Tomato consumption and health: its association with cardiovascular diseases and effects on traditional and novel cardiovascular risk factors

Ho Ming CHENG

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Ho Ming Cheng

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Abstract

Cardiovascular diseases (CVD) are the leading causes of deaths representing 31% of global deaths. A healthy dietary eating pattern characterised by a greater intake of fruit and vegetables is associated with a reduction in the risk of CVD. Whether specific fruit and vegetables differ in the health benefits need further research.

The systematic reviews and meta-analysis of previous relevant literature that were undertaken for this PhD programme showed positive associations between higher tomato and/or lycopene intake or status were associated with significant reductions of 26% in stroke (p=0.001), 14% in CVD (p=0.003); while high serum lycopene concentration was associated with significant reduction of 37% in mortality (p>0.001). In addition, previous dietary intervention studies have shown that supplementing tomatoes were associated with significant reductions of LDL-cholesterol by 0.22 mmol/L (p=0.006) and IL-6 by 0.25 pg/mL (p=0.03) and improvement of FMD by 2.53% (p=0.01); while supplementing lycopene was associated with significant reduction in SBP by 5.66 mmHg (p=0.002), but its effects on other markers of endothelial function has not been fully investigated.

These literature reviews informed further research in this PhD programme. Previous RCT investigating the effects of tomato consumption have focused only on red tomatoes, red-tomato products, or lycopene supplementations, and the effects of different tomato varieties on markers of cardiovascular risk had not been previously evaluated, and was therefore identified as a gap in the literature. With new varieties of tomato available to the public, it is important to evaluate whether other varieties exert the same effect, and this was the aim of further work on this research programme.

A 12-week, cross-over, randomised controlled dietary interventional study was undertaken to compare the effects of supplementing 300 g of two tomato varieties, red cherry tomato (Piccolo) and orange cherry tomato (Oranjstar) on cardiovascular health. Overall, this study provided evidence on the benefits of consumption Piccolo, for four weeks, has a significant impact on reducing both nighttime ambulatory SBP by 4.52 mmHg (p=0.039) and DBP by 3.82 mmHg (p=0.016), improving FMD by 3.43% (p=0.026), while Oranjstar has significant impact on reducing adhesion molecules sICAM-1, sVCAM-1 and E-selectin by 59.14 ng/mL (p<0.001), 67.35 ng/mL (p=0.003) and 6.65 ng/mL(p<0.016) respectively. Therefore, lowering the risk of cardiovascular disease in healthy, particularly in older, adult males.

The findings reported in the present thesis could be valuable to different health professionals to develop more effective interventions and could also help the public to make better informed food choices relating to cardiovascular health.

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Declaration

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others.

Any ethical clearance for the research presented in this thesis has been approved. Approval has been sought and granted by the Northumbria University Ethics Committee (Project ref: PG02_Cheng_121216) on January 2017.

I declare that the Word Count of this Thesis is 44,301 words

Name: Ho Ming CHENG

Signature:

Date:

List of Abbreviations

ACN	Acetonitrile
ABP	Ambulatory blood pressure
ACh	Acetylcholine chloride
AF	Atrial fibrillation
AgeCat	Age category
AIx	Augmentation index
AP	Arterial pressure
BHT	Relative centrifugal force
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BP	Blood pressure
cf-PWV	Carotid-femoral PWV
CHD	Coronary heart disease
CHF	Congestive heart failure
CI	Confidence interval
CRP	C-reactive protein
CVD	Cardiovascular diseases
℃	Degrees centigrade
DALYs	Disability-adjusted life-years
DBP	Diastolic blood pressure
DCM	Dichloromethane
EA	Ethyl acetate
EF	Endothelial function
FMD	Flow mediated dilation
HDL	High density lipoprotein
Hex	Hexane
HPLC	High performance liquid chromatography
HR	Hazard Ratio
HR	Heart rate
IgSF	Immunoglobulin superfamily
IHD	Ischaemic heart disease
IL-6	Interleukin-6
LDI	Laser Doppler imaging
LDL	Low-density lipoprotein
m/s	Metre per second
MD	Mediterranean diet

MeOH	Methanol
mg	Milligram
MI	Myocardial infarction
min	minute (s)
mL	Millilitre (s)
mmHg	Millimetre of mercury
mmol	Millimole
MTBE	Methyl t-butyl ether
MUFA	Monounsaturated fatty acids
NaCl	Sodium chloride
NCDs	Non-communicable diseases
nm	Nanometre
NO	Nitric oxide
OR	Odd Ratio
oxLDL	Oxidised LDL
PAD	Peripheral Arterial Disease
PP	Pulse pressure
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROSPERO	International Prospective Register of Systematic Reviews
PTFE	Polytetrafluoroethylene
PU	Perfusion units
PUFA	Polyunsaturated fatty acids
PVD	Peripheral vascular disease
PWA	Pulse wave analysis
PWV	Pulse wave velocity
RCF	Relative centrifugal force
RCT	Randomised controlled trials
RR	Relative Ratio
SBP	Systolic blood pressure
SD	Standard deviation
SEM	Standard Error of mean
SFA	Saturated fatty acid
sICAM-1	Soluble intercellular adhesion molecules-1
SMD	Standardised mean differences
SNP	Sodium nitroprusside
sVCAM-1	Soluble vascular cell adhesion molecules-1
TC	Total cholesterol

TG	Triglyceride
TIA	Transient ischaemic attack
TNF-α	Tumour necrosis factor alpha
USDA	United States Department of Agriculture
	Agricultural Research Service
VSMC	Vascular smooth muscle cell
YLLs	Years of life lost
µg	Microgram (s)
µL	Microliter (s)

Publication

Publications derived from this PhD dissertation

1. **Cheng HM**, Koutsidis G, Lodge JK, Ashor AW, Siervo M and Lara J. (2017) Lycopene and tomato and risk of cardiovascular diseases: A systematic review and meta-analysis of epidemiological evidence. **Crit Rev Food Sci Nutr.** 11:1-18. doi: 10.1080/10408398.2017.1362630. [Epub ahead of print]
2. **Cheng HM**, Koutsidis G, Lodge JK, Ashor A, Siervo M and Lara J (2017) Tomato and lycopene supplementation and cardiovascular risk factors: A systematic review and meta-analysis. **Atherosclerosis** 257:100-108.

Original Communications in Academic and Scientific

1. The Nutrition Society Spring Meeting, Abertay University, UK, 1-2nd April 2019. **Cheng HM**, Farsi D and Lara J. "The effects of food intervention on measures of arterial stiffness and microvascular vasodilation: Systematic Review and meta-analysis." Oral session 1, Room 2517, OC06.
2. The Nutrition Society Spring Meeting, Abertay University, UK, 1-2nd April 2019. **Cheng HM**, Koutsidis G, Lodge JK, Keane, k, Siervo M, and Lara J. "The effect of consuming red and orange cherry tomatoes on blood-borne circulating biomarkers in free-living normotensive males: a randomised cross-over trial." Oral session 2, Room 2522, OC08.
3. The Nutrition Society Summer Meeting, University of Leeds, UK, 10-12th July 2018. **Cheng HM**, Koutsidis G, Lodge JK, Keane, k, Siervo M, and Lara J. "The effect of consuming red and orange cherry tomato on endothelial function in free-living normotensive males: a randomised cross-over trial." Oral session 5, Room RS01, OC94.
4. The Nutrition Society Summer Meeting, University of Leeds, UK, 10-12th July 2018. **Cheng HM**, Kazi A, Mavroudis N, and Lara J. "Effect of supplementation with antihypertensive peptides from food proteins on cardiovascular risk factors: A systematic review and meta-analysis of randomised controlled trials." Poster session 8, Poster Pod 4, Sports Hall One, OC90.
5. The Nutrition Society Summer Meeting, University of Nottingham, UK, 6-9th July 2015. **Cheng HM**, Cheng HK and George TW. "The effect of red and yellow beetroot juices on blood pressure in free-living normotensive males: a pilot study." Oral session 9, Room A03, OC55.

Chapter 1 Introduction

1.1 Cardiovascular Diseases: Aetiology and Epidemiology

1.1.1 Definition of cardiovascular diseases

Cardiovascular diseases (CVD) commonly involve the degeneration and interruption of arteries which subsequently result in inadequate supply of blood to the heart muscle (coronary heart disease, CHD), the brain (cerebrovascular disease) and the extremities, especially lower limbs (peripheral vascular disease) (**Figure 1.1**) (Frayn et al., 2005).

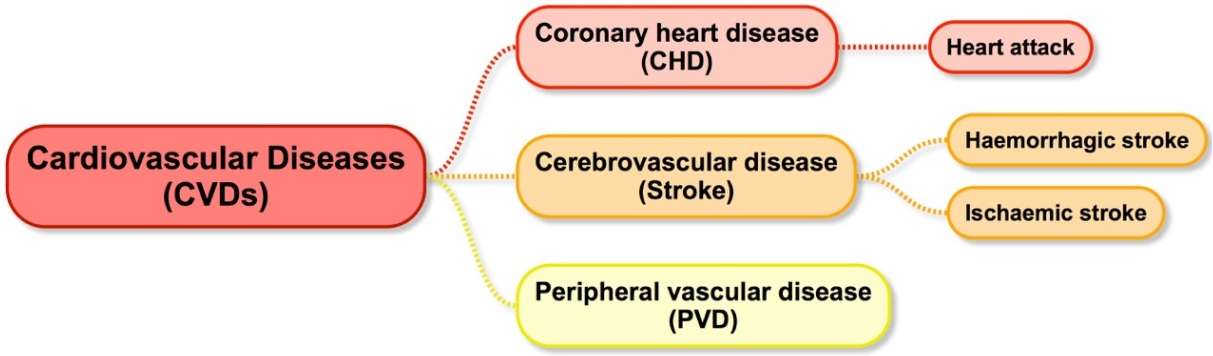


Figure 1.1 Types of Cardiovascular diseases.

1.1.1.1 Coronary heart disease (CHD)

CHD, also known as ischaemic heart disease (IHD), is the most common type of CVD (Townsend et al., 2015). CHD is a condition in which coronary arteries supplying blood to the heart muscle become thickened by a build-up of atheroma; a deposit of fatty material and/or scar tissue in the lining of the arteries wall. Constriction or blockage of coronary arteries interrupts the supply of blood to the heart muscle (the myocardium) and produces chest discomfort and pain on exertion, known as angina (Townsend et al., 2015). In more severe cases, when the fibrous cap covering the plaque ruptures, exposing the underlying tissue and lipid to the circulation, thrombosis (blood clotting) may occur within arteries and obstruct the blood flow completely, causing a heart attack (myocardial infarction, MI) (Bentzon et al., 2014). Sudden cardiac death may be due to MI or cardiac arrhythmia (irregular heart rate). Stress or illness may aggravate cardiac arrhythmia which is more common and more frequently lethal for those individuals who have IHD or any other causes of cardiac dysfunction such as hypertension or excess alcohol consumption. Therefore, the main risk factors observed for cardiac arrhythmia and sudden cardiac death are similar (Frayn et al., 2005).

1.1.1.2 Cerebrovascular diseases

Cerebrovascular disease involves disorders of the arteries which supply the brain and the meninges (membranes covering the brain), causing a stroke or transient ischaemic attack (TIA). Stroke occurs when the blood flow to the brain is interrupted, causing damages to brain cells. Therefore, a stroke may affect body function permanently. TIA, also known as a mini-stroke, is a temporary disruption in blood supply causing non-permanent damage to the brain and the associated symptoms usually disappear within 24 hours.

There are two types of stroke, haemorrhagic stroke and ischaemic stroke. Haemorrhagic stroke occurs when a blood vessel in the brain bursts and the pressure of the leaked blood damages brain cells. High blood pressure (hypertension) is a major risk for haemorrhagic stroke (Frayn et al., 2005). Ischaemic stroke, which is the most common type of stroke in Western countries, is a blockage of the blood supply to the brain. The reduced blood supply to a specific region of the brain may lead to irreversible damage to that part of brain tissue. The blockage most commonly begins from the process of thromboembolism, in which a blood clot is formed in the carotid artery and is subsequently lodged into other sites and impedes blood flow within the brain (cerebral arteries). Atherosclerotic plaques narrowing the intracerebral arteries may also increase the risk of blood clot formation. The main risk factors for stroke are thus similar to those for CHD (Frayn et al., 2005).

1.1.1.3 Peripheral vascular disease (PVD)

The narrowing of arteries supplying other regions excluding the myocardium and brain is classified as peripheral vascular disease (PVD). It commonly occurs in the arteries supplying blood to the lower limbs, resulting in pain on exertion (claudication). More seriously, inadequate blood supply may lead to the death of limb tissues, which may require amputation (Frayn et al., 2005). Functional PVD and organic PVD are the main types of PVD. Functional PVDs do not cause defects in blood vessel structure, while organic PVDs are caused by structural changes in the blood vessels, including inflammation and tissue damage. Peripheral artery disease (PAD) is a type of organic PVD caused by the build-up of fatty deposits (atherosclerosis) in the inner walls of arteries supplying blood to parts of the body (Townsend et al., 2015).

According to the Quality and Outcomes Framework (QOF) data (2013/14) in the UK, around 13.8% of people lived with hypertension, 3.4% with CHD, 1.8% with stroke, 1.6% was diagnosed with atrial fibrillation (AF), 0.7% of people lived with heart failure and 0.7% suffered from PAD (**Table 1.1**).

Table 1.1 Prevalence of cardiovascular conditions in the UK (2013/14).

Total UK population	66953292	100.0%
Hypertension	9252607	13.8%
CHD	2286532	3.4%
Stroke	1184551	1.8%
Atrial Fibrillation	1063093	1.6%
Heart Failure	492814	0.7%
PAD	446018	0.7%

1.1.2 Epidemiology of CVD

Worldwide, mortality due to CVD is still prominent, even though when cardiovascular medicine has advanced in the past few decades (Oikonomou et al., 2016). Among the top 25 causes of death in the world in both 1990 and 2010, IHD and stroke were the top two causes of death during this period, the percentage change in numbers of deaths with stroke and IHD remarkably increased by 26% and 35% respectively (**Figure 1.2**) (GBD 2010 Causes of death Collaborators, 2012). The top three leading causes of years of life lost (YLLs) globally were IHD, lower respiratory infections and stroke in 2010; the total number of YLLs from IHD and stroke increased by 28% and 177% respectively in the interval of 1990-2010 (**Figure 1.3**) (GBD 2010 Causes of death Collaborators, 2012). Globally, in 2015, 39.5 million, or 70% of global deaths (56.4 million) were due to non-communicable diseases (NCDs) (World Health Organization, 2016). CVD were the leading causes of NCDs deaths (17.7 million deaths, or 45% of all NCDs deaths), and represented 31% of global deaths (World Health Organization, 2016) (**Figure 1.4**). In the UK, CHD and stroke were the main forms of CVD, CHD being the largest single cause of death; 45% of CVD deaths were caused by CHD and 25% were caused by stroke in 2014 (Townsend et al., 2015).

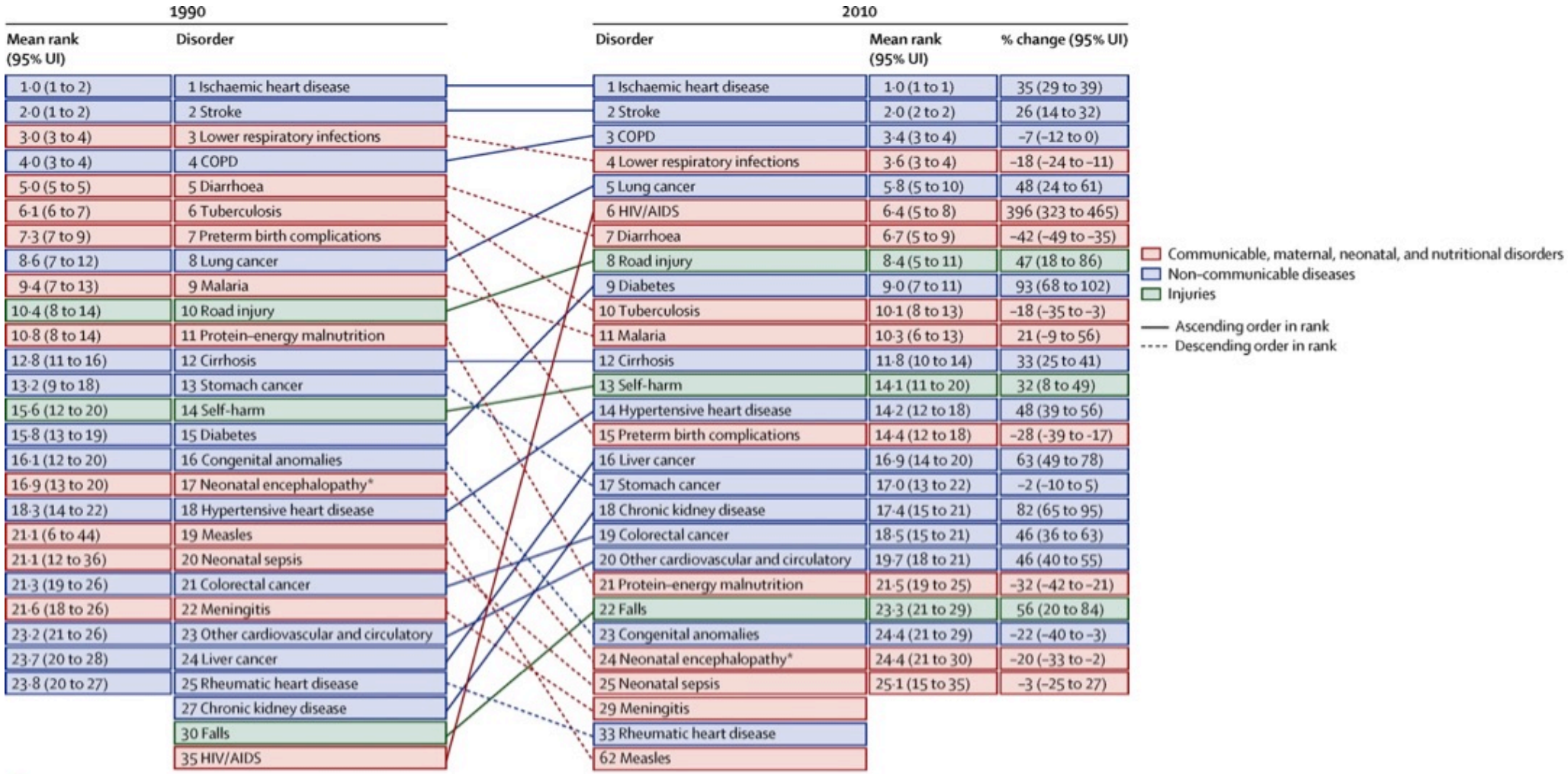


Figure 1.2 Global death ranks with 95% UIs for the top 25 causes in 1990 and 2010, and the percentage change with 95% UIs between 1990 and 2010. (GBD 2010 Causes of death Collaborators, 2012).

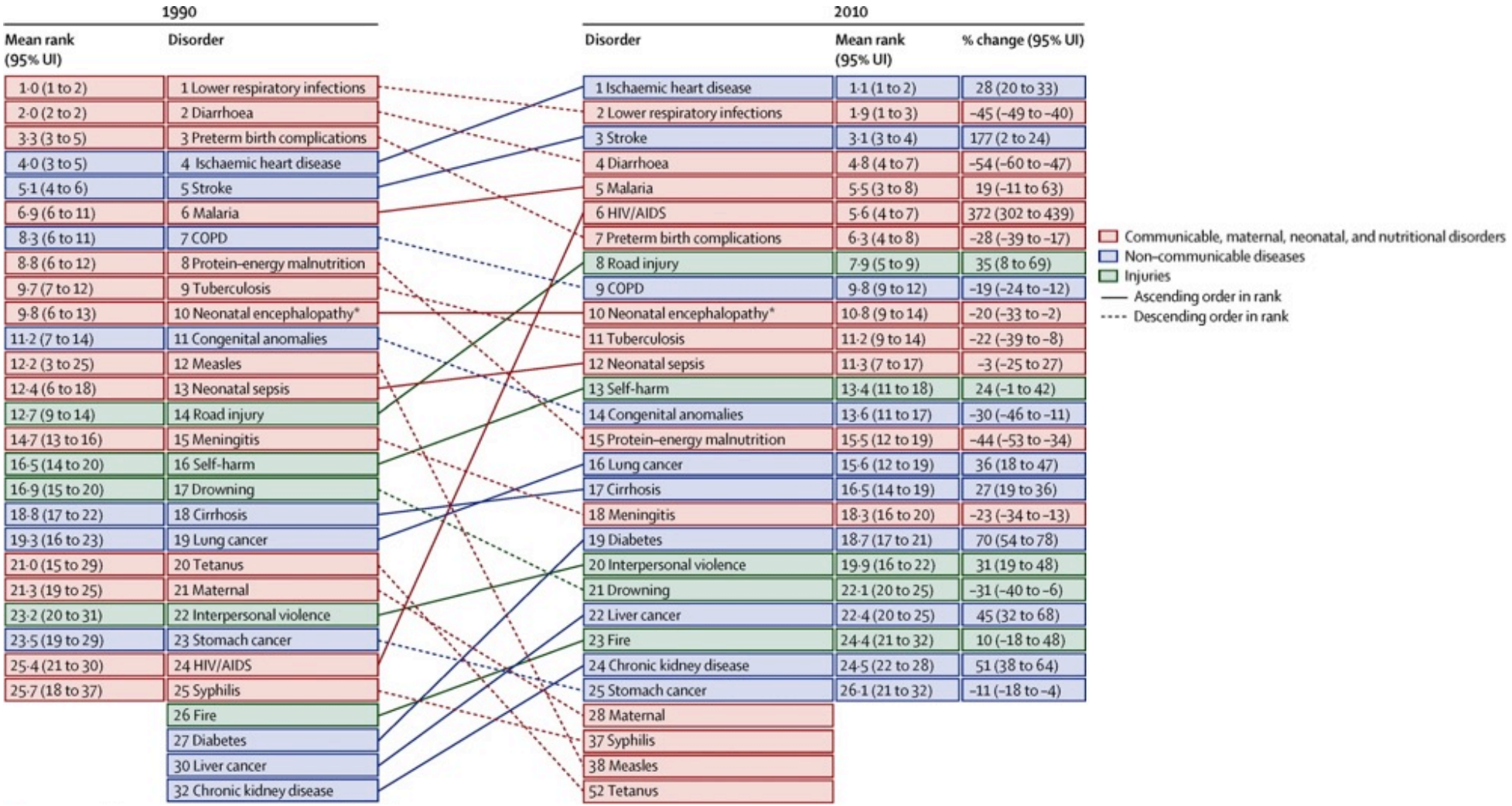


Figure 1.3 Global years of life lost (YLLs) ranks with 95% UIs for the top 25 causes in 1990 and 2010, and the percentage change with 95% UIs between 1990 and 2010. (GBD 2010 Causes of death Collaborators, 2012).

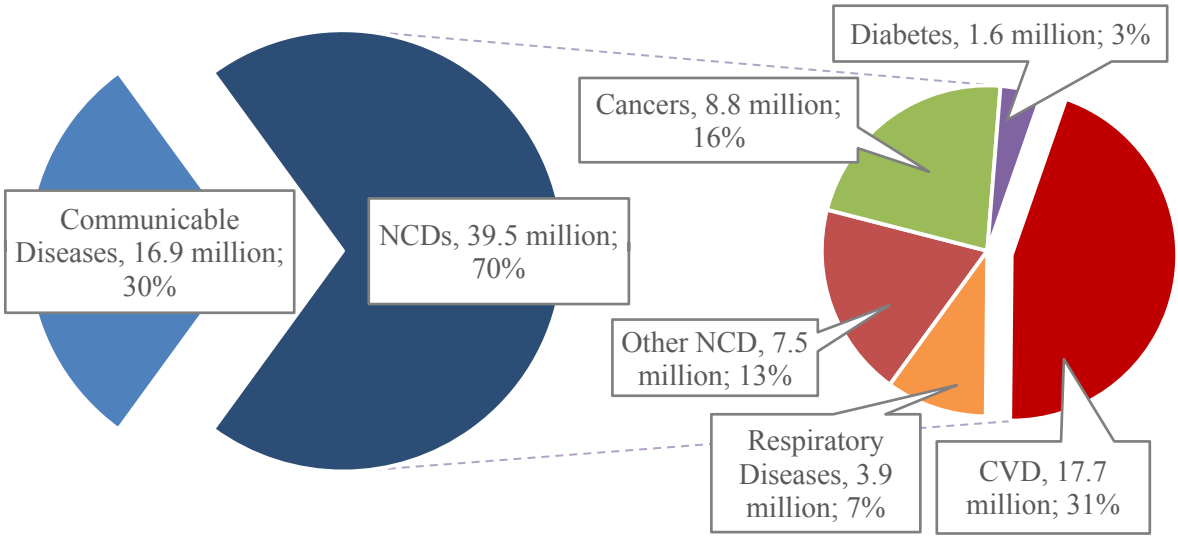


Figure 1.4 Global deaths in 2015 for individuals.

The largest causal category among CVD deaths was CHD (13.1%), closely followed by stroke (11.9%) (**Table 1.2**). Together, CHD and stroke killed an estimate of 14.1 million people in 2015, which was a quarter of the global deaths that year, compared with one in five deaths worldwide 20 years earlier (World Health Organization, 2015).

Table 1.2 Global death population caused by CVD in 2015.

	Population (millions)	% total
Total Global death (million)	56.4	100.0%
Non-Communicable Diseases (NCDs)	39.5	70.0%
CVD	17.7	31.4%
CHD deaths	7.4	13.1%
Stroke deaths	6.7	11.9%

The burden of CVD was rising disproportionately among lower income countries and populations. Over three quarters of NCDs deaths, 30.7 million, occurred in low and middle-income countries in which early death are high with about 48% of deaths happening before the age of 70 years (World Health Organization, 2016).

The burden of CVD was not only reflected in the mortality rate, but also by non-fatal cardiovascular events and their long-term consequences of having survived a damaging heart attack or stroke on health and social service (Dahlöf, 2010). According to British Heart Foundation (BHF), in the UK, over 1.2 million people have survived a stroke and nearly 200,000 hospital visits were due to heart attacks and at least 7 out of 10 people survived (Townsend et al., 2015). The total healthcare costs of CVD in the UK in 2014 were estimated at £9 billion (Townsend et al., 2015). Therefore, the prevention and management of CVD are major public health challenges.

1.1.3 Pathogenesis of CVD

CVD, influencing the coronary, cerebral or peripheral arteries, involve the same pathophysiology: atherosclerosis and thrombosis, with the addition of changes to the function of the blood vessel lining (Frayn et al., 2005). Atherosclerosis is the chronic deposition of inflammatory plaques in blood vessel wall leading to hardened and narrowed blood vessels. This is the first step in development of CVD (**Figure 1.5**) (Kraakman et al., 2016; Ray et al., 2014).

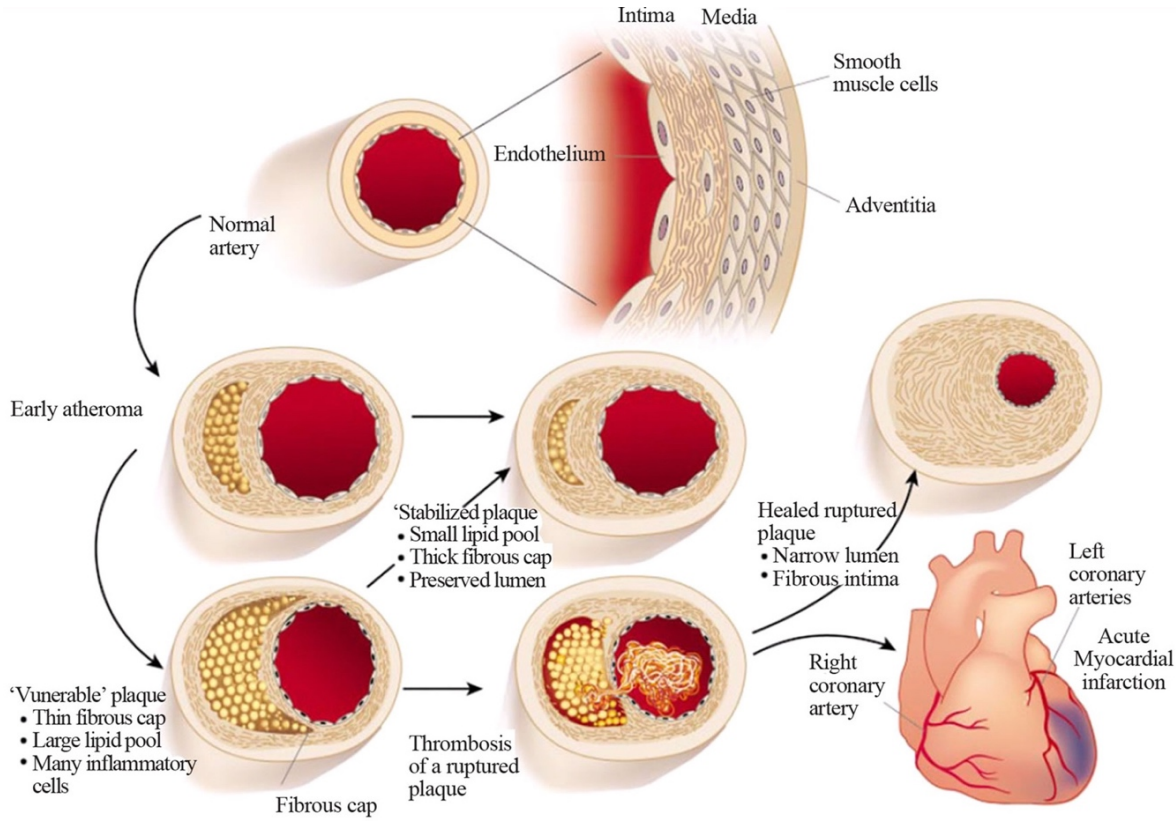


Figure 1.5 Illustration of Inflammation in Atherosclerosis. (Libby, 2002).

Lipid, especially low-density lipoprotein (LDL) cholesterol, from the circulation enters the intima (subendothelial space) through the endothelium (Bentzon et al., 2014). LDL-cholesterol is susceptible to be attacked by free radicals or other reactive oxygen species and becomes oxidised-LDL (oxLDL) upregulating adhesion molecules and cytokines which recruit monocytes and T-lymphocytes (Palmefors et al., 2014). Monocytes uptake oxLDL and differentiate to macrophages. As macrophages fail to break down the oxLDL, they become lipid-laden foam cells after taking up numerous amounts of oxLDL and continue to accumulate in the intima, developing an atherosclerotic plaque (Libby et al., 2011). The growth of plaque stimulates inflammatory responses including the secretion of cytokines, which stimulates proliferation and migration of smooth muscle cells to the plaque and collagen accretion causing the narrowing of arteries, known as fibrous cap or fibrin cap

(**Figure 1.5**). Plaques with gradual accumulation of foam cells have a thicker fibrous cap on the lesion matures plaques, which tend to be stable and are not prone to rupture. However, if the plaque grows more quickly as a result of more rapid lipid deposition which have thinner fibrin caps then it is susceptible to rupture. When a plaque ruptures, it initiates acute thrombosis by activating platelets and the clotting cascade (Libby, 2002).

1.1.4 Endothelial function in cardiovascular disease

The vascular endothelium is one of the largest organs by area lining inside the surface of blood vessels in both arteries and veins. The vascular endothelium has a main role in the pathophysiology of diseases such as atherosclerosis, diabetes and hypertension, which consequently translates into cardiovascular mortality and morbidly. It is an active flattened monolayer cell regulating the blood flow in micro- and macrovascular of the body and is recognised to be essential to the regulation of vascular homeostatic and organ perfusion (Ray et al., 2014). The endothelial layer secretes numerous vasoactive mediators regulating a state of balance in vascular tone, cell growth, vascular homeostatic, leucocyte adhesion, thrombosis, and platelet aggregation (Donato et al., 2015). The endothelium senses and responds to local mechanical stimuli and metabolic conditions through complex cell membrane receptors and signal transduction mechanisms, leading to the synthesis and release of mediators, such as nitric oxide and Prostaglandin. Nitric oxide (NO) in particular has an important role in protecting against the initiation and progression of atherosclerosis. NO has vasodilatory activity and inhibitory activity against growth of vascular smooth muscle cell, nuclear transcription of cell adhesion molecules, platelet aggregation and leukocyte adhesion to vascular endothelial cells (Ray et al., 2014; Tomiyama and Yamashina, 2010).

1.1.4.1 Nitric Oxide synthase (NOS)

Nitric oxide (NO) is a highly reactive, diffusible and dissolved gas with vasodilator properties through the guanylate cyclase-cGMP-PKG pathway and synthesised from L-arginine by nitric oxide synthase (NOS) (**Figure 1.6**); NOS comprises of three enzyme isoforms, the neuronal (nNOS, NOS I), the inducible (iNOS, NOS II) and endothelial NOS (eNOS, NOS III) forms (Levick, 2010). The nNOS, predominantly expressed in the central and peripheral nervous systems, mediates central control of BP, smooth muscle relaxation, and vasodilatation and penile erection via peripheral nitrergic nerves (Forstermann and Sessa, 2012). The iNOS is mostly expressed in the immune system cells, such as macrophages, and expression of iNOS is only induced by bacterial infection and secretion

of cytokines such as tumour necrosis factor alpha (TNF-α) (Leifeld et al., 2002). The eNOS is constitutively expressed in endothelial cells mediated vasodilation in response to shear stress exerted by flowing blood and agonists such as ACh and substance P (**Figure 1.6**) (Levick, 2010).

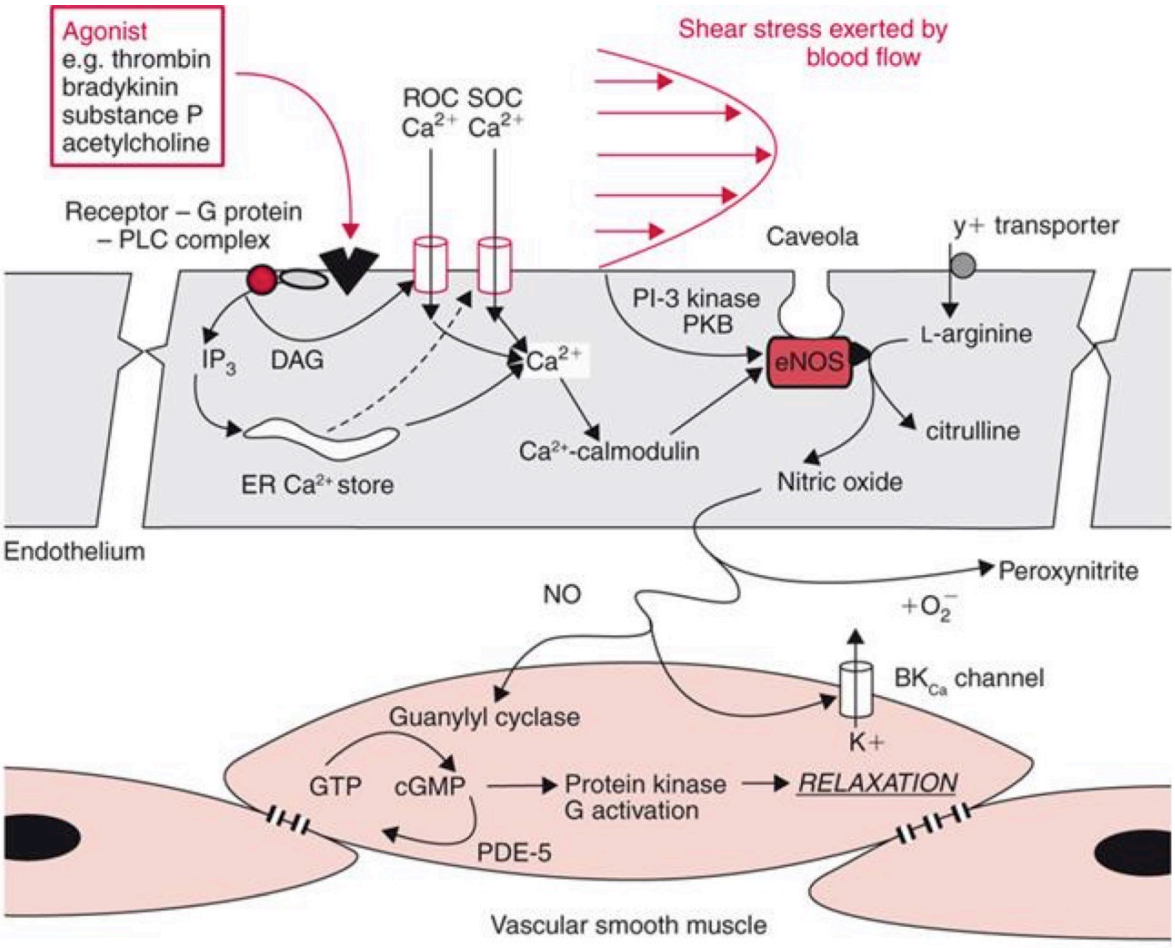


Figure 1.6 Regulation of NO production and its effects on neighbouring vascular smooth muscle.

eNOS, endothelial nitric oxide synthase; ER, endoplasmic reticulum; GTP, guanylyl triphosphate; cGMP, cyclic guanosine monophosphate; PDE-5, phosphodiesterase 5 (inhibited by sildenafil, Viagra); PI-3 kinase, phosphatidyl inositol-3 kinase; PKB, protein kinase B (akt); BKca, big calcium-activated potassium channel; ROC Ca^{2+}, receptor-operated Ca^{2+}channel; SOC Ca^{2+}, store-operated Ca^{2+} channel; PLC, phospholipase in membrane; IP3, cytosolic trisphosphate; DAG, diacyl glycerol. (Levick, 2010).

1.1.4.2 Synthesis of NO from NOS

All NOS monomers include a C-terminal reductase domain and N-terminal oxygenase domain (**Figure 1.6**). The C-terminal reductase domain is structurally homologous to cytochrome P450 reductase (CPR) containing a reductase domain including flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN) and nicotinamide adenine dinucleotide phosphate (NADPH) (Forstermann and Sessa, 2012). The N-terminal oxygenase domain contains a ferric heme complex and binding sites for tetrahydrobiopterin (BH_4) and arginine. The C-terminal reductase domain and N-terminal oxygenase domain are connected via a calmodulin (CaM)-binding amino acid sequence (Forstermann and Sessa, 2012). The NOS enzymes synthesises NO in two sequential monooxygenase reactions. Firstly, L-arginine is hydroxylated to N-hydroxy-L-arginine. Secondly, N-hydroxy-L-arginine is subsequently further oxidised to generate NO and L-citrulline (Andrew and Mayer, 1999). Electrons derived from the NADPH are transferred to the flavins (FAD and FMN) in the C-terminal reductase domain and CaM transfers electrons to the heme complex located in the oxygenase domain. The conversion from a ferric (Fe^{3+}) to ferrous (Fe^{2+}) heme allows the binding of oxygen to synthesise NO (**Figure 1.7**) (Forstermann and Sessa, 2012). Ca^{2+} interacts with CaM (Ca^{2+}-calmodulin complex) which enhances nNOS and eNOS activity and hence NO synthesis (**Figure 1.6**) (Levick, 2010). For iNOS, due to a different amino acid structure of the CaM-binding site, CaM already binds at extremely low intracellular Ca^{2+} concentrations, thus iNOS is not regulated by intracellular Ca^{2+} concentrations (Levick, 2010).

1.1.4.3 Physiological function of NO

NO released by the eNOS is one of the major determinants of vascular tone, vascular smooth muscle cell (VSMC) proliferation, platelet and leukocyte aggregation and adhesion to the vascular wall (Forstermann and Sessa, 2012).

There are two mechanisms of NO action in vasodilation, cGMP dependent and cGMP independent. The eNOS derived NO is highly membrane permeable and freely diffuses to neighbouring VSMC binding the haem group of soluble cytosolic soluble guanylate cyclase (Levick, 2010). As NO is chemically similar to O_2, it has high affinity for haem and activate guanylate cyclase to synthesise cyclic guanosine monophosphate (cGMP) from guanylyl triphosphate (GTP) (**Figure 1.6**). cGMP then activates cGMP-dependent protein kinase (cGMP-PKG) which promotes vascular relaxation. NO can also regulate intracellular Ca^{2+} levels. High concentrations of NO directly activate conductance BK_{Ca} channels in the smooth muscle membrane reducing intracellular Ca^{2+} concentrations which hyperpolarises the smooth muscle leading to vascular relaxation (**Figure 1.6**) (Levick, 2010).

The eNOS-derived NO reduces the expression of monocyte chemoattractant protein (MCP-1) and inhibits the leukocyte adhesion to vascular endothelium and migration into the intima by inhibiting CD11/CD18 expression on leukocytes (Forstermann and Sessa, 2012). Furthermore, NO decreases permeability and uptake of lipoprotein into vascular wall via endothelium and inhibition of lipoprotein oxidation. This protects against the onset of atherogenesis, an early stage in the development of atherosclerosis (Libby et al., 2011).

In addition, NO released towards the vascular wall can protect against thrombosis by inhibiting platelet aggregation and adhesion to the blood vessels which is an early phase of atherogenesis. NO also inhibits the release of platelet-derived growth factors stimulating VSMC proliferation (Forstermann and Sessa, 2012). In addition, NO also inhibit the migration of VSMC to the plaque and collagen accretion causing the narrowing of arteries (Forstermann and Sessa, 2012). This protects against the final stage of atherogenesis (Libby et al., 2011).

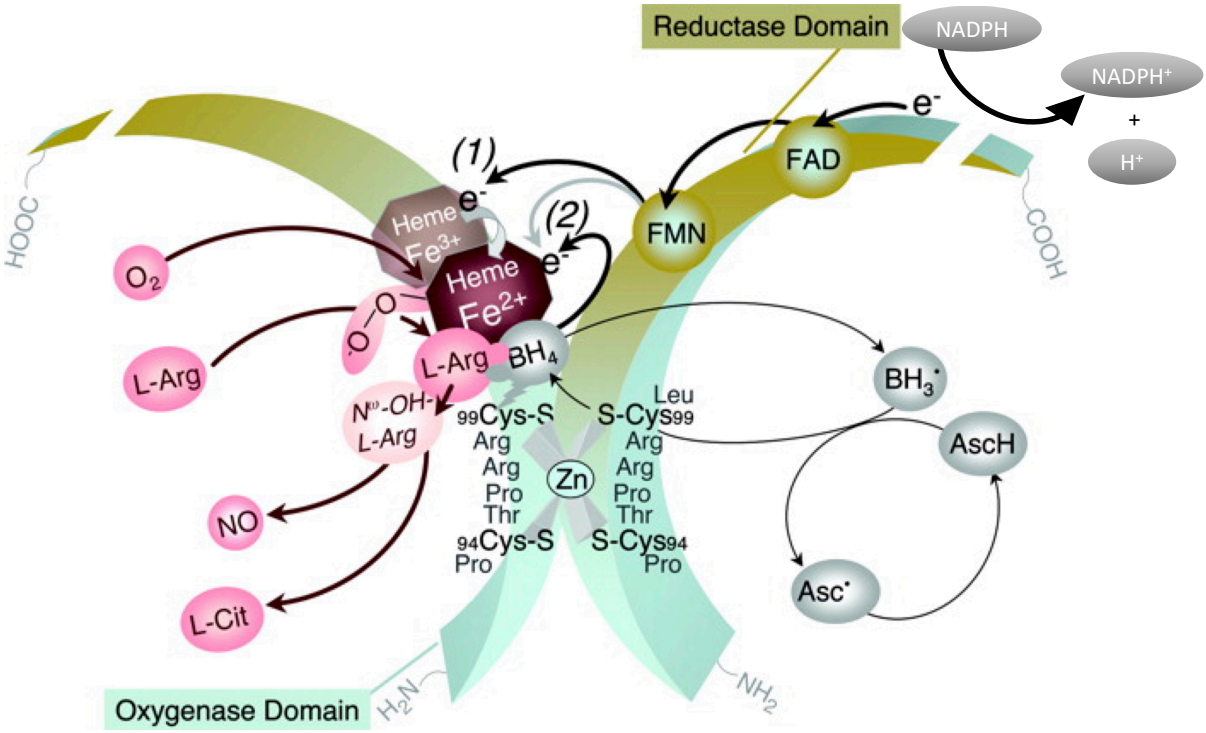


Figure 1.7 Structure and catalytic mechanisms of NOS.

FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; e-, electron; L-Arg, L-arginine; CaM, calmodulin; BH_4, tetrahydrobiopterin; Fe^{3+}, ferric; Fe^{2+}, ferrous.
(Forstermann and Sessa, 2012).

1.1.5 Endothelial dysfunction

Physical or biochemical injury coupled with genetic predisposition can lead to impaired production or dysregulation of pivotal mediators of NO (Mudau et al., 2012). Excess production of reactive oxygen species (ROS) is one fundamental change that triggers vascular endothelial dysfunction which is a pathologic state characterised by reduced NO bioavailability due to increase the production of reactive oxygen species (ROS) and superoxide anion radical. L-arginine and the cofactors BH_4 and FAD are readily oxidised in the presence of ROS (**Figure 1.7**). As discussed above, L-arginine and the cofactors BH_4 and FAD, are necessary for the production of NO by the eNOS; eNOS uncoupling occurs under lower than optimal concentrations of these substrates (Forstermann and Munzel, 2006). When uncoupling of eNOS occurs, eNOS switches its function from a primarily NO-synthesizing enzyme, to a superoxide-producing enzyme generating superoxide anion radical ($O_2^{-\bullet}$) (Forstermann and Sessa, 2012), and thus it exacerbates endothelial oxidative stress levels. Decreased NO production results in a proinflammatory and expression of proadhesive phenotype with the loss of the ability of the endothelial cells to interact with each other (Comba et al., 2016). Furthermore, endothelial dysfunction enhances LDL oxidation, vasoconstriction, upregulate the expression of adhesion molecules, increases cytokine secretion, pro-coagulative, increases cellular permeability, and promotes the proliferation and migration of VSMC (Comba et al., 2016; Donato et al., 2015; Mudau et al., 2012). Therefore, endothelial dysfunction is one of the initial steps in the development of atherosclerotic lesion formation and progression, as well as pathogenesis of atherosclerosis.

In addition to traditional risk factors, blood pressure and blood lipid, endothelial dysfunction is a significant predictor of atherosclerosis, CVD and cardiovascular events (Seals et al., 2014). Thus, a direct measure of endothelial function may provide a powerful tool to guide diagnosis, treatment, and prognosis of patients with pre-existing CVD. There are several non-invasive assessments to measure the vascular function.

1.1.5.1 Endothelial-dependent vasodilation – Flow mediated dilation (FMD)

Flow mediated dilation (FMD) is the most commonly used method for measuring EF (Mahmud and Feely, 2001). It involves ultrasonic measurement of brachial artery dilation in response to increased blood flow (five- to sevenfold) and shear stress generated by hyperaemia of the brachial artery (Flammer et al., 2012). Although it is unusual for the brachial artery to have significant atheroma, the brachial artery is of similar size to coronary arteries. The responses of brachial artery on hyperaemic stimulus have been shown to

correlate with responses in the coronary circulation (Takase et al., 1998). The hyperaemic stimulus is commonly induced through inflation of a pneumatic cuff. On deflation of the cuff, the increased blood flow results in shear stress, which activates eNOS to release NO. The NO diffuses to the vascular smooth muscles, resulting in vasodilation (Arrebola-Moreno et al., 2012). FMD is measured as the percentage change in the diameter of the brachial artery from baseline to the maximum increase in diameter. FMD is mediated by NO and impaired by classical risk factors for CVD. FMD is a widely used non-invasive measurement, but it is technically demanding and requires considerable training, expertise and expensive equipment for its assessment.

1.1.5.2 Arterial stiffness – Pulse Wave Velocity (cf-PWV) and Pulse Wave Analysis (PWA)

Arteries, with elastic walls, are able to buffer rapid changes in blood volume taken up during systole is returned during diastole (Avolio, 2013). Arterial stiffness, also known as the loss of arterial elasticity, which is an important in the pathophysiology of CVD and manifestation of vascular ageing (Frayn et al., 2005). The loss of elasticity of the artery wall leads to stiffening of the conduit vessels, reducing arterial storage capacity as well as increasing the speed of the propagating pulse along the blood vessel wall (Avolio, 2013). For a given ventricular stroke volume, increases in arterial stiffness is a major contributor of high pulse pressure due to the combined influence on the capacitive effects of the artery wall to absorb the pulsatile energy and the wave propagation effects that influence peripheral wave reflection (Avolio, 2013).

Pulse wave velocity (PWV), a direct measure of arterial stiffness, is calculated as the propagation speed of pulse wave in the artery by simultaneously detecting the arrival of the wave at both locations with gating to a contemporaneously electrocardiogram (Arrebola-Moreno et al., 2012; McCall et al., 2011). Thus, PWV, which is used to directly measure the regional stiffness, is generally accepted as the simplest, most robust, reproducible and non-invasive method of detecting arterial stiffness. Aortic PWV is the gold standard for assessing arterial stiffness as aorta makes the largest contribution to the buffering function and is responsible for most of the pathophysiological effects of arterial stiffness (Chen et al., 2017). Aortic PWV is usually measured between the carotid and femoral artery (cf-PWV) (Tomiyama and Yamashina, 2010). Increased propagation speed of pulse wave is associated with increased arterial stiffness (Levick, 2010).

Pulse wave analysis (PWA) is used to measure systemic arterial stiffness indirectly and expressed as the augmentation index (AIx, %). Augmentation pressure is the result of the sum of the forward wave, which is the cardiac pressure impulse and the reflected wave generated by the peripheral vascular system at the interface between large arteries and resistance vessels (arterioles) (Arrebola-Moreno et al., 2012). AIx is calculated as the percentage of the augmentation pressure (AG) to central pulse pressure (AP) (AG/PP) (**Figure 1.8**) (Hashimoto et al., 2008; Stoner et al., 2012). Augmentation pressure (AG) is the pressure difference between second systolic peak and first systolic peak (i.e. Ps-Pr in **Figure 1.8**). Central pulse pressure (PP) is the pressure difference between the maximum systolic pressure (Ps) and minimum diastolic pressure (Pd) (**Figure 1.8**). Therefore, arterial stiffness is associated particularly with increased systolic pressure rather than diastolic pressure (Keane et al., 2016; Mahmud and Feely, 2001).

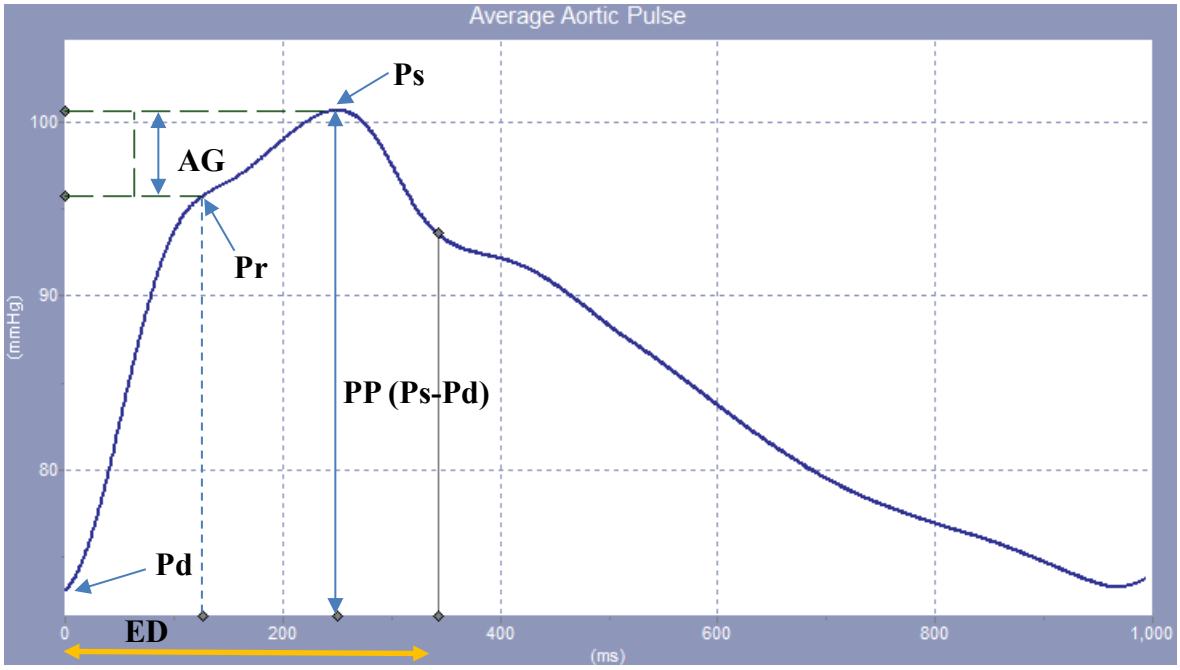


Figure 1.8 Aortic pulse pressure waveform in old healthy man.

Pd, minimum diastolic pressure; P_f; the forward pressure wave; Ps; peak systolic pressure (P2); ED, left ventricular ejection duration; PP, central pulse pressure; AG, Augmented pressure; AIx, Aortic augmentation index (AG/PP).

The location of P_f peak differs depending on the age and disease status. In young individuals, P_f peak is mostly the second peak (**Figure 1.9A**) which give negative value on AIx. This slow return of the reflective wave boosts coronary artery perfusion (Levick, 2010). By contrast, in older or hypertensive individuals, P_f peak is mostly the first peak (**Figure 1.9B**) which gives a positive value on AIx. The early return of reflecting wave increases the systole

pressure and therefore the left ventricle has to eject blood against an increase afterload (Levick, 2010). Increased arterial stiffness results in a high velocity of the forward wave as well as hastens the return of reflected wave (Keane et al., 2016). A high AIx is associated with arterial stiffness and has been shown to be a predictor of adverse cardiovascular events in a variety of patient populations (Shimizu and Kario, 2008).

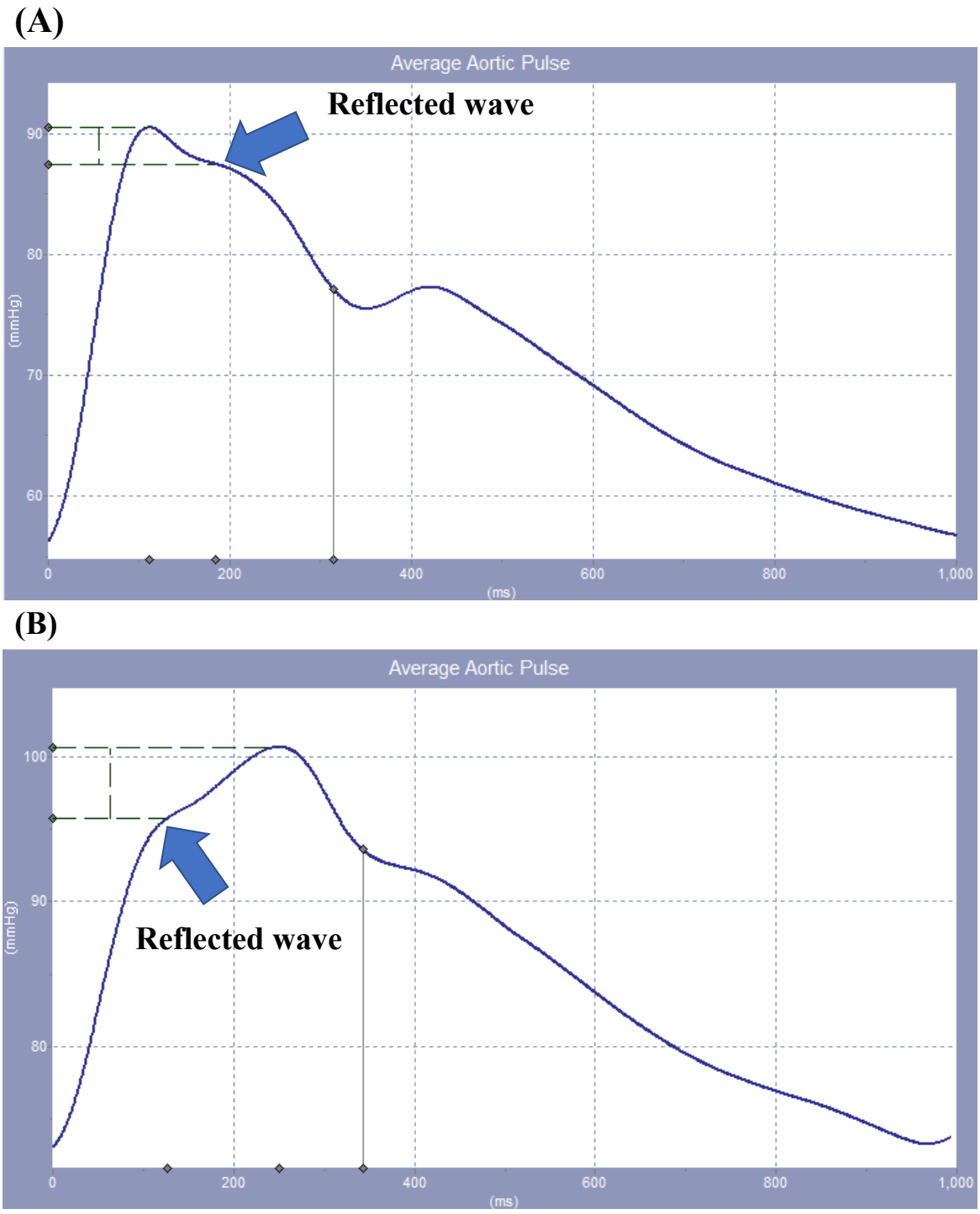


Figure 1.9 Pulse waveform of young healthy volunteer (A) and old healthy volunteer (B). Arrow show the arrival of reflective wave.

1.1.5.3 Microvascular vasodilation – Laser Doppler Imaging (LDI)

Laser Doppler imaging (LDI) with iontophoresis is another modern measurement of vascular function by assessing skin microvascular function, which measures cutaneous perfusion accompanied by iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP) (Ferrell et al., 2002). ACh, which measures endothelium-dependent vasodilation, activates the endothelial nitric oxide synthase (NOS) to produce NO. SNP, which measures endothelium-independent vasodilation, directly causes relaxation of SMCs without the endothelium (**Figure 1.10**) (Turner et al., 2008). Iontophoresis is the use of electric current to drive charged chemicals into the skin. ACh, positively charged, is driven into skin at the positive anode while SNP, negatively charged, is driven into skin at the cathode (Ferrell et al., 2002). It is a safe procedure and has been used to measure blood vessel function in children and pregnant women successfully and without difficulty (Frayn et al., 2005). An infrared laser beam over the skin, which penetrates two to three millimetres below the skins surface and reflects light with red blood cells, produces a signal detecting the magnitude of blood flow. The degree of endothelial dysfunction occurring in the peripheral circulation has been shown to be proportional to that occurring in the coronary arteries (Turner et al., 2008).

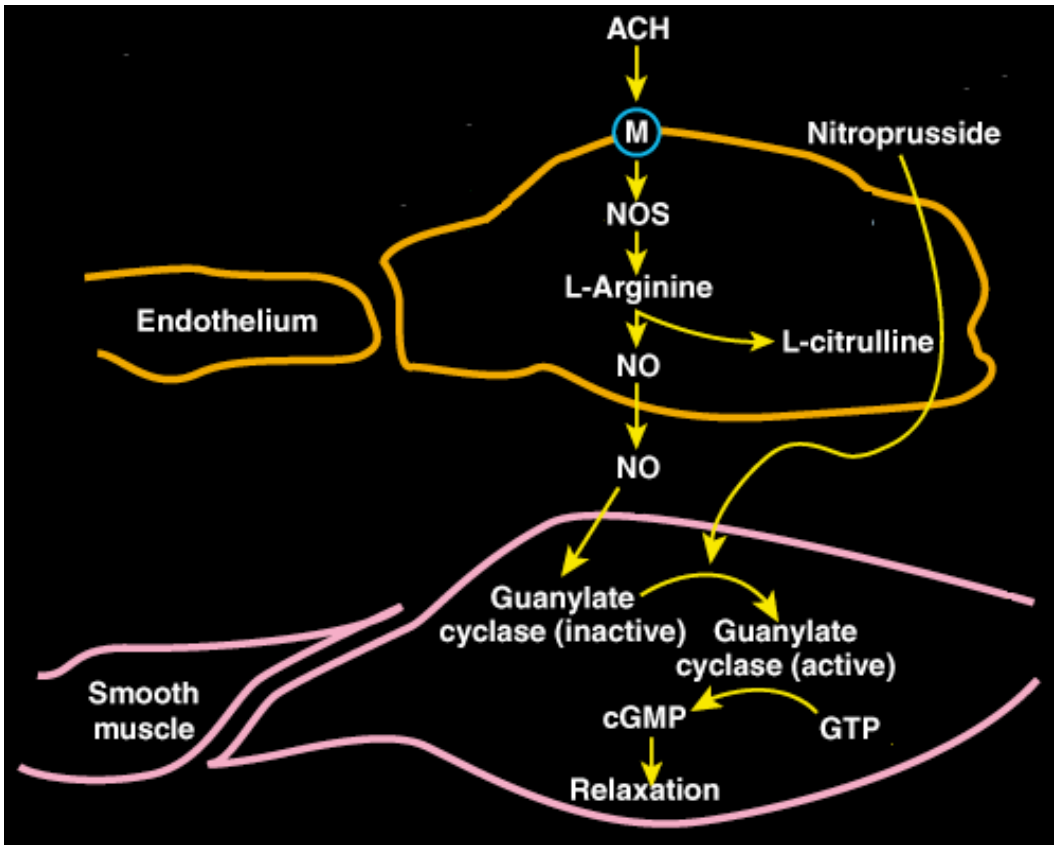


Figure 1.10 Endothelium-dependent and endothelium-independent vasodilation.

1.2 Risk Factors of CVD

CVD and atherosclerosis are the results of complex interaction between environmental and genetic factors rather than just a single significant risk factor. However, wide differences in CHD incidence and mortality rates between populations or ethnic groups are not fully explained by their respective distribution of modifiable traditional cardiovascular risk factors (TCRFs) such as hypertension, dyslipidaemia, diabetes, obesity, and smoking. Indeed, some populations may have a higher burden of CHD than others despite similar rates of TCRFs. In Afro-Caribbeans, patients with CHD had higher rates of hypertension (78.7% vs 30.1%), hypercholesterolemia (52.8% vs 15.0%), and diabetes (53.9% vs 14.8%) and were more often men (64.0% vs 43.7%) and smokers (27.5% vs 13.4%) compared with non-CHD controls (all $p<0.001$) (Larifla et al., 2016). TCRFs can predict the risk of CHD in most populations around the world. It is estimated that in the USA population, 54% of the men's and 49.6% of the women's CVD deaths could be prevented through the complete elimination of increased cholesterol levels, diabetes, hypertension, obesity, and smoking (Patel et al., 2015). Different types of cardiovascular risk scores, such as the Framingham Risk Score, the European Systematic COronary Risk Evaluation (SCORE) algorithm, the German Prospective Cardiovascular Münster (PROCAM) model, the UK QRISK2 equations, are used to estimate the risks of developing CVD in individuals before its clinical onset. The CVD risk scores mainly incorporate the effects of well-established (or traditional) risk factors including age, sex, blood pressure, total cholesterol, LDL-cholesterol, high density lipoprotein (HDL)-cholesterol, smoking status, diabetes mellitus and family history of CHD to estimate the prospect of developing coronary death, angina pectoris, coronary insufficiency or myocardial infarction in future of 10 years. All these factors were well established and highly related to CVD risk. These risk scores aid in decision making for primary prevention in individuals who have not developed the clinical manifestations of CVD (Karmali et al., 2017).

1.2.1 Traditional CVD risk factors

1.2.1.1 Age

Ageing is one of the non-modifiable independent predictors for CVD in risk scores in middle-aged persons. Starting from the age of 50, cardiovascular and circulatory diseases begin to rise steadily to become the largest cause of death in 2010 (**Figure 1.11**) (GBD 2010 Causes of death Collaborators, 2012). As the average lifespan of humans has extended, there is a trend in the increase of population aged 65 and older that will continue over the next 20

years (North and Sinclair, 2012). In this age group, CVD result in 40% of all deaths and rank as the leading cause of death years (North and Sinclair, 2012). Although ageing is an unavoidable part of human life, many of the risks associated with ageing are modifiable. Therefore, it is widely acknowledged that ageing is plastic (Kirkwood, 2008) and therefore by targeting the modifiable factors healthy ageing is a realistic and achievable target (Mathers, 2015).

1.2.1.2 Blood lipid profile

Blood lipid, total cholesterol (TC), LDL-cholesterol, HDL-cholesterol and triglyceride (TC), are well-recognised risk factors for CVD and used to predict overall CVD. Cholesterol is a fatty substance, found in blood and most tissues especially nerve tissues. Cholesterol, the main component of cell membranes, is also a precursor of many hormones and bile acid, synthesised mainly in the liver. As cholesterol is a lipid, it requires a transport vesicle to shield it from blood, which is aqueous in nature (Daniels et al., 2009). A complex of various proteins and lipids achieve cholesterol transport through the vascular system. These complexes, intuitively known as lipoproteins, are heterogeneous in size, shape, composition and function and are classified according to their density. Low-density lipoproteins (LDL) cholesterol and high-density lipoproteins (HDL) cholesterol are perhaps most frequently related to CVD. Homeostasis of cholesterol is centred on the metabolism of lipoproteins, which mediate transport of the lipid to and from tissues (Daniels et al., 2009).

High total cholesterol (TC) was the ninth and tenth-leading risk factor attributable to disability-adjusted life-years (DALYs) for men and women respectively in 2015 (**Figure 1.12**) (GBD 2015 Risk Factors Collaborators, 2016). A positive association between a reduction of 1 mmol/L of TC with lower IHD mortality was observed in both genders at middle age (40-49years) (HR 0.44, 95% CI 0.42 to 0.48) and older ages (70-89years) (HR 0.83, 95% CI 0.81 to 0.85) (Lewington et al., 2007).

LDL-cholesterol, known as “bad cholesterol”, carries cholesterol to the tissues that need it. Excessive accumulation of blood LDL-cholesterol at the arterial wall can lead to the build-up of atheroma, eventually causing atherosclerosis and CVD. Lower LDL-cholesterol levels were independently associated with a lower IHD mortality. On average, a reduction of 1 mmol/L of LDL-cholesterol was associated with a reduction in about a third of IHD mortality within every age group and in both sexes (Gupta and Smith, 2014; Lewington et al., 2007).

HDL-cholesterol, known as “good cholesterol”, carries cholesterol away from the tissues and delivers it back to the liver, where it is broken down and excreted in the bile (Daniels et al., 2009). High levels of HDL-cholesterol had cardioprotective effect, while low levels of HDL-cholesterol were an independent predictor of cardiovascular risk and were associated with an increased risk of atherosclerosis (Townsend et al., 2015). A study has shown that reduced HDL-cholesterol was an independent risk factor for IHD (Linton et al., 2009). On average, an increase of 0.33 mmol/L HDL-cholesterol was associated with a reduction of IHD mortality by about a third within every age group and in both genders (Lewington et al., 2007).

Triglyceride (TG) is the main form of dietary fat consisting of glycerol combined with three fatty acids. TG plays an important role in metabolism, synthesis of hormones and building cells. However, high levels of TG in the bloodstream increase the risk atherosclerosis. Current evidence indicates that elevated TG is an independent risk factor for IHD risk among different ethnic groups (Linton et al., 2009). A meta-analysis of 29 studies has shown that there was a significant association between TG values and CHD risk in Western populations (Odds Ratio (OR) 1.72; 95% confidence interval (CI), 1.56 to 1.90) (Sarwar et al., 2007). Raised circulating TG levels were associated with increased CHD risk, adjustment for established coronary risk factors, especially HDL-cholesterol, substantially attenuated the magnitude of this association. Therefore, levels of cholesterol and related lipids circulating in blood are essential predictive tools employed clinically to measure risk of a cardiac event.

In high CVD risk patients, rigorous control of blood cholesterol is recommended. The optimal TC target is less than 4 mmol/L, LDL-cholesterol less than 2 mmol/L, HDL-cholesterol higher than 1 mmol/L and triglyceride less than 1.7 mmol/L (British Cardiac Society, 2005).

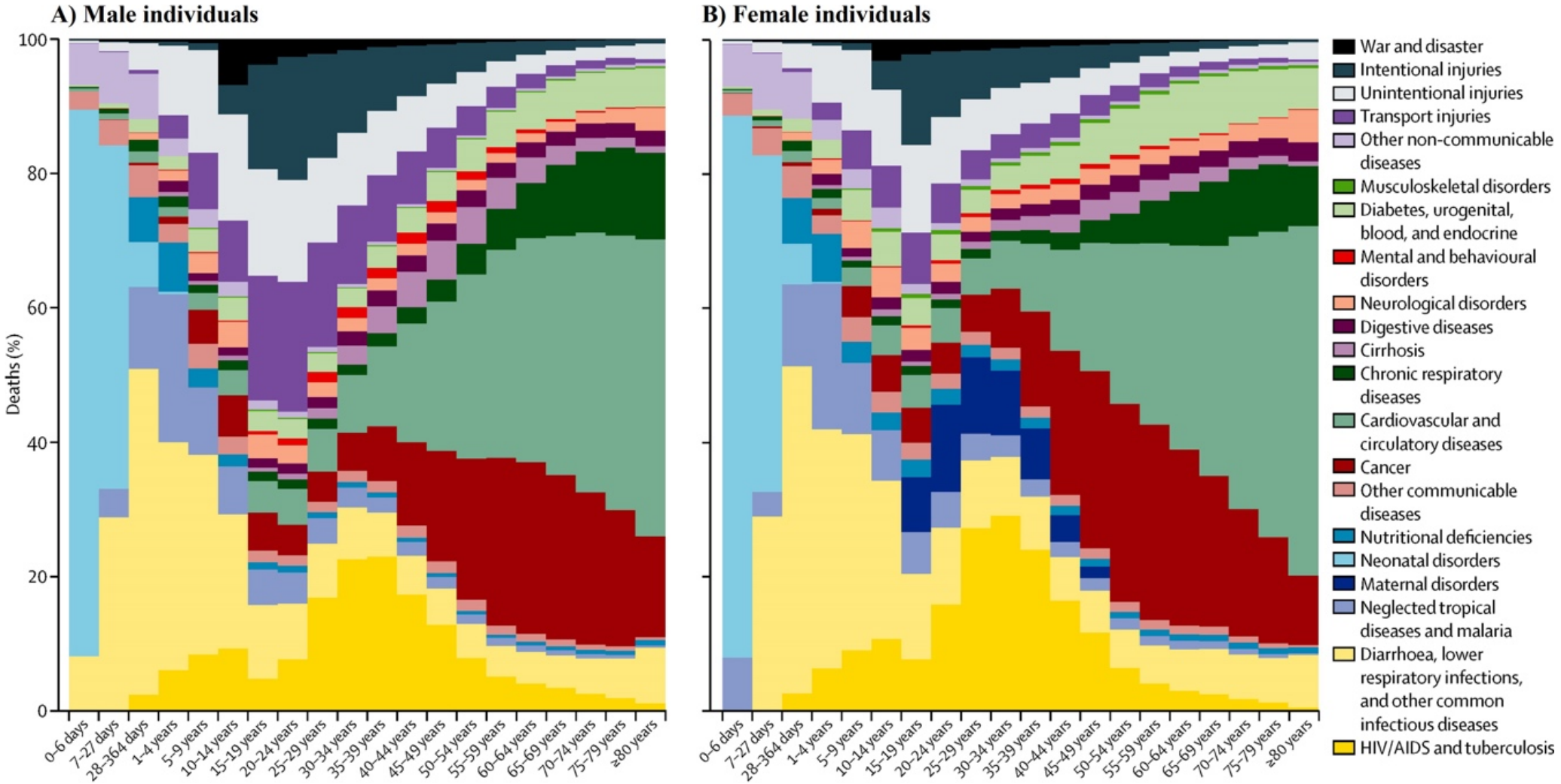


Figure 1.11 Percentage of global deaths for male (A) and female (B) individuals in 2010 by cause and age.

(GBD 2010 Causes of death Collaborators, 2012).

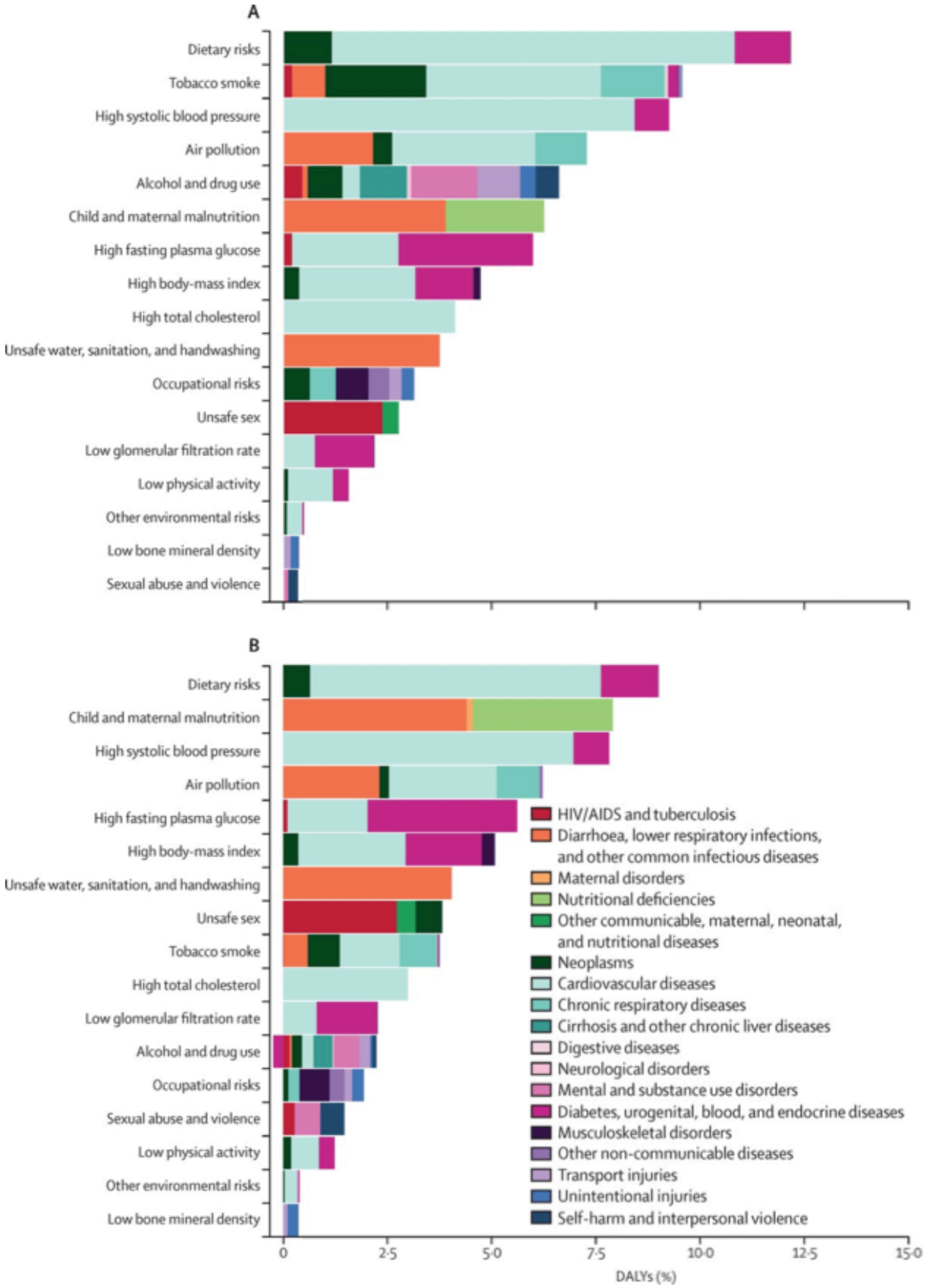


Figure 1.12 Global disability-adjusted life-years attributable to risk factors for men (A) and women (B) in 2015.

(GBD 2015 Risk Factors Collaborators, 2016).

1.2.1.3 High blood pressure

High blood pressure including both systolic blood pressure (SBP) and diastolic blood pressure (DBP), are directly related to the progression of CVD (Lewington et al., 2002). Elevated BP was a well-established risk factor for CVD. An increase of every 20 mmHg in SBP and 10 mmHg in DBP doubled the risk of CVD, across the BP range from SBP <115 mmHg to >180 mmHg and from DBP <75 mmHg to >105 mmHg (Lewington et al., 2002). According to the WHO, normal adult BP is defined as 120/80 mmHg, while BP equal to or above 140/90 mmHg is indicative of hypertension. Individuals with hypertension are at four times the risk of developing CVD. In 2015, high SBP was the top third leading risk factors among other leading risks for both sexes, contributing to 9.2% (8.3 to 10.2) of DALYs for men and 7.8% (6.9 to 8.7) of DALYs for women (**Figure 1.12**) (GBD 2015 Risk Factors Collaborators, 2016). Additionally, from 1990-2015, high BP were the leading three risk factors for attributable DALYs and from 2005 to 2015, the numbers of DALYs due to high SBP increased 11.7% (**Figure 1.13**) (GBD 2015 Risk Factors Collaborators, 2016). In the UK, nearly 30% of adults have high BP (Townsend et al., 2015). The World Health Report 2002 estimated that nearly 11% of all burden of diseases in developed countries were leaded by high BP (World Health Organization, 2002). In addition, about 49% of IHD and 62% of cerebrovascular disease were attributable to suboptimal SBP (>115 mmHg) (World Health Organization, 2002). Throughout middle and old age, usual blood pressure was strongly and directly related to vascular mortality. Meta-analysis of prospective data on over one million adults aged 40-69 years has shown that every 20 mmHg increase to normal SBP (115 mmHg) or 11 mmHg to normal DBP (75 mmHg), had a doubling of mortality from both IHD and stroke (**Figure 1.14**) (Lewington et al., 2002). Therefore, the WHO voluntary targets for NCDs have called for a 25% reduction in the prevalence of high BP by 2025 (GBD 2013 Risk Factors Collaborators, 2015).

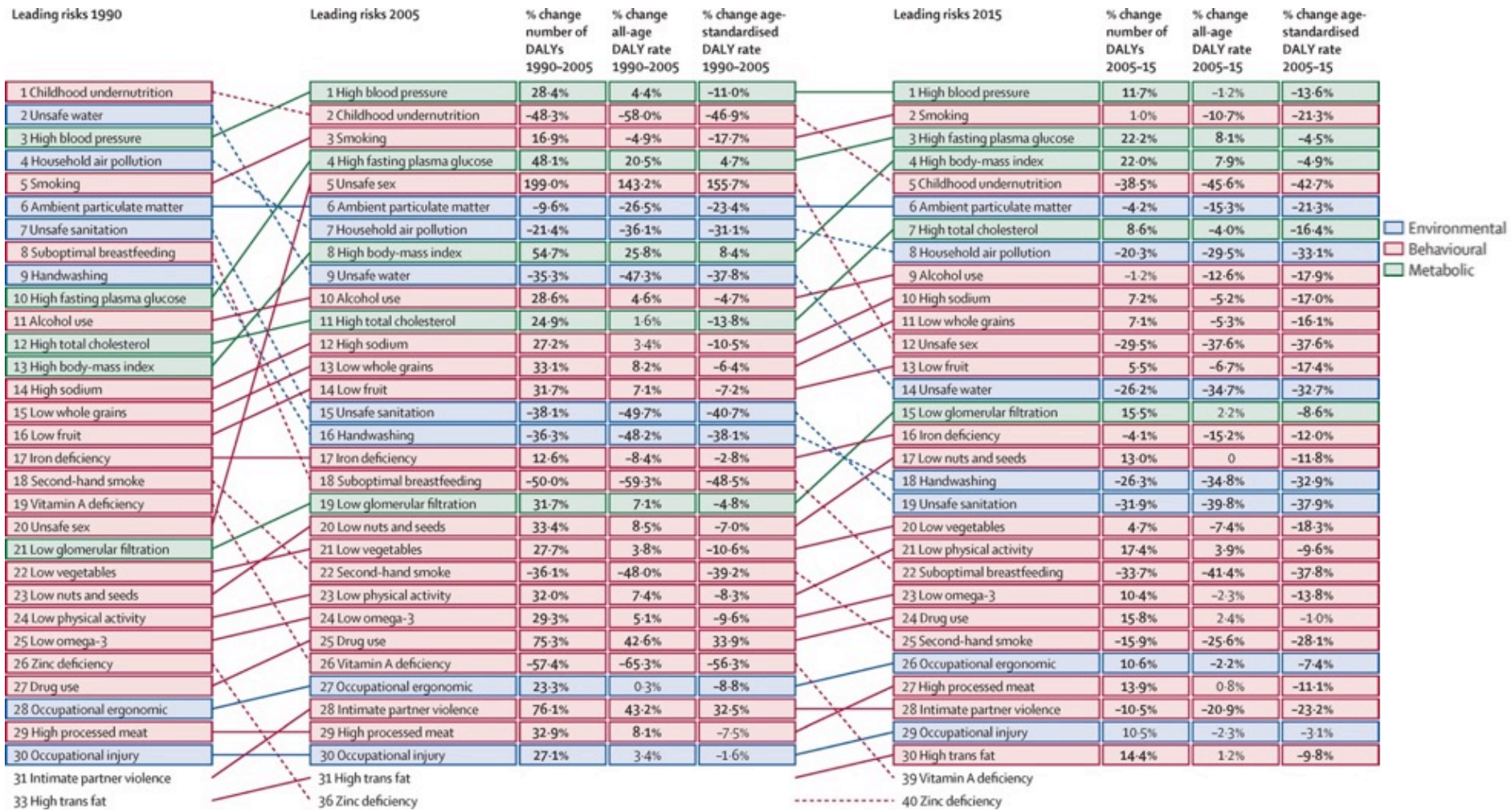


Figure 1.13 Leading 30 Level 3 global risk factors for DALYs for both sexes combined, 1990, 2005, and 2015, with percentage change in number of DALYs, and all-age, and age-standardised rates.

(GBD 2015 Risk Factors Collaborators, 2016).

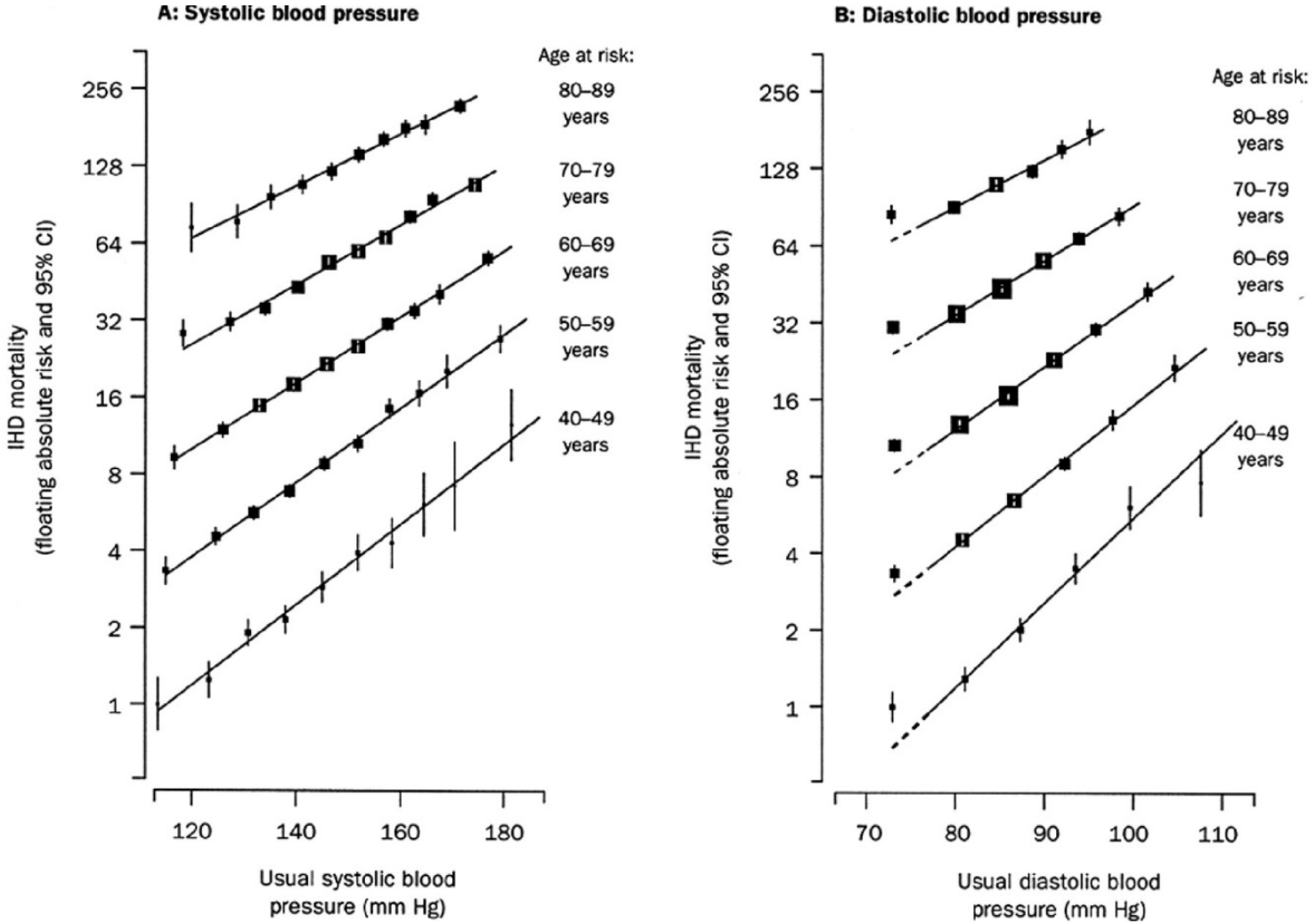


Figure 1.14 Ischemic heart disease (IHD) mortality rate in each decade of age versus usual blood pressure at the start of that decade. (Lewington et al., 2002).

Compared with the traditional method of taking a BP reading under clinical setting, ambulatory blood pressure monitoring (ABPM) offers the ability to collect BP readings across a 24-Hour period which can be aggregated to yield overall 24-Hour BP pattern or grouped to reflect daytime and nighttime BP patterns (Turner et al., 2015). The various BP categorisations facilitated by ABPM are valuable for clinical management of hypertension since they increase accuracy for diagnosis and the prediction of cardiovascular risk (Krakoff, 2013). In addition, ABPM enables us to rule out white-coat effect hypertension and identify masked hypertension (Turner et al., 2015). A meta-analysis has shown a two-fold higher incidence of CVD (HR 2.00, 95% CI 1.58 to 2.52) in people with masked hypertension compared with those with normal BP and ABP (Fagard and Cornelissen, 2007). Strong evidence have shown oxidative stress, inflammatory processes, endothelial dysfunction and subsequent vascular remodelling are associated with the pathogenesis of hypertension (Widlansky et al., 2003).

1.2.2 Other risk factors

The role of plasma lipids, especially LDL-cholesterol, in atherosclerosis showed the reason of elevated serum TC level has long been recognised as a predisposing factor to CVD. However, over the past few decades, more epidemiological evidence has highlighted the importance of other processes, including endothelial dysfunction, the tendency of oxidation in the subendothelial space, the inflammatory processes involved in formation of plaque, and blood clotting. In addition, recent research has been directed to identify and develop biomarkers, other than blood lipids, of CVD risks that could be useful in stratifying individuals and also in measuring the impact of interventions (Lara et al., 2015; Morrow and de Lemos, 2007). These biomarkers are involved at various stages of the atherosclerosis process.

1.2.2.1 Inflammation-related factors

Inflammation is a basal element of atherogenesis through all stages of its development, from initiation, through progression to its thrombotic complication (Frayn et al., 2005). Inflammatory cytokines are protein molecules secreted by various immune cells and vascular cells that act as enhancing mediators for immune responses. Endothelial cells are major targets of cytokines that could mediate endothelial dysfunction and vascular inflammation (Sprague and Khalil, 2009). Cytokines promote adhesion of leukocytes to endothelial cells and cause an increase in vascular permeability. They also stimulate VSMC to migrate from the medial portion of the arterial wall towards the intima (Sprague and Khalil, 2009).

Interleukin-6 (IL-6)

IL-6 is a major pro-inflammatory glycosylated cytokine released by activated leukocytes, adipocytes, and endothelial cells with diverse humoral and cellular immunomodulatory effects. IL-6, one of the plasma cytokines that stimulates the production of C-reactive protein (CRP) and fibrinogen, is secreted by monocytes and macrophages in inflammatory response (**Figure 1.15**) (Frayn et al., 2005). The study of Schnabel et al. (2013) found that there were significant associations between high levels of IL-6 and increased CVD mortality risk (HR 1.16, 95% CI 1.01 to 1.32, p=0.03 (Schnabel et al., 2013). In addition, the study of Danesh et al. (2008) has shown that increased IL-6 levels were associated with progressively increased CHD risk (OR 1.61, 95% CI 1.42 to 1.83) (Danesh et al., 2008).

Tumour necrosis factor alpha (TNF-α)

TNF-α, also known as Cachectin and TNFSF1A, is a pro-inflammatory cytokine that is essential in the initiation of an inflammatory response. TNF-α is expressed by a variety of cells, including macrophages, foam cells, monocytes, T-cells, VSMCs, adipocytes, and fibroblasts. TNF-α regulates and interferes with lipid metabolism, suppresses free fatty acid (FFA) uptake, promotes lipogenesis, induces lipolysis, inhibits lipid-metabolism-related enzymes activity, regulates cholesterol metabolism and regulates other adipocyte-derived adipokines (Chen et al., 2009). In patients with hyperlipidaemia, high TNF-α levels correlated significantly to the concentrations of very-low-density lipoprotein (VLDL) cholesterol, TG and TC and negatively to HDL-cholesterol (Jovinge et al., 1998). TNF-α also stimulates additional cytokine and adhesion molecules expression, which increases plaque instability and augments VSMCs proliferation and migration (Libby, 2006).

C-reactive protein (CRP)

CRP, an acute phase reactant, is synthesised in the liver and is secreted in response to secretion of the cytokine, IL-6. However, CRP has also been found in the endothelium of atherosclerotic plaques, in smooth muscles cells, macrophages and in adipocytes (**Figure 1.15**) (Frayn et al., 2005; Kressel et al., 2009; Ridker and Silvertown, 2008). Like other acute phase reactants, CRP reflects the inflammatory response to atherosclerotic damage, in addition to enhancing clot formation, lipid oxidation and cell activation (Frayn et al., 2005). CRP is a predictor of risk for CVD, even in individuals with low LDL-cholesterol levels and at all levels of the Framingham Risk Score (Danesh et al., 2000; Ridker and Silvertown, 2008). Meta-analysis of a prospective study with 18,569 participants has shown that participants in the top third of the group (2 mg/L) with respect to baseline CRP values had increased risks of CHD compared with those in the bottom third (0.78 mg/L) (OR 1.45, 95%

1.25 to 1.68) (Danesh et al., 2004). In addition, meta-analyses of CVD mortality risk has shown significant associations with CRP (HR 1.31, 95% CI 1.02 to 1.68, p=0.033) (Barron et al., 2015).

Fibrinogen

Fibrinogen is the precursor of fibrin and plays a dual role in atherosclerosis as the major coagulation factor in the blood and a pro-inflammatory molecule (**Figure 1.15**) (Frayn et al., 2005). Like CRP, fibrinogen is an acute phase reactant synthesised in liver, whose circulating levels can fluctuate enormously during acute responses to tissue damage or infection. Many studies have found that fibrinogen is a strong predictor of CHD and associates strongly with other inflammation factors. Meta-analysis of 18 studies with 4018 CHD cases, comparing individuals in the top third with those in the bottom third of the baseline measurements yielded a combined risk ratio of 1.8 (95% CI 1.6 to 2.0), and showed differences in long-term usual mean fibrinogen levels of 2.9 µmol/L between the two groups (10.3 vs 7.4 µmol/L) (Danesh et al., 1998).

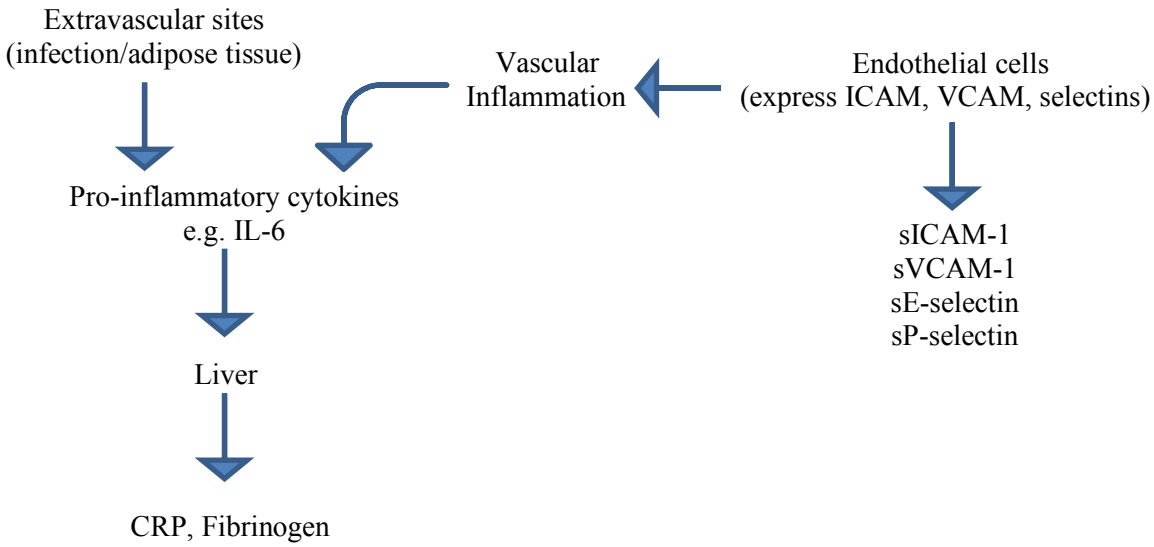


Figure 1.15 Interrelationship between inflammatory risk factors.

1.2.2.2 Adhesion molecules

Adhesion molecules are involved in the initiation and progression of atherosclerosis. They mediate the attachment and transmigration of leukocytes into the subendothelial space (Frayn et al., 2005). Endothelial dysfunction increases the expression of adhesion molecules which plays a crucial role in the recruitment of leukocytes to the endothelium, triggers transmigration of leukocyte into the subendothelial space and consequently leads to atherosclerosis (Comba et al., 2016). The key adhesion molecules related to atherosclerosis are the selectins and Immunoglobulin (Ig) superfamily (Frayn et al., 2005). Their expression

and therefore their concentrations are influenced by oxidised LDL and other inflammatory cytokines including IL-6 and TNF-α (Frayn et al., 2005).

Selectins

The selectins (cluster of differentiation 62, CD62) are transmembrane glycoproteins expressed on leukocytes (L-selectin), endothelial cells (E-selectin), and platelets (P-selectin) (**Figure 1.15**) (Krieglstein and Granger, 2001). Selectins mediate tethering and rolling of leukocytes on endothelial cells, platelet-leukocyte aggregation, and ultimately atherosclerosis (Bonaterra et al., 2010; Krieglstein and Granger, 2001).

E-Selectin, also known as endothelial leukocyte adhesion molecule-1, ELAM-1, or CD62E, is expressed only on endothelial cells and after activation by inflammatory cytokines (Bonaterra et al., 2010). A prospective study and meta-analysis has shown a non-significant increase in the incidence of CHD among individuals in the top third of soluble E-selectin levels (75 ng/mL) compared with those in the bottom third of sE-selectin levels (52 ng/mL) (OR 1.08, 95% CI 0.84 to 1.39) after adjustments for age, town, smoking and risk factors (Malik et al., 2001).

P-Selectin, also known as GMP-140, LECAM-3, PADGEM, and CD62P, is an adhesion receptor expressed on activated endothelial cells, which mediates the so called 'rolling' of leukocyte along the endothelium (Bonaterra et al., 2010). A prospective study and meta-analysis has shown a non-significant increase in the incidence of CHD among individuals in the top third of soluble P-selectin levels (150 ng/mL) compared to those in the bottom third of sP-selectin levels (95 ng/mL) (OR 1.02, 95% CI 0.79 to 1.32) after adjustments for age, town, smoking and risk factors (Malik et al., 2001). There was a positive linear association between sP-selectin levels and the rate of incidence of CHD (HR 1.63, 95% CI 1.15 to 2.30) after adjustments for traditional risk factors (Bielinski et al., 2015).

Immunoglobulin (Ig) superfamily

The Ig superfamily, including a wide range of molecules with multiple Ig like domains, is relevant to CVD (Krieglstein and Granger, 2001). They play a key role in the process of atherosclerosis by reinforcing leukocyte binding via its integrin ligands and trans-endothelial migration (Bonaterra et al., 2010).

Intercellular Adhesion Molecule 1 (ICAM-1, CD54), a glycoprotein receptor, is part of the Ig superfamily (Krieglstein and Granger, 2001). It functions as mediators of leukocyte adhesion. Expression of ICAM-1 increases during infection and upon stimulation of cytokines. The main binding site of ICAM-1 are the leukocyte integrins LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) (Jun et al., 2001). Soluble forms of ICAM-1 (sICAM-1) is generated via proteolytic cleavage and hence be detected with significantly raised levels under various disease states (Krieglstein and Granger, 2001). Elevated levels of sICAM-1 were associated with the development of atherosclerosis in healthy men (Pradhan et al., 2002). A prospective study and meta-analysis has shown an significant increase in the incidence of CHD among middle age men within the top third of sICAM-1 levels (338 ng/mL) compared to those in the bottom third of sICAM-1 levels (261 ng/mL) (OR 1.49, 95% CI 1.14 to 1.94) after adjustments for age, town, smoking and risk factors (Malik et al., 2001). However, sICAM-1 was not associated with events of acute thrombosis or vessel occlusion (Malik et al., 2001).

Vascular Cell Adhesion Molecules (VCAM-1, CD106) are part of the Ig superfamily (Krieglstein and Granger, 2001). They can be expressed on endothelial cells, smooth muscle cells, fibroblasts and macrophages. VCAM-1 binds to both α4β1 (VLA-4) and α4β7 (LPAM-1) integrin which are expressed on all leukocytes (Bonaterra et al., 2010). VCAM-1 mediates the adhesion of circulating leukocytes to the endothelium and their migration into subendothelial spaces. Similar to ICAM-1, VCAM-1 was also associated with the extent of atherosclerosis (Ley and Huo, 2001). A prospective study and meta-analysis has shown a significant increase in the incidence of CHD among individuals in the top third of sVCAM-1 levels (516 ng/mL) compared with those in the bottom third of sVCAM-1 levels (37 ng/mL) (OR 1.35, 95% CI 1.05 to 1.75) after adjustments for age, town, smoking and risk factors (Malik et al., 2001).

1.2.3 Behavioural risk factors

Epidemiological evidence indicates that health-related behaviours such as smoking, a poor diet, excessive alcohol, or obesity, linked to fourfold increase in risk for dying early. The mortality risk for those with four compared to zero health behaviours was equivalent to being 14 years younger in chronological age (Khaw et al., 2008).

In developed countries, around 12% of major burdens of disease were caused by tobacco smoking which was the top leading risk (World Health Organization, 2002). Tobacco smoking increases risk of CHD by elevating blood pressure and the tendency of blood clotting while lowering exercise tolerance and blood levels of HDL-cholesterol (Townsend

et al., 2015). One 50 years cohort study has shown that the risk of CHD mortality of a current Smoker was around 60% higher compared to a lifelong non-smoker (Doll et al., 2004). In England in 2013, around 17% (estimated 78,200) of deaths among adults aged 35 and over were attributed to smoking, including around 13% (estimated 16,700) of deaths from circulatory diseases (Health and Social Care Information Centre, 2015).

Physical activity means all forms of physical movement including activities of daily life and exercise (Frayn et al., 2005). A physically inactive lifestyle contributes to traditional cardiovascular risk factors, such as high BP, higher triglycerides, lower HDL-cholesterol, diabetes and obesity (Townsend et al., 2015). Physical activity has also been shown to decrease levels of novel cardiovascular risk factors and improve EF, such as IL-6, sICAM-1 and sVCAM-1 (Palmefors et al., 2014). Physical activity was associated with a 35% reduction in CVD mortality and 33% reduction in all-cause mortality in comparison with sedentary lifestyle (Nocon et al., 2008). Compared with low physical activity, moderate and high physical activity were associated with graded reduction in mortality (hazard ratio 0.80, 95% CI 0.74-0.87 and 0.65, 0.60-0.71; $p<0.0001$ for trend), and major CVD (0.86, 0.78-0.93; $p<0.001$) (Lear et al., 2017).

1.2.3.1 Dietary patterns

Dietary patterns also influence risk of CVD and associate strongly with other cardiovascular risk factors. Dietary habits in developing countries have changed from consumption of carbohydrate rich foods with modest fat content to, the so-called “Western” diet with a high amount of meat and milk products. Western diet tends to include high intakes of saturated fatty acid (SFA), trans-fat, salt, free sugar and excess calories; conversely, intakes of polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), fibre, fruit and vegetables are insufficient (Martin et al., 2012). A poor diet comprising high intakes of saturated fatty acids, trans fatty acids and free sugar (processed starches and added sugars) can lead to an energy imbalance, where energy intake is greater than energy expenditure, thus resulting in overweight and obesity (Romieu et al., 2017). Obesity is also a driver of cardiovascular risk. In developed countries, over 7% of all disease burden was caused by raised body mass index (BMI) (World Health Organization, 2002). It associates with other cardiovascular risk factors, such as hypertension, diabetes, and high cholesterol. Around a third of CHD and ischaemic stroke and almost 60% of hypertensive disease in developed countries was due to overweight (World Health Organization, 2002). In 2012, more than 75% of mortality caused by CVD in developing countries was driven by the changes of industrialisation, urbanisation, related lifestyle changes, and dietary habits (Dahlöf, 2010;

Gupta et al., 2016). A diet with a low intake of fruit and vegetables was estimated to cause about 31% of CHD and 11% of stroke worldwide; overall, low fruit and vegetables intake contributed to 2.7 million (4.9%) deaths and 26.7 million (1.8%) of DALYs respectively and of those burdens that were attributable to low fruit and vegetable intake, about 85% was from CVD and 15% from cancers (World Health Organization, 2002). Contrastingly, higher consumption of total fruit and vegetable was inversely associated with major cardiovascular disease, myocardial infarction, cardiovascular mortality, non-cardiovascular mortality, and total mortality in the models adjusted for age, sex, and centre (**Figure 1.17**) (Miller et al., 2017). Lock et al. (2005) estimated that inadequate consumption of fruit and vegetable contributed to 2.6 million deaths per year around the world and increasing fruit and vegetable to 600 g/d could reduce 1.8% of total global burden of disease. Globally, 31% and 19% of IHD and ischaemic stroke could be reduced by achieving this dietary target (Lock et al., 2005). Therefore, a healthy dietary pattern and eating habits can reduce the burden of CVD. There are different types of healthy dietary patterns such as Japanese diet, the traditional Asian diet and the Mediterranean diet (MD). Among these dietary patterns, MD has received the most attention. The MD is characteristic of populations from countries bordering the Mediterranean Sea, including Italy, France, Greece, and Spain. Although the MD varies by region, it is largely based on vegetable, fruit, nuts, beans, cereal grains, olive oil and fish.

A meta-analysis of 15 studies has shown a significant reduction in total cholesterol in those trials describing the intervention as a MD (-0.23 mmol/L, 95% CI -0.27 to -0.2) when compared with control (-0.06 mmol/L, 95% CI -0.13 to 0.01) (Rees et al., 2013). A prospective cohort study has shown that higher adherence to a MD (higher Mediterranean diet score) was associated with a statistically significant reduction in total mortality (0.864, 95% CI 0.802 to 0.932). When vegetables were excluded from the Mediterranean diet score of the mortality ratio, it reduced the apparent effect by 16.2% (Trichopoulou et al., 2009). It indicated that vegetables influence and lead to the health beneficial effects.

However, not all fruit and vegetables have equal cardioprotective effects. Specific fruits and vegetables found in Mediterranean diet were individually associated with significant reductions of CVD. Fruit and vegetables with a high content of various antioxidants were suggested to have cardioprotective effects (Ruxton et al., 2006). High intake of tomato and lycopene have shown improvements in cardiovascular health. As mentioned in the above section, oxidation of LDL-cholesterol has been considered as one of the mechanisms causing CVD. Tomato is one of the main ingredients in the MD that plays a key role in reducing the prevalence of CVD. The antioxidant activity of lycopene in tomato has been investigated as one preventive factor of CVD (Bohm, 2012).

1.3 Tomato and Cardiovascular Health

Carotenoids, the yellow, orange and red coloured pigments of several fruit and vegetables are one class of compounds being discussed for a long time as cardioprotective food ingredients (**Figure 1.16**) (Natalia Di Pietro et al., 2016; Voutilainen et al., 2006).

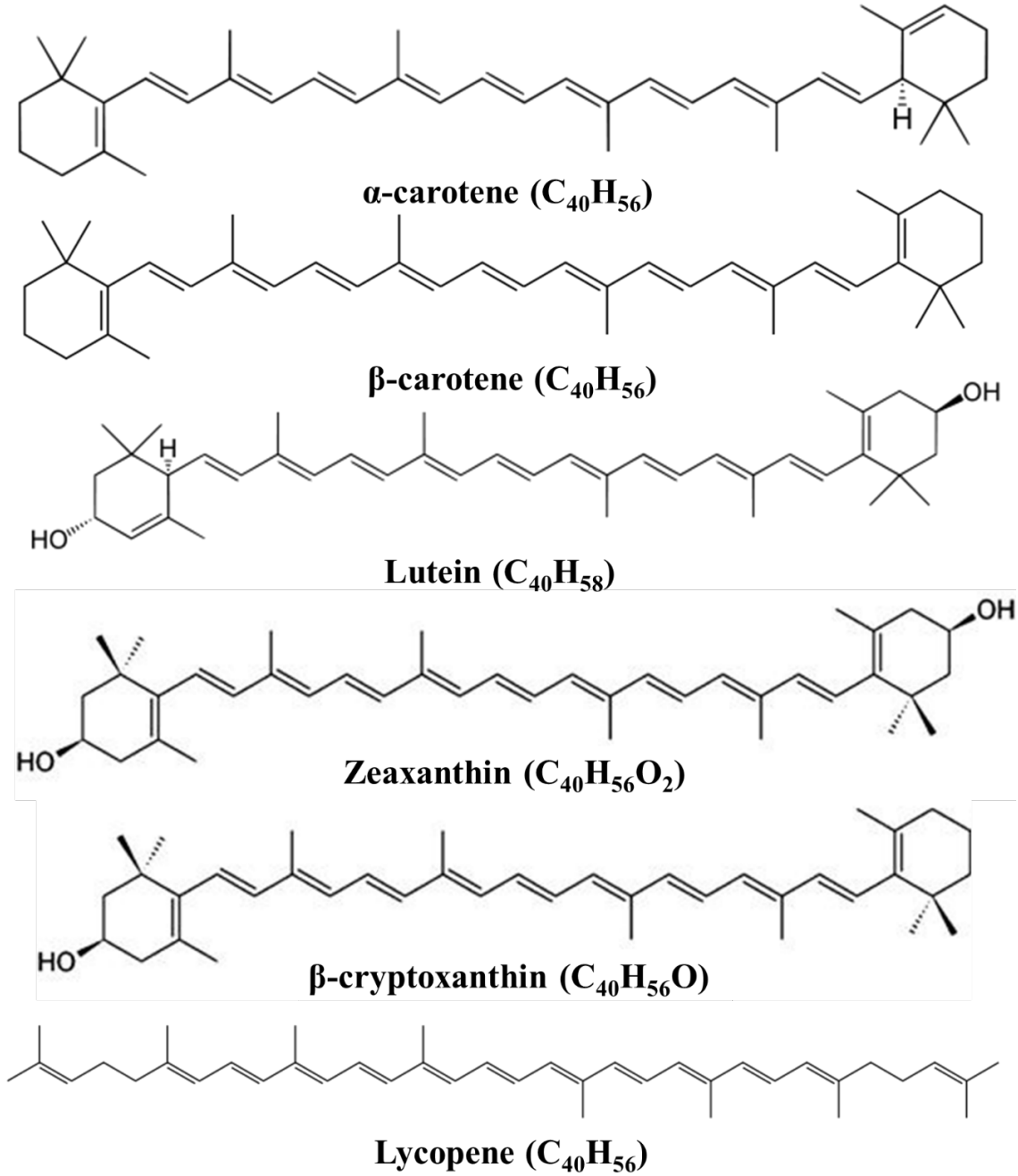


Figure 1.16 Chemical structure of the main carotenoids in the human diet.

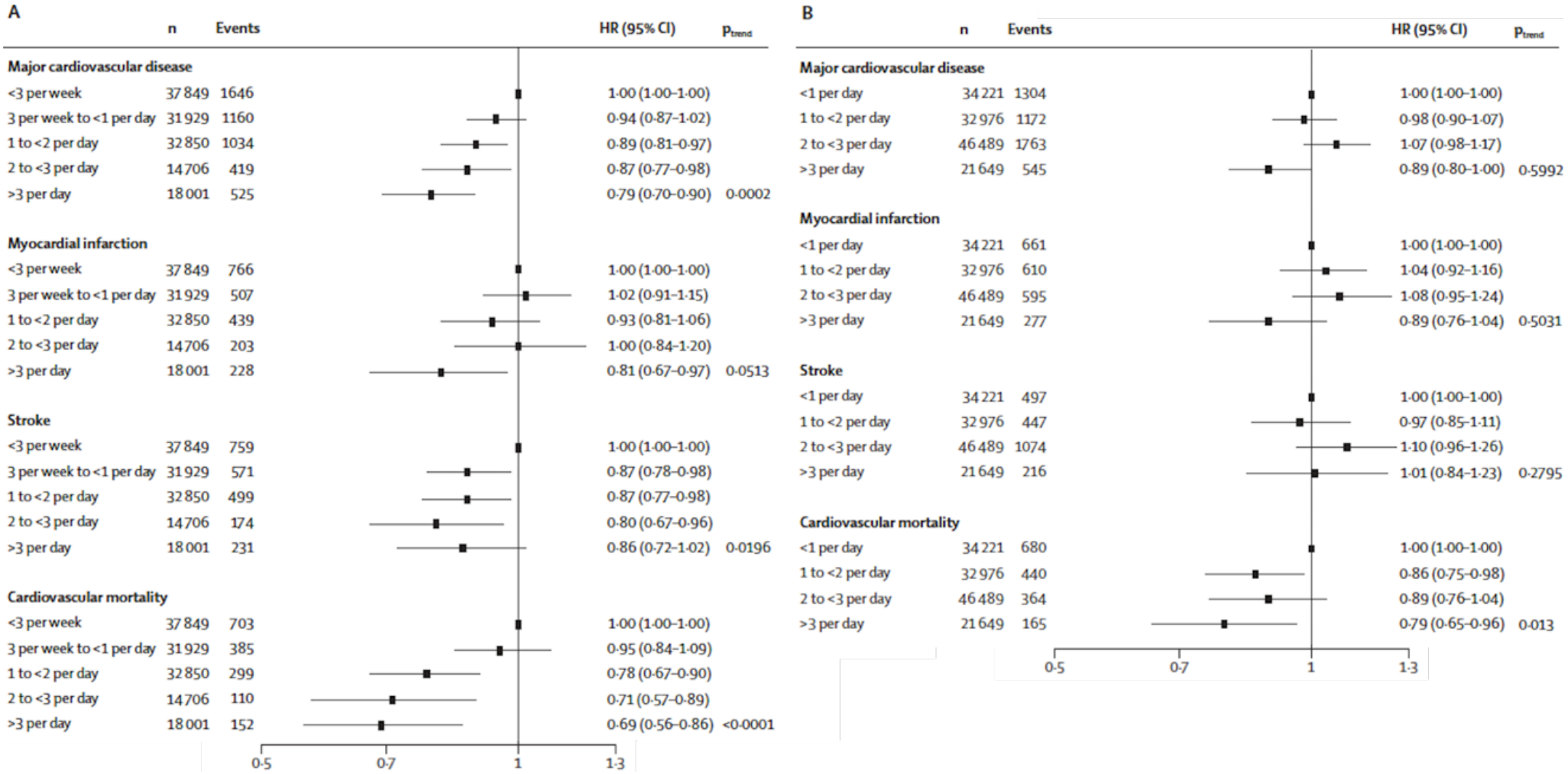


Figure 1.17 Association of fruit intake (A) and vegetable (B) with cardiovascular outcomes and mortality (Adjusted for age, sex, and centre (random effect)). (Miller et al., 2017).

Tomatoes and tomatoes product are a rich source of carotenoids, especially lycopene in our diet (Bohm, 2012). Tomatoes and tomato product provided an estimated 85% of lycopene in the American diet and are an essential component of the MD (Basu and Imrhan, 2007). Tomatoes are top vegetable and vegetable products consumed in USA per capita which just below the consumption of potatoes, lettuce/vegetable salads and onions (FAOSTAT, 2015a). According to USDA's loss-adjusted food availability data, Americans consumed 13.4 kg of tomatoes in 2016 and 42% (5.7 kg) of tomato were consumed in fresh and 58% (7.7 kg) consumed as processed tomatoes (USDA, 2018). In the UK, the average consumption of fresh tomatoes was 8.32 kg per capita per year (British Tomato Growers' Association, 2015) while the consumption of tomatoes in European countries were 14.4 kg per capita per year (European Commission, 2016). Among European countries, Mediterranean countries were particularly high consumption in tomato (**Figure 1.18**).

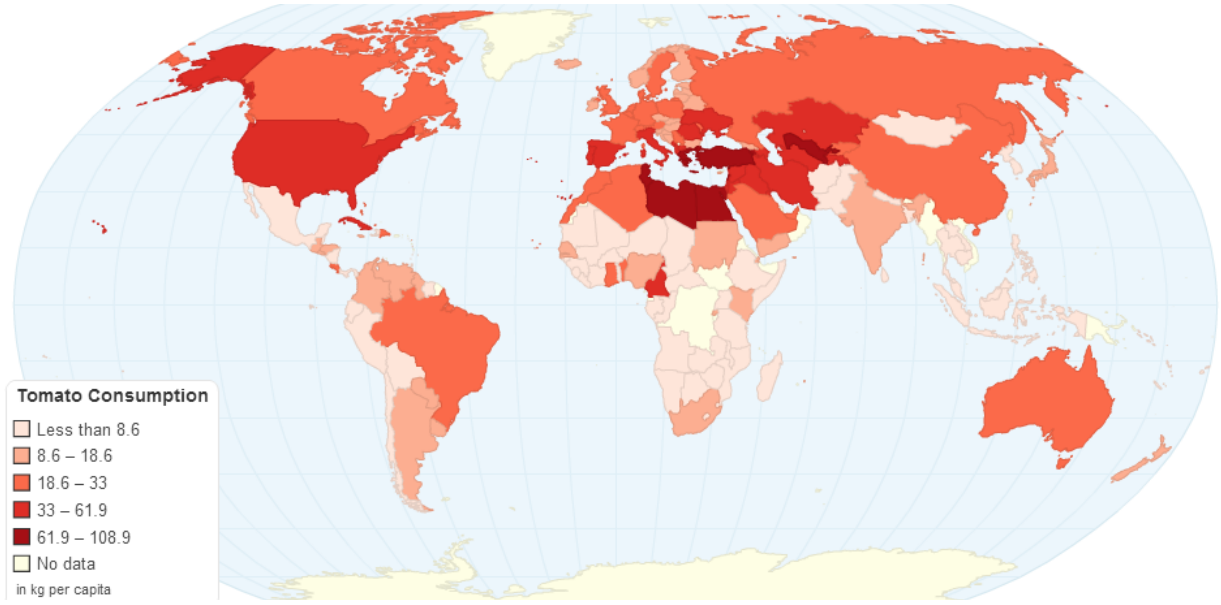


Figure 1.18 Fresh tomato and tomato products consumption in 2013 (kg per capita) (FAOSTAT, 2015b).

Lycopene is a polyunsaturated carotenoid with 11 liner conjugated and two non-conjugated double bonds. Therefore, lycopene exists in a variety of geometric isomers including all-trans and various forms of cis-isomers (the most common are 5-cis, 9-cis, 13-cis and 15-cis) (**Figure 1.19**) (Agarwal and Rao, 2000). The all-trans conformation is the most predominant isomer found naturally in plants and is the most thermodynamically stable form (Shi and Le Maguer, 2000). When exposed to heat during food processing, especially with the presence of fat, lycopene is converted to cis-isomer forms (Kong et al., 2010). Apart from food processing, under acidic condition in gastric milieu also enhance isomerisation of the all trans lycopene to cis-isomers (Re et al., 2001).

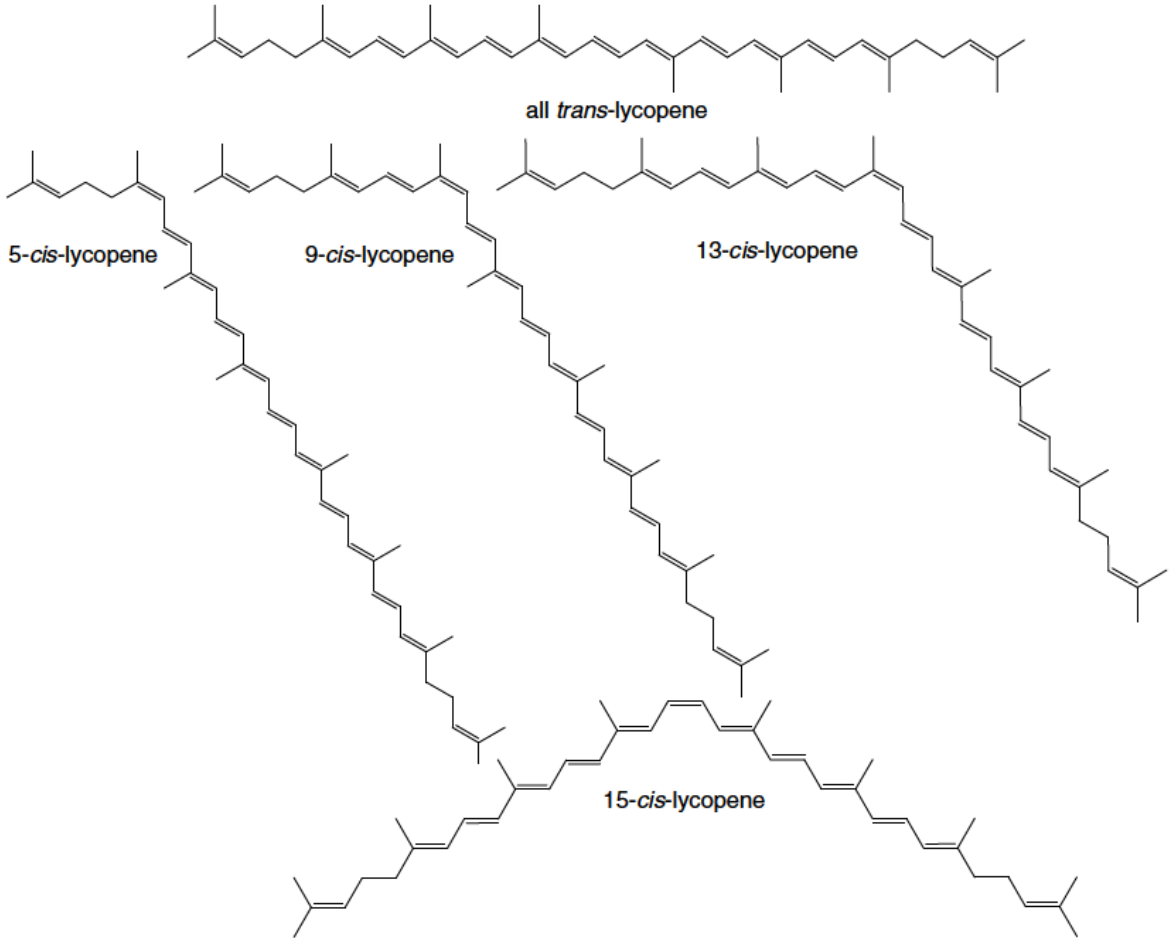


Figure 1.19 Structures of all-trans and cis-isomers of lycopene.

Cis-isomers of lycopene have distinct physical characteristics and chemical behaviours from all-trans-form, including decreased colour intensity, lower melting points, greater polarity, lesser tendency to crystallisation, and greater solubility in oil and hydrocarbon solvents (Edge and Truscott, 2018). Cis-lycopene isomers are more readily absorbed by enterocytes and preferentially accumulate in tissues than all-trans lycopene due to the reduced length of cis-isomers more readily allows them to be incorporated into micelles and cis-isomers less readily form crystals, severely increase their uptake by micelles (Boileau et al., 2002). As well as influencing lycopene absorption via isomerisation of trans- to cis-form, food processing may improve its bioavailability by breaking down cell walls, which weakens the bonding forces between lycopene and the tissue matrix, thus making lycopene more accessible (Shi and Le Maguer, 2000).

The highly conjugated double bonds give lycopene antioxidant properties scavenging radical species ($R^{\bullet}$) such as singlet oxygen ($^1O_2$) and peroxyl radical (ROO•) (Edge and Truscott, 2018). The mechanism of action for lycopene towards the radical species ($R^{\bullet}$) scavenging activity were predicted through three possible mechanisms: (I) Radical adduct formation (RAF), (II) electron transfer (EF) to the radical and (III) Hydrogen atom transfer (HAT) (Galano and Francisco-Marquez, 2009).

I. Radical adduct formation (RAF): $R^{\bullet} + \text{Lycopene} \rightarrow [\text{R-Lycopene}]^{\bullet}$

II. Electron transfer (EF): $R^{\bullet} + \text{Lycopene} \rightarrow R^{-} + \text{Lycopene}^{\bullet+}$

III. Hydrogen atom transfer (HAT): $R^{\bullet} + \text{Lycopene} \rightarrow RH + \text{Lycopene}(-H)^{\bullet}$

Among all of the carotenoid pigments, lycopene is the most potent singlet oxygen quencher (Sinha and Dua, 2015). In vitro study, singlet oxygen quenching ability of lycopene was found to be twice more than that of β-carotene and 10 times more than that of α-tocopherol (Shi and Le Maguer, 2000). The potent antioxidant chemical properties of lycopene enable to inhibit LDL oxidant, which is central to the initiation of atherosclerosis.

Accumulating epidemiological findings have established that high consumption of tomato (4.4 servings/day) (Jacques et al., 2013) and lycopene (1450-7900 μg/day) (Hirvonen et al., 2000; Jacques et al., 2013) inversely associated with CVD and mortality. High plasma lycopene level (0.27-0.45 μmol/L) has been associated with reductions in CVD risk (Ford et al., 2014; Hirvonen et al., 2000; Rissanen et al., 2001) and has also been reported to improve biomarkers associated with CVD, including LDL-cholesterol, interleukin-6, flow mediated dilation (FMD), and systolic blood pressure (SBP) in healthy population (Cheng et al., 2017).

1.4 Research Questions Raised from The Present Chapter

The work described in this chapter raised the following questions:

1. What is the epidemiological evidence on tomato consumption and its association with CVD risk?
2. What is the epidemiological evidence on lycopene consumption and its association with CVD risk?

These questions will be addressed in Chapter 2 by systematically and quantitatively evaluating the evidence from epidemiological cohort studies reporting the associations between tomato intake and risk of CVD. We hypothesised that lycopene and tomato may play a beneficial role in preventing cardiovascular diseases and early death.

Chapter 2 Lycopene and Tomato and Risk of Cardiovascular Diseases: A Systematic Review and Meta-Analysis of Epidemiological Evidence

This study was designed to answer the research questions in **section 1.4**.

This chapter is presented as originally published in: Critical Reviews in Food Science and Nutrition 2017; 1-18.

2.1 Introduction

Low intakes of fruit and vegetables are important global risk factors for the development of morbidity, acceleration of the ageing process and occurrence of early mortality (GBD 2015 Risk Factors Collaborators, 2016). A number of epidemiological studies of disease endpoints provide strong and consistent evidence for a beneficial effect of fruit and vegetables, combined or separately, on cardiovascular health (Mozaffarian et al., 2011). Fruit and vegetables intake in the range commonly recommended (e.g. >5 servings) is associated with a 21 to 26% reduction in the risk of stroke (He et al., 2006; Hu et al., 2014) and 17 to 25% in the risk of CHD (Dauchet et al., 2006; He et al., 2007; Ness and Powles, 1997).

In addition, epidemiological studies have shown that specific fruit and vegetables are individually associated with significant reductions for different types of cancers. On this area, recent systematic reviews and meta-analyses of epidemiological studies have reported that higher consumption of tomato products is associated with a significantly reduced risk of gastric cancer (27%) (Yang et al., 2013). Tomato is a rich source of lycopene, a major carotenoid in human plasma with strong antioxidant properties (Mein et al., 2008). Both higher consumption of, and higher blood levels of, lycopene, are also associated with a lower risk of prostate cancer (Chen et al., 2013b; Chen et al., 2015b; Etminan et al., 2004). These findings have triggered an interest on the effects of lycopene and tomato consumption on health outcomes.

A recent systematic review of interventions trials by our group has shown that tomato or lycopene supplementation successfully improved important cardiovascular risk factors, including LDL-cholesterol, interleukin-6, flow mediated dilation (FMD), and systolic blood pressure (SBP) in healthy population (Cheng et al., 2017). In addition, observational cohort studies seem to add support by reporting positive associations between higher tomato and/or lycopene intake or status and lower risk of cardiovascular diseases (CVD) (Jacques et al., 2013).

A previous meta-analysis on the association of lycopene and stroke reported a reduction in risk of stroke (19.3%) only (Li and Xu, 2014). However, it is now 4 years old and further epidemiological evidence on consumption of lycopene and tomatoes associated with other CVD and mortality are likely to be available. Therefore, here we present an updated systematic review of the literature and meta-analysis on the associations between lycopene or tomato consumption and CVD risk and mortality in epidemiological studies. In addition, the present systematic review aimed to explore the impact of important covariates as potential sources of heterogeneity in studies.

2.2 Methods

This systematic review was undertaken following standard guidance by the Cochrane collaboration (Higgins and Green, 2011) and the Centre for Reviews and Dissemination (Centre for Reviews and Dissemination, 2009). This manuscript is reported according to the PRISMA guidelines (**Figure 2.1** and **Appendix A1**) (Moher et al., 2010). The systematic review protocol was registered in PROSPERO, the International Prospective Register of Systematic Reviews (CRD42016049526).

In July 2017, three databases including Medline, Web of Science, and Scopus were searched from inception. In addition, reference lists of identified publications were screened in an attempt to identify further relevant studies.

The searches included the following terms/keywords related to the exposures and outcomes of interest: tomato, lycopene, cardiovascular disease (CVD), coronary heart disease (CHD), myocardial infarction (MI), stroke, atherosclerosis, atrial fibrillation (AF), congestive heart failure (CHF), sudden cardiac death, mortality, and morbidity. The terms related to outcome measures thus included a number of conditions under the umbrella of CVD, as well as associated disorders such AF. Data for each of these outcomes was extracted if explicitly reported in the original papers. The present systematic review was restricted to articles published in English.

Two authors (HMC, JL) screened articles independently for eligibility. The decision to include/exclude studies was hierarchical and consisted on screening firstly the titles and abstracts of studies; if the authors were unable to reach decision at this stage, then the full-text of the article was evaluated.

2.2.1 Inclusion/exclusion criteria

The following specific inclusion criteria were used to identify eligible articles: 1) Study Design: longitudinal and cross-sectional studies; 2) Subjects: Adult subjects >18 years of age; 3) Exposure: data related to tomato or lycopene dietary intakes or serum concentration; 4) Outcomes: CVD morbidity and associated disorders and mortality outcomes (CVD, CHD, MI, stroke, atherosclerosis, AF, CHF, sudden cardiac death, mortality) expressed as Hazard Ratio (HR), Relative Ratios (RR), and Odd Ratios (OR) with 95% confidence interval (CI).

Exclusion criteria included: 1) Study Design: Non-epidemiological studies; 2) Subjects: Subjects <18 years of age; 3) Exposure: different exposures; 4) Outcomes: risk factors, non-CVD related outcomes.

2.2.2 Data extraction

Extracted information included: study design (country, assessment of tomato and/or lycopene intake, serum lycopene concentrations, cohort name, follow-up length); participant characteristics (sample size, population, mean age, body mass index and ethnicity); outcome measures (CVD and associated disorders, and mortality, as stated above); adjustments for covariates.

Information related to the outcomes of interest was extracted and analysed as reported in the original papers. If studies reported associations of tomato or lycopene with CVD, but also reported associations for specific CVD such as stroke or CHD, all these were extracted and analysed separately. This procedure was adopted in order to provide differential associations between the exposures and specific outcomes of interest.

2.2.3 Outcome measures and exposures

Primary outcomes of interest were risk of stroke, CVD, CHD, MI, atherosclerosis, AF, CHF, all-cause mortality, cardiovascular mortality and sudden cardiac death.

We were interested in studies associating these outcomes with exposures such as intakes of lycopene or tomato products, and lycopene concentrations in blood or any other body tissues such adipose tissue.

2.2.5 Statistical analysis

Meta-analysis of results was undertaken using the Review Manager (RevMan Version 5.1 for Windows Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011). Random effects models, accounting for inter-study variation and minimizing potential bias due to methodological differences between studies, were used.

Results are informed as HR or OR with 95% confidence intervals (CI) and two-sided *P*-values. In this meta-analysis, five studies evaluated multiple arms of cardiovascular diseases and associated disorders. Meta-regression analysis was undertaken to explore the association of effect size (Q-test) with continuous variables such as age and BMI of study participants, or length of follow-up. In addition, we undertook subgroup analysis to explore the impact of variables such as sex, or country of origin.

Statistical heterogeneity was evaluated using the I^2 statistic describing the percentage of variation across studies (Centre for Reviews and Dissemination, 2009; Higgins and Green, 2011), with I^2 values greater than 50% representing high levels of heterogeneity. Publication bias was assessed by inspecting the funnel plot of effect size against the standard error (SE), with asymmetry assessed formally with Egger's regression test (Egger et al., 1997).

2.3 Results

A total of 3970 articles were identified (**Figure 2.1**). Twenty-eight articles fulfilled our inclusion criteria (**Table 2.1**) (Ascherio et al., 1999; Ford et al., 2014; Hak et al., 2004; Hak et al., 2003; Han and Han, 2016; Han et al., 2016; Hirvonen et al., 2000; Iribarren et al., 1997; Ito et al., 2006; Jacques et al., 2013; Kabagambe et al., 2005; Karppi et al., 2013a; Karppi et al., 2013b; Karppi et al., 2012a; Karppi et al., 2012b; Karppi et al., 2013c; Karppi et al., 2012c; Klipstein-Grobusch et al., 2000; Kohlmeier et al., 1997; Mayne et al., 2004; Osganian et al., 2003; Rissanen et al., 2001; Sesso et al., 2004; Sesso et al., 2005; Sesso et al., 2003; Street et al., 1994; Tavani et al., 2006; Wood and Johnson, 2004) were included in this review, and 25 of these provided quantitative results for meta-analysis (Ascherio et al., 1999; Ford et al., 2014; Hak et al., 2004; Hak et al., 2003; Han et al., 2016; Hirvonen et al., 2000; Iribarren et al., 1997; Ito et al., 2006; Jacques et al., 2013; Kabagambe et al., 2005; Karppi et al., 2013a; Karppi et al., 2013b; Karppi et al., 2012a; Karppi et al., 2012b; Karppi et al., 2013c; Karppi et al., 2012c; Klipstein-Grobusch et al., 2000; Kohlmeier et al., 1997; Mayne et al., 2004; Osganian et al., 2003; Rissanen et al., 2001; Sesso et al., 2004; Sesso et al., 2005; Sesso et al., 2003; Tavani et al., 2006).

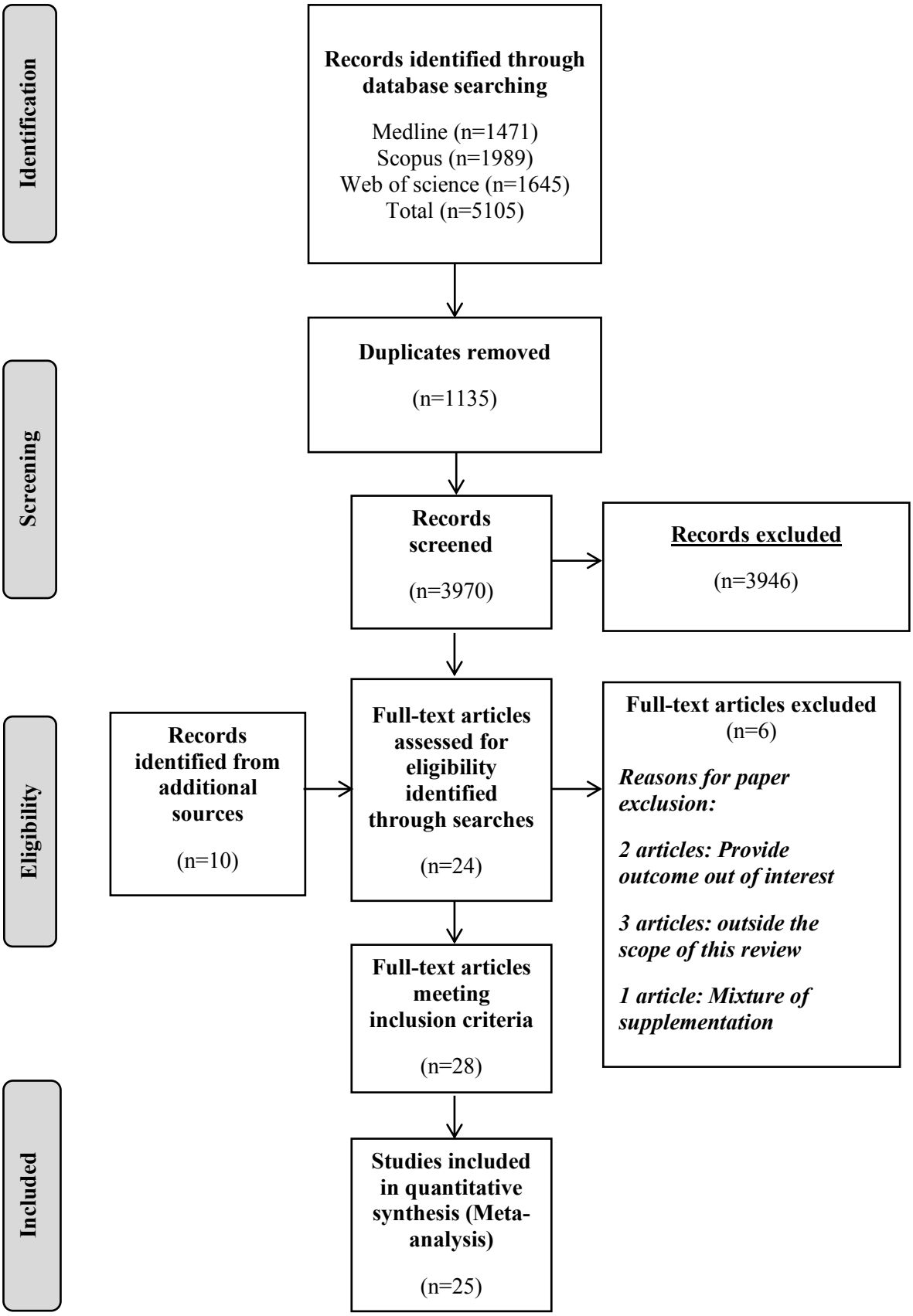


Figure 2.1 PRISMA flow diagram of selection of studies on lycopene or tomato consumption and vascular risk factors.

Table 2.1 Characteristics of studies with exposure tomato/lycopene included in systematic review.

Ref (Country of origin)	Exposure	Study design (Cohort name)	Sample size (Sex)	Mean length of follow-up (years)	Mean Age±SD (Range)	BMI (kg/m^2)	Effect size (95% CI)	Confounders adjusted for	Ethnicity
Lycopene associations									
Ascherio et al., 1999 (USA)	Intake of lycopene from diet Food frequency questionnaire	Prospective study (The Health Professionals Follow-up Study)	43738 (Male)	8	40-75	25.5	Total Stroke RR 0.96 (0.68, 1.36) Highest (Median value 18798 µg/d) and lowest (Median value 3442 µg/d) (Ref) quintiles for intake of lycopene Ischaemic Stroke RR 1.01 (0.65, 1.57) Highest (Median value 18798 µg/d) and lowest (Median value 3442 µg/d) (Ref) quintiles for intake of lycopene Haemorrhagic Stroke RR 1.04 (0.52, 2.07) Highest (Median value 18798 µg/d) and lowest (Median value 3442 µg/d) (Ref) quintiles for intake of lycopene	Total energy intake, smoking, alcohol consumption, history of hypertension, parental history of MI, profession, and quintiles of BMI and physical activity, age	N/A

(Table 2.1 continues on next page)

Ref (Country of origin)	Exposure	Study design (Cohort name)	Sample size (Sex)	Mean length of follow-up (years)	Mean Age±SD (Range)	BMI (kg/m^2)	Effect size (95% CI)	Confounders adjusted for	Ethnicity
Ford et al., 2014 (USA)	Serum level of lycopene	Prospective study (National Health and Nutrition Examination Survey III)	1429 (Mixed Sex)	14	55.7 (20-79)	26.5	All-cause Mortality HR 0.8 (0.67, 0.95) (inverse association) for serum level of lycopene as continuous variable (Mean±SE, for total participants 0.45±0.01 μmol/L)	Age, sex, race, ethnicity, education, smoking, alcohol consumption, leisure-time physical activity, use of vitamin or mineral supplements, SBP, HDL-cholesterol, non-HDL-cholesterol, BMI, CRP, albumin: creatinine ratio, health status, diabetes, history of MI and history of stroke	White: 83.5%; Black American: 7.5%; Other: 6.8%; Mexican American: 2.2%
Hak et al., 2003 (USA)	Plasma level of lycopene	Prospective nested case-control (Physicians' Health Study)	1061 (Male)	13	58±8.5	25.2	MI OR 1.43 (0.87, 2.35) Highest (Median value 578.8 ng/mL) and lowest (Median value 217.7 ng/mL) (Ref) quintiles for plasma of lycopene	Age, smoking, BMI, total and HDL-cholesterol, history of hypertension, diabetes mellitus, and parental history of MI < age 60, physical activity, alcohol consumption; multivitamin use, and assignment to aspirin or β-carotene treatment or placebo	N/A
Hak et al., 2004 (USA)	Plasma level of lycopene	Prospective nested case-control (Physicians' Health Study)	594 (Male)	13	60.5 (40-84)	25.3	Ischaemic stroke OR 0.72 (0.38, 1.37) Highest (Median value 606.8 ng/mL) and lowest (Median value 216.4 ng/mL) (Ref) quintiles for plasma level of lycopene	Age, smoking, BMI, total and HDL-cholesterol, history of hypertension, diabetes mellitus, and parental history of MI < age 60, physical activity, alcohol consumption, multivitamin use, and assignment to aspirin or β-carotene treatment or placebo	N/A

(Table 2.1 continues on next page)

Ref (Country of origin)	Exposure	Study design (Cohort name)	Sample size (Sex)	Mean length of follow-up (years)	Mean Age±SD (Range)	BMI (kg/m^2)	Effect size (95% CI)	Confounders adjusted for	Ethnicity
Han and Han, 2016 (USA)	Serum level of lycopene	Prospective study (National Health and Nutrition Examination Survey III)	37 (Mixed Sex)	17.5	45±16.8	26±7	Mortality rate for low lycopene level group 33% Mortality rate for high lycopene level group 5.3%	N/A	non-Hispanic white: 43%; non-Hispanic black: 30%; Mexican American: 27%
Han et al., 2016 (USA)	Serum level of lycopene	Prospective study (National Health and Nutrition Examination Survey)	2499 (Mixed Sex)	5-10	Three aged group (20-39; 40-59; ≥60)	Three groups (<24.9; 25-29.9; ≥30)	All-cause Mortality HR 0.61 (0.42, 0.89) Highest (Mean value 0.626 μmol/L) and lowest (Mean value 0.204 μmol/L) (Ref) tertiles for serum level of lycopene	Race, sex, age, BMI, smoking, alcohol consumption, physical activity, fruit consumption, vegetable consumption, and cancer	non-Hispanic white: 55.9%; non-Hispanic black: 13.5%; Mexican American: 23.9%; Other: 6.7%

(Table 2.1 continues on next page)

Ref (Country of origin)	Exposure	Study design (Cohort name)	Sample size (Sex)	Mean length of follow-up (years)	Mean Age±SD (Range)	BMI (kg/m^2)	Effect size (95% CI)	Confounders adjusted for	Ethnicity
Hirvonen et al., 2000 (Finland)	Intake of lycopene from diet Food frequency questionnaire	Prospective study (The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study)	26593 (Male)	6.1	57 (50-69)	26.6	Cerebral Infarction RR 0.74 (0.59, 0.92) Highest (Median value 1.45 mg/d) and lowest (Median value 0.14 mg/d) (Ref) quartiles for intake of lycopene	Age, supplementation group, SBP, DBP, total and HDL-cholesterol, BMI, height, smoking, number of cigarettes daily, history of diabetes or CHD, alcohol consumption, and education	N/A
						26.8	Intracerebral Haemorrhage RR 0.45 (0.24, 0.86) Highest (Median value 1.45 mg/d) and lowest (Median value 0.14 mg/d) (Ref) quartiles for intake of lycopene		
						26	Subarachnoid Haemorrhage RR 0.63 (0.33, 1.20) Highest (Median value 1.45 mg/d) and lowest (Median value 0.14 mg/d) (Ref) quartiles for intake of lycopene		
Iribarren et al., 1997 (USA)	Serum level of lycopene	Case-control (the Atherosclerosis Risk in Communities (ARIC) study)	462 (Mixed sex)	N/A	59±5	27.3	Asymptomatic Carotid Atherosclerosis OR 0.81 (0.60, 1.08) Per 1-SD increase in serum lycopene (Mean value 0.44 μmol/L)	Age, blood storage time, total cholesterol, triglycerides, education level, smoking, BMI, alcohol consumption, hypertension, diabetes mellitus and vitamin supplementation use	White: 90%; Black: 10%

(Table 2.1 continues on next page)

Ref (Country of origin)	Exposure	Study design (Cohort name)	Sample size (Sex)	Mean length of follow-up (years)	Mean Age±SD (Range)	BMI (kg/m^2)	Effect size (95% CI)	Confounders adjusted for	Ethnicity
Ito et al., 2006 (Japan)	Serum level of lycopene	Prospective study	3061 (Mixed sex)	11.9	39-80	N/A	CVD HR 0.77 (0.57, 1.04) Highest and lowest (Ref) serum level of lycopene per each logarithmically transformed value of serum	Age, sex, smoking, alcohol consumption, BMI, SBP, total cholesterol, triglyceride and alanine transaminase activity	Japanese population
							Stroke HR 0.78 (0.51, 1.19) Highest and lowest (Ref) serum level of lycopene per each logarithmically transformed value of serum		
							CHD HR 0.74 (0.48, 1.13) Highest and lowest (Ref) serum level of lycopene per each logarithmically transformed value of serum		

(Table 2.1 continues on next page)

Ref (Country of origin)	Exposure	Study design (Cohort name)	Sample size (Sex)	Mean length of follow-up (years)	Mean Age±SD (Range)	BMI (kg/m^2)	Effect size (95% CI)	Confounders adjusted for	Ethnicity
Jacques et al., 2013 (USA)	Intake of lycopene from diet Harvard semi-quantitative food frequency questionnaire	Longitudinal (Framingham Heart Study)	5135 (Mixed sex)	9	54 (26-79)	27.2	CVD HR 0.83 (0.70, 0.98) 75th and 25th (Ref) percentiles intake of lycopene (Mean 7.9 mg/d)	Age, sex, SBP, total and cholesterol/HDL ratio, BMI, smoking, hypertension treatment, diabetes, energy intake and intake of saturated fat, β-carotene, flavonol, vitamin C and vitamin E	N/A
				9			Stroke HR 0.82 (0.59, 1.16) 75th and 25th (Ref) percentiles intake of lycopene (Mean 7.9 mg/d)		
				11			CHD HR 0.74 (0.58, 0.94) 75th and 25th (Ref) percentiles intake of lycopene (Mean7.9 mg/d)		

(Table 2.1 continues on next page)

Ref (Country of origin)	Exposure	Study design (Cohort name)	Sample size (Sex)	Mean length of follow-up (years)	Mean Age±SD (Range)	BMI (kg/m^2)	Effect size (95% CI)	Confounders adjusted for	Ethnicity
Kabagambe et al., 2005 (Costa Rica)	Lycopene level in adipose tissue Intake of lycopene from diet Semi-quantitative food-frequency questionnaire	Case-control	2912 (Mixed sex)	9	58±11	N/A	MI OR 0.91 (0.67, 1.24) Highest (Median value 1.47 μmol/kg) and lowest (Median value 0 μmol/kg) (Ref) quintiles for intake of lycopene MI OR 1.05 (0.78, 1.42) Highest (Median value 0.62 μg/g) and lowest (Median value 0.11 μg/g) (Ref) quintiles for intake of lycopene	Smoking, alcohol intake, history of diabetes, history of hypertension, abdominal obesity, physical activity, income, intake of saturated fat, polyunsaturated fat, trans-fat, total energy, and dietary fibre	Hispanic Americans
Karppi et al., 2012a (Finland)	Serum level of lycopene	Prospective study (Kuopio Ischaemic Heart Disease Risk Factor (KIHD) cohort)	1031 (Male)	11.5	56.2±6.6	27.5	Acute MI RR 1.55 (1.05, 2.30) Lowest (<0.08 μmol/L) and highest (>0.19 μmol/L) (Ref) tertiles for serum level of lycopene	Age, examination year, BMI, SBP, smoking, alcohol intake, LDL-cholesterol, years of education, physical activity, symptomatic CHD or CHD history, diabetes, antihypertensive medication, drug for high cholesterol and any b-adrenergic blocking agent	Finnish

(Table 2.1 continues on next page)

Ref (Country of origin)	Exposure	Study design (Cohort name)	Sample size (Sex)	Mean length of follow-up (years)	Mean Age±SD (Range)	BMI (kg/m^2)	Effect size (95% CI)	Confounders adjusted for	Ethnicity
Karppi et al., 2012b (Finland)	Serum level of lycopene	Prospective study (Kuopio Ischaemic Heart Disease Risk Factor (KIHD) cohort)	1031 (Male)	15.9	56.4±6.5	27.4	CVD mortality HR 1.51 (0.86, 2.67) Lowest (≤0.03 μmol/L) and highest (>0.22 μmol/L) (Ref) quartiles for serum level of lycopene	Age, examination year, BMI, SBP, smoking, alcohol consumption, physical activity, years of education, LDL-cholesterol, symptomatic CHD or CHD history, use of antihypertensive drugs, use of any b-blockers, serum hs-CRP and diabetes	Finnish
Karppi et al., 2012c (Finland)	Serum level of lycopene	Prospective study (Kuopio Ischaemic Heart Disease Risk Factor (KIHD) cohort)	1031 (Male)	12.1	56.2 (46-65)	27.5	Stroke HR 0.45 (0.21, 0.95) Highest (>0.22 μmol/L) and lowest (≤0.03 μmol/L) (Ref) quartiles for serum level of lycopene	Age, examination year, BMI, SBP, smoking, LDL-cholesterol, diabetes, and stroke	Finnish
Karppi et al., 2013a (Finland)	Plasma level of lycopene	Prospective study (Kuopio Ischaemic Heart Disease Risk Factor (KIHD) cohort)	1847 (Mixed sex)	2.8	71.1 (61-82)	27.4	Risk of AF HR 1.21 (0.68, 2.13) Lowest (≤0.05 μmol/L) and highest (>0.11 μmol/L) (ref) tertiles for serum level of lycopene	Age, examination year, gender, education, SBP, smoking, alcohol consumption, diabetes and the use of antihypertensive medication, CHF, recurrent AF, prevalent CHD and baseline prevalence of MI	Finnish

(Table 2.1 continues on next page)

Ref (Country of origin)	Exposure	Study design (Cohort name)	Sample size (Sex)	Mean length of follow-up (years)	Mean Age±SD (Range)	BMI (kg/m^2)	Effect size (95% CI)	Confounders adjusted for	Ethnicity
Karppi et al., 2013b (Finland)	Serum level of lycopene	Prospective study (Kuopio Ischaemic Heart Disease Risk Factor (KIHD) cohort)	1031 (Male)	17.8	56.2±6.6	27.6	CHF HR 1.99 (0.83, 4.77) Lowest (≤0.03 μmol/L) and highest (>0.22 μmol/L) (Ref) quartiles for serum level of lycopene	Age, examination year, BMI, years of education, smoking, alcohol consumption, physical activity, serum hs-CRP and serum LDL-cholesterol, diabetes, hypertension with antihypertensive medication, prevalent CHD and HF at baseline examination	Finnish
Karppi et al., 2013c (Finland)	Serum level of lycopene	Prospective study (Kuopio Ischaemic Heart Disease Risk Factor (KIHD) cohort)	1031 (Male)	15.9	56.2 (46-65)	27.5	Sudden cardiac death (SCD) HR 1.93 (0.92, 4.04) Lowest (≤0.08 μmol/L) and highest (>0.19 μmol/L) (ref) tertiles for serum level of lycopene	Age, SBP, waist circumference, smoking, alcohol consumption, years of education, LDL-cholesterol, CRP, diabetes, prevalent CHD and CHF	Finnish
Klipstein-Grobusch et al., 2000 (Netherland)	Serum level of lycopene	Case-control (Subsample of the Rotterdam Study)	217 (Mixed sex)	N/A	66.8 (7.2)	26.4	Atherosclerosis OR 0.66 (0.29, 1.49) Highest (>0.166 μmol/L) and lowest (<0.058 μmol/L)) (ref) quartiles for serum level of lycopene	Age, sex, cholesterol, season, waist-to-hip ratio, pack-years smoked, alcohol consumption	N/A

(Table 2.1 continues on next page)

Ref (Country of origin)	Exposure	Study design (Cohort name)	Sample size (Sex)	Mean length of follow-up (years)	Mean Age±SD (Range)	BMI (kg/m^2)	Effect size (95% CI)	Confounders adjusted for	Ethnicity
Kohlmeier et al., 1997 (USA)	Lycopene level in adipose tissue	Case-control (EURAMIC study)	1379 (Male)	N/A	54	26.3	MI OR 0.42 (0.25, 0.7) Highest (Median value 0.62 μg/g) and lowest (Median value 0.11 μg/g) (Ref) quintiles for intake of lycopene	Age, BMI, smoking, maternal, paternal history of disease, history of high blood pressure, study site and α-tocopherol intake	N/A
Mayne et al., 2004 (USA)	Plasma level of lycopene	Longitudinal study (Yale University cancer prevention trial)	259 (Mixed sex)	7.5	61.8 (20-79)	N/A	All-cause Mortality HR 0.53 (0.30, 0.93) Above versus below (Ref) Median plasma level of lycopene (Median value 280 μg/l) CHD Mortality HR 0.42 (0.14, 1.30) Above versus below (Ref) Median plasma level of lycopene (Median value 280 μg/l)	Age, gender, treatment arm, time-dependent smoking, baseline plasma cholesterol, study site	N/A

(Table 2.1 continues on next page)

Ref (Country of origin)	Exposure	Study design (Cohort name)	Sample size (Sex)	Mean length of follow-up (years)	Mean Age±SD (Range)	BMI (kg/m^2)	Effect size (95% CI)	Confounders adjusted for	Ethnicity
Osganian et al., 2003 (USA)	Intake of lycopene from diet Semi-quantitative food-frequency questionnaire	Longitudinal study (Nurses' Health Study)	73286 (Female)	12	50 (30-55)	25	Coronary artery disease RR 0.93 (0.77, 1.14) Highest (Median value 15830 µg/d) and lowest (Median value 3570 µg/d) (ref) quintiles for serum level of lycopene	Age, smoking, postmenopausal hormone use, parental history of MI, history of high blood pressure, history of high cholesterol, diabetes, BMI, physical activity, aspirin use, alcohol consumption, total energy intake and intake of saturated fat, polyunsaturated fat, trans unsaturated fat, cereal fibre, folate, dietary glycaemic load, and vitamin B-6	N/A
Rissanen et al., 2001 (Finland)	Serum level of lycopene	Prospective study (Kuopio Ischaemic Heart Disease Risk Factor (KIHD) cohort)	725 (Male)	4	46-64	27	Acute coronary events or Stroke HR 3.3 (1.7, 6.4) Lowest quarter (≤0.07 µmol/L) versus every other quarter of serum level of lycopene (≥0.08 µmol/L) (Ref) Acute coronary events HR 2.8 (1.4, 5.4) Lowest quarter (≤0.07 µmol/L) versus every other quarter of serum level of lycopene (≥0.08 µmol/L) (Ref)	Age, examination years, SBP and three nutritional factors (serum b-carotene, folate and plasma vitamin C)	Finnish

(Table 2.1 continues on next page)

Ref (Country of origin)	Exposure	Study design (Cohort name)	Sample size (Sex)	Mean length of follow-up (years)	Mean Age±SD (Range)	BMI (kg/m^2)	Effect size (95% CI)	Confounders adjusted for	Ethnicity
Sesso et al., 2003 (USA)	Intake of lycopene from diet Semi-quantitative food-frequency questionnaire	Prospective study (Women's Health Study)	38445 (Female)	7.2	54.0±7.0	26	CVD RR 0.90 (0.69, 1.17) Highest (Median value 16741 μg/d) and lowest (Median value 3326 μg/d) (Ref) quintiles for intake of lycopene	Age, randomized aspirin, randomized vitamin E, randomized β-carotene, BMI, exercise, smoking, postmenopausal hormone use, parental history of MI < age 60, diabetes, hypertension, high cholesterol, and the intake of fruit and vegetables, alcohol, fibre, folate, nonsupplemental vitamin E and saturated fat	N/A
							Stroke RR 0.91 (0.57, 1.45) Highest (Median value 16741 μg/d) and lowest (Median value 3326 μg/d) (Ref) quintiles for intake of lycopene		
							MI RR 0.69 (0.41, 1.15) Highest (Median value 16741 μg/d) and lowest (Median value 3326 μg/d) (Ref) quintiles for intake of lycopene		

(Table 2.1 continues on next page)

Ref (Country of origin)	Exposure	Study design (Cohort name)	Sample size (Sex)	Mean length of follow-up (years)	Mean Age±SD (Range)	BMI (kg/m^2)	Effect size (95% CI)	Confounders adjusted for	Ethnicity
Sesso et al., 2004 (USA)	Plasma level of lycopene	Nested case-control (Women's Health Study)	966 (Female)	4.8	58.8±8.4	26.6	CVD RR 0.67 (0.41, 1.11) Highest (≥21.0 μg/dL) and lowest (<11.7 μg/dL) (Ref) quartiles for plasma of lycopene	Age, smoking, adjusted for randomized aspirin treatment, randomized vitamin E treatment, randomized β-carotene treatment, plasma cholesterol concentration, BMI, physical activity, postmenopausal hormone use, parental history of MI < age 60, diabetes, hypertension, high cholesterol, alcohol, fibre, folate, saturated fat, and fruit and vegetable intakes	N/A
Sesso et al., 2005 (USA)	Plasma level of lycopene	Nested case-control (Physicians' Health Study)	499 (Male)	2.1	69.7±8.1	24.5	CVD RR 1.03 (0.65, 1.64) Highest (>12.7 μg/dL) and lowest (≤6.4 μg/dL) (Ref) quartiles for plasma of lycopene	Age, smoking, adjusted for randomized aspirin treatment, randomized vitamin E treatment, randomized β-carotene treatment, plasma cholesterol concentration, BMI, physical activity, postmenopausal hormone use, parental history of MI < age 60, diabetes, hypertension, high cholesterol, alcohol, fibre, folate, saturated fat, and fruit and vegetable intakes	N/A

(Table 2.1 continues on next page)

Ref (Country of origin)	Exposure	Study design (Cohort name)	Sample size (Sex)	Mean length of follow-up (years)	Mean Age±SD (Range)	BMI (kg/m^2)	Effect size (95% CI)	Confounders adjusted for	Ethnicity
Street et al., 1994 (USA)	Serum level of lycopene	Nested case-control	369 (Mixed sex)	14	23-58	N/A	MI OR 1.33 Lowest and highest (Ref) quintiles for serum level of lycopene	N/A	N/A
Tavani et al., 2006 (Italy)	Intake of lycopene Food-frequency questionnaire	Nested case-control	1442 (Mixed sex)	9	50-70	N/A	Acute MI OR 1.19 (0.82, 1.70) Highest and lowest (Ref) quartiles for intake of lycopene (Mean lycopene intake 7189.5 µg/d)	Age, sex, study site, education, smoking, alcohol consumption, coffee, non-alcohol total energy, BMI, physical activity, cholesterol level, diabetes, hyperlipidemia hypertension, and family history of acute MI in first degree relatives	N/A
Wood and Johnson, 2004 (USA)	Serum level of lycopene	Cross-sectional analysis (Third National Health and Nutrition Examination Survey (NHANES III))	4087 with periodontitis (Mixed Sex)	N/A	47.7 (SEM 0.4)	23.9	CHF RR 0.65 (0.21, 2.03) Lowest (≤14 µg/dL) and highest (>29 µg/dL) (Ref) quartiles for serum level of lycopene	Age, race, gender, BMI, waist to hip ratio, serum CRP, WBC count, smoking, history of diabetes, hypertension, socioeconomic status, and education level	Caucasian, African-American, Other
			1443 without periodontitis (Mixed Sex)		47.7 (SEM 0.6)	23.6	CHF RR 0.59 (0.15, 2.24) Lowest (≤14 µg/dL) and highest (>29 µg/dL) (Ref) quartiles for serum level of lycopene		

(Table 2.1 continues on next page)

Ref (Country of origin)	Exposure	Study design (Cohort name)	Sample size (Sex)	Mean length of follow-up (years)	Mean Age±SD (Range)	BMI (kg/m^2)	Effect size (95% CI)	Confounders adjusted for	Ethnicity
Tomato associations									
Jacques et al., 2013 (USA)	Intake of tomatoes and tomato-based products Harvard semi-quantitative food frequency questionnaire	Longitudinal study (Framingham Heart Study)	5135 (Mixed sex)	9	54 (26-79)	27.2	CVD HR 0.94 (0.878, 0.995) 75th and 25th (Ref) percentiles for intake of tomatoes and tomato-based products (Mean 4.4 servings/wk)	Age, sex, SBP, total cholesterol, total cholesterol/HDL ratio, BMI, smoking, hypertension treatment, diabetes, saturated fat, β-carotene, flavonol, vitamin C and vitamin E intakes and energy intake	N/A
				9			Stroke HR 0.99 (0.90, 1.10) 75th and 25th (Ref) percentiles for intake of tomatoes and tomato-based products (Mean 4.4 servings/wk)		
				9			CHD HR 0.90 (0.83, 0.99) 75th and 25th (Ref) percentiles for intake of tomatoes and tomato-based products (Mean 4.4 servings/wk)		

(Table 2.1 continues on next page)

Ref (Country of origin)	Exposure	Study design (Cohort name)	Sample size (Sex)	Mean length of follow-up (years)	Mean Age±SD (Range)	BMI (kg/m^2)	Effect size (95% CI)	Confounders adjusted for	Ethnicity
Sesso et al., 2003 (USA)	Intake of tomato based product Semi-quantitative food-frequency questionnaire	Prospective study (Women's Health Study)	38445 (Female)	7.2	54.0±7.0	26	CVD RR 0.71 (0.42, 1.17) Highest (12 servings/wk) and lowest (1.4 servings/wk) (Ref) quintiles for intake of tomato based product per week Stroke RR 0.20 (0.05, 0.84) Highest (12 servings/wk) and lowest (1.4 servings/wk) (Ref) quintiles for intake of tomato based product per week MI RR 0.39 (0.12, 1.30) Highest (12 servings/wk) and lowest (1.4 servings/wk) (Ref) quintiles for intake of tomato based product per week	Age, randomized aspirin, randomized vitamin E, randomized β-carotene, BMI, exercise, smoking, postmenopausal hormone use, parental history of MI <60 y, diabetes, hypertension, high cholesterol, and the intake of fruit and vegetables, alcohol, fibre, folate, nonsupplemental vitamin E and saturated fat	N/A

(Table 2.1 continues on next page)

Ref (Country of origin)	Exposure	Study design (Cohort name)	Sample size (Sex)	Mean length of follow-up (years)	Mean Age±SD (Range)	BMI (kg/m^2)	Effect size (95% CI)	Confounders adjusted for	Ethnicity
Wood and Johnson, 2004 (USA)	Intake of tomato Semi-quantitative food-frequency questionnaire	Cross-sectional analysis (Third National Health and Nutrition Examination Survey (NHANES III))	4087 with periodontitis (Mixed Sex)	N/A	47.7 (SEM 0.4)	23.9	CHF RR 5.10 (1.67, 15.57) Lowest (≤3) and highest (>17) (Ref) quartiles for monthly tomato consumption	Age, race, gender, BMI, waist to hip ratio, serum CRP, WBC count, smoking, history of diabetes, hypertension, socioeconomic status, and education level	Caucasian, African-American, Other
			1443 without periodontitis (Mixed Sex)		47.7 (SEM 0.6)	23.6	CHF RR 1.68 (0.6, 4.76) Lowest (≤3) and highest (>17) (Ref) quartiles for monthly tomato consumption		

Ref, Reference; CI, confidence intervals; CVD, Cardiovascular diseases; CHD, Coronary heart disease; CHF, Congestive heart failure; AF, Atrial fibrillation; Intima-media thickness, IMT; HR, Hazard ratio; RR, Relative ratio; OR, Odd ratio; BMI, Body mass index; MI, Myocardial Infarction; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; HDL, High density lipoprotein; LDL, Low density lipoprotein; CRP, C-reactive protein; WBC, White blood cell; d, Day; wk, week

2.3.1 Characteristics of studies

Twenty-five of the included studies assessed lycopene alone (Ascherio et al., 1999; Ford et al., 2014; Hak et al., 2004; Hak et al., 2003; Han and Han, 2016; Han et al., 2016; Hirvonen et al., 2000; Iribarren et al., 1997; Ito et al., 2006; Kabagambe et al., 2005; Karppi et al., 2013a; Karppi et al., 2013b; Karppi et al., 2012a; Karppi et al., 2012b; Karppi et al., 2013c; Karppi et al., 2012c; Klipstein-Grobusch et al., 2000; Kohlmeier et al., 1997; Mayne et al., 2004; Osganian et al., 2003; Rissanen et al., 2001; Sesso et al., 2004; Sesso et al., 2005; Street et al., 1994; Tavani et al., 2006), and three studies assessed both lycopene and tomato (Jacques et al., 2013; Sesso et al., 2003; Wood and Johnson, 2004). These studies varied according to length of follow-up (2 to 17.5 years), sample size (range: 37 to 73286 participants), mean age (45 to 71y), and mean BMI (24 to 28). The studies included in this review originated from USA (n=15), Finland (n=8) and single studies from Costa Rica, Italy, Japan and Netherland, and a multicentre European study. According to sex, 12 studies included only men and three included only women, while 13 studies were including mixed sex. Overall, the mean intakes of lycopene were 0.14 mg/d (lowest categories) and 18.8 mg/d (highest categories) (**Table 2.2**); mean serum concentration of lycopene were 0.0012 µmol/L (lowest categories) and 1.13 µmol/L (highest categories) (**Table 2.3**).

Table 2.2 The mean intake of tomato/lycopene included in systematic review.

Ref (Country of origin)		Lowest category	Highest category	Unit
Intake of tomatoes and/or tomato-based products				
Jacques et al., 2013 (USA)	Mean❖	4.4		Servings/ wk
Sesso et al., 2003 (USA)	Median*	1.4	12	Servings/ wk
Wood and Johnson, 2004 (USA)	Mean‡	≤3	>17	Tomato intake per month
Intake of lycopene				
Ascherio et al., 1999 (USA)	Median*	3442	18798	µg/day
Hirvonen et al., 2000 (Finland)	Median‡	140	1450	µg/day
Jacques et al., 2013 (USA)	Mean¤	7900		µg/day
Kabagambe et al., 2005 (Costa Rica)	Median*	0	1.47	µmol/kg of adipose tissue
Osganian et al., 2003 (USA)	Median*	3570	15830	µg/day
Sesso et al., 2003 (USA)	Median*	3326	16741	µg/day
Tavani et al., 2006 (Italy)	Mean‡	7190		µg/day

‡ Quartile
* Quintile
❖ Percentile

Table 2.3 The mean serum concentration of lycopene included in systematic review.

Ref (Country of origin)		Lowest category (µmol/L)	Highest category (µmol/L)
Ford et al., 2014 (USA)	Mean	0.45	
Hak et al., 2003 (USA)	Median*	0.41	1.08
Hak et al., 2004 (USA)	Median*	0.40	1.13
Han and Han, 2016 (USA)	Mean	0.23	0.55
Han et al., 2016 (USA)	Mean†	0.20	0.63
Iribarren et al., 1997 (USA)	Mean▲	0.44	
Ito et al., 2006 (Japan)	Mean#	0.33	
Karppi et al., 2012a (Finland)	Mean†	<0.08	>0.19
Karppi et al., 2012b (Finland)	Mean‡	≤0.03	>0.22
Karppi et al., 2012c (Finland)	Mean‡	≤0.03	>0.22
Karppi et al., 2013a (Finland)	Mean†	≤0.05	>0.11
Karppi et al., 2013b (Finland)	Mean‡	≤0.03	>0.22
Karppi et al., 2013c (Finland)	Mean†	≤0.08	>0.19
Klipstein-Grobusch et al., 2000 (Netherland)	Mean‡	<0.058	>0.166
Kohlmeier et al., 1997 (USA)	Median*	0.11	0.62
Mayne et al., 2004 (USA)	Median	0.52	
Rissanen et al., 2001 (Finland)	Mean‡	≤0.07	≥0.24
Sesso et al., 2004 (USA)	Mean‡	<0.0022	≥0.0039
Sesso et al., 2005 (USA)	Median‡	≤0.0012	>0.0024
Street et al., 1994 (USA)	Mean*	N/A	
Wood and Johnson, 2004 (USA)	Mean‡	≤0.0026	>0.0054

† Tertile
‡ Quartile
* Quintile
▲1-SD increase
Logarithmically transformed value

2.3.2 Meta-analysis: Lycopene and CVD

Twenty-five studies, including 211704 participants, evaluated the association between lycopene and a number of cardiovascular diseases reported below (Ascherio et al., 1999; Ford et al., 2014; Hak et al., 2004; Hak et al., 2003; Han et al., 2016; Hirvonen et al., 2000; Iribarren et al., 1997; Ito et al., 2006; Jacques et al., 2013; Kabagambe et al., 2005; Karppi et al., 2013a; Karppi et al., 2013b; Karppi et al., 2012a; Karppi et al., 2012b; Karppi et al., 2013c; Karppi et al., 2012c; Klipstein-Grobusch et al., 2000; Kohlmeier et al., 1997; Mayne et al., 2004; Osganian et al., 2003; Rissanen et al., 2001; Sesso et al., 2004; Sesso et al., 2005; Sesso et al., 2003; Tavani et al., 2006).

2.3.4 Stroke

Eight studies, including 119322 participants, evaluated intake or serum concentration of lycopene and incidence of stroke (Ascherio et al., 1999; Hak et al., 2004; Hirvonen et al., 2000; Ito et al., 2006; Jacques et al., 2013; Karppi et al., 2012c; Rissanen et al., 2001; Sesso et al., 2003). Overall, meta-analysis showed that highest category intake or serum concentration of lycopene were significantly associated with reduced incidence of stroke (HR 0.74, 95% CI 0.62 to 0.89, p=0.001). Heterogeneity levels assessed by the I^2 test were low at 32% (**Figure 2.2A**). Results from studies evaluating intake of lycopene showed that highest category intake was significantly associated with reduced incidence of stroke (HR 0.79, 95% CI 0.64 to 0.97, p=0.02) (Ascherio et al., 1999; Hirvonen et al., 2000; Jacques et al., 2013; Sesso et al., 2003). Heterogeneity levels assessed by the I^2 test were low at 44% (**Figure 2.2A**). Similarly, there was strong evidence that high serum concentration of lycopene was associated with lower risk of stroke (HR 0.61, 95% CI 0.40 to 0.92, p=0.02) (Hak et al., 2004; Ito et al., 2006; Karppi et al., 2012c; Rissanen et al., 2001). Heterogeneity levels assessed by the I^2 test were low at 31% (**Figure 2.2A**).

Visual inspection of the funnel plot and calculation of the Egger's regression test indicated no publication bias (p=0.70) (**Appendix A2**) (Egger et al., 1997).

In addition, meta-regression analysis on the association between high intake or serum concentration of lycopene and incidence of stroke showed no significant associations with the length of follow-up (8 studies), age (5 studies) or BMI (7 studies) (**Appendix A3-Appendix A5**). However, this result should be interpreted cautiously due to the small number of studies.

Subgroup analysis (**Table 2.4**) showed that four studies originating from Japan and Finland reported significant reduction in the incidence of stroke with high intake and serum concentrations of lycopene (HR 0.63, 95% CI 0.48 to 0.82, p<0.001; I^2=30%); while four studies from the USA reported non-significant lower risk of stroke (HR 0.88, 95% CI 0.71 to 1.07, p=0.20; I^2=0%). Between-group comparison of these results according to geographical origins were not significantly different between groups (p=0.06).

Subgroup analysis (**Table 2.4**) according to sex showed that high intake and serum concentrations of lycopene were significantly associated with reduced incidence of stroke in studies including men only (HR 0.71, 95% CI 0.51 to 0.99, p=0.04; I^2=56%) and studies including both sexes (HR 0.75, 95% CI 0.58 to 0.98, p=0.03; I^2=7%). One study including women only showed that high intake of lycopene was non-significantly associated with

reduced incidence of stroke (HR 0.91, 95% CI 0.57 to 1.45, p=0.69). The between-group comparisons according to sex was not significantly different between groups (p=0.69).

Subgroup analysis (**Table 2.4**) by follow-up time showed that in studies with ≤ 8y follow-up, high intake or serum concentration of lycopene were associated with non-significant reductions in the risk of stroke with high heterogeneity levels (HR 0.75, 95% CI 0.54 to 1.05, p=0.09; I^2=62%). In studies with a follow-up >8y, high intake or serum concentration of lycopene were associated with significant reductions in the risk of stroke with low heterogeneity levels (HR 0.75, 95% CI 0.60 to 0.95, p=0.02; I^2=0%). Between-group comparison of these results according to length of follow-up were not significantly different between groups (p=0.98).

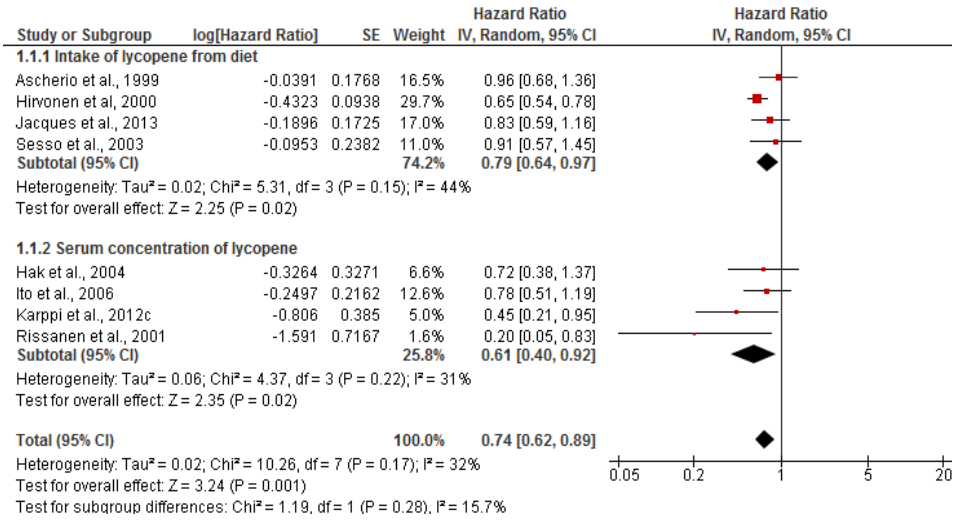
Subgroup analysis (**Table 2.4**) according to age showed that in studies with mean age ≤55y, high intake of lycopene was associated with non-significant lower risk of stroke (HR 0.85, 95% CI 0.65 to 1.12, p=0.26; I^2=0%); however, studies with mean age >55y, high intake or serum concentration of lycopene were associated with significantly lower risk of stroke (HR 0.66, 95% CI 0.56 to 0.77, p<0.001; I^2=0%). In addition, two studies in which mean age was not reported showed that high intake of lycopene was associated with non-significant reduction in the risk of stroke with high heterogeneity levels (HR 0.52, 95% CI 0.12 to 2.29, p=0.39; I^2=77%).

Subgroup analysis (**Table 2.4**) by BMI showed that in studies with mean BMI <26, high intake or serum concentration of lycopene were associated with non-significant lower risk of stroke (HR 0.90, 95% CI 0.70 to 1.16, p=0.44; I^2=0%), while in studies with mean BMI ≥26, high intake or serum concentration of lycopene were associated with significant lower risk of stroke with low heterogeneity levels (HR 0.64, 95% CI 0.48 to 0.86, p=0.003; I^2=44%). In addition, one study not reporting mean BMI showed that high serum concentration of lycopene was associated with non-significant lower risk of stroke with high heterogeneity levels (HR 0.78, 95% CI 0.51 to 1.19, p=0.25; I^2=77%).

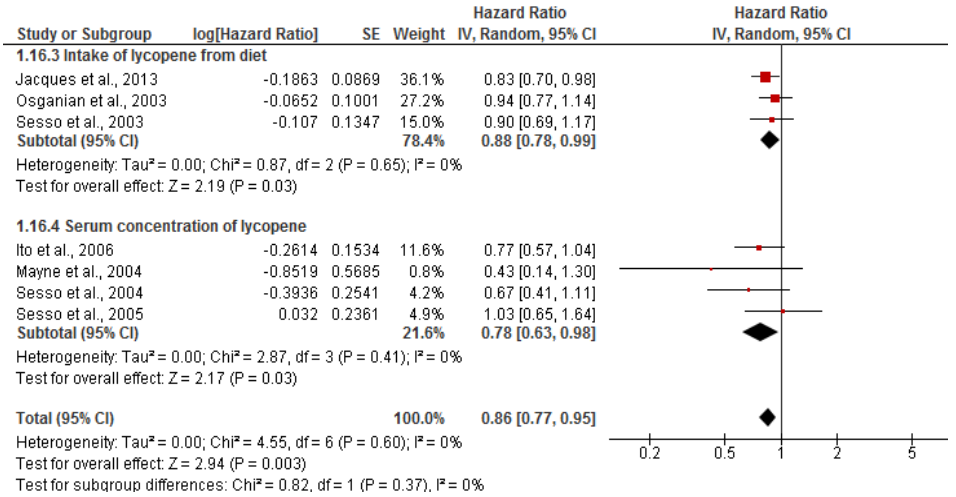
Table 2.4 Subgroup analysis for risk of stroke.

Variable (Number of studies or subgroups)	HR (95% CI)	P (Z-test)	Heterogeneity I^2%	Between group comparisons (Chi^2)
Geographical origins				
USA (n=4) (Ascherio et al., 1999; Hak et al., 2004; Jacques et al., 2013; Sesso et al., 2003)	0.88 (0.71, 1.07)	p=0.20	0	p=0.06
Japan and Finland (n=4) (Hirvonen et al., 2000; Ito et al., 2006; Karppi et al., 2012c; Rissanen et al., 2001)	0.63 (0.48, 0.82)	p<0.001	30	
Sex				
Men (n=4) (Ascherio et al., 1999; Hak et al., 2004; Hirvonen et al., 2000; Rissanen et al., 2001)	0.71 (0.51, 0.99)	p=0.04	56	
Mixed (n=3) (Ito et al., 2006; Jacques et al., 2013; Karppi et al., 2012c)	0.75 (0.58, 0.98)	p=0.03	7	p=0.69
Women (n=1) Sesso et al., 2003	0.91 (0.57, 1.45)	p=0.69	n/A	
Follow-up time				
≤8y (n=4) (Ascherio et al., 1999; Hirvonen et al., 2000; Rissanen et al., 2001; Sesso et al., 2003)	0.75 (0.54, 1.05)	p=0.09	62	p=0.98
>8y (n=4) (Hak et al., 2004; Ito et al., 2006; Jacques et al., 2013; Karppi et al., 2012c)	0.75 (0.60, 0.95)	p=0.02	0	
Age				
≤55y (n=2) (Jacques et al., 2013; Sesso et al., 2003)	0.85 (0.65, 1.12)	p=0.26	0	p=0.25
>55y (n=4) (Hak et al., 2004; Hirvonen et al., 2000; Ito et al., 2006; Karppi et al., 2012c)	0.66 (0.56, 0.77)	p<0.001	0	
N/A (n=2) (Ascherio et al., 1999; Rissanen et al., 2001)	0.52 (0.12, 2.29)	p=0.39	77	
BMI				
<26 (n=3) (Ascherio et al., 1999; Hak et al., 2004; Sesso et al., 2003)	0.90 (0.70, 1.17)	p=0.44	0	p=0.23
≥26 (n=4) (Hirvonen et al., 2000; Jacques et al., 2013; Karppi et al., 2012c; Rissanen et al., 2001)	0.64 (0.48, 0.86)	p=0.003	45	
N/A (n=1) (Ito et al., 2006)	0.78 (0.51, 1.19)	p=0.25	N/A	

(A) Stroke



(B) CVD



(C) Mortally

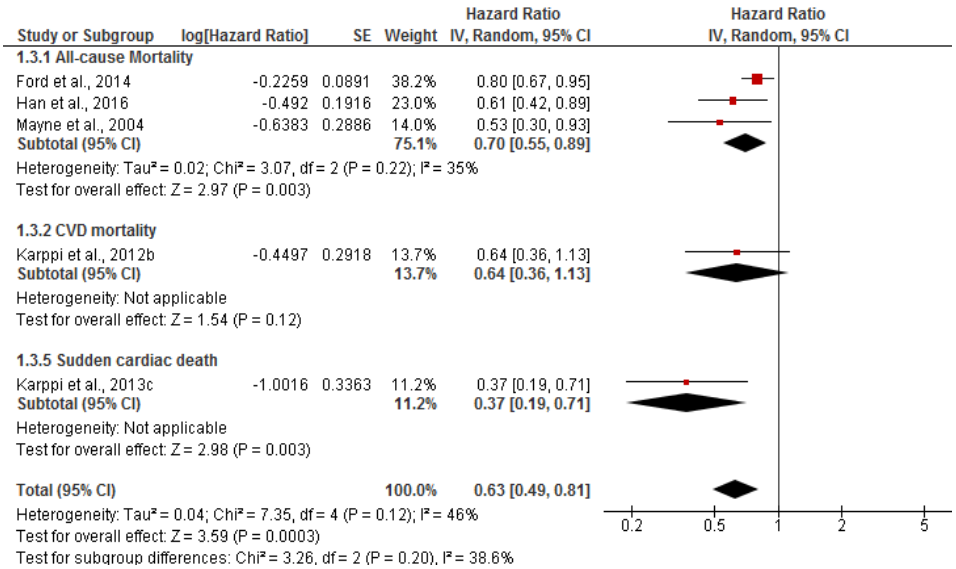


Figure 2.2 Forest plots of epidemiological studies evaluating associations between high serum level/intake of lycopene and risk of stroke (A); CVD (B) and mortality (C).

The forest plots show individual studies, the weight allocated to each of them, and shown in the form of black diamonds, the subgroups and overall effect size as hazards ratios with 95% confidence intervals.

2.3.5 CVD

Seven studies, including 121651 participants, evaluated intake or serum concentration of lycopene and incidence of CVD (Ito et al., 2006; Jacques et al., 2013; Mayne et al., 2004; Osganian et al., 2003; Sesso et al., 2004; Sesso et al., 2005; Sesso et al., 2003). Overall, meta-analysis showed that high intake or serum concentration of lycopene were significantly associated with a reduced incidence of CVD (HR 0.86, 95% CI 0.77 to 0.95, p=0.003). Heterogeneity levels assessed by the I^2 test were low at 0% (**Figure 2.2B**).

Results from studies evaluating intake of lycopene showed that higher intake was significantly associated with reduced incidence of CVD (HR 0.88, 95% CI 0.78 to 0.99, p=0.03) (Jacques et al., 2013; Osganian et al., 2003; Sesso et al., 2003). Heterogeneity levels assessed by the I^2 test were low at 0% (**Figure 2.2B**). In addition, studies evaluating serum lycopene concentrations showed that high concentration was associated with non-significant lower risk of CVD (HR 0.78, 95% CI 0.63 to 0.98, p=0.03) (Ito et al., 2006; Mayne et al., 2004; Sesso et al., 2004; Sesso et al., 2005). Heterogeneity levels assessed by the I^2 test were low at 0% (**Figure 2.2B**).

Meta-analysis of four studies (Ito et al., 2006; Jacques et al., 2013; Mayne et al., 2004; Osganian et al., 2003), including 81741 participants, indicated that there was strong evidence that high intake or serum concentration of lycopene were associated with lower incidence of CHD (HR 0.81, 95% CI 0.67 to 0.98, p=0.03). Heterogeneity levels assessed by the I^2 test were low at 26% (**Appendix A6**).

2.3.6 Mortality

Five studies, including 6249 participants, evaluated serum concentration of lycopene and risk of mortality (Ford et al., 2014; Han et al., 2016; Karppi et al., 2012b; Karppi et al., 2013c; Mayne et al., 2004). Overall, meta-analysis showed that high serum concentration of lycopene was significantly associated with a reduced risk of mortality (HR 0.63, 95% CI 0.49 to 0.81, p>0.001). Heterogeneity levels assessed by the I^2 test were low at 46% (**Figure 2.2C**).

In addition, two studies assessing mortality were not included in the meta-analysis. One study was not included in meta-analysis because the results were based on the use of a different reference group, reported that high serum concentration of lycopene was associated with non-significant lower risk of CHD mortality (HR 0.42, 95% CI 0.14 to 1.30, p=0.42) (Mayne et al., 2004). The second study reported that among people with lupus erythematosus, high serum concentration of lycopene were associated with a lower mortality

rate (5.3%) while the group with low serum lycopene concentration had a mortality rate of 33% (Han and Han, 2016).

2.3.7 Others cardiovascular-related disorders

One study evaluating CHF, including 1031 participants, indicated that there was strong evidence that high serum concentration of lycopene was associated with lower incidence of CHF (HR 0.24, 95% CI 0.10 to 0.56, p=0.001) (**Appendix A7**) (Karppi et al., 2013b). In addition, Wood and Johnson (2004) evaluating CHF reported that lower lycopene levels was associated with non-significant reduction of CHF among individuals with periodontitis (HR 0.65, 95% CI 0.21 to 2.03) and without periodontitis (HR 0.59, 0.15-2.24) (Wood and Johnson, 2004). One study by Karppi et al. (2013) evaluated AF and reported that high serum concentration of lycopene was associated with significant increased risk of AF (HR 0.45, 95% CI 0.26 to 0.78, p=0.004) (**Appendix A7**) (Karppi et al., 2013a).

Meta-analysis of five studies, including 7825 participants, indicated that there was not strong evidence that high serum concentration (Hak et al., 2003; Karppi et al., 2012a), or either adipose tissue (Kabagambe et al., 2005; Kohlmeier et al., 1997) or intake (Tavani et al., 2006) of lycopene was associated with lower incidence of MI (OR 0.84, 95% CI 0.57 to 1.23, p=0.37) (**Appendix A8**). In addition, another study on MI that was not included in meta-analysis because the results were based on the use of a different reference group, reported a non-significant higher risk of MI with lower serum lycopene concentration (OR 1.33, CI not provided, p=0.54) (Street et al., 1994).

Meta-analysis of two studies, including 679 participants, indicated that high serum concentration of lycopene was associated with a non-significant reduction in the risk of incidence of atherosclerosis (OR 0.79, 95% CI 0.60 to 1.04, p=0.09). Heterogeneity levels assessed by the I^2 test were low at 0% (**Appendix A8**) (Iribarren et al., 1997; Klipstein-Grobusch et al., 2000).

2.3.8 Meta-analysis: Tomato and cardiovascular diseases

Three studies, including 49110 participants, evaluated the association between consumption of tomato products and CVD reported below (Jacques et al., 2013; Sesso et al., 2003; Wood and Johnson, 2004).

Jacques et al. (2013) and Sesso et al. (2003), evaluated intake of tomato and incidence of stroke and CVD (Jacques et al., 2013; Sesso et al., 2003). Meta-analysis of these studies, including 43580 participants, showed that high intake of tomato was associated with non-

significant reductions in stroke (HR 0.53, 95% CI 0.12 to 2.42, *p*=0.41), and non-significant reductions in CVD (HR 0.91, 95% CI 0.77 to 1.07, *p*=0.26) (**Appendix A9**).

Jacques et al. (2013) including 5135 participants, evaluated tomato product intake and incidence of CHD (Jacques et al., 2013). Higher intake of tomato product was significantly associated with a reduced incidence of CHD (HR 0.91, 95% CI 0.83 to 0.99, *p*=0.03).

Sesso et al. (2003), including 38445 participants, reported that high intake of tomato based product was non-significantly associated with reduced risk of MI (RR 0.39, 95% CI 0.12 to 1.30, *p*=0.13) (**Appendix A9**) (Sesso et al., 2003).

In addition, Wood and Johnson (2004) reported that lower consumption of tomato were associated with significant increased risk of CHF among individuals with periodontitis (HR 5.10, 95% CI 1.67 to 15.57) and without periodontitis (HR 1.68, 95% CI 0.60 to 4.76) (Wood and Johnson, 2004).

2.4 Discussion

2.4.1 Principal findings

This systematic review and meta-analysis of epidemiological studies revealed that there is strong evidence indicating that lycopene intake or serum concentrations were associated with significant reductions of 26% in stroke, 14% in CVD; while high serum lycopene concentration were associated with significant reduction of 37% in mortality. In addition, subgroup analysis of the studies evaluating stroke revealed that the association of high intakes of lycopene and lower risk of stroke were significant among older and overweight individuals; studies with longer follow-up were also more likely to report significant associations (**Table 2.4**). This systematic review revealed a dearth of evidence on the associations with other cardiovascular outcomes such as atherosclerosis, MI, CHF, AF. Scarce evidence also indicated that high dietary and plasma lycopene, or high intakes of tomato were not associated with significant reduction of stroke, CVD, CHD, MI and CHF. These results have important public health implications given the high prevalence of CVD globally.

2.4.2 Scientific analysis of findings and implications for health

A focus on the health impacts of dietary patterns, as a whole, has been emphasised over the past couple of decades (Hu, 2002), and the important health benefits of major dietary patterns, such as the Mediterranean dietary pattern, are well documented (Estruch et al., 2013). However, it is evident that the impact of dietary patterns is a result of the additive

effects of different food groups and previous evidence highlights this relative contribution of specific food groups to dietary patterns (Trichopoulou et al., 2009).

CVD are the leading cause of death, followed by cancer (GBD 2015 Mortality and Causes of Death Collaborators, 2016). In addition, results from the Global burden of disease collaboration (GBD 2015 Risk Factors Collaborators, 2016), indicates that metabolic risk factors such as high blood pressure (1st) and blood cholesterol (7th), and behavioural risk factors such as low fruit (13th) and vegetable (20th) intakes are among the top 30 leading causes of death and disability. This meta-analysis showed that high lycopene consumption and serum concentrations were associated with significant reductions of 37% in mortality, 26% in stroke, and 14% in CVD. The results of the present review on the association of lycopene and stroke are in line with those from a previous meta-analysis reporting a reduction in risk of stroke (19.3%) (Li and Xu, 2014). In addition, a recent meta-analysis on 14 studies reporting significant reductions of stroke (RR 0.83, 95% CI 0.69 to 0.96), CVD (RR 0.83, 95% CI 0.76 to 0.90), and CHD (RR 0.87, 95% CI 0.76 to 0.98) with higher lycopene intake (Song et al., 2017). Compared with these previous meta-analyses, present systematic review identified 28 studies and 25 of those were meta-analysed on the association of lycopene and tomato and CVD and mortality. Also, this systematic review explored the impact of important covariates such as age, and BMI as sources of heterogeneity by means of meta-regression and subgroup analysis.

Taken together, the results of this study and the results from systematic review and meta-analysis on the impact of interventions on tomato and lycopene consumption on cardiovascular risk factors (Cheng et al., 2017), provide reasonable and consistent evidence supporting the health benefits and important role of tomato products and lycopene as part of a healthy cardioprotective diet. This is an important and reassuring finding given the criticism to which observational evidence is sometimes subjected (Taubes, 1995; Young and Karr, 2011).

Inadequate consumption of fruit and vegetable contributes to 2.6 million deaths per year around the world, and increasing fruit and vegetable to 600 g/d could reduce 1.8% of total global burden of disease (Lock et al., 2005). Diets with high consumption of fruit and vegetables provide vitamins, minerals and fibre, as well as phytochemicals which contribute significantly to improvements in several risk factors, including blood pressure (BP), lipid levels, insulin resistance, inflammatory biomarker levels, endothelial function, and weight control (Mozaffarian et al., 2011). Tomatoes are one of the most consumed vegetable and vegetable products, just below the consumption of potatoes, lettuce and vegetable salads,

and onions (FAOSTAT, 2015a). Tomatoes are the primary source of lycopene, the most powerful antioxidant, and are therefore likely to lower oxidative stress induced by reactive oxygen species, inflammation and platelet aggregation, decrease lipid peroxidation and reduced low-density-lipoprotein (Bohm, 2012). All these factors play critical roles in development of atherosclerosis and CVD. The results of the present systematic review should encourage the development of well-designed randomised controlled trials, which may potentially have important implications in primary and secondary prevention of cardiovascular mortality and the global burden of disease.

2.4.3 Strengths and limitations

The strengths of this study include the consistency of findings across the studies which is reflected by low levels of heterogeneity surrounding the findings. In addition, the validity of the results is strengthened by a comprehensive search of the literature adhering to pre-specified criteria.

The potential limitations of these findings merit consideration. These are associated with the widely acknowledged limitations of self-reported intakes of lycopene according to food frequency questionnaire (FFQ) relying on dietary intakes assessed many years before the occurrence of the outcome, which allow for unavoidable changes in dietary habits. The uncertainty associated with the assessment of dietary exposure are unavoidable because of the lack of accurate information and are likely to affect the results (Prentice, 2010). Usual intakes and their distribution in a given population are difficult to ascertain given the inaccuracy of data collected by dietary methods. Sources of uncertainty associated with dietary methods such as FFQ, used by most studies reviewed in this paper, include measurement error such as portion sizes used to calculate amounts, frequency and duration of consumption (exposure), the levels and frequency of occurrence of compounds in foods, and the relationship between these variables and those unidentified. Recent studies on the evaluation of uncertainty in dietary intakes suggest that uncertainty in portion sizes may vary in relation with different food groups (Souverein et al., 2011).

Dietary underreporting is a conspicuous and pervasive problem to most dietary studies, thus affecting the relationship between dietary variables and health outcomes (Rosell et al., 2003). Uncertainty, results in the overestimation or underestimation of risk. Significant debate exist over the value of self-reported methods traditionally used in nutritional epidemiology (Dhurandhar et al., 2015; Satija et al., 2015). Therefore, growing interest in identifying objective biomarkers of food intake is a priority in the field of nutrition and health to overcome some of these limitations (Fave et al., 2011).

Another potential limitation might be the decision to pool findings from studies using dietary intakes and studies using plasma/serum concentrations of lycopene since high serum lycopene levels might not necessarily mean high lycopene/tomato intakes. In addition, tomatoes also contain other antioxidants such as vitamin C which has also been associated with potential cardio protective effects in some individuals (Ashor et al., 2016). Bioavailability and absorption of lycopene might be affected by the degree of processing and preparation as well as on the composition of the food or diet an individual consumes. However, in order to ensure clarity of the current findings results are presented as subgroups in the forest plots. In addition, most studies included in the systematic review originated from the USA and their applicability to other populations and other ethnic groups is uncertain. Although this study undertook meta-regression analysis, it was not able to conduct subgroup analysis to explore the impact of important factors such as ethnicity.

2.5 Conclusions

Current evidence from epidemiological studies support the hypothesis that lycopene and tomato may play a beneficial role in preventing CVD and early death. These results complement previous findings on intervention trials indicating that lycopene and tomato supplementation significantly reduced important CVD risk factors. The current systematic review revealed that there is a limited number of studies assessing cardiovascular disorders such as MI, CHF, AF and atherosclerosis; the associations between lycopene and tomato consumption and these disorders need to be investigated in future studies. Overall, these results should encourage the development of promising individualised nutritional strategies to tackle CVD risk factors and diseases.

2.6 Research Questions Raised from The Present Chapter

The work described in this chapter raised the following questions:

1. What is the interventional evidence on the effects of tomato supplementation on CVD risk factors?
2. What is the interventional evidence on the effects of lycopene supplementation on CVD risk factors?

These questions will be addressed in Chapter 3 by evaluating the evidence from randomised controlled trials reporting the effects of tomato/lycopene supplementation on CVD risk factors. We hypothesised that tomato/lycopene consumption improves cardiovascular risk factors.

Chapter 3 Tomato and Lycopene Supplementation and Cardiovascular Risk Factors: A Systematic Review and Meta-Analysis

This study was designed to answer the research questions in **section 2.6**.

This chapter is presented as originally published in: Atherosclerosis 2017; 257, 100-08.

3.1 Introduction

Globally, behavioural risk factors including a range of dietary risks, e.g. low intakes of fruit and vegetables, have the greatest potential to promote disease and impair human health (GBD 2015 Risk Factors Collaborators, 2016). A wealth of epidemiological evidence indicates that particularly cardiovascular health is strongly influenced by a healthy diet; fruit and vegetables are considered an important element of a cardioprotective diet (Mozaffarian et al., 2011).

The benefit of consuming fruit and vegetables is often ascribed to specific components of food. Recent systematic reviews of the literature indicate that supplementation with dietary nitrates, or foods rich in these compounds such as beetroot, have the potential to lower blood pressure (Siervo et al., 2013) and improve endothelial function (Lara et al., 2016), both regarded as early indicators of CVD. These benefits are valuable when developing effective nutritional strategies targeting specific key metabolic risk factors for the prevention and management of CVD.

Vegetables such as tomato are ubiquitous in most dietary patterns across the world and their contribution to health has been documented in longitudinal epidemiologic studies. High self-reported intakes of tomato and tomato products, and of dietary lycopene (a carotenoid compound) are associated with lower risk for CVD (Jacques et al., 2013; Sesso et al., 2003). Lycopene is one of the most potent antioxidants and the most predominant carotenoid in human plasma and it is assumed to be one of the active compounds on the health benefits of tomato (Mein et al., 2008). While the epidemiological evidence indicates a consistent association between tomato products and/or lycopene and lower CVD risk (Jacques et al., 2013; Sesso et al., 2003), the effects of nutritional interventions on tomato products and lycopene have been studied only recently and their efficacy on improving vascular function remains to be evaluated. The reduction in mortality associated with tomatoes consumption is due to the positive effects on a number of cardiovascular risk factors. Tomatoes consumption can potentially modify both traditional and well-established markers, such as blood pressure, lipids and glucose, and novel markers such as inflammatory factors, IL-6 and FMD. The evidence from human intervention trials on the efficacy of tomato products

or lycopene supplementations on cardiovascular risk biomarkers, for example blood lipids (total-, HDL-, LDL-cholesterol, triglycerides, oxLDL) and vascular function (FMD, Pulse wave velocity, PWV), has not been meta-analysed and systematically reviewed previously and a critical appraisal of the literature should be useful in testing this hypothesis. Here, the present study reported the findings of a systematic review and meta-analysis of the evidence from intervention trials investigating the efficacy of tomato products or lycopene supplementations on cardiovascular risk factors in adult human individuals. This systematic review was decided to focus particularly on blood lipids (total-, HDL-, LDL-cholesterol, triglycerides, oxLDL), vascular function (FMD, PWV), inflammatory factors (CRP, IL-6) and adhesion molecules (ICAM-1).

3.2 Method

This systematic review was undertaken in adherence with guidance from Cochrane (Higgins and Green, 2011) and the Centre for Reviews and Dissemination guidelines (Centre for Reviews and Dissemination, 2009) and is reported according to PRISMA guidelines (**Figure 3.1** and **Appendix B1**) (Moher et al., 2010). The protocol of this systematic review has been previously registered with PROSPERO, the International Prospective Register of Systematic Reviews (Registration number CRD42016042092).

In August 2016, Medline, Web of science, and Scopus were searched systematically from inception. Reference lists of identified publications were hand searched to identify other studies potentially eligible for inclusion.

The search strategies included the following terms 1) tomato; 2) lycopene; 3) trial/clinical trials; 4) vascular risk factors; 5) biomarkers; 6) vascular function; 7) endothelial function; 8) blood lipids. The systematic review was restricted to articles published in English.

Two researchers (HMC, JL) assessed articles independently for eligibility. The decision to include studies was hierarchical and was made initially on the basis of screening the studies' titles and abstracts, and if a decision was not reached at this stage, then the full-text of the article was evaluated to make such a judgement. The full text of the selected articles was independently assessed by the same researchers.

3.2.1 Inclusion/exclusion criteria

The selection of references during the search strategy and the data extraction was performed according to specific criteria which are delineated below:

Inclusion criteria included: 1) Study Design: intervention studies; 2) Subjects: adult subjects >18 years of age; 3) Interventions: nutritional/dietary interventions (tomato and tomato-based products or lycopene supplements versus a control or placebo group); 4) Outcomes: cardiovascular health-related outcome measures (described below). Exclusion criteria included 1) Study Design: non-interventional studies; 2) Subjects: subjects <18 years of age; 3) Interventions: interventions not involving tomato or lycopene, or interventions combined interventions in which the effects of tomato and lycopene cannot be singled out; 4) Outcomes: non-vascular outcome measures.

3.2.2 Data extraction

A standardised, pre-piloted form was used to extract data from the included studies for assessment of study quality and evidence synthesis. Extracted information included: study design (country of origin, randomisation, duration and length of follow-up, methods of analysis, completion rates); participant characteristics (population, settings of interventions, baseline characteristics); outcome measures (dietary and/or nutritional intake, BMI, cardiovascular biomarkers); intervention details (i.e. tomato or lycopene). Study quality was assessed using the Cochrane risk of bias tool (Cuevas-Ramos et al., 2013).

3.2.3 Outcome measures

The primary outcomes of this review were changes in cardiovascular risk factors after tomato or lycopene supplementation. Measures included blood lipids (Total-, LDL-, and HDL-cholesterol, Triglycerides, oxLDL), assessment of vascular function by FMD, PWV, resting SBP and DBP, inflammatory factors (CRP, IL-6) and adhesion molecules (ICAM-1).

3.2.4 Statistical analysis

The Review Manager (RevMan Version 5.1 for Windows Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011) was used to synthesise and meta-analyse results from the individual studies. Pooled results are mostly reported as weighted mean differences with 95% CI and with two-sided *P*-values. However, variables (such as IL-6 or CRP, and ICAM) were reported on different scales, and standardised mean differences (SMD) were therefore used as a summary statistic for comparing effect sizes across studies.

A random effects model accounting for inter-study variation was used. Multiple dietary intervention arms from three studies were included in the meta-analysis. Following previous guidance (Higgins and Green, 2011), excessive weightings from "double counts" arising from the "shared" group (in this case, the control group) were controlled by splitting the sample size of the shared group into approximately equal smaller groups for the comparisons. In this analysis, reviewers sought to extract and analyse adjusted results from multivariate models, if reported in the studies.

Heterogeneity was evaluated using the I^2 statistic (Centre for Reviews and Dissemination, 2009; Higgins and Green, 2011). Levels of heterogeneity are commonly regarded as high when I^2 values are >50%. Publication bias was appraised by visually inspecting the funnel plot, and supplemented with calculations of the Egger's regression test (Egger et al., 1997). Quality of studies was assessed using the Jadad system (Jadad et al., 1996).

3.3 Results

The searches yielded 1189 publications after de-duplication and results of the screening process are described in **Figure 3.1**. Twenty-two publications that met the inclusion criteria were included in the present systematic review and meta-analysis (**Table 3.1**) (Biddle et al., 2015; Blum et al., 2006; Blum et al., 2007; Burton-Freeman et al., 2012; Collins et al., 2004; Cuevas-Ramos et al., 2013; Devaraj et al., 2008; Engelhard et al., 2006; Gajendragadkar et al., 2014; Ghavipour et al., 2013; Kim et al., 2011; Markovits et al., 2009; Misra et al., 2006; Paran et al., 2009; Ried et al., 2009; Samaras et al., 2014; Silaste et al., 2007; Stangl et al., 2011; Thies et al., 2012; Tsitsimpikou et al., 2014; Upritchard et al., 2000; Xaplanteris et al., 2012), and 21 of those publications were included in the meta-analysis (Biddle et al., 2015; Blum et al., 2006; Blum et al., 2007; Burton-Freeman et al., 2012; Collins et al., 2004; Cuevas-Ramos et al., 2013; Devaraj et al., 2008; Engelhard et al., 2006; Gajendragadkar et al., 2014; Ghavipour et al., 2013; Kim et al., 2011; Markovits et al., 2009; Misra et al., 2006; Paran et al., 2009; Ried et al., 2009; Samaras et al., 2014; Silaste et al., 2007; Stangl et al., 2011; Thies et al., 2012; Tsitsimpikou et al., 2014; Xaplanteris et al., 2012).

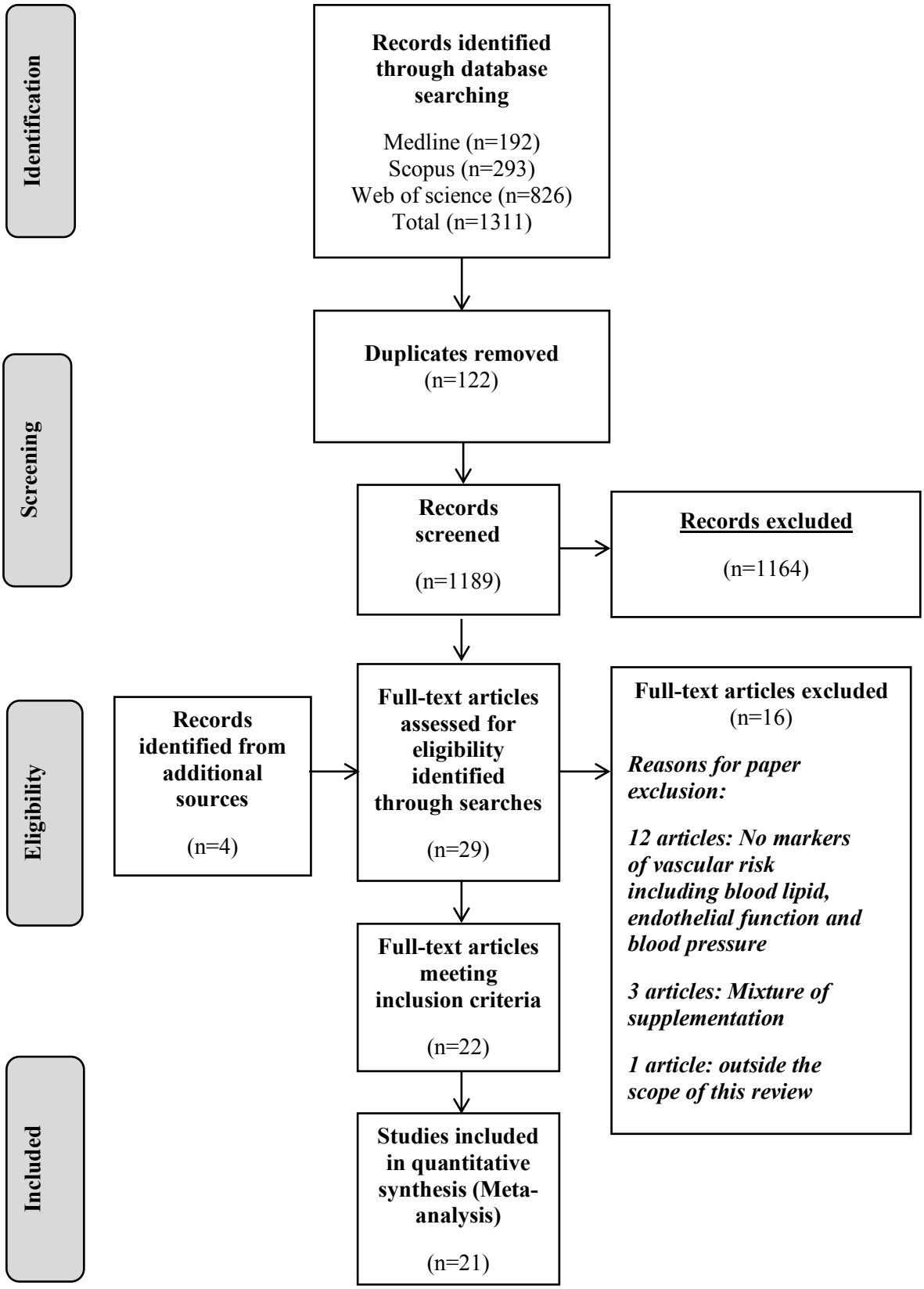


Figure 3.1 PRISMA flow diagram of selection of studies on lycopene or tomato consumption and vascular risk factors.

Table 3.1 Characteristics of studies included in systematic review.

Ref (Country of origin)	Health status	Sample/Sex	Age (SD)	BMI	Study design	Duration	Intervention (Active component)	Dose	Control group	Outcomes studied
Biddle et al., 2015 (USA)	Patients with Heart Failure	Intervention group (M=8, F=10); Control group (M=15, F=7)	Intervention group (65±11); Control group (65±9)	M=30.7 F=31.9	Parallel, RCT	30 d	Usual diet supplemented with tomato Juice	11.5 ounces of V8 juice per day	Usual diet without tomato juice (no placebo)	CRP
Blum et al., 2006 (Israel)	Healthy	Tomato (M=16, F=34); Control (M=16, F=32)	45.5±14.1	N/A	Parallel, controlled	4 wk	Usual diet supplement with tomato	300 g tomato products (including tomato sauce, tomato juice, fresh tomatoes, or soup) per day	Usual diet without tomato (no placebo)	TC, TG, HDL, LDL

(**Table 3.1** continues on next page)

Ref (Country of origin)	Health status	Sample/Sex	Age (SD)	BMI	Study design	Duration	Intervention (Active component)	Dose	Control group	Outcomes studied
Blum et al., 2007 (Israel)	Healthy overweight	Intervention group (M=17, F=33); Control group (M=18, F=35)	Intervention group (45.5±14); Control group (45.1±13.5)	N/A	Parallel, controlled	4 wk	Usual diet supplement with tomato	300 g tomato products (including tomato sauce, tomato juice, fresh tomatoes, or soup) per day	Usual diet without tomato (no placebo)	CRP, ICAM-1
Burton-Freeman et al., 2012 (USA)	Healthy	M=13; F=12	27±8	22	Cross-over, RCT	360 min	Usual diet with tomato-containing meal	Around 85 g of tomato paste	Usual diet without tomato-containing meal	TG, oxLDL, FMD, IL-6
Collins et al., 2004 (USA)	Healthy	M=5, F=5	M (49 (43-68)); F (51 (35-63))	M=26.3 F=29.1	Cross-over, RCT	3 wk	Low lycopene diet supplemented tomato juice	18.4 mg lycopene per day	Low lycopene diet	TC, TG, HDL

(**Table 3.1** continues on next page)

Ref (Country of origin)	Health status	Sample/Sex	Age (SD)	BMI	Study design	Duration	Intervention (Active component)	Dose	Control group	Outcomes studied
Cuevas-Ramos et al., 2013 (Mexico)	Healthy	Tomato (M=6, F=20); Control (M=5, F=19)	42±15.5	27.1	Parallel, RCT, single-blind	4 wk	Usual diet supplemented with raw tomato	300 g of raw tomato per day	Usual diet with 300 g of raw cucumber	TC, TG, HDL, LDL
Devaraj et al., 2008 (USA)	Healthy	6.5 mg lycopene (M=4, F=17); 15 mg lycopene (M=4, F=13); 30 mg lycopene (M=7, F=14); Placebo (M=4, F=14)	6.5 mg lycopene (51.1±5.9); 15 mg lycopene (49.8±8.2); 30 mg lycopene (51.2±9.7); Placebo (49.9±15.8)	6.5 mg lycopene=29.6; 15 mg lycopene=25.4; 30 mg lycopene=26.4; Placebo=28.1	Parallel, RCT, doubled-blind	8 wk	Lycopene restricted diet with supplemented lycopene capsules	6.5, 15, or 30 mg per day	Placebo capsules (Lycopene restricted diet)	TC, TG, HDL, LDL
Engelhard et al., 2006 (Israel)	Hypertension without concomitant diseases	M=18, F=12	48	29.5	Non-randomised single-blind placebo-controlled trial	8 wk	Usual diet supplemented with lycopene capsule (Lyc-O-Mato)	250 mg of tomato extract capsule containing 15 mg lycopene per day	Usual diet with identical-looking placebo capsule	TC, TG, HDL, LDL, SBP, DBP

(**Table 3.1** continues on next page)

Ref (Country of origin)	Health status	Sample/Sex	Age (SD)	BMI	Study design	Duration	Intervention (Active component)	Dose	Control group	Outcomes studied
Gajendragadkar et al., 2014 (UK)	Healthy and CVD patients	Healthy (7 mg M=23, F=1; control M=10, F=2); CVD patients (7 mg M=15, F=9; Control (M=10, F=2)	Healthy (7 mg=61±13, control=68±5); CVD patients (7 mg =67±6, control=68±5)	Healthy (7 mg=25.2; control=26.7); CVD patients (7 mg 28.6; control=28.4)	Parallel, RCT, double-blind	8 wk	Usual diet with lycopene capsule	7 mg lycopene daily per day	Usual diet with identical-looking placebo capsule	HDL, LDL, oxLDL, SBP, DBP, PWV, CRP, IL-6
Ghavipour et al., 2013 (Iran)	Obese and overweight	Intervention group (F=53); Control group (F=51)	Intervention group (23.3±0.5 (SEM)); Control group (23.2±0.4 (SEM))	Intervention group=28.2; Control group=28.3	Parallel, RCT	20 d	Usual diet supplemented with tomato juice	330 mL tomato juice per day	Usual diet with water	CRP, IL-6
Kim et al., 2011 (Korea)	Healthy	6 mg lycopene (n=41); 15 mg lycopene (n=37); Control (n=38)	6 mg lycopene (34.8±1.28); 15 mg lycopene (34.7±1.23); Control (33.5±1.13)	6 mg lycopene=25.3; 15 mg lycopene=23.9; Control=24.9	Parallel, RCT, double-blind	8 wk	Usual diet supplemented with lycopene capsules (Lyc-O-Mato)	6 mg or 15 mg lycopene per day	Usual diet with placebo containing soybean oil capsules	SBP, CRP, ICAM-1

(**Table 3.1** continues on next page)

Ref (Country of origin)	Health status	Sample/Sex	Age (SD)	BMI	Study design	Duration	Intervention (Active component)	Dose	Control group	Outcomes studied
Markovits et al., 2009 (Israel)	Obesity and healthy	Obesity (M=4, F=4); Healthy (M=4, F=4)	47±6 (SEM)	Obesity =37.5; Healthy=21.6	Single treatment	8 wk	Usual diet supplemented with lycopene capsules (Lyc-O-Mato)	30 mg lycopene per day	Usual diet with placebo capsules, made of edible soya oil	CRP, IL-6
Misra et al., 2006 (India)	Healthy postmenopausal women	Lycopene supplement (F=20); Hormone replacement therapy (HRT) (F=21)	46	Lycopene=25.8 HRT=25.3	Parallel, RCT	6 months	Lycopene capsule (LycoRed)	2 Lycopene capsule containing 2 mg of lycopene per day	Hormone replacement therapy (estradiol valerate 2 mg and norethisterone acetate 1 mg)	TC, TG, HDL, LDL
Paran et al., 2009 (Israel)	Hypertension patient	M=26, F=24	61.4±8.9	N/A	Cross-over, RCT	6 wk	Usual diet supplemented with lycopene capsules (Lyc-O-Mato)	250 mg of tomato extract capsule containing 15 mg lycopene per day	Usual diet with identical-looking placebo capsule	SBP, DBP
Ried et al., 2009 (Australia)	Pre-hypertensive	Lycopene (n=15); Control (n=10)	Tomato extract (51.2±12.1); Control (57.9±13.4)	N/A	Parallel, RCT	8 wk	Usual diet supplemented with lycopene capsules (Lyc-O-Mato)	Tomato extract capsule containing 15 mg lycopene per day	Usual diet supplemented with identical-looking placebo capsule containing mainly soy oil	SBP, DBP

(**Table 3.1** continues on next page)

Ref (Country of origin)	Health status	Sample/Sex	Age (SD)	BMI	Study design	Duration	Intervention (Active component)	Dose	Control group	Outcomes studied
Samaras et al., 2014 (Greece)	Ultra-marathon runners	Tomato Juice (n=15); Control (n=12)	Tomato Juice (44.9±8.53); Control (46.6±15.3)	Tomato Juice=24.1; Control=24.3	Parallel, controlled	8 wk	Tomato juice	Amount required to match subject's usual carbohydrate supplementation	Continued any usual supplementation consumption	TC, TG, HDL, LDL, FMD
Silaste et al., 2007 (Finland)	Healthy	M=5; F=16	30	23.5	Sequential 3 weeks low tomato, 3 weeks high tomato	6 wk	Usual diet supplemented with tomato juice	400 ml tomato juice containing 27 mg lycopene)	Usual diet without tomato products	TC, LDL
Stangl et al., 2011 (Germany)	Healthy postmenopausal women	F=19	58.9±6.3	25	Cross-over, RCT	7 d	Tomato free diet supplemented with buttered roll with tomato puree	70 g tomato puree	Tomato free diet supplemented with buttered roll without tomato puree	FMD
Thies et al., 2012 (UK)	Moderately overweight	High tomato (M=35, F=46); Lycopene (M=28, F=40); Control (M=30, F=46)	High tomato (51±0.7 (SEM)); Lycopene (51.1±0.9 (SEM); Control (51±0.7 (SEM))	High tomato=26.4; Lycopene=26.7; Control=26.8	Parallel, RCT, single-blind	12 wk	1, High tomato based diet or 2, low tomato diet supplemented with lycopene capsule	1, High tomato diet were above the minimum target of 7 mg per day 2, Low tomato diet with lycopene capsules containing 10 mg lycopene	Low tomato diet (low in tomato-based foods)	TC, TG, HDL, LDL, oxLDL, SBP, DBP, PWV, CRP, IL-6, ICAM-1,

(**Table 3.1** continues on next page)

Ref (Country of origin)	Health status	Sample/Sex	Age (SD)	BMI	Study design	Duration	Intervention (Active component)	Dose	Control group	Outcomes studied
Tsitsimpikou et al., 2014 (Greece)	Patients with metabolic syndrome	Tomato (M=13, F=2); Control (M=11, F=1)	Tomato (53.5±9.8); Control (56.6±10.2)	N/A	Parallel, controlled	8 wk	Usual diet supplemented with tomato juice	Tomato juice with 2.51 mg lycopene per 100 ml	Usual diet without tomato juice	TC, TG, HDL, LDL, IL-6
Upritchard et al., 2000 (New Zealand)	Patients with Type 2 diabetes	Tomato juice (M=10, F=5); Placebo (M=10, F=3)	Tomato (63±8); Placebo (60±6)	Tomato=30.9; Control=31.8	Parallel, RCT	4 wk	Usual diet supplemented with tomato juice	250 mL of tomato juice twice a day	Usual diet with gelatin placebo capsule containing pharmaceutical starch	CRP
Xaplanteris et al., 2012 (Greece)	Healthy	M=8, F=11	39±13	24.8	Cross-over, RCT, single-blinded	2 wk	usual diet supplemented with tomato paste	70 g of tomato paste containing 33.3 mg lycopene per day	Usual diet without tomato paste	FMD

Ref, Reference; M, Male; F, Female; RCT, randomised controlled trial; wk, weeks; BMI, Body mass index; CRP, C-reactive protein; TC, Total cholesterol; TG, Triglyceride; HDL, High density lipoprotein; LDL, Low density lipoprotein; ICAM-1, Intercellular adhesion molecule 1; oxLDL, Oxidised low-density lipoproteins; FMD, Flow-mediated dilation; IL-6, Interleukin 6; SBP, Systolic blood pressure, DBP, Diastolic blood pressure; PWV, Pulse wave velocity

3.3.1 Study characteristics

Fifteen studies used RCT design while seven were controlled trials using a non-randomised design. These 22 studies originated from the USA (n=4), UK (n=2), Greece (n=3), Israel (n=5), and single studies from Finland, Germany, Korea, Mexico, Iran, India, Australia and New Zealand.

The pooled study population meta-analysed included 1197 participants who were followed-up for 4 months on average (range from 1 day to 6 months). The mean ages of the samples in these studies ranged from 27 to 68 years. Three studies recruited women only (Ghavipour et al., 2013; Misra et al., 2006; Stangl et al., 2011). Mean BMI in these studies was 26.8 kg/m^2 (**Table 3.1**). Twelve of the included studies assessed tomato products alone as the intervention agent (Biddle et al., 2015; Blum et al., 2006; Blum et al., 2007; Burton-Freeman et al., 2012; Collins et al., 2004; Cuevas-Ramos et al., 2013; Ghavipour et al., 2013; Samaras et al., 2014; Silaste et al., 2007; Stangl et al., 2011; Tsitsimpikou et al., 2014; Xaplanteris et al., 2012), eight studies used lycopene alone (Devaraj et al., 2008; Engelhard et al., 2006; Gajendragadkar et al., 2014; Kim et al., 2011; Markovits et al., 2009; Misra et al., 2006; Paran et al., 2009; Ried et al., 2009) and one study used both tomato products and lycopene (Thies et al., 2012). The dose of tomato-products ranged from 70 to 400g/d, and lycopene dose ranged from 4 to 30 mg/d.

Approximately half (n=13) of the reports included in the systematic review came from studies involving healthy participants (**Table 3.1**). The remaining studies reported the recruitment of participants with underlying cardiometabolic disorders (n=11), such as metabolic syndrome (1 study), CVD (1 study), heart failure (1 study), Type 2 diabetes (1 study), overweight (3 studies), obesity (2 studies), hypertension (2 studies) and prehypertension (1 study).

3.3.2 Meta-analysis of studies supplementing tomato

3.3.2.1 LDL-cholesterol

Six studies, including 401 participants, evaluated the impact of tomato supplementation on LDL-cholesterol. Overall, tomato supplementation significantly reduced LDL-cholesterol by 0.22 mmol/L (95% CI -0.37 to -0.06, p=0.006). Heterogeneity levels assessed by the I^2 test were low at 0% (**Figure 3.2**).

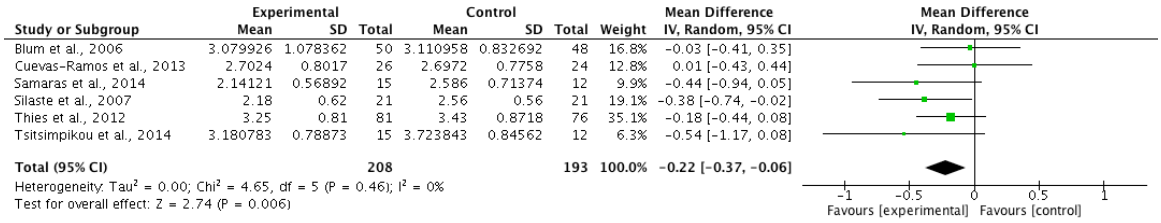


Figure 3.2 Meta-analysis of effect of interventions supplementing tomato on LDL-cholesterol (mmol/L).

3.3.2.2 FMD

Six studies, including 233 participants evaluated the impact of tomato supplementation on FMD. Three studies measured FMD acutely (≤24hours) and three studies evaluated FMD in the short-term (≥1 week). Overall, short-term tomato supplementation significantly increased FMD by 2.53% (95% CI 0.56 to 4.50, *p*=0.01). Heterogeneity levels assessed by the I^2 test were low at 0%; however acute studies did not show any improvements (1.46%; 95% CI -0.33 to 3.26, *p*=0.11) (**Figure 3.3**).

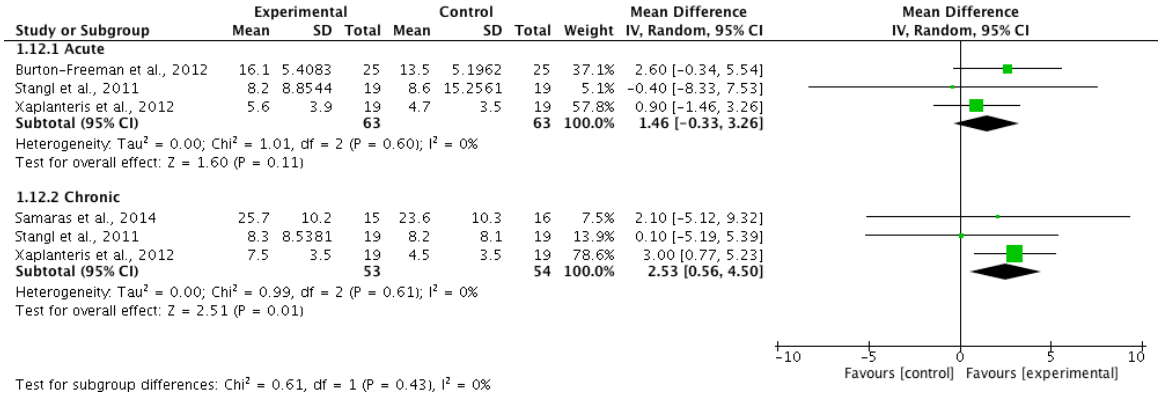


Figure 3.3 Meta-analysis of effect of interventions supplementing tomato on FMD (%).

3.3.2.3 IL-6

Three studies, including 288 participants, reported IL-6 showing tomato supplementation significantly reduced by 0.25 pg/mL (95% CI -0.49 to -0.02, *p*=0.03). Heterogeneity levels assessed by the I^2 test were low at 1% (**Figure 3.4**).

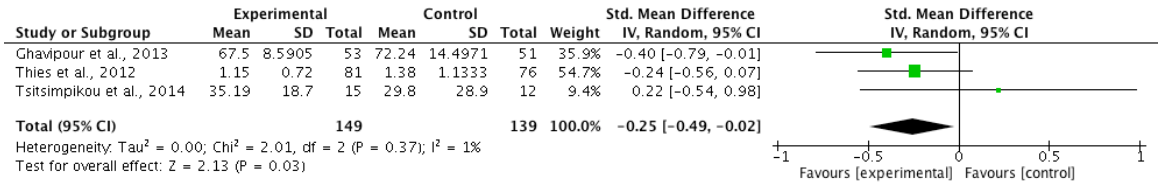


Figure 3.4 Meta-analysis of effect of interventions supplementing tomato on IL-6 (pg/mL).

There was only weak evidence that tomato interventions had an effect on other cardiovascular factors including SBP, DBP, Total- or HDL-cholesterol, triglycerides, oxLDL, PWV, or CRP and ICAM-1.

3.3.2.4 Resting blood pressure

Three studies/arms, including 207 participants, reported resting SBP and DBP which showed no differences between interventions and controls, mean is -0.84mmHg (95% CI -5.10 to 3.42, *p*=0.70) (**Figure 3.5**) and 1.12mmHg (95% CI -1.34 to 3.59, *p*=0.37) (**Figure 3.6**) respectively. Both heterogeneity levels assessed by the I^2 test were low at 0%.

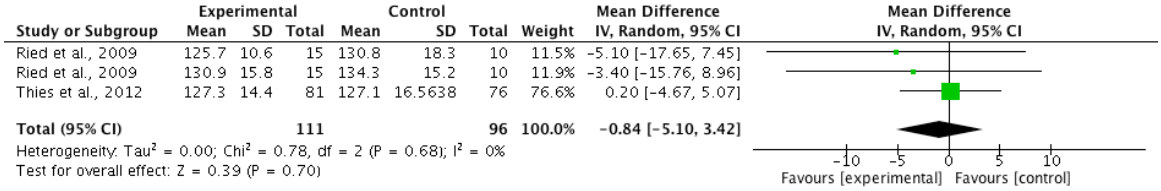


Figure 3.5 Meta-analysis of effect of interventions supplementing tomato on resting SBP (mmHg).

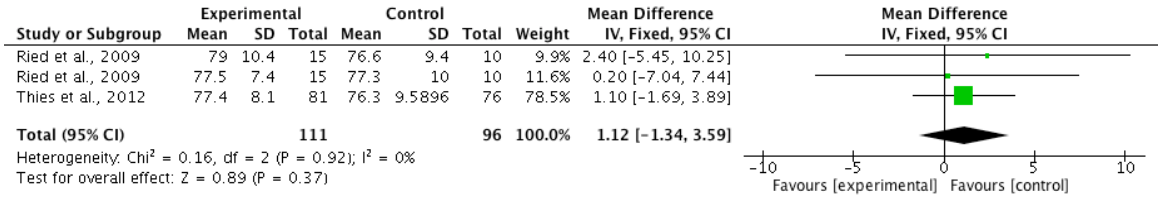


Figure 3.6 Meta-analysis of effect of interventions supplementing tomato on resting DBP (mmHg).

3.3.2.5 Total-cholesterol

Nine studies/arms, including 421 participants, reported total cholesterol which showed no differences between interventions and controls, mean is -0.01mmol/L (95% CI -0.16 to -0.13, *p*=0.87). Heterogeneity levels assessed by the I^2 test were low at 0% (**Figure 3.7**).

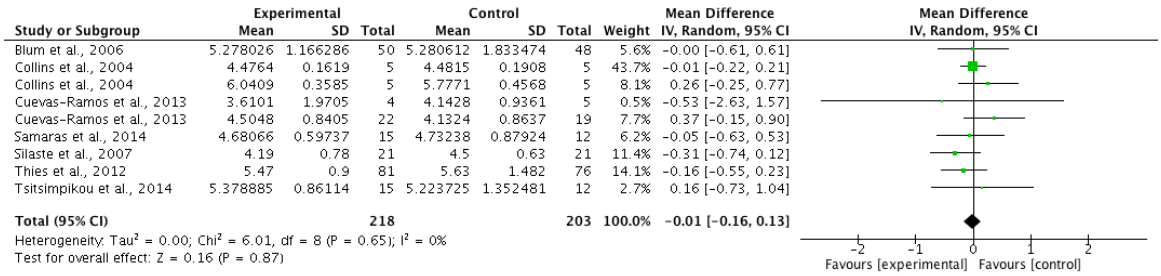


Figure 3.7 Meta-analysis of effect of interventions supplementing tomato on total-cholesterol (mmol/L).

3.3.2.7 Triglycerides

Seven studies/arms, including 379 participants, reported triglycerides which showed no differences between interventions and controls, mean is 0.05mmol/L (95% CI -0.07 to 0.17, p=0.41) (**Figure 3.8**). Heterogeneity levels assessed by the I^2 test were low at 10%.

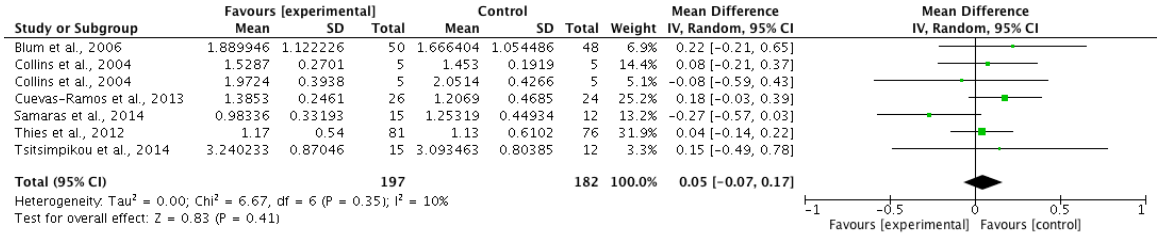


Figure 3.8 Meta-analysis of effect of interventions supplementing tomato on triglyceride (mmol/L).

3.3.2.8 HDL-cholesterol

Eight studies/arms, including 379 participants, reported HDL-cholesterol which showed no differences between interventions and controls, mean is 0.11mmol/L (95% CI -0.01 to 0.23, p=0.08). Heterogeneity levels assessed by the I^2 test were high at 80% (**Figure 3.9**).

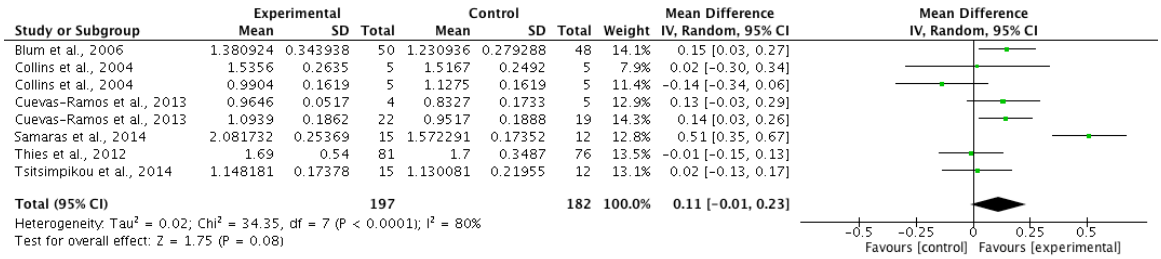


Figure 3.9 Meta-analysis of effect of interventions supplementing tomato on HDL-cholesterol (mmol/L).

3.3.2.9 OxLDL

One study, including 157 participants, reported oxLDL which showed no differences between interventions and controls, mean is 0.80mmol/L (95% CI -9.04 to 10.64, p=0.87) (**Figure 3.10**).

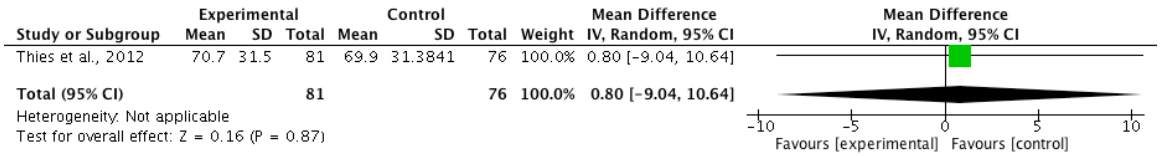


Figure 3.10 Meta-analysis of effect of interventions supplementing tomato on oxLDL (mmol/L).

3.3.2.11 PWV

One study, including 157 participants, reported PWV which showed no differences between interventions and controls, mean is 0.93m/s (95% CI -0.20 to 2.06, *p*=0.11) (**Figure 3.11**).

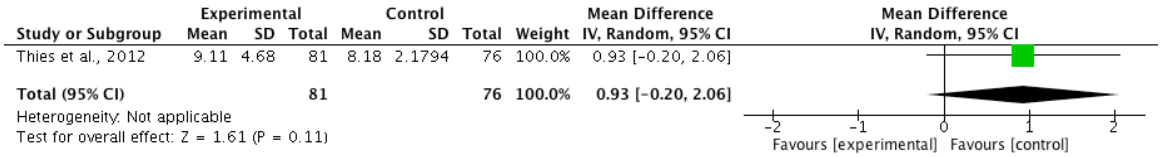


Figure 3.11 Meta-analysis of effect of interventions supplementing tomato on PWV (m/s).

3.3.2.12 CRP

Four studies, including 404 participants, reported CRP which showed no differences between interventions and controls, mean is -0.14mg/L (95% CI -0.34 to 0.05, *p*=0.15). Heterogeneity levels assessed by the I^2 test were low at 0% (**Figure 3.12**).

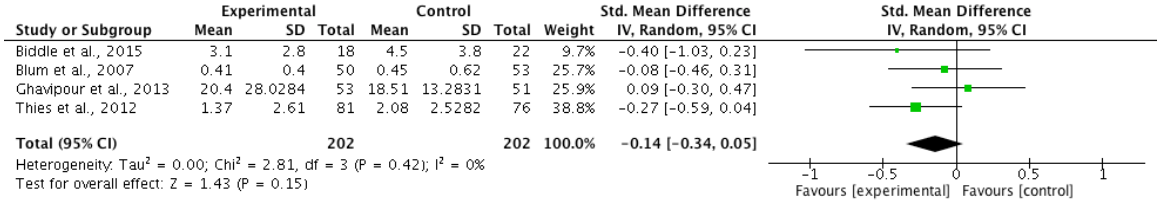


Figure 3.12 Meta-analysis of effect of interventions supplementing tomato on CRP (mg/L).

3.3.2.13 ICAM-1

Two studies, including 260 participants, reported ICAM-1 which showed no differences between interventions and controls, mean is 0.20pg/mL (95% CI -0.47 to 0.88, *p*=0.56). Heterogeneity levels assessed by the I^2 test were high at 86% (**Figure 3.13**).

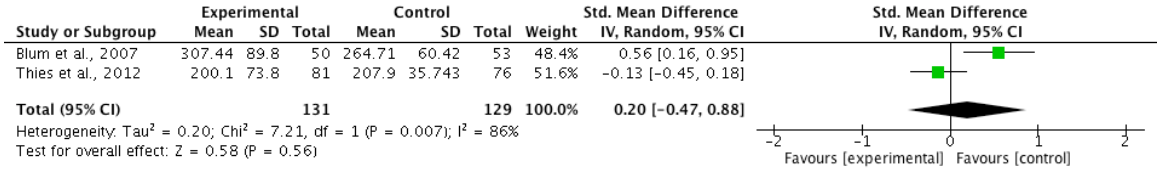


Figure 3.13 Meta-analysis of effect of interventions supplementing tomato on ICAM-1 (pg/mL).

3.3.3 Meta-analysis of studies supplementing lycopene

3.3.3.1 Resting SBP

Seven studies/arms, including 492 participants, evaluated the impact of lycopene supplementation on resting SBP. Overall lycopene supplementation significantly reduced SBP by 5.66 mmHg (95% CI -9.31 to -2.01, *p*=0.002). Heterogeneity levels assessed by the I^2 test were high at 65% (**Figure 3.14**).

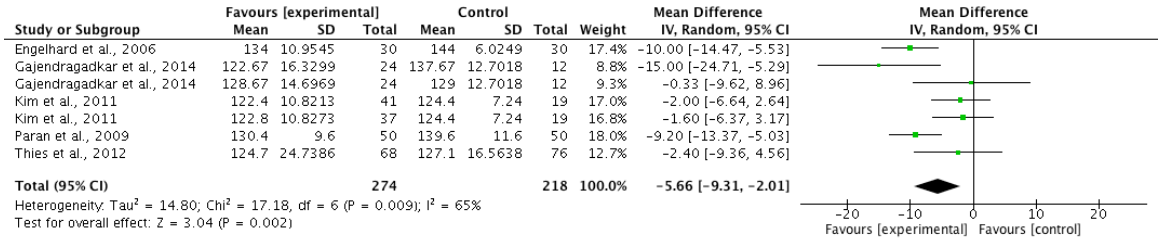


Figure 3.14 Meta-analysis of effect of interventions supplementing lycopene on systolic blood pressure (mmHg).

There was only weak evidence that lycopene interventions had an effect on other cardiovascular factors including DBP, Total- LDL- or HDL-cholesterol, triglycerides, oxLDL, PWV, CRP, IL-6 or ICAM-1.

3.3.3.2 Total-cholesterol

Six studies/arms, including 363 participants, reported total-cholesterol which showed no differences between interventions and controls, mean is 0.14mmol/L (95% CI -0.06 to 0.34, *p*=0.16). Heterogeneity levels assessed by the I^2 test were low at 0% (**Figure 3.15**).

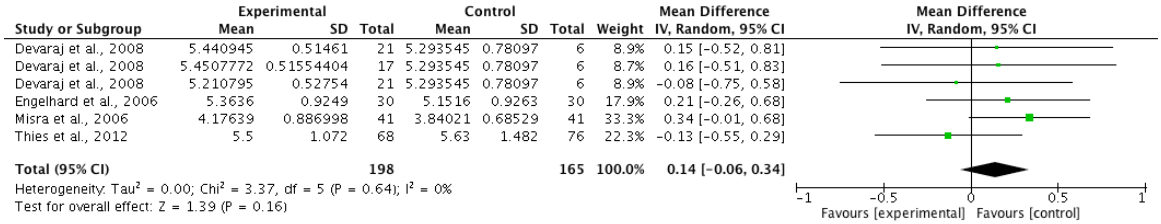


Figure 3.15 Meta-analysis of effect of interventions supplementing lycopene on total-cholesterol (mmol/L).

3.3.3.3 Triglyceride

Six studies/arms, including 363 participants, reported triglyceride which showed no differences between interventions and controls, mean is -0.05mmol/L (95% CI -0.14 to 0.04, *p*=0.24). Heterogeneity levels assessed by the I^2 test were low at 0% (**Figure 3.16**).

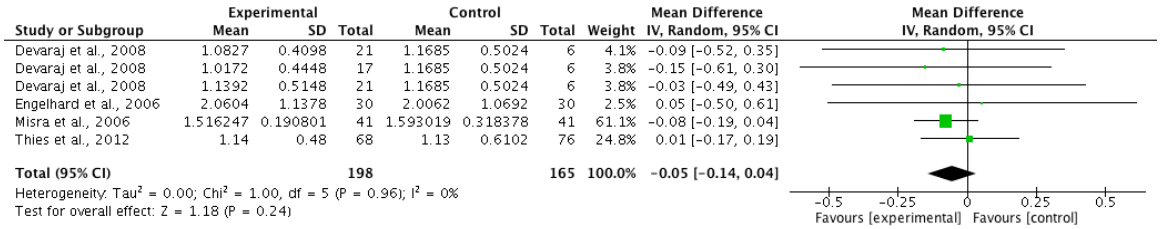


Figure 3.16 Meta-analysis of effect of interventions supplementing lycopene on triglyceride (mmol/L).

3.3.3.4 HDL-cholesterol

Eight studies/arms, including 435 participants, reported HDL-cholesterol which showed no differences between interventions and controls, mean is -0.02mmol/L (95% CI -0.12 to 0.09, *p*=0.76). Heterogeneity levels assessed by the I^2 test were high at 64% (**Figure 3.17**).

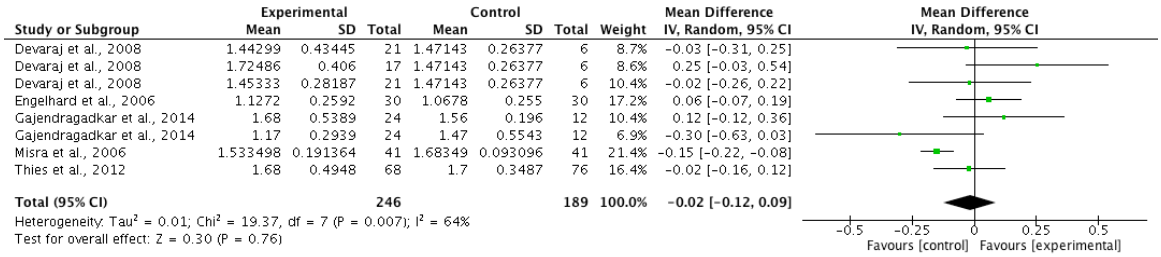


Figure 3.17 Meta-analysis of effect of interventions supplementing lycopene on HDL-cholesterol (mmol/L).

3.3.3.5 LDL-cholesterol

Eight studies/arms, including 441 participants, reported LDL-cholesterol which showed no differences between interventions and controls, mean is 0.04mmol/L (95% CI -0.08 to 0.16, *p*=0.54). Heterogeneity levels assessed by the I^2 test were low at 0% (**Figure 3.18**).

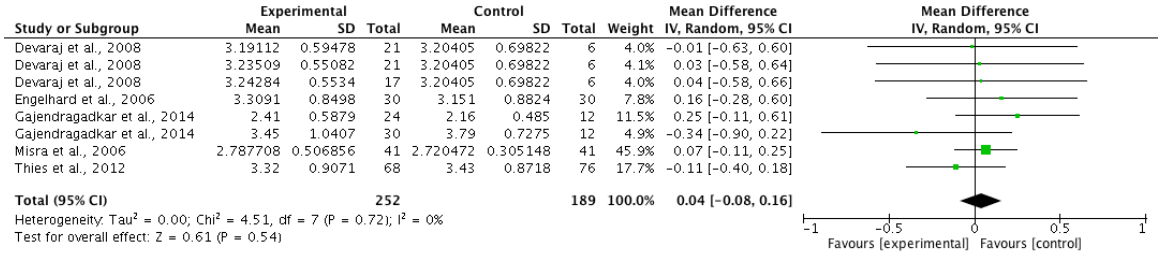


Figure 3.18 Meta-analysis of effect of interventions supplementing lycopene on LDL-cholesterol (mmol/L).

3.3.3.6 OxLDL

Three studies/arms, including 216 participants, reported OxLDL which showed no differences between interventions and controls, mean is 5.20 mmol/L (95% CI -4.11 to 14.51, *p*=0.27). Heterogeneity levels assessed by the I^2 test were low at 0% (**Figure 3.19**).

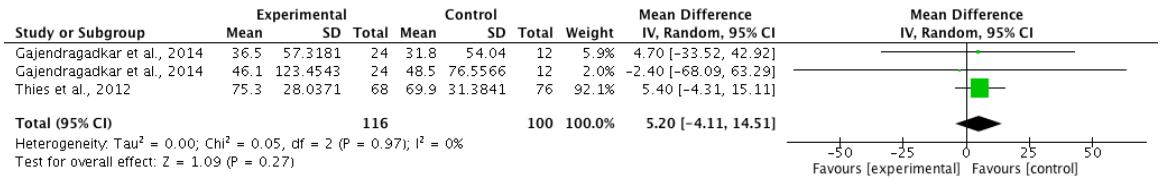


Figure 3.19 Meta-analysis of effect of interventions supplementing lycopene on OxLDL (mmol/L).

3.3.3.7 DBP

Five studies/arms, including 376 participants, reported DBP which showed no differences between interventions and controls, mean is -1.45mmHg (95% CI -4.02 to 1.13, *p*=0.27). Heterogeneity levels assessed by the I^2 test were high at 53% (**Figure 3.20**).

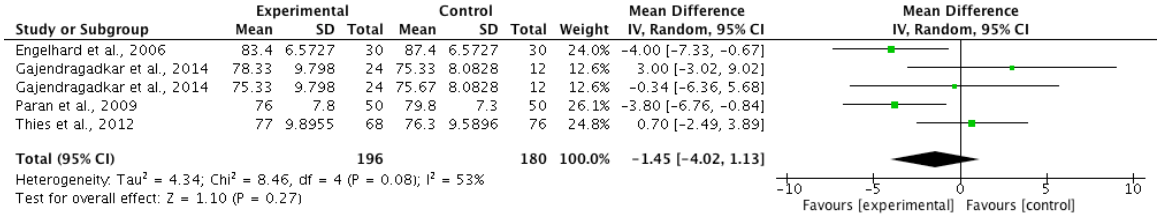


Figure 3.20 Meta-analysis of effect of interventions supplementing lycopene on DBP (mmHg).

3.3.3.8 PWV

Three studies/arms, including 216 participants, reported PWV which showed no differences between interventions and controls, mean is 0.39m/s (95% CI -0.20 to 0.97, p=0.19). Heterogeneity levels assessed by the I^2 test were low at 0% (**Figure 3.21**).

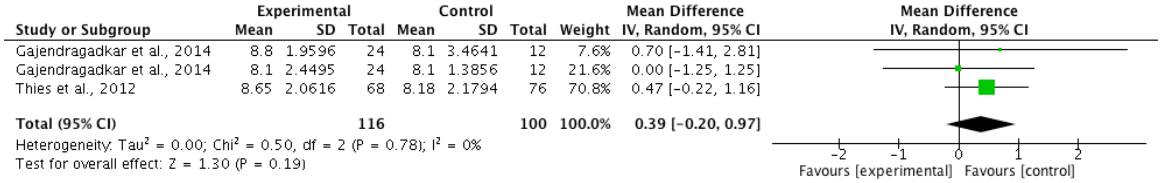


Figure 3.21 Meta-analysis of effect of interventions supplementing lycopene on PWV (m/s).

3.3.3.9 CRP

Six studies/arms, including 344 participants, reported CRP which showed no differences between interventions and controls, mean is -0.03mg/L (95% CI -0.28 to 0.23, p=0.83). Heterogeneity levels assessed by the I^2 test were low at 17% (**Figure 3.22**).

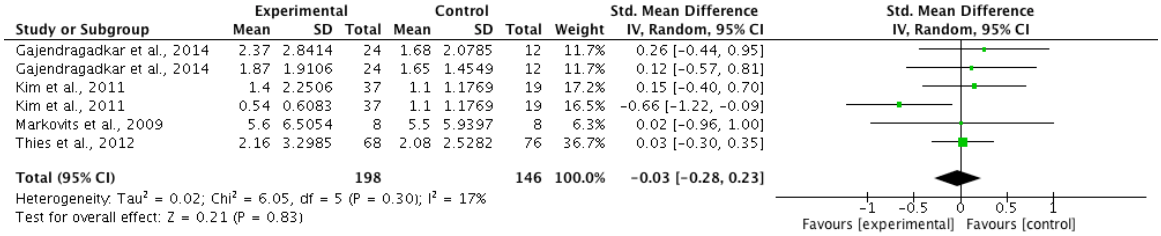


Figure 3.22 Meta-analysis of effect of interventions supplementing lycopene on CRP (mg/L).

3.3.3.10 IL-6

Four studies/arms, including 232 participants, reported IL-6 which showed no differences between interventions and controls, mean is -0.02pg/mL (95% CI -0.42 to 0.37, p=0.90). Heterogeneity levels assessed by the I^2 test were low at 0% (**Figure 3.23**).

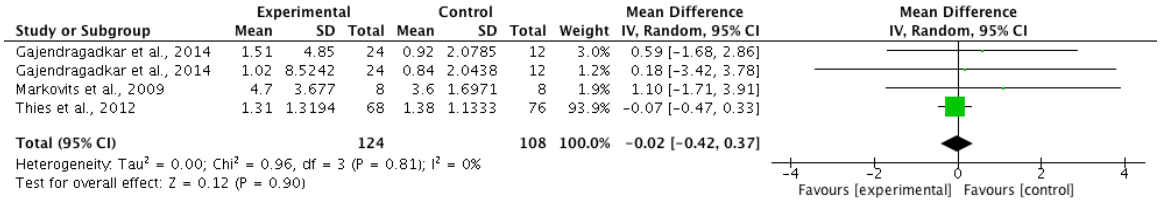


Figure 3.23 Meta-analysis of effect of interventions supplementing lycopene on IL-6 (pg/mL).

3.3.3.11 ICAM-1

Three studies/arms, including 260 participants, reported ICAM-1 which showed no differences between interventions and controls, mean is -0.05pg/ml (95% CI -0.30 to 0.20, p=0.71). Heterogeneity levels assessed by the I^2 test were low at 0% (**Figure 3.24**).

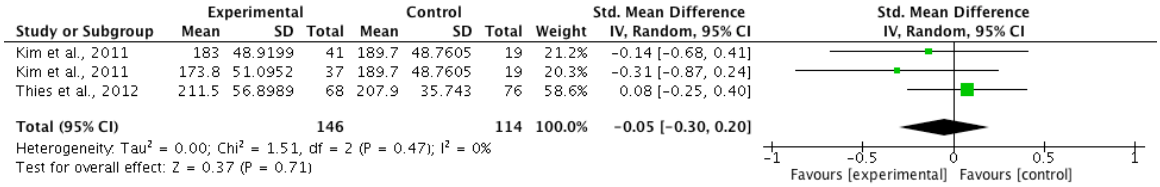


Figure 3.24 Meta-analysis of effect of interventions supplementing lycopene on ICAM-1 (pg/ml).

3.3.3.12 Subgroup analysis

Subgroup analysis according to country of origin was undertaken. Studies originating from western countries with relatively similar dietary patterns such as the UK, USA, Australia, Germany, and Finland were combined and compared with studies originating from countries with non-western dietary patterns such as Mexico, Israel, Iran, India, Korea, and Greece. Results showed that tomato supplementation significantly increased HDL-cholesterol by 0.19mmol/L (95% CI 0.04 to 0.33, p=0.01) in studies from non-western dietary patterns. Heterogeneity levels assessed by the I^2 test were significant at 81% (**Figure 3.25**).

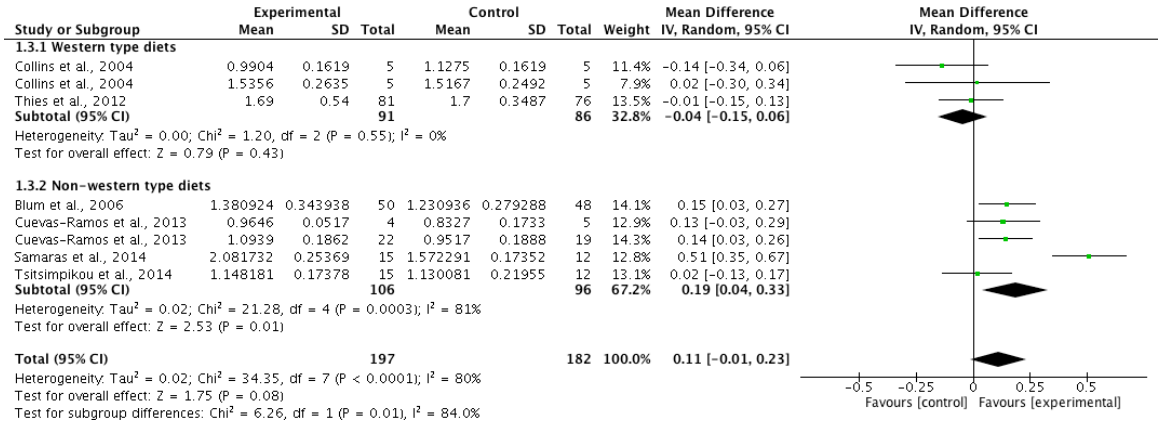


Figure 3.25 Meta-analysis of effect of supplementing tomato on HDL-cholesterol (mmol/L) according to two dietary pattern groups.

In addition, tomato supplementation significantly reduced LDL-cholesterol by -0.25mmol/L (95% CI -4.60 to -0.04, p=0.02) in studies from western dietary patterns. Heterogeneity levels assessed by the I^2 test were low at 0% (**Figure 3.26**).

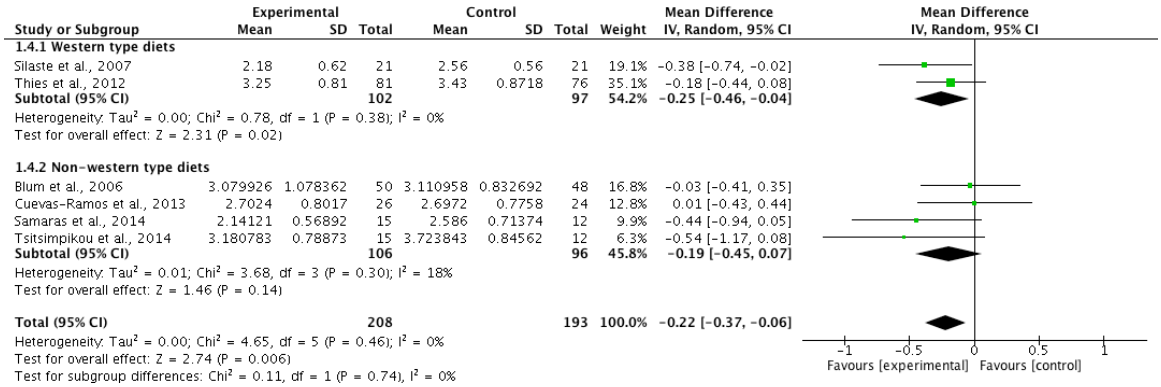


Figure 3.26 Meta-analysis of effect of supplementing tomato on LDL-cholesterol (mmol/L) according to two dietary pattern groups.

Lycopene supplementation significantly reduced SBP and DBP by -5.78mmHg (95% CI -10.31 to -1.38, p=0.01) (**Figure 3.27**) and -3.89mmHg (95% CI -6.10 to -1.68, p=0.0006) respectively in studies from non-western countries (**Figure 3.28**). Heterogeneity levels of SBP and DBP subgroup from non-western countries assessed by the I^2 test were 74% and 0% respectively (**Figure 3.27** and **Figure 3.28**).

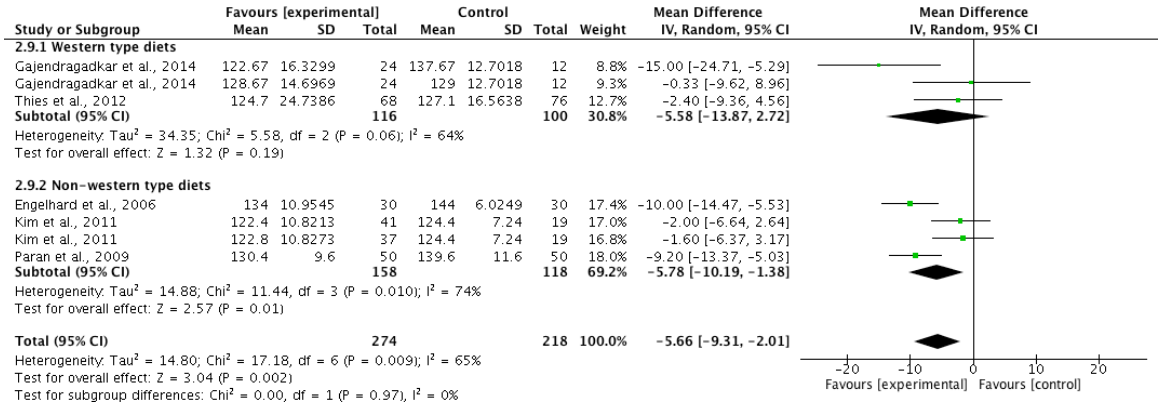


Figure 3.27 Meta-analysis of effect of supplementing lycopene on SBP (mmHg) according to two dietary pattern groups.

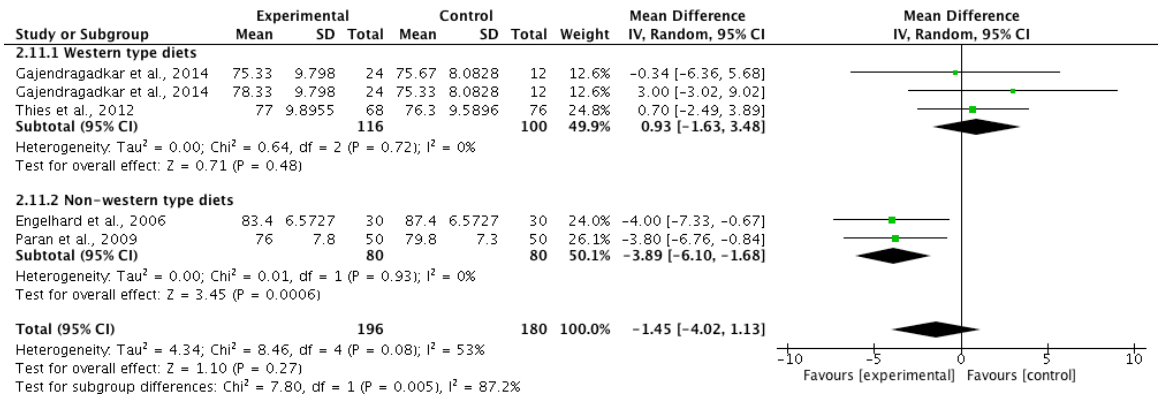


Figure 3.28 Meta-analysis of effect of supplementing lycopene on DBP (mmHg) according to two dietary pattern groups.

3.3.4 Publication bias

Funnel plots of results were derived and are reported in **Figure 3.29**, **Figure 3.30**, **Figure 3.31** and **Figure 3.32**. These suggested absences of publication bias. Egger's tests supported this for LDL-cholesterol (p=0.467), FMD (p=0.43) and SBP (p=0.62). Egger's test was not calculated for IL-6 given that only 3 studies were identified.

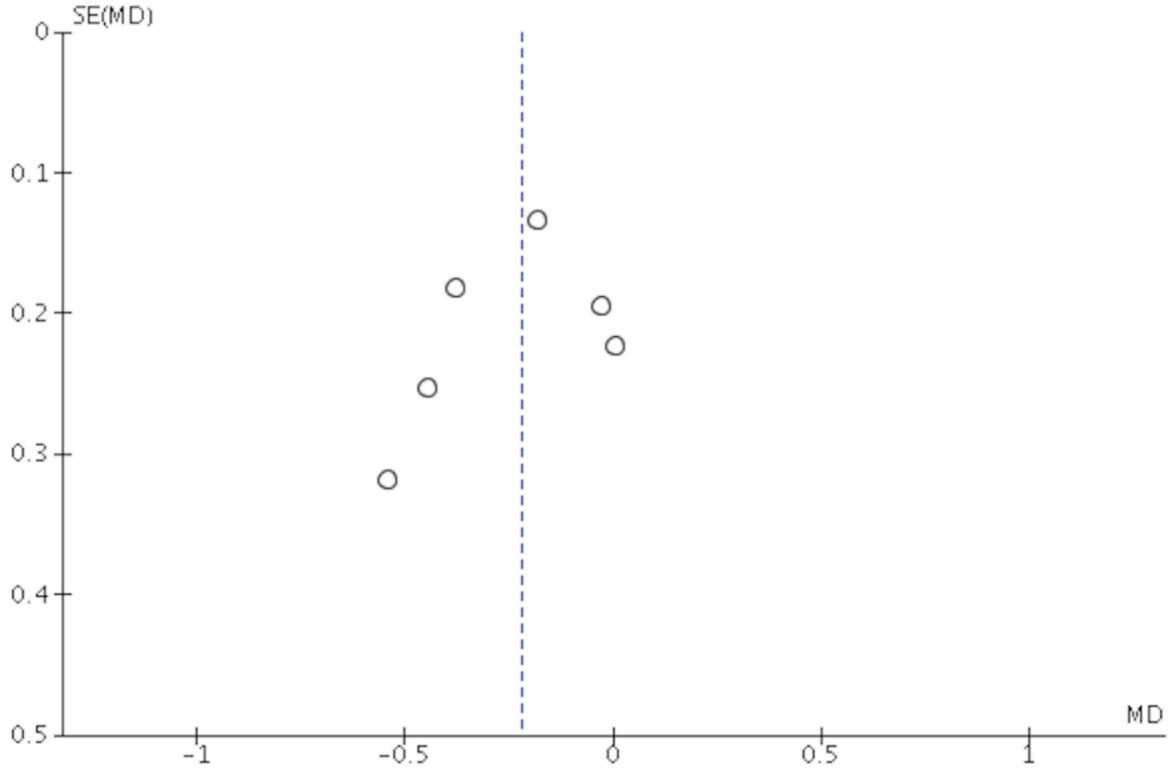


Figure 3.29 Funnel plot of standard error for the effect of interventions supplementing tomato on LDL.

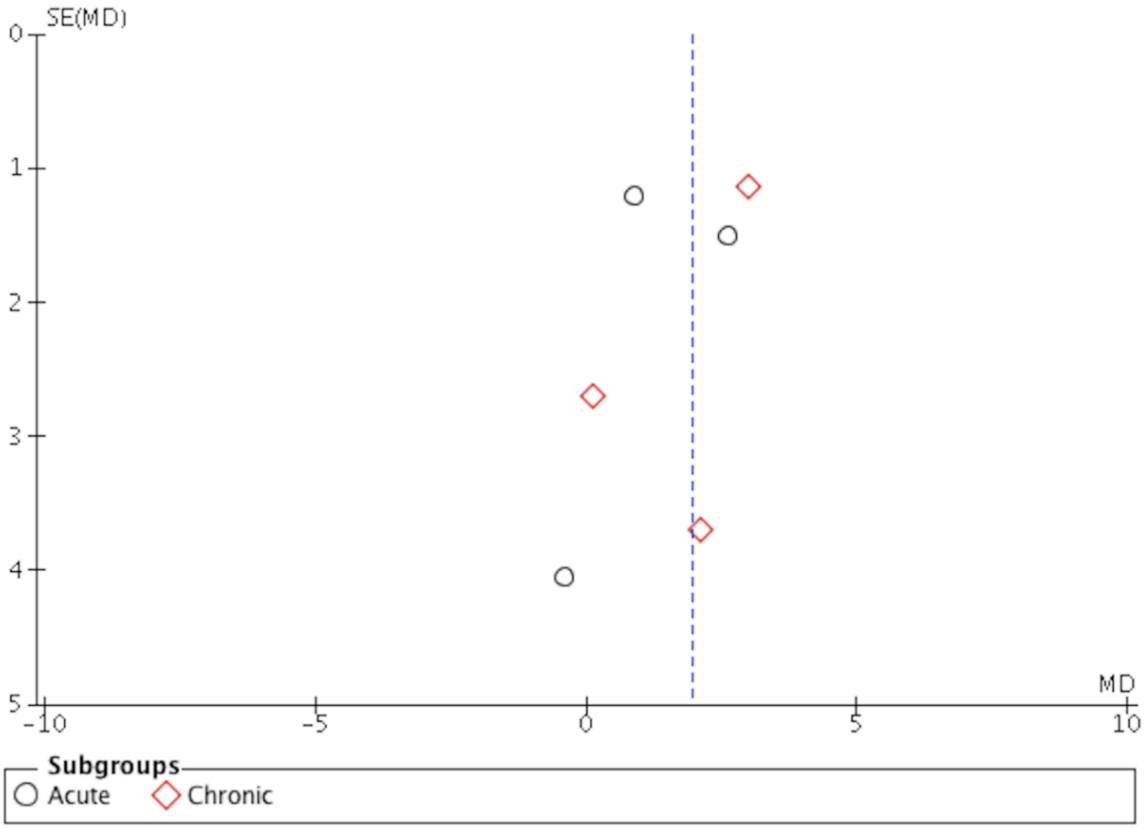


Figure 3.30 Funnel plot of standard error for the effect of interventions supplementing tomato on FMD.

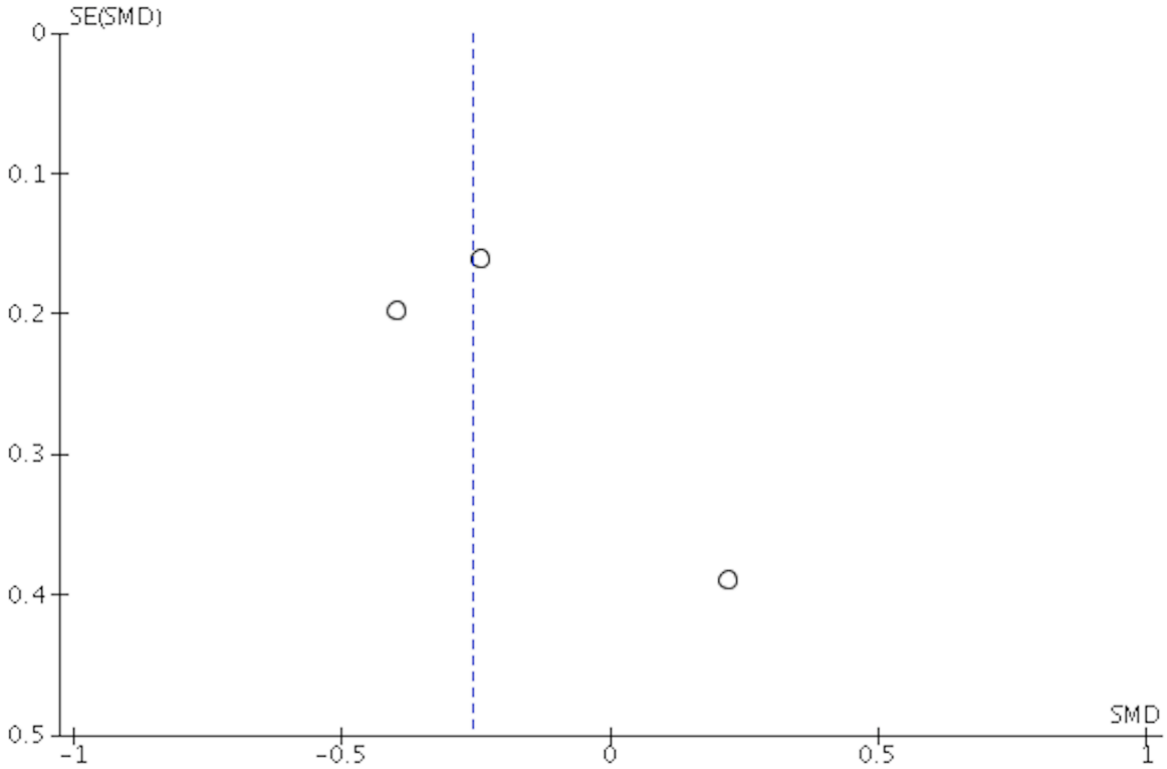


Figure 3.31 Funnel plot of standard error for the effect of interventions supplementing tomato on IL-6.

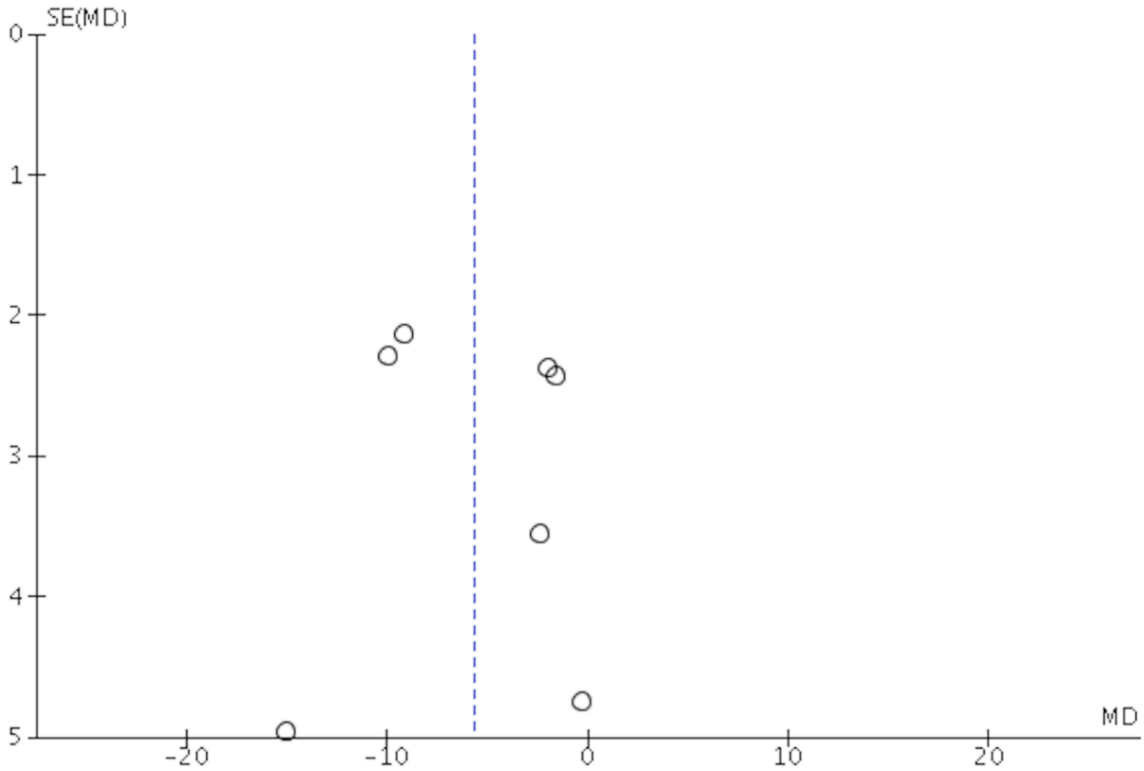


Figure 3.32 Funnel plot of standard error for the effect of interventions supplementing lycopene on SBP.

3.3.5 Quality of studies

We assessed the methodological quality and risk bias of the studies included in this review. The average retention rate for the RCT included in this review was >90% for all studies and the reason for the dropouts were often not related to the interventions themselves. Therefore, the majority of the included studies satisfied the criteria of the quality assessment tool. Blinding of participants and researchers delivering the intervention was implemented in interventions testing lycopene supplements. In addition, the included studies provided an adequate description of methods and randomisation procedures, thus no studies were excluded from analysis based on quality assessment.

3.4 Discussion

3.4.1 Principal findings

This systematic review and meta-analysis assessed the effectiveness of tomato and lycopene supplementation on cardiovascular risk factors, including blood lipids (total-, HDL-, LDL-cholesterol, triglycerides, oxLDL) vascular function (FMD, PWV), inflammatory factors (CRP, IL-6) and adhesion molecules (ICAM-1), among adult subjects >18 years of age within RCT. This systematic review revealed that there is strong evidence indicating that tomato supplementation is associated with significant reductions in LDL-cholesterol and improvements in FMD, but not on other blood lipids or measures of vascular function. In addition, lycopene supplementation was associated with significant reductions in SBP but not on any other cardiovascular marker. In subgroup analysis tomato supplementation improved HDL-cholesterol in non-western dietary patterns but not in western dietary patterns while LDL-cholesterol decreased in western dietary patterns but not in non-western dietary patterns. Lycopene supplementation significantly reduced SBP and DBP in non-western diets. The reduction of SBP and DBP may due to the HDL-antiatherogenic properties. Thies et al. (2017) suggested that increased lycopene intake improvement in HDL functionality, as measured by the activity of HDL-associated enzymes such as paraoxonase 1, lecithin cholesterol acyl transferase and cholesterol ester transfer protein, potentially enhancing HDL-antiatherogenic properties (Thies et al., 2017). It is noteworthy that the changes of the subgroup outcome reported in these studies could be due to the characteristics of the studied population. In addition, the heterogeneity of non-western diets in HDL-cholesterol is high at 81% which showed there are high variability among the studies. More research needed to identify tomato supplementation that improved HDL-cholesterol.

Together, these results indicate that consuming tomato products and/or supplementing lycopene may have important health implications. This systematic review has also revealed that current studies have mostly focused on studying well-established cardiovascular biomarkers such as blood lipids and blood pressure, with less evidence on FMD and other measures of vascular function, however the evidence on the effects of tomato products or lycopene supplementation on novel biomarkers of vascular risk is scarce at present and requires further investigation. The results are in agreement with earlier reports such as a meta-analysis carried out in 2010 showing that lycopene supplementation from different sources significantly reduced LDL-cholesterol (Ried and Fakler, 2011) and a meta-analysis carried out on 2012 indicating that lycopene supplementation significantly lowered SBP (Li and Xu, 2013).

3.4.2 Strengths and limitations

The strengths of these findings include a rigorous methodology in the systematic review of the literature, the low levels of heterogeneity between the studies included in this meta-analysis were very low, as well as the risk of publications bias was low, adding validity to the findings of this meta-analysis. It is of note that the intervention studies included in this review were consistently, and significantly, successful in modifying the outcomes above described. The overall quality of the studies was high. All studies were randomised, and double-blind in the case of lycopene supplementation. The studies reported a high compliance with the interventions, which may be explained by the relatively short duration of the studies.

This study is not without limitations. Due to the limited number of studies available, the analysis focused on examining the overall effects of interventions on the pre-specified outcomes preventing us of exploring the effects of common moderator variables such as age, gender, or other aspects of the interventions. Overall, studies were characterised by a small sample size, short duration, and over-representation of young, healthy men. It is important to acknowledge that there might be important heterogeneity on the bioavailability and concentrations of lycopene given that the products used in these studies varied in the degree of processing. Furthermore, the present study was not able to conduct subgroup analysis due to the limited number of studies. Studies included in meta-analysis originated from different geographic regions, and the dietary patterns, ethnicity of participants, genetic and sensitivity to lycopene might be influenced by these factors. The present study limited the choice of databases to three and although there might be a risk to have overlooked some studies, the databases included were considered the most relevant to the topic of study.

3.4.3 Scientific analysis of findings

This meta-analysis showed that tomato products supplementation was associated with significant reductions in LDL-cholesterol and improvements in short-term FMD, while lycopene supplementations reduced SBP. The beneficial effects of these interventions support the epidemiological evidence indicating an association between lower CVD risk and tomato (Jacques et al., 2013; Sesso et al., 2003) and suggest that these epidemiological findings may be explained by a potential combination of improvements in blood lipids, BP, and vascular function an early marker of cardiovascular risk (Gonzalez and Selwyn, 2003).

A number of potential mechanisms are probably responsible behind the findings of this systematic review. Lycopene, the major carotenoid in tomato, might be more important than

other carotenoids in preventing atherosclerosis and cardiovascular diseases, however the findings from supplementing lycopene this systematic review did not support a role in reducing LDL-cholesterol. This may be related to the fact that tomatoes contain also other compounds (e.g. antioxidants such as vitamin C) possessing lipid-lowering properties associated with increased faecal excretion and reduced intestinal absorption of cholesterol, in addition to increasing cellular LDL receptor activity (Ashor et al., 2016; Silaste et al., 2007).

In addition, the results of the present meta-analysis are comparable to those achieved by other nutritional interventions such as dietary nitrates modifying SBP and FMD. Two previous systematic reviews of the literature have shown that inorganic nitrate or beetroot juice consumption were associated with significant reductions in SBP (4.4 mmHg 95% CI -5.9 to -2.8, p<0.01) (Siervo et al., 2013), and improvements in FMD (SMD 0.52; 95% CI 0.15 to 0.68, p=0.002) (Lara et al., 2016).

Evidence from the effects of lycopene supplementation on oxidative stress is scarce at present and suggests the possibility of a positive effect on DNA damage but not on markers such as lag-time of LDL (Chen et al., 2013a). The results of the present study on oxLDL are in line with this evidence.

3.4.4 Implications for health and future research

Our results showed a significant decline in vascular risk factors after short-term (1 day to 6 months) tomato-products and lycopene (tomato-products doses from 70 to 400 g/d, and lycopene dose ranged from 4 to 30 mg/d) supplementation, which may potentially have important implications in primary and secondary prevention of atherosclerosis, cardiovascular diseases and cardiovascular mortality. The seventh report of the Joint National Committee on BP estimated that a systolic BP reduction of at least 5 mmHg (similar to the observed decline in SBP after Lycopene supplementation) could decrease the risk of mortality due to stroke by 13-14% (Chobanian et al., 2003; Reboldi et al., 2011) and mortality from cardiovascular diseases by 9% (Chobanian et al., 2003). In addition, reductions of 1 mmol in LDL-cholesterol have been associated with a 23% reduction in myocardial infarction or coronary death and 12% reduction in all-cause mortality (Baigent et al., 2005). In relation with the findings on FMD, several recent systematic reviews and meta-analysis indicate that increases in FMD of 1% increase, independently of confounding factors, are associated with reductions ranging from 10-13% in the risk of cardiovascular events (Inaba et al., 2010; Matsuzawa et al., 2015; Xu et al., 2014).

3.5 Conclusions

The available evidence on the effects of tomato and lycopene supplementation on vascular risk factors supports the view that increasing the intake of these has positive effects on blood lipids, blood pressure, some inflammatory factors, and vascular function. These results have potential public health implications and support the development of promising individualised nutritional strategies to tackle cardiovascular diseases.

3.6 Research Questions Raised from The Present Chapter

The work described in this chapter raised the following questions:

1. Does the carotenoid content vary in different varieties of tomato?
2. Does the carotenoid content change during postharvest storage?

These questions will be addressed in Chapter 4 by using HPLC to determine the lycopene content in different tomato varieties. We also investigate the effect of postharvest storage on carotenoid content. We hypothesised that carotenoid content varies in different tomato varieties.

Chapter 4 Method for Determine Lycopene Content in Tomato

This study was designed to answer the research questions in **section 3.6**.

4.1 Introduction

Nutritional value and appearance of tomato fruit are primary determined by carotenoid content (Kang et al., 2014). Carotenoid biosynthesis pathway begins with formation of phytoene from geranylgeranyl pyrophosphate (GGPP) (**Figure 4.1**) (Kang et al., 2014). Phytoene is further metabolised through desaturations, cyclisation and hydroxylation to from other carotenoid such as lycopene, carotenes, lutein and xanthophyll. There are only two recessive mutation available in tomato, *yellow-flesh* and *tangerine*, affecting lycopene biosynthesis from GGPP (Fantini et al., 2013; Kachanovsky et al., 2012; Liu et al., 2015). Mutation *yellow-flesh* is a loss-of-function mutant of the phytoene synthase (PSY1) gene while *tangerine* is a mutation in carotenoid cis-trans isomerase (CRTISO) gene (Liu et al., 2015). Fruits of tomato with the recessive mutation *yellow-flesh* appear pale yellow fruit (Liu et al., 2015). The *tangerine* phenotype of the mutant tomato fruits is associated with an orange fruit due to the accumulation of 7,9,9',7'-tetra-cis-lycopene (prolycopene) as the result of mutation in the CRTISO (Kachanovsky et al., 2012). CRTISO, a specific enzyme, produces all-trans-lycopene from prolycopene (**Figure 4.1**).

Lycopene is the predominant carotenoid in tomatoes and tomato products responsible for the red colour. It is a polyunsaturated hydrocarbon with 11 liner conjugated double bond and two non-conjugated double bond, which give lycopene antioxidant properties and singlet oxygen quenching ability (Agarwal and Rao, 2000; Bohm, 2012). During process of tomato product, it usually involves thermal treatment and/or homogenisation, which can release lycopene from the food matrix and initiates cis-isomerisation of lycopene (Cooperstone et al., 2015; Petyaev, 2016; Shi and Le Maguer, 2000; Story et al., 2010; Valderas-Martinez et al., 2016). Cis-isomerisation can also be facilitated under light and oxidation (Shi and Le Maguer, 2000; Story et al., 2010). In fruit and vegetables, lycopene is present predominately in the all-trans configuration (approximately 95%), while lycopene in human plasma and tissue consists of 50-90% cis-lycopene (**Figure 4.2**) (Cooperstone et al., 2015; Sinha and Dua, 2015; Unlu et al., 2007b). The observation has led to the hypothesis that isomerisation from all-trans-isomers to cis-isomers increases the bioavailability and enhance the lycopene biological benefits (Cooperstone et al., 2015; Gärtner et al., 1997; Stahl and Sies, 1992).

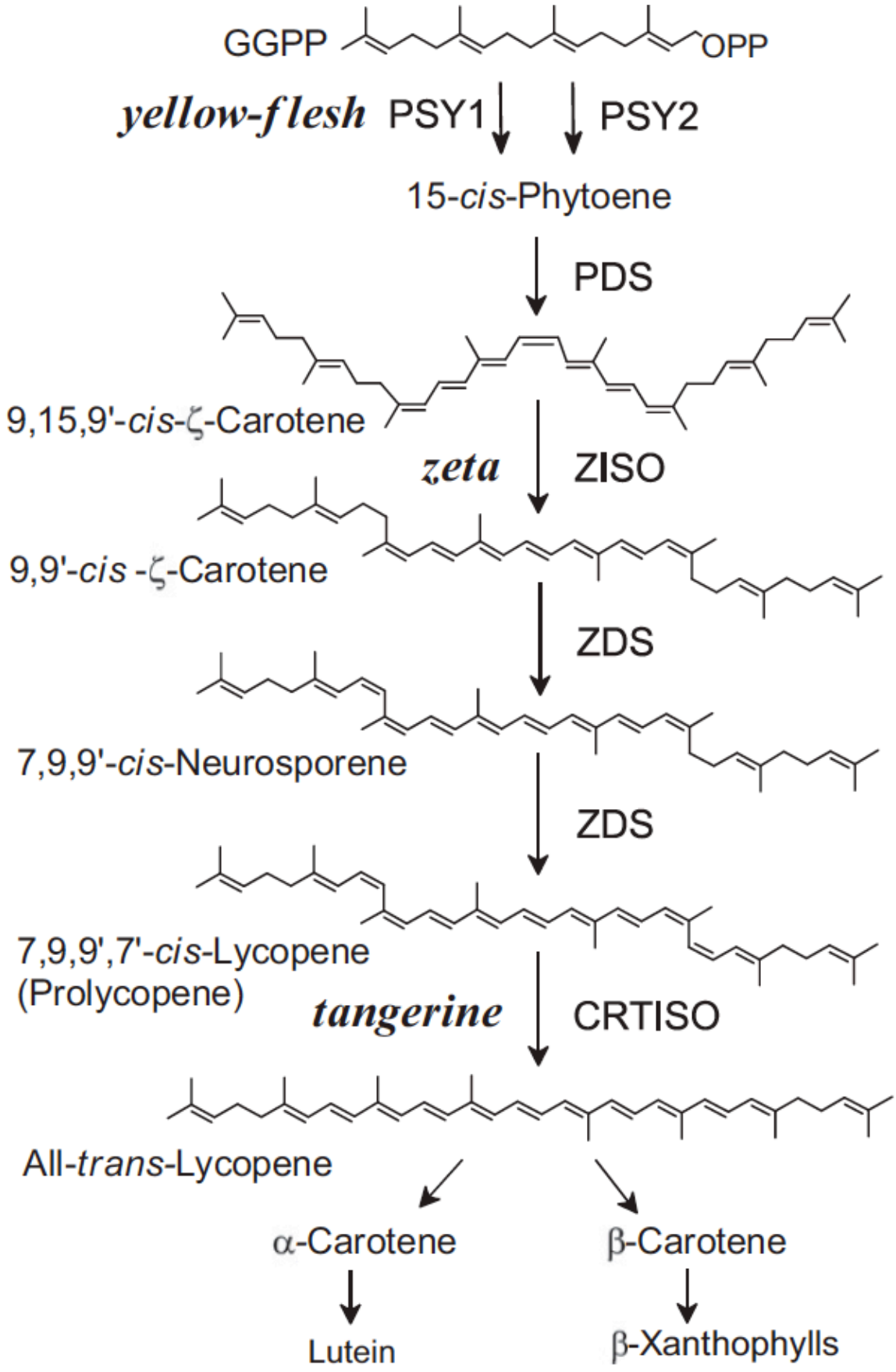


Figure 4.1 The carotenoid biosynthesis pathway in plants.

4.1.1 Determination of lycopene content using HPLC

Studies have reported various assay methods for the determination of lycopene content in food products using HPLC, which is a technique in analytical chemistry used to separate, identify, and quantify a compound in a solution mixture (Lindsay, 1992). In addition to all-trans-lycopene and cis-lycopene, tomato and its products contain other types of carotenoids such as β-carotene. Therefore, HPLC is used to separate the different carotenoid components of the tomato sample from each other so that they may be identified and/or quantified.

There are two main objectives of this chapter. First, to analysis the lycopene content in both Piccolo (red cherry tomato) and Oranjstar (orange cherry tomato) tomatoes. Second, to variate the effect of storage time under darkness condition on carotenoids content of cherry tomato during postharvest storage.

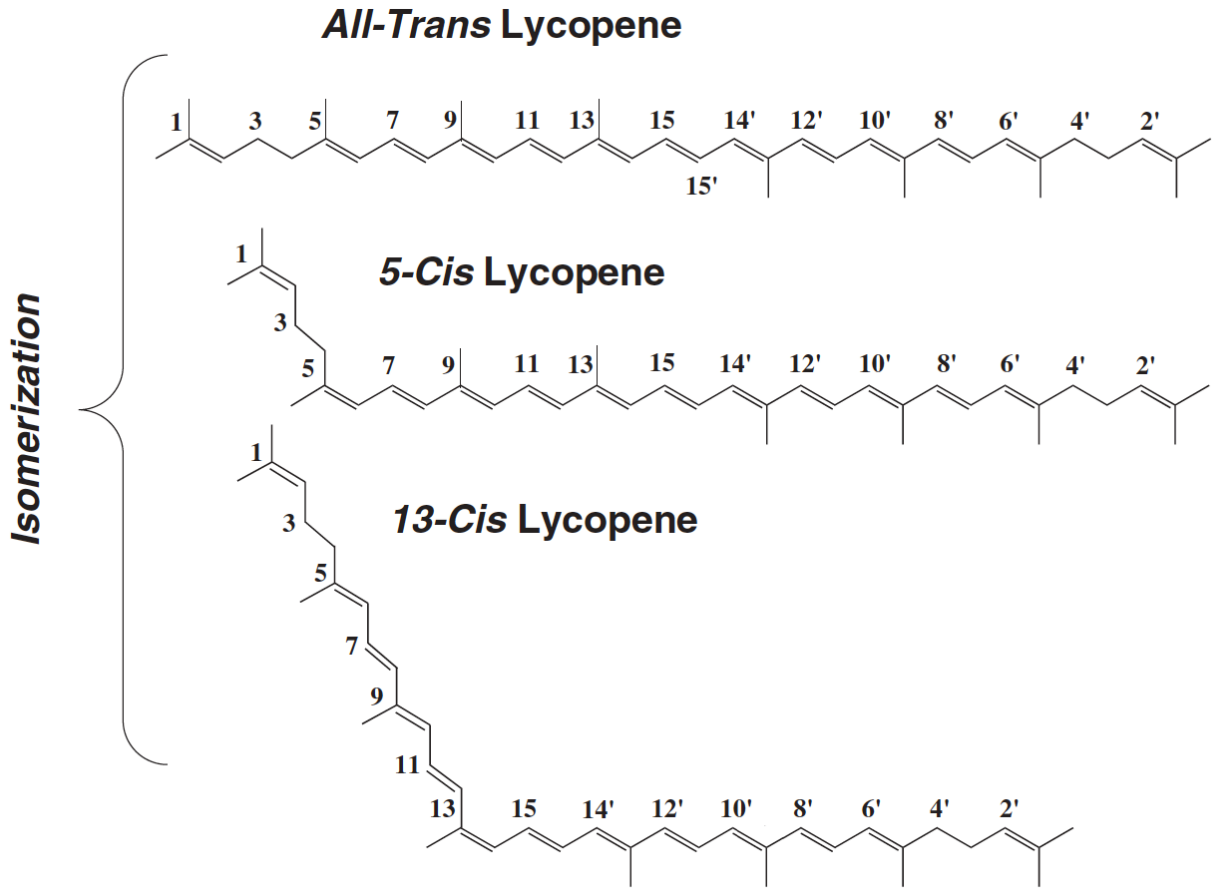


Figure 4.2 Structures of all-trans and cis isomers of lycopene. (Wang, 2012).

4.2 Method

4.2.1 Measurement of carotenoids in tomato fruit

All organic solvents (methanol (MeOH), methyl t-butyl ether (MTBE), butylated hydroxytoluene (BHT), ethyl acetate (EA), dichloromethane (DCM), acetonitrile (ACN), and hexane (Hex)) used for separation of tomato carotenoids were HPLC grade and purchased from Fisher Scientific (Pittsburgh, PA, USA). Lycopene standard was purchased from Sigma-Aldrich Chemicals (St Louis, MO, USA). To protect carotenoids from degradation and oxidation, all steps of extraction and determination were conducted under limited light and using amber Eppendorf.

4.2.1.1 Plant material sampling and experimental design

A total of three varieties of tomatoes, Piccolo (red cherry tomato), Oranjstar (orange cherry tomato), and Red Comet (red cherry tomato), were submitted to this experiment. Two varieties of Piccolo and Oranjstar were provided by Thanet Earth (**Figure 4.3**). Healthy cherry tomato fruits provided by Thanet Earth were hand harvested from each plant when they had reached the mature ripe stage. Red cherry tomatoes purchased from supermarket (Marks & Spencer, Piccolo grown by Thanet Earth (**Appendix C1**) and by Andy Roe (**Appendix C2**) respectively, Sainsbury's, Piccolo grown by Thanet Earth (**Appendix C3**), and Tesco, Red Comet grown in Spain (**Appendix C4**)). Changes in carotenoids content were measured by HPLC on day zero and after 4, 7 and 10 days of storage in temperature controlled incubator (15 ℃) under darkness condition.

Six biological replicates of tomatoes, Piccolo and Oranjstar, provided by Thanet Earth, were analysed on each day, (day of harvest (day 0), after 4, 7 and 10 days of storage). Position 1, 4 and 8 of each tomato trusses were taken for analysis (**Figure 4.3**) to confirm the carotenoids content not vary in the different position. Tomato samples purchased from supermarkets, were analysed by randomly picking 3 tomatoes from each pack and the process was repeated twice for each supermarket.

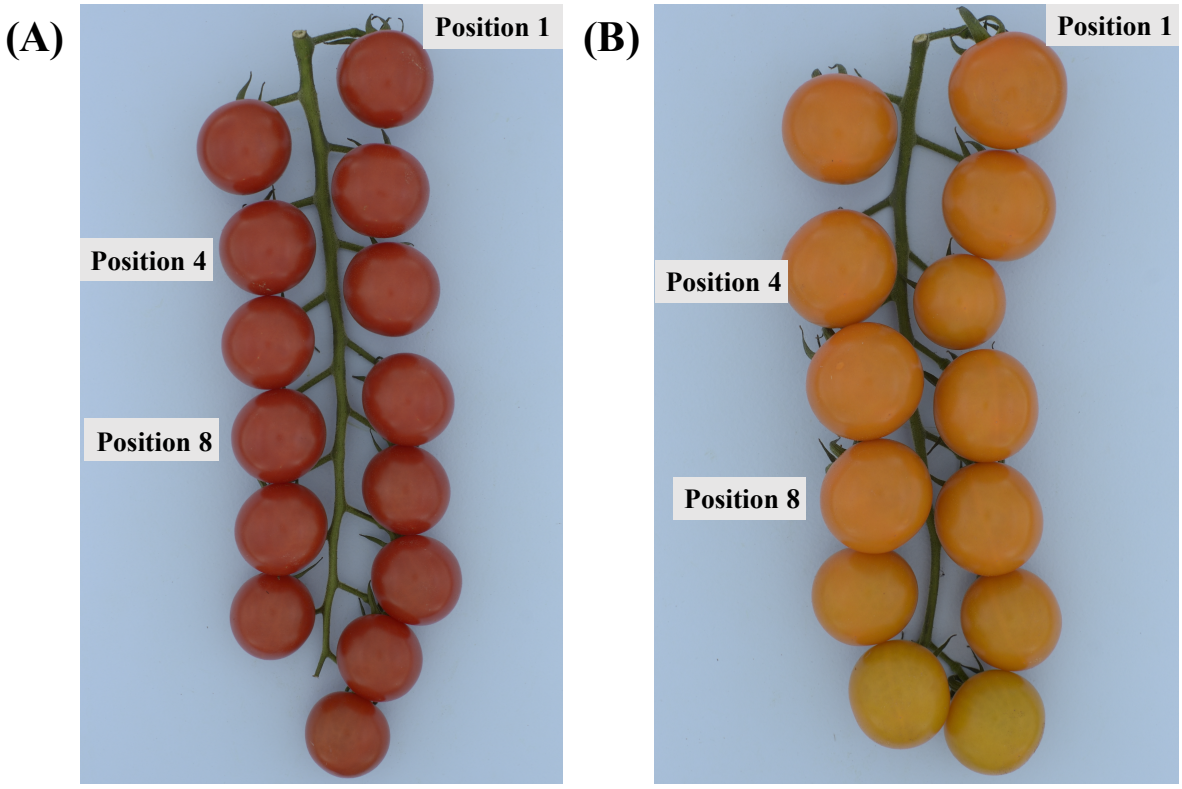


Figure 4.3 Tomato samples provided by Thanet Earth, Piccolo (A) and Oranjstar (B).

4.2.1.2 Carotenoid extraction methods

The micro-extraction technique was employed to extract the carotenoids from the samples (Serino et al., 2009). Tomatoes were washed, dried and cut into halves. Liquid nitrogen was used to snap-freeze the samples followed by pulverisation using a mechanical blender. Carotenoid extraction was performed in a 2 mL amber Eppendorf tube containing 400-600 mg of accurately weighed tomato powder. Saturated aqueous NaCl solution (100 µL) and 50 µL of hexane was added, followed by vortex mixing for 30s; addition of 200 µL of DCM, vortex for 30s; addition of 1 mL EA, vortex for 30s, and centrifugation for 5 minutes at 9800 RCF to separate the organic and aqueous phase. The organic phase was recovered and filtered with a 0.2 µm PTFE microfilter. The detailed procedure for carotenoid extraction is shown in **Figure 4.4**.

4.2.1.3 Preparation of the lycopene and β-carotene standard

The lycopene and β-carotene standard used in HPLC (100 µg/mL) was originally prepared in the lab and stored at -80 ℃ in DCM. Eight different concentrations of lycopene standard solutions were prepared from the 100 µg/mL lycopene and β-carotene stock solution as shown in **Table 4.1**.

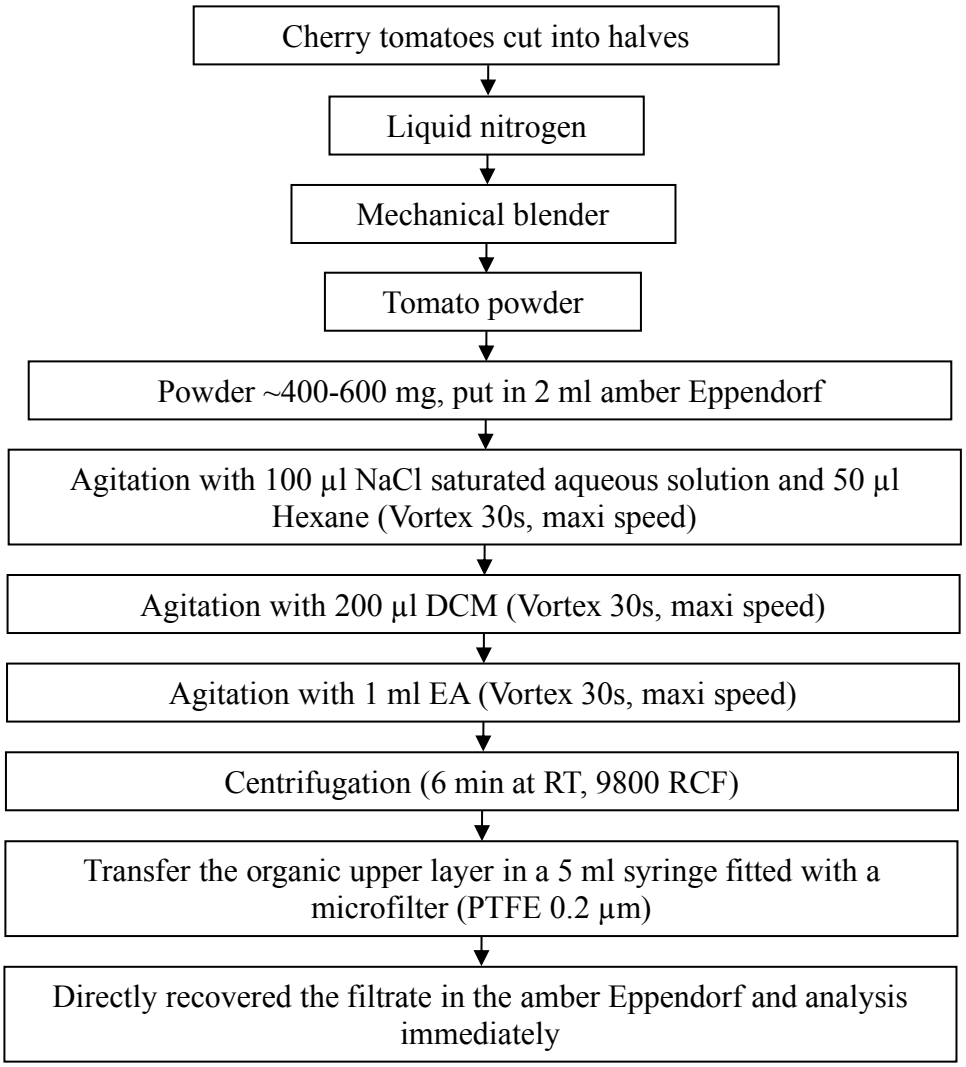


Figure 4.4 Carotenoids extraction procedures for analytical HPLC. (Serino et al., 2009).

Table 4.1 Lycopene and β-carotene standard preparation.

Lycopene and β-carotene (µg/mL)	**Volume of lycopene and β-carotene standard stock solution (100 µg/mL) (µL)**	**DCM added in vial (µL)**
100	No dilution needed	0
75	375	125
50	250	250
25	125	375
10	50	450
5	25	475
Lycopene and β-carotene (µg/mL)	**Volume of lycopene and β-carotene standard stock solution (10 µg/mL) (µL)**	**DCM added in vial (µL)**
2.5	125	375
1	50	450

4.2.3 Analytical HPLC

A modified carotenoid determination method was used for lycopene and β-carotene quantification in this study (Müller et al., 2008).

HPLC analysis was performed on an analytical HPLC Dionex Ultimate 3000 HPLC system (Thermo Fisher, Dreieich, Germany), consisting of a WPS-3000 TPL RSWell Plate autosampler, a HPG-3000 RS binary pump with online solvent degasser and a 3000 RS diode array detector (DAD). Separation was operated on a Sigma SUPLEX PKB-100 amide column (5 µm particle size, 250 mm length x 4.6 mm inner diameter) and connected to a SUPLEX PKB-100 Supelguard Cartridge (5 µm particle size, 20 mm length x 4.6 mm inner diameter). Isocratic elution was used at a flow rate of 1.5 mL/min. Injection volume was 5 µL. Column temperature was set at 30 ℃ and the autosampler temperature was at 4 ℃. The presumptive peaks were identified based on HPLC retention times and published absorbance spectral data (**Table 4.2**) (Serino et al., 2009). Analysis was performed through the Chromeleon Chromatography Data System (CDS) Software (version 7.1.1) (Thermo Fisher Scientific, USA).

Table 4.2 Presumptive peak identification of HPLC peaks in extracts of tomato.

Carotenoids	Peak retention time (min)	Absorbance maxima (nm)
All-trans-lycopene	5.78	474
cis-lycopene	6.62	474
β-Carotene	7.92	454

4.2.3.1 Preparation of mobile phases

Mobile phase A: BHT (50 mg) dissolved in 20 mL of 2-propanol were added in a 1 L volumetric flask, followed by the addition of a 0.2 mL N-ethyldiisopropylamine and 50 mg of ammonium acetate dissolved in 25 mL of water. Acetonitrile (455 mL) and 450 mL of MeOH were added and the solution was diluted to volume with methanol and filtered through filter paper (0.2 µm). Mobile phases B was MTBE with BHT (50 mg/L). All solutions were used within 2 days of preparation (Müller et al., 2008).

4.2.4 Statistical analysis

All data are reported as mean (SD) of the average for two or six replications. One-way analysis of variance (ANOVA) was used to compare the means of all evaluated parameters. Differences were considered significant at $p<0.05$. T-test for two averages was also used to study the interactions between pure standards of antioxidants.

4.3 Result

4.3.1 Carotenoid Content of different tomato varieties

In **Figure 4.5** and **Figure 4.6**, show the chromatograms of carotenoids standard and carotenoid extracted from Piccolo and Oranjstar under absorbance 484nm and 474nm respectively. The mean trans-lycopene content of Piccolo over 10 days were 8.32±0.14 mg/100 g while trans-lycopene content of Oranjstar were untraceable (<0.05 mg/100 g) (**Figure 4.7**). The mean cis-lycopene content of Piccolo over 10 days were 0.42±0.01 mg/100 g while cis-lycopene content of Oranjstar were 2.64±0.08 mg/100 g (**Figure 4.7**). The mean β-carotene content of Piccolo over 10 days were 0.89±0.02 mg/100 g while β-carotene content of Oranjstar were 0.68±0.03 mg/100 g (**Figure 4.7**). The content of trans-lycopene, cis-lycopene and β-carotene of the red tomato purchased in the supermarket were shown in **Figure 4.8**.

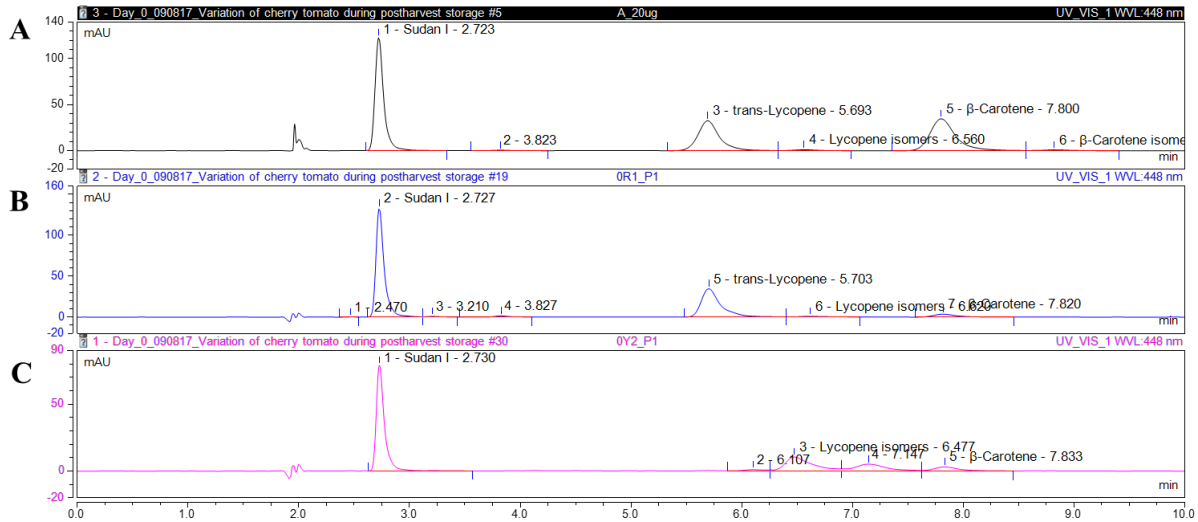


Figure 4.5 HPLC chromatograms of carotenoids standard (A) and carotenoids extracted from Piccolo (B) and Oranjstar (C) under 448nm wavelength detection.

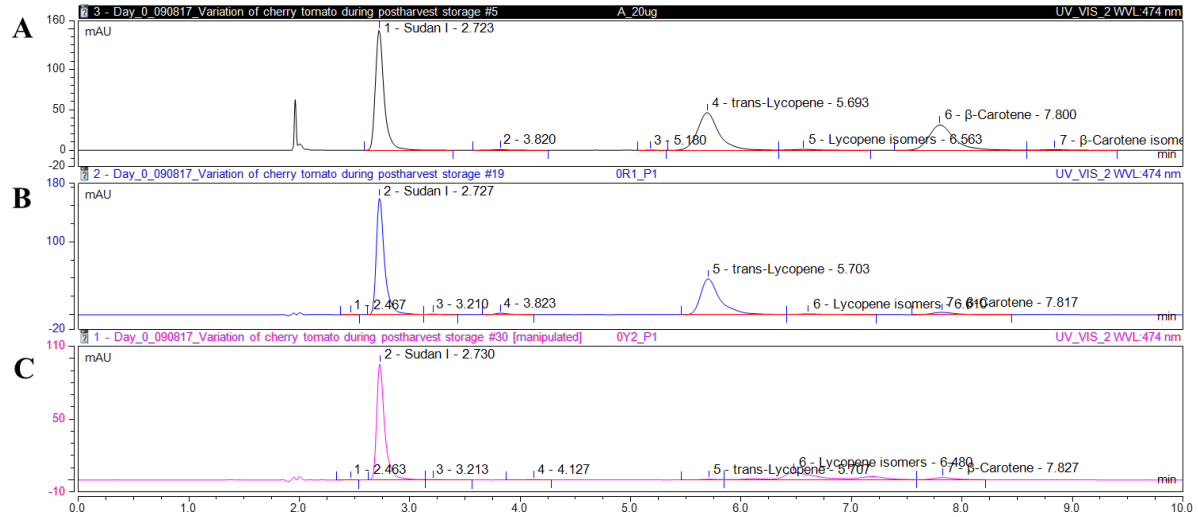


Figure 4.6 HPLC chromatograms of carotenoids standard (A) and carotenoids extracted from Piccolo (B) and Oranjstar (C) under 448nm wavelength detection.

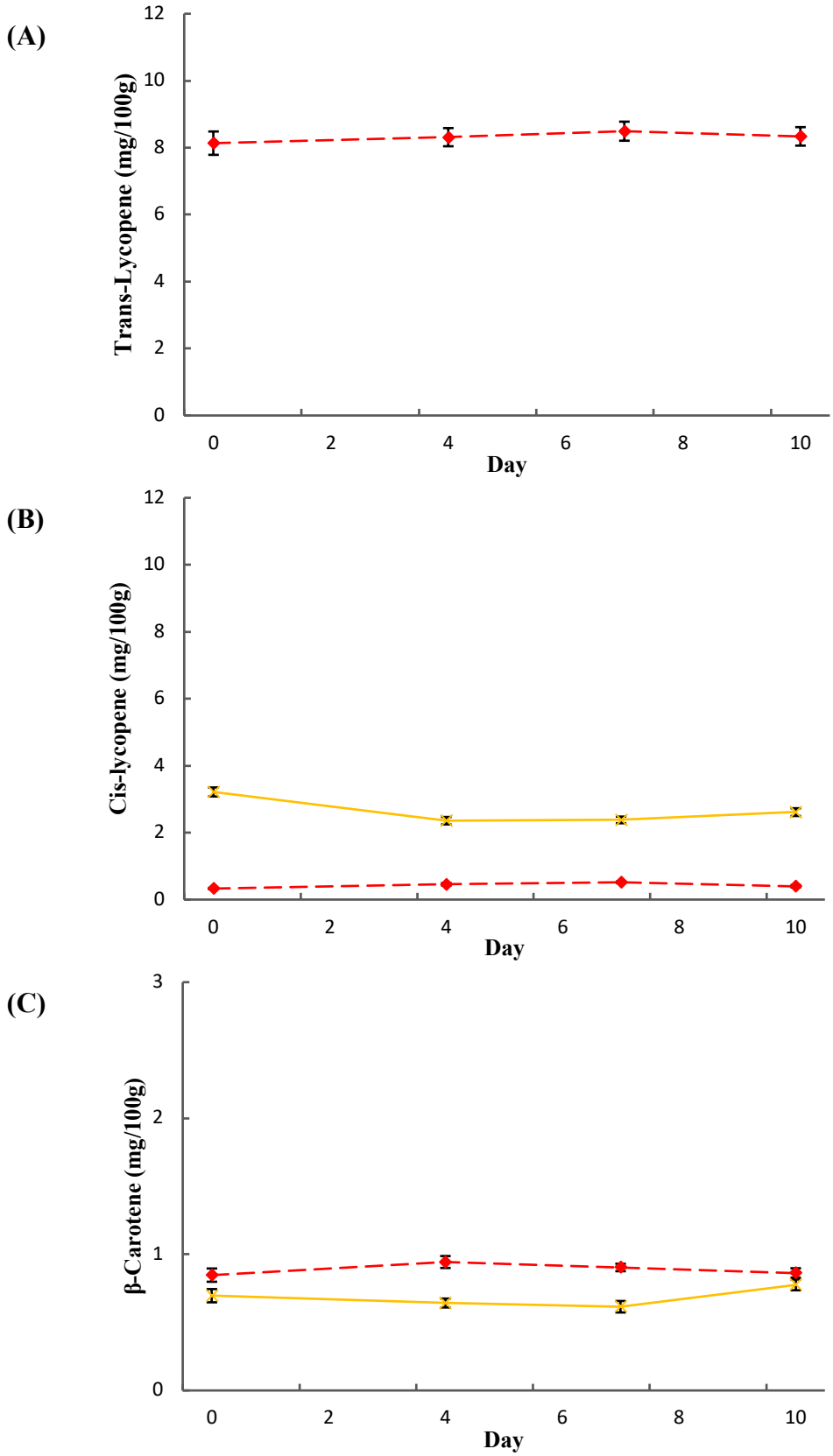


Figure 4.7 Trans-lycopene content (A), cis-lycopene content (B) and β-carotene (C) of different varieties of cherry tomato provided by Thanet Earth.

Piccolo (6 replications); Oranjstar (6 replications).

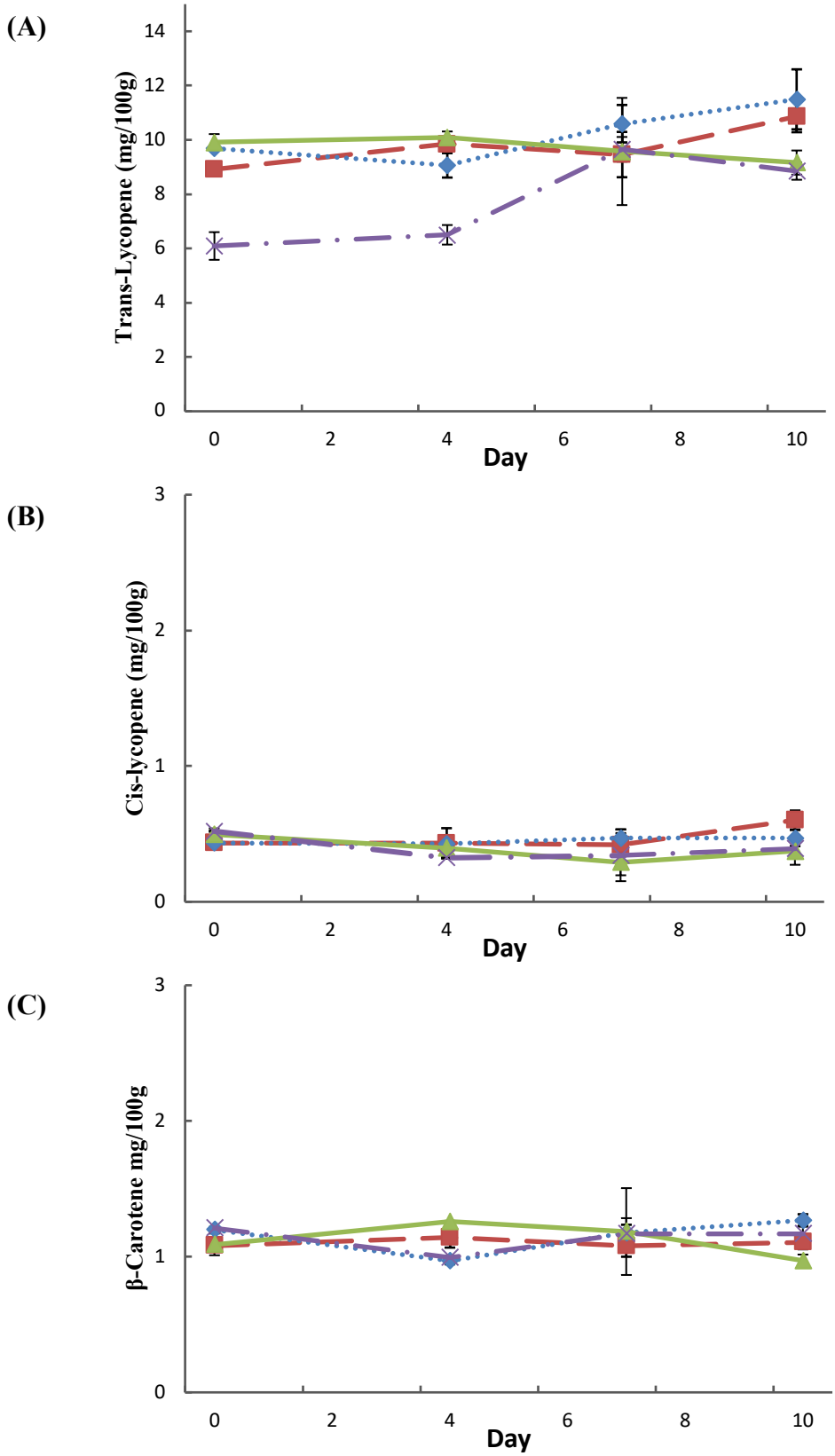


Figure 4.8 Trans-lycopene content (A), cis-lycopene content (B) and β-carotene (C) of different varieties of cherry tomato purchased from supermarket.

Piccolo, purchased from M&S grown by Andy Roe (2 replications); Piccolo, purchased from M&S grown by Thanet Earth (2 replications); Piccolo, purchased from Sainsbury's grown by Thanet Earth (2 replications); Red Comet, purchased from Tesco grown in Spain (2 replications).

4.4 Discussion

The carotenoid content of Piccolo and Oranjstar did not change significantly over the 10 days of storage. The mean trans-lycopene content of Piccolo over 10 days were 8.32±0.14 mg/100 g while the mean cis-lycopene content of Piccolo over 10 days were 0.42±0.01 mg/100 g while cis-lycopene content of Oranjstar were 2.64±0.08 mg/100 g. Therefore, intake of 300 g of Piccolo and Oranjstar provided 26.22 mg and 7.92 mg of total lycopene respectively. Our analysis of the lycopene content showed no variations over time, therefore adding confidence that participants received a constant dose of lycopene throughout the study. Overall the carotenoid content of tomato used in the present study were in agreement with previous studies, as well as with tomatoes of the same variety available in supermarket (**Table 4.3**, **Figure 4.7** and **Figure 4.8**). Therefore, population supplementing tomato purchased from supermarket could have the same beneficial effects as present study (in Chapter 5-7). Nutritional composition data obtained from the USDA Food Composition Databases, indicates that overall, the macronutrient content between different varieties of tomato is fairly similar as reported in **Table 4.4**. However, some differences at the micronutrient level can be observed. For example, the content of vitamin C in green tomato varieties is reportedly higher than other tomato varieties. In relation with the colour of tomatoes used in this study (i.e. red vs orange), only the content of vitamin A and sodium are reportedly higher in the orange variety (**Table 4.4**).

4.5 Conclusion

The Piccolo was rich in trans-lycopene while the Oranjstar was rich in cis-lycopene as well as low content of trans-lycopene.

4.6 Research Questions Raised from The Present Chapter

The work described in this chapter raised the following questions:

1. What are the gaps in the literature in relation with RCT supplementing tomato on cardiovascular risk factors?
2. Do different varieties of cherry tomato have the same extent of health beneficial effects?

The following chapters present the work undertaken to answer this research question. A study protocol was designed to investigate whether red cherry tomatoes (Piccolo) and orange cherry tomatoes (Oranjstar), improve vascular function and other cardiovascular risk factors to the same extent. This trial has been registered in the National Library of Medicine (ClinicalTrials.gov identifier: NCT03209817).

Table 4.3 Carotenoid Content (mg/100 g fresh weight) of different tomato varieties

	No. of varieties	All trans-lycopene (mg/100 g fresh weight)	SD	Cis-lycopene (mg/100 g fresh weight)	SD	β-carotene (mg/100 g fresh weight)	SD
Tomato used in the clinical trial							
Red cherry tomato (Piccolo, provided by Thanet Earth)	1	8.32	0.14 (SEM)	0.42	0.01 (SEM)	0.89	0.02 (SEM)
Orange cherry tomato (Oranjstar, provided by Thanet Earth)	1	<0.05	N/A	2.64	0.08 (SEM)	0.68	0.03 (SEM)
Tomato purchased from supermarket							
Red cherry tomato (Piccolo, purchased from M&S grown by Andy Roe)	1	9.68	0.42 (SEM)	0.38	0.04 (SEM)	1.13	0.08 (SEM)
Red cherry tomato (Piccolo, purchased from M&S grown by Thanet Earth)	1	9.77	0.35 (SEM)	0.47	0.04 (SEM)	1.1	0.03 (SEM)
Red cherry tomato (Piccolo, purchased from Sainsbury's grown by Thanet Earth)	1	10.21	0.49 (SEM)	0.45	0.03 (SEM)	1.15	0.05 (SEM)
Red cherry tomato (Red Comet, purchased from Tesco grown in Spain)	1	7.85	0.52 (SEM)	0.39	0.03 (SEM)	1.13	0.04 (SEM)
Data from previous studies							
Red salad tomato (including different varieties) (Abushita et al., 2000)	12	6.27	0.95	0.10	0.02	0.42	0.11
Red tomato (including different varieties) (Kavitha et al., 2014)	14	8.60	2.24	N/A	N/A	N/A	N/A
Red cherry tomato (including different varieties) (Kavitha et al., 2014)	9	8.14	2.22	N/A	N/A	N/A	N/A
Red cherry tomato (including different varieties) (Kuti and Konuru, 2005)	4	8.69	1.59	0.50	0.43	N/A	N/A
Tangerine (Orange cherry tomato) (Unlu et al., 2007a)	1	0.10	0.01 (SEM)	3.15	0.05 (SEM)	2.32	0.24 (SEM)

Table 4.4 Nutrient Content (mg/100 g fresh weight) of different tomato varieties. Data obtained from USDA Food Composition Databases.

Nutrient (unit)	Red, ripe, raw, year round	Green, raw	Orange, raw	Yellow, raw	Red cherries, raw
Macronutrients					
Water (g)	94.5	93.0	94.8	95.3	86.1
Energy (kcal)	18.0	23.0	16.0	15.0	50.0
Protein (g)	0.9	1.2	1.2	1.0	1.0
Total fat (g)	0.2	0.2	0.2	0.3	0.3
Carbohydrate (g)	3.9	5.1	3.2	3.0	12.2
Fibre, total dietary (g)	1.2	1.1	0.9	0.7	1.6
Sugars					
Sugars, total (g)	2.6	4.0	N/A	N/A	8.5
Sucrose (g)	0.0	N/A	N/A	N/A	0.8
Glucose (dextrose) (g)	1.3	N/A	N/A	N/A	4.2
Fructose (g)	1.4	N/A	N/A	N/A	3.5
Minerals					
Calcium, Ca (mg)	10.0	13.0	5.0	11.0	16.0
Iron, Fe (mg)	0.3	0.5	0.5	0.5	0.3
Magnesium, Mg (mg)	11.0	10.0	8.0	12.0	9.0
Phosphorus, P (mg)	24.0	28.0	29.0	36.0	15.0
Potassium, K (mg)	237.0	204.0	212.0	258.0	173.0
Sodium, Na (mg)	5.0	13.0	42.0	23.0	3.0
Zinc, Zn (mg)	0.2	0.1	0.1	0.3	0.1
Copper, Cu (mg)	0.1	0.1	0.1	0.1	0.1
Manganese, Mn (mg)	0.1	0.1	0.1	0.1	0.1
Selenium, Se (mg)	0.0	0.4	0.4	0.4	0.0
Vitamins					
Vitamin C, total ascorbic acid (mg)	13.7	23.4	16.0	9.0	10.0
Thiamine (mg)	0.0	0.1	0.0	0.0	0.0
Riboflavin (mg)	0.0	0.0	0.0	0.0	0.0
Niacin (mg)	0.6	0.5	0.6	1.2	0.4
Pantothenic acid (mg)	0.1	0.5	0.2	0.1	0.1
Vitamin B-6 (mg)	0.1	0.1	0.1	0.1	0.0
Folate, total (mg)	15.0	9.0	29.0	30.0	8.0
Choline, total (mg)	6.7	8.6			6.1
Vitamin A (IU)	833.0	642.0	1496.0	0.0	1283.0
Vitamin E (mg)	0.5	0.4	N/A	N/A	0.1
Vitamin K (µg)	N/A	10.1	N/A	N/A	2.1

Chapter 5 Protocol for A Randomised Controlled Trial on The Effects of Two Tomato Varieties on Cardiovascular Function in Young and Older Adults

This study was designed to answer the research questions in **section 4.6**.

5.1 Introduction

In order to inform the design of this protocol, systematic reviews and meta-analysis of the relevant literature were undertaken. Overall, the analysis of epidemiological and experimental studies consistently showed that tomato consumption is associated with reduced CVD risk and mortality (Cheng et al., 2019) as well as CVD risk markers (Cheng et al., 2017). This evidence therefore supports the inclusion of tomato as an important food within dietary recommendations.

The systematic reviews and meta-analyses in Chapter 2 and 3 revealed that previous controlled experimental studies on the effects of tomato consumption have focused on red tomatoes, red tomato products or lycopene supplementations. However, a comprehensive study examining different varieties of tomatoes and its effects on both traditional and novel CVD risk factors is currently lacking in the literature. With new varieties of tomatoes, Oranjstar (rich in cis-lycopene with low trans-lycopene content), available to the public, it is important to evaluate whether other varieties exert the same effect.

Previous human trials have shown the protective effects of the tomato on blood lipid. However, there are numerous emergent circulating biomarkers including interleukin 6 (IL-6), tumour necrosis factor alpha (TNF-α), intercellular adhesion molecules-1 (ICAM-1), vascular adhesion molecules-1 (VCAM-1), E-selectin and P-selectin. However, these inflammatory markers were less examined. We aimed to determine the beneficial effects of tomato on blood lipid profile as well as these inflammatory markers including TNF-α, IL-6, E-selectin, P-selectin, sICAM-1, and sVCAM-1. Furthermore, previous nutrition interventions were focused on BP and blood lipid while the impact of tomato on vascular function, especially on microvascular vasodilation and arterial stiffness were less evaluated.

This chapter outlines the methods used throughout this tomato human trial study. Specific methods used in individual experimental chapters are discussed within the corresponding chapters. The present study investigated whether red cherry tomatoes (Piccolo) characterised by high content of trans-lycopene, and orange cherry tomatoes (Oranjstar) rich in cis-lycopene, improve vascular function to the same extent (**Table 4.3**). A 4-week period of intervention was chosen to evaluate chronic effects of the interventions tested. This study included three arm treatments including 300 g fresh Piccolo and Oranjstar supplementation

and a washout period (without tomatoes supplementation). We aimed to compare the beneficial effects of two different varieties of tomato on blood pressure, vascular function, blood lipid and inflammatory markers in young and older adults.

5.2 Method

5.2.1 Subjects

Thirty non-smoking healthy male volunteers aged 18-60 years of age, with a BMI between 25 and 40kg/m^2, were recruited and a total of 27 participants completed the intervention. The study participants were recruited from Northumbria University and the local community between January and May 2017.

5.2.1.1 Inclusion criteria

Participants were selected if they met the following inclusion criteria: BP <150/90 mmHg; not on a weight-reducing diet or taking dietary supplements; not diagnosed with diabetes, high blood cholesterol and heart problems (e.g. arrhythmia, high-grade stenosis of the carotid artery or carotid sinus syndrome); alcohol intake <21 units/week for men; non-allergic to tomatoes or tomato products; and were healthy non-smokers. The study was subject to ethical review by the Northumbria University Ethics Committee (Project ref: PG02_Cheng_121216) and was given a favourable ethical opinion to proceed. Informed signed consent was provided by each participant. This trial has been registered in the National Library of Medicine (ClinicalTrials.gov identifier: NCT03209817).

5.2.1.2 Sample size

Sample size calculation was performed for the primary endpoint: change in SBP response. Data for this calculation was derived from a previous systematic review of the literature carried out as part of this PhD program (Cheng et al., 2017). Sample size was calculated using G*Power version 3.1.3 (Program written, concept and design by Franz, Universitat Kiel, Germany; freely available windows application software) (Faul et al., 2007). The two-tails t-test to compare the differences between two dependent means was used. Calculations considered a prior power of 0.8, a 0.05 significance level and a 0.5 correlation coefficient between groups. The estimated participants required to allow detection a difference of 6 mmHg between the responses to the intervention and control, based on the systematic review in Chapter 3, was estimated to be 24 as shown in **Figure 5.1**. This sample size is a conservative one, given that in a cross-over study design, the correlation between the groups

would be higher which lower the sample size. For example, a correlation of 0.7 would result in a sample of 16. This study aimed to recruit 30 participants to allow for 20% drop out rate.

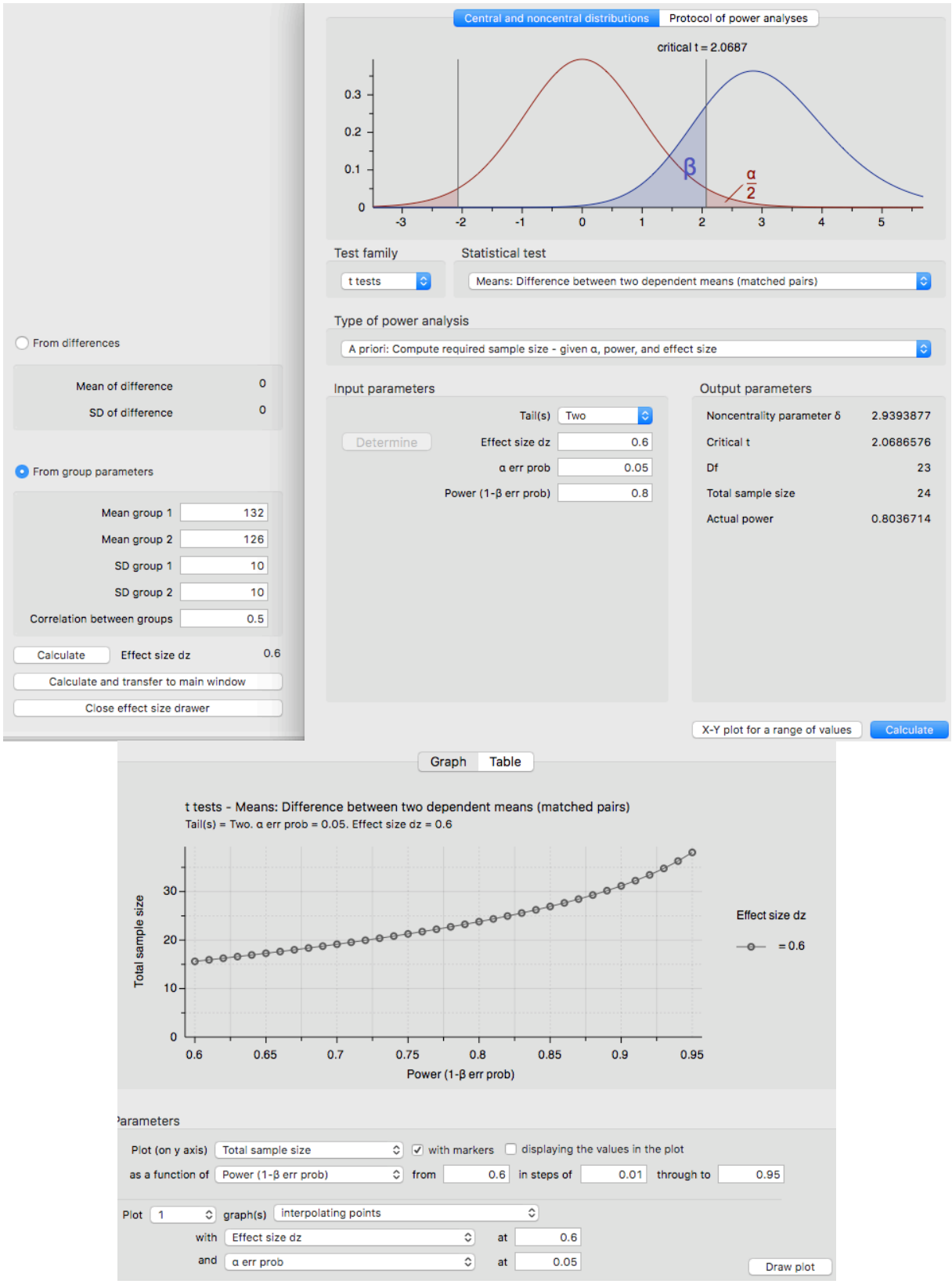


Figure 5.1 Power calculation from change in SBP response considering a priori power of 0.8, a 0.05 significance level and a 0.5 correlation coefficient between groups.

5.2.1.3 Randomisation

The randomisation plan was carried out using www.randomization.com by the principal supervisor, Dr Jose Lara. The randomisation sequence defined the order of the tomato interventions in counterbalance, half participants started with Piccolo and half participants started with Oranjstar. Participants were randomised either to consumed 300 g of fresh Piccolo or Oranjstar for a 4-week period. After a 4-week washout, they repeated the above process consuming the other tomato intervention **(Figure 5.2**). Participants were unaware of the randomisation sequence; but they were not blind to intervention, once they began each phase of the intervention, they were aware of the variety of tomato to be consumed.

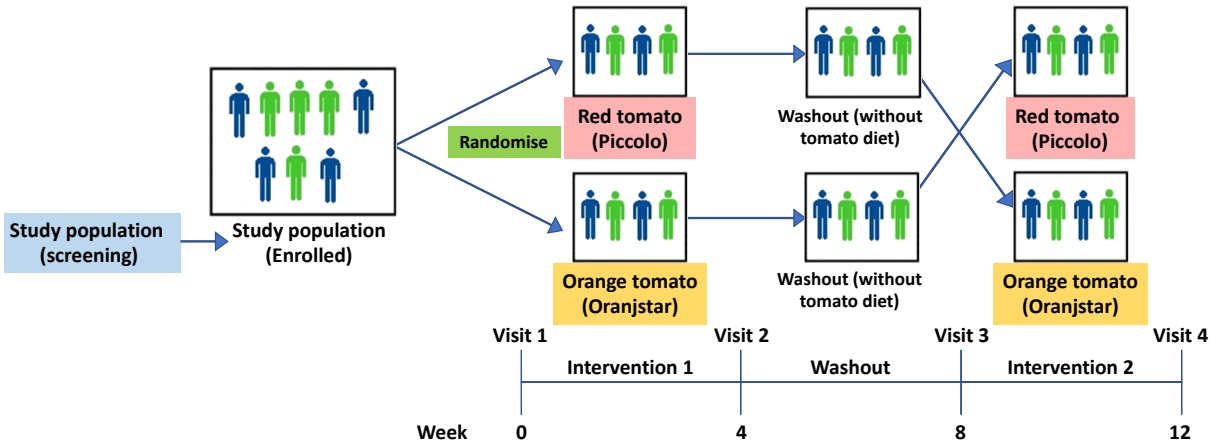


Figure 5.2 Flow diagram of tomato intervention.

5.2.2 Study design

Based on the systematic review in Chapter 3, a daily intake of 15 mg lycopene in 4-week would be sufficient to show the effect on the vascular function and cardiovascular factors (Cheng et al., 2017). In Chapter 4, intake of 300 g of Piccolo and Oranjstar provided 26.22 mg and 7.92 mg of total lycopene respectively which inform the maximum does in order to increase the chances to observe effects of intervention. Therefore, a 4-week period of intervention was chosen to evaluate chronic effects of the interventions tested. According to Gustin et al. (2004), the half-life for the lycopene ranged between 1 day and 2.5 day. Therefore, in corporation of 4 week washout (non-tomato diet) could diminish the impact of carryover effects (Gustin et al., 2004).

This study was designed as a 12-week, cross-over design, randomised controlled dietary interventional study. The study was conducted at the Northumbria University and it was undertaken according to the CONSORT guidelines (**Figure 5.3** and **Appendix D1**) (Schulz et al., 2010). Participants completed a questionnaire including socio-demographic data at the beginning of the study. Participants were also asked to record their dietary intake for 2 non-

consecutive days (i.e. type of foods, preparation and amount of food/drink consumed), one day on weekday and one day on weekend.

During the 12 weeks of the study intervention, participants were asked to consume two different fresh cherry tomato varieties (Piccolo or Oranjstar, 300 g per day according to the randomisation order) for 4 weeks each and 4 weeks of non-tomato diet in between these tomato intervention periods. The participants were instructed to consume tomatoes anytime as snack or with meal and not to consume additional tomato sources. The researcher (HMC) weighted the tomatoes and packed them into in individual food bags the amount to be consumed each day (300g). As in Chapter 4, it had shown that the carotenoid content did not change significantly over 10 days storage, participants received 7 days portions of tomatoes every week and were asked to keep the tomatoes under cool condition.

On each study day, participants arrived at either 0800 or 1000 at the research facility at Northumbria University, UK after a 12-hour fasting. Participants attended four 2-hour study visits (baseline, end of the first, second and last study period). Fasting plasma samples were collected on each of study visit. Additionally, anthropometric measurements, peripheral microvascular vasodilation using Laser Doppler Imaging (LDI) with iontophoresis, arterial stiffness using carotid-femoral pulse wave velocity (cf-PWV) and pulse wave analysis (PWA) and endothelium-dependent vasodilation using FMD were also assessed. During the study days, participants were asked to refrain from strenuous exercise. Participants returned at same time point for the study visit every 4 weeks and repeated all of the measurement mentioned above.

In order to control for other dietary factors, participants were also asked to record food diaries throughout the first study period (4 weeks) and recommended to repeat their own dietary patterns for the washout period (4 weeks) and second study period to match up the food and nutrition intake throughout the whole study. Participants were encouraged to not alter their usual diet and exercise patterns when completing the food diary or study (in conjunction with daily consumption of tomatoes). Before and after each 4 weeks intervention or washout period, the participants attended the study visit for measurements. Compliance with interventions was assessed by asking participants to self-report consumption using daily checklist. In addition, body weight and body composition, and handgrip strength (see below) were measured on each study visit as indicators of evidence of maintenance of habitual lifestyles. Participants were also contacted regularly to discuss any problems related to the supplementation of tomato and to provide encouragement and support.

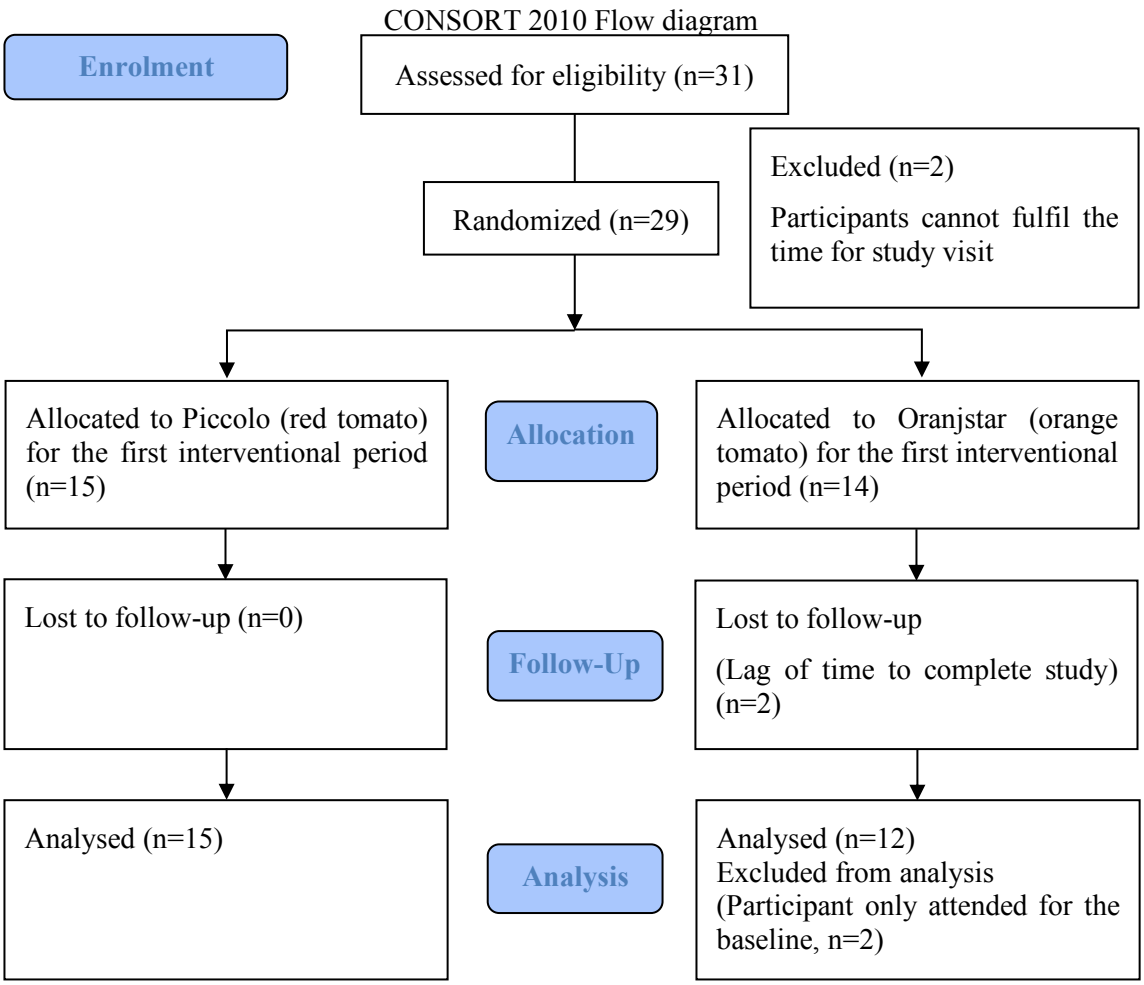


Figure 5.3 CONSORT Flow diagram for reporting of trials.

5.2.3 Outcome measures

The outcome measures of this study included resting (laboratory) blood pressure measurements, basic anthropometric measurements, and plasma samples, microvascular vasodilation by LDI with iontophoresis, endothelium dependent vasodilation by FMD, arterial stiffness by PWA and cf-PWV, 24-Hour ambulatory blood pressure (ABP) and home blood pressure (**Table 5.1**). All measurements were carried in a temperature-controlled room in which the ambient temperature was 23±1°C.

To minimise inter-observer variability all outcome measures in this study were measured by the researcher (Ho Ming Cheng) every time.

Table 5.1 Outcome measure at baseline, 4, 8 and 12 weeks

Primary Outcome Measures	
A. Vascular Function	1. Peripheral microvascular vasodilation by Laser Doppler Imaging (LDI) 2. Endothelium-dependent vasodilation by Flow-mediated dilation (FMD) 3. Arterial stiffness by Carotid-femoral Pulse wave velocity (cf-PWV) and Pulse wave analysis (PWA)
B. Blood pressure	1. Resting (Laboratory) Blood pressure 2. 24hr ambulatory blood pressure 3. Home blood pressure
C. Blood lipid	1. Total cholesterol 2. HDL 3. LDL 4. Triglyceride
D. New established biomarker	1. Interleukin-6 2. Tumour Necrosis Factor Alpha (TNF-α) 3. E-Selectin 4. P-Selectin 5. Intercellular Adhesion Molecule 1 (ICAM-1) 6. Vascular Cell Adhesion Molecules (VCAM-1)
Secondary Outcome Measures	
Anthropometric measurements Body composition assessment Handgrip strength assessment	

5.2.3.1 Resting blood pressure measurements

Resting blood pressure was measured in a quiet room after participants have rested for 10 minutes in a seated position with arm resting on a firm surface and feet flat on the floor, using a non-invasive digital automatic blood pressure monitor (Carescape™ V100: GE Healthcare, UK). Blood pressure measurements were taken in the non-dominant upper arm in triplicate, and the average of the last two measurements was used for subsequent analyses (Pickering et al., 2005).

5.2.3.2 Anthropometric measurements

Anthropometric measurements were taken including: height and weight without shoes (World Health Organization, 1995), which were then used to calculate BMI by dividing body mass (kg) by body height^2 (m) (kg/m^2). A stadiometer was used to measure height to the nearest centimetre (cm). Participants were asked to remove their shoes and position their head in the Frankfurt plane position. Participants were positioned looking straight ahead with the lower border of the left orbit and the tragus of the ear lying on the horizontal plane (Raine and Twomey, 1994). Body weight was measured using Tanita scales after participants

removed outer garments and any personal objects affecting body weight (keys, coins, jewellery etc.). Participants were bare-foot for both height and weight measurements.

5.2.3.3 Body composition assessment

Bioelectrical impedance analysis (BIA) was performed using a single frequency (50 kHz) device (Bodystat 1500, Bodystat Ltd; Isle of Man, UK). Estimations of fat in percentage (%) and in weight (kg), lean weight in percentage (%) and (kg), total water in percentage (%) and litre (L) were recorded. Before measurement, participants were rested in the supine position and have their limbs abducted to avoid current shunting. Bodystat electrode pads were placed in the middle of the dorsal surface of the left hand just proximal to the metacarpophalangeal joints and left foot proximal to the metatarsophalangeal joints; a second set of electrodes was placed between the distal prominence of the radius and the ulnar styloid and between the medial and lateral malleoli at the ankle (**Figure 5.4**) (Simpson et al., 2001).

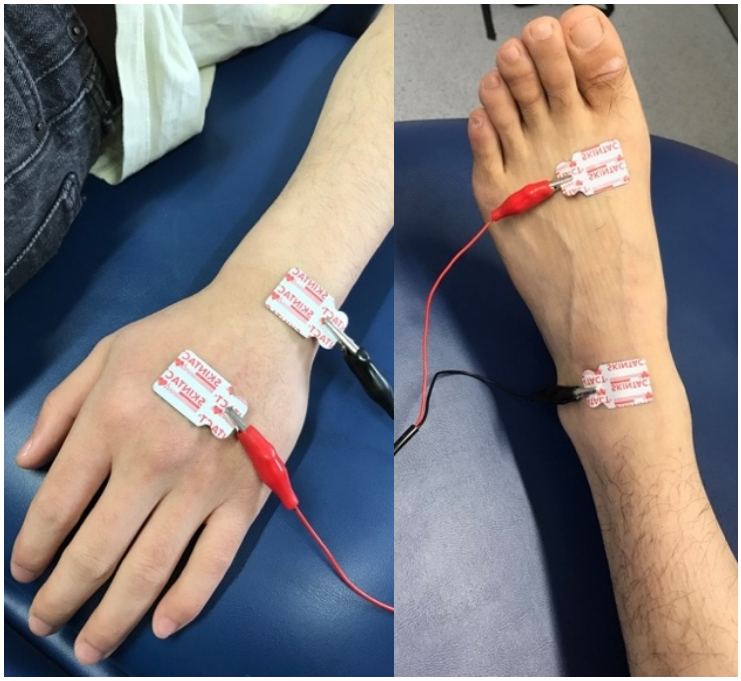


Figure 5.4 Electrodes position on hand and foot.

5.2.3.4 Handgrip strength

Handgrip strength was measured using a CAMRY-EH101 hand dynamometer (range 0 to 90 kg; accuracy 0.1 kg) (EH101; Camry, Guangdong Province, China). In a standing position, participants held the dynamometer in their dominant hand (Meng et al., 2015). The handle of the dynamometer was adjusted if required. When participants were ready, they were instructed to squeeze the dynamometer with maximum effort and hold for approximately 5 seconds without other body movements. The participants were strongly

encouraged by the researcher to give a maximum effort. Handgrip strength was assessed in triplicate and the average of all readings were taken as the final score.

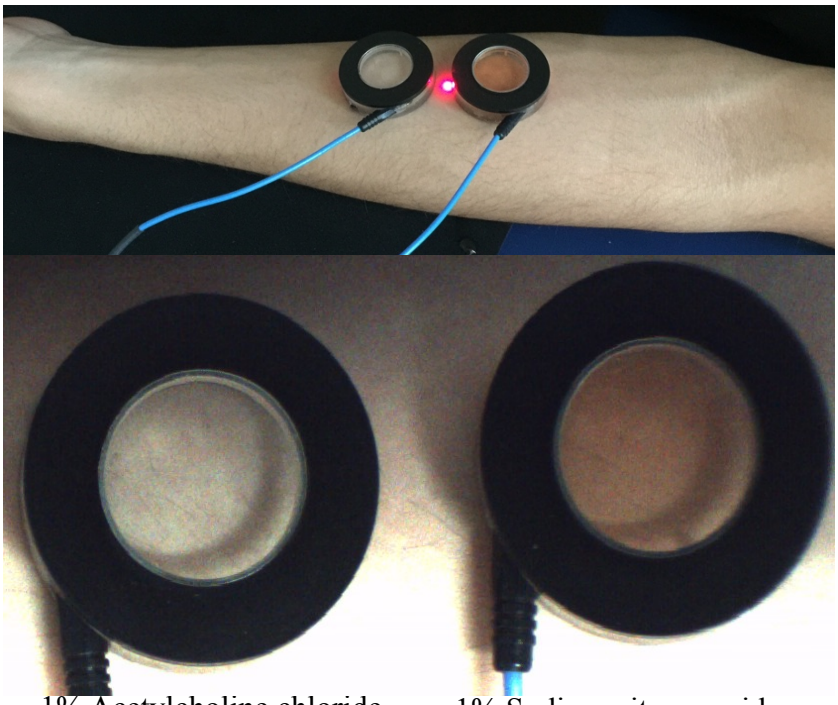
5.2.3.5 Fasting blood sample collection

Fasting blood samples were collected using a butterfly needle inserted into the antecubital vein in right arm; blood was drawn into separate 6 mL Lithium Heparin vacutainer tubes (Becton Dickinson). Tubes were inverted 10 times to mix the blood and anticoagulant inside the tube, and were centrifuged immediately for 10 min at 1200rpm. Plasma samples were immediately stored at -80 ℃ until analysis. Analyses of plasma samples were commenced after the intervention study was completed and all samples for each participant were analysed without knowledge of the treatments, within one batch to reduce inter-batch variation.

5.2.3.6 Measurement of vascular function

Peripheral microvascular vasodilation – Laser Doppler Imaging (LDI)

Peripheral microvascular vasodilation was assessed by Laser Doppler Imaging (LDI) with iontophoresis using a moorLDI2-IR Laser Doppler Imager (Moor Instruments Ltd, Axminster, UK). All measurements were carried out on the left arm after a 30-min rest in a supine position in a quiet room.



1% Acetylcholine chloride (ACh) 1% Sodium nitroprusside (SNP)

Figure 5.5 Iontophoresis set-up for LDI.

Two Perspex iontophoresis chambers (MIC-ION6; Moor Instruments) with an internal platinum wire electrode were attached to the skin with the use of double sided adhesive discs (MIC-1AD; Moor Instruments) on the ventral aspect of the left forearm of the participants avoiding hair, broken skin, scar tissue, skin blemishes and superficial veins (**Figure 5.5**). Both Perspex iontophoresis chambers were connected to the iontophoresis controller (MIC2; Moor Instruments) (Keane et al., 2016). The iontophoresis method of Ferrell et al. (2002) was used to administer acetylcholine chloride, ACh (2.5 mL; 1%; Sigma-Aldrich) in 0.5% NaCl solution at the anodal chamber and sodium nitroprusside, SNP (2.5 mL of 1%; Sigma-Aldrich) in 0.5% NaCl solution at the cathedral chamber, to assess endothelium-dependent and endothelium-independent vasodilation, respectively (Ferrell et al., 2002). Circular glass coverslips were placed over each chamber after addition of the corresponding solution to ensure there is no air bubbles within the chambers and prevent the evaporation of solutions (**Figure 5.5**). Current delivery was controlled by moorLDI Review software version 6.1 (Moor Instruments). Skin perfusion was measured with the use of a moorLDI2-IR Laser Doppler Imager (Moor Instruments). The LDI scanner head was sited 30cm above the chambers and the laser beam was directed via a moving mirror to execute a raster pattern across. A total of twenty repeat scans were measured, the first without current to act as a control, then four scans at 5 μA, four at 10 μA, four at 15 μA and two at 20 μA, giving a total charge of 8 mC; the final five scans were recorded without any current (**Figure 5.6**) (George et al., 2012a). All twenty scans were performed on the left arm after a 30min rest in the supine position in a quiet. The flux compared with time area under the curve over the 20 scans was calculated as a measure of microvascular response to ACh (endothelium-dependent vasodilation) and SNP (endothelium-independent vasodilation) (George et al., 2012a). During the measurement, participants were not allowed to speak or sleep.

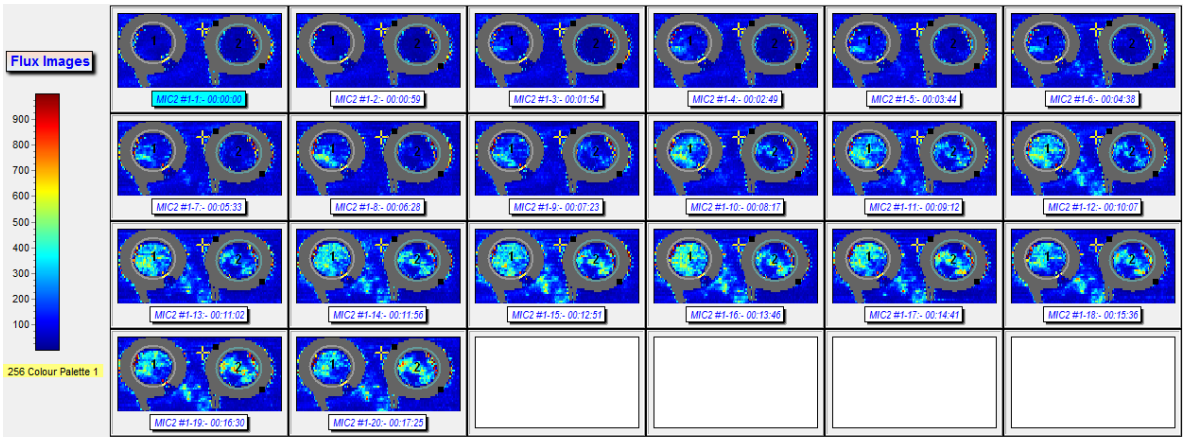


Figure 5.6 Flux images acquired with the moorLDI2-IR Laser Doppler Imager in young healthy man.

Endothelial-dependent vasodilation – Flow mediated dilation (FMD)

Endothelium-dependent vasodilation, as partly nitric oxide mediated dilation, was assessed by noninvasively high-resolution ultrasonography as the percentage of flow-mediated dilation (FMD %) of the brachial artery in the right arm. Participants rested in the supine position for at least 15 minutes to facilitate baseline assessment of heart rate and blood flow (Corretti et al., 2002). To examine brachial artery FMD, the right arm was extended and positioned at an angle of ~90° from the torso (**Figure 5.7**) (Schreuder et al., 2014). Rapid inflation and deflation of the sphygmomanometer cuff is fitted on the right forearm to create a blood flow stimulus in the brachial artery (Corretti et al., 2002).

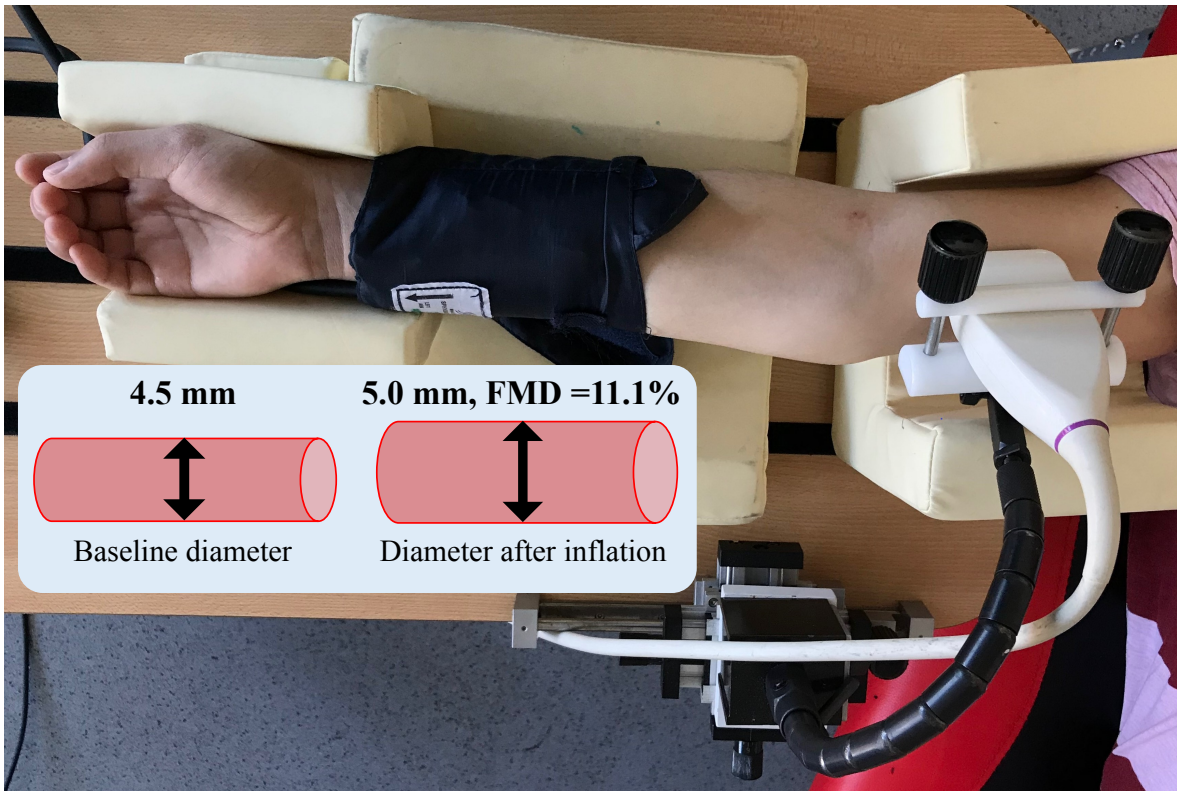


Figure 5.7 Ultrasound imaging of the brachial artery with upper versus lower cuff placement and transducer position above the antecubital fossa.

A 10-MHz multifrequency linear array probe (L12-5 38 mm Linear Array: Bavaria, Germany) connected to a high-resolution ultrasound machine, ATL HDI 5000 ultrasound system (ATL, Philips Medical Systems, Bothell, Washington, USA), was used to image the brachial arteries in the distal one-third of the upper arm (Corretti et al., 2002). The ultrasound parameters were set to optimise the longitudinal, B-mode images of lumen brachial arterial wall interface. Continuous doppler velocity assessment was simultaneously obtained using the ultrasound machine, and was collected using the lowest possible insonation angle (always <60°), which did not vary during each study (**Figure 5.7**) (Corretti et al., 2002). The brachial artery was imaged above the antecubital fossa in the longitudinal plane (Corretti et al., 2002). When an optimal image was obtained, the probe was held stable with a mechanical

arm (**Figure 5.7**). Images of baseline diameter and blood flow were recorded 1 frame per second for 1 minute, using the software Vascular Imager version 6.0.3 (Medical Imaging Applications LLC, USA) (**Figure 5.8A**). The forearm cuff was then inflated to 200 mmHg for 5 minutes, to create forearm ischemia, and images were recorded 1 flame per 10 second. Subsequently, the rapid deflation and image of diameter, to allow reactive hyperaemia, and blood flow were recorded 1 flame per second for 5 minutes (**Figure 5.8B**).

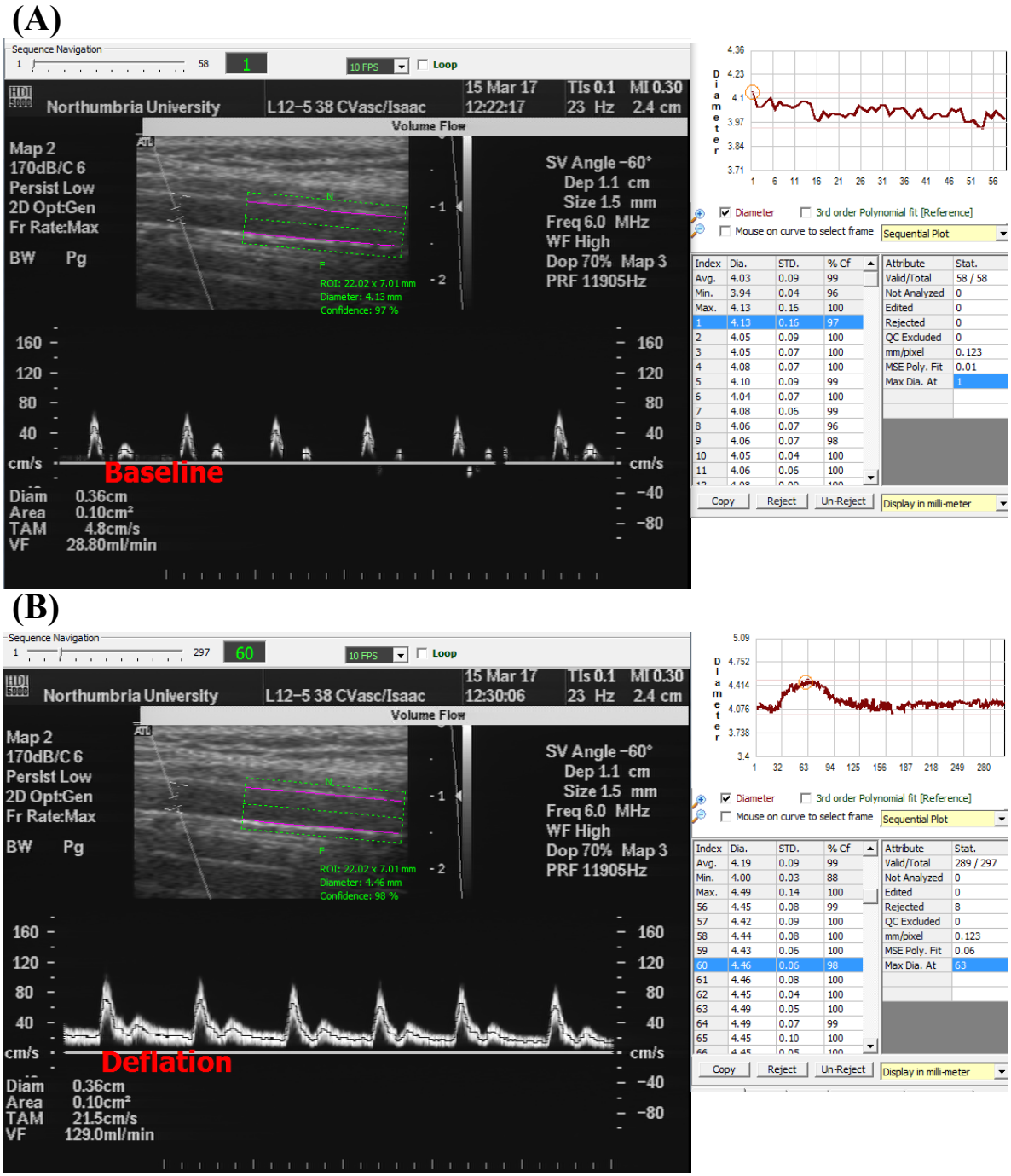


Figure 5.8 Diameter of brachial artery diameter baseline (A) and deflation (B) acquired with the ultrasound in a young healthy man.

Brachial arterial diameter was analysed and measured from the recorded image using the Brachial Analyzer for Research version 5.10.9 (Medical Imaging Applications LLC, USA). During the measurement, participants were not allowed to speak or sleep.

The FMD were all taken by the same researcher for every visit to reduce the possibility of bias. Evaluation of FMD reproducibility prior to the study showed low variability within and between days. Intra-researcher reproducibility for FMD was good with a coefficient of variation (CV) of 5.22% (n= 20). There was no systematic bias between the first and second reading of the same researcher. The reproducibility was in agreement with previous study (Corretti et al., 2002).

Arterial stiffness – Pulse Wave Velocity (cf-PWV) and Pulse Wave Analysis (PWA)

Arterial stiffness was assessed by carotid-femoral pulse wave velocity (cf-PWV) and pulse wave analysis (PWA) using a tonometer of SphygmoCor® CPV System (AtCor Medical Pty. Ltd., Australia) by a single trained researcher (HMC). All measurements were carried out after a 15-min rest in the supine position in a quiet. During the measurement, participants not allowed to speak or sleep.

cf-PWV

The carotid pulse (on the neck) and femoral pulse (on the groin) were located with both middle and index fingers by the researcher (HMC) and their position were highlighted with a marker. The distance from the sternal notch (the "V" in their chest) to the carotid and femoral arteries were measured using measuring tape. The researcher measured the distance from the sternal notch to the mark on the neck in a straight-line and the distance from the sternal notch straight down to the umbilicus (belly button) and then diagonally across to the mark on the groin. Three electrocardiogram (ECG) lead were placed at the sternal notch, sternum and left-hand side of the patient halfway down the rib cage (**Figure 5.9**).

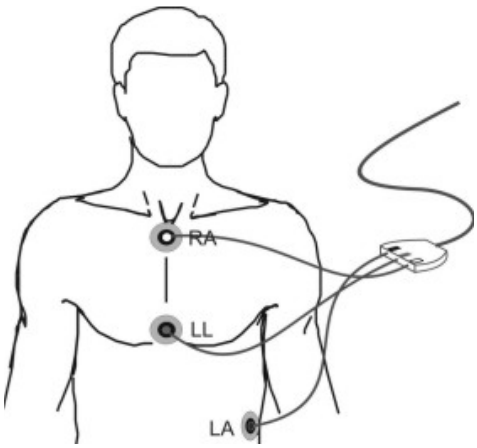


Figure 5.9 Position of ECG lead for PWV measurement.

Pressure wave form in carotid and femoral arteries were measured by a sensitive tonometer. The transit time was calculated as the time between the R-spike in the ECG and the arrival of the pulse wave at the carotid and femoral recording site respectively. After the pulse wave of both sites were captured, the SphygmoCor® software (version 9.0, AtCor Medical, Sydney, Australia) automatically calculated the carotid-femoral PWV (cf-PWV) (**Figure 5.10**).

Figure 5.10 ECG signal (yellow line) and arterial pulse wave at carotid and femoral artery acquired with the SphygmoCor CPV System, in a young health man.

PWA

The atrial pressure (**Figure 5.11A**) in the right radials was recorded using the same tonometer as cf-PWV (SphygmoCor, AtCor Medical) and transformed to central aortic pressure waveform (**Figure 5.11B**) by the general transfer function in a validated software, SphygmoCor® (version 9.0, AtCor Medical, Sydney, Australia) (Bressendorff et al., 2016). The augmentation index (AIx) was calculated as the pressure difference between the second (P_2) and first (P_1) systolic peak as a percentage of the central pulse pressure (PP, difference between central SBP and DBP) (**Figure 5.11B**). All AIx data in this trial were corrected for heart rate at 75bpm (AIx@HR75) (**Figure 5.12**).

Both cf-PWV and PWA were measured in duplicate and averaged. The SphygmoCor® (version 9.0, AtCor Medical, Sydney, Australia) software provides a quality control of the recorded pressure waveforms. If the measurement did not meet these control criteria, it was discarded and replaced by a new measurement.

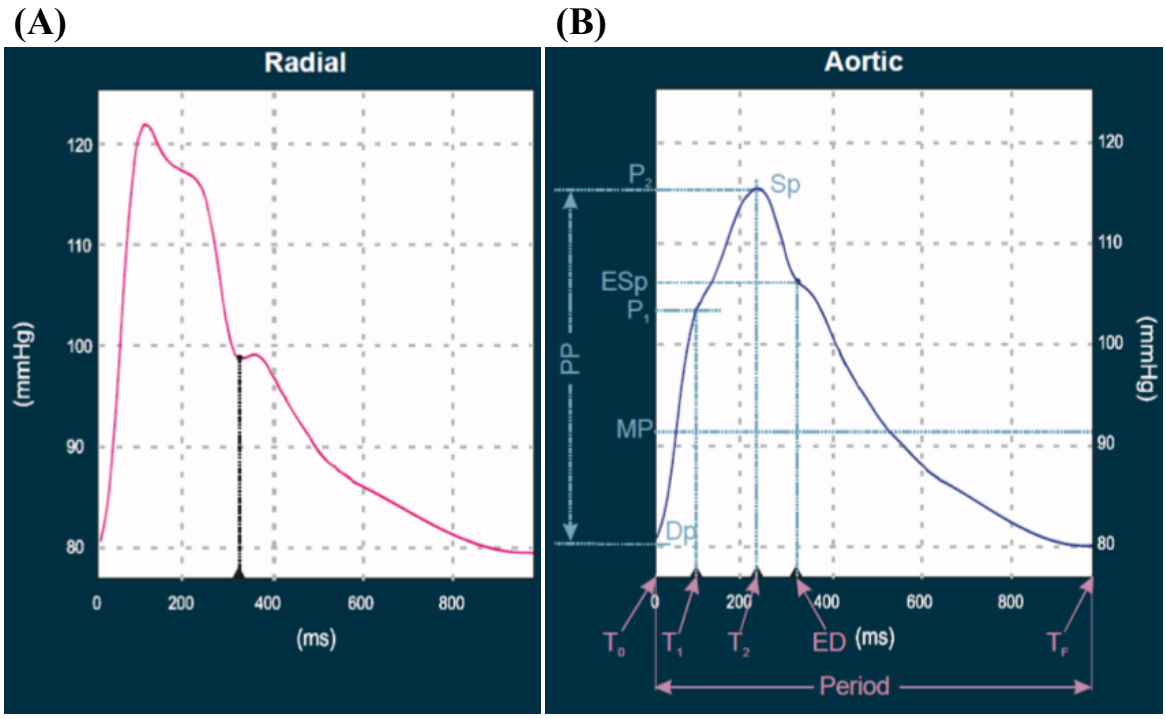


Figure 5.11 Pulse pressure waveforms from the radial artery (A) and the derived waveform in the central aorta by SphygmoCor software (B). PP, Pulse pressure (Sp-Dp); P_1, first systolic peak/shoulder; P_2, systolic peak/shoulder; AIx= (P_2-P_1)/PP.

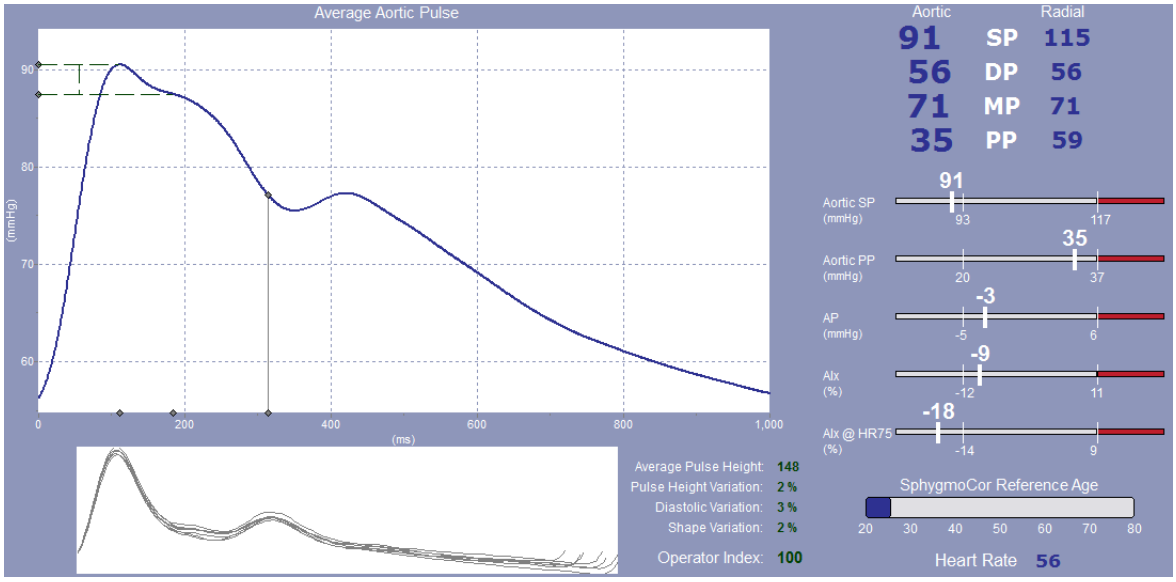


Figure 5.12 Pulse pressure waveforms from the radial artery and the derived waveform in the central aorta acquired with the SphygmoCor CPV System in a young healthy man.

5.2.3.7 24-Hour ambulatory blood pressure (ABP)

24-Hour ABP was recorded by a non-invasive ABP device (Medical 90217-1Q: Spacelabs, Inc. Richmond, Washington, USA). After completing all other measurements, an ABP cuff was fitted by researcher and participants were asked to wear the monitors on for a period of 24-Hours. All readings were performed on the non-dominant upper arm of the participant on each study day. The monitor was programmed (ABP Report Management System version

3.0.3 Spacelabs, Inc. Richmond, Washington, USA) to inflate automatically, at 30-min intervals during 0800-2200 and 60-min intervals during 2200-0800, for a total period of 24-Hours. Participants were instructed to act and work as normal between 08:00 and 22:00 and rest or sleep between 22:00 and 08:00 and avoid any strenuous cardiovascular exercise during the 24 hour period. Participants were instructed to keep their arm relaxed down the side of their body, and keep still until the end of each subsequent measurement. While in walking, the participants were asked to stop and stand still for a minute until the measurement finished (Hobbs et al., 2012). Separate averages were obtained for the 24-Hour, daytime (10:00 to 20:00) and nighttime (00:00 to 06:00) values.

5.2.3.8 Home blood pressure

All participants were asked to measure their blood pressure at home in the morning every day during the last week of each study period. Blood pressure and HR were measured in the non-dominant upper arm in triplicate with the use of fully automatic blood pressure recorder (Omron® M3: HEM-7200-E, Omron Healthcare, Kyoto, Japan) while sitting and after having rested for 10 minutes.

5.2.3.9 Plasma samples analysis

All plasma samples were frozen at -80 ℃ and kept until analysis.

Plasma soluble vascular and intracellular adhesion molecules (sVCAM-1 and sICAM-1), were analysed by commercial ELISA kits for Human sVCAM-1 (Cat. No.: DDVC00) (**Appendix D2**) and sICAM-1 (Cat. No.: DCD540) (Quantikine®, R&D Systems, Inc., USA) (**Appendix D3**), respectively according to the manufacturer's specifications. Plasma soluble E-selectin (sE-selectin) and P-selectin (sP-selectin) were measured by ELISA kits for Human E-selectin (Cat. No.: RAB0422) and P-selectin (Cat. No.: RAB0426) (Sigma-Aldrich, St Louis, MO, USA) (**Appendix D4**). Plasma pro- and anti-inflammatory cytokines, IL-6 and TNF-α, were measured by commercial ELISA kits for human IL-6 (Cat. No.: KHC0061) (**Appendix D5**) and TNF-α (Cat. No.: KHC3012) (Invitrogen®, Camarillo, CA) (**Appendix D6**), respectively following the procedures of the manufacturers.

Lipid profile, (including total cholesterol, triglycerides, HDL and LDL), insulin and glucose content in plasma were measured by The Laboratories of the Integrated Laboratory Medicine in Freeman Hospital at Newcastle Upon Tyne. Plasma triglycerides were measured by an enzymatic, colorimetric method with glycerol phosphate oxidase and peroxidase (Trigl, Cobas Roche Diagnostics, Indianapolis, IN, USA). Total plasma cholesterol was obtained by an enzymatic, colorimetric method through the cholesterol esterase/cholesterol

oxidase/peroxidase reaction (CHOL2, Cobas Roche Diagnostics, Indianapolis, IN, USA). The plasma concentration of HDL-cholesterol was determined by a homogeneous enzymatic colorimetric assay through the cholesterol esterase/cholesterol oxidase/peroxidase reaction (HDLC3, Cobas Roche Diagnostics, Indianapolis, IN, USA). Plasma glucose concentrations were determined with a colorimetric assay (GLUC3, Cobas, Roche Diagnostics GmbH, Mannheim, Germany). Commercial enzyme-linked immunosorbent assay (ELISA) kits were used to measure insulin (Iso-Insulin ELISA, Mercodia AB, Uppsala, Sweden) according to the manufacturer's specifications.

5.2.3.10 Nutritional analysis

Participants were asked to record their dietary intake for 2 non-consecutive days (i.e. type of foods, preparation and amount of food/drink consumed), one day on week day and one day on weekend. The record of the dietary intake allowed a nutritional analysis with the aid of the ''Microdiet'' software (Microdiet System, Version 6.4, Salford University, UK). In addition, Participants were required to keep record of their dietary intake during the first 4 weeks of the study and then asked to repeat their food consumption during the next 8 weeks.

5.2.4 Statistical analysis

Participant data and recorded measurements were inputted into and statistically analysed using IBM SPSS Statistics version 24. Data were evaluated for normality of distribution using the Shapiro-Wilk test.

The General Linear Model (GLM) for repeated measures was used to compare treatments. Unadjusted results (mean±SEM) as well as results adjusting for covariates including baseline values, results obtained after the washout period, and randomisation order are presented with Bonferroni correction test for multiple comparisons. Paired t-tests were used to compare changes from baseline.

Analysis was carried out on the participants as a whole as well as subgrouped by age: <30 (AgeCat=1) and >30 (AgeCat=2), to identify patterns in data.

A p value less than 0.05 was classified as significant.

5.3 Result

The study was completed at 9th August 2018. Overall, 27 participants completed the entire trial (93.2%) after baseline assessment. Self-reported daily checklist of tomato supplementation showed a good acceptability and compliance with tomato supplementation. No side-effect of interventions was reported about supplementation of tomato. The results of the study were reported in detail in Chapter 6 and 7.

5.4 Discussion

The study outcomes were discussed in detail in Chapter 6 and 7.

5.5 Conclusion

The present protocol for RCT showed the consumption of 300 g cherry tomato on the health effects on the vascular function, blood pressure, blood lipid and CVD risk biomarker.

5.6 Research Questions Raised from The Present Chapter

The work described in this chapter raised the following questions:

1. Do different varieties of cherry tomato have the same extent of beneficial effects on BP and vascular function?
2. Do these health benefits differ according to age?

This question will be addressed in Chapter 6 by examining the effects of Piccolo and Oranjstar on blood pressure and vascular function. We also aimed to assess whether different varieties of cherry tomato would have same extent of beneficial effects on different age groups.

Chapter 6 The Effects of Piccolo and Oranjstar on Vascular Function and Cardiovascular Factors

This study was designed to answer the research questions in **section 5.6**.

6.1 Introduction

Cardiovascular disease is a multifactorial disease, which is a result of interactive effects of different combinations of risk factors such as high blood pressure, high blood cholesterol, smoking, obesity, diabetes and lack of physical activity. The systematic reviews and meta-analyses in Chapter 3 showed that previous RCT on the effects of tomato on resting BP, however only few studies have evaluated 24-Hour BP, a more accurate marker of BP (Cheng et al., 2017). In contrast, less is known on whether there are beneficial effects of tomato on vascular function especially on microvascular vasodilation and arterial stiffness (Cheng et al., 2017). We aimed to determine the beneficial effects of tomato on BP measured using different methods and outcomes of vascular function. We also aimed to compare whether two varieties of cherry tomato, Piccolo and Oranjstar, have the same extent of beneficial effects on BP and vascular function.

6.2 Method

6.2.1 Measurement of BP and vascular function

The method of measuring BP and non-invasive assessment of vascular function were described in detail in Chapter 5 (**section 5.2.3.1** and **5.2.3.6-5.2.3.8**).

6.2.2 Statistical analysis

The statistical analysis was described in detail in Chapter 5 (**section 5.2.4**).

6.3 Results

6.3.1 Participants baseline characteristic

The characteristics of the sample studied are shown in **Table 6.1**. In total 27 participants completed the intervention study, ranging from an age of 19 to 60 years. The participants were statistically analysed as a full sample size as well as being split into two different age groups. These were categorised as below 30 years of age, which included 16 participants, and above 30 years of age, including 11 participants. BMI values ranged from 16.5 kg/m^2 to 32.5 kg/m^2 among all participants, but the mean BMI difference between the two age groups was small (2 kg/m^2) (**Table 6.1**).

Table 6.1 Participants baseline characteristic.

Baseline characteristics	All participants (n=27)		Below 30yrs (n=16)		Above 30yrs (n=11)	
	Mean	SEM	Mean	SEM	Mean	SEM
Age (year)	32.1	2.64	21.9	0.68	46.9	2.54
Height (m)	1.8	0.01	1.8	0.02	1.8	0.02
Weight (kg)	72.2	2.28	69.4	3.22	76.1	2.83
BMI (kg/m^2)	23.3	0.65	22.5	0.88	24.5	0.88
Resting SBP (mmHg)	116.2	2.15	118.2	3.11	113.4	2.63
Resting DBP (mmHg)	71.1	1.51	70.1	2.09	72.6	2.16
HR (bpm)	68.4	1.62	68.4	2.21	68.4	2.50
24-Hour BP						
SBP (mmHg)	123.7	1.88	124.5	2.46	122.6	3.04
DBP (mmHg)	74.3	1.20	73.0	1.45	76.1	2.01
Daytime BP						
SBP (mmHg)	128.0	1.94	129.1	2.64	126.5	2.91
DBP (mmHg)	78.3	1.34	76.8	1.79	80.6	1.89
Nighttime BP						
SBP (mmHg)	112.6	2.10	113.9	2.69	110.8	3.41
DBP (mmHg)	64.2	1.23	63.9	1.71	64.6	1.81
Handgrip strength (kg)	42.76	1.33	41.52	2.03	44.56	1.34
Body fat (%)	17.13	0.82	14.68	0.80	20.68	0.90
Body fat (kg)	12.65	0.90	10.43	0.99	15.87	1.13
Lean (kg)	59.52	1.62	59.01	2.41	60.26	2.00
Water (%)	59.22	0.83	60.11	1.24	57.94	0.91
Water (L)	42.36	0.96	41.24	1.29	43.99	1.35
Impedance 50KHz	525.52	12.06	541.06	16.95	502.91	14.66
LDI-ACh	158.50	10.36	168.25	12.86	144.31	17.02
LDI-SNP	187.02	12.73	175.62	14.78	203.60	22.60
Baseline brachial artery diameter (mm)	4.03	0.11	3.89	0.13	4.23	0.20
Post-release brachial artery diameter (mm)	4.23	0.11	4.11	0.13	4.41	0.20
FMD%	5.26	0.68	5.85	1.02	4.40	0.78
cf-PWV (m/s)	6.15	0.18	5.71	0.18	6.80	0.25
PWA (AIx, %)	4.49	2.63	-4.31	2.34	17.30	2.16
PWA@75bpm (AIx, %)	-2.22	2.60	-10.75	2.36	10.18	2.27

6.3.2 Study compliance

The average energy intake of participants from 2-d diet diaries were in line with the DRV (**Table 6.2**). The weight, BMI, handgrip strength and body composition, including body fat, water mass and impedance of the participants did not differ significantly between baseline, washout period and after intake of both Piccolo and Oranjstar (**Table 6.3**).

Table 6.2 The average daily intakes of nutrients by participants from 2-d diet diaries comparable with the RNI's.

Nutrients	Reference Nutrient intake (RNIs)	Mean	SD
Protein (g)	50	107.6	35.4
Fat (g)	<70	86.6	29.0
Carbohydrate (g)	>260	253.1	76.3
Energy (kcal)	2000	2201.0	574.8
Energy (kJ)		9249.5	2401.0
Total Sugars (g)	90	78.1	42.2
Total Saturates (g)	<20	30.5	14.3
Total Monounsaturate (g)		23.3	10.7
Total Polyunsaturate (g)		9.6	4.0
Tot Trans Fatty Acid (g)		0.9	0.9
Total n3 fatty acid (g)		0.5	0.4
Total n6 fatty acid (g)		4.8	5.2
Non-starch Polysacch (g)		9.6	5.8
Starch (g)		116.5	59.1
Potassium (mg)		2181.9	840.2
Calcium (mg)		512.6	269.8
Magnesium (mg)		229.7	137.0
Iron (mg)		7.9	3.3
Copper (mg)		1.0	0.4
Zinc (mg)		8.1	3.9
Thiamin (B1) (mg)		26.0	13.6
Riboflavin (B2) (mg)		4.3	1.7
Vitamin B6 (mg)		15.0	6.8
Vitamin B12 (µg)		168.5	90.8
Vitamin C (mg)		8.6	43.3
Vitamin D (µg)		334.0	700.4
Vitamin E (alpha-t) (mg)		1329.5	2328.1
Beta-carotene (µg)		0.8	1.6

Table 6.3 Effects of Piccolo and Oranjstar on anthropometric data in healthy men.

Variable	N	Unadjusted				*P*	Adjustment for baseline and washout				*P†*	Adjustment for baseline, washout and randomisation order				*P‡*
		Piccolo		Oranjstar			Piccolo		Oranjstar			Piccolo		Oranjstar		
		Mean	SEM	Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
Grip strength (kg)	27	44.69	1.29	45.14	1.38	0.51	44.69	0.57	45.14	0.48	0.50	44.69	0.58	45.14	0.48	0.49
Body fat (%)	27	17.16	0.89	17.25	0.86	0.69	17.16	0.16	17.25	0.18	0.66	17.16	0.17	17.25	0.18	0.66
Body fat (kg)	27	12.68	0.98	12.74	0.98	0.78	12.68	0.17	12.74	0.27	0.78	12.68	0.17	12.74	0.28	0.78
Water (%)	27	59.12	0.90	59.13	0.84	0.97	59.12	0.16	59.13	0.22	0.97	59.12	0.16	59.13	0.22	0.96
Water (It)	27	42.11	0.91	42.01	0.91	0.55	42.11	0.15	42.01	0.19	0.55	42.11	0.15	42.01	0.20	0.56
Impedance (50KHz)	27	527.81	10.49	529.00	9.64	0.73	527.82	3.38	529.00	4.13	0.72	527.82	3.45	52.00	4.20	0.72

†Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline and washout as covariate.
‡Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline, washout and randomisation order as covariate with Bonferroni correction test.
LSM= Least Squared Means (Marginal means).

6.3.3 Blood pressure

6.3.3.1 Resting BP

The resting SBP was significantly reduced by 3.3 mmHg (p=0.021) after consumption of Piccolo, and no change was observed after consumption of Oranjstar in the group of all participants when compared to the baseline. There was no significant difference in resting SBP between Piccolo and Oranjstar intervention in either the younger or older groups, or when these were combined (**Table 6.4**, **Table 6.5** and **Table 6.6**).

After the consumption of Piccolo and Oranjstar, there were no change in resting DBP in the group of all participants when compared to the baseline. However, there was no significant difference in resting DBP changes between Piccolo and Oranjstar intervention in either the younger or older groups, or when these were combined (**Table 6.4**, **Table 6.5** and **Table 6.6**).

6.3.3.2 Ambulatory blood pressure (ABP)

After the consumption of Piccolo and Oranjstar, there were no change in the 24-Hour ambulatory SBP in the group of all participants when compared to the baseline. There was no significant difference in the 24-Hour ambulatory SBP between Piccolo and Oranjstar intervention in either the younger or older groups, or when these were combined (**Table 6.4**, **Table 6.5** and **Table 6.6**).

After the consumption of Piccolo and Oranjstar, the 24-Hour ambulatory DBP was significantly reduced by 2.33 mmHg (p=0.006) and 1.56 mmHg (p=0.049) respectively in the group of all participants when compared to the baseline. After adjusting for covariate (baseline, washout and randomisation order), 24-Hour ambulatory DBP was significantly lower 1.8 mmHg (p=0.029) after Piccolo consumption compared with Oranjstar in the age group above 30 years of age (**Table 6.6**). The age group below 30 years of age showed no significant differences in the comparison of unadjusted or adjusted results.

After the consumption of Piccolo and Oranjstar, there were no change in the daytime ambulatory SBP in the group of all participants when compared to the baseline. In age group above 30 years of age, daytime ambulatory SBP was significantly lower 3.3 mmHg (p=0.043) after Piccolo consumption compared with Oranjstar and the significance remained after adjusting for covariate (**Table 6.6**). Age group below 30 years of age showed no significant differences in the comparison of unadjusted or adjusted results.

After consumption of Piccolo, the daytime ambulatory DBP was significantly reduced by 1.78 mmHg (p=0.047) and no change was observed after consumption of Oranjstar in the group of all participants when compared to the baseline. In age group above 30 years of age, daytime ambulatory DBP was significantly lower 3.0 mmHg (p=0.025) followed by Piccolo consumption compared with Oranjstar and the significance remained after adjusting for covariate (**Table 6.6**). Age group below 30 years of age showed no significant differences in the comparison of unadjusted or adjusted results.

The nighttime ambulatory SBP was significantly reduced by 4.52 mmHg (p=0.039) after consumption of Piccolo and no change was observed after consumption of in the group of all participants when compared to the baseline. Nighttime ambulatory SBP was significantly lower 2.9 mmHg (p=0.042) followed by Piccolo consumption in the group of all participants, compared with Oranjstar and the significance remained after adjusting for covariate (**Table 6.4**).

After the consumption of Piccolo and Oranjstar, the nighttime ambulatory DBP was significantly reduced 3.82 mmHg (p=0.016) and 2.63 mmHg (p=0.026) respectively in the group of all participants when compared to the baseline. However, there were no significant differences in nighttime ambulatory DBP changes between Piccolo and Oranjstar intervention groups in either the younger or older groups, or when these were combined (**Table 6.4**, **Table 6.5** and **Table 6.6**).

6.3.3.3 Home BP

After the consumption of Piccolo and Oranjstar, the home SBP did not change in the group of all participants when compared with washout period. There were no significant differences in the home SBP between Piccolo and Oranjstar intervention in either the younger or older groups, or when these were combined (**Table 6.4**, **Table 6.5** and **Table 6.6**).

After the consumption of Piccolo and Oranjstar, the home DBP did not change in the group of all participants when compared with washout period. There were no significant differences in the home DBP between Piccolo and Oranjstar intervention in either the younger or older groups, or when these were combined (**Table 6.4**, **Table 6.5** and **Table 6.6**).

Table 6.4 Effects of Piccolo and Oranjstar on blood pressure in all healthy men.

Variable	N	Unadjusted					Adjustment for baseline and washout					Adjustment for baseline, washout and randomisation order				
		Piccolo		Oranjstar		*P*	Piccolo		Oranjstar		*P†*	Piccolo		Oranjstar		*P‡*
		Mean	SEM	Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
Resting SBP (mmHg)	27	112.9	1.58	113.6	1.72	0.44	112.9	0.75	113.6	1.08	0.46	112.9	0.75	113.6	1.10	0.46
Resting DBP (mmHg)	27	68.2	1.33	68.8	1.38	0.46	68.2	0.97	68.8	0.89	0.47	68.2	0.99	68.8	0.90	0.48
24-Hour ABP																
SBP (mmHg)	27	121.7	1.85	122.6	1.86	0.32	121.7	1.07	122.6	0.80	0.33	121.7	1.02	122.6	0.80	0.33
DBP (mmHg)	27	71.9	1.31	72.7	1.46	0.32	71.9	0.80	72.7	0.73	0.30	71.9	0.80	72.7	0.74	0.30
Daytime ABP																
SBP (mmHg)	27	126.8	2.00	128.0	1.82	0.34	126.8	1.12	128.0	0.99	0.35	126.8	1.10	128.0	1.01	0.34
DBP (mmHg)	27	76.6	1.38	78.0	1.46	0.17	76.6	0.78	78.0	0.86	0.17	76.6	0.71	78.0	0.88	0.15
Nighttime ABP																
SBP (mmHg)	27	108.1	2.24	111.0	1.91	0.042*	108.1	1.92	111.0	1.45	0.048*	108.1	1.93	111.0	1.48	0.049*
DBP (mmHg)	27	60.4	1.42	61.6	1.33	0.27	60.4	1.36	61.6	1.08	0.27	60.4	1.36	61.6	1.10	0.27
Home BP																
SBP (mmHg)	27	116.9	2.02	117.7	2.05	0.41	116.9	0.91	117.7	0.94	0.42	116.9	0.93	117.7	0.89	0.39
DBP (mmHg)	27	73.5	1.11	72.3	1.23	0.08	72.3	0.64	73.5	0.54	0.08	72.3	0.64	73.5	0.52	0.06

†Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline and washout as covariate.
‡Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline, washout and randomisation order as covariate with Bonferroni correction test.
LSM= Least Squared Means (Marginal means).
*$p<0.05$.

Table 6.5 Effects of Piccolo and Oranjstar on blood pressure in healthy men below 30 years of age.

Variable	N	Unadjusted					Adjustment for baseline and washout					Adjustment for baseline, washout and randomisation order				
		Piccolo		Oranjstar		*P*	Piccolo		Oranjstar		*P†*	Piccolo		Oranjstar		*P‡*
		Mean	SEM	Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
Resting SBP (mmHg)	16	113.1	2.14	113.7	2.39	0.68	113.1	0.91	113.7	1.55	0.70	113.1	0.95	113.7	1.58	0.70
Resting DBP (mmHg)	16	65.9	1.38	65.9	1.28	1.00	65.9	1.26	65.9	0.99	1.00	65.9	1.31	65.9	0.99	1.00
24-Hour ABP																
SBP (mmHg)	16	122.2	2.62	122.3	2.27	0.92	122.2	1.61	122.3	1.12	0.94	122.2	1.64	122.3	1.16	0.92
DBP (mmHg)	16	69.7	1.69	69.8	1.49	0.95	69.7	1.13	69.8	0.99	0.96	69.7	1.15	69.8	1.03	0.96
Daytime ABP																
SBP (mmHg)	16	127.9	2.89	127.6	2.09	0.86	127.9	1.57	127.6	1.38	0.85	127.9	1.60	127.6	1.41	0.84
DBP (mmHg)	16	74.4	1.90	74.8	1.54	0.81	74.4	1.22	74.8	1.26	0.81	74.4	1.11	74.8	1.30	0.78
Nighttime ABP																
SBP (mmHg)	16	109.3	3.09	111.1	2.64	0.29	109.3	2.74	111.1	1.75	0.31	109.3	2.85	111.1	1.76	0.31
DBP (mmHg)	16	59.6	1.90	59.1	1.55	0.66	59.6	1.64	59.1	0.97	0.67	59.6	1.70	59.1	1.00	0.69
Home BP																
SBP (mmHg)	16	117.3	3.00	118.7	2.94	0.28	117.3	1.17	118.7	0.98	0.30	117.3	1.20	118.7	0.91	0.26
DBP (mmHg)	16	71.8	1.16	70.4	1.14	0.15	70.4	0.78	71.8	0.80	0.17	70.4	0.80	71.8	0.68	0.14

†Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline and washout as covariate.
‡Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline, washout and randomisation order as covariate with Bonferroni correction test.
LSM= Least Squared Means (Marginal means).

Table 6.6 Effects of Piccolo and Oranjstar on blood pressure in healthy men above 30 years of age.

Variable	N	Unadjusted					Adjustment for baseline and washout					Adjustment for baseline, washout and randomisation order				
		Piccolo		Oranjstar		*P*	Piccolo		Oranjstar		*P†*	Piccolo		Oranjstar		*P‡*
		Mean	SEM	Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
Resting SBP (mmHg)	11	112.6	2.42	113.4	2.53	0.22	112.6	0.80	113.4	1.28	0.26	112.6	0.84	113.4	1.37	0.30
Resting DBP (mmHg)	11	71.6	2.29	73.0	2.37	0.28	71.5	1.43	73.0	1.68	0.24	71.5	1.42	73.0	1.79	0.25
24-Hour ABP																
SBP (mmHg)	11	121.1	2.62	123.1	3.30	0.13	121.1	1.12	123.1	0.90	0.08	121.1	1.01	123.1	0.76	0.10
DBP (mmHg)	11	75.2	1.71	77.0	2.36	0.10	75.2	0.73	77.0	0.78	0.02*	75.2	0.71	77.0	0.73	0.029*
Daytime ABP																
SBP (mmHg)	11	125.3	2.61	128.6	3.39	0.043*	125.3	1.15	128.6	1.09	0.011*	125.3	1.16	128.6	1.08	0.017*
DBP (mmHg)	11	79.6	1.61	82.6	2.20	0.025*	79.6	0.71	82.6	1.00	0.026*	79.6	0.61	82.6	1.02	0.038*
Nighttime ABP																
SBP (mmHg)	11	106.5	3.27	110.9	2.84	0.08	106.5	2.70	110.9	1.90	0.12	106.5	2.57	110.9	1.91	0.14
DBP (mmHg)	11	61.6	2.19	65.2	1.95	0.07	61.5	1.96	65.2	0.97	0.09	61.5	1.90	65.2	1.00	0.10
Home BP																
SBP (mmHg)	11	116.4	2.50	116.3	2.73	0.90	116.4	0.82	116.3	1.26	0.90	116.4	0.84	116.3	1.29	0.90
DBP (mmHg)	11	76.0	1.97	75.2	2.34	0.35	75.2	1.15	76.0	0.69	0.36	75.2	1.10	76.0	0.72	0.34

†Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline and washout as covariate.
‡Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline, washout and randomisation order as covariate with Bonferroni correction test.
LSM= Least Squared Means (Marginal means).
*$p<0.05$.

6.3.4 Vascular function

6.3.4.1 FMD

After consumption of Piccolo and Oranjstar, FMD did not change in the group of all participants when compared to the baseline. In the age group above 30 years, FMD was significantly improved by 3.43% (p=0.026) after intake of Piccolo when compared with Oranjstar and the significant change remained after the covariate adjustment (**Table 6.9**). Age group below 30 years of age showed no significant differences in the comparison of unadjusted or adjusted results (**Table 6.8**).

6.3.4.2 Arterial stiffness

After the consumption of Piccolo and Oranjstar, cf-PWV did not change in the group of all participants when compared to the baseline. There were no significant differences in cf-PWV between Piccolo and Oranjstar intervention in either the younger or older groups, or when these were combined (**Table 6.7**, **Table 6.8** and **Table 6.9**).

After the consumption of Piccolo and Oranjstar, PWA did not change in the group of all participants when compared to the baseline. In the age group above 30 years, consumption of Piccolo significantly improved PWA by 2.48% (p=0.013) and no change was observed after intake of Oranjstar, when compared to the baseline. There were no significant differences in PWA between Piccolo and Oranjstar intervention in either the younger or older groups, or when these were combined (**Table 6.7**, **Table 6.8** and **Table 6.9**).

6.3.4.3 LDI

After the consumption of Piccolo and Oranjstar, LDI-ACh did not change in the group of all participants when compared to the baseline. There were no significant differences in LDI-ACh between Piccolo and Oranjstar intervention in either the younger or older groups, or when these were combined (**Table 6.7**, **Table 6.8** and **Table 6.9**). After the consumption of Piccolo and Oranjstar, LDI-SNP did not change in the group of all participants when compared to the baseline. There were no significant differences in LDI-SNP between Piccolo and Oranjstar intervention in either the younger or older groups, or when these were combined (**Table 6.7**, **Table 6.8** and **Table 6.9**).

Table 6.7 Effects of Piccolo and Oranjstar on non-invasive assessment in all healthy men.

Variable	N	Unadjusted					Adjustment for baseline and washout					Adjustment for baseline, washout and randomisation order				
		Piccolo		Oranjstar		*P*	Piccolo		Oranjstar		*P†*	Piccolo		Oranjstar		*P‡*
		Mean	SEM	Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
FMD (%)	27	6.38	0.70	5.48	0.69	0.26	6.38	0.72	5.48	0.68	0.24	6.38	0.72	5.48	0.68	0.25
PWV (m/s)	27	6.03	0.17	5.91	0.17	0.26	6.03	0.09	5.91	0.12	0.26	6.03	0.92	5.91	0.12	0.26
PWA (AIx, %)	27	3.62	2.22	3.58	2.69	0.98	3.62	0.80	3.58	1.06	0.98	3.62	0.82	3.58	1.06	0.98
PWA@75bpm (AIx, %)	27	-3.34	2.30	-3.72	2.74	0.78	-3.34	0.80	-3.72	1.13	0.77	-3.34	0.82	-3.72	1.15	0.78
LDI-Ach (PU)	27	160.00	10.06	168.49	10.90	0.39	160.00	9.86	168.49	10.09	0.40	160.00	9.90	168.49	10.28	0.41
LDI-SNP (PU)	27	191.39	15.81	209.42	15.01	0.21	191.39	11.04	209.42	12.76	0.22	191.39	10.58	209.42	12.49	0.23

†Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline and washout as covariate.
‡Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline, washout and randomisation order as covariate with Bonferroni correction test.
LSM= Least Squared Means (Marginal means).

Table 6.8 Effects of Piccolo and Oranjstar on non-invasive assessment in healthy men below 30 years of age.

Variable	N	Unadjusted					Adjustment for baseline and washout					Adjustment for baseline, washout and randomisation order				
		Piccolo		Oranjstar		*P*	Piccolo		Oranjstar		*P†*	Piccolo		Oranjstar		*P‡*
		Mean	SEM	Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
FMD (%)	16	5.61	0.75	6.44	1.01	0.25	5.61	0.81	6.44	1.03	0.26	5.61	0.76	6.44	0.98	0.27
PWV (m/s)	16	5.48	0.13	5.35	0.14	0.32	5.48	0.11	5.35	0.12	0.32	5.48	0.11	5.35	0.12	0.31
PWA (AIx, %)	16	-4.09	1.82	-5.10	2.76	0.65	-4.09	1.27	-5.10	1.65	0.61	-4.09	1.28	-5.10	1.68	0.62
PWA@75bpm (AIx, %)	16	-11.44	1.76	-12.64	2.74	0.58	-11.44	1.14	-12.64	1.80	0.57	-11.44	1.15	-12.64	1.85	0.58
LDI-Ach (PU)	16	165.30	10.51	169.97	12.56	0.64	165.30	10.91	169.97	13.02	0.64	165.30	11.35	169.97	12.89	0.61
LDI-SNP (PU)	16	171.13	16.43	179.41	14.08	0.28	171.13	12.26	179.41	12.83	0.20	171.13	12.40	179.41	13.20	0.22

†Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline and washout as covariate.
‡Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline, washout and randomisation order as covariate with Bonferroni correction test.
LSM= Least Squared Means (Marginal means).

Table 6.9 Effects of Piccolo and Oranjstar on non-invasive assessment in healthy men above 30 years of age.

Variable	N	Unadjusted					Adjustment for baseline and washout					Adjustment for baseline, washout and randomisation order				
		Piccolo		Oranjstar		*P*	Piccolo		Oranjstar		*P†*	Piccolo		Oranjstar		*P‡*
		Mean	SEM	Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
FMD (%)	11	7.51	1.30	4.08	0.73	0.026*	7.51	1.33	4.08	0.73	0.042*	7.51	1.38	4.08	0.66	0.035*
PWV (m/s)	11	6.83	0.20	6.72	0.18	0.59	6.83	0.15	6.72	0.18	0.57	6.83	0.16	6.72	0.20	0.59
PWA (AIx, %)	11	14.82	1.76	16.21	1.63	0.19	14.82	0.68	16.21	0.94	0.23	14.82	0.63	16.21	0.99	0.21
PWA@75bpm (AIx, %)	11	8.44	1.90	9.26	1.81	0.45	8.44	0.94	9.26	1.13	0.49	8.44	0.91	9.26	1.19	0.51
LDI-Ach (PU)	11	152.30	19.86	166.33	20.32	0.49	152.30	18.67	166.34	10.69	0.38	152.30	16.61	166.34	10.70	0.36
LDI-SNP (PU)	11	220.86	29.30	253.07	26.15	0.35	220.86	22.77	253.07	24.91	0.39	220.86	22.06	253.07	24.98	0.43

†Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline and washout as covariate.
‡Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline, washout and randomisation order as covariate with Bonferroni correction test.
LSM= Least Squared Means (Marginal means).
*$p<0.05$.

6.4 Discussion

The aim of this study was to evaluate the impact of a 4-week dietary intervention of two varieties of tomato on BP and vascular function in healthy male subjects.

6.4.1 Principle findings

6.4.1.1 Blood pressure

The present study showed that the consumption of 300 g of Piccolo (containing 26.22 mg of total lycopene) and Oranjstar (7.92 mg of total lycopene) over a 4-week period produced a positive effect on BP. The resting SBP was significantly reduced by 3.3 mmHg (p=0.021) after consumption of Piccolo when compared to the baseline. After the consumption of Piccolo and Oranjstar, the 24-Hour ambulatory DBP was significantly reduced by 2.33 mmHg (p=0.006) and 1.56 mmHg (p=0.049) respectively when compared to the baseline. However, Piccolo showed a greater reduction in BP. Specifically in the age group above 30 years of age, there was a significant reduction in daytime ambulatory SBP and DBP compared with Oranjstar. In addition, a substantial decrease in nighttime ambulatory SBP by 4.52 mmHg (p=0.039) and nighttime ambulatory DBP reduced by 3.82 mmHg (p=0.016) and a significant decrease in daytime ambulatory DBP by 1.78 mmHg (p=0.047) following the consumption of Piccolo for group of all participants when compared to the baseline. No significant differences in the younger group, or in any other BP measurements were observed. These results indicate that consuming tomato as part of an overall diet brings benefits that in the long-term may translate into reducing the risk of CVD. These results remained after adjustments for baseline values.

6.4.1.2 Vascular function

The present study showed that the consumption of 300 g of Piccolo and Oranjstar over a 4-week period produced a positive effect on EF. However, Piccolo showed greater improvements in EF. In the age group above 30 years, consumption of Piccolo significantly improved PWA by 2.48% (p=0.013) from baseline and a borderline-significant trend to improvement of FMD by 3.11% (p=0.054) was observed when compared to the baseline. No significant differences in the younger group, or in any other vascular measurements were observed. These results indicate that consuming tomato as part of an overall diet brings benefits that in the long-term may translate into reducing the risk of CVD. In the age group above 30 years, consumption of Piccolo significantly improved FMD by 3.43% (p=0.026)

when compared to intake of Oranjstar. These results were robust and statistical significance remained after adjustments for baseline FMD values.

6.4.2 Meaning of the current findings

The results of BP reduction were significant given the fact that high BP remains ranked 1st among the leading 30 level-3 global risk factors for DALYs (**Figure 1.13**) (GBD 2015 Risk Factors Collaborators, 2016). The results from the Framingham Heart Study (in 1948-1966) showed that a 2 mmHg decrease in DBP could reduce the prevalence of hypertension by 17% and reduce the risk of CHD by 6% (Hardy et al., 2015). Furthermore, a meta-analysis has shown a reduction in SBP by 10 mmHg and 5 mmHg DBP can reduce the risk of stroke by around 41% (33-38%) (Gaciong et al., 2013). A similar study reported that a 10 mmHg SBP and 5 mmHg DBP reduction also reduces chance of stroke by a third, CHD events by a quarter and heart failure by a quarter, regardless of the health of the patient prior to reduction (Law et al., 2009). In addition, one third of CHD and all-cause mortality can be attributed to elevated SBP (Hardy et al., 2015). These previous studies provide evidence on the significant effect of lowering BP on reducing prevalence of hypertension, CVD, CHD, heart failure, stroke and therefore a reduction in mortality. Consequently, the results of this dietary intervention indicate that a higher intake of trans-lycopene found in Piccolo was associated with causing a significant reduction in both SBP and DBP. Therefore, if Piccolo is included in the diet regularly, it could help to prevent BP related conditions and in the long-term reduce the levels of mortality. The cis-lycopene in the Oranjstar showed non-significant reduction on BP. It may be due to the lower total lycopene content compared to that of Piccolo.

As several methods of BP measurement were used during this study, comparisons between BP measurements can be made. For example, the highest measurement recorded was daytime 24hr ambulatory SBP/DBP (126.8/76.6 mmHg). This is expected and in agreement with other data available as measurements taken during this time are when the subject is awake and active during the day when the BP is likely to be at its highest. The lowest values recorded were nighttime SBP/DBP (108.1/60.4 mmHg), which would be expected as during the nighttime when participants were inactive BP would be lower. The second lowest measurement was resting office BP (112.9/68.2 mmHg) while home SBP/DBP (116.9/73.5 mmHg) was higher than resting office BP. This is not expected as commonly under clinical settings patient's BP tends to be slightly higher due to the uncomfortable and unfamiliar environment (white coat hypertension); while under relaxed environment (at home), the patient's BP was expected to be lower.

FMD in the present study also have significant health implications. A recent systematic review reporting continuous risk estimates, CVD risk was RR 0.90 per 1% higher FMD (95% CI 0.86 to 0.94, $p<0.01$) (Ras et al., 2013). Regarding health status, the observed association between FMD and CVD risk was stronger for diseased populations RR 0.87 (95% CI 0.83 to 0.92, $p<0.01$) than for asymptomatic populations RR 0.96 (95% CI 0.92 to 1.00, $p>0.03$) (Ras et al., 2013). The RR for every 10% increase in AIx was 1.51 (95% CI 1.23 to 1.86, $p<0.0001$) for all-cause mortality and 1.48 (95% CI 1.16 to 1.90, $p<0.0001$) for CV mortality (London et al., 2001). Another systematic review showed that age- and sex-adjusted HR per 1-SD change in loge PWV were 1.35 (95% CI 1.22 to 1.50; $p<0.001$) for CHD, 1.54 (95% CI 1.34 to 1.78, $p<0.001$) for stroke, and 1.45 (95% CI 1.30 to 1.61, $p<0.001$) for CVD (Ben-Shlomo et al., 2014). Additionally, an increase in every 1 m/s in PWV was associated with a 15% higher CVD risk (Verbeke et al., 2011).

6.4.3 Comparison to previous studies

The systematic review in Chapter 3 showed that, lycopene supplementation significantly reduced SBP by 5.66 mmHg, but no significant reduction in DBP (Cheng et al., 2017). When comparing these results to the findings of the present study where there was a significant reduction in daytime SBP by 4.52 mmHg as well as in DBP by 3.82 mmHg. This could be due to the splitting of age groups in the present study. The significant changes were only found in the older group whereas the systematic review covered all different ages.

A study into the effect of tomato products on CVD resulted in a significant reduction in DBP after a 6 week high tomato diet when compared to a low tomato diet but no significance in SBP reduction (Burton-Freeman et al., 2015). Again, with non-significant reduction in SBP and DBP, this difference could be due to the splitting into age categories in this study. There were not many current studies regarding tomatoes and BP. Therefore, comparing the results overall with the studies available, this study seems to be in line with the data.

In Chapter 3, it has shown that, chronic (>1 week) tomato supplementation significantly increased FMD by 2.53% (95% CI 0.56 to 4.50, $p=0.01$, $I^2=0\%$) (Cheng et al., 2017). However acute studies (<24 hours) did not show any improvements (1.46%; 95% CI -0.33 to 3.26, $p=0.11$) (Cheng et al., 2017). Previous results were compared to the findings of the present study where there was a non-significant trend to improvement of FMD by 3.11% ($p=0.054$) after Piccolo supplementation when compared to the baseline and FMD was significantly improved by 3.43% ($p=0.026$) after intake of Piccolo when compared with Oranjstar. The significant change remained after the covariate adjustment. In addition, the

meta-analysis in Chapter 3 showed that PWV did not differ between high intake of tomato interventions and washout period, mean difference was 0.93 m/s (95% CI -0.20 to 2.06, p=0.11) (Cheng et al., 2017). This result was in line with the findings of the present study where Piccolo and Oranjstar non-significantly reduced PWV by 0.12 m/s (p=0.357) and 0.25 m/s (p=0.161) respectively when compared to the baseline.

6.4.4 Retrospective power calculation for future study

The retrospective sample size calculation was derived from the changes in nighttime SBP response after consumption of Piccolo and Oranjstar. The two-tails t-test to compare the differences between two dependent means was used. Calculations considered a Post hoc, a 0.05 significance level and a 0.8 correlation coefficient between groups. The estimated participants required to allow detection a difference of 3 mmHg between the responses to the Piccolo and Oranjstar intervention, based on current study, was estimated to be 37 to give power of 0.8 as shown in **Figure 6.1**.

However, if retrospective sample size calculation was derived from the changes in FMD response after consumption of Piccolo and Oranjstar, the estimated participants required to allow detection a difference of 3.42%, was estimated to be 15 to give power of 0.8 as shown in **Figure 6.2**. The two-tails t-test to compare the differences between two dependent means was used. Calculations considered a Post hoc, a 0.05 significance level and a 0.263 correlation coefficient between groups.

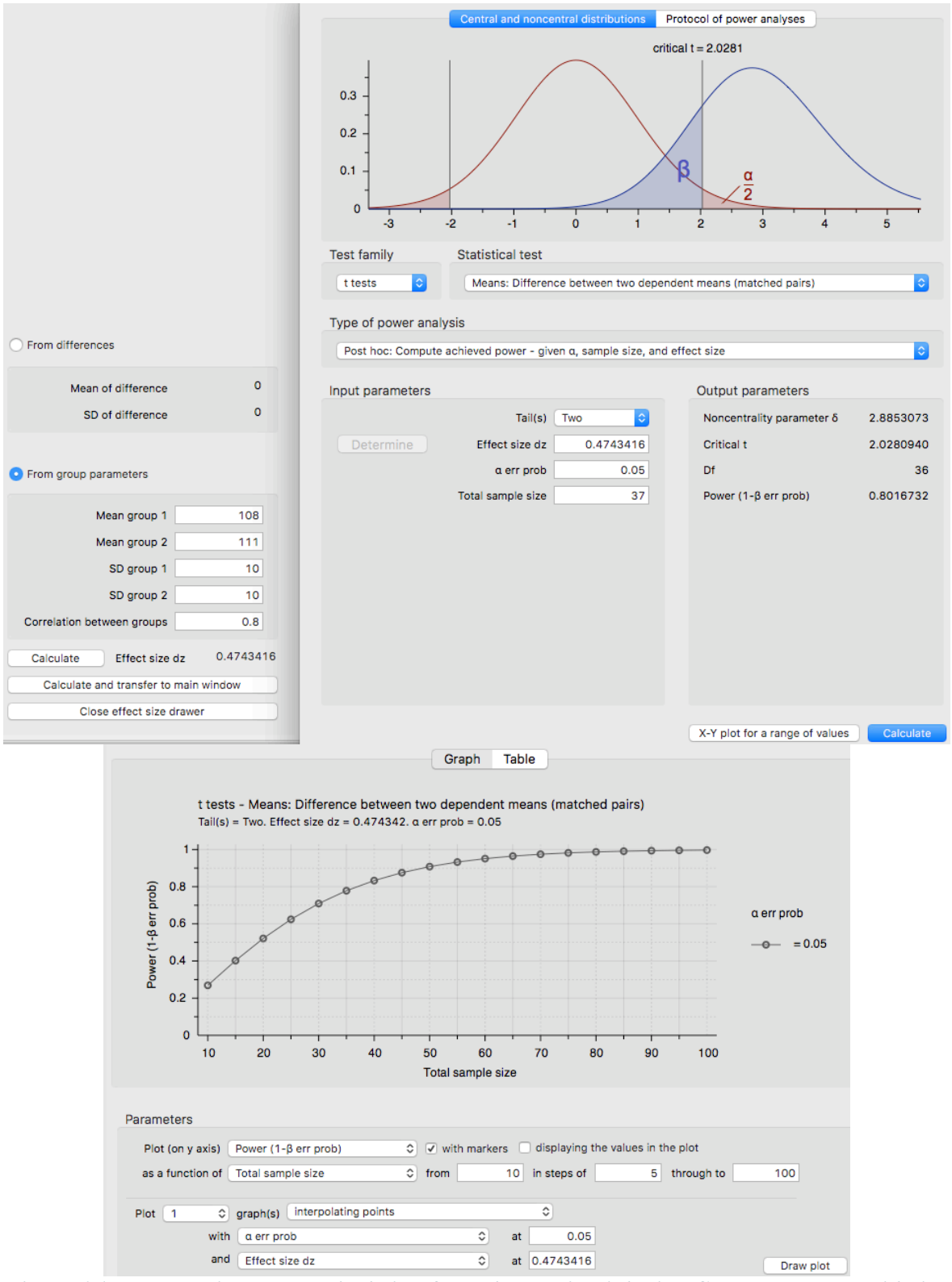


Figure 6.1 Retrospective power calculation from changes in night time SBP response considering a Post hoc, a 0.05 significance level, a correlation coefficient between groups of 0.47.

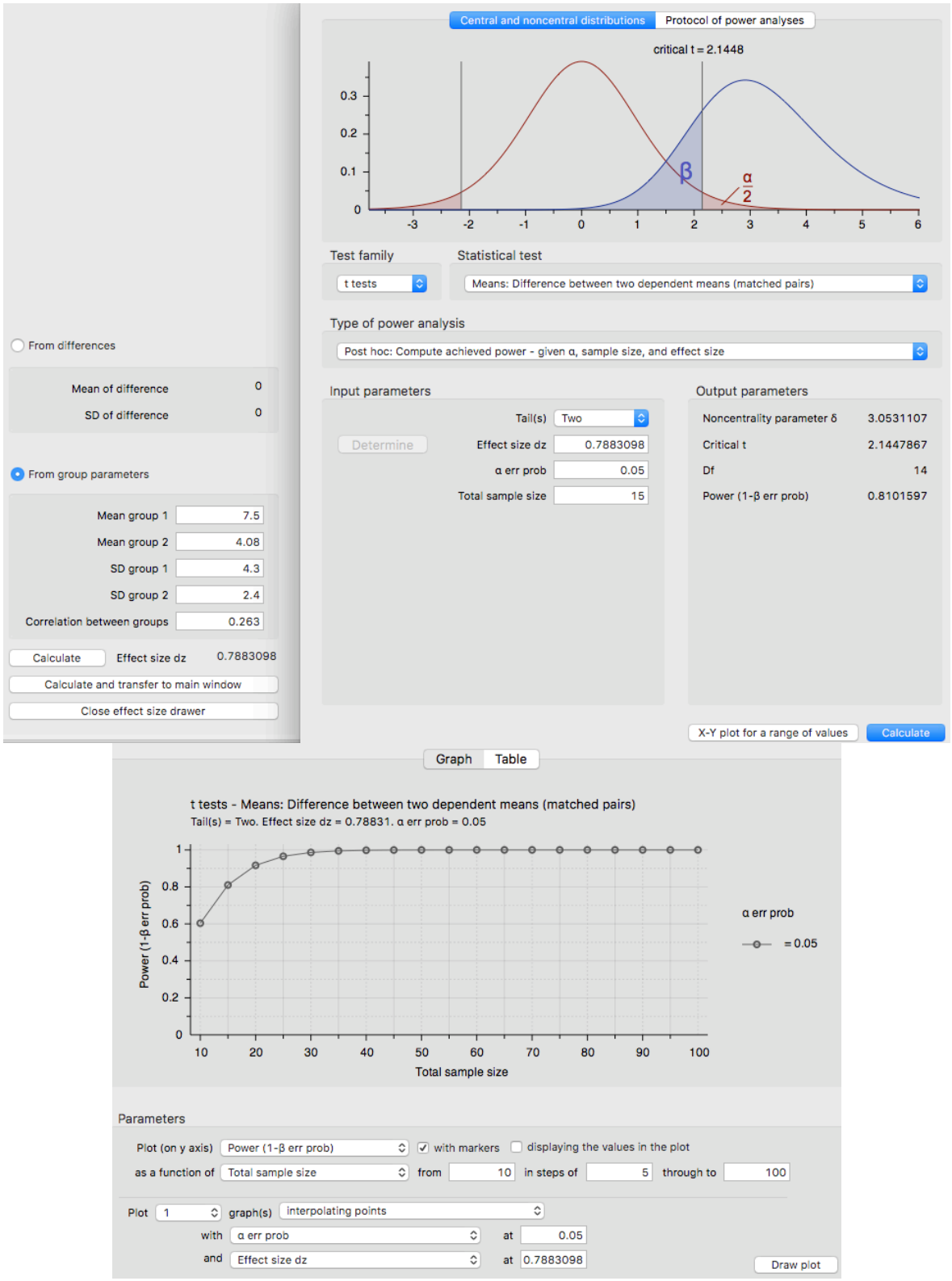


Figure 6.2 Retrospective power calculation from the changes in FMD response considering a Post hoc, a 0.05 significance level, a correlation coefficient between groups of 0.47.

6.4.5 Strengths

This study has a number of strengths. Firstly, the improvements observed in SBP and FMD reported in this study were documented using robust methodology. BP changes were documented using ambulatory 24-hr BP rather than resting BP (more likely to be affected by external factors to the intervention).

The fact that the amount/dose of tomato to be consumed in this study was accurately weighed by the researcher and provided as separate parcels, and the fact that the two tomato varieties were provided by Thanet earth, reduces the risk of variability in consumption and composition of the tomato. Our analysis of the lycopene content showed no variations over time, therefore adding confidence that participants received a constant dose of lycopene throughout the study. The lycopene content and β-carotene content of cherry tomato used in present study were in line with previous studies (**Table 4.3**). In addition, Piccolo purchased from supermarket had similar carotenoid content to the Piccolo used in this study (**Table 4.3**). Therefore, population supplementing Piccolo purchased from supermarket could have the same beneficial effects as present study.

The measurements were all taken by the same researcher, so it would be consistent for every visit and reduce the possibility of bias. Furthermore, evaluation of FMD reproducibility prior to the study showed low variability within and between days. Intra-researcher reproducibility for FMD was good with a coefficient of variation (CV) of 5.22% (n= 20). Moreover, there was low systematic bias between the measurements from the same the researcher. This study implemented randomised allocation achieving a balanced order of interventions across groups which would reduce the influence of order effects. Subjects were not screened or selected on the basis of metabolic risk factors, so regression to the mean could not have confounded the results.

As mentioned in Chapter 5, of the 31 enrolled subjects, 29 completed attended the baseline assessment and 27 completed the entire trial (93.2%); drop outs were due to inability to fulfil the time for study visits (**Figure 5.3)**. Participants were contacted regularly to discuss any problems related to the supplementation of tomato. Self-reported daily checklist of tomato supplementation showed a good acceptability and compliance with tomato supplementation. No side-effect of interventions was reported about supplementation of tomato. In addition, the fact that BMI, body composition and handgrip strength remained unchanged during the whole clinical trial, supports the presence of good compliance with maintaining usual diet and physical activity patterns.

The fact that only men were studied, excludes the confounding associated with hormonal changes during menstrual cycles affecting outcome measures in women.

6.4.6 Limitations

This study is, however, not without limitations. For example, only males took part in the study, and results cannot be extrapolated to women. Although that is the case, female BP can vary through their menstrual cycle, therefore conducting the study with females could produce skewed results. This study was an "open label" study that could not blind participants and researchers. However, this is a common situation in dietary interventions. Although, participants were taking the same amount of tomato for both varieties, the total lycopene content of both tomatoes were not the same. This could be the reason Oranjstar showed lower improvement in BP and FMD. This level of tomato intake in this study was not intended to represent an amount to be recommended but to show that consumption of tomatoes was clearly sufficient to induce significant risk factor improvements.

6.4.7 Possible mechanisms and implications for clinicians or policymakers.

Tomatoes are not only rich in essential vitamins and minerals but also very low in fats, cholesterol or sodium, and thus, are great for cardiovascular health (**Table 4.4**) (Willcox et al., 2003). Lycopene is one of the most powerful antioxidants and free radical quenchers. Our results indicate that the role of lycopene in lowering SBP might be attributed to its role as an antioxidant and free radical quencher, which could inhibit oxidative stress, indirectly stimulate production of NO in the endothelium and improve vascular function. The antioxidant nature of lycopene found in tomatoes can act as an anticarcinogenic, chemopreventive agent helping to inhibit development of cancer cells. There has been research into the effects of lycopene and specifically prostate cancer prevention (Sporn and Liby, 2013; Zu et al., 2014). Therefore, this intervention could have also had other possible health benefits on the participants but have not been monitored. Lycopene could also help to reduce synthesis of cholesterol and increase low-density lipoprotein degradation (Cheng et al., 2017; Story et al., 2010). LDL-cholesterol builds up in the arteries and contributes to the development of atherosclerosis. The results of this study are important to health-related professionals. Nutritionists and dietitians should encourage a diet with an increased lycopene intake through tomato consumption to reduce BP and further reduce the risk of CVD, and also potentially decrease the risk of cancer, specifically prostate cancer. Especially for patients with hypertension or borderline hypertension, the intake of fruit and vegetables should be encouraged and the positive effects of Piccolo consumption should be highlighted.

6.4.8 Unanswered questions and future research

In this study, only raw tomato was used for the dietary intervention, rather than tomato products such as tomato paste, juice or cooked tomatoes, which are associated with enhanced bioavailability of lycopene. These other forms of tomato products and lycopene supplements could be evaluated and ascertain the effects of varying lycopene doses and their effects on BP. Cooking vegetables changes their chemical composition and could affect the bioaccessibility of the vitamins and minerals (Chan et al., 2014). Future studies could also alter the dose (e.g. lower doses) of tomato supplemented and the length of intervention (longer periods). The possibility of combining another food with the lycopene rich tomatoes is also an attractive approach to test whether additional effects could be observed from food combinations such as beetroots alongside tomato. Beetroots have been shown to help lower BP but have never been trialled alongside tomato consumption and this could have a positive effect on the body (Hobbs et al., 2013a; Lara et al., 2016; Siervo et al., 2013). This study was conducted with only male participants, and so there would be room for further research with lycopene supplementation and effects on female BP.

6.5 Conclusion

In conclusion, this study provided evidence on the benefits of the consumption Piccolo, for four weeks, which had a significant impact on reducing both ambulatory SBP and DBP, and improving FMD, therefore lowering the risk of cardiovascular disease in healthy, particularly in older, adult males.

6.6 Research Questions Raised from The Present Chapter

The work described in this chapter raised the following questions:

1. Do different varieties of cherry tomato have the same extent of beneficial effects on blood-borne biomarkers of vascular function?
2. Do these health benefits differ according to age?
3. Are changes in FMD associated with improvements on blood-borne biomarkers of vascular function?

These questions will be addressed in Chapter 7 by examining the effects of Piccolo and Oranjstar on blood-borne cardiovascular biomarkers. We also aimed to assess whether different varieties of cherry tomato would have the same extent of beneficial effects.

Chapter 7 The Effects of Piccolo and Oranjstar on Blood-Borne Circulating Biomarkers and Other Cardiovascular Factors

This study was designed to answer the research questions in **section 6.7**.

7.1 Introduction

In addition to vascular function and BP, a large number of traditional and emergent circulating biomarkers are significant predictors of CVD. A number of biomarkers have become well-established (also called traditional) markers of atherosclerosis, CVD, disability, and mortality. The traditional circulating biomarkers include blood lipid profile (total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride (TG)). Some of these have been included in a number of algorithms, such as the Framingham Risk Score and the German Prospective Cardiovascular Münster (PROCAM) model, to calculate estimated risks of CHD and CVD.

Previous human trials have shown the protective effects of tomato on blood lipid. There are numerous emergent circulating biomarkers including interleukin 6 (IL-6), tumour necrosis factor alpha (TNF-α), soluble intercellular adhesion molecules (sICAM-1), soluble vascular adhesion molecules (sVCAM-1), E-selectin and P-selectin. However, these inflammatory markers have been less studied. We aimed to determine the beneficial effects of tomato on blood lipid profile including TG, TC, LDL-cholesterol, HDL-cholesterol and inflammatory markers including TNF-α, IL-6, E-selectin, P-selectin, sICAM-1, and sVCAM-1. We also aimed to assess whether different varieties of cherry tomato would have the same extent of beneficial effects.

7.2 Method

7.2.1 Blood sample collection and measurement of blood circulating CVD risk markers

The method of the collection of blood samples and the measurement of blood circulating CVD risk markers were described in detail in Chapter 5 (**section 5.2.3.9**).

7.2.2 Statistical analysis

The statistical analysis was described in detail in Chapter 5 (**section 5.2.4**).

7.3 Result

7.3.1 Participants baseline characteristic

In total 27 participants completed the intervention study, ranging from an age of 19 to 60 years. The participants were statistically analysed as a full sample size as well as being split into two different age groups. These were categorised as below 30 years of age, which included 16 participants, and above 30 years of age, including 11 participants. BMI values ranged from 16.5 kg/m^2 to 32.5 kg/m^2 among all participants, but the mean BMI difference between the two age groups was small (2 kg/m^2) (**Table 6.1**). The baseline blood-borne biomarkers characteristics of the participants are shown in **Table 7.1**. The blood-borne marker level of all participants was in normal range.

Table 7.1 Participants baseline characteristic of blood-borne biomarkers.

Baseline characteristics	All participants (n=27)		Below 30yrs (n=16)		Above 30yrs (n=11)	
	Mean	SEM	Mean	SEM	Mean	SEM
Fasting glucose (mmol/L)	5.46	0.20	5.23	0.08	5.78	0.46
Total-cholesterol (mmol/L)	4.53	0.14	4.51	0.16	4.55	0.25
HDL-cholesterol (mmol/L)	1.26	0.06	1.31	0.07	1.18	0.10
LDL (mmol/L)	3.42	0.16	3.21	0.17	3.72	0.28
Triglycerides (mmol/L)	1.06	0.09	1.07	0.14	1.05	0.11
Interleukin 6 (ng/mL)	3.35	0.36	3.56	0.55	3.06	0.39
TNF-α (pg/mL)	25.01	1.76	27.35	2.52	21.51	1.88
sICAM (ng/mL)	210.38	14.63	189.09	16.72	239.41	24.18
sVCAM (ng/mL)	432.75	25.56	445.48	39.12	416.55	31.36
P-selectin (ng/mL)	45.08	1.72	41.95	1.73	49.34	2.93
E-selectin (ng/mL)	30.45	2.63	26.46	2.57	35.88	4.79

7.3.2 Blood lipid profile

7.3.2.1 Triglyceride

After the consumption of Piccolo and Oranjstar, TG did not change in the group of all participants when compared to the baseline. There were no significant differences in TG between Piccolo and Oranjstar intervention in either the younger or older groups, or when these were combined (**Table 7.2**, **Table 7.3** and **Table 7.4**).

7.3.2.2 Total cholesterol

After the consumption of Piccolo and Oranjstar, TC did not change in the group of all participants when compared to the baseline. In the age group above 30 years, consumption of Piccolo and Oranjstar significantly increased TC by 0.423 mmol/L (p=0.013) and 0.45 mmol/L (p=0.02) respectively, when compared to the baseline. There were no significant differences in TC between Piccolo and Oranjstar intervention in either the younger or older groups, or when these were combined (**Table 7.2**, **Table 7.3** and **Table 7.4**).

7.3.2.3 LDL-cholesterol

After the consumption of Piccolo and Oranjstar, LDL-cholesterol did not change in the group of all participants when compared to the baseline. There were no significant differences in LDL-cholesterol between Piccolo and Oranjstar intervention in either the younger or older groups, or when these were combined (**Table 7.2**, **Table 7.3** and **Table 7.4**).

7.3.2.4 HDL-cholesterol

After the consumption of Piccolo and Oranjstar, HDL-cholesterol did not change in the group of all participants when compared to the baseline. After adjusting for baseline, HDL-cholesterol was significantly increase 0.04 mmol/L (p=0.048) followed by Piccolo consumption compared with Oranjstar in the group of all participants (**Table 7.2**).

7.3.3 Blood glucose level

After the consumption of Piccolo and Oranjstar, blood glucose level did not change in the group of all participants when compared to the baseline. There were no significant differences in blood glucose level between Piccolo and Oranjstar intervention in either the younger or older groups, or when these were combined (**Table 7.2**, **Table 7.3** and **Table 7.4**).

7.3.4 Blood insulin level

Insulin was only measured after each tomato intervention. There were no significant differences in blood insulin level between Piccolo and Oranjstar intervention in either the younger or older groups, or when these were combined (**Table 7.2**, **Table 7.3** and **Table 7.4**).

Table 7.2 Effects of Piccolo and Oranjstar on blood-borne biomarkers in healthy men.

Variable	Unadjusted						Adjustment for baseline and washout					Adjustment for baseline, washout and randomisation order				
	N	Piccolo		N	Oranjstar		*P*	Piccolo		Oranjstar		*P†*	Piccolo		Oranjstar		*P‡*
		Mean	SEM		Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
Glucose (mmol/l)	16	5.25	0.08	16	5.14	0.12	0.14	5.25	0.07	5.14	0.11	0.16	5.25	0.06	5.14	0.08	0.10
Total cholesterol (mmol/l)	16	4.40	0.17	16	4.53	0.22	0.33	4.40	0.11	4.53	0.11	0.30	4.40	0.11	4.53	0.10	0.14
HDL (mmol/l)	16	1.31	0.05	16	1.26	0.07	0.09	1.31	0.03	1.26	0.04	0.05	1.31	0.03	1.26	0.03	0.06
Non-HDL (mmol/l)	16	3.09	0.18	16	3.21	0.19	0.26	3.09	0.10	3.21	0.10	0.27	3.10	0.10	3.26	0.11	0.06
Triglycerides (mmol/l)	16	1.04	0.09	16	1.28	0.29	0.32	1.04	0.07	1.28	0.26	0.30	1.04	0.62	1.28	0.25	0.28

Variable	Unadjusted						Adjustment for baseline					Adjustment for baseline and randomisation order				
	N	Piccolo		N	Oranjstar		*P*	Piccolo		Oranjstar		*P❖*	Piccolo		Oranjstar		*P▲*
		Mean	SEM		Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
Insulin (pmol/l)	16	30.65	4.72	16	28.90	3.44	0.59						30.65	4.873	28.96	3.56	0.60
IL-6 (pg/ml)	16	2.73	0.68	16	2.78	0.61	0.90	2.66	0.73	2.89	0.69	0.60	2.66	0.73	2.89	0.70	0.61
TNF-α (pg/ml)	16	23.40	2.22	16	22.67	1.88	0.81	23.86	2.40	22.60	2.05	0.71	23.86	2.10	22.60	1.96	0.63
sICAM-1 (ng/ml)	15	141.56	13.28	15	152.96	15.37	0.39	144.99	11.85	158.55	12.29	0.35	144.99	11.53	158.55	12.67	0.32
sVCAM-1 (ng/ml)	15	357.81	29.39	15	364.69	29.73	0.74	369.04	20.21	373.11	25.81	0.85	369.04	20.58	373.11	26.51	0.84
P-selectin (ng/ml)	16	44.82	2.36	16	46.13	2.34	0.54	44.71	2.58	46.02	2.57	0.58	44.71	2.68	46.02	2.39	0.52
E-selectin (ng/ml)	16	25.01	28.01	16	20.14	2.59	0.77	22.56	4.09	20.52	2.59	0.71	22.56	4.25	20.52	2.69	0.72

†Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline and washout as covariate.
‡Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline, washout and randomisation order as covariate with Bonferroni correction test.
❖Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline value as covariate.
▲Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline and randomisation order as covariate with Bonferroni correction test.
LSM= Least Squared Means (Marginal means).
Insulin levels were only measured in post-intervention.
IL-6, TNF-α, sICAM-1, sVCAM-1, P-selectin and E-selectin level were only measured in baseline and post-intervention.

Table 7.3 Effects of Piccolo and Oranjstar on blood-borne biomarkers in healthy men below 30 years of age.

Variable	Unadjusted						Adjustment for baseline and washout					Adjustment for baseline, washout and randomisation order				
	N	Piccolo		N	Oranjstar		*P*	Piccolo		Oranjstar		*P*†	Piccolo		Oranjstar		*P*‡
		Mean	SEM		Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
Glucose (mmol/l)	16	5.25	0.08	16	5.14	0.12	0.14	5.25	0.07	5.14	0.11	0.16	5.25	0.06	5.14	0.08	0.10
Total cholesterol (mmol/l)	16	4.40	0.17	16	4.53	0.22	0.33	4.40	0.11	4.53	0.11	0.30	4.40	0.11	4.53	0.10	0.14
HDL (mmol/l)	16	1.31	0.05	16	1.26	0.07	0.09	1.31	0.03	1.26	0.04	0.05	1.31	0.03	1.26	0.03	0.06
Non-HDL (mmol/l)	16	3.09	0.18	16	3.21	0.19	0.26	3.09	0.10	3.21	0.10	0.27	3.10	0.10	3.26	0.11	0.06
Triglycerides (mmol/l)	16	1.04	0.09	16	1.28	0.29	0.32	1.04	0.07	1.28	0.26	0.30	1.04	0.62	1.28	0.25	0.28

Variable	Unadjusted						Adjustment for baseline					Adjustment for baseline and randomisation order				
	N	Piccolo		N	Oranjstar		*P*	Piccolo		Oranjstar		*P*❖	Piccolo		Oranjstar		*P*▲
		Mean	SEM		Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
Insulin (pmol/l)	16	30.65	4.72	16	28.90	3.44	0.59						30.65	4.873	28.96	3.56	0.60
IL-6 (pg/ml)	16	2.73	0.68	16	2.78	0.61	0.90	2.66	0.73	2.89	0.69	0.60	2.66	0.73	2.89	0.70	0.61
TNF-α (pg/ml)	16	23.40	2.22	16	22.67	1.88	0.81	23.86	2.40	22.60	2.05	0.71	23.86	2.10	22.60	1.96	0.63
sICAM-1 (ng/ml)	15	141.56	13.28	15	152.96	15.37	0.39	144.99	11.85	158.55	12.29	0.35	144.99	11.53	158.55	12.67	0.32
sVCAM-1 (ng/ml)	15	357.81	29.39	15	364.69	29.73	0.74	369.04	20.21	373.11	25.81	0.85	369.04	20.58	373.11	26.51	0.84
P-selectin (ng/ml)	16	44.82	2.36	16	46.13	2.34	0.54	44.71	2.58	46.02	2.57	0.58	44.71	2.68	46.02	2.39	0.52
E-selectin (ng/ml)	16	25.01	28.01	16	20.14	2.59	0.77	22.56	4.09	20.52	2.59	0.71	22.56	4.25	20.52	2.69	0.72

†Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline and washout as covariate.
‡Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline, washout and randomisation order as covariate with Bonferroni correction test.
❖Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline value as covariate.
▲Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline and randomisation order as covariate with Bonferroni correction test.
LSM= Least Squared Means (Marginal means).
Insulin levels were only measured in post-intervention.
IL-6, TNF-α, sICAM-1, sVCAM-1, P-selectin and E-selectin level were only measured in baseline and post-intervention.

Table 7.4 Effects of Piccolo and Oranjstar on blood-borne biomarkers in healthy men above 30 years of age.

Variable	Unadjusted						Adjustment for baseline and washout					Adjustment for baseline, washout and randomisation order				
	N	Piccolo		N	Oranjstar		*P*	Piccolo		Oranjstar		*P*†	Piccolo		Oranjstar		*P*‡
		Mean	SEM		Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
Glucose (mmol/l)	11	5.75	0.42	11	5.73	0.39	0.81	5.75	0.08	5.73	0.13	0.79	5.75	0.87	5.73	0.13	0.78
Total cholesterol (mmol/l)	11	4.98	0.22	11	5.00	0.24	0.87	4.98	0.13	5.01	0.16	0.87	4.98	0.05	5.01	0.10	0.84
HDL (mmol/l)	11	1.24	0.10	11	1.21	0.09	0.36	1.24	0.04	1.21	0.02	0.39	1.24	0.02	1.21	0.03	0.14
Non-HDL (mmol/l)	11	3.74	0.23	11	3.80	0.25	0.66	3.74	0.13	3.80	0.08	0.66	3.74	0.67	3.80	0.96	0.63
Triglycerides (mmol/l)	11	1.13	0.14	11	1.25	0.20	0.31	1.13	0.09	1.26	0.13	0.29	1.13	0.87	1.26	0.13	0.22

Variable	Unadjusted						Adjustment for baseline					Adjustment for baseline and randomisation order				
	N	Piccolo		N	Oranjstar		*P*	Piccolo		Oranjstar		*P*❖	Piccolo		Oranjstar		*P*▲
		Mean	SEM		Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
Insulin (pmol/l)	11	32.89	6.69	11	37.00	5.23	0.49						32.89	6.86	37.00	5.44	0.52
IL-6 (pg/ml)	11	2.55	0.32	11	1.73	0.24	0.026*	2.54	0.34	1.84	0.24	0.027*	2.54	0.35	1.84	0.25	0.034*
TNF-α (pg/ml)	11	20.54	1.29	11	22.36	1.60	0.46	20.98	1.40	21.99	1.67	0.69	20.98	1.16	21.99	0.89	0.25
sICAM-1 (ng/ml)	11	184.69	21.07	11	147.78	18.77	0.14	184.70	16.40	147.78	16.87	0.15	184.70	17.38	147.78	17.76	0.18
sVCAM-1 (ng/ml)	11	444.58	42.78	11	356.27	27.01	0.08	444.58	37.04	356.72	25.21	0.09	444.58	38.52	356.27	24.48	0.09
P-selectin (ng/ml)	11	50.41	3.02	11	46.91	3.11	0.42	50.41	2.95	46.91	3.01	0.44	50.41	2.15	46.91	2.84	0.46
E-selectin (ng/ml)	11	25.01	28.01	11	28.26	4.56	0.82	30.08	5.38	28.26	4.25	0.82	30.08	4.11	28.26	4.50	0.81

†Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline and washout as covariate.
‡Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline, washout and randomisation order as covariate with Bonferroni correction test.
❖Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline value as covariate.
▲Estimated from repeated measures ANOVA of the Piccolo and Oranjstar with baseline and randomisation order as covariate with Bonferroni correction test.
LSM= Least Squared Means (Marginal means).
*$p<0.05$.
Insulin levels were only measured in post-intervention.
IL-6, TNF-α, sICAM-1, sVCAM-1, P-selectin and E-selectin level were only measured in baseline and post-intervention.

7.3.5 Other circulating biomarkers

7.3.5.1 Inflammatory cytokines

After the consumption of Piccolo and Oranjstar, IL-6 did not change in the group of all participants when compared to the baseline. The age group above 30 years of age showed significant reduction in IL-6 by 1.22 pg/mL (p=0.035) after intake of Oranjstar when compared to the baseline. IL-6 was significantly reduced by 0.82 pg/mL (p=0.026) following Oranjstar consumption compared with Piccolo in age group above 30 years of age and the significant change remained after the covariate adjustment (**Table 7.4**). Age group below 30 years of age showed no significant change after adjusting for covariate (**Table 7.3**).

After the consumption of Piccolo and Oranjstar, TNF-α did not change in the group of all participants when compared to the baseline. There were no significant differences in TNF-α between Piccolo and Oranjstar intervention in either the younger or older groups, or when these were combined (**Table 7.2**, **Table 7.3** and **Table 7.4**).

7.3.5.2 Adhesion molecules

E-selectin was significantly reduced by 6.651 ng/mL (p=0.016) after consumption of Oranjstar and no change was observed after consumption of Piccolo in the group of all participants when compared to the baseline. There were no significant differences in E-selectin between Piccolo and Oranjstar intervention in either the younger or older groups, or when these were combined (**Table 7.2**, **Table 7.3** and **Table 7.4**).

After the consumption of Piccolo and Oranjstar, P-selectin did not change in the group of all participants when compared to the baseline. There were no significant differences in P-selectin between Piccolo and Oranjstar intervention in either the younger or older groups, or when these were combined (**Table 7.2**, **Table 7.3** and **Table 7.4**).

After the consumption of Piccolo and Oranjstar, sICAM-1 was significantly reduced by 49.56 ng/mL (p<0.001) and 59.14 ng/mL (p<0.001) respectively in the group of all participants when compared to the baseline. There were no significant differences in sICAM-1 between Piccolo and Oranjstar intervention in either the younger or older groups, or when these were combined (**Table 7.2**, **Table 7.3** and **Table 7.4**).

sVCAM-1 was significantly reduced by 67.35 ng/mL (p=0.003) after consumption of Oranjstar and no change was observed after consumption of Piccolo in the group of all participants when compared to the baseline. There were no significant differences in

sVCAM-1 between Piccolo and Oranjstar intervention in either the younger or older groups, or when these were combined (**Table 7.2**, **Table 7.3** and **Table 7.4**).

7.4 Discussion

Previous interventional studies have focused on explaining the effects of tomatoes, tomato by-products, or lycopene supplementations on blood lipid profile and inflammatory biomarkers (Blum et al., 2006; Collins et al., 2004; Cuevas-Ramos et al., 2013; Devaraj et al., 2008; Engelhard et al., 2006; Misra et al., 2006). In this study, we measured the blood lipid profile and a wide range of molecules that involved in the initiation and progression of atherosclerosis, such as the inflammatory cytokines TNF-α and IL-6, as well as adhesion molecules sICAM-1, and sVCAM-1, after 4-week interventions with different varieties of tomatoes. We also elucidated the role of major carotenoids in tomatoes.

7.4.1 Principle findings

The present study showed that the consumption of 300 g of Piccolo (containing 26.22 mg of total lycopene) and Oranjstar (7.92 mg of total lycopene) over a 4-week period produced a positive effect on inflammatory biomarkers. Supplementation of Piccolo and Oranjstar significantly reduced sICAM-1 by 49.56 ng/mL (p<0.001) and 59.14 ng/mL (p<0.001) respectively from baseline values for all participants. After the consumption of Oranjstar, all participants showed a significant reduction of sVCAM-1 and E-selectin by 67.35 ng/mL (p=0.003) and 6.65 ng/mL (p=0.016) respectively, when compared to the baseline.

In the age group above 30 years of age, supplementation of Oranjstar was a significant reduction in IL-6 by 1.22 pg/mL (p=0.035) when compared with baseline values; Oranjstar consumption was also significantly lower (by 0.82 pg/mL, p=0.026) than the changes observed with Piccolo.

Moreover, these effects were unrelated to differences in blood lipid profile, body fats and BMI.

7.4.2 Meaning of the current findings

The adhesion molecules ICAM-1 and VCAM-1 were well recognised for their role in the development of atherosclerotic plaque. Its expression was related to pro-inflammatory stimuli such as oxidised LDL and CRP (Galkina and Ley, 2007; Ley and Huo, 2001). A prospective study and meta-analysis showed a significant increase in the incidence of CHD among individuals within the top third of sICAM-1 levels (338 ng/mL) when compared with those in the bottom third of sICAM-1 levels (261 ng/mL) (OR 1.49, 95% CI 1.14 to 1.94)

after adjustments for age, town, smoking and risk factors (Malik et al., 2001). In addition, this study also reported a significant increase in the incidence of CHD among individuals in the top third of sVCAM-1 levels (516 ng/mL) when compared with those in the bottom third of sVCAM-1 levels (37 ng/mL) (OR 1.35, 95% CI 1.05 to 1.75) after adjustments for age, town, smoking and risk factors (Malik et al., 2001). Consequently, the results of the present dietary intervention study indicated that a higher intake of Oranjstar was associated with significant reductions in both ICAM-1 and VCAM-1, independent to the changes in blood lipids.

7.4.3 Comparison to previous studies

Human intervention trials that have evaluated the effects of lycopene on inflammatory biomarker were scarce. In agreement with our results, Colman-Martinez et al. (2017) showed a 10-fold reduction in sICAM-1 (Colman-Martinez et al., 2017) after tomato juice supplementation for 2 weeks. In addition, García-Alonso et al. (2012) observed a reduction of sVCAM-1 after tomato juice supplementation for 2 weeks (García-Alonso et al., 2012). It is noteworthy that the differences in magnitudes of the changes reported in these studies when compared to our study could be due to the characteristics of the studied population. Both trials mentioned above included participants with high cardiovascular risks (Colman-Martinez et al., 2017; García-Alonso et al., 2012).

In contrast, two previous studies showed no significant changes in sICAM-1 after dietary intervention with tomatoes, tomato by-products or lycopene supplements (Blum et al., 2007; Cheng et al., 2017; Thies et al., 2012).

The present study also showed a reduction of plasma IL-6 after consumption of Oranjstar when compared with baseline (1.22 pg/mL, p=0.035) in age group above 30 years. In line with our results, previously reviewed studies supplementing tomato were also associated with significant reductions in IL-6 (SMD -0.25; p=0.03) (Cheng et al., 2017).

The evidence of the impact of tomato or lycopene on blood lipids is contrasting. Several studies have shown an improvements on lipid parameters such as TC (Li et al., 2015; Silaste et al., 2007; Vinha et al., 2014), TG (Vinha et al., 2014), HDL-cholesterol (Bohn et al., 2013; Cuevas-Ramos et al., 2013; Madrid et al., 2006) and LDL-cholesterol (Silaste et al., 2007) after tomato consumption. However, other studies such as the one conducted by Petyaev et al. (2018) showed that tomato did not significantly affect parameters of lipid profile in patients with coronary vascular disease (Petyaev et al., 2018). The systematic review and meta-analysis in Chapter 3 showed that overall, interventions supplementing tomato were

associated with significant reductions in LDL-cholesterol (-0.22 mmol/L; p=0.006) but no significant changes were observed in other lipid parameters (Cheng et al., 2017).

7.4.4 Strengths

Cis-lycopene isomers were well recognised for their better bioavailability and greater proportion in human plasma and tissues when compared with the trans-lycopene. It was therefore suggested that cis-lycopene has a more important effect than trans-lycopene on reducing risk factors of CVD (Böhm and Bitsch, 1999; Rao and Agarwal, 1999; Unlu et al., 2007b). One of the strong points of the present study was the elucidation of the beneficial effects of both trans-lycopene and cis-lycopene on the decrease of adhesion molecules after tomato consumption, which were in agreement with a previous study (Colman-Martinez et al., 2017). These beneficial effects were independent to blood lipids. A possible mechanism is that lycopene may modulate the concentration of adhesion molecules, and probably other inflammatory molecules. This is achieved through inhibiting the translocation of the nuclear factor kappa B (NF-κB) (Hung et al., 2008; Yang et al., 2017). NF-κB is involved in the activation of the inflammatory cascade that could stimulate adhesion molecule and inflammatory molecules expression (Hung et al., 2008; Yang et al., 2017). In addition, lycopene may increase the intracellular glutathione (GSH) level and glutamate-cysteine ligase expression. Subsequently, lycopene induces nuclear factor-erythroid 2 related factor 2 (Nrf2) activation, leading to the increased expression of downstream heme oxygenase-1 (HO-1). The use of siRNA to target HO-1 blocks the inhibitory effects of lycopene on IκB degradation and sICAM-1 expression. The inhibitory effects of lycopene thus appear to be mediated through its induction of Nrf2-mediated HO-1 expression (Yang et al., 2017).

7.4.5 Limitations

In the present study, we did not have a control arm but previous studies have proven that tomato intervention has improved cardiovascular risk factors when compared against control or placebo groups (Cheng et al., 2017). We did not involve a control arm because the purpose of the present intervention is to compare the effects of two tomato varieties on cardiovascular risk factors. Furthermore, the volunteers enrolled in this trial were perhaps too healthy to allow detection of significant changes in blood lipid. In 2013, 67% of men were measured as overweight (BMI>25kg/m^2) and 26% of men were defined as obese (BMI>30kg/m^2) in England (Townsend et al., 2015), which is higher than the mean BMI (23.3kg/m^2) of participants in this study. Although not all previous intervention studies carried out in obese individuals and/or patients with disease with lycopene/tomato-based products showed

beneficial effects on blood lipid (Cheng et al., 2017), recruiting participants with established elevated risk markers for CVD and/or with a higher BMI may increase the probability of detecting such changes.

7.4.6 Unanswered questions and future research

To the best of our knowledge, no previous studies have compared the two different varieties of tomato (rich in trans-lycopene and cis-lycopene). The positive impacts of lycopene on inflammatory biomarkers encourage further studies with a larger population to investigate the effects of carotenoid consumption. In addition, influences of other components present in tomato, such as vitamin C, polyphenols or other micronutrients, which could act in a synergistic/antagonistic way in regulating the activity of these molecules, could also be studied. This dietary intervention was conducted with only healthy male participants, and so there would be room for further research with lycopene supplementation and effects on high CVD risk groups as well as patients with CVD and diabetes.

7.5 Conclusion

In conclusion, this study has proven that the consumption of Piccolo and Oranjstar for four weeks has a significant impact on reducing adhesion molecules, such as sICAM-1 and sVCAM-1, and therefore lowers the risk of developing cardiovascular disease in healthy males.

7.6 Research Questions Raised from The Chapter 5

Regarding the result of Chapter 5, tomato supplementation significantly improved BP and FMD but showed less beneficial effects on other non-invasive methods assessing vascular function. Microvascular vasodilation and arterial stiffness assessing vascular function are still novel markers for estimating the risks of developing CVD. Both techniques are increasingly being adopted as clinical measures of vascular function but the evidence on the impact of food interventions on vascular function are scattered and has not been systematically reviewed nor meta-analysed. This raised the following questions:

1. What is the evidence for nutritional interventions enhancing microvascular vasodilation and arterial stiffness?

This question will be addressed in the Chapter 8 where the evidence from the literature on the effectiveness and impact of nutritional intervention on microvascular vasodilation and arterial stiffness will be evaluated.

Chapter 8 The Effects of Food Interventions on Measures of Arterial Stiffness and Microvascular Vasodilation: Systematic Review and Meta-Analysis

This study was designed to answer the research questions in **section 7.6**.

8.1 Introduction

The tomato clinical trial in Chapter 5 showed that although FMD and SBP improved after tomato supplementation, but other vascular function, such as microvascular vasodilation and arterial stiffness, showed no effect. Given the fact that the systematic searches of the literature identified no interventional studies evaluating the effects of tomato or lycopene on these outcomes, the work presented in this chapter intended to identify dietary and nutritional interventions affecting arterial stiffness and microvascular blood flow.

Recent research studies showed clear benefits of specific foods and dietary patterns in improving cardiovascular function. There is evidence that supplementation with inorganic nitrates, or foods rich in inorganic nitrate can increase NO and improve risk factors such as BP and endothelial dysfunction (Siervo et al., 2013). Reduction in NO production is thus a proposed mechanism of endothelial dysfunction and a contributor to atherosclerosis. NO prevents oxidative modification of low-density lipoprotein (LDL) cholesterol; this oxidation process is proposed as a major mechanism of the atherosclerotic process (Rubbo et al., 2002; Steinberg and Witztum, 2002). Increased blood LDL can be linked to a high fat diet and through this, a link between diet and vascular function can be made.

Traditionally, FMD has been the golden method to evaluate vascular function (Greyling et al., 2016; Ras et al., 2013). A recent systematic review of 8 interventional studies (involving 652 participants) has shown that nut consumption significant improved FMD (Mean different 0.79% (95% CI 0.35 to 1.23)) (Neale et al., 2017). Each 1% increase of FMD significantly reduces the risk of CVD events (Inaba et al., 2010). In comparison, Neale et al. 2017 study found an effect estimate of 0.79% for nut consumption compared with controls suggesting these results are likely to be of clinical relevance in predicting future CVD risk (Neale et al., 2017). The assessments for microvascular vasodilation and arterial stiffness are non-invasive methods to measure vascular function that have been developed in the past two decades. Both techniques are increasingly being adopted as clinical measures of vascular function. However, the evidence of the impact of dietary and nutritional interventions on these methods has not been systematically reviewed nor meta-analysed. Systematic reviews allow us to establish the effectiveness of interventions, which is done by thoroughly reviewing primary research for a focussed question. In this particular case, previous

nutritional interventions and their impact on arterial stiffness and microvascular vasodilation as assessed through reviewing their methodological quality and execution (Pearson et al., 2005). Along with this, performing meta-analysis allows us to combine the results from many interventions, thus determines whether there is significant correlation between the testing methods in nutritional interventions. This helps to clarify the position of these methods in the effect of nutritional interventions on vascular function. It is important to systematically review primary articles in this field, as there is uncertainty and a lack of clarity in the effectiveness of PWV, PWA and LDI as testing methods. Here, a systematic review and meta-analysis will be reported on the current literature surrounding effects of on nutritional interventions vascular function assessed by PWV, PWA and LDI.

8.2 Methods

This systematic review was undertaken following standard guidance by the Cochrane collaboration (Higgins and Green, 2011) and the Centre for Reviews and Dissemination (Centre for Reviews and Dissemination, 2009). This review is reported according to the PRISMA guidelines (**Figure 8.1** and **Appendix E1**) (Moher et al., 2010). The protocol of this systematic review has been previously registered with PROSPERO, the International Prospective Register of Systematic Reviews (Registration number CRD42016042092).

In March 2019, three databases including PubMed, Medline, and Web of Science were searched from inception. In addition, reference lists of identified publications were screened in an attempt to identify further relevant studies. The searches included the following terms/keywords related to the exposures and outcomes of interest: 1) “PWV”; 2) “Pulse Wave Velocity”; 3) “PWA”; 4) “Pulse Wave Analysis”; 5) “Augmentation Index”; 6) “Arterial Stiffness”; 7) “Microvascular blood flow”; 8) “ LDI”; 9) “ Laser Doppler Imaging”; 10) “ Endothelial Function”; 11) “Vascular Function”; 12) “Microcirculation”; 13) “Iontophoresis”; 14) “Nutrition”; 15) “Diet”; 16) “Potassium”; 17) “Trial”. The terms related to outcome measures of vascular function, based on nutritional interventions. Data for each of these outcomes was extracted if explicitly reported in the original papers. The present systematic review was restricted to articles published in English. Two authors (AOR, HMC) screened articles independently for eligibility. The decision to include/ exclude studies was hierarchical and consisted on screening firstly the titles and abstracts of studies; if the authors were unable to reach decision at this stage, then the full-text of the article was evaluated.

8.2.2 Inclusion/Exclusion Criteria

The following specific inclusion criteria were used to identify eligible articles: 1) Study Design: Intervention Studies; 2) Subjects: Adult subjects >18 years of age; 3) Interventions: Nutritional/dietary interventions; 4) Outcomes: Vascular function. Exclusion criteria included: 1) Study design: Non-intervention studies; 2) Subjects: Subjects <18 years of age; 3) Interventions: Non-Nutritional dietary interventions; 4) Outcomes: Non-vascular function.

8.2.3 Data extraction

A standardised, pre-piloted form was used to extract data from the included studies for assessment of study quality and evidence synthesis. Extracted information included: Study design (country, method of recruitment, follow-up length); participants (population and sample size, health status, mean age, body mass index and ethnicity); measurement description (PWV (m/s), augmentation index (%), LDI (PU)); outcome measure (vascular function); features of intervention (controlled conditions, study methodology, completion rates, outcomes and times of measurement).

8.2.4 Outcome measures and exposures

The primary outcome of interest was changes in arterial stiffness and microvascular blood flow with varying dietary compounds believed to be linked to improved vascular function. This included a high nitrate diet, fish oil and flavones.

8.2.5 Statistical analysis

Meta-analysis of results was undertaken using the Review Manager (RevMan Version 5.1 for Windows Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011). Random effects models, accounting for inter-study variation and minimizing potential bias due to methodological differences between studies, were used. For outcomes measured with the same technique and same units (pulse wave velocity, m/s), results were expressed as mean difference between groups with 95% confidence intervals and with two-sided *P*-values. For comparisons where dissimilar units (LDI and augmentation index (AIx, %)) were reported, results were expressed as standardized mean difference (SMD). In addition, we undertook subgroup analysis to explore the impact of intervention type.

A random effects model accounting for inter-study variation was used. Multiple dietary intervention arms from three studies were included in the meta-analysis. Following previous guidance (Higgins and Green, 2011), excessive weightings from “double counts” arising

from the "shared" group (in this case, the control group) were controlled by splitting the sample size of the shared group into approximately equal smaller groups for the comparisons. In this analysis, reviewers sought to extract and analyse adjusted results from multivariate models, if reported in the studies.

Statistical heterogeneity was evaluated using the I^2 statistic (Centre for Reviews and Dissemination, 2009; Higgins and Green, 2011), with I^2 values greater than 50% representing high levels of heterogeneity. Publication bias was assessed by inspecting the funnel plot , and supplemented with calculations of the Egger's regression test (Egger et al., 1997). Quality of studies was assessed using the Jadad system (Jadad et al., 1996).

8.3 Results

A total of 1774 publications after de-duplication and results of the screening process are described in **Figure 8.1**. One hundred and thirteen publications met our inclusion criteria were included in the present systematic reviews (**Table 8.1**) and 93 of those publications were included in the meta-analysis.

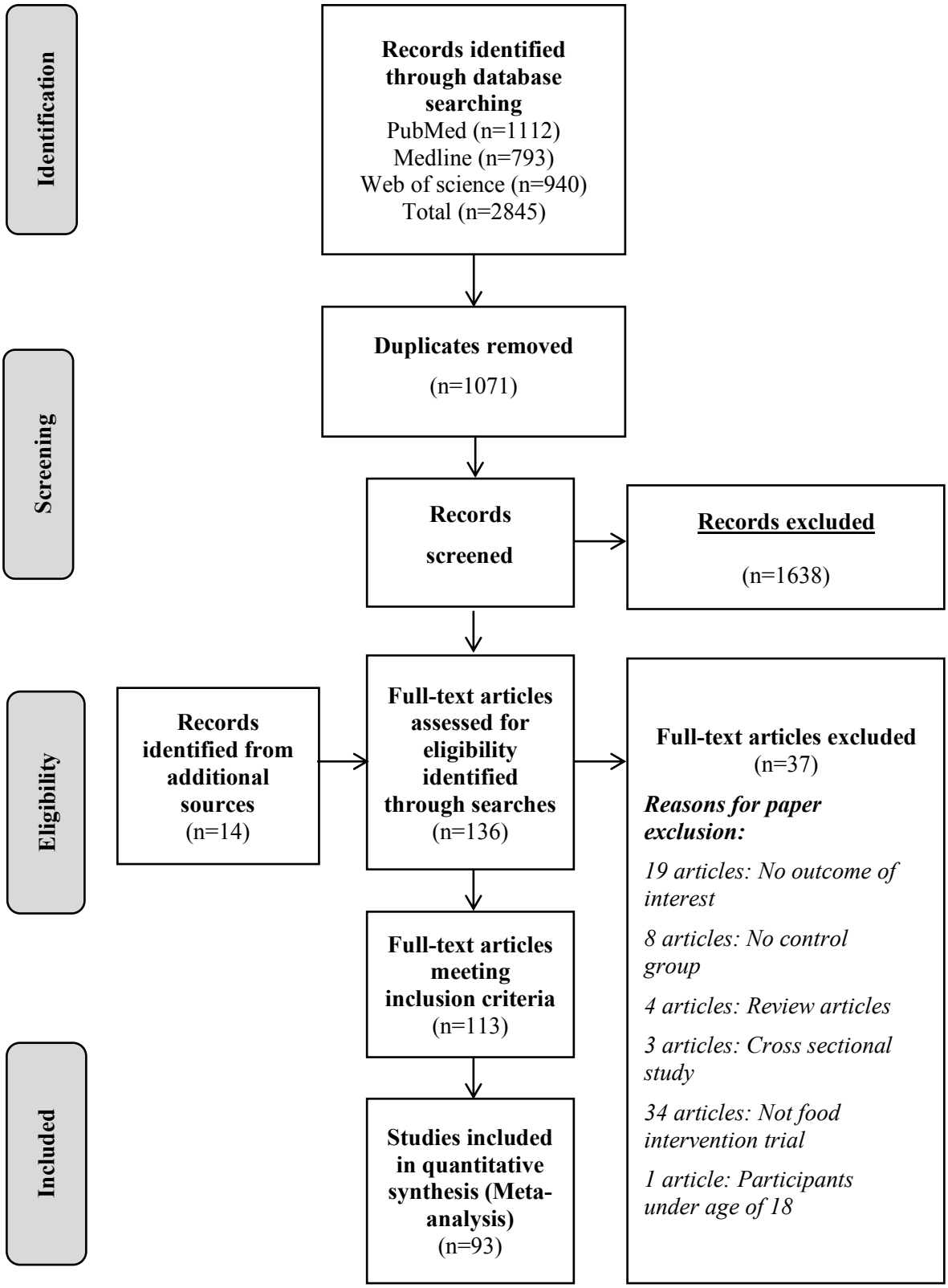


Figure 8.1 PRISMA flow diagram of selection of studies on food intervention and vascular function.

Table 8.1 Characteristics of studies included in systematic review.

Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
Ahuja et al., 2007 (Australia)	RCT, Cross-over	46±12	100%	26.4±4.8	Healthy	N= 14	4 wk	Chilli blend	30 g/d	Without chilli blend	AIx_HR75
			0%			N= 20					
Al-Solaiman et al., 2010 (USA)	RCT, Cross-over	40.3±1.7 (SEM)	20%	34.5±1.2 (SEM)	Obese/ HTN	N= 15	3 wk	Usual diet, with average of one fruit and one vegetable supplemented with potassium, magnesium and fibre	Potassium 4100 mg/d, magnesium 500 mg/d, fibre-31 mg/d	Usual diet, with average of one fruit and one vegetable	AIx
								DASH	2 Fruit/Veg/d - Dash		
		36.7±1.8 (SEM)		22.8±0.4 (SEM)	Healthy	N= 15		Usual diet, with average of one fruit and one vegetable supplemented with potassium, magnesium and fibre	Potassium 4100 mg/d, magnesium 500 mg/d, fibre-31 mg/d		
								DASH	2 Fruit/Veg/d - Dash		
Al-Solaiman et al., 2009 (USA)	RCT, Cross-over	44.1±1.4 (SEM)	22%	26.5±1.6 (SEM)	Healthy, salt sensitive	N= 9	3 wk	DASH	3000 mg Na/d	Usual low fruits and vegetables diet	AIx
								Low sodium-DASH	1500 mg Na/d		
		34.3±2.5 (SEM)	30%	30.1±2.7 (SEM)	Healthy, salt resistant	N= 10		DASH	3000 mg Na/d		
								Low sodium-DASH	1500 mg Na/d		

(**Table 8.1** continues on next page)

Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
Armah et al., 2008 (UK)	RCT, Cross-over	46±4	100%	25.5±0.8	Healthy	N= 25	4 hr	Fish oil meal	31 g of mixed fat and 9 g of fish oil	Placebo oil	LDI in ACh LDI in SNP
Babar et al., 2018 (Canada)	RCT, Parallel	Intervention= 28.7±3.76 Control= 28.5±3.46	0%	Intervention= 23.57±4.46 Control= 23.55±3.89	Healthy pregnant	Intervention= 66 Control= 65	12 wk	High flavanol chocolate	171 mg of flavanols	Low flavanol chocolate	AIx AIx_HR75
Berry et al., 2008 (UK)	RCT, Cross-over	27.1±5.3	100%	24.3±3.0	Healthy	N= 17	3 hr	Shea butter blend rich in stearic acid meal	50 g shea butter	Oleic acid	PAIx CAIx cf-PWV
Blanch et al., 2014 (Australia)	RCT, Cross-over	31±11	26%	21.7±3.0	Healthy	N= 35	6 d	High potassium diet	5690 mg/d	Usual potassium	AIx cf-PWV
Blumenthal et al., 2010 (USA)	RCT, Parallel	Intervention= 51.8±10 Control= 51.8±9	33%	Intervention= 32.8±3.4 Control= 33±3.9	HTN	Intervention= 46 Control= 49	16 wk	DASH	/	Usual diet	cf-PWV
Bondonno et al., 2014 (Australia)	RCT, Cross-over	60±7.0	32%	27.1±3.8	Healthy	N= 38	1 wk	High-nitrate diet by consuming spinach and other green leafy vegetable	400 mg/d of nitrate	Low nitrate diet	AIx_HR75 cf-PWV
Breslavsky et al., 2013 (Israel)	RCT, Parallel	Intervention= 66.8±9.2 Control= 65.8±9.7	Intervention= 46% Control= 48%	Intervention= 27.9±5.2 Control= 30.5±5.1	T2D	Intervention= 24 Control= 23	12 m	Oral daily supplementation with cholecalciferol	1000 u/d	Placebo capsules	AIx
Bressendorff et al., 2016 (Denmark)	RCT, Parallel	Intervention= 41±9.05 Control= 44.5±8.5	58%	Intervention= 24.7±3.8 Control= 25.1±4.3	Healthy	Intervention= 22 Control= 18	16 wk	Vitamin D precursors (Cholecalciferol)	3000 IU/d	Placebo	AIx_HR75 cf-PWV

(**Table 8.1** continues on next page)

Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
Chen et al., 2015a (USA)	RCT, Cross-over	61.8±8.6	40%	30.2±5.1	CAD	N= 45	6 wk	Almonds	85 g/d	No Nuts	cf-PWV
											cr-PWV
Chong et al., 2010 (UK)	RCT, Cross-over	48±18	48%	24.7±3.2	Healthy	N= 25	4 hr	Milkshake with EPA and DHA oil	2 g EPA and 2.7g DHA	Milkshake with Palm oil	AIx_HR75
Chuengsamarn et al., 2014 (Thailand)	RCT, Parallel	Intervention= 59.16±1.067 (SEM) Control= 59.58±1.04 (SEM)	45%	Intervention= 27.09±0.52 (SEM) Control= 26.84±0.42 (SEM)	T2D	Intervention= 107 Control= 106	6 m	Curcuminoid capsule	250 mg/d	Placebo capsules	ba-PWV (Right)
											ba-PWV (Left)
Coombes et al., 2016 (Australia)	RCT, Parallel	Intervention= 49.1±11.2 Control= 50.9±13.4	74%	Intervention= 26.2±4.1 Control= 27.7±7.6	Renal transplant patients	Intervention= 33 Control= 28	12 m	Astaxanthin capsules	12 mg/d	Placebo capsules	AIx
											AIx_HR75
											cf-PWV
Curtis et al., 2013 (UK)	RCT, Parallel	Intervention= 62.1±0.7 (SEM) Control= 63±0.8 (SEM)	0%	Intervention= 32.7±1.1 (SEM) Control= 31.9±0.9 (SEM)	T2D	Intervention= 47 Control= 46	12 m	27 g flavonoid-enriched chocolate/d	90 mg epicatechin (850 mg total flavan-3-ols) and 100 mg isoflavone	Placebo chocolate	AIx_HR75
											cf-PWV
Dalan et al., 2016 (Singapore)	RCT, Parallel	Intervention= 52.2±8.2 Control= 54.8±10.8	Intervention= 14% Control= 19%	Intervention= 27.3±5.8 Control= 28.9±6	T2D	Intervention= 33 Control= 31	16 wk	Cholecalciferol	Cholecalciferol 2000-4000 IU daily depending om baseline 25(OH)D and response	Placebo	AIx_HR75
Dickinson et al., 2014a (Australia)	RCT, Cross-over	18-70	44%	N/A	Healthy	N= 16	2 hr	Low sodium soup	5 mmol Na/d	High sodium soup (65mmol Na /d)	AIx

(**Table 8.1** continues on next page)

Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
Dickinson et al., 2014b (Australia)	RCT, Cross-over	N/A	32%	27-40	Overweight	N= 25	6 wk	Salt reduction diet	6 g Na/d	Usual salt diet (9 g/d)	AIx
											cf-PWV
Din et al., 2011 (UK)	RCT, Cross-over	23±3	100%	24.5±2.3	Healthy	N= 30	4 wk	Walnut	15 g/d	Normal diet	AIx
Dower et al., 2016 (Netherlands)	RCT, Cross-over	61.8±9.3	100%	25.1±2.1	Healthy	N= 20	2 hr	Dark chocolate	150 mg epicatechin /d	75g white chocolate	AIx_HR75
								Epicatechin capsules	100 mg epicatechin /d		
Dreyer et al., 2014 (UK)	RCT, Parallel	Intervention= 45.8±10 Control= 48.8±12.2	Intervention= 14% Control= 14%	Intervention= 30.4±7.1 Control= 29.2±3.4	CKD	Intervention= 20 Control= 18	6 m	Ergocalciferol	5000 IU weekly for 1 m and then monthly for 5 m	Placebo	cf-PWV
Duthie et al., 2018 (UK)	RCT, Parallel	Intervention= 48.3±5.6 Control= 48.5±4.8	42%	Intervention= 26.6±3.9 Control= 26.0±3.4	Healthy	Intervention= 21 Control= 24	12 wk	Additional 480 g of fruit and vegetables and fruit juice (300 mL) daily		Usual diet	PWV
Esser et al., 2013 (Netherlands)	RCT, Cross-over	61.8±5.9	100%	23.8±0.8	Lean	N= 18	4 hr	Milkshake rich in MUFA	95 g/d	Milkshake rich in SFA	AIx
								Milkshake rich in PUFA			
		62.6±3.2		32.4±3.0	Obese	N= 11		Milkshake rich in MUFA			
								Milkshake rich in PUFA			
Fahs et al., 2009 (USA)	RCT, Cross-over	24.2±0.7 (SEM)	100%	27±1.2 (SEM)	Healthy	N= 17	1 hr	L-Arginine capsule	7 g/d	Placebo capsule	AIx
											AIx_HR75

(**Table 8.1** continues on next page)

Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
Feresin et al., 2017 (USA)	RCT, Parallel	Intervention= 59±1 (SEM) Control= 58±1 (SEM)	0.0%	Intervention= 32.7±1.1 (SEM) Control= 32.1±0.7 (SEM)	Healthy	Intervention= 20 Control= 20	8 wk	Freeze-dried strawberry powder	50 g	Placebo powder	ba-PWV
		Intervention= 61±1 (SEM) Control= 58±1 (SEM)		Intervention= 31.0±1.0 (SEM) Control= 32.1±0.7 (SEM)		Intervention= 20 Control= 20			25 g		
Figueroa et al., 2011 (USA)	RCT, Cross-over	54±3 (SEM)	44%	28.4±0.9 (SEM)	Healthy	N= 9	6 wk	Watermelon powder	4 g L-Citrulline/L-Arginine /d	Placebo	AIx
											AIx_HR75
											cf-PWV
Figueroa et al., 2012 (USA)	RCT, Cross-over	58±1 (SEM)	21%	37.3±1.8 (SEM)	HTN	N= 14	6 wk	Watermelon extract	6 g L-Citrulline/L-Arginine /d	Placebo	CAIx
Figueroa et al., 2014 (USA)	RCT, Cross-over	57.4±1.4 (SEM)	23%	36.8±2.1 (SEM)	HTN, Obese	N= 13	6 wk	Watermelon powder	6 g L-Citrulline/L-Arginine /d	Placebo	AIx
											AIx_HR75
Forouhi et al., 2016 (UK)	RCT, Parallel	Intervention= 52.5±8.2 Control= 52.4±8.5	Intervention= 51% Control= 58%	Intervention= 29±5.5 Control= 28.3±5	elevated risk of T2D	Intervention= 114 Control= 114	16 wk	Vigantol oil	Cholecalciferol 20000 IU/ m	Miglyol oil	cf-PWV
		Intervention= 52.5±8.2 Control= 52.4±8.5	Intervention= 50% Control= 58%	Intervention= 28.9±5.5 Control= 28.3±5		Intervention= 112 Control= 114		Sterogyl	Ergocalciferol 20000 IU/ m		

(**Table 8.1** continues on next page)

Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
Fulton et al., 2016 (UK)	RCT, Parallel	76±4.4	53%	N/A	Diabetes/ HTN	Intervention= 38 Control= 39	6 m	Vitamin K2 capsule	500 mg/d	Placebo capsule	cf-PWV AIx
Garg et al., 2015 (India)	RCT, Parallel	Intervention= 22±4.61 Control= 22.8±4.56	0%	Intervention= 26.8±4.56 Control= 26.7±6.11	PCOS	Intervention= 15 Control= 17	6 m	1.5 g Metformin and vitamin D	4000 IU/d	1.5 g Metformin and placebo	AIx_75HR cf-PWV
George et al., 2012a (UK)	RCT, Cross-over	45±10	38%	24.4±3.2	Healthy	N= 39	6 wk	Fruit and vegetable puree based drink	2 x 100 mL/d	2x100 mL/d fruit-flavoured cordial	LDI in ACh LDI in SNP
George et al., 2013 (UK)	RCT, Cross-over	M= 46±11 F= 49±4	84%	M= 25.6±3.2 F= 24±2.6	Healthy	N= 24	6 hr	Fruit and vegetable puree based drink	400 mL	400 mL fruit-flavoured cordial	LDI in ACh
Gepner et al., 2012 (USA)	RCT, Parallel	64.1±3.0	0%	27.1±4.7	Healthy	Intervention= 38 Control= 37	16 wk	Cookies rich in vitamin D	2500 IU vitamin D3/d	Placebo cookies	cf-PWV AIx
Gijsbers et al., 2015 (Netherlands)	RCT, Cross-over	M= 66±9.3 F= 65.4±8.2	66%	M= 27.3±4.8 F= 27.0±4.6	Pre-HTN	M= 24 F= 12	4 wk	Placebo capsule	Cellulose	Sodium capsule (371mg of Na/d)	AIx cf-PWV
								Potassium capsule	353 mg/d	Placebo capsule	AIx cf-PWV
Hazim et al., 2016 (UK)	RCT, Cross-over	62±2 (SEM)	100%	25±1 (SEM)	Healthy-Non EP	N= 14	1 d	Isoflavone Supplement	80 mg/d	Placebo	AIx
		57±1 (SEM)		26±1 (SEM)	Healthy-Equol Producer (EP)						
		62±2 (SEM)		25±1 (SEM)	Healthy-Non EP						cf-PWV
		57±1 (SEM)		26±1 (SEM)	Healthy-Equol Producer (EP)						

(**Table 8.1** continues on next page)

Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
He et al., 2010 (UK)	RCT, Cross-over	51±10	71%	29.7±4.8	HTN	N= 42	4 wk	10 Potassium chloride capsules	6.4 mmol potassium per capsule/d	Placebo capsule	cf-PWV
								10 Potassium bicarbonate capsules			
He et al., 2009 (UK)	RCT, Cross-over	52±12	79%	28±5	HTN	N= 71	6 wk	Sodium matching capsule	90 mmol	Slow sodium capsule	cf-PWV
		50±9	49%	31±5		N= 69					
		47±10	79%	27±5		N= 29					
Hewitt et al., 2013 (Australia)	RCT, Parallel	Intervention= 60 Control= 67	Intervention= 53% Control= 43%	Intervention= 26.6±6.4 Control= 31.3±9.5	Haemodialysis	Intervention= 30 Control= 30	6 m	Cholecalciferol	50000 IU/ wk	Placebo	PWV
Hin et al., 2017 (UK)	RCT, Parallel	Intervention= 71±6 Control= 72±6	Intervention= 51% Control= 51%	Intervention= 27±5 Control= 28±5	Healthy	Intervention= 193 Control= 95	12 m	Cholecalciferol	4000 IU/d or 2000 IU/d	Placebo	AIx
											cf-PWV
Hobbs et al., 2013b (UK)	RCT, Cross-over	31±2 (SEM)	100%	23.3±0.5 (SEM)	Healthy	N= 24	6 hr	Beetroot bread	1.1 mmol nitrate/d	White bread	AIx
											AIx_HR75
											cf-PWV
											LDI in SNP
											LDI in ACh
Hollands et al., 2018a (UK)	RCT, Cross-over	52.2±13.6	50.0%	29±5.1	Healthy overweight	N= 41	4 wk	Anthocyanin-rich blood orange juice	500 mL (50 mg anthocyanins)	blonde orange juice (without anthocyanins	cf-PWV

(**Table 8.1** continues on next page)

Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
Hollands et al., 2018b (UK)	RCT, Cross-over	63±7	35.7%	25.9±3.1	Healthy	N= 42	4 wk	Apple flavanol extract	70 mg monomeric flavanols, 65 mg PCs	Placebo capsules	AIx_HR75
											cf-PWV
								Apple flavanol extract	140 mg monomeric flavanols, 130 mg PCs		AIx_HR75
											cf-PWV
								Apple PC extract	130 mg PCs, 6.5 mg monomeric flavanols		AIx_HR75
											cf-PWV
Hu et al., 2009 (China)	RCT, Parallel	59±10	46%	27±3.9	Vascular disease/ T2D	Intervention= 93 Control= 94	12 m	Salt substitute	65% sodium chloride, 25% potassium chloride and 10% magnesium sulfate	Normal salt	AIx
Jablonski et al., 2013(USA)	RCT, Cross-over	60±2 (SEM)	73%	81.8±5.4 (SEM)	HTN	N= 11	5 wk	Low sodium intake	2.5 g/d	Normal diet	cf-PWV
Jauhiainen et al., 2010 (Finland)	RCT, Parallel	49±5	60%	27.6±3.6	HTN	Intervention= 45 Control= 44	12 wk	200 mL lactobacillus helveticus fermented milk	Ile-Pro-Pro 5.8 mg/ 100 mL and Val-Pro-Pro 6.6 mg/ 100 mL	Placebo milk drink	AIx

(**Table 8.1** continues on next page)

Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
Jennings et al., 2019 (European Multi-Centre)	RCT, Parallel	Intervention= 70.7±14 Control= 71±3.9	Intervention= 55.7% Control= 53.1%	Intervention= 70.7±14 Control= 71±3.9	Healthy	Intervention= 114 Control= 116	12 m	Mediterranean-Style Diet		Usual diet	AIx_HR75
						Intervention= 115 Control= 122					cf-PWV
Jin et al., 2011 (UK)	RCT, Cross-over	M= 44±5.2 (SEM) F= 45±5.2 (SEM)	45%	M= 24±0.83 (SEM) F= 23±0.91 (SEM)	Healthy	M= 9 F= 11	2 hr	Blackcurrant Juice	250 mL/d	Placebo Juice	LDI in ACh
											LDI in SNP
Johnson et al., 2015 (UK)	RCT, Parallel	Intervention= 59.7±4.58 Control= 57.3±4.76	0%	Intervention= 30.1±5.94 Control= 32.7±6.79	HTN	Intervention= 20 Control= 20	8 wk	Freeze-dried blueberry powder	22 g/d (Phenolics 845 mg, Anthocyanins 470 mg)	Control powder	cf-PWV
											ba-PWV
Joris et al., 2016 (Netherlands)	RCT, Parallel	Intervention= 62±5 Control= 62±6	37%	29.6±2.8	Overweight	Intervention= 26 Control= 25	6 m	Magnesium capsules	350 mg/d	Placebo capsules	AIx_HR75
											cf-PWV
Jovanovski et al., 2014 (Canada)	RCT, Cross-over	25±2	39%	22±0.6	Healthy	N= 23	3 hr	Ginsenoside Rg3-Korean red ginseng extract	400 mg/d	Wheat bran control	AIx_HR75
Jovanovski et al., 2015 (Canada)	RCT, Cross-over	24.5±11	41%	22.8±3.7	Healthy	N= 27	2 wk	Spinach	845 mg Nitrate /d	Asparagus	AIx_HR75

(**Table 8.1** continues on next page)

Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
Jovanovski et al., 2010 (Canada)	RCT, Cross-over	30±9	59%	25±3	Healthy	N= 17	3 hr	Korean red ginseng rootlet extract	3 g of dried Korean red ginseng	3 g of cornstarch control	AIx
								Ginsenoside extract	1.22 g of ginsenoside extract delivering 105 mg total saponin-equivalent to the total ginsenosides from 3 g of KRG rootlets		
								Polysaccharide extract	0.21 g of polysaccharide extract delivering 172 mg of polysaccharide equivalent to the polysaccharide content of 3 g of KRG rootlets		
Karatzi et al., 2013 (Greece)	RCT, Cross-over	28.5±5.2	100%	24.4±2.5	Healthy	N= 17	2 hr	Beer	20 g alcohol	Dealcohol beer	AIx
								Vodka			
								Beer			cf-PWV
								Vodka			

(**Table 8.1** continues on next page)

Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
Karatzi et al., 2005 (Greece)	RCT, Cross-over	52.4±9.7	100%	28.3±1.8	Mix	N= 15	1.5 hr	Red wine	250 mL	Dealcohol red wine	AIx
											AIx_HR75
Kasliwal et al., 2015 (India)	RCT, Parallel	Intervention= 40.4±8.2 Control= 37.7±7.6	74%	Intervention= 27.8±4.7 Control= 26.1±2.9	Dyslipidaemia	Intervention= 21 Control= 21	12 wk	Pistachio	80 g	No nuts	ba-PWV
											cf-PWV
Keane et al., 2016 (UK)	RCT, Cross-over	31±9	100%	27.0±3.8	Early HTN	N= 15	8 hr	Montmorency tart cherry	60 mL	Placebo	AIx
											cf-PWV
											LDI in ACh
											LDI in SNP
Keheyan et al., 2011 (UK)	RCT, Cross-over	22±5	100%	22±3.2	T2D/ CVD/ Thrombosis	N= 14	6 hr	6 Ginkgo biloba extract capsules	Each capsule contains 60 mg GBE	Placebo capsules	AIx
Kendall et al., 2014 (Canada)	RCT, Cross-over	54±8	40%	37.5±7.9	Metabolic Syndrome	N= 20	3 hr	Pistachio	3 oz/d	White bread	AIx
Kouguchi et al., 2013 (Japan)	RCT, Parallel	Intervention= 54.3±9.2 Control= 51.2±7.8	52%	Intervention= 24.6±2.9 Control= 24.8±3	HTN	Intervention= 29 Control= 29	12 wk	120 mL lactic acid drink	Contain 2.9 g Chicken collagen hydrolysate	120 mL lactic acid drink	ba-PWV

(**Table 8.1** continues on next page)

Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
Krantz et al., 2015 (USA)	RCT, Parallel	60.2±10.8	37%	31.5±7.1	CVD Risk	Intervention= 27 Control= 35	12 wk	4 Omega 3 fatty acids capsules	Each capsule contains 465 mg of EPA and 375 mg of DHA	Placebo capsules	ba-PWV
Kristensen et al., 2016 (Denmark)	RCT, Parallel	Intervention= 53.2±11.4 Control= 50.7±11.5	60%	Intervention= 28.6±5.7. Control= 28.0±5	Patients psoriatic arthritis	Intervention= 58 Control= 56	6 m	6 omega 3 fatty acids capsules	3 g of n-3 PUFA (50% EPA and 50% DHA)	Olive oil capsules	AIx cr-PWV
Kumar et al., 2017 (Australia)	RCT, Parallel	Intervention= 43.2±11.8 Control= 45.2±11.6	Intervention= 67.8% Control= 70.7%	Intervention= 23.6±2.7 Control= 23.5±2.9	CKD	Intervention= 59 Control= 58	16 wk	Cholecalciferol	300000 IU single dose	Placebo	cf-PWV
Larijani et al., 2013 (USA)	RCT, Parallel	Intervention= 54±5 Control= 55±6	100%	Intervention= 29±4 Control= 28±3	Healthy	Intervention= 32 Control= 33	12 m	4 Ageing garlic extract capsules	4 capsules contain 300 mg/AGE and 30 mg/CoQ10	Placebo capsules	cr-PWV
Larsen et al., 2012 (Denmark)	RCT, Parallel	Intervention= 60±12 Control= 61±9	Intervention= 32% Control= 30%	Intervention= 27.7±4.2 Control= 28.3±3.7	HTN	Intervention= 55 Control= 57	6 m	Cholecalciferol	3000 IU (75 µg)/d	Placebo	AIx cf-PWV
Levin et al., 2017 (Canada)	RCT, Parallel	Intervention= 65.9±15.3 Control= 64.5±12.2	Intervention= 70% Control= 73%	Intervention= 29±5 Control= 28.8±5.3	CKD	Intervention= 29 Control= 30	6 m	Calcifediol	5000 IU thrice weekly	Placebo	AIx PWV
		Intervention= 66.9±11.7 Control= 64.5±12.2	Intervention= 72% Control= 73%	Intervention= 29.7±5.9 Control= 28.8±5.3		Intervention= 28 Control= 30		Calcitriol	0.5 µg thrice weekly		AIx cf-PWV

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Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
Lithander et al., 2013 (Ireland)	RCT, Cross-over	38.7±14.4	100%	24.1±2.3	Healthy	N= 20	4 hr	Monounsaturated fat meal	MUFA meal and SFA meal	SFA Meal	AIx
											cf-PWV
Liu et al., 2013 (Australia)	RCT, Cross-over	58.8±7.6	23%	25.4±3.3	Healthy	N= 26	3.5 hr	Nitrate rich meal (spinach)	High nitrate 220 mg twice/d	Low nitrate	AIx
											cf-PWV
Lucey et al., 2018 (UK)	RCT, Cross-over	56.9±5.2	56.9%	28.3±3.5	Healthy	N= 65	6 wk	Powdered egg ovalbumin-derived protein hydrolysate	3 g/d	Maltodextrin powder	AIx_HR75
											cf-PWV
Lundwall et al., 2015 (Sweden)	RCT, Parallel	Intervention= 70.8±10 Control= 59.1±11.6	Intervention= 67% Control= 75%	Intervention= 28.1±2.4 Control= 26.8±2.8	CKD	Intervention= 12 Control= 12	12 wk	Paricalcitol	2 µg/d	Placebo	AIx
											PWV
		Intervention= 66.1±7.9 Control= 59.1±11.6	Intervention= 92% Control= 75%	Intervention= 26.4±3.5 Control= 26.8±2.8		Intervention= 12 Control= 12			1 µg/d		LDI in ACh
											LDI in SNP
Lynn et al., 2012 (UK)	RCT, Parallel	Intervention= 39.0±1.24 Control= 36.1±0.92	33%	Intervention= 24.99±1.26 Control= 24.99±1.06	Healthy	Intervention= 24 Control= 24	4 wk	Pomegranate juice drink	330 mL/d	Placebo	bf-PWV

(**Table 8.1** continues on next page)

Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
Macready et al., 2014 (UK)	RCT, Parallel	Intervention= 52±1 (SEM) Control= 53±1 (SEM)	0%	Intervention= 28.2±0.6 (SEM) Control= 27±0.6 (SEM)	Healthy	Intervention= 22 Control= 21	6 wk	High flavonoid Diet	>15 mg/100 g flavonoid food	Habitual diet	AIx_HR75
											cf-PWV
		Intervention= 47±1 (SEM) Control= 50±2 (SEM)	100%	Intervention= 26.8±0.6 (SEM) Control= 27.9±0.6 (SEM)		Intervention= 36 Control= 36					AIx_HR75
											cf-PWV
		Intervention= 52±2 (SEM) Control= 53±1 (SEM)	0%	Intervention (LF, F)= 28.4±0.5 (SEM) Control (F)= 27±0.6 (SEM)		Intervention= 25 Control= 21		Low flavonoid Diet	>5 mg/100 g of total flavonoids		AIx_HR75
											cf-PWV
		Intervention= 49±1 (SEM) Control= 50±2 (SEM)	100%	Intervention= 27.5±0.5 (SEM) Control= 27.9±0.6 (SEM)		Intervention= 34 Control= 36					AIx_HR75
											cf-PWV
Marckmann et al., 2012 (Danmark)	RCT, Parallel	Intervention= 71 Control= 68	Intervention= 73% Control= 77%	Intervention= 25.9 Control= 24.6	CKD	Intervention= 13 Control= 17	8 wk	Cholecalciferol	40000 UI/wk	Placebo	AIx_HR75
						Intervention= 14 Control= 17					cf-PWV

(**Table 8.1** continues on next page)

Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
Marques et al., 2018 (Brazil)	RCT, Cross-over	54±1SEM	42%	30±2	HTN	24	1.5 hr	Trans-resveratrol capsule	300 mg	Placebo	AIx AIx_HR75
Martins et al., 2014 (USA)	RCT, Parallel	18-60	Intervention= 63.1% Control= 58.5%	25-30	HTN	Intervention= 60 Control= 55	12 wk	Vitamin D	100000 IU/m	Placebo	AIx
McAnulty et al., 2014 (USA)	RCT, Parallel	Intervention= 46.15±11.92 Control= 24.23±3.44	N/A	Intervention= 27.8±5.46 Control= 24.23±7.79	Healthy (sedentary)	Intervention= 13 Control= 12	6 wk	Whole blueberry powder	38 g/d of dehydrated blueberry powder	Placebo	AIx cf-PWV
McGreevy et al., 2015 (Ireland)	RCT, Parallel	Intervention= 79.3±7 Control= 80.5±6.6	Intervention= 55% Control= 51%	Intervention= 26.6±6.4 Control= 26.9±9.5	Vitamin D insufficient patients	Intervention= 51 Control= 51	8 wk	Vitamin D (Cholecalciferol) supplementation	100,000 IU Vitamin D/d	50,000 IU Vitamin D /d	AIX cf-PWV
Mose et al., 2014 (Denmark)	RCT, Parallel	Intervention= 68±9 Control= 67±13	Intervention= 60% Control= 68%	Intervention= 24±4.5 Control= 23.8±4.4	Haemodialysis	Intervention= 25 Control= 25	6 m	Cholecalciferol	3000 IU (75 µg)/d	Placebo	AIx cf-PWV
Mucalo et al., 2013 (USA)	RCT, Parallel	Intervention= 62.1±8.8 Control= 63.910.93	Intervention= 54% Control= 50%	Intervention= 33.4±5.6 Control= 29.9±4.95	T2DM, HTN	Intervention= 30 Control= 34	6 wk	American ginseng extract capsules	6 capsules /d	Placebo capsules	AIx
Mullan et al., 2016 (UK)	RCT, Parallel	Intervention= 60.3±4.60 Control= 62.21±4.26	Intervention= 50% Control= 53%	Intervention= 33.08±6.68 Control= 31.49±4.38	Overweight	Intervention= 20 Control= 19	4 wk	250 mL Beverage rich in polyphenol	361 mg of (poly)phenols and 120 mg of vitamin C	Placebo drink	cf-PWV AIx
Mullan et al., 2004 (UK)	RCT, Cross-over	25.2±4.1	100%	24.4±2.8	Healthy	N= 12	2 hr	Hyperglycaemia meal, with Vitamin C	2 g Vitamin C	Hyperglycaemia meal, without ascorbic acid	AIx_HR75

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Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
Mullan et al., 2002 (UK)	RCT, Parallel	Intervention= 61±6.5 Control= 57.9±6.6	Intervention= 80% Control= 67%	Intervention= 28.6±4.4 Control= 28.6 4.2	T2DM	Intervention= 15 Control= 15	4 wk	Vitamin C	500 mg/d	Placebo	AIx_HR72
Muth et al., 2017 (USA)	RCT, Cross-over	27±1 (SEM)	61.2%	23.6±0.4 (SEM)	Healthy	N= 49	1 wk	Low Sodium	20 mmol/d	High Sodium (300 mmol/d)	AIx_HR75
											cf-PWV
		52±1 (SEM)	36%	25.1±0.5 (SEM)		N= 36					AIx_HR75
											cf-PWV
Noad et al., 2016 (UK)	RCT, Parallel	Intervention= 54.0±6.7 Control= 55.6±6.8	25%	Intervention= 31.6±6.5 Control= 29.8±5	HTN	Intervention= 48 Control= 47	8 wk	High Polyphenol diet	6 portions of fruit and vegetables/d	Low polyphenol diet	LDI in ACh
											LDI in SNP
Ochiai et al., 2012 (Japan)	RCT, Parallel	Intervention= 58.5±5.0 Control= 58±3.9	100%	Intervention= 25.2±2.4 Control= 24.9±2.2	Healthy	Intervention= 8 Control= 7	1 wk	L-Citrulline	L-Citrulline 5.6 g/d	Placebo	ba-PWV
Otsuki et al., 2015 (Japan)	RCT, Parallel	Intervention= 62.2±1.5 (SEM) Control= 63.1±2.3 (SEM)	40%	Intervention= 23.6±0.6 (SEM) Control= 22.8±0.8 (SEM)	Healthy	Intervention= 15 Control= 17	4 wk	Chlorella-derived supplementation	200 mg Chlorella	Placebo	ba-PWV

(**Table 8.1** continues on next page)

Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
Pal and Ellis, 2010 (Australia)	RCT, Parallel	Intervention= 48.0±2.1 (SEM) Control= 48.4±1.5 (SEM)	N/A	Intervention= 31.3±0.9 (SEM) Control= 30.6±0.8 (SEM)	Healthy	Intervention= 20 Control= 25	12 wk	Casein supplement	Two sachets in 250 mL of water - twice a day	Glucose control	AIx
		Intervention= 48.5±2.0 (SEM) Control= 48.4±1.5 (SEM)		Intervention= 32.0±0.8 (SEM) Control= 30.6±0.8 (SEM)		Intervention= 25 Control= 25		Whey supplement	Two sachets in 250 mL of water - twice a day		
Pilz et al., 2015 (Denmark)	RCT, Parallel	60.1±11.3	53.0%	N/A	HTN	Intervention= 100 Control= 100	8 wk	Cholecalciferol	2800 IU/d	Placebo	PWV
Pimenta et al., 2009 (Australia)	RCT, Cross-over	55.5±9.4	33%	32.9±6.3	Healthy	N= 12	4 wk	Low sodium intake	Low Sodium (1 wk)= 50 mmol	High sodium (1 wk)= 250 mmol	AIx
											cf-PWV
Plat et al., 2019 (Netherlands)	RCT, Cross-over	60±9	75%	28.7±2.7	T2D	N= 41	3 d	Egg protein hydrolysate	10 capsules with 5 g egg protein hydrolysate	Placebo	AIx_HR75
											cf-PWV
Rathnayake et al., 2018 (UK)	RCT, Cross-over	58±1 (SEM)	0%	25.9±0.7 (SEM)	Healthy	N= 31	480 min	High-fat meals at breakfast and lunch	33-36 g MUFAs	33-36 g SFAs	LDI in ACh
									33-36 g n-6 PUFAs		LDI in SNP

(**Table 8.1** continues on next page)

Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
Richter et al., 2017a (USA)	RCT, Cross-over	51.6±6.6 (SEM)	45%	26.4±0.9 (SEM)	Healthy	N= 20	6 wk	Soya protein	25 g/d	Placebo	AIx_HR75
											cf-PWV
									50 g/d		AIx_HR75
											cf-PWV
Richter et al., 2017b (USA)	RCT, Cross-over	28±2 (SEM)	56.7%	31.5±0.5 (SEM)	Healthy and Overweight	N= 30	4hr	Freeze-dried strawberry powder to a high-fat (50 g total fat) meal	40 g	macro- and micronutrient-matched control meal	AIx_HR75
											cf-PWV
Ried et al., 2018 (Australia)	RCT, Parallel	Intervention= 28.6±7.7 Control= 29.7±6.2	45%	24.6±2.6	HTN	Intervention= 23 Control= 26	12 wk	Kyolic-aged-garlic-extract	2 capsules/d (1.2 g of the vaso-active S-allylcysteine (SAC))	Placebo capsules	cf-PWV
Ruel et al., 2013 (Canada)	RCT, Cross-over	45±10	100%	28.3±2.4	Healthy - Overweight	N= 35	4 wk	500 mL Cranberry juice cocktail	Contained 400 mg total polyphenols, 20.8 mg anthocyanins, and 21.84 g carbohydrates	500 mL Placebo juice	AIx
Ryu et al., 2014 (Korea)	RCT, Parallel	Intervention= 54.5±7.4 Control= 56.7±7.9	N/A	Intervention= 24.4±5 Control= 25.3±3.4	T2D	Intervention= 32 Control= 30	6 m	Cholecalciferol	2000 IU/d	Placebo	AIx_HR75
											ba-PWV
Saban-Ruiz et al., 2017 (Spain)	RCT, Parallel	Intervention= 39.9±8.5 Control= 40.5±8.9	23.2%	Intervention= 23.6±3.2 Control= 23.8±2.9	Healthy	Intervention= 48 Control= 51	6 wk	Ad libitum diet with Iberian cured ham	50 g	Libitum diet	AIx_HR75

(**Table 8.1** continues on next page)

Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
Sanders et al., 2013 (UK)	RCT, Parallel	Intervention= 51±9.2 Control= 51±7.9	Intervention= 37% Control= 40%	Intervention= 27.7±3.8 Control= 28.5±4.3	Healthy - At risk of metabolic syndrome	Intervention= 38 Control= 30	4 wk	Low saturated fat and high carbohydrate	SFA:MUFA:PUFA= 21:52:24	SFA rich diet SFA:MUFA:PUFA= 50:26:24	AIx
											cf-PWV
		Intervention= 51±10.2 Control= 51±7.9	Intervention= 32% Control= 40%	Intervention= 28.5±4.5 Control= 28.5±4.3		Intervention= 44 Control= 30		Low saturated fat and high MUFA	SFA:MUFA:PUFA= 18:60:23		AIx
											cf-PWV
Smolders et al., 2018 (Netherlands)	RCT, Cross-over	45-70	63.6%	25-35	Healthy overweight	N= 44	4 wk	Theobromine	500 mg	Placebo	AIx_HR75
											cf-PWV
Stricker et al., 2012 (Switzerland)	RCT, Parallel	Intervention= 72.9±8.7 Control= 74.8±14.6	Intervention= 61% Control= 61%	N/A	PAD	Intervention= 31 Control= 31	4 wk	Cholecalciferol	10000 IU single dose	Placebo	AIx
Todd et al., 2010 (USA)	RCT, Cross-over	51.8±7.6	38%	25.7±5.2	HTN	N= 34	4 wk	Tomato Juice	500 mL of tomato (90 mmol Na)	Tomato juice with 0 mmol Na	AIx
									500 mL of tomato (140 mmol Na)		
									500 mL of tomato (90 mmol Na)		cf-PWV
									500 mL of tomato (140 mmol Na)		
Tripkovic et al., 2015 (UK)	RCT, Cross-over	39.8±9	100%	30.2±3	Overweight	N= 10	4 wk	Inulin	15 g/d	Refined grain (15g/d)	cf-PWV
											cr-PWV
								Wheat fibre	15 g/d		cf-PWV
											cr-PWV

(**Table 8.1** continues on next page)

Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
Turner et al., 2005 (Denmark)	RCT, Parallel	Intervention= 49.6±5.5 Control= 50.9±4.6	39%	Intervention= 24.2±3.9 Control= 24.7±2.8	Healthy	Intervention= 30 Control= 32	12 wk	Garlic powder tablets	Garlic Powder Tablets= 920 mg/d	Placebo capsules	PWV
Wang et al., 2015 (China)	Not RCT	49±7.9	48%	23.6±2.9	Healthy - Smoking	N= 48	1 wk	Low salt meal	Low salt (1 wk)= 3 g/d	High salt (1 wk)= 18g/d	ba-PWV
								High salt meal with potassium supplementation	High salt with potassium supplementation (1 Wk)= 18 g/d + 4.5 g/d(NaCl)		
Weech et al., 2018 (UK)	RCT, Parallel	Intervention= 43±1 (SEM) Control= 45±1 (SEM)	43%	Intervention= 27±0.5 Control= 26.5±0.5	Individual with moderate CVD risk	Intervention= 62 Control= 64	16 wk	MUFA-rich diet		SFAs rish diet	AIx_HR75
		Intervention= 45±1 (SEM) Control= 45±1 (SEM)		Intervention= 27±0.5 Control= 26.5±0.5		Intervention= 66 Control= 64		n–6 PUFA-rich diet			cf-PWV
West et al., 2014 (USA)	RCT, Cross-over	51.7±1.2 (SEM)	51%	27.8±0.6 (SEM)	Healthy and Overweight	N= 30	4 wk	Chocolate bar and cocoa beverage	Chocolate bar= 37 g/d	Low-flavanol chocolate bar and a cocoa-free beverage mix with no added sugar	AIx
											AIx_HR75
Witham et al., 2013a (UK)	RCT, Parallel	Intervention= 39.4±11.8 Control= 41.7±13.4	0%	Intervention= 28.7±5.5 Control= 24.9±3.3	Healthy	Intervention= 25 Control= 25	8 wk	Cholecalciferol	100000 IU single dose	Placebo	AIx_HR75
											cr-PWV
											LDI in ACh
											LDI in SNP

(**Table 8.1** continues on next page)

Ref (Country of origin)	Study design	Age±SD	Sex (%male)	BMI±SD	Healthy status	Sample size	Period	Intervention type	Dose	Control/ comparator	Outcome
Witham et al., 2013b (UK)	RCT, Parallel	Intervention= 76.7±4.8 Control= 76.9±4.5	Intervention= 50% Control= 53%	Intervention= 27.9±4.5 Control= 28.5±3.5	HTN	Intervention= 79 Control= 80	12 m	Cholecalciferol	100000 IU/3m	Placebo	AIx_HR75
											cr-PWV
Witham et al., 2015 (UK)	RCT, Parallel	Intervention= 48.1±12 Control= 50.7±13.1	Intervention= 28% Control= 20%	Intervention= 28.8±7.9 Control= 29.8±5.4	Chronic fatigue syndrome	Intervention= 25 Control= 25	6 m	Cholecalciferol	100000 IU/2m	Placebo	AIx_HR75
											cf-PWV
Xing et al., 2018 (China)	Not RCT	53.4±6.6	60%	25.1±3.4	Healthy	N= 99	7 wk	Low sodium	51.3 mmol sodium/d	High-sodium (307.8 mmol sodium/d)	AIx_HR75
								High-sodium with potassium supplementation	60.0 mmol potassium/d		
Yiu et al., 2013 (HK)	RCT, Parallel	Intervention= 65.8±7.3 Control= 64.9±8.9	Intervention= 54% Control= 46%	Intervention= 25.8±4.3 Control= 25.1±3.4	T2D	Intervention= 50 Control= 50	12 wk	Cholecalciferol	5000 IU/d	Placebo	ba-PWV
Yui et al., 2017 (Japan)	RCT, Parallel	Intervention= 45±7.8 Control= 44.8±9.6	50%	N/A	Healthy	Intervention= 14 Control= 14	6 wk	Lemon balm leaf extract		Barley tea	ba-PWV

Ref, Reference; M, Male; F, Female; RCT, randomised controlled trial; hr, hour; d, day; wk, week; m, month; BMI, Body mass index; LDI, Laser Doppler Imaging; Ach, Acetylcholine; SNP, Sodium nitroprusside; AIx, Augmentation index; AIx@HR75, Augmentation index adjusted to a heart rate of 75 beats/min; PAIx, Peripheral augmentation index; CAIx, central augmentation index; PWV, Pulse wave velocity; cf, Carotid-femoral; cr, Carotid-radial; ba, brachial-ankle; HTN, Hypertension; CAD, Coronary artery disease; CKD, Chronic kidney disease; T2D, Type 2 diabetes; PCOS, Polycystic ovarian syndrome

8.3.1 Study characteristics

Fifty-eight studies were RCT parallel design while fifty-three studies used a RCT cross-over design and two study used non-RCT design. These 113 studies originated from the UK (n= 36), USA (n= 21), Australia (n= 12), Canada (n= 7), Denmark (n= 7), Netherlands (n= 6), Japan (n= 4), China (n= 3), Greece (n= 2), Ireland (n= 2), India (n= 2) and single study from Brazil, Finland, Spain, Sweden, Switzerland, European multi-centre, Israel, Hong Kong, Singapore, Thailand, and Korea. These studies included a range of acute and chronic studies (1 day to 1 year), mean age of the samples studied ranged from 22 to 80 years, and BMI from 21 to 37 kg/m^2, with mixed health status. Eleven studies evaluated the impact of LDI after intervention. Sixty-nine studies evaluated the impact of PWA after intervention. Sixty-one studies evaluated the impact of PWV after intervention. Thirty-eight studies evaluated the impact of both PWA and PWV after intervention.

8.3.2 Microvascular vasodilation with LDI measurement

Ten studies assessed the effect of nutritional intervention on microvascular vasodilation using LDI techniques (**Table 8.2**) (Armah et al., 2008; Dreyer et al., 2014; George et al., 2012a; George et al., 2013; Hobbs et al., 2013b; Jin et al., 2011; Keane et al., 2016; Noad et al., 2016; Rathnayake et al., 2018; Witham et al., 2013a). The meta-analysis of 9 studies with 467 participants evaluated the effect of nutritional intervention on endothelium dependent microcirculation vasodilation (ACh) (Armah et al., 2008; Dreyer et al., 2014; George et al., 2012a; George et al., 2013; Hobbs et al., 2013b; Keane et al., 2016; Noad et al., 2016; Rathnayake et al., 2018; Witham et al., 2013a). Overall, nutritional intervention did not change endothelium dependent microcirculation vasodilation (0.30 (SMD); 95% CI -0.03 to 0.63; p=0.07; I^2 =66%) (**Figure 8.2**). Subgroup analysis according to supplementation of polyphenols (George et al., 2012a; George et al., 2013; Keane et al., 2016; Noad et al., 2016), fatty acid (Armah et al., 2008; Rathnayake et al., 2018), nitrate/nitrite (Hobbs et al., 2013b) and vitamin D (Dreyer et al., 2014; Witham et al., 2013a) was undertaken. Supplementation of polyphenols, fatty acid, nitrate/nitrite and vitamin D showed no differences on endothelium dependent microcirculation vasodilation (ACh) (**Figure 8.2**).

The meta-analysis of 6 studies with 331 participants evaluated the effect of nutritional intervention on endothelium independent microcirculation vasodilation (SNP) (Armah et al., 2008; George et al., 2012a; Hobbs et al., 2013b; Keane et al., 2016; Noad et al., 2016; Rathnayake et al., 2018). Overall, nutritional intervention did not change endothelium

independent microcirculation vasodilation (0.02 (SMD); 95% CI -0.32 to 0.37; p=0.89; I^2 =60%) (**Figure 8.3**). Subgroup analysis according to supplementation of polyphenols (George et al., 2012a; Keane et al., 2016; Noad et al., 2016), fatty acid (Armah et al., 2008; Rathnayake et al., 2018), nitrate/nitrite (Hobbs et al., 2013b) and vitamin D (Dreyer et al., 2014) was undertaken. Supplementation of polyphenols, fatty acid, and nitrate/nitrite showed no differences on endothelium independent microcirculation vasodilation (SNP) (**Figure 8.3**).

In addition, one study assessing consumption of blackcurrant juice (Jin et al., 2011) was not included in the meta-analysis because the study did not provide the raw data. It showed no effect on endothelium dependant or independent microcirculation after consumption of blackcurrant juice (Jin et al., 2011).

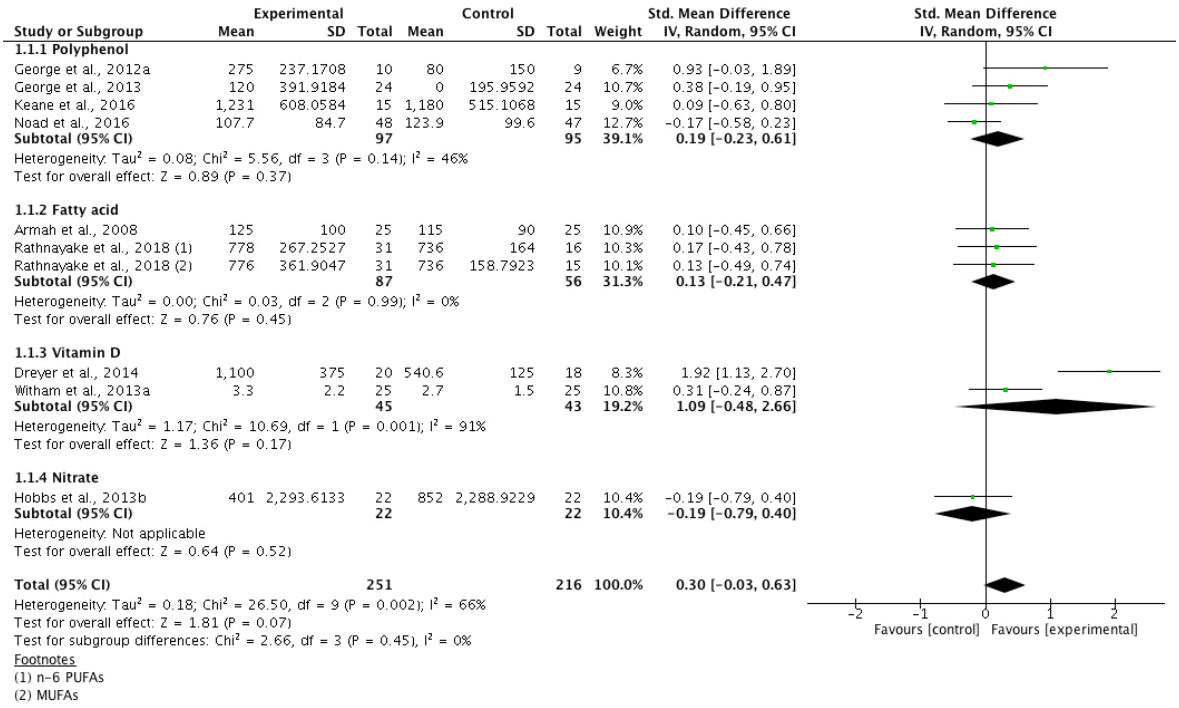


Figure 8.2 Meta-analysis of effect of food interventions on endothelium dependant microcirculation (LDI-ACh).

Table 8.2 Effects of nutritional intervention on endothelial dependant and independent vasodilation.

NS: Non-significant change; N/A: Not available; AU: Arbitrary units; PU: Perfusion units.

Study	Duration	Type of intervention	Active component	Effect on LDI-ACh (SEM)	Effect on LDI-SNP (SEM)
Armah et al., 2008	Acute (4 hours)	Fish oil	n-3 PUFAs	NS (Intervention 125 PU±20 vs Placebo 100±18)	NS (*Intervention 140 PU±20 vs Placebo 115 ±18) *Compare to baseline (p=0.024)
Dreyer et al., 2014	Chronic (6 month)	Vitamin D	Ergocalciferol	^Intervention 1100%±375 (SD) vs Placebo 540.6%±125 (SD)) ^Compare to placebo (p=0.012)	NS (Intervention 445.7%±187.5 (SD)vs Placebo 585.9%±85(SD))
George et al., 2012a	Chronic (6 weeks)	Fruit and vegetable puree	Polyphenol	NS (Intervention 275%±75 vs Placebo 80% ±50)	NS (Intervention 250%± 75 vs Placebo 50%± 50)
George et al., 2013	Acute (6 hours)	Fruit and vegetable puree	Polyphenol	NS (Intervention 120% ±80 vs Placebo 0% ±40)	N/A
Hobbs et al., 2013b	Acute (4 hours)	Beetroot bread	Nitrate	NS (Intervention 401 PU±489 vs White bread 852±488)	†Intervention 2270 PU±600 vs White bread 661±639 †Compare to white bread (p=0.018)
Jin et al., 2011	Acute (2 hours)	Blackcurrant juice	Polyphenol	NS	NS
Keane et al., 2016	Acute (8 hours)	Montmorency tart cherry	Polyphenol	NS (Intervention 1231 PU±157 vs Placebo 1180±133)	NS (Intervention 1424 PU±178 vs Placebo 1690±187)
Noad et al., 2016	Chronic (4 weeks)	High polyphenol diet	Polyphenol	NS (‡Intervention 107.7% ±84.7 (SD) vs Low polyphenol diet 123.9%±99.6 (SD)) ‡Compare to baseline (p=0.02)	NS (Intervention 107.7%±70.1 (SD) vs Low polyphenol diet 126.3%±120.4 (SD))
Rathnayake et al., 2018	Acute (4 hours)	PUFA-rich meals	n-6 PUFAs	NS (Intervention 778 AU±48 vs SFAs 736±41)	NS (Intervention 721 AU±55 vs SFAs 745±45)
		MUFA-rich meals	MUFAs	NS (Intervention 776 AU±65 vs SFAs 736±41)	NS (Intervention 813 AU±63 vs SFAs 745±45)
Witham et al., 2013a	Chronic (8 weeks)	Vitamin D	Cholecalciferol	NS (Intervention 3.3±2.2 vs Placebo 2.7±1.5 Ratio)	N/A

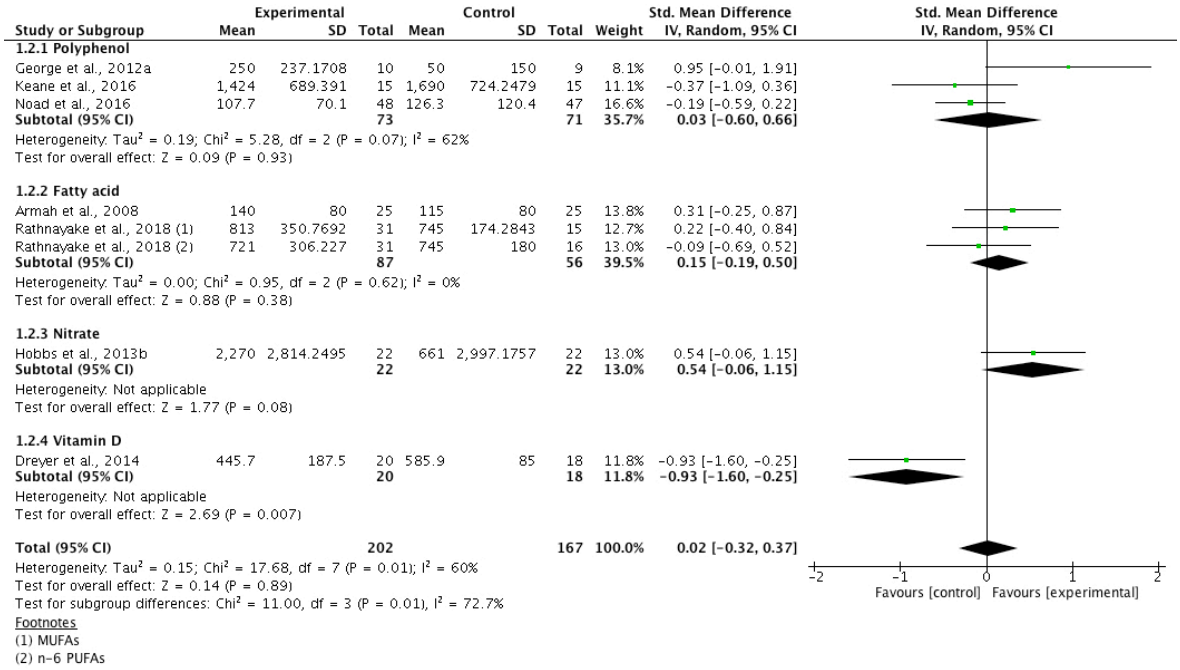


Figure 8.3 Meta-analysis of effect of food interventions on endothelium independent microcirculation (LDI-SNP).

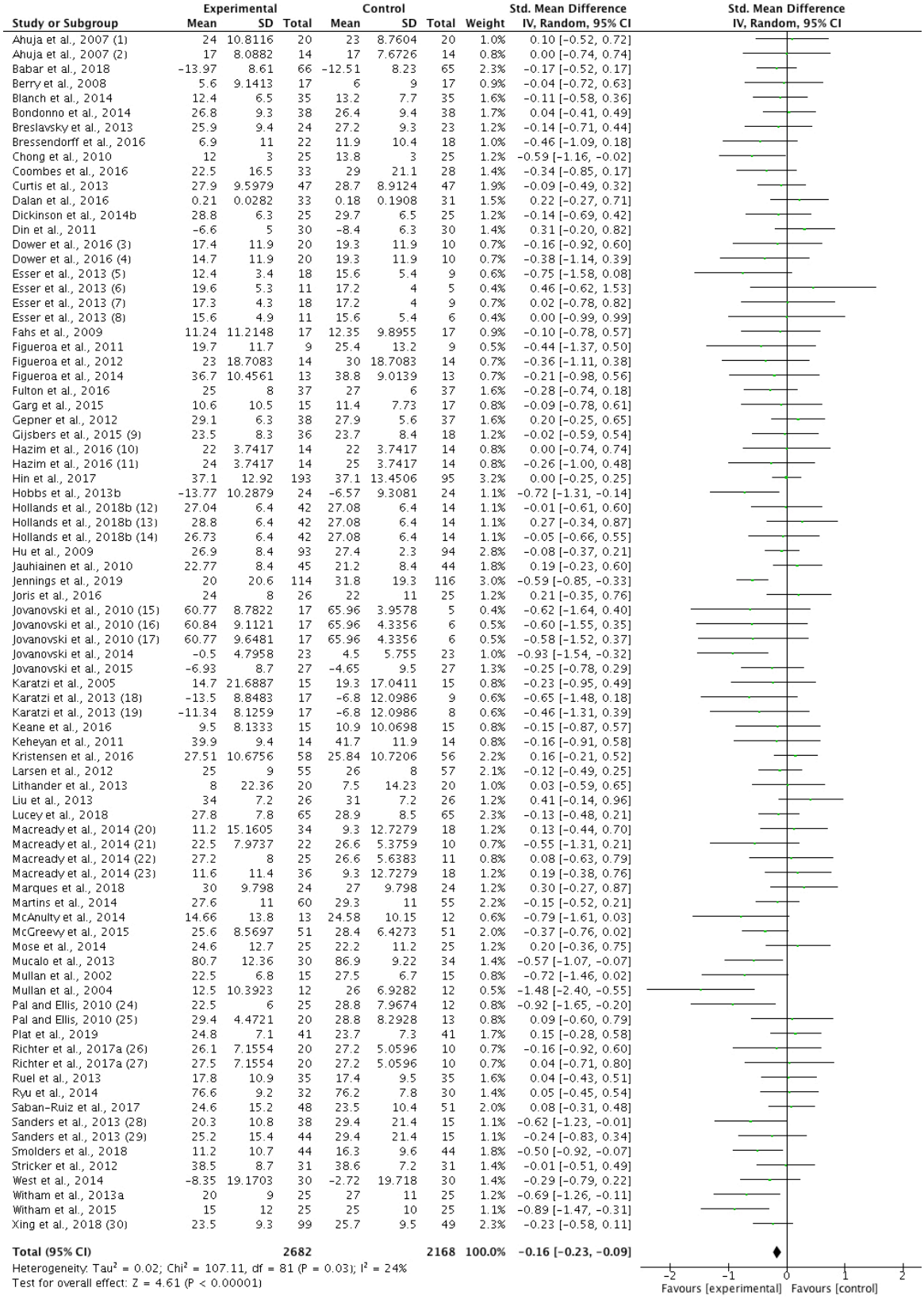
8.3.3 Meta-analysis of studies evaluated the effect of nutritional intervention on arterial stiffness by PWA measurement

Sixty-six studies, including 4850 participants, evaluated the effect of nutritional intervention on arterial stiffness by PWA. Overall, nutritional intervention significantly reduced PWA by 0.16 (95% CI -0.23 to -0.09; p<0.0001). Heterogeneity levels assessed by the I^2 test were low at 24% (**Figure 8.4**).

Subgroup analysis according to food type intervention was undertaken. Studies were separated according to change of diet style and supplementation of polyphenols, fatty acid, salt (extra salt, low sodium and high potassium intake), nitrate/nitrite, vitamin, alcohol, watermelon/L-citrulline, Ginseng and protein (**Table 8.3**).

Two studies, including 342 participants, evaluated the effect of changing of diet style on arterial stiffness by PWA (Jennings et al., 2019; Sanders et al., 2013). Changing of diet style significantly reduced PWA by 0.54 (95% CI -0.77 to -0.32; p<0.0001). Heterogeneity levels assessed by the I^2 test were low at 0% (**Table 8.3**).

Four studies, including 184 participants, evaluated the effect of supplementing Ginseng on arterial stiffness by PWA (Jovanovski et al., 2014; Jovanovski et al., 2010; Keheyan et al., 2011; Mucalo et al., 2013). Supplementing Ginseng significantly reduced PWA by 0.59 (95% CI -0.90 to -0.29; p<0.0001). Heterogeneity levels assessed by the I^2 test were low at 0% (**Table 8.3**).



Footnotes

(1) Women
(2) Men
(3) Epicatechin Capsules
(4) Dark chocolate
(5) Lean, MUFA
(6) Obese, PUFA
(7) Obese, MUFA
(8) Lean, PUFA
(9) Potassium
(10) Equol producer
(11) Non Equol producer
(12) 130 mg PCs, 6.5 mg monomeric flavanols
(13) 70 mg monomeric flavanols, 65 mg PCs
(14) 140 mg monomeric flavanols, 130 mg PCs
(15) Polysaccharide extract
(16) Korean red ginseng rootlet extract
(17) Ginsenoside extract
(18) Vodka
(19) Beer
(20) Low flavonoid, Male
(21) High flavonoid, Female
(22) Low flavonoid, Female
(23) High flavonoid, Male
(24) Whey supplement
(25) Casein supplement
(26) 25 g Soya protein
(27) 50 g Soya protein
(28) Low saturated fat and high carbohydrate
(29) Low saturated fat and high MUFA
(30) 60.0 mmol potassium/day

Figure 8.4 Meta-analysis of effect of nutritional intervention on arterial stiffness by PWA measurement.

Table 8.3 Subgroup analysis of nutritional intervention on arterial stiffness by PWA measurement.

Subgroup	Standard Mean Difference (SMD) IV, Random (95%CI)	*P* (Z-test)	Heterogeneity I^2%
Change of diet style (2 studies, n=342)	-0.54 (-0.77, -0.32)	<0.0001	0
Ginseng (4 studies, n=184)	-0.59 (-0.90, -0.29)	<0.0001	0
Vitamin C (2 studies, n=54)	-1.05 (-1.78, -0.31)	0.005	36
Vitamin D (13 studies, n=1149)	-0.13 (-0.28, 0.02)	0.09	34
Alcohol (2 studies, n=81)	-0.42 (-0.88, 0.03)	0.07	0
Nitrate/ Nitrite (4 studies, n=230)	-0.12 (-0.56, 0.32)	0.60	64
Protein (4 studies, n=371)	-0.06 (-0.37, 0.25)	0.69	50
Watermelon/ L-Citrulline (4 studies, n=106)	-0.25 (-0.64, 0.13)	0.20	0

Two studies, including 54 participants, evaluated the effect of supplementing Vitamin C on arterial stiffness by PWA (Mullan et al., 2004; Mullan et al., 2002). Supplementing Vitamin C significantly reduced PWA by -1.05 (95% CI -(-1.78 to -0.31; p=0.005). Heterogeneity levels assessed by the I^2 test were low at 36% (**Table 8.3**).

Supplementation of Vitamin D, alcohol, Nitrate/Nitrite, protein, and watermelon/L-Citrulline showed no differences between interventions and controls on PWA (**Table 8.3**).

Four studies, including 291 participants, evaluated the effect of fatty acid on arterial stiffness by PWA (Chong et al., 2010; Esser et al., 2013; Kristensen et al., 2016; Lithander et al., 2013). The subgroup analysis of polyunsaturated fatty acid (PUFA) showed no differences on PWA (-0.05; 95% CI -0.50 to 0.40; p=0.82; I^2 =46%) (Chong et al., 2010; Esser et al., 2013; Kristensen et al., 2016) (**Figure 8.5**). Monounsaturated fatty acid (MUFA) showed no effect on PWA (-0.19; 95% CI -0.66 to 0.29; p=0.44; I^2 =19%) (Esser et al., 2013; Lithander et al., 2013) (**Figure 8.5**).

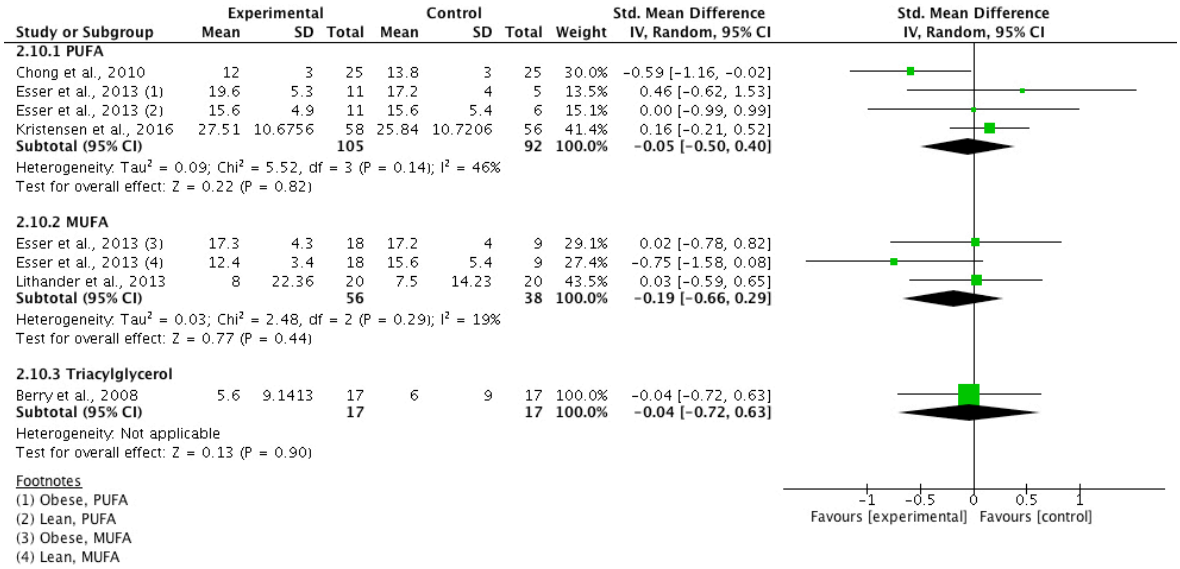


Figure 8.5 Meta-analysis of effect of fatty acid on arterial stiffness by PWA measurement.

Twelve studies, including 976 participants, evaluated the effect of polyphenol on arterial stiffness by PWA (Babar et al., 2018; Curtis et al., 2013; Dower et al., 2016; Hazim et al., 2016; Hollands et al., 2018b; Keane et al., 2016; Macready et al., 2014; Marques et al., 2018; McAnulty et al., 2014; Richter et al., 2017a; Ruel et al., 2013; West et al., 2014). Overall, the supplementation of polyphenol showed no differences between interventions and controls on PWA (-0.54; 95% CI -1.53 to 0.44; p=0.28; I^2=0%) (**Figure 8.6**).

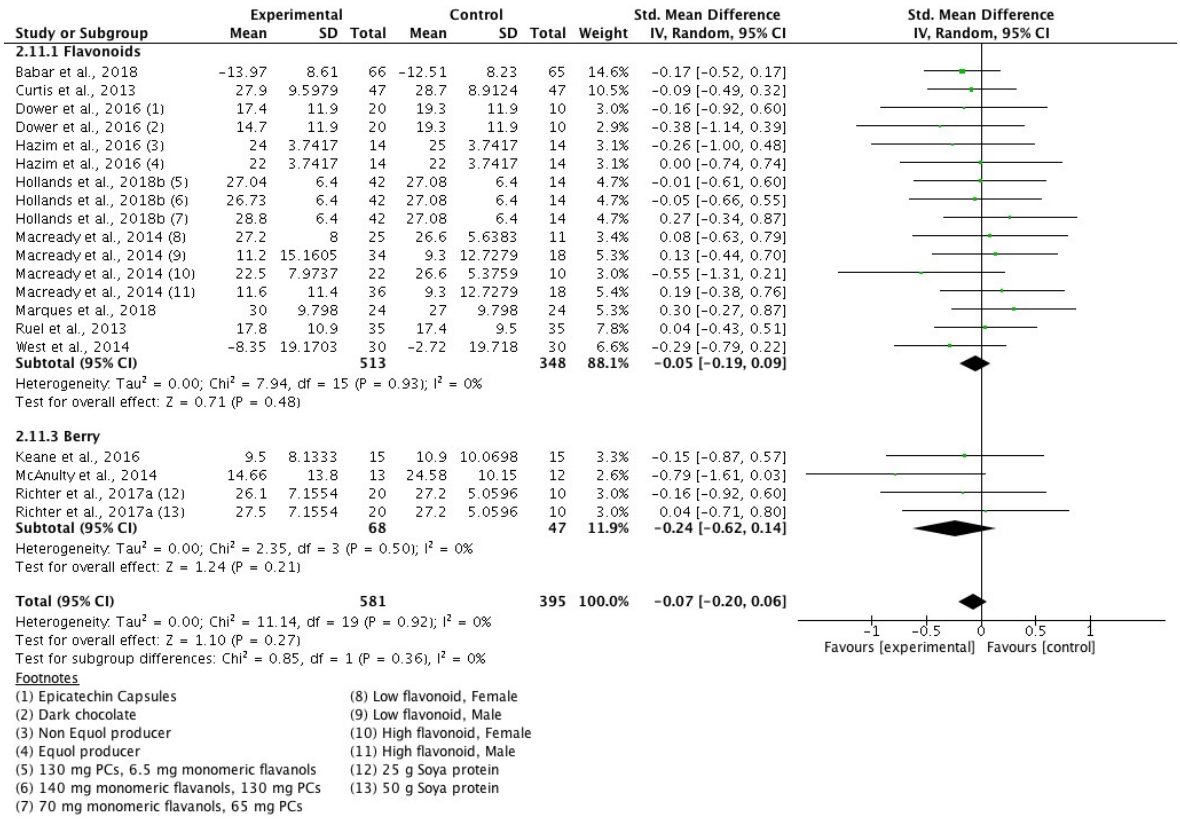


Figure 8.6 Meta-analysis of effect of polyphenol on arterial stiffness by PWA measurement.

Three studies, including 339 participants, showed no effect on PWA after low sodium (Na) intake (-0.11; 95% CI -0.32 to 0.11; p=0.32; I^2 =0%) (Dickinson et al., 2014b; Hu et al., 2009; Todd et al., 2010) (**Figure 8.7**). Three studies, including 272 participants, showed no effect on PWA after high potassium (K) intake (-0.16; 95% CI -0.41 to 0.09; p=0.21; I^2 =0%) (Blanch et al., 2014; Gijsbers et al., 2015; Xing et al., 2018) (**Figure 8.7**).

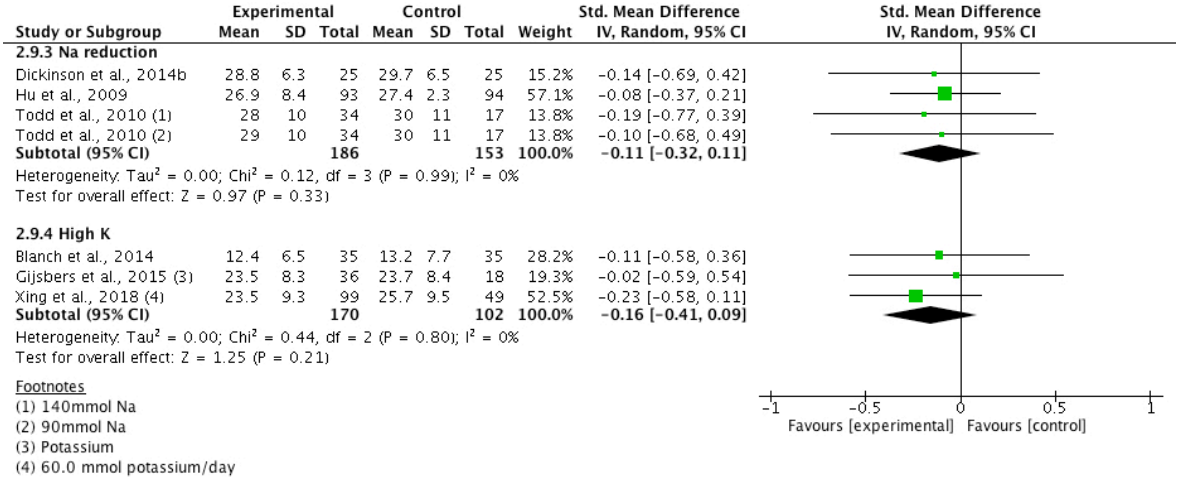


Figure 8.7 Meta-analysis of effect of low Na intake and high K intake on arterial stiffness by PWA measurement.

In addition, six studies, including 531 participants, evaluated the effect of extra Na intake on arterial stiffness by PWA (Dickinson et al., 2014a; Gijsbers et al., 2015; Muth et al., 2017; Pimenta et al., 2009; Todd et al., 2010; Xing et al., 2018). Extra Na intake (experimental group) significantly increased PWA by 0.22 (95% CI 0.04 to 0.39; p=0.02) (**Figure 8.8**). Heterogeneity levels assessed by the I^2 test were low at 0%.

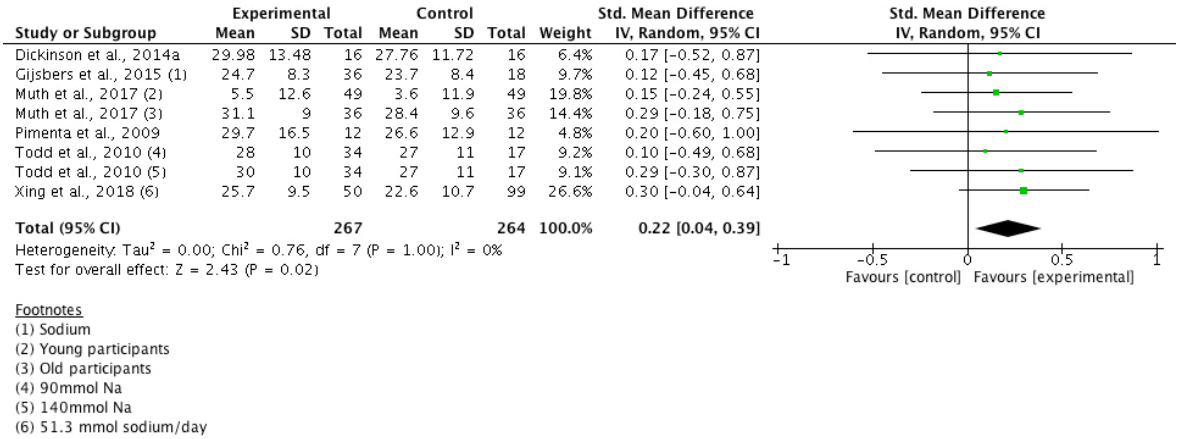


Figure 8.8 Meta-analysis of effect of extra Na intake on arterial stiffness by PWA measurement.

8.3.4 Meta-analysis of studies evaluated the effect of nutritional intervention on arterial stiffness by PWV measurement

Fifty-seven studies, including 5126 participants, evaluated the impact of PWV after intervention. Overall, nutritional intervention significantly reduced PWV by -0.26 m/s (95% CI -0.39 to -0.13; p<0.0001). Heterogeneity levels assessed by the I^2 test were high at 53% (**Figure 8.9**).

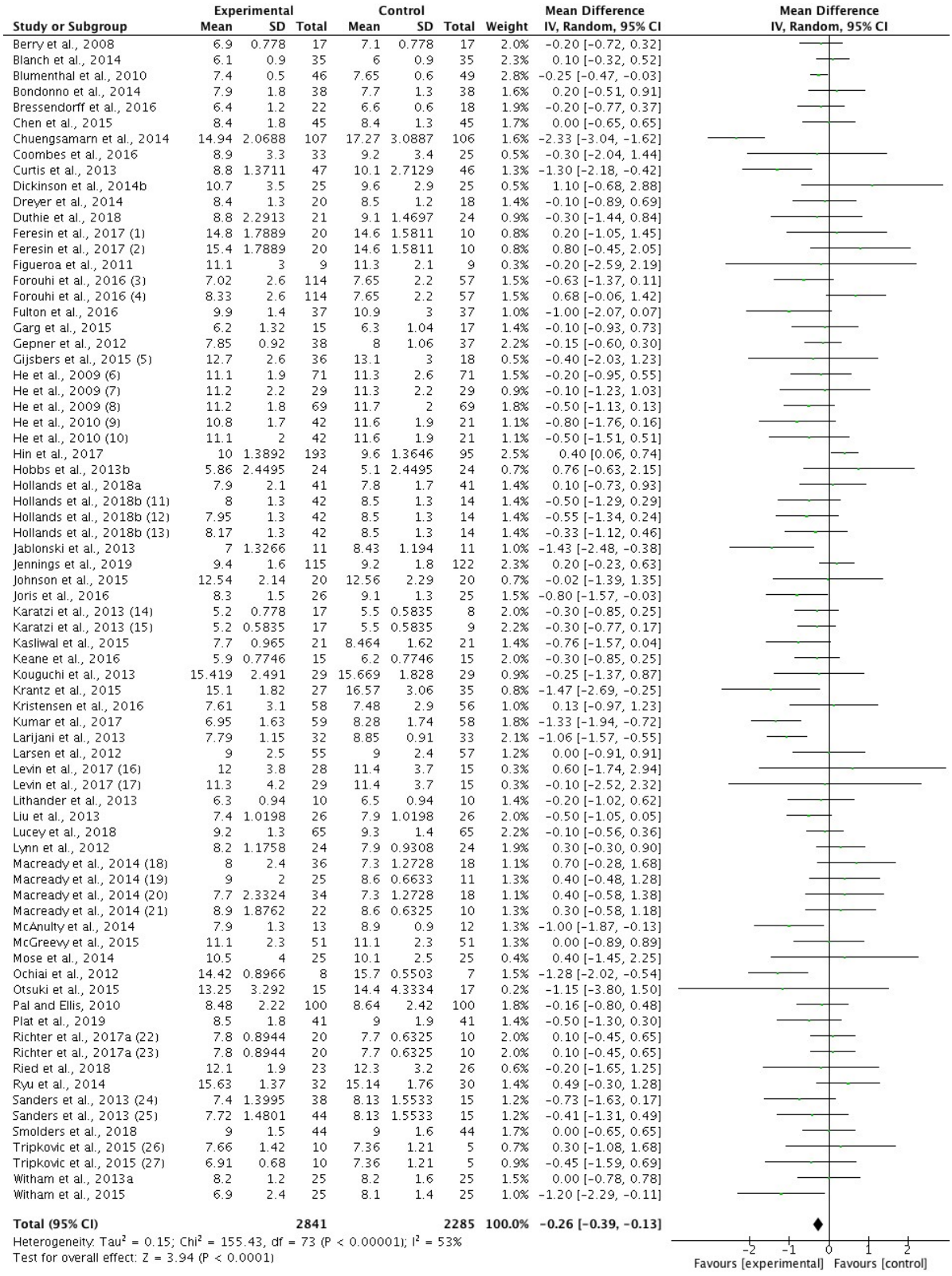
Subgroup analysis according to food type intervention was undertaken. Studies were separated according to change of diet style and supplementation of polyphenols, fatty acid, salt (extra salt, low sodium and high potassium intake), nitrate/nitrite, vitamin, nut, alcohol, watermelon/L-citrulline, aging garlic extract and protein (**Table 8.4**).

Table 8.4 Subgroup analysis of nutritional intervention on arterial stiffness by PWV measurement.

Subgroup	Mean Difference IV, Random (95%CI)	*P* (Z-test)	Heterogeneity I^2%
Watermelon/ L-Citrulline (2 studies, n=33)	-1.18 m/s (-1.89, -0.48)	0.001	0
Alcohol (1 study, n=51)	-0.30 m/s (-0.66, 0.06)	0.10	0
Aging garlic extract (2 studies, n=114)	-0.20 m/s (-0.92, 0.52)	0.58	0
Nitrate/ Nitrite (3 studies, n=176)	-0.02 m/s (-0.68, 0.64)	0.95	52
Protein (3 studies, n=270)	-0.21 m/s (-0.58, 0.17)	0.29	0
Change of diet style (3 studies, n=444)	-0.18 m/s (-0.51, 0.14)	0.26	40
Nut (2 studies, n=122)	-0.34 m/s (-1.09, 0.40)	0.37	52
Vitamin D (14 studies, n=1645)	-0.12 m/s (-0.40, 0.16)	0.41	57

Two studies, including 33 participants, evaluated the effect of supplementing watermelon/ L-Citrulline on arterial stiffness by PWV (Figueroa et al., 2011; Ochiai et al., 2012). supplementing watermelon/ L-Citrulline significantly reduced PWV by 1.18 m/s (95% CI -1.89 to -0.48; p=0.001) (**Table 8.4**). Heterogeneity levels assessed by the I^2 test were low at 0%.

Supplementation of alcohol, aging garlic extract, nitrate/ nitrite, protein, change of diet style, nut and vitamin D showed no differences between interventions and controls on PWV (**Table 8.4**).



Footnotes

(1) 25 g
(2) 50 g
(3) Ergocalciferol 20000 IU
(4) Cholecalciferol 20000 IU
(5) Potaasium
(6) White
(7) Asian
(8) Black
(9) Potassium Chloride
(10) Potassium Biarbonate
(11) 70 mg monomeric flavanols, 65 mg PCs
(12) 130 mg PCs, 6.5 mg monomeric flavanols
(13) 140 mg monomeric flavanols, 130 mg PCs
(14) Beer
(15) Vodka
(16) Calcitriol
(17) Calcifediol
(18) High flavonoid, Male
(19) Low flavonoid, Female
(20) Low flavonoid, Male
(21) High flavonoid, Female
(22) 50 g/d soya protein
(23) 25 g/d soya protein
(24) Low saturated fat and high carbohydrate
(25) Low saturated fat and high MUFA
(26) Wheat Fibre
(27) Inulin

Figure 8.9 Meta-analysis of effect of nutritional intervention on arterial stiffness by PWV measurement.

Two studies, including 176 participants, evaluated the effect of polyunsaturated fatty acid (PUFA) (Karatzi et al., 2013; Kristensen et al., 2016). There was no change in PWV after consumption of PUFA (-0.65; 95% CI -2.21 to 0.92; p=0.42; I^2=72%) (**Figure 8.10**).

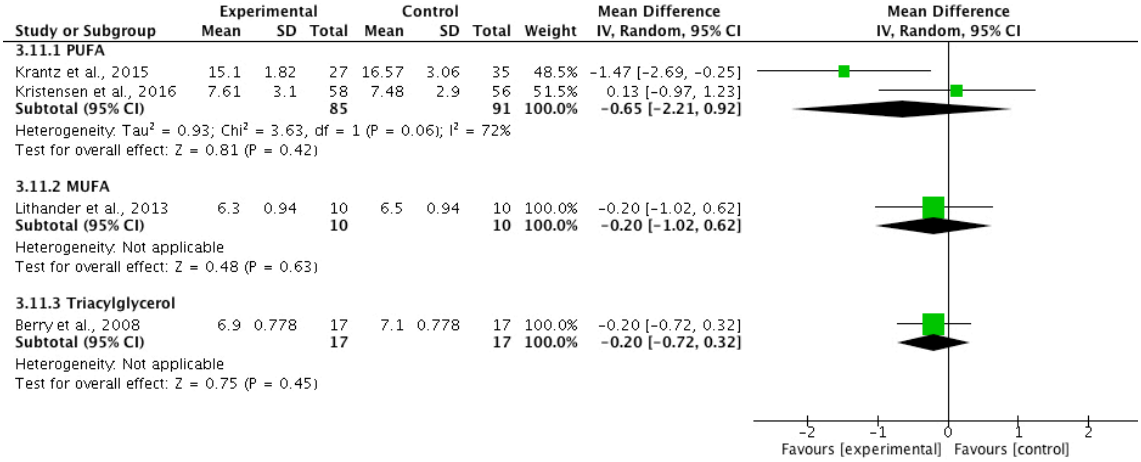


Figure 8.10 Meta-analysis of effect of fatty acid on arterial stiffness by PWV measurement.

Eleven studies, including 825 participants, evaluated the effect of polyphenol on arterial stiffness by PWV (Curtis et al., 2013; Duthie et al., 2018; Feresin et al., 2017; Hollands et al., 2018a; Hollands et al., 2018b; Johnson et al., 2015; Keane et al., 2016; Lynn et al., 2012; Macready et al., 2014; McAnulty et al., 2014; Richter et al., 2017a). Overall, the supplementation of polyphenol showed no differences between interventions and controls on PWV (-0.08 m/s; 95% CI -0.33 to -0.16; p=0.52; I^2=32%) (**Figure 8.11**).

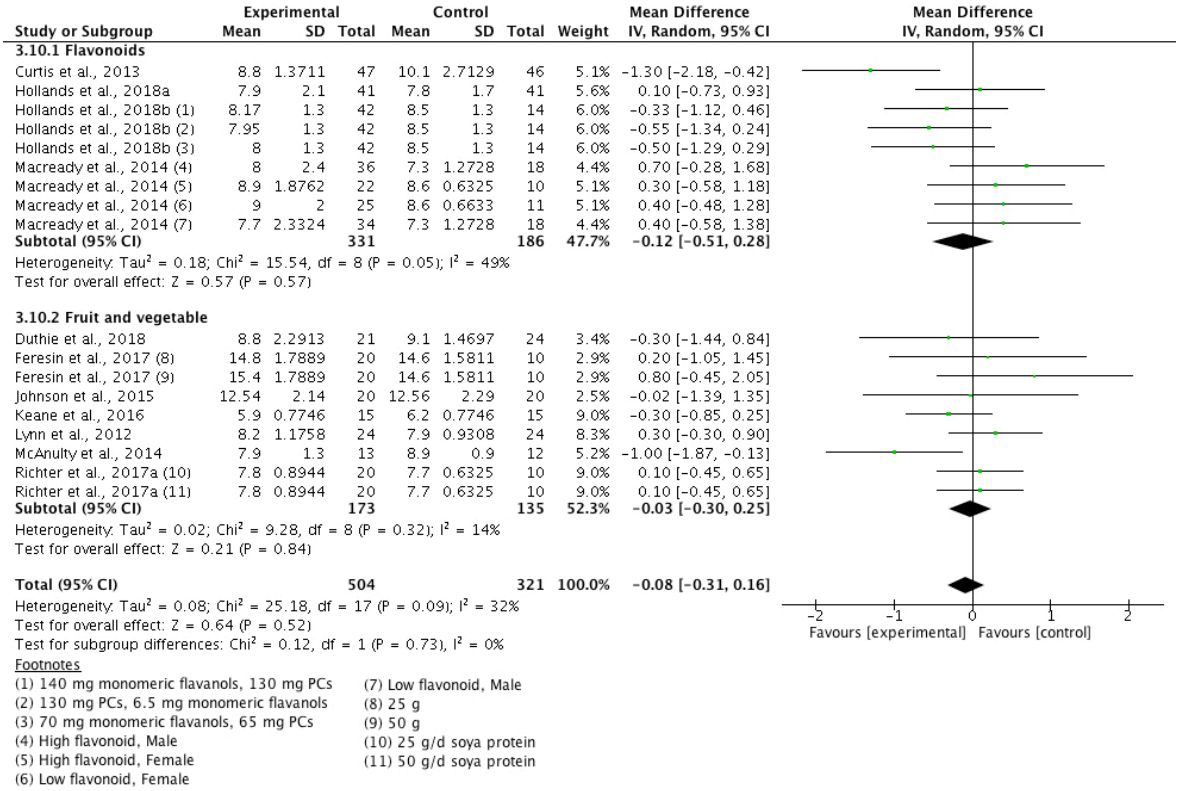


Figure 8.11 Meta-analysis of effect of polyphenol on arterial stiffness by PWV measurement.

Three studies, including 410 participants, evaluated the effect of low sodium (Na) intake on arterial stiffness by PWV (Dickinson et al., 2014b; He et al., 2009; Jablonski et al., 2013). The low Na intake showed a no effect on PWV reduction (-0.39 m/s; 95% CI -0.96 to 0.18; *p*=0.18) (**Figure 8.12**). Heterogeneity levels assessed by the I^2 test were low at 43%.

Three studies, including 410 participants, evaluated the effect of high potassium intake on arterial stiffness by PWV (Blanch et al., 2014; Gijsbers et al., 2015; He et al., 2010). The low Na intake showed a no effect on PWV reduction (-0.20 m/s; 95% CI -0.64 to 0.25; *p*=0.38) (**Figure 8.12**). Heterogeneity levels assessed by the I^2 test were low at 17%.

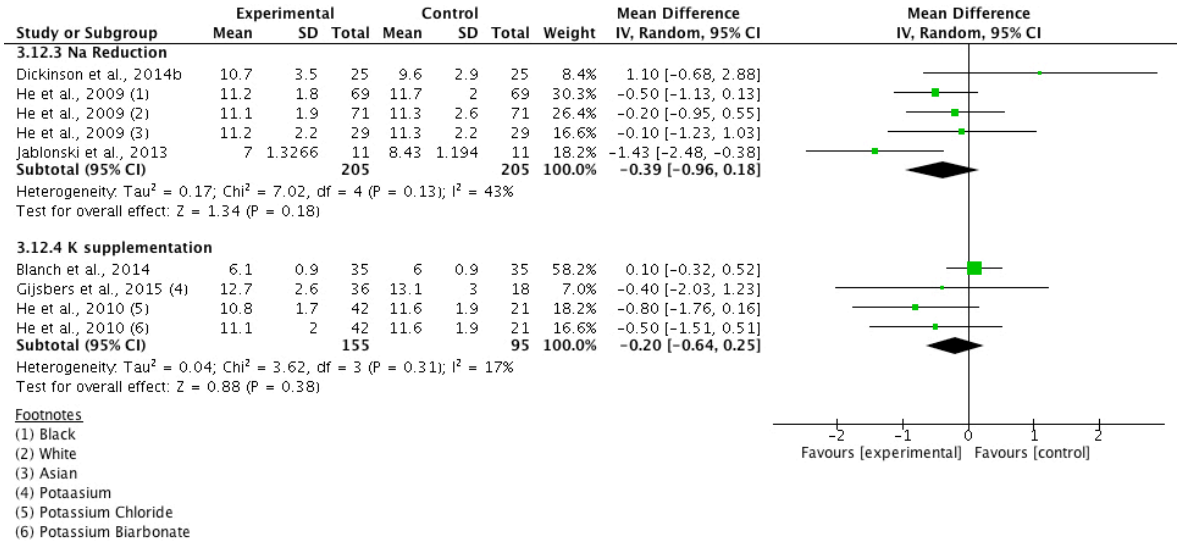


Figure 8.12 Meta-analysis of effect of low Na intake and high P intake on arterial stiffness by PWV measurement.

In addition, four studies, including 350 participants, evaluated the effect of extra Na intake on arterial stiffness by PWV (Gijsbers et al., 2015; Muth et al., 2017; Pimenta et al., 2009; Todd et al., 2010). Extra Na intake (experimental group) had no effect on PWV (0.20 m/s; 95% CI -0.12 to 0.53; *p*=0.22) (**Figure 8.13**). Heterogeneity levels assessed by the I^2 test were low at 0%.

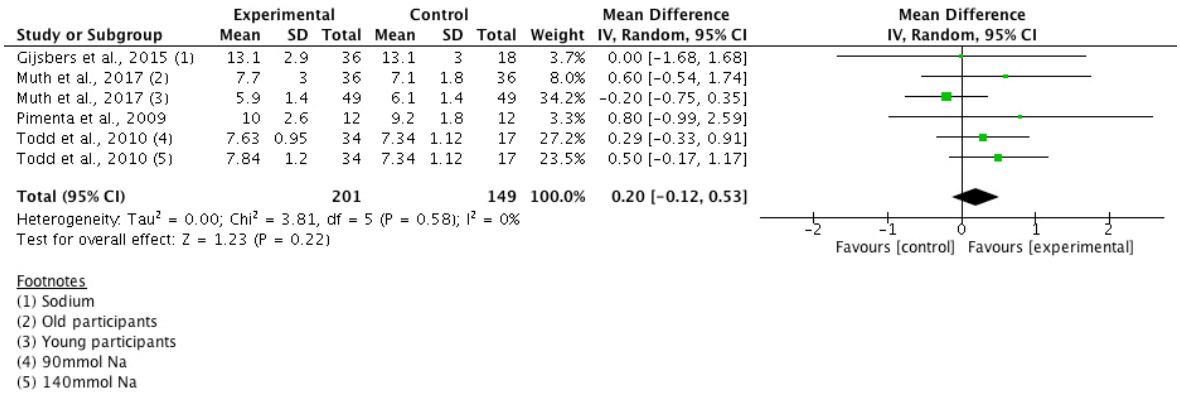


Figure 8.13 Meta-analysis of effect of extra Na intake on arterial stiffness by PWV measurement.

8.4 Discussion

8.4.1 Principal findings

This systematic review and meta-analysis evaluated that nutrition intervention significantly improved arterial stiffness, PWA by 0.16 (SMD) and PWV by 0.26 m/s (MD), among adult subjects >18 years of age. Intervention type was determined to be a significant influencing factor. Extra Na intake was significantly increase PWA. Dietary supplement showed significant reduction in arterial stiffness. PWA was significantly reduced after changes of diet and supplementation of Ginseng and Vitamin C while PWV was significantly reduced after supplementation of watermelon/L-Citrulline. Nineteen of the studies included in the present review measured flow-mediated dilation (FMD) concurrently with PWV and/or PWA (Berry et al., 2008; Blanch et al., 2014; Blumenthal et al., 2010; Chen et al., 2015a; Curtis et al., 2013; Dickinson et al., 2014b; Dower et al., 2016; Fulton et al., 2016; Gepner et al., 2012; Karatzi et al., 2013; Kasliwal et al., 2015; Marques et al., 2018; Rathnayake et al., 2018; Smolders et al., 2018; West et al., 2014; Witham et al., 2013a; Witham et al., 2015; Witham et al., 2013b; Yiu et al., 2013). Eight of them showed significant improvement in FMD after the nutritional intervention but not in PWV or PWA (Berry et al., 2008; Blanch et al., 2014; Blumenthal et al., 2010; Dickinson et al., 2014b; Karatzi et al., 2013; Kasliwal et al., 2015; Marques et al., 2018; Smolders et al., 2018). Four chronic studies showed supplementation of vitamin D were no improvement in FMD, as well as PWV and/or PWA (Witham et al., 2013a; Witham et al., 2015; Witham et al., 2013b; Yiu et al., 2013). Another study on pistachio supplementation (3 months) showed significant improvement in FMD and PWV (Kasliwal et al., 2015). Only one study on acute intake of alcohol showed significant improvement in FMD, PWV and PWA (Karatzi et al., 2013). Concurrent improvements in indices of arterial stiffness, PWV and PWA measuring different aspects of vascular function, were not always observed.

We reviewed microvascular vasodilation with the use of LDI, which measured the response to cutaneous perfusion of the forearm with acetylcholine and SNP. The meta-analysis of microvascular vasodilation with the use of LDI showed nutrition intervention did not change the response to cutaneous perfusion of the forearm with ACh and SNP. Majority interventions were investigating polyphenol supplementation and showed no effect on microvascular vasodilation. A potential explanation to the lack of effect of polyphenols studies could be the fact that the response of polyphenol on microcirculation vasodilation has been associated with certain genotypes such as the Glu298Asp (George et al., 2012b).

George et al., 2012b showed that acute consumption of a flavonoid-rich drink increased dilation of the microcirculation in the forearm in response to acetylcholine after 180 min in homozygous for guanine (GG) genotype individuals had no effect on endothelium-dependent vasodilation or endothelium-independent vasodilation in the heterozygous for guanine (GT) genotype individuals (George et al., 2012b).

The high heterogeneity level (I^2>50%) in LDI-ACh, LDI-SNP, PWA (protein and nitrate/nitrite) and PWV (all and PUFA) showed that there is inconsistency and high variation across the studies. Sensitivity analysis showed that two studies using vitamin D (Dreyer et al., 2014) and curcuminoid (Chuengsamarn et al., 2014) supplementation contributed to the heterogeneity. The SMD of LDI-ACh and LDI-SNP were 0.11 (95% CI -0.09 to 0.30; p=0.92; I^2=0%) and 0.13 (95% CI -0.16 to 0.42; p=0.37; I^2=38%) respectively after excluding the vitamin D study (Dreyer et al., 2014). This analysis also showed that food interventions still had no effect on both LDI-ACh and LDI-SNP with low heterogeneity level. The MD of overall PWV was 0.23 m/s (95% CI -0.34 to -0.11; p=0.0002; I^2=40%) after excluding the curcuminoid study (Chuengsamarn et al., 2014). It showed that the food intervention still significantly reduced PWV with low heterogeneity level.

8.4.2 Scientific analysis of findings and implications for health

A focus on the health impact of dietary patterns on cardiovascular risk factors has been a growing area of research in recent decades (Mozaffarian et al., 2011). There was significant research showing that major dietary patterns, such as the Mediterranean diet, can decrease mortality rates and improve cardiovascular health (Knoops et al., 2004). Growing evidence now shows that the impact of dietary patterns was the result of the accumulative effects of different food groups (Trichopoulou et al., 2009).

Globally, CVD is one of the leading causes of death. Evidences from this systematic review with meta-analysis showed that vascular function was affected by various aspects of the diet. Results from this study suggested that vascular function can be improved by dietary changes and a cardioprotective diet can reverse or limit previous damage, perhaps caused from previous poor diet. It is noteworthy that statistical analysis showed that the nutrition interventions significantly improved arterial stiffness, PWA by 0.16 (SMD) and PWV by 0.26 m/s (MD), however such low improvements may not have significant clinical relevance. According to previous studies each 1 m/s increase in PWV was associated with a 15% higher CVD risk (Verbeke et al., 2011) and each 10% increase in AIx was 1.48 for CV mortality (95% CI 1.16 to 1.90, p<0.0001) (London et al., 2001).

Organic compounds such as polyphenols and concentrated extracts showed the greatest improvements on arterial stiffness, PWV and PWA, while fish oil supplements and fruit and vegetables were both borderline significant in improving vascular function. Together these results gain traction and improve significance, following previous research, especially in relation to the recent research of the cardiovascular benefits of fish oils and omega-3 fatty acids (Kris-Etherton et al., 2002). It is notable that the significant improvements to vascular function were observed in studies originating from North America and Europe, whereas those from Asia and Oceania reported non-significant improvements to vascular function.

8.4.3 Strengths and Limitations

To our knowledge, this is the first systematic review and meta-analysis of nutritional interventions on vascular function determined by microvascular vasodilation and arterial stiffness. The strengths of this study include the consistency of findings across the studies and the results reported seem valid and robust due to the low levels of heterogeneity surrounding the findings in this study. The validity of these results is further strengthened by a comprehensive search of literature across multiple major scientific databases using pre-specified criteria.

There are limitations that merit consideration. There are very few amino acids and alcohol studies present, while they showed significant changes, reliability was limited due to a small sample of studies. Many of the studies were conducted on older participants, and this widely omitted the knowledge on how vascular function is affected in youths. The studies had overall small sample sizes, with a narrow range of ethnicities. Larger and longer-term studies could give more concrete evidence for the effects of different dietary interventions on EF.

8.5 Conclusion

Evidence from the papers analysed showed significant effects of nutritional intervention on endothelial dysfunction. PWV and PWA can be accurately and reliably reproduced as methods assessing the selected vascular function markers of these studies. Such results are however of low clinical value. The systematic review revealed there are a limited number of studies for LDI testing methods, and they do show signs of improvement from nutritional intervention. More research is needed to identify interventions that are effective in improving these outcomes. Future studies are needed to identify the key connections between nutrition intervention and endothelial dysfunction to allow intervention study to be designed and promulgated.

Chapter 9 General Discussion

9.1 Thesis Summary

Dietary factors are well recognised contributors to common diseases, including heart disease, stroke, type 2 diabetes and cancer. Therefore, our food choices are directly affecting our health. Conventional dietary guidelines use a "one size fits all" approach to achieve dietary change to improve health (Kiefte-de Jong et al., 2014). However, each individual in population has different genotype and different lifestyle which play an essential role in modulating the likelihood of develop of disease. Therefore, the use of dietary patterns and personalised dietary recommendations are becoming more effective in promotion of health (Celis-Morales et al., 2017). Among different dietary patterns, Mediterranean diet, which is characterised by high intake of vegetables, fruits, grain, fish and olive oil, has received the most attention in relation to chronic disease prevalence (Celis-Morales et al., 2017). Specific fruits and vegetables found in Mediterranean diet are individually associated with significant reductions of CVD. Tomato is one of the main ingredients in the Mediterranean diet that plays a key role in reducing the prevalence of CVD. High intake of tomato and lycopene have shown improvements in cardiovascular health.

The main aim of this thesis was to investigate the effect of tomato supplementation on cardiovascular health and to evaluate the effects of the two varieties of tomato on vascular health. This thesis followed a series of logical steps presented on separated chapters and summarised in **Figure 9.1**. Furthermore, the research questions posed in this thesis are summarised in **Table 9.1**.

Phase one of the present PhD programme involved an overview of the topic. In phase two, the literature on the association between tomato and cardiovascular health was explored (**Figure 9.1**). The first aim of this thesis was to evaluate the evidence from previous epidemiological studies on the association of the consumption of lycopene and tomatoes and other CVD and mortality. Systematic review and meta-analysis in Chapter 2 showed that high tomato/lycopene intake significantly reduced the risk of stroke and CVD by 26% and 14% respectively (**Table 9.1**). The second aim of this thesis was to evaluate the evidence from previous RCT on the effects of tomato/lycopene supplementation on cardiovascular risk factors. Systematic review and meta-analysis in Chapter 3 showed that tomato supplementation was associated with significant reductions in LDL-cholesterol and improvements in FMD while, lycopene supplementation was associated with significant reductions in SBP (**Table 9.1**). It showed that the consumption of tomato has important

public health implications towards the prevalence of CVD. These results showed that dietary habits are one of the important modifiable factors that can affect the maintenance of a healthy phenotype. Chapter 3 also indicated that other markers of vascular function have not been fully investigated and there are no RCT investigating the effects of other varieties of tomato apart from red tomato. The results from both systematic reviews can be incorporated into the future development of personalised nutrition.

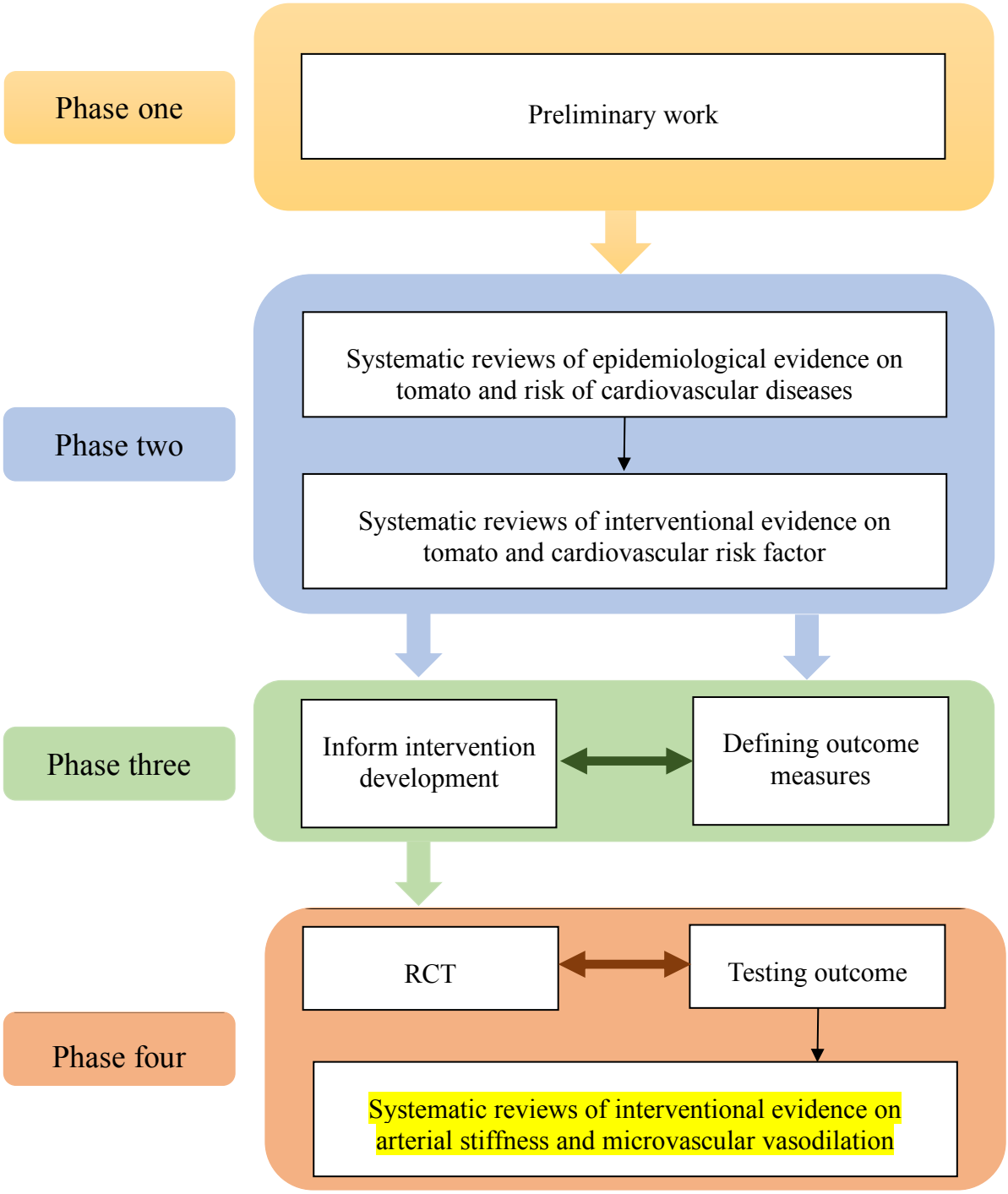


Figure 9.1 Overview of this PhD programme

Vegetables are now available in a variety of colours. Cauliflower heads, commonly white in colour, can now be found in shades of purple, yellow, and green, and bags of potatoes are popping up in yellow, red, and purple varieties. Some dietary guidelines suggested eating different colours of the same vegetable, as they were believed to have different nutritional value. However, whether they have different nutritional benefits and have the same beneficial effect as the original variety remain unknown. Chapter 3 indicated that previous RCT investigated the effects of red tomato on cardiovascular health only. Therefore, it is worth to evaluate whether other varieties exert the same effect. Therefore, it could be of value to investigate if other colours exert the same effect and this could be an area for potential research.

Phase three of the present PhD programme was the development of dietary intervention on comparing the two different tomato varieties (**Figure 9.1**). In Chapter 3, it showed that previous interventions on tomato-based or lycopene supplementation were mainly long-term (4-8 weeks) and lycopene doses were mainly 15-30 mg/d. The systematic reviews of the scientific literature also identified clinical outcome measures relevant to cardiovascular risk. In adherence to previous literature, the selection criteria for the measures included biomarkers that responded to dietary interventions (Cheng et al., 2017), particularly to tomato-based or lycopene supplementation. We thus selected a tentative panel of biomarkers of cardiovascular risk to be tested in our study. Chapter 5 detailed the protocol for dietary intervention to examine the effects of Piccolo and Oranjstar on BP, vascular function and blood-borne biomarkers.

In phase four of the present PhD programme, an RCT was conducted to evaluate the effect of two varieties of tomato, Piccolo and Oranjstar on vascular health (**Figure 9.1**). The results of the dietary intervention on BP and vascular function were examined in Chapter 6. Both Piccolo and Oranjstar reduced BP and improved FMD from baseline values (**Table 9.1**). Moreover, Piccolo is more superior to Oranjstar in reducing BP and improving FMD (**Table 9.1**). Chapter 7 examined the effects of Piccolo and Oranjstar on blood lipids profile and new established vascular risk markers. Both showed no improvement in blood lipids profile. However, both significantly reduced adhesion molecules, sICAM-1 and sVCAM-1, from baseline values independent of the blood lipids profile. Oranjstar is more effective at reducing adhesion molecules than Piccolo (**Table 9.1**). The systematic review in Chapter 8 showed that nutritional intervention significantly improved arterial stiffness. However, the results, although statistically significant, might have low clinical value (**Table 9.1**). The

results presented in Chapter 6 showed that both tomato varieties have no significant effects on microvascular vasodilation and arterial stiffness.

9.2 Novelty and Strengths

The work presented in this thesis contributes to our understanding of the impact of food and diet on cardiovascular health. This work it is novel in several ways and has a number of strengths.

Strengths of this work include, adopting a rigorous methodology and a series of logical steps on investigating the effect of tomato on cardiovascular health. The design of the clinical trial presented here was developed based on evidence from previous relevant literature. Previous RCTs focused on investigating the effects of tomato consumption on red tomatoes and lycopene supplementations while the effects of different tomato varieties on markers of cardiovascular risk had not been previously evaluated. The present work thus fills a gap in the literature by reporting results comparing the health impacts of two varieties of cherry tomato, Piccolo and Oranjstar. The result of the clinical trial were consistent with previous evidence. Furthermore, this is the first clinical trial investigating the effect of tomato on LDI, as well as other cardiovascular risk factors which have been less studied such as PWV, PWA, sICAM-1, sVCAM-1, E-selectin and P-selectin.

9.3 Limitations of Present Thesis

Work presented in this thesis examined only two varieties of cherry tomato. Therefore, the effects of other tomato varieties on vascular function require further investigation. In addition, only raw tomato was used in this study for the dietary intervention. Thus, the findings cannot be extrapolated to tomato products such as tomato paste, juice or cooked tomatoes, in which the thermal food processing method is known to enhance the bioavailability of lycopene (Dewanto et al., 2002). Future studies could also test whether different doses (e.g. lower doses) of tomato or different length of intervention (longer periods) are effective.

Further research is needed to determine the effects of tomatoes on the cardiovascular health of other populations. This study was conducted only in healthy male participants. Hence, further research is needed to evaluate whether tomato supplementation is equally effective on female individuals, population with high CVD risk and patients with CVD and diabetes. Finally, compliance, albeit good, was self-reported and there were no objective methods in place to measure compliance.

9.4 Relevance for Stakeholders

The results of the present work are of relevