE^{-/-}$ mice fed a Western diet containing both low (0.2%), or high (2%) amounts of cocoa powder, had smaller lesion size in their total aorta (*en face*) respectively by 42% and 63%, and less lipid deposits respectively by 47% and 32%, compared with the controls. The dosage of cocoa powder used in Guan et al. (2016) study was equivalent to respectively 4.2 (low), or 42 (high) g daily consumption by humans, which is in accordance with the amounts previously reported by other human studies (Monagas et al., 2009; Khan et al., 2012). However, the dosage of chocolate applied in the current study (250 g/kg diet) related to humans' daily consumption equals to one bar of chocolate, which is rather high but not irrelevant amounts (**Paper III**).

In order to find the underlying cause for VSMCs migration and proliferation, the degree of local (descending aorta) and systemic (serum) inflammation was further investigated. The messenger ribonucleic acid (mRNA) concentrations of ICAM-1, CD_{36}, TNF-α, MCP-1, MMP-9 were not different between the groups ($p > 0.05$) whereas mice fed chocolate liquor had almost 5-fold higher expression of IL-6 in their descending aorta compared with those on control paste diet ($p < 0.05$). The higher gene expression of IL-6 on the aorta was not supported by higher serum concentration of IL-6, henc suggesting a local action of chocolate liquor but not systemic (**Paper III**). It has been previously shown that IL-6 cytokines and their signaling events may contribute to both, atherosclerotic plaque development and lesion destabilization through several mechanisms (Schuett et al., 2009; Yoshimoto and Yoshimoto, 2014), among which provoked migration and proliferation of VSMCs seem to prevail (Ikeda et al., 1991; Nabata et al., 1990; Klouche et al., 2000). In return, VSMCs stimulate the secretion of more IL-6 at the local sites (Orr et al., 2010); hence a vicious circle occurs which ultimately leads to plaque expansion and complication.

Thus, it can be speculated that the intake of chocolate liquor (**Paper III**) has stimulated the expression of IL-6 in the vasculature, and IL-6 has further provoked VSMC migration and proliferation, while in return VSMC has played its role in collagen production. Contrary to own

findings, several *in vivo* and *ex vivo* studies have reported repressive roles of cocoa and its polyphenols in markers of inflammation, in particular IL-6 expression. Gu and colleagues (2014) showed that male mice fed a high-fat diet supplemented with 8% cocoa powder, for 10 weeks, presented a significantly reduced plasma IL-6 concentration (30.4%), compared with mice that consumed only a high fat diet. Also, a mice model of experimental myocarditis showed that treatments with the cocoa polyphenol extract markedly reduced mRNA expressions of IL-1β, IL-6, E-selectin, VCAM-1 in the heart (Zempo et al., 2016). Administration of a high-cocoa diet (4.8 g/kg/day) for seven days lowered the production of pro-inflammatory cytokines IL-6 and TNF-α in rat peritoneal macrophages *ex vivo* (Ramos-Romero et al., 2012). The dissimilar outcomes concerning the role of cocoa/chocolate intake on inflammatory responses, can be as a result of differences in composition of nutrients in cocoa/chocolate (e.g. polyphenol content, fiber, added milk and sugar), the dosage and duration of administration, the polyphenols bioavailability and even the composition of the control diet.

Chocolate liquor applied in this work comprised high levels of SFAs (mainly palmitic and stearic acids) and MUFAs (mainly oleic acid), but low levels of PUFAs (**Paper III**). The analysis of liver FAs concentrations correspondingly showed lower concentrations of n-3 (p=0.009) and n-6 (p=0.011) PUFAs in mice fed chocolate liquor, but comparable amounts of SFA and MUFA in both groups. The significant difference in single fatty acid (PUFA concentration) led to a significantly higher hepatic ration of SFA/PUFA in mice fed chocolate liquor in contrast to the control group. In addition, the circulating ApoB level, as an indicative for total cholesterol-carrying lipoprotein was not affected by the total fat and FAs composition of the diets. Also, circulating levels of fructosamine yielded no significant difference between the two groups (**Paper III**). Serum fructosamine is a biomarker of glycated serum proteins and higher blood glucose (Mosca et al., 1987) and recently has been shown to be associated with severity of coronary artery atherosclerosis in insulin resistant pigs (Nichols et al., 2015). Ealier in 2007, Tomaru and colleagues fed mice with cocoa liquor comprising various doses of proanthocyanidins and similarly did not reach any significant changes in blood fructosamine levels in the healthy mice; however, in obese-diabetic animals, cocoa liquor procyanidins declined the blood levels of fructosamine significantly and dose-dependently.

Chocolate liquor also comprised slight amounts of serotonin (2.7 mg/kg) (**Paper III**), which was in accordance with the concentrations reported previously (Guillén-Casla et al., 2012). Serotonin is a decarboxylated derivative of the amino acid tryptophan, known as a neurotransmitter and also as a vasoactive substance, which participates in local vascular injury and inflammation associated with atherosclerosis (Katz et al., 1994; Willerson et al., 1989). It is found that serotonin stimulates VSMC proliferation, contraction and migration (Tamura et al., 1997) and enhances the secretion of interleukin-6 in VSMCs, through its effects on 5-HT_{2A} receptors (Ito et al., 2000). It was tempting to assume that serotonin in chocolate liquor may have provoked inflammatory signals in VSMCs, however the serum serotonin concentration was not affected by the low amounts of dietary serotonin in chocolate liquor and remained comparable across the two groups (**Paper III**).

A possible explanation for the observed results may concern the vitamin D_2 content of the chocolate liquor. The current study was first to report a substantial amounts of vitamin D_2 (14.1 μg) in chocolate liquor (**Paper III**). Nevertheless, the serum concentration of 25-hydroxy vitamin D $(25(OH)D)_2$ was not detectable in both groups. A recent meta-analysis of several RCTs reported that vitamin D_2 does not increase the serum total 25(OH)D concentrations similarly effective as vitamin D_3 (Tripkovic et al., 2012). The biological reason behind this phenomenon may be the lower affinity of $25(OH)D_2$ molecule for binding to vitamin D binding protein, therefore leads to its increased catabolism and lower concentrations in the circulating blood (Hollis et al., 1984). Surprisingly, the serum concentration of $25(OH)D_3$ was 25% lower in mice fed chocolate liquor than the controls (**Paper III**). Several studies have similarly reported a decline in serum $25(OH)D_3$ levels after oral administration of vitamin D_2 (Glendenning et al., 2009; Lehmann et al., 2013; Baur et al., 2016). It seems that vitamin D_2 impairs hydroxylation of vitamin D_3 molecules that are already present in the circulation (Holmberg et al., 1986). A large body of research from large epidemiological (Cigolini et al., 2006; Martins et al., 2007; Dobnig et al., 2008) and clinical studies (Carrelli et al., 2011; Cheraghi et al., 2012; Ellam et al., 2014) in mice and humans, has indicated that low levels of circulating 25(OH)D are associated with atherosclerosis and other cardiovascular disorders in humans. In an *in vivo* study, vitamin D-deficient mice developed 2-fold larger lesions in aortic arch and 2-8-fold larger lesions in thoracic and abdominal aorta when compared with vitamin D-sufficient mice (Weng et al., 2013).

There are also reports indicating negative associations between serum vitamin D levels and pro-inflammatory cytokines, such as TNF-α and IL-6 (Mateen et al., 2017). In diabetes mice, vitamin D_3 deficiency has been associated with enhanced IL-6 expression in the liver (Labudzynskyi et al., 2016). Besides, crossing vitamin D receptors null ($VDR^{-/-}$) and $Ldlr^{-/-}$ mice has resulted in a mouse model ($Ldlr^{-/-}VDR^{-/-}$) with accelerated atherogenesis (Szeto et al., 2012). Similarly, $ApoE^{-/-}VDR^{-/-}$mice have shown higher levels of serum IL-6 and extensive aortic lesions compared with $ApoE^{-/-}VDR^{+/+}$ mice (Bozic et al., 2015). Whether the reason behind the vascular IL-6 expression and the subsequent lesion development is the higher levels of vitamin D_2 in chocolate liquor or the subsequent lower $25(OH)D_3$ in mice fed chocolate liquor (**Paper III**) warrants further investigation. Also due to the animal nature of the study, further studies in humans are needed to investigate the direct effects of chocolate liquor and its various components on vascular lesion development and composition. The findings from the current study did not show cardioprotective effects of the chocolate liquor.

4.3 Conclusions

The findings from the performed animal studies answered the main questions addressed in this work.

I. **What is the role of fish protein in vascular lesion development and composition? Does it engage the local or systemic risk profile?** Fish protein induced 2-fold larger vascular lesion area in $ApoE^{-/-}$ compared with casein and soy protein. Compared with the control groups, aortic roots of the mice fed fish protein exhibited a larger necrotic core and extensive calcifications, both known as features of advanced lesions. Higher expressions of CD_{68}, CD_{36} and IL-6 on aortic valves were detected in mice fed fish protein compared with the controls, but no significant differences in systemic inflammation or even serum lipids, suggesting a local atherogenic activity of fish protein.

II. **What are the underlying mechanisms for fish protein vascular effects?** Fish protein had a 21-fold higher amounts of TMA in contrast to casein and soy protein. Accordingly, the mice fed fish protein had a higher serum concentration of TMAO compared with the control groups. TMAO is responsible for higher expressions of several SRs such as CD_{36}

which in turn contribute to internalization of lipids and foam cells formation, hence atherosclerosis development.

III. **Do morphological assessments of atherosclerotic lesions confirm vascular protective role of fish oil supplementation? What are the underlying mechanisms?** Diet supplemented with fish oil significantly inhibited the lesion development and lipid accumulations in $Ldlr^{-/-}$ mice compared with lard fed counterparts hence, suggesting its vascular protective effects. Fish oil reduced the release of ADAMs substrates (JAM-A, ICAM-1 and VE-cadherin) in endothelial cells, hence its vascular benefits were reflected through improved endothelial barrier function.

IV. **Does chocolate liquor affect the atherosclerotic plaque development and composition?** Mice fed the chocolate liquor had larger plaque areas in the descending aorta and aortic root compared with the control animals. The plaques in mice fed chocolate liquor were characterized with higher mass of VSMCs and higher collagen content, both known to stabilize the atherosclerotic plaques. Mice fed chocolate liquor had almost 5-fold higher expression of IL-6 in their descending aorta compared with the control animals. However, no significant difference was detected in systemic inflammatory parameters also not in ApoB and fructosamine serum concentrations, suggesting a local but not a systemic effect of chocolate liquor.

V. **What are the underlying mechanisms for chocolate liquor vascular effects?** Higher amounts of vitamin D_2 in chocolate liquor than control paste but a lower serum vitamin D_3 levels in mice fed chocolate liquor than those fed control paste were detected. Lower levels of serum vitamin D_3 has been associated with atherogenesis and even enhanced IL-6 expressions. IL-6 has been shown to induce VSMCs migration and proliferation. Also, VSMCs produce most of the collagen within atherosclerotic plaques. Whether all these interactions have been the underlying cause for the observed atherogenic effects of chocolate liquor demands further investigations.

5 Summary

Atherosclerotic cardiovascular disease is among the leading causes of death worldwide. In recent decades several food components such as unsaturated fatty acids have been identified to be effective in reducing the risk of atherosclerosis. Most of the studies evaluating the role of dietary food and components on atherosclerosis have been primarily restricted to the analysis of traditional risk factors such as serum lipids, blood pressure, *etc*. and the contribution of morphological and histological analyses has been uncommon. On the other hand, in the studies assessing atherosclerotic plaque development and composition, the effect of complex foods has been less investigated since most of these studies have applied either single nutrients or the extraction of food active ingredients.

Most of the studies suggesting atheroprotective effects of fish have mainly focused on the role of fish oil, while data on the vascular effects of fish protein are rather scarce. Therefore, the current work, in a mouse model of atherosclerosis, investigated the vascular effect of fish protein compared with casein and soy protein. After 16 wk consumption of a *"Western diet"* containing 20% fish (turbot) protein, casein, or soy protein, the animals were terminated, and their blood vessels were subjected to several morphological analyses. The data from *en face* and histochemical analyses revealed that mice fed fish protein had larger lesion area respectively in aorta and aortic roots than those on casein- or soy protein-diets. The atherosclerotic plaques in fish protein fed mice were characterized by larger area of necrotic cores and calcification deposits, compared with the other groups. Also, higher aortic expressions of CD_{68}, CD_{36}, and IL-6 were detected in animals fed fish protein than casein or soy protein. Since fish protein contained relatively higher amounts of trimethylamine (TMA), the serum concentration of TMA oxide (TMAO) was determined. It was found that mice that received fish protein had significantly higher serum concentrations of proatherogenic TMAO than the casein or soy protein supplement groups. These results suggest that different components of the fish diet may have various effects on cardiovascular health.

In the second work, as a part of a project investigating the link between atheroprotective effects of fish oil and ADAMs function (University of Kiel), $Ldlr^{-/-}$ and WT mice were fed a *"Western diet"* containing either 20% lard or 10% lard combined with 10% fish oil for 12 wk. Successively, the morphological and histological assessments of atherosclerotic plaques were performed (Martin

Luther University), to confirm the protective effects of fish oil on atherosclerosis. The results showed that a fish oil-enriched diet prevented lesion development and lipid accumulations in $Ldlr^{-/-}$ mice compared with the lard-rich diet. ADAMs expression in the aorta or liver were not affected by fish oil supplementation. Also, these positive effects were not supported by changes in plasma and liver concentrations of inflammatory markers (e.g. IL-6 and MCP-1). However, fish oil diet significantly reduced the circulating levels of endothelial cell adhesion molecules such as JAM-A, ICAM-1 and VE-cadherin. These results suggest that antiatherogenic role of fish oil is reflected in reduced release of ADAMs substrates in endothelial cells, hence improved endothelial barrier function.

Chocolate is another complex food with well-known cardiovascular health effects, mainly because of its high polyphenol content. However, the role of chocolate liquor on vasculature is not yet conclusive. Thus, the current study investigated the vascular actions of chocolate liquor in a mouse model of atherosclerosis. Animals ate a semi-synthetic *"Western diet"* containing 25% either chocolate liquor or a polyphenol-free isoenergetic control paste. Mice receiving chocolate liquor exhibited a larger plaque area in the descending aorta and aortic root, characterized with greater number of VSMCs and extensive collagen, compared with the mice who ate control paste diet. Compared with the controls, mice fed chocolate liquor had a 5-fold higher expression of IL-6 in their aortic roots. The higher SFAs in chocolate liquor resulted in higher hepatic SFA/PUFA ratio in mice consuming chocolate liquor than the control paste. Despite the considerable amounts of vitamin D_2 in chocolate liquor, the mice did not have measurable circulating $25(OH)D_2$ after consumption of chocolate liquor. Surprisingly, even a significant reduction (25%) in circulating level of $25(OH)D_3$ was observed in mice fed chocolate liquor compared with the control mice. Data from the current study did not show any positive effects of the chocolate liquor on the vascular system.

6 Zusammenfassung

Herz-Kreislauferkrankungen, die vornehmlich als direkte Folge der Arteriosklerose entstehen, gehören weltweit zu den Hauptursachen für Tod. In den letzten Jahrzehnten konnten einige Nahrungsmittelinhaltsstoffe identifiziert werden, die das Risiko für vaskuläre Läsionen senken. Dazu gehören beispielsweise ungesättigte Fettsäuren. Die meisten dieser Studien konzentrierten sich in erster Linie auf die Untersuchung traditioneller Risikofaktoren wie Serumlipide, Blutdruck, usw. und trugen darüber hinaus kaum zur morphologischen und histologischen Analyse dieser bei. Des Weiteren haben Studien zu atherosklerotischen Plaques und deren Zusammensetzung meist nur ungenügend den Einfluss von regelmäßigen Verzehr von komplexen Lebensmitteln untersucht, da sie sich oft nur auf einen einzelnen Nährstoff oder einen Extrakt von dessen aktiven Bestandteilen bezogen. Die meisten Studien zur atheroprotektiven Wirkungen von Nahrungsmitteln aus Fischen befassten sich, zum Beispiel, vorwiegend mit Fischöl, während kaum Daten zu den vaskulären Effekten von Fischprotein verfügbar sind. Mit Hilfe eines Atherosklerose-Mausmodells wurde in der ersten Studie die Wirkung von Fischprotein im Vergleich zu Kasein und Sojaprotein untersucht. Nach 16-wöchigen Verzehr einer Diät, die der westlichen Ernährungsweise entspricht, mit 20% Fisch (Steinbutt)-Protein, Kasein oder Sojaprotein, wurden in den Mäusen morphologische Analysen von deren Blutgefäßen durchgeführt. Die Daten von en-Face- und histochemischen Analysen zeigten, dass Mäuse, denen Fischprotein verabreicht wurde, größere Läsionen in der Aorta und Aortenwurzel aufwiesen als Mäuse, die Kasein- oder Sojaprotein mit der Diät erhielten. Die Mäuse, die mit Fischprotein gefüttert wurde, hatten außerdem größere nekrotische Bereiche, mehr Kalkablagerungen und eine höhere Expression von CD_{68}, CD_{36} und IL-6. Da das Fischprotein relative hohe Gehalte an Trimethylamin (TMA) aufwies, wurde die Serumkonzentration an TMA-Oxid (TMAO) bestimmt. Dabei zeigte sich, dass die Gruppe, welche Fischprotein erhielt, deutlich höhere Serumkonzentrationen des proatherogenen TMAO aufwiesen als die Gruppen mit Zugabe von Kasein oder Sojaprotein. Die Ergebnisse weisen somit darauf hin, dass verschiedene Komponenten des Lebensmittels Fisch ganz unterschiedliche Effekt auf die kardiovaskuläre Gesundheit haben können. In der zweiten Studie, die Teil eines Projektes zur Unterstuchung dse Zusammenhanges zwischen der atheroprotektiven Wirkung von Fischöl und ADAMs (in Zusammenarbeit mit der Universität Kiel) war, wurden $Ldlr^{-/-}$ und WT-Mäuse für 12 Wochen mit einer westlichen Diät

gefüttert, die entweder 20% Schweineschmalz oder 10% Schweineschmalz zusammen mit 10% Fischöl enthielt. Aufeinander aufbauend wurden morphologische und histologische Messungen der atheorskleortischen Plaque-Flächen (von der Martin-Luther Universität) durchgeführt. Die Ergebnisse wiesen darauf hin, dass mit Fischöl angereicherte Diäten die Entwicklung von Läsionen in $Ldlr^{-/-}$ Mäusen reduzieren können. Dieser positive Effekt auf die Läsionen konnte leider nicht bei der ADAMs-Expression in der Aorta oder Leber bestätigt werden. Ebenfalls wurden die in Plasma und Leber aufgefundene Konzentration von Leukozyten-abgeleiteten Entzündungsmarkern (z.B. IL-6 und MCP-1) durch Fischölergänzung nicht messbar beeinflusst. Allerdings wurde die im Blut zirkulierender Konzentration an Endothelzelladhäsionsmoleküle wie JAM-A, ICAM-1 und VE-Cadherin verringert. Diese Ergebnisse deuten darauf hin, dass ein antiatherogener Effekt von Fischöl durch die reduzierte Freisetzung von ADAMs -Substraten in Endothelzellen besteht und somit zu einer verbesserten endothelialen Barrierefunktion beiträgt.

Schokolade is ein weiteres Beispiel für ein komplex zusammengesetztes Lebensmittel, das aufgrund seines hohen Polyphenolgehalt als günstiges Lebensmittel für die Herz-Kreislaufgesundheit erachtet wird. Daher wurde in jener Studie die Wirkung einer Schokoladenmasse auf Gefäßparameter in einem Mausmodell untersucht. Die Tiere erhielten eine westliche Diät, die entweder zu 25% aus Schokoladenmasse oder aus einer Polyphenol-freien isoenergetischen Vergleichsmasse mit ähnlichen Gehalten an Makronährstoffen bestand. Mäuse, welche die Schokoladenflüssigkeit erhielten, wiesen im Vergleich zu Mäusen aus der Kontrollgruppe in der absteigenden Aorta und der Aortenwurzel größere Plaque-Flächen auf, mit einer größeren Anzahl von VSMCs und mehr Kollagen. Im Aortengewebe von Mäusen der Schokoladenmasse-Gruppe wurde außerdem eine 5-fach höhere Expression von IL-6 nachgewiesen. Die höheren Konzentrationen von SFAs in der Schokoladenmasse resultierten in einem höheren SFA/PUFA-Verhältnis in der Leber dieser Mäuse als in den Lebern der Vergleichstiere. Obgleich die Schokoladenmasse nachweisbare Mengen an Vitamin D_2 aufwies, zeigten die Mäuse nach deren Verzehr keine höheren Serumkonzentrationen an $25(OH)D_2$. Überraschenderweise wurde für diese Tiere sogar eine signifikante Verringerung (25%) ihres zirkulierenden $25(OH)D_3$-Niveaus im Vergleich zu den Kontrollmäusen gemessen. Daten aus der aktuellen Studie konnten keine positiven Effekte der Schokoladenmasse auf das Gefäßsystem zeigen.

References

Aarsetoey H, Grundt H, Nygaard O, Nilsen DWT. The role of long-chained marine N-3 polyunsaturated Fatty acids in cardiovascular disease. Cardiology research and practice 2012; 2012: Article ID 303456.

Abedin M, Tintut Y, Demer LL. Vascular calcification: Mechanisms and clinical ramifications. Arteriosclerosis, thrombosis, and vascular biology 2004; 24(7):1161–70.

Adler AJ, Taylor F, Martin N, Gottlieb S, Taylor RS, Ebrahim S. Reduced dietary salt for the prevention of cardiovascular disease. The Cochrane database of systematic reviews 2014; (12):CD009217.

Akesson A, Larsson SC, Discacciati A, Wolk A. Low-risk diet and lifestyle habits in the primary prevention of myocardial infarction in men: A population-based prospective cohort study. Journal of the American college of cardiology 2014; 64(13):1299–306.

Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, Franklin B, Kris-Etherton P, Harris WS et al. Diet and Lifestyle Recommendations Revision 2006: A Scientific Statement From the American Heart Association Nutrition Committee. Circulation 2006; 114(1):82–96.

Alissa EM, Ferns GA. Functional foods and nutraceuticals in the primary prevention of cardiovascular diseases. Journal of nutrition and metabolism 2012; 2012:569486.

Alkerwi A'a, Sauvageot N, Buckley JD, Donneau A-F, Albert A, Guillaume M, Crichton GE. The potential impact of animal protein intake on global and abdominal obesity: Evidence from the Observation of Cardiovascular Risk Factors in Luxembourg (ORISCAV-LUX) study. Public health nutrition 2015; 18(10):1831–8.

Almoosawi S, Fyfe L, Ho C, Al-Dujaili E. The effect of polyphenol-rich dark chocolate on fasting capillary whole blood glucose, total cholesterol, blood pressure and glucocorticoids in healthy overweight and obese subjects. The British journal of nutrition 2010; 103(6):842–50.

Al-Obaide MAI, Singh R, Datta P, Rewers-Felkins KA, Salguero MV, Al-Obaidi I, Kottapalli KR, Vasylyeva TL. Gut Microbiota-Dependent Trimethylamine-N-oxide and Serum Biomarkers in Patients with T2DM and Advanced CKD. Journal of clinical medicine 2017; 6(9).

Altorf-van der Kuil W, Engberink MF, Brink EJ, van Baak MA, Bakker SJL, Navis G, van 't Veer P, Geleijnse JM. Dietary protein and blood pressure: A systematic review. PloS one 2010; 5(8):e12102.

Al-Waiz M, Mikov M, Mitchell SC, Smith RL. The exogenous origin of trimethylamine in the mouse. Metabolism: clinical and experimental 1992; 41(2):135–6.

American Heart Association (AHA). The American Heart Association's Diet and Lifestyle Recommendations; 2015. Available from: URL: http://www.heart.org/HEARTORG/HealthyLiving/HealthyEating/Nutrition/The-American-Heart-Associations-Diet-and-Lifestyle-Recommendations_UCM_305855_Article.jsp#.WoXUMajOVPY.

Anitschkow NN, Chalatov SS. Über experimentelle Cholesterinsteatose und ihre Bedeutung für die Entstehung einiger pathologischer Prozesse. Zbl. allg. Path. path. Anat. 1913; 24:1–9.

Appel LJ. The effects of protein intake on blood pressure and cardiovascular disease. Current opinion in lipidology 2003; 14(1):55–9.

Armstrong B, van Merwyk AJ, Coates H. Blood pressure in Seventh-day Adventist vegetarians. American journal of epidemiology 1977; 105(5):444–9.

Arts ICW, Hollman PCH. Polyphenols and disease risk in epidemiologic studies. The American journal of clinical nutrition 2005; 81(1 Suppl):317S-325S.

Ashok Kumar S, editor. Hypercholesterolemia: InTech; 2015.

Asset G, Baugé E, Fruchart JC, Dallongeville J. Lack of triglyceride-lowering properties of fish oil in apolipoprotein e-deficient mice. Arteriosclerosis, thrombosis, and vascular biology 2001; 21(3):401–6.

Astrup A, Dyerberg J, Elwood P, Hermansen K, Hu FB, Jakobsen MU, Kok FJ, Krauss RM, Lecerf JM et al. The role of reducing intakes of saturated fat in the prevention of cardiovascular disease: Where does the evidence stand in 2010? The American journal of clinical nutrition 2011; 93(4):684–8.

Baba S, Natsume M, Yasuda A, Nakamura Y, Tamura T, Osakabe N, Kanegae M, Kondo K. Plasma LDL and HDL cholesterol and oxidized LDL concentrations are altered in normo- and hypercholesterolemic humans after intake of different levels of cocoa powder. The Journal of nutrition 2007; 137(6):1436–41.

Badimon L, Vilahur G. Thrombosis formation on atherosclerotic lesions and plaque rupture. Journal of internal medicine 2014; 276(6):618–32.

Bain MA, Faull R, Fornasini G, Milne RW, Evans AM. Accumulation of trimethylamine and trimethylamine-N-oxide in end-stage renal disease patients undergoing haemodialysis. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association 2006; 21(5):1300–4.

Bang HO, Dyerberg J. Lipid Metabolism and Ischemic Heart Disease in Greenland Eskimos. In: Draper HH, editor. Advances in nutritional research. New York: Springer Science+Business Media; 1980: 1–22.

Barbieri SS, Cavalca V, Eligini S, Brambilla M, Caiani A, Tremoli E, Colli S. Apocynin prevents cyclooxygenase 2 expression in human monocytes through NADPH oxidase and glutathione redox-dependent mechanisms. Free radical biology and medicine 2004; 37(2):156–65.

Baur AC, Brandsch C, König B, Hirche F, Stangl GI. Plant Oils as Potential Sources of Vitamin D. Frontiers in nutrition 2016; 3:29.

Bazzano LA, Hu T, Reynolds K, Yao L, Bunol C, Liu Y, Chen C-S, Klag MJ, Whelton PK, He J. Effects of low-carbohydrate and low-fat diets: A randomized trial. Annals of internal medicine 2014; 161(5):309–18.

Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, Ferranti SD de, Floyd J, Fornage M et al. Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. Circulation 2017; 135(10):e146-e603.

Bennett MR, Sinha S, Owens GK. Vascular Smooth Muscle Cells in Atherosclerosis. Circulation research 2016; 118(4):692–702.

Bent S, Bertoglio K, Hendren RL. Omega-3 fatty acids for autistic spectrum disorder: A systematic review. Journal of autism and developmental disorders 2009; 39(8):1145–54.

Bentzon JF, Otsuka F, Virmani R, Falk E. Mechanisms of plaque formation and rupture. Circulation research 2014; 114(12):1852–66.

Berger S, Raman G, Vishwanathan R, Jacques PF, Johnson EJ. Dietary cholesterol and cardiovascular disease: A systematic review and meta-analysis. The American journal of clinical nutrition 2015; 102(2):276–94.

Bernstein AM, Sun Q, Hu FB, Stampfer MJ, Manson JE, Willett WC. Major dietary protein sources and risk of coronary heart disease in women. Circulation 2010; 122(9):876–83.

Bhattacharya A, Rahman M, Sun D, Fernandes G. Effect of fish oil on bone mineral density in aging C57BL/6 female mice. The Journal of nutritional biochemistry 2007; 18(6):372–9.

Bjerregaard P, Young TK, Hegele RA. Low incidence of cardiovascular disease among the Inuit-what is the evidence? Atherosclerosis 2003; 166(2):351–7.

Blobel CP. ADAMs: Key components in EGFR signalling and development. Nature reviews. Molecular cell biology 2005; 6(1):32–43.

Bosch J, Gerstein HC, Dagenais GR, Díaz R, Dyal L, Jung H, Maggiono AP, Probstfield J, Ramachandran A et al. n-3 fatty acids and cardiovascular outcomes in patients with dysglycemia. The New England journal of medicine 2012; 367(4):309–18.

Bouzan C, Cohen JT, Connor WE, Kris-Etherton PM, Gray GM, König A, Lawrence RS, Savitz DA, Teutsch SM. A quantitative analysis of fish consumption and stroke risk. American journal of preventive medicine 2005; 29(4):347–52.

Bozic M, Álvarez Á, Pablo C de, Sanchez-Niño M-D, Ortiz A, Dolcet X, Encinas M, Fernandez E, Valdivielso JM. Impaired Vitamin D Signaling in Endothelial Cell Leads to an Enhanced Leukocyte-Endothelium Interplay: Implications for Atherosclerosis Development. PloS one 2015; 10(8):e0136863.

Bracco U. Effect of triglyceride structure on fat absorption. The American journal of clinical nutrition 1994; 60(6 Suppl):1002S–1009S.

Braganza DM, Bennett MR. New insights into atherosclerotic plaque rupture. Postgraduate Medical Journal 2001; 77(904):94–8.

Brown AL, Zhu X, Rong S, Shewale S, Seo J, Boudyguina E, Gebre AK, Alexander-Miller MA, Parks JS. Omega-3 fatty acids ameliorate atherosclerosis by favorably altering monocyte subsets and limiting monocyte recruitment to aortic lesions. Arteriosclerosis, thrombosis, and vascular biology 2012; 32(9):2122–30.

Brown JM, Shelness GS, Rudel LL. Monounsaturated fatty acids and atherosclerosis: Opposing views from epidemiology and experimental animal models. Current atherosclerosis reports 2007; 9(6):494–500.

Buckley ML, Ramji DP. The influence of dysfunctional signaling and lipid homeostasis in mediating the inflammatory responses during atherosclerosis. Biochimica et biophysica acta 2015; 1852(7):1498–510.

Buijsse B, Weikert C, Drogan D, Bergmann M, Boeing H. Chocolate consumption in relation to blood pressure and risk of cardiovascular disease in German adults. European heart journal 2010; 31(13):1616–23.

Buitrago-Lopez A, Sanderson J, Johnson L, Warnakula S, Wood A, Di Angelantonio E, Franco OH. Chocolate consumption and cardiometabolic disorders: Systematic review and meta-analysis. The British medical journal (Clinical research ed.) 2011; 343:d4488.

Burr ML, Ashfield-Watt PAL, Dunstan FDJ, Fehily AM, Breay P, Ashton T, Zotos PC, Haboubi NAA, Elwood PC. Lack of benefit of dietary advice to men with angina: results of a controlled trial European Journal of Clinical Nutrition 2003; 57:193–200.

Butcovan D, Mocanu V, Baran D, Ciurescu D, Tinica G. Assessment of vulnerable and unstable carotid atherosclerotic plaques on endarterectomy specimens. Experimental and therapeutic medicine 2016; 11(5):2028–32.

Cam A, Mejia EG de. Role of dietary proteins and peptides in cardiovascular disease. Molecular nutrition & food research 2012; 56(1):53–66.

Canault M, Peiretti F, Kopp F, Bonardo B, Bonzi M-F, Coudeyre J-C, Alessi M-C, Juhan-Vague I, Nalbone G. The TNF alpha converting enzyme (TACE/ADAM17) is expressed in the atherosclerotic lesions of apolipoprotein E-deficient mice: Possible contribution to elevated plasma levels of soluble TNF alpha receptors. Atherosclerosis 2006; 187(1):82–91.

Carlson SJ, Nandivada P, Chang MI, Mitchell PD, O'Loughlin A, Cowan E, Gura KM, Nose V, Bistrian BR, Puder M. The addition of medium-chain triglycerides to a purified fish oil-based diet alters inflammatory profiles in mice. Metabolism: clinical and experimental 2015; 64(2):274–82.

Carrelli AL, Walker MD, Lowe H, McMahon DJ, Rundek T, Sacco RL, Silverberg SJ. Vitamin D deficiency is associated with subclinical carotid atherosclerosis: The Northern Manhattan study. Stroke 2011; 42(8):2240–5.

Carroll KK, Hamilton RMG. Effects of dietary protein and carbohyadrate on plasma cholesterol levels in relation to atherosclerosis. Journal of food science 1975; 40(1):18–23.

Casós K, Sáiz MP, Ruiz-Sanz JI, Mitjavila MT. Atherosclerosis prevention by a fish oil-rich diet in apoE(-/-) mice is associated with a reduction of endothelial adhesion molecules. Atherosclerosis 2008; 201(2):306–17.

Cavusoglu E, Kornecki E, Sobocka MB, Babinska A, Ehrlich YH, Chopra V, Yanamadala S, Ruwende C, Salifu MO et al. Association of plasma levels of F11 receptor/junctional adhesion molecule-A (F11R/JAM-A) with human atherosclerosis. Journal of the American college of cardiology 2007; 50(18):1768–76.

Chalamaiah M, Dinesh Kumar B, Hemalatha R, Jyothirmayi T. Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: a review. Food chemistry 2012; 135(4):3020–38.

Chan HM, Egeland GM. Fish consumption, mercury exposure, and heart diseases. Nutrition reviews 2004; 62(2):68–72.

Chang CL, Deckelbaum RJ. Omega-3 fatty acids: Mechanisms underlying 'protective effects' in atherosclerosis. Current opinion in lipidology 2013; 24(4):345–50.

Chang CL, Torrejon C, Jung UJ, Graf K, Deckelbaum RJ. Incremental replacement of saturated fats by n-3 fatty acids in high-fat, high-cholesterol diets reduces elevated plasma lipid levels and arterial lipoprotein lipase, macrophages and atherosclerosis in LDLR-/- mice. Atherosclerosis 2014; 234(2):401–9.

Chen IS, Subramaniam S, Vahouny GV, Cassidy MM, Ikeda I, Kritchevsky D. A comparison of the digestion and absorption of cocoa butter and palm kernel oil and their effects on cholesterol absorption in rats. The Journal of nutrition 1989; 119(11):1569–73.

Cheng Y-C, Sheen J-M, Hu WL, Hung Y-C. Polyphenols and Oxidative Stress in Atherosclerosis-Related Ischemic Heart Disease and Stroke. Oxidative medicine and cellular longevity 2017; 2017:8526438.

Cheraghi N, Dai H, Raghuveer G. Vitamin D deficiency is associated with atherosclerosis-promoting risk factor clustering but not vascular damage in children. Medical science monitor: international medical journal of experimental and clinical research 2012; 18(12):CR687-92.

Chevrier G, Mitchell PL, Rioux L-E, Hasan F, Jin T, Roblet CR, Doyen A, Pilon G, St-Pierre P et al. Low-Molecular-Weight Peptides from Salmon Protein Prevent Obesity-Linked Glucose Intolerance, Inflammation, and Dyslipidemia in LDLR-/-/ApoB100/100 Mice. The Journal of nutrition 2015; 145(7):1415–22.

Chiesa G, Busnelli M, Manzini S, Parolini C. Nutraceuticals and Bioactive Components from Fish for Dyslipidemia and Cardiovascular Risk Reduction. Marine drugs 2016; 14(6).

Cho CE, Taesuwan S, Malysheva OV, Bender E, Tulchinsky NF, Yan J, Sutter JL, Caudill MA. Trimethylamine-N-oxide (TMAO) response to animal source foods varies among healthy young men and is influenced by their gut microbiota composition: A randomized controlled trial. Molecular nutrition & food research 2017; 61(1).

Chowdhury R, Warnakula S, Kunutsor S, Crowe F, Ward HA, Johnson L, Franco OH, Butterworth AS, Forouhi NG et al. Association of dietary, circulating, and supplement fatty acids with coronary risk: A systematic review and meta-analysis. Annals of internal medicine 2014; 160(6):398–406.

Ciccone MM, Marzullo A, Mizio D, Angiletta D, Cortese F, Scicchitano P, Carbonara S, Ricci G, Regina G et al. Can carotid plaque histology selectively predict the risk of an acute coronary syndrome? International heart journal 2011; 52(2):72–7.

Cienfuegos-Jovellanos E, Quiñones MdM, Muguerza B, Moulay L, Miguel M, Aleixandre A. Antihypertensive effect of a polyphenol-rich cocoa powder industrially processed to preserve the original flavonoids of the cocoa beans. Journal of agricultural and food chemistry 2009; 57(14):6156–62.

Cigolini M, Iagulli MP, Miconi V, Galiotto M, Lombardi S, Targher G. Serum 25-hydroxyvitamin D3 concentrations and prevalence of cardiovascular disease among type 2 diabetic patients. Diabetes care 2006; 29(3):722–4.

Cimadon HMS, Geremia R, Pellanda LC. Dietary habits and risk factors for atherosclerosis in students from Bento Gonçalves (state of Rio Grande do Sul). Arquivos brasileiros de cardiologia 2010; 95(2):166–72.

Collie-Duguid ES, Wahle KW. Inhibitory effect of fish oil N-3 polyunsaturated fatty acids on the expression of endothelial cell adhesion molecules. Biochemical and biophysical research communications 1996; 220(3):969–74.

Collins HL, Drazul-Schrader D, Sulpizio AC, Koster PD, Williamson Y, Adelman SJ, Owen K, Sanli T, Bellamine A. L-Carnitine intake and high trimethylamine N-oxide plasma levels correlate with low aortic lesions in ApoE(-/-) transgenic mice expressing CETP. Atherosclerosis 2016; 244:29–37.

Cooper KA, Donovan JL, Waterhouse AL, Williamson G. Cocoa and health: A decade of research. The British journal of nutrition 2008; 99(1):1–11.

Czarnecki SK, Krichevsky D. The effect of dieatary proteins in lipoprotein metabolism and atherosclerosis in rabbits. Journal of the Amrican Oil Chemists' Society 1979; 56:388A.

Damianou C, Couppis A. Feasibility study for removing calcified material using a planar rectangular ultrasound transducer. Journal of ultrasound 2016; 19(2):115–23.

Danaei G, Ding EL, Mozaffarian D, Taylor B, Rehm J, Murray CJL, Ezzati M. The preventable causes of death in the United States: Comparative risk assessment of dietary, lifestyle, and metabolic risk factors. PLoS medicine 2009; 6(4):e1000058.

David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014; 505(7484):559–63.

De Caterina R, Cybulsky MI, Clinton SK, Gimbrone MA, Libby P. The omega-3 fatty acid docosahexaenoate reduces cytokine-induced expression of proatherogenic and proinflammatory proteins in human endothelial cells. Arteriosclerosis and thrombosis 1994; 14(11):1829–36.

De Caterina R. n-3 fatty acids in cardiovascular disease. The New England journal of medicine 2011; 364(25):2439–50.

De Caterina R, Madonna R, Zucchi R, La Rovere MT. Antiarrhythmic effects of omega-3 fatty acids: From epidemiology to bedside. American heart journal 2003; 146(3):420–30.

Deckelbaum RJ, Torrejon C. The omega-3 fatty acid nutritional landscape: Health benefits and sources. The Journal of nutrition 2012; 142(3):587S–591S.

Degirolamo C, Shelness GS, Rudel LL. LDL cholesteryl oleate as a predictor for atherosclerosis: Evidence from human and animal studies on dietary fat. Journal of lipid research 2009; 50 Suppl:S434-9.

Dehghan M, Mente A, Zhang X, Swaminathan S, Li W, Mohan V, Iqbal R, Kumar R, Wentzel-Viljoen E et al. Associations of fats and carbohydrate intake with cardiovascular disease and mortality in 18 countries from five continents (PURE): A prospective cohort study. The Lancet 2017; 390(10107):2050–62.

Di Renzo L, Rizzo M, Sarlo F, Colica C, Iacopino L, Domino E, Sergi D, Lorenzo A de. Effects of dark chocolate in a population of normal weight obese women: A pilot study. European review for medical and pharmacological sciences 2013; 17(16):2257–66.

Dobnig H, Pilz S, Scharnagl H, Renner W, Seelhorst U, Wellnitz B, Kinkeldei J, Boehm BO, Weihrauch G, Maerz W. Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. Archives of internal medicine 2008; 168(12):1340–9.

Doherty TM, Detrano RC. Coronary arterial calcification as an active process: A new perspective on an old problem. Calcified tissue international 1994; 54(3):224–30.

Donners MMPC, Wolfs IMJ, Olieslagers S, Mohammadi-Motahhari Z, Tchaikovski V, Heeneman S, van Buul JD, Caolo V, Molin DGM et al. A disintegrin and metalloprotease 10 is a novel mediator of vascular endothelial growth factor-induced endothelial cell function in angiogenesis and is associated with atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology 2010; 30(11):2188–95.

Donovan JL, Crespy V, Oliveira M, Cooper KA, Gibson BB, Williamson G. (+)-Catechin is more bioavailable than (-)-catechin: Relevance to the bioavailability of catechin from cocoa. Free radical research 2006; 40(10):1029–34.

Doran AC, Meller N, McNamara CA. Role of smooth muscle cells in the initiation and early progression of atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology 2008; 28(5):812–9.

Draper HH, editor. Advances in nutritional research. New York: Springer Science+Business Media; 1980.

Dumas M-E, Maibaum EC, Teague C, Ueshima H, Zhou B, Lindon JC, Nicholson JK, Stamler J, Elliott P et al. Assessment of analytical reproducibility of 1H NMR spectroscopy based metabonomics for large-scale epidemiological research: The INTERMAP Study. Analytical chemistry 2006; 78(7):2199–208.

Edwards DR, Handsley MM, Pennington CJ. The ADAM metalloproteinases. Molecular aspects of medicine 2008; 29(5):258–89.

Eilertsen K-E, Mæhre HK, Cludts K, Olsen JO, Hoylaerts MF. Dietary enrichment of apolipoprotein E-deficient mice with extra virgin olive oil in combination with seal oil inhibits atherogenesis. Lipids in health and disease 2011; 10:41.

Ellam T, Hameed A, ul Haque R, Muthana M, Wilkie M, Francis SE, Chico TJA. Vitamin D deficiency and exogenous vitamin D excess similarly increase diffuse atherosclerotic calcification in apolipoprotein E knockout mice. PloS one 2014; 9(2):e88767.

Elvevoll EO, Barstad H, Breimo ES, Brox J, Eilertsen K-E, Lund T, Olsen JO, Osterud B. Enhanced incorporation of n-3 fatty acids from fish compared with fish oils. Lipids 2006; 41(12):1109–14.

European Food Safety Authority Panel on Dietetic Products, Nutrition, and Allergies (NDA); Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA Journal 2010a; 8(3):1461.

European Food Safety Authority Panel. Scientific Opinion on the substantiation of health claims related to coenzyme Q10 and contribution to normal energy-yielding metabolism (ID 1508, 1512, 1720, 1912, 4668), maintenance of normal blood pressure (ID 1509, 1721, 1911), protection of DNA, prote. EFSA Journal 2010b; 8(10):1793.

European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on the substantiation of a health claim related to cocoa flavanols and maintenance of normal endothelium-dependent vasodilation pursuant to Article 13(5) of Regulation (EC) No 1924/2006. EFSA Journal 2012; 10(7):2141.

Expert Dyslipidemia Panel of the International Atherosclerosis Society (IAS). An International Atherosclerosis Society Position Paper: Global recommendations for the management of dyslipidemia-full report. Journal of clinical lipidology 2014; 8(1):29–60.

Falk E, Shah PK, Fuster V. Coronary Plaque Disruption. Circulation 1995; 92(3):657–71.

Falk E. Pathogenesis of Atherosclerosis. Journal of the American College of Cardiology 2006; 47(8):C7-C12.

Feil S, Fehrenbacher B, Lukowski R, Essmann F, Schulze-Osthoff K, Schaller M, Feil R. Transdifferentiation of vascular smooth muscle cells to macrophage-like cells during atherogenesis. Circulation research 2014; 115(7):662–7.

Ferdowsian HR, Barnard ND. Effects of plant-based diets on plasma lipids. The American journal of cardiology 2009; 104(7):947–56.

Fernández-Ortiz A, Badimon JJ, Falk E, Fuster V, Meyer B, Mailhac A, Weng D, Shah PK, Badimon L. Characterization of the relative thrombogenicity of atherosclerotic plaque components: Implications for consequences of plaque rupture. Journal of the American college of cardiology 1994; 23(7):1562–9.

Feskens EJM, Sluik D, van Woudenbergh GJ. Meat consumption, diabetes, and its complications. Current diabetes reports 2013; 13(2):298–306.

Field D, Newton G. The antihypertensive actions of cocoa polyphenols - A review. Current Topics in Nutraceutical Research 2013; 11(4):113–28.

Finn AV, Nakano M, Narula J, Kolodgie FD, Virmani R. Concept of vulnerable/unstable plaque. Arteriosclerosis, thrombosis, and vascular biology 2010; 30(7):1282–92.

Fodor JG, Helis E, Yazdekhasti N, Vohnout B. "Fishing" for the origins of the "Eskimos and heart disease" story: Facts or wishful thinking? The Canadian journal of cardiology 2014; 30(8):864–8.

Food and Agriculture Organization of the United Nations (FAO). The state of world fisheries and aquaculture 2012. Rome, London: FAO; Eurospan [distributor]; 2012.

Fraga CG, Actis-Goretta L, Ottaviani JI, Carrasquedo F, Lotito SB, Lazarus S, Schmitz HH, Keen CL. Regular consumption of a flavanol-rich chocolate can improve oxidant stress in young soccer players. Clinical & developmental immunology 2005; 12(1):11–7.

Freeman MP, Hibbeln JR, Wisner KL, Davis JM, Mischoulon D, Peet M, Keck PE, Marangell LB, Richardson AJ et al. Omega-3 fatty acids: Evidence basis for treatment and future research in psychiatry. The Journal of clinical psychiatry 2006; 67(12):1954–67.

Gabrielsson BG, Wikström J, Jakubowicz R, Marmon SK, Carlsson N-G, Jansson N, Gan L-M, Undeland I, Lönn M et al. Dietary herring improves plasma lipid profiles and reduces atherosclerosis in obese low-density lipoprotein receptor-deficient mice. International journal of molecular medicine 2012; 29(3):331–7.

García-Lafuente A, Guillamón E, Villares A, Rostagno MA, Martínez JA. Flavonoids as anti-inflammatory agents: Implications in cancer and cardiovascular disease. Inflammation research 2009; 58(9):537–52.

Garton KJ, Gough PJ, Philalay J, Wille PT, Blobel CP, Whitehead RH, Dempsey PJ, Raines EW. Stimulated shedding of vascular cell adhesion molecule 1 (VCAM-1) is mediated by tumor necrosis factor-alpha-converting enzyme (ADAM 17). The Journal of biological chemistry 2003; 278(39):37459–64.

Gascon A, Jacques H, Moorjani S, Deshaies Y, Brun LD, Julien P. Plasma lipoprotein profile and lipolytic activities in response to the substitution of lean white fish for other animal protein sources in premenopausal women. The American journal of clinical nutrition 1996; 63(3):315–21.

Getz GS, Reardon CA. Animal models of atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology 2012; 32(5):1104–15.

Ginsberg GL, Toal BF. Quantitative approach for incorporating methylmercury risks and omega-3 fatty acid benefits in developing species-specific fish consumption advice. Environmental health perspectives 2009; 117(2):267–75.

Giroux I, Kurowska EM, Carroll KK. Role of dietary lysine, methionine, and arginine in the regulation of hypercholesterolemia in rabbits. The Journal of nutritional biochemistry 1999; 10(3):166–71.

Glass CK, Witztum JL. Atherosclerosis. the road ahead. Cell 2001; 104(4):503–16.F

Glendenning P, Chew GT, Seymour HM, Gillett MJ, Goldswain PR, Inderjeeth CA, Vasikaran SD, Taranto M, Musk AA, Fraser WD. Serum 25-hydroxyvitamin D levels in vitamin D-insufficient hip fracture patients after supplementation with ergocalciferol and cholecalciferol. Bone 2009; 45(5):870–5.

Goldbourt U, Yaari S, Medalie JH. Factors Predictive of Long-Term Coronary Heart Disease Mortality among 10,059 Male Israeli Civil Servants and Municipal Employees. Cardiology 1993; 82(2-3):100–21.

Gomez D, Shankman LS, Nguyen AT, Owens GK. Detection of histone modifications at specific gene loci in single cells in histological sections. Nature methods 2013; 10(2):171–7.

Gonçalves I, Andersson Georgiadou E, Mattsson S, Skog G, Pedro L, Fernandes E Fernandes J, Dias N, Engström G, Nilsson J, Stenström K. Direct association between diet and the stability of human atherosclerotic plaque. Scientific reports 2015; 5:15524.

Gooz M. ADAM-17: The enzyme that does it all. Critical reviews in biochemistry and molecular biology 2010; 45(2):146–69.

Goulding NJ, Gibney MJ, Taylor TG, Gallagher PJ. Reversible hypercholesterolaemia produced by cholesterol-free fish meal protein diets. Atherosclerosis 1983; 49(2):127–37.

Goya L, Martín MÁ, Sarriá B, Ramos S, Mateos R, Bravo L. Effect of Cocoa and Its Flavonoids on Biomarkers of Inflammation: Studies of Cell Culture, Animals and Humans. Nutrients 2016; 8(4):212.

Graham I, Atar D, Borch-Johnsen K, Boysen G, Burell G, Cifkova R, Dallongeville J, Backer G de, Ebrahim S et al. European guidelines on cardiovascular disease prevention in clinical practice: Executive summary: Fourth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (Constituted by representatives of nine societies and by invited experts). European heart journal 2007; 28(19):2375–414.

Grassi D, Necozione S, Lippi C, Croce G, Valeri L, Pasqualetti P, Desideri G, Blumberg JB, Ferri C. Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. Hypertension (Dallas, Tex. : 1979) 2005; 46(2):398–405.

Greenberg JA, Buijsse B. Habitual chocolate consumption may increase body weight in a dose-response manner. PloS one 2013; 8(8):e70271.

Greenberg JA, Manson JE, Buijsse B, Wang L, Allison MA, Neuhouser ML, Tinker L, Waring ME, Isasi CR et al. Chocolate-candy consumption and 3-year weight gain among postmenopausal U.S. women. Obesity (Silver Spring, Md.) 2015; 23(3):677–83.

Gu Y, Yu S, Lambert JD. Dietary cocoa ameliorates obesity-related inflammation in high fat-fed mice. European journal of nutrition 2014; 53(1):149–58.

Guan H, Lin Y, Bai L, An Y, Shang J, Wang Z, Zhao S, Fan J, Liu E. Dietary Cocoa Powder Improves Hyperlipidemia and Reduces Atherosclerosis in apoE Deficient Mice through the Inhibition of Hepatic Endoplasmic Reticulum Stress. Mediators of inflammation 2016; 2016:1937572.

Gudbrandsen OA, Wergedahl H, Liaset B, Espe M, Berge RK. Dietary proteins with high isoflavone content or low methionine–glycine and lysine–arginine ratios are hypocholesterolaemic and lower the plasma homocysteine level in male Zucker fa/fa rats. The British journal of nutrition 2005; 94(03):321.

Guillén-Casla V, Rosales-Conrado N, León-González ME, Pérez-Arribas LV, Polo-Díez LM. Determination of serotonin and its precursors in chocolate samples by capillary liquid chromatography with mass spectrometry detection. Journal of chromatography. A 2012; 1232:158–65.

Habauzit V, Morand C. Evidence for a protective effect of polyphenols-containing foods on cardiovascular health: An update for clinicians. Therapeutic advances in chronic disease 2012; 3(2):87–106.

Halton TL, Willett WC, Liu S, Manson JE, Albert CM, Rexrode K, Hu FB. Low-Carbohydrate-Diet Score and the Risk of Coronary Heart Disease in Women. The New England journal of medicine 2006; 355(19):1991–2002.

Hamed MS, Gambert S, Bliden KP, Bailon O, Singla A, Anand S, Antonino MJ, Hamed F, Tantry US, Gurbel PA. Dark chocolate effect on platelet activity, C-reactive protein and lipid profile: A pilot study. Southern medical journal 2008; 101(12):1203–8.

Hammer A, Koppensteiner R, Steiner S, Niessner A, Goliasch G, Gschwandtner M, Hoke M. Dark chocolate and vascular function in patients with peripheral artery disease: A randomized, controlled cross-over trial. Clinical hemorheology and microcirculation 2015; 59(2):145–53.

Hampton T. How Useful Are Mouse Models for Understanding Human Atherosclerosis? Review Examines the Available Evidence. Circulation 2017; 135(18):1757–8.

Hannum SM, Erdman JW. Emerging Health Benefits from Cocoa and Chocolate. Journal of Medicinal Food 2000; 3(2):73–5.

Hansson GK, Libby P. The immune response in atherosclerosis: A double-edged sword. Nature reviews immunologz 2006; 6(7):508–19.

Hara M, Sakata Y, Nakatani D, Suna S, Usami M, Matsumoto S, Hamasaki T, Doi Y, Nishino M et al. Low levels of serum n-3 polyunsaturated fatty acids are associated with worse heart failure-free survival in patients after acute myocardial infarction. Circulation Journal 2013; 77(1):153-62.

Haring B, Gronroos N, Nettleton JA, Von Ballmoos MCW, Selvin E, Alonso A. Dietary protein intake and coronary heart disease in a large community based cohort: Results from the Atherosclerosis Risk in Communities (ARIC) study corrected. PloS one 2014; 9(10):e109552.

Harman NL, Leeds AR, Griffin BA. Increased dietary cholesterol does not increase plasma low density lipoprotein when accompanied by an energy-restricted diet and weight loss. European journal of nutrition 2008; 47(6):287–93.

Harris WS. Are n-3 fatty acids still cardioprotective? Current opinion in clinical nutrition and metabolic care 2013; 16(2):141–9.

Harris WS, Von Schacky C. The Omega-3 Index: A new risk factor for death from coronary heart disease? Preventive medicine 2004; 39(1):212–20.

He, K., Song, Y., Daviglus, M. L., Liu, K., Van Horn, L., Dyer, A. R., & Greenland, P. Accumulated Evidence on Fish Consumption and Coronary Heart Disease Mortality: A Meta-Analysis of Cohort Studies. Circulation 2004; 109(22):2705–11.

Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. The Journal of nutritional biochemistry 2002; 13(10):572–84.

Herron KL, Lofgren IE, Sharman M, Volek JS, Fernandez ML. High intake of cholesterol results in less atherogenic low-density lipoprotein particles in men and women independent of response classification. Metabolism 2004; 53(6):823–30.

Hlebowicz J, Drake I, Gullberg B, Sonestedt E, Wallström P, Persson M, Nilsson J, Hedblad B, Wirfält E. A high diet quality is associated with lower incidence of cardiovascular events in the Malmö diet and cancer cohort. PloS one 2013; 8(8):e71095.

Holdt LM, Thiery J, Breslow JL, Teupser D. Increased ADAM17 mRNA expression and activity is associated with atherosclerosis resistance in LDL-receptor deficient mice. Arteriosclerosis, thrombosis, and vascular biology 2008; 28(6):1097–103.

Hollis BW. Comparison of equilibrium and disequilibrium assay conditions for ergocalciferol, cholecalciferol and their major metabolites. Journal of steroid biochemistry 1984; 21(1):81–6.

Holmberg I, Berlin T, Ewerth S, Björkhem I. 25-Hydroxylase activity in subcellular fractions from human liver. Evidence for different rates of mitochondrial hydroxylation of vitamin D2 and D3. Scandinavian journal of clinical and laboratory investigation 1986; 46(8):785–90.

Hooper L, Kay C, Abdelhamid A, Kroon PA, Cohn JS, Rimm EB, Cassidy A. Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: A systematic review and meta-analysis of randomized trials. The American journal of clinical nutrition 2012; 95(3):740–51.

Hooper L, Kroon PA, Rimm EB, Cohn JS, Harvey I, Le Cornu KA, Ryder JJ, Hall WL, Cassidy A. Flavonoids, flavonoid-rich foods, and cardiovascular risk: A meta-analysis of randomized controlled trials. The American journal of clinical nutrition 2008; 88(1):38–50.

Houston DK, Ding J, Lee JS, Garcia M, Kanaya AM, Tylavsky FA, Newman AB, Visser M, Kritchevsky SB. Dietary fat and cholesterol and risk of cardiovascular disease in older adults: The Health ABC Study. Nutrition, metabolism, and cardiovascular diseases 2011; 21(6):430–7.

Howard DP, van Lammeren GW, Rothwell PM, Redgrave JN, Moll FL, Vries J-PP de, Kleijn DP de, den Ruijter HM, Borst GJ de, Pasterkamp G. Symptomatic carotid atherosclerotic disease: Correlations between plaque composition and ipsilateral stroke risk. Stroke 2015; 46(1):182–9.

Howell WH, McNamara DJ, Tosca MA, Smith BT, Gaines JA. Plasma lipid and lipoprotein responses to dietary fat and cholesterol: A meta-analysis. The American journal of clinical nutrition 1997; 65(6):1747–64.

Hu FB. Protein, body weight, and cardiovascular health. The American journal of clinical nutrition 2005; 82(1 Suppl):242S–247S.

Hu FB, Stampfer MJ, Manson JE, Ascherio A, Colditz GA, Speizer FE, Hennekens CH, Willett WC. Dietary saturated fats and their food sources in relation to the risk of coronary heart disease in women. The American journal of clinical nutrition 1999; 70(6):1001–8.

Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Rosner BA, Hennekens CH, Willett WC. Dietary fat intake and the risk of coronary heart disease in women. The New England journal of medicine 1997; 337(21):1491–9.

Hu FB, Bronner L, Willett WC, Stampfer MJ, Rexrode KM, Albert CM, Hunter D, Manson JE. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. Journal of the American medical association 2002a; 287(14):1815–21.

Hu FB, Willett WC. Optimal diets for prevention of coronary heart disease. Journal of the American medical association 2002b; 288(20):2569–78.

Hughes DA, Pinder AC, Piper Z, Johnson IT, Lund EK. Fish oil supplementation inhibits the expression of major histocompatibility complex class II molecules and adhesion molecules on human monocytes. The American journal of clinical nutrition 1996; 63(2):267–72.

Ikeda U, Ikeda M, Oohara T, Oguchi A, Kamitani T, Tsuruya Y, Kano S. Interleukin 6 stimulates growth of vascular smooth muscle cells in a PDGF-dependent manner. The American journal of physiology 1991; 260(5 Pt 2):H1713-7.

Ito T, Ikeda U, Shimpo M, Yamamoto K, Shimada K. Serotonin increases interleukin-6 synthesis in human vascular smooth muscle cells. Circulation 2000; 102(20):2522–7.

Jacques H, Noreau L, Moorjani S. Effects on plasma lipoproteins and endogenous sex hormones of substituting lean white fish for other animal-protein sources in diets of postmenopausal women. The American journal of clinical nutrition 1992; 55(4):896–901.

Jakobsen MU, O'Reilly EJ, Heitmann BL, Pereira MA, Bälter K, Fraser GE, Goldbourt U, Hallmans G, Knekt P et al. Major types of dietary fat and risk of coronary heart disease: A pooled analysis of 11 cohort studies. The American journal of clinical nutrition 2009; 89(5):1425–32.

Janszky I, Mukamal KJ, Ljung R, Ahnve S, Ahlbom A, Hallqvist J. Chocolate consumption and mortality following a first acute myocardial infarction: The Stockholm Heart Epidemiology Program. Journal of internal medicine 2009; 266(3):248–57.

Jayedi A, Shab-Bidar S, Eimeri S, Djafarian K. Fish consumption and risk of all-cause and cardiovascular mortality: A dose-response meta-analysis of prospective observational studies. Public health nutrition 2018:1–10.

Jensen I-J, Abrahamsen H, Maehre HK, Elvevoll EO. Changes in antioxidative capacity of saithe (Pollachius virens) and shrimp (Pandalus borealis) during in vitro digestion. Journal of agricultural and food chemistry 2009; 57(22):10928–32.

Jensen I-J, Walquist M, Liaset B, Elvevoll EO, Eilertsen K-E. Dietary intake of cod and scallop reduces atherosclerotic burden in female apolipoprotein E-deficient mice fed a Western-type high fat diet for 13 weeks. Nutrition & metabolism 2016; 13:8.

Jia L, Liu X, Bai YY, Li SH, Sun K, He C, Hui R. Short-term effect of cocoa product consumption on lipid profile: A meta-analysis of randomized controlled trials. The American journal of clinical nutrition 2010; 92(1):218–25.

Johnston WA, Nicholson FJ, Roger A, Stroud GD. Freezing and refrigerated storage in fisheries. Rome; 1994. (FAO fisheries technical paper; vol 340).

Kanadaşı M, Çaylı M, Şan M, Aikimbaev K, Alhan CC, Demir M, Demirtaş M. The Presence of a Calcific Plaque in the Common Carotid Artery as a Predictor of Coronary Atherosclerosis. Angiology 2006; 57(5):585–92.

Karimi S, Dadvar M, Modarress H, Dabir B. Kinetic modeling of low density lipoprotein oxidation in arterial wall and its application in atherosclerotic lesions prediction. Chemistry and physics of lipids 2013; 175-176:1–8.

Karwowski W, Naumnik B, Szczepański M, Myśliwiec M. The mechanism of vascular calcification - A systematic review. Medical Science Monitor 2012;18:RA1–11.

Katz DL, Doughty K, Ali A. Cocoa and chocolate in human health and disease. Antioxidants & redox signaling 2011; 15(10):2779–811.

Katz MF, Farber HW, Dodds-Stitt Z, Cruikshank WW, Bear DJ. Serotonin-stimulated aortic endothelial cells secrete a novel T lymphocyte chemotactic and growth factor. Journal of leukocyte biology 1994; 55(5):567–73.

Kenny TP, Keen CL, Schmitz HH, Gershwin ME. Immune effects of cocoa procyanidin oligomers on peripheral blood mononuclear cells. Experimental biology and medicine (Maywood, N.J.) 2007; 232(2):293–300.

Khan N, Khymenets O, Urpí-Sardà M, Tulipani S, Garcia-Aloy M, Monagas M, Mora-Cubillos X, Llorach R, Andres-Lacueva C. Cocoa polyphenols and inflammatory markers of cardiovascular disease. Nutrients 2014; 6(2):844–80.

Khan N, Monagas M, Andres-Lacueva C, Casas R, Urpí-Sardà M, Lamuela-Raventós RM, Estruch R. Regular consumption of cocoa powder with milk increases HDL cholesterol and reduces oxidized LDL levels in subjects at high-risk of cardiovascular disease. Nutrition, metabolism, and cardiovascular diseases 2012; 22(12):1046–53.

Kim Y, Keogh J, Clifton P. A review of potential metabolic etiologies of the observed association between red meat consumption and development of type 2 diabetes mellitus. Metabolism: clinical and experimental 2015; 64(7):768–79.

Klouche M, Rose-John S, Schmiedt W, Bhakdi S. Enzymatically degraded, nonoxidized LDL induces human vascular smooth muscle cell activation, foam cell transformation, and proliferation. Circulation 2000; 101(15):1799–805.

Koenen RR, Pruessmeyer J, Soehnlein O, Fraemohs L, Zernecke A, Schwarz N, Reiss K, Sarabi A, Lindbom L et al. Regulated release and functional modulation of junctional adhesion molecule A by disintegrin metalloproteinases. Blood 2009; 113(19):4799–809.

Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, Fu X, Wu Y et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nature medicine 2013; 19(5):576–85.

König A, Bouzan C, Cohen JT, Connor WE, Kris-Etherton PM, Gray GM, Lawrence RS, Savitz DA, Teutsch SM. A quantitative analysis of fish consumption and coronary heart disease mortality. American journal of preventive medicine 2005; 29(4):335–46.

Krajcovicova-Kudlackova M, Babinska K, Valachovicova M. Health benefits and risks of plant proteins. Bratislavske lekarske listy 2005; 106(6-7):231–4.

Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, Erdman JW, Kris-Etherton P, Goldberg IJ et al. AHA Dietary Guidelines: Revision 2000: A statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Circulation 2000; 102(18):2284–99.

Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. The American journal of medicine 2002; 113(9):71–88.

Kritchevsky D. Vegetable protein and atherosclerosis. Journal of the American oil chemists' society 1979; 56(3):135–40.

Kritchevsky D, Tepper SA. Experimental atherosclerosis in rabbits fed cholesterol-free diets: Influence of chow components. Journal of atherosclerosis research 1968; 8(2):357–69.

Kritchevsky D, Tepper SA, Czarnecki SK, Klurfeld DM. Atherogenicity of animal and vegetable protein. Influence of the lysine to arginine ratio. Atherosclerosis 1982; 41(2-3):429–31.

Kritchevsky D, Trepper SA, Story JA. Infuelcne of soy protein and casein on Atherosclerosis in rabbits. Federation proceedings 1978; 37:747.

Kromhout D, Yasuda S, Geleijnse JM, Shimokawa H. Fish oil and omega-3 fatty acids in cardiovascular disease: Do they really work? European heart journal 2012; 33(4):436–43.

Kurlandsky SB, Stote KS. Cardioprotective effects of chocolate and almond consumption in healthy women. Nutrition research 2006; 26(10):509–16.

Kwak SM, Myung S-K, Lee YJ, Seo HG. Efficacy of omega-3 fatty acid supplements (eicosapentaenoic acid and docosahexaenoic acid) in the secondary prevention of cardiovascular disease: A meta-analysis of randomized, double-blind, placebo-controlled trials. Archives of internal medicine 2012; 172(9):686–94.

Kwok CS, Loke YK, Welch AA, Luben RN, Lentjes MAH, Boekholdt SM, Pfister R, Mamas MA, Wareham NJ et al. Habitual chocolate consumption and the risk of incident heart failure among healthy men and women. Nutrition, metabolism, and cardiovascular diseases 2016; 26(8):722–34.

Labudzynskyi D, Shymanskyy I, Veliky M. Role of vitamin D3 in regulation of interleukin-6 and osteopontin expression in liver of diabetic mice. European review for medical and pharmacological sciences 2016; 20(13):2916–9.

Lacaille B, Julien P, Deshaies Y, Lavigne C, Brun L-D, Jacques H. Responses of Plasma Lipoproteins and Sex Hormones to the Consumption of Lean Fish Incorporated in a Prudent-Type Diet in Normolipidemic Men. Journal of the American college of nutrition 2000; 19(6):745–53.

Lamuela-Raventós RM, Romero-Pérez AI, Andrés-Lacueva C, Tornero A. Review: Health Effects of Cocoa Flavonoids. Food sci. technol. int. 2005; 11(3):159–76.

Landfald B, Valeur J, Berstad A, Raa J. Microbial trimethylamine-N-oxide as a disease marker: Something fishy? Microbial ecology in health and disease 2017; 28(1):1327309.

Lang DH, Yeung CK, Peter RM, Ibarra C, Gasser R, Itagaki K, Philpot RM, Rettie AE. Isoform specificity of trimethylamine N-oxygenation by human flavin-containing monooxygenase (FMO) and P450 enzymes: Selective catalysis by FMO3. Biochemical pharmacology 1998; 56(8):1005–12.

Larsen R, Eilertsen K-E, Mæhre H, Jensen I-J, Elvevoll EO. Taurine Content in Marine Foods: Beneficial Health Effects. In: Hernández-Ledesma B, Herrero M, editors. Bioactive Compounds from Marine Foods: Plant and Animal Sources. Chichester, UK: John Wiley & Sons Ltd; 2013:249-268.

Larsson SC, Virtamo J, Wolk A. Chocolate consumption and risk of stroke in women. Journal of the American college of cardiology 2011; 58(17):1828–9.

Lecumberri E, Mateos R, Ramos S, Alía M, Rúperez P, Goya L, Izquierdo-Pulido M, Bravo L. Caracterización de la fibra de cacao y su efecto sobre la capacidad antioxidante en suero de animales de experimentación. Nutricion hospitalaria 2006; 21(5):622–8.

Lecumberri E, Goya L, Mateos R, Alía M, Ramos S, Izquierdo-Pulido M, Bravo L. A diet rich in dietary fiber from cocoa improves lipid profile and reduces malondialdehyde in hypercholesterolemic rats. Nutrition (Burbank, Los Angeles County, Calif.) 2007; 23(4):332–41.

Lee CC, Watkins SM, Lorenzo C, Wagenknecht LE, Il'yasova D, Chen Y-DI, Haffner SM, Hanley AJ. Branched-Chain Amino Acids and Insulin Metabolism: The Insulin Resistance Atherosclerosis Study (IRAS). Diabetes care 2016; 39(4):582–8.

Lee YT, Lin HY, Chan YWF, Li KHC, To OTL, Yan BP, Liu T, Li G, Wong WT et al. Mouse models of atherosclerosis: A historical perspective and recent advances. Lipids in health and disease 2017; 16(1):12.

Lehmann U, Hirche F, Stangl GI, Hinz K, Westphal S, Dierkes J. Bioavailability of vitamin D(2) and D(3) in healthy volunteers, a randomized placebo-controlled trial. The Journal of clinical endocrinology and metabolism 2013; 98(11):4339–45.

Leidy HJ, Clifton PM, Astrup A, Wycherley TP, Westerterp-Plantenga MS, Luscombe-Marsh ND, Woods SC, Mattes RD. The role of protein in weight loss and maintenance. The American journal of clinical nutrition 2015.

Leiva E, Wehinger S, Guzmán L, Orrego R. Role of Oxidized LDL in Atherosclerosis. In: Ashok Kumar S, editor. Hypercholesterolemia: InTech; 2015: 199-206

Lenz EM, Bright J, Wilson ID, Hughes A, Morrisson J, Lindberg H, Lockton A. Metabonomics, dietary influences and cultural differences: A 1H NMR-based study of urine samples obtained from healthy British and Swedish subjects. Journal of pharmaceutical and biomedical analysis 2004; 36(4):841–9.

Leung Yinko SSL, Stark KD, Thanassoulis G, Pilote L. Fish consumption and acute coronary syndrome: A meta-analysis. The American Journal of Medicine 2014; 127(9):848-57.e2.

Levy D. Combating the epidemic of heart disease. Journal of the American medical association 2012; 308(24):2624–5.

Li Y, Hruby A, Bernstein AM, Ley SH, Wang DD, Chiuve SE, Sampson L, Rexrode KM, Rimm EB et al. Saturated Fats Compared With Unsaturated Fats and Sources of Carbohydrates in Relation to Risk of Coronary Heart Disease: A Prospective Cohort Study. Journal of the American college of cardiology 2015; 66(14):1538–48.

Lichtman AH, Clinton SK, Iiyama K, Connelly PW, Libby P, Cybulsky MI. Hyperlipidemia and atherosclerotic lesion development in LDL receptor-deficient mice fed defined semipurified diets with and without cholate. Arteriosclerosis, thrombosis, and vascular biology 1999; 19(8):1938–44.

Lidbury I, Murrell JC, Chen Y. Trimethylamine N-oxide metabolism by abundant marine heterotrophic bacteria. Proceedings of the National Academy of Sciences of the United States of America 2014; 111(7):2710–5.

Liu W, Zhang Y, Yu C-M, Ji Q-W, Cai M, Zhao Y-X, Zhou Y-J. Current understanding of coronary artery calcification. Journal of geriatric cardiology 2015; 12(6):668–75.

Lopez-Garcia E, Schulze MB, Manson JE, Meigs JB, Albert CM, Rifai N, Willett WC, Hu FB. Consumption of (n-3) fatty acids is related to plasma biomarkers of inflammation and endothelial activation in women. The Journal of nutrition 2004; 134(7):1806–11.

Lusis AJ. Atherosclerosis. Nature 2000; 407(6801):233–41.

Ma G, Pan B, Chen Y, Guo C, Zhao M, Zheng L, Chen B. Trimethylamine N-oxide in atherogenesis: Impairing endothelial self-repair capacity and enhancing monocyte adhesion. Bioscience reports 2017; 37(2).

Madamanchi NR, Vendrov A, Runge MS. Oxidative Stress and Vascular Disease. Arteriosclerosis, thrombosis, and vascular biology 2005.

Madsen T, Skou HA, Hansen VE, Fog L, Christensen JH, Toft E, Schmidt EB. C-reactive protein, dietary n-3 fatty acids, and the extent of coronary artery disease. The American journal of cardiology 2001; 88(10):1139–42.

Mallika V, Goswami B, Rajappa M. Atherosclerosis Pathophysiology and the Role of Novel Risk Factors: A Clinicobiochemical Perspective. Angiology 2007; 58(5):513–22.

Mancia G, Fagard R, Narkiewicz K, Redón J, Zanchetti A, Böhm M, Christiaens T, Cifkova R, De Backer G et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Journal of hypertension 2013; 31(7):1281–357.

Manoharan G, Ntalianis A, Muller O, Hamilos M, Sarno G, Melikian N, Vanderheyden M, Heyndrickx GR, Wyffels E et al. Severity of coronary arterial stenoses responsible for acute coronary syndromes. The American journal of cardiology 2009; 103(9):1183–8.

Marckmann P, Grønbaek M. Fish consumption and coronary heart disease mortality. A systematic review of prospective cohort studies. European journal of clinical nutrition 1999; 53(8):585–90.

Martins D, Wolf M, Pan D, Zadshir A, Tareen N, Thadhani R, Felsenfeld A, Levine B, Mehrotra R, Norris K. Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States: Data from the Third National Health and Nutrition Examination Survey. Archives of internal medicine 2007; 167(11):1159–65.

Massaro M, Martinelli R, Gatta V, Scoditti E, Pellegrino M, Carluccio MA, Calabriso N, Buonomo T, Stuppia L et al. Correction: Transcriptome-Based Identification of New Anti-Anti-Inflammatory and Vasodilating Properties of the n-3 Fatty Acid Docosahexaenoic Acid in Vascular Endothelial Cell Under Proinflammatory Conditions. PloS one 2016; 11(4):e0154069.

Mateen S, Moin S, Shahzad S, Khan AQ. Level of inflammatory cytokines in rheumatoid arthritis patients: Correlation with 25-hydroxy vitamin D and reactive oxygen species. PloS one 2017; 12(6):e0178879.

Mathiesen EB, Bonaa KH, Joakimsen O. Echolucent Plaques Are Associated With High Risk of Ischemic Cerebrovascular Events in Carotid Stenosis: The Tromso Study. Circulation 2001; 103(17):2171–5.

Matsumoto M, Sata M, Fukuda D, Tanaka K, Soma M, Hirata Y, Nagai R. Orally administered eicosapentaenoic acid reduces and stabilizes atherosclerotic lesions in ApoE-deficient mice. Atherosclerosis 2008; 197(2):524–33.

Matter CM, Stuber M, Nahrendorf M. Imaging of the unstable plaque: How far have we got? European heart journal 2009; 30(21):2566–74.

Matthews AL, Noy PJ, Reyat JS, Tomlinson MG. Regulation of A disintegrin and metalloproteinase (ADAM) family sheddases ADAM10 and ADAM17: The emerging role of tetraspanins and rhomboids. Platelets 2017; 28(4):333–41.

McCombie G, Browning LM, Titman CM, Song M, Shockcor J, Jebb SA, Griffin JL. omega-3 oil intake during weight loss in obese women results in remodelling of plasma triglyceride and fatty acids. Metabolomics 2009; 5(3):363–74.

McGee DL, Reed DM, Yano K, Kagan A, Tillotson J. Ten-year incidence of coronary heart disease in the Honolulu Heart Program. Relationship to nutrient intake. American journal of epidemiology 1984; 119(5):667–76.

McGill HC. The relationship of dietary cholesterol to serum cholesterol concentration and to atherosclerosis in man. The American journal of clinical nutrition 1979; 32(12 Suppl):2664–702.

McLaren JE, Michael DR, Ashlin TG, Ramji DP. Cytokines, macrophage lipid metabolism and foam cells: Implications for cardiovascular disease therapy. Progress in lipid research 2011; 50(4):331–47.

De Mello VDF, Erkkilä AT, Schwab US, Pulkkinen L, Kolehmainen M, Atalay M, Mussalo H, Lankinen M, Oresic M et al. The effect of fatty or lean fish intake on inflammatory gene expression in peripheral blood mononuclear cells of patients with coronary heart disease. European journal of nutrition 2009; 48(8):447–55.

Mellor DD, Sathyapalan T, Kilpatrick ES, Beckett S, Atkin SL. High-cocoa polyphenol-rich chocolate improves HDL cholesterol in Type 2 diabetes patients. Diabetic medicine 2010; 27(11):1318–21.

Mendis S, Puska P, Norrving B. Global atlas on cardiovascular disease prevention and control: Shanthi Mendis, Pekka Puska and Bo Norrving. Geneva: World Health Organization in collaboration with the World Heart Federation and the World Stroke Organization; 2011.

Mensink RP, Katan MB. Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. The New England journal of medicine 1990; 323(7):439–45.

Mente A, Chalcraft K, Ak H, Davis AD, Lonn E, Miller R, Potter MA, Yusuf S, Anand SS, McQueen MJ. The Relationship Between Trimethylamine-N-Oxide and Prevalent Cardiovascular Disease in a Multiethnic Population Living in Canada. The Canadian journal of cardiology 2015; 31(9):1189–94.

Mente A, Koning L de, Shannon HS, Anand SS. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. Archives of internal medicine 2009; 169(7):659–69.

Mercando AD, Lai HM, Aronow WS, Kalen P, Desai HV, Gandhi K, Sharma M, Amin H, Lai TM. Reduction in atherosclerotic events: A retrospective study in an outpatient cardiology practice. Archives of medical science 2012; 8(1):57–62.

Merkel M, Velez-Carrasco W, Hudgins LC, Breslow JL. Compared with saturated fatty acids, dietary monounsaturated fatty acids and carbohydrates increase atherosclerosis and VLDL cholesterol

levels in LDL receptor-deficient, but not apolipoprotein E-deficient, mice. Proceedings of the National Academy of Sciences of the United States of America 2001; 98(23):13294–9.

Miles EA, Thies F, Wallace FA, Powell JR, Hurst TL, Newsholme EA, Calder PC. Influence of age and dietary fish oil on plasma soluble adhesion molecule concentrations. Clinical science 2001; 100(1):91–100.

Mink PJ, Scrafford CG, Barraj LM, Harnack L, Hong C-P, Nettleton JA, Jacobs DR. Flavonoid intake and cardiovascular disease mortality: A prospective study in postmenopausal women. The American journal of clinical nutrition 2007; 85(3):895–909.

Monagas M, Khan N, Andres-Lacueva C, Casas R, Urpí-Sardà M, Llorach R, Lamuela-Raventós RM, Estruch R. Effect of cocoa powder on the modulation of inflammatory biomarkers in patients at high risk of cardiovascular disease. The American journal of clinical nutrition 2009; 90(5):1144–50.

Moreno PR. Vulnerable plaque: Definition, diagnosis, and treatment. Cardiology clinics 2010; 28(1):1–30.

Mosca A, Carenini A, Zoppi F, Carpinelli A, Banfi G, Ceriotti F, Bonini P, Pozza G. Plasma protein glycation as measured by fructosamine assay. Clinical chemistry 1987; 33(7):1141–6.

Mozaffarian D, Appel LJ, van Horn L. Components of a cardioprotective diet: New insights. Circulation 2011; 123(24):2870–91.

Mursu J, Voutilainen S, Nurmi T, Rissanen TH, Virtanen JK, Kaikkonen J, Nyyssönen K, Salonen JT. Dark chocolate consumption increases HDL cholesterol concentration and chocolate fatty acids may inhibit lipid peroxidation in healthy humans. Free radical biology & medicine 2004; 37(9):1351–9.

Myhrstad MCW, Retterstøl K, Telle-Hansen VH, Ottestad I, Halvorsen B, Holven KB, Ulven SM. Effect of marine n-3 fatty acids on circulating inflammatory markers in healthy subjects and subjects with cardiovascular risk factors. Inflammation research 2011; 60(4):309–19.

Nabata T, Morimoto S, Koh E, Shiraishi T, Ogihara T. Interleukin-6 stimulates c-myc expression and proliferation of cultured vascular smooth muscle cells. Biochemistry international 1990; 20(3):445–53.

Najafian L, Babji AS. A review of fish-derived antioxidant and antimicrobial peptides: Their production, assessment, and applications. Peptides 2012; 33(1):178–85.

Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. Arteriosclerosis and thrombosis 1994; 14(1):133–40.

Ness AR, Hughes J, Elwood PC, Whitley E, Smith GD, Burr ML. The long-term effect of dietary advice in men with coronary disease: follow-up of the Diet and Reinfarction Trial (DART). European Journal of Clinical Nutrition 2002; 56:512–8.

Neves PO, Andrade J, Monção H. Coronary artery calcium score: Current status. Radiologia brasileira 2017; 50(3):182–9.

Nichols M, Townsend N, Scarborough P, Rayner M. Cardiovascular disease in Europe 2014: Epidemiological update. European heart journal 2014; 35(42):2950–9.

Nichols TC, Merricks EP, Bellinger DA, Raymer RA, Yu J, Lam D, Koch GG, Busby WH, Clemmons DR. Oxidized LDL and Fructosamine Associated with Severity of Coronary Artery Atherosclerosis in Insulin Resistant Pigs Fed a High Fat/High NaCl Diet. PloS one 2015; 10(7):e0132302.

Nicolaides A, Beach KW, Kyriacou E, Pattichis CS, editors. Ultrasound and Carotid Bifurcation Atherosclerosis. London: Springer London; 2012.

Nilausen K, Meinertz H. Lipoprotein(a) and dietary proteins: Casein lowers lipoprotein(a) concentrations as compared with soy protein. The American journal of clinical nutrition 1999; 69(3):419–25.

Oba S, Nagata C, Nakamura K, Fujii K, Kawachi T, Takatsuka N, Shimizu H. Consumption of coffee, green tea, oolong tea, black tea, chocolate snacks and the caffeine content in relation to risk of diabetes in Japanese men and women. The British journal of nutrition 2010; 103(3):453–9.

Oksala N, Levula M, Airla N, Pelto-Huikko M, Ortiz RM, Järvinen O, Salenius J-P, Ozsait B, Komurcu-Bayrak E et al. ADAM-9, ADAM-15, and ADAM-17 are upregulated in macrophages in advanced human atherosclerotic plaques in aorta and carotid and femoral arteries--Tampere vascular study. Annals of medicine 2009; 41(4):279–90.

Orr AW, Hastings NE, Blackman BR, Wamhoff BR. Complex regulation and function of the inflammatory smooth muscle cell phenotype in atherosclerosis. Journal of vascular research 2010; 47(2):168–80.

Otsuka F, Sakakura K, Yahagi K, Joner M, Virmani R. Has our understanding of calcification in human coronary atherosclerosis progressed? Arteriosclerosis, thrombosis, and vascular biology 2014; 34(4):724–36.

Ouellet V, Weisnagel SJ, Marois J, Bergeron J, Julien P, Gougeon R, Tchernof A, Holub BJ, Jacques H. Dietary cod protein reduces plasma C-reactive protein in insulin-resistant men and women. The Journal of nutrition 2008; 138(12):2386–91.

Paradis G, Fodor JG. Diet and the prevention of cardiovascular diseases. Canadian journal of cardiology 1999; 15:81–8.

Park YC, Rimbach G, Saliou C, Valacchi G, Packer L. Activity of monomeric, dimeric, and trimeric flavonoids on NO production, TNF-alpha secretion, and NF-kappaB-dependent gene expression in RAW 264.7 macrophages. FEBS letters 2000; 465(2-3):93–7.

Parolini C, Vik R, Busnelli M, Bjørndal B, Holm S, Brattelid T, Manzini S, Ganzetti GS, Dellera F et al. A salmon protein hydrolysate exerts lipid-independent anti-atherosclerotic activity in ApoE-deficient mice. PloS one 2014; 9(5):e97598.

Petrone AB, Gaziano JM, Djoussé L. Chocolate consumption and risk of heart failure in the Physicians' Health Study. European journal of heart failure 2014; 16(12):1372–6.

Petursdottir DH, Olafsdottir I, Hardardottir I. Dietary fish oil increases tumor necrosis factor secretion but decreases interleukin-10 secretion by murine peritoneal macrophages. The Journal of nutrition 2002; 132(12):3740–3.

Phinikaridou A, Qiao Y, Hamilton JA. Stable and Vulnerable Atherosclerotic Plaques. In: Nicolaides A, Beach KW, Kyriacou E, Pattichis CS, editors. Ultrasound and Carotid Bifurcation Atherosclerosis. London: Springer London; 2012: 3–25.

Picard-Deland E, Lavigne C, Marois J, Bisson J, Weisnagel SJ, Marette A, Holub B, Chu E, Frohlich J et al. Dietary supplementation with fish gelatine modifies nutrient intake and leads to sex-dependent responses in TAG and C-reactive protein levels of insulin-resistant subjects. Journal of nutritional science 2012; 1:e15.

Pletcher MJ, Tice JA, Pignone M, Browner WS. Using the Coronary Artery Calcium Score to Predict Coronary Heart Disease Events. Archives of internal medicine 2004; 164(12):1285.

Ponnuchamy B, Khalil RA. Role of ADAMs in endothelial cell permeability: Cadherin shedding and leukocyte rolling. Circulation research 2008; 102(10):1139–42.

Ramos-Romero S, Pérez-Cano FJ, Pérez-Berezo T, Castellote C, Franch A, Castell M. Effect of a cocoa flavonoid-enriched diet on experimental autoimmune arthritis. The British journal of nutrition 2012; 107(4):523–32.

Ramsden CE, Zamora D, Leelarthaepin B, Majchrzak-Hong SF, Faurot KR, Suchindran CM, Ringel A, Davis JM, Hibbeln JR. Use of dietary linoleic acid for secondary prevention of coronary heart disease and death: Evaluation of recovered data from the Sydney Diet Heart Study and updated meta-analysis. The British medical journal (Clinical research ed.) 2013; 346:e8707.

Reddy KS, Katan MB. Diet, nutrition and the prevention of hypertension and cardiovascular diseases. PHN 2004; 7(1a):154.

Rekhter M. Collagen synthesis in atherosclerosis: Too much and not enough. Cardiovascular research 1999; 41(2):376–84.

Richter CK, Skulas-Ray AC, Champagne CM, Kris-Etherton PM. Plant protein and animal proteins: Do they differentially affect cardiovascular disease risk? Advances in nutrition (Bethesda, Md.) 2015; 6(6):712–28.

Ried K, Sullivan TR, Fakler P, Frank OR, Stocks NP. Effect of cocoa on blood pressure. The Cochrane database of systematic reviews 2012; (8):CD008893.

Rimbach G, Egert S, Pascual-Teresa S de. Chocolate: (un)healthy source of polyphenols? Genes & nutrition 2011; 6(1):1–3.

Rimm EB, Ascherio A, Giovannucci E, Spiegelman D, Stampfer MJ, Willett WC. Vegetable, fruit, and cereal fiber intake and risk of coronary heart disease among men. Journal of the American medical association 1996; 275(6):447–51.

Rizos EC, Ntzani EE, Bika E, Kostapanos MS, Elisaf MS. Association between omega-3 fatty acid supplementation and risk of major cardiovascular disease events: A systematic review and meta-analysis. Journal of the American medical association 2012; 308(10):1024–33.

Rocnik EF, Chan BM, Pickering JG. Evidence for a role of collagen synthesis in arterial smooth muscle cell migration. The Journal of clinical investigation 1998; 101(9):1889–98.

Rodriguez NR, Miller SL. Effective translation of current dietary guidance: Understanding and communicating the concepts of minimal and optimal levels of dietary protein. The American journal of clinical nutrition 2015.

Rohrmann S, Linseisen J, Allenspach M, Von Eckardstein A, Müller D. Plasma Concentrations of Trimethylamine-N-oxide Are Directly Associated with Dairy Food Consumption and Low-

Grade Inflammation in a German Adult Population. The Journal of nutrition 2016; 146(2):283–9.

Rudijanto A. The role of vascular smooth muscle cells on the pathogenesis of atherosclerosis. Acta medica Indonesiana 2007; 39(2):86–93.

Sacks FM, Rosner B, Kass EH. Blood pressure in vegetarians. American journal of epidemiology 1974; 100(5):390–8.

Sacks FM, Castelli WP, Donner A, Kass EH. Plasma lipids and lipoproteins in vegetarians and controls. The New England journal of medicine 1975; 292(22):1148–51.

Sadeghi S, Wallace FA, Calder PC. Dietary lipids modify the cytokine response to bacterial lipopolysaccharide in mice. Immunology 1999; 96(3):404–10.

Saftig P, Reiss K. The "A Disintegrin And Metalloproteases" ADAM10 and ADAM17: Novel drug targets with therapeutic potential? European journal of cell biology 2011; 90(6-7):527–35.

Saraswathi V, Gao L, Morrow JD, Chait A, Niswender KD, Hasty AH. Fish oil increases cholesterol storage in white adipose tissue with concomitant decreases in inflammation, hepatic steatosis, and atherosclerosis in mice. The Journal of nutrition 2007; 137(7):1776–82.

Satoh M, Ishikawa Y, Itoh T, Minami Y, Takahashi Y, Nakamura M. The expression of TNF-alpha converting enzyme at the site of ruptured plaques in patients with acute myocardial infarction. European journal of clinical investigation 2008; 38(2):97–105.

Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L. Dietary polyphenols and the prevention of diseases. Critical reviews in food science and nutrition 2005; 45(4):287–306.

Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. The Journal of nutrition 2000; 130(8S Suppl):2073S–85S.

Scheller J, Chalaris A, Garbers C, Rose-John S. ADAM17: A molecular switch to control inflammation and tissue regeneration. Trends in immunology 2011; 32(8):380–7.

Schuett H, Luchtefeld M, Grothusen C, Grote K, Schieffer B. How much is too much? Interleukin-6 and its signalling in atherosclerosis. Thrombosis and haemostasis 2009; 102(2):215–22.

Schulz B, Pruessmeyer J, Maretzky T, Ludwig A, Blobel CP, Saftig P, Reiss K. ADAM10 regulates endothelial permeability and T-Cell transmigration by proteolysis of vascular endothelial cadherin. Circulation research 2008; 102(10):1192–201.

Schulze MB. Sugar-Sweetened Beverages, Weight Gain, and Incidence of Type 2 Diabetes in Young and Middle-Aged Women. Journal of the American medical association 2004; 292(8):927.

Schwartz SM, Virmani R, Rosenfeld ME. The good smooth muscle cells in atherosclerosis. Curr Atheroscler Rep 2000; 2(5):422–9

Seldin MM, Meng Y, Qi H, Zhu W, Wang Z, Hazen SL, Lusis AJ, Shih DM. Trimethylamine N-Oxide Promotes Vascular Inflammation Through Signaling of Mitogen-Activated Protein Kinase and Nuclear Factor-κB. Journal of the American heart association 2016; 5(2).

Sena CM, Matafome P, Crisóstomo J, Rodrigues L, Fernandes R, Pereira P, Seiça RM. Methylglyoxal promotes oxidative stress and endothelial dysfunction. Pharmacological research 2012; 65(5):497–506.

Senthong V, Li XS, Hudec T, Coughlin J, Wu Y, Levison B, Wang Z, Hazen SL, Tang WHW. Plasma Trimethylamine N-Oxide, a Gut Microbe-Generated Phosphatidylcholine Metabolite, Is Associated With Atherosclerotic Burden. Journal of the American college of cardiology 2016; 67(22):2620–8.

Shankman LS, Gomez D, Cherepanova OA, Salmon M, Alencar GF, Haskins RM, Swiatlowska P, Newman AAC, Greene ES et al. KLF4-dependent phenotypic modulation of smooth muscle cells has a key role in atherosclerotic plaque pathogenesis. Nature medicine 2015; 21(6):628–37.

Shekelle RB, Shryock AM, Paul O, Lepper M, Stamler J, Liu S, Raynor WJ. Diet, serum cholesterol, and death from coronary heart disease. The Western Electric study. The New England journal of medicine 1981; 304(2):65–70.

Shimokawa H. Beneficial effects of eicosapentaenoic acid on endothelial vasodilator functions in animals and humans. World review of nutrition and dietetics 2001; 88:100–8.

Shively CA, Tarka SM. Methylxanthine composition and consumption patterns of cocoa and chocolate products. Progress in clinical and biological research 1984; 158:149–78.

Shrime MG, Bauer SR, McDonald AC, Chowdhury NH, Coltart CEM, Ding EL. Flavonoid-rich cocoa consumption affects multiple cardiovascular risk factors in a meta-analysis of short-term studies. The Journal of nutrition 2011; 141(11):1982–8.

Shukla A, Bettzieche A, Hirche F, Brandsch C, Stangl GI, Eder K. Dietary fish protein alters blood lipid concentrations and hepatic genes involved in cholesterol homeostasis in the rat model. The British journal of nutrition 2006; 96(4):674–82.

Simionescu M, Sima AV. Morphology of Atherosclerotic Lesions. In: Wick G, Grundtman C, editors. Inflammation and atherosclerosis. Wien: Springer; 2012:19–37.

Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. Saturated fat, carbohydrate, and cardiovascular disease. The American journal of clinical nutrition 2010a; 91(3):502–9.

Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. The American journal of clinical nutrition 2010b; 91(3):535–46.

Skulas-Ray AC, Kris-Etherton PM, Harris WS, Vanden Heuvel JP, Wagner PR, West SG. Dose-response effects of omega-3 fatty acids on triglycerides, inflammation, and endothelial function in healthy persons with moderate hypertriglyceridemia. The American journal of clinical nutrition 2011; 93(2):243–52.

Slavin JL, Lloyd B. Health benefits of fruits and vegetables. Advances in nutrition (Bethesda, Md.) 2012; 3(4):506–16.

Slevin M, Elasbali AB, Miguel Turu M, Krupinski J, Badimon L, Gaffney J. Identification of differential protein expression associated with development of unstable human carotid plaques. The American journal of pathology 2006; 168(3):1004–21.

Smit HJ, Gaffan EA, Rogers PJ. Methylxanthines are the psycho-pharmacologically active constituents of chocolate. Psychopharmacology 2004; 176(3-4):412–9.

Song Y, Shen H, Schenten D, Shan P, Lee PJ, Goldstein DR. Aging enhances the basal production of IL-6 and CCL2 in vascular smooth muscle cells. Arteriosclerosis, thrombosis, and vascular biology 2012; 32(1):103–9.

De Souza RJ, Mente A, Maroleanu A, Cozma AI, Ha V, Kishibe T, Uleryk E, Budylowski P, Schünemann H et al. Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: Systematic review and meta-analysis of observational studies. The British medical journal 2015; 351.

Spagnoli LG, Bonanno E, Sangiorgi G, Mauriello A. Role of Inflammation in Atherosclerosis. Journal of nuclear medicine 2007; 48(11):1800–15.

Spencer JPE, Abd El Mohsen MM, Minihane A-M, Mathers JC. Biomarkers of the intake of dietary polyphenols: Strengths, limitations and application in nutrition research. The British journal of nutrition 2008; 99(01):1911.

Statistisches Bundesamt. Gesundheit. Todesursachen in Deutschland - 2013; 2015. Available from: URL: https://www.destatis.de/DE/Publikationen/Thematisch/Gesundheit/Todesursachen/Todesursachen2120400137004.pdf?__blob=publicationFile

Stary HC. Natural history and histological classification of atherosclerotic lesions: An update. Arteriosclerosis, thrombosis, and vascular biology 2000; 20(5):1177–8.

Stone GW, Maehara A, Lansky AJ, Bruyne B de, Cristea E, Mintz GS, Mehran R, McPherson J, Farhat N et al. A prospective natural-history study of coronary atherosclerosis. The New England journal of medicine 2011; 364(3):226–35.

Sudano I, Flammer AJ, Roas S, Enseleit F, Ruschitzka F, Corti R, Noll G. Cocoa, blood pressure, and vascular function. Current hypertension reports 2012; 14(4):279–84.

Sun R, Wang X, Liu Y, Xia M. Dietary supplementation with fish oil alters the expression levels of proteins governing mitochondrial dynamics and prevents high-fat diet-induced endothelial dysfunction. The British journal of nutrition 2014; 112(2):145–53.

Szeto FL, Reardon CA, Yoon D, Wang Y, Wong KE, Chen Y, Kong J, Liu SQ, Thadhani R et al. Vitamin D receptor signaling inhibits atherosclerosis in mice. Molecular endocrinology (Baltimore, Md.) 2012; 26(7):1091–101.

Szondy Z, Garabuczi E, Joós G, Tsay GJ, Sarang Z. Impaired clearance of apoptotic cells in chronic inflammatory diseases: Therapeutic implications. Frontiers in immunology 2014; 5:354.

Tabas I, García-Cardeña G, Owens GK. Recent insights into the cellular biology of atherosclerosis. The Journal of cell biology 2015; 209(1):13–22.

Takashima A, Fukuda D, Tanaka K, Higashikuni Y, Hirata Y, Nishimoto S, Yagi S, Yamada H, Soeki T et al. Combination of n-3 polyunsaturated fatty acids reduces atherogenesis in apolipoprotein E-deficient mice by inhibiting macrophage activation. Atherosclerosis 2016; 254:142–50.

Tamura K, Kanzaki T, Saito Y, Otabe M, Morisaki N. Serotonin (5-hydroxytryptamine, 5-HT) enhances migration of rat aortic smooth muscle cells through 5-HT2 receptors. Atherosclerosis 1997; 132(2):139–43.

Tanaka K, Sata M. Visualization of the human coronary vasa vasorum in vivo. Circulation journal 2015; 79(6):1211–2.

Tang WHW, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, Wu Y, Hazen SL. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. The New England journal of medicine 2013; 368(17):1575–84.

Tang D, Kamm RD, Yang C, Zheng J, Canton G, Bach R, Huang X, Hatsukami TS, Zhu J et al. Image-based modeling for better understanding and assessment of atherosclerotic plaque progression and vulnerability: Data, modeling, validation, uncertainty and predictions. Journal of biomechanics 2014; 47(4):834–46.

Thies F, Garry JMC, Yaqoob P, Rerkasem K, Williams J, Shearman CP, Gallagher PJ, Calder PC, Grimble RF. Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: A randomised controlled trial. The Lancet 2003; 361(9356):477–85.

Tokede OA, Gaziano JM, Djoussé L. Effects of cocoa products/dark chocolate on serum lipids: A meta-analysis. European journal of clinical nutrition 2011; 65(8):879–86.

Tomaru M, Takano H, Osakabe N, Yasuda A, Inoue K-i, Yanagisawa R, Ohwatari T, Uematsu H. Dietary supplementation with cacao liquor proanthocyanidins prevents elevation of blood glucose levels in diabetic obese mice. Nutrition 2007; 23(4):351–5.

Tripkovic L, Lambert H, Hart K, Smith CP, Bucca G, Penson S, Chope G, Hyppönen E, Berry J et al. Comparison of vitamin D2 and vitamin D3 supplementation in raising serum 25-hydroxyvitamin D status: A systematic review and meta-analysis. The American journal of clinical nutrition 2012; 95(6):1357–64.

Trumbo P, Schlicker S, Yates AA, Poos M. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids. Journal of the American dietetic association 2002; 102(11):1621–30.

Tsakadze NL, Sithu SD, Sen U, English WR, Murphy G, D'Souza SE. Tumor necrosis factor-alpha-converting enzyme (TACE/ADAM-17) mediates the ectodomain cleavage of intercellular adhesion molecule-1 (ICAM-1). The Journal of biological chemistry 2006; 281(6):3157–64.

Ueshima H, Stamler J, Elliott P, Chan Q, Brown IJ, Carnethon MR, Daviglus ML, He K, Moag-Stahlberg A et al. Food Omega-3 Fatty Acid Intake of Individuals (Total, Linolenic Acid, Long-Chain) and Their Blood Pressure: INTERMAP Study. Hypertension 2007; 50(2):313–9.

United States Department of Agriculture (USDA). Dietary guidelines for Americans. Washington D.C.: United States Department of Agriculture (USDA); 2010. Available from: URL: https://health.gov/dietaryguidelines/dga2010/DietaryGuidelines2010.pdf.

United States Department of Agriculture (USDA). Scientific Report of the 2015 Dietary Guidelines Advisory Committee. Washington D.C.: United States Department of Agriculture (USDA); 2015. Available from: URL: https://health.gov/dietaryguidelines/2015-scientific-report/PDFs/Scientific-Report-of-the-2015-Dietary-Guidelines-Advisory-Committee.pdf.

VanderLaan PA, Reardon CA, Getz GS. Site specificity of atherosclerosis: Site-selective responses to atherosclerotic modulators. Arteriosclerosis, thrombosis, and vascular biology 2004; 24(1):12–22.

Vasdev S, Stuckless J. Antihypertensive effects of dietary protein and its mechanism. The International journal of angiology 2010; 19:7–20.

Vengrenyuk Y, Carlier S, Xanthos S, Cardoso L, Ganatos P, Virmani R, Einav S, Gilchrist L, Weinbaum S. A hypothesis for vulnerable plaque rupture due to stress-induced debonding around cellular microcalcifications in thin fibrous caps. Proceedings of the National Academy of Sciences 2006; 103(40):14678–83.

Vestweber D. VE-cadherin: The major endothelial adhesion molecule controlling cellular junctions and blood vessel formation. Arteriosclerosis, thrombosis, and vascular biology 2008; 28(2):223–32.

Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons From Sudden Coronary Death: A Comprehensive Morphological Classification Scheme for Atherosclerotic Lesions. Arteriosclerosis, thrombosis, and vascular biology 2000; 20(5):1262–75.

Virmani R, Burke AP, Farb A, Kolodgie FD. Pathology of the Vulnerable Plaque. Journal of the American college of cardiology 2006; 47(8):C13-C18.

Vita JA. Polyphenols and cardiovascular disease: Effects on endothelial and platelet function. The American journal of clinical nutrition 2005; 81(1 Suppl):292S-297S.

Mafune A, Iwamoto T, Tsutsumi Y, Nakashima A, Yamamoto I, Yokoyama K, Yokoo T, Urashima M. Associations among serum trimethylamine-N-oxide (TMAO) levels, kidney function and infarcted coronary artery number in patients undergoing cardiovascular surgery: A cross-sectional study. Clinical and experimental nephrology 2016; 20(5):731–9.

Wallace FA, Miles EA, Calder PC. The effect of dietary fat on cytokine production by murine macrophages in different activation states. Lipids 1999; 34(S1):S145-S145.

Wang H-H, Hung T-M, Wei J, Chiang A-N. Fish oil increases antioxidant enzyme activities in macrophages and reduces atherosclerotic lesions in apoE-knockout mice. Cardiovascular research 2004; 61(1):169–76.

Wang J, Uryga AK, Reinhold J, Figg N, Baker L, Finigan A, Gray K, Kumar S, Clarke M, Bennett M. Vascular Smooth Muscle Cell Senescence Promotes Atherosclerosis and Features of Plaque Vulnerability. Circulation 2015a; 132(20):1909–19.

Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011; 472(7341):57–63.

Wang Z, Roberts AB, Buffa JA, Levison BS, Zhu W, Org E, Gu X, Huang Y, Zamanian-Daryoush M et al. Non-lethal Inhibition of Gut Microbial Trimethylamine Production for the Treatment of Atherosclerosis. Cell 2015b; 163(7):1585–95.

Weber C, Erl W, Pietsch A, Danesch U, Weber PC. Docosahexaenoic Acid Selectively Attenuates Induction of Vascular Cell Adhesion Molecule 1 and Subsequent Monocytic Cell Adhesion to Human Endothelial Cells Stimulated by Tumor Necrosis Factor. Arteriosclerosis, thrombosis, and vascular biology 1995; 15(5):622–8.

Weber C, Noels H. Atherosclerosis: Current pathogenesis and therapeutic options. Nature medicine 2011; 17(11):1410–22.

Weisburger JH. Chemopreventive effects of cocoa polyphenols on chronic diseases. Experimental biology and medicine 2001; 226(10):891–7.

Weng S, Sprague JE, Oh J, Riek AE, Chin K, Garcia M, Bernal-Mizrachi C. Vitamin D deficiency induces high blood pressure and accelerates atherosclerosis in mice. PloS one 2013; 8(1):e54625.

Whelton SP, He J, Whelton PK, Muntner P. Meta-analysis of observational studies on fish intake and coronary heart disease. The American journal of cardiology 2004; 93(9):1119–23.

Wick G, Grundtman C, editors. Inflammation and atherosclerosis. Wien: Springer; 2012.

Willerson JT, Golino P, Eidt J, Campbell WB, Buja LM. Specific platelet mediators and unstable coronary artery lesions. Experimental evidence and potential clinical implications. Circulation 1989; 80(1):198–205.

Willett WC, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Rosner BA, Sampson LA, Hennekens CH. Intake of trans fatty acids and risk of coronary heart disease among women. The Lancet 1993; 341(8845):581–5.

Willett W. Nutritional epidemiology. 2nd ed. New York, Oxford: Oxford University Press; 1998.

World Health Organization (WHO), publisher. Research for universal health coverage. Geneva: World Health Organization; 2013. (The world health report, 1020-3311; vol 2013).

World Health Organisation (WHO). Cardiovascular diseases (CVDs): Fact sheet; 2017. Available from: URL: http://www.who.int/mediacentre/factsheets/fs317/en/.

Wu B, Pei X, Li Z-Y. How does calcification influence plaque vulnerability? Insights from fatigue analysis. The Scientific World Journal 2014; 2014:ID417324.

Xu J, Lu X, Shi G-P. Vasa vasorum in atherosclerosis and clinical significance. International journal of molecular sciences 2015; 16(5):11574–608.

Xun P, Qin B, Song Y, Nakamura Y, Kurth T, Yaemsiri S, Djousse L, He K. Fish consumption and risk of stroke and its subtypes: Accumulative evidence from a meta-analysis of prospective cohort studies. European journal of clinical nutrition 2012; 66(11):1199–207.

Yagi S, Fukuda D, Aihara K-I, Akaike M, Shimabukuro M, Sata M. n-3 Polyunsaturated Fatty Acids: Promising Nutrients for Preventing Cardiovascular Disease. Journal of atherosclerosis and thrombosis 2017; 24(10):999–1010.

Yakala GK, Wielinga PY, Suarez M, Bunschoten A, van Golde JM, Arola L, Keijer J, Kleemann R, Kooistra T, Heeringa P. Effects of chocolate supplementation on metabolic and cardiovascular parameters in ApoE3L mice fed a high-cholesterol atherogenic diet. Molecular nutrition & food research 2013; 57(11):2039–48.

Yamada H, Yoshida M, Nakano Y, Suganami T, Satoh N, Mita T, Azuma K, Itoh M, Yamamoto Y et al. In vivo and in vitro inhibition of monocyte adhesion to endothelial cells and endothelial adhesion molecules by eicosapentaenoic acid. Arteriosclerosis, thrombosis, and vascular biology 2008; 28(12):2173–9.

Yamazaki KG, Taub PR, Barraza-Hidalgo M, Rivas MM, Zambon AC, Ceballos G, Villarreal FJ. Effects of (-)-epicatechin on myocardial infarct size and left ventricular remodeling after permanent coronary occlusion. Journal of the American College of Cardiology 2010; 55(25):2869–76.

Yancey PH, Siebenaller JF. Co-evolution of proteins and solutions: Protein adaptation versus cytoprotective micromolecules and their roles in marine organisms. The Journal of experimental biology 2015; 218(Pt 12):1880–96.

Yang Q, Wang S, Ji Y, Chen H, Zhang H, Chen W, Gu Z, Chen YQ. Dietary intake of n-3 PUFAs modifies the absorption, distribution and bioavailability of fatty acids in the mouse gastrointestinal tract. Lipids in health and disease 2017; 16(1):10.

Yang Z-H, Bando M, Sakurai T, Chen Y, Emma-Okon B, Wilhite B, Fukuda D, Vaisman B, Pryor M et al. Long-chain monounsaturated fatty acid-rich fish oil attenuates the development of atherosclerosis in mouse models. Molecular nutrition & food research 2016; 60(10):2208–18.

Yang Z-H, Miyahara H, Iwasaki Y, Takeo J, Katayama M. Dietary supplementation with long-chain monounsaturated fatty acids attenuates obesity-related metabolic dysfunction and increases expression of PPAR gamma in adipose tissue in type 2 diabetic KK-Ay mice. Nutrition & metabolism 2013; 10(1):16.

Yang Z-H, Miyahara H, Takemura S, Hatanaka A. Dietary saury oil reduces hyperglycemia and hyperlipidemia in diabetic KKAy mice and in diet-induced obese C57BL/6J mice by altering gene expression. Lipids 2011; 46(5):425–34.

Yates CM, Tull SP, Madden J, Calder PC, Grimble RF, Nash GB, Rainger GE. Docosahexaenoic acid inhibits the adhesion of flowing neutrophils to cytokine stimulated human umbilical vein endothelial cells. The Journal of nutrition 2011; 141(7):1331–4.

Yoshimoto T, Yoshimoto T. Cytokine Frontiers: Regulation of Immune Responses in Health and Disease. Tokyo: Springer Japan; 2014.

Yu E, Rimm E, Qi L, Rexrode K, Albert CM, Sun Q, Willett WC, Hu FB, Manson JE. Diet, Lifestyle, Biomarkers, Genetic Factors, and Risk of Cardiovascular Disease in the Nurses' Health Studies. American journal of public health 2016; 106(9):1616–23.

Zadelaar S, Kleemann R, Verschuren L, Vries-Van der Weij J de, van der Hoorn J, Princen HM, Kooistra T. Mouse Models for Atherosclerosis and Pharmaceutical Modifiers. Arteriosclerosis, thrombosis, and vascular biology 2007; 27(8):1706–21.

Zampolli A, Bysted A, Leth T, Mortensen A, De Caterina R, Falk E. Contrasting effect of fish oil supplementation on the development of atherosclerosis in murine models. Atherosclerosis 2006; 184(1):78–85.

Zempo H, Suzuki J-i, Watanabe R, Wakayama K, Kumagai H, Ikeda Y, Akazawa H, Komuro I, Isobe M. Cacao polyphenols ameliorate autoimmune myocarditis in mice. Hypertension research: official journal of the Japanese Society of Hypertension 2016; 39(4):203–9.

Zhang AQ, Mitchell SC, Smith RL. Dietary precursors of trimethylamine in man: A pilot study. Food and chemical toxicology 1999b; 37(5):515–20.

Zhang J, Sasaki S, Amano K, Kesteloot H. Fish consumption and mortality from all causes, ischemic heart disease, and stroke: An ecological study. Preventive medicine 1999a; 28(5):520–9.

Zhang X, Beynen AC. Influence of dietary fish proteins on plasma and liver cholesterol concentrations in rats. The British journal of nutrition 1993; 69(03):767.

Zhang Z, Xu G, Yang F, Zhu W, Liu X. Quantitative analysis of dietary protein intake and stroke risk. Neurology 2014; 83(1):19–25.

Zhao G, Etherton TD, Martin KR, West SG, Gillies PJ, Kris-Etherton PM. Dietary alpha-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. The Journal of nutrition 2004; 134(11):2991–7.

Zheng J, Huang T, Yu Y, Hu X, Yang B, Li D. Fish consumption and CHD mortality: An updated meta-analysis of seventeen cohort studies. Public health nutrition 2012; 15(4):725–37.

Zhou J, Møller J, Danielsen CC, Bentzon J, Ravn HB, Austin RC, Falk E. Dietary supplementation with methionine and homocysteine promotes early atherosclerosis but not plaque rupture in ApoE-deficient mice. Arteriosclerosis, thrombosis, and vascular biology 2001; 21(9):1470–6.

Zhu W, Gregory JC, Org E, Buffa JA, Gupta N, Wang Z, Li L, Fu X, Wu Y et al. Gut Microbial Metabolite TMAO Enhances Platelet Hyperreactivity and Thrombosis Risk. Cell 2016; 165(1):111–24.

Acknowledgments

Completion of this doctoral dissertation was possible with support and inspiration of several people hereby, I would like to express my sincere gratitude to all of them.

First and foremost, I would like to gratefully and sincerely acknowledge the guidance, support and encouragements of my doctoral supervisor, Prof. Dr. Gabriele I. Stangl who has been a tremendous mentor for me during my Ph.D studies and helped me add a scientific value to my academic career and move forward with investigation in depth.

I would like to express my very sincere gratitude to Prof. Dr. Gerald Rimbach and his team at the Institute of Human Nutrition and Food Science, University of Kiel, for their wonderful collaboration and the scholarly inputs into this work.

With great appreciation I shall acknowledge the unconditional support from Dr. Corinna Brandsch who helped me at every stage of my personal and academic life. With her amicable and positive disposition, and the warmth she extended to me, my Ph.D journey became less obstacle ridden.

I am thankful to all my colleagues at the Institute of Agriculture and Nutritional Sciences at MLU, for their support during my study, especially Dr. Frank Hirche for his technical contribution to this project, and Frau Kerstin Isaak for her care and precious kindness.

I find words inadequate to express my profound sense of gratitude to Prof. George Fodor who provided his invaluable support at various phases of my life ever since I met him. I cannot thank him enough for his great attitude, wise counsel and sympathetic ear which helped me improve myself in many ways and overcome many hardships.

I would like to say a heartfelt and sincere thank to my husband Dr. Aaron Grau for his continued and unfailing love, support and understanding during my pursuit of Ph.D. His inspirational words of encouragement have lifted me up whenever I was down or doubted myself and have helped me keep moving forward.

I am indebted to all my family and my extended family, for helping me in whatever way they could during this challenging period and longed to see this achievement come true; also, to my father who is not with me today to share this joy; and finally, to darling Avid who is the sunshine and greatest motive in my life. With his delightful presence, he has given me happiness, love and power to overcome every obstacle and hardship during the last three years.

Curriculum vitae

Personal Details

Name	Narges Yazdekhasti-Grau
Date of birth	18/06/1981
Place of birth	Isfahan, Iran
Marital status	Married, one child

Education

2007-2009	Master of Nutritional Sciences, Thesis "*The effects of roasted soy-nut supplementation on lipid profile of iranian postmenopausal women. A Randomized Control Trial study*", Universiti Putra Malaysia, Serdang, Malaysia
2000-2004	Bachelor of Nutritional Science, Isfahan University of Medical Sciences, Isfahan, Iran
1995-1999	Diploma & Foundation, Saffora High School, Isfahan, Iran

Work Experience

2011-2016	Research Assistant Isfahan Cardiovascular Research Institute, WHO center, Isfahan, Iran
2012-2013	Food & Nutrition Supervisor Parsa Ghaem Zagros Company, Reg.#2091, Isfahan, Iran
2004- 2006	Nutritionist & Diet Counsellor Dr Najafpour's Clinic of Nutrition & Diet Therapy, Boushehr, Iran

Publications

Yazdekhasti N, Brandsch C, Hirche F, Kühn J, Schloesser A, Esatbeyoglu T, Huebbe P, Wolffram S, Rimbach G, Stangl GI: Impact of chocolate liquor on vascular lesions in apoE-knockout mice. *Clin Sci (Lond)* 2017; 131(20): 2549-2560

Yazdekhasti N, Brandsch C, Schmidt N, Schloesser A, Huebbe P, Rimbach G, Stangl GI: Fish protein increases circulating levels of trimethylamine-*N*-oxide and accelerates aortic lesion formation in apoE null mice. *Mol. Nutr. Food Res* 2016; 60(2): 358–368

N Mohammadifard, M Mansurian, F Sajjadi, M Maghrun, A Pourmoghaddas, **N Yazdekhasti**, N Sarrafzadegan. Association of glycaemic index and glycaemic load with metabolic syndrome in an Iranian adult population: Isfahan Healthy Heart Program. *Nutr Diet* 2017; 74(1):61-66

Speck N, Brandsch C, Schmidt N, **Yazdekhasti N**, Hirche F, Lucius R, Rimbach G, Stangl GI, Reiss K: The Antiatherogenic Effect of Fish Oil in Male Mice Is Associated with a Diminished Release of Endothelial ADAM17 and ADAM10 Substrates. *J Nutr* 2015; 145(6):1218-26

G Fodor, E Helis, **N Yazdekhasti**, B Vohnout. "Fishing" for the origins of the "Eskimos and heart disease" story. Facts or wishful thinking? A review. *Can J Cardiol* 2014; 30(8):864-8

G Askari, **N Yazdekhasti**, N Mohammadifard, N Sarrafzadegan, A Bahonar, M Badiei, F Sajjadi and M Taheri. Inverse association between the frequency of nut consumption and obesity among Iranian population: Isfahan Healthy Heart Program. *Eur J Nutr* 2015; 54(6):925-31

N Yazdekhasti, N Mohammadifard, N Sarrafzadegan, D Mozaffarian, M Taheri, F Sajjadi, M Maghroun. The relationship of nut consumption and blood pressure among Iranian adult population: Isfahan Healthy Heart Program. *Nutr Metab Cardiovasc Dis* 2013; 23(10):929-36

G Askari, **N Yazdekhasti**, N Mohammadifard, N Sarrafzadegan, A Bahonar, M Badiei, F Sajjadi and M Taheri. The relationship of nut consumption and lipid profile among Iranian adult population: Isfahan Healthy Heart Program. *Eur J Clin Nutr* 2013; 67(4):385-9

S Najafpour Boushehri, R M Yusof, M Mohamad Taib, Z Bt Yassin, N Sarrafzadegan, K Mirzaei, I Nabipour and **N Yazdekhasti**. Effect of Vitamin Supplementation on Serum Oxidised Low-Density Lipoprotein Levels in Male Subjects with Cardiovascular Diseases Risk Factors. *Iran J Basic Med Sci* 2012; 15(4):958-64

S N Boushehri, R M Yusof, M Mohamad Taib, Z Bt Yassin, N Sarrafzadegan, K Mirzaei, I Nabipour and **N Yazdekhasti**. Effects of Antioxidant Vitamins (C, E, Beta- Carotene) Supplementation on Serum 8-iso-Prostaglandin PGF2" in Male Subjects with Risk Factors for Cardiovascular Diseases. *MEJSR* 2011; 10 (5):577-580

N Yazdekhasti, Y Zaitun, M E Norhaizan and S Najafpour Boushehri. Effects of Roasted Soy-Nut Supplementation on Lipid Profile of Iranian Postmenopausal Women. *MEJSR* 2011; 8 (4):816-822

Oral Presentation

N Yazdekhasti, C Brandsch, N Schmidt, A Schloesser, P Huebbe, G Rimbach, G I Stangl. Dietary Fish Protein Modifies Aortic Plaque Composition - A 4 Months Feeding Study with Apo E-/- Mice. Wissenschaftlicher Kongress der Deutschen Gesellschaft für Ernährung (DGE), Universität Paderborn, Germany, March 2014

Poster Presentation

N Yazdekhasti, C Brandsch, N Schmidt, A Schloesser, P Huebbe, G Rimbach, G Stangl. Cocoa differentially affects atherogenesis in apoE-deficient mice. Wissenschaftlicher Kongress der Deutschen Gesellschaftfür Ernährung (DGE), Martin-Luther-Universität Halle-Wittenberg, Halle, Germany, March 2015

Narges Y, Zaitun Y, Norhaizan ME, Mohammad Hassan E. Effect of soy-nut consumption on lipid profile of postmenopausal Iranian women. Symposium on Plant Polyphenols: Nutrition, Health and Innovations, Kuala Lumpur, Malaysia, June 22-23, 2009

_____ _____

Place, Date Signature

Eidesstattliche Erklärung

Hiermit versichere ich an Eides statt, dass ich die vorliegende Arbeit selbstständig angefertigt und diese nicht bereits für eine Promotion oder ähnliche Zwecke an einer anderen Universität eingereicht habe. Weiterhin versichere ich, dass ich die verwendeten wissenschaftlichen Arbeiten und Hilfsmittel genau und vollständig angegeben habe.

_____ _____

Ort, Datum Unterschrift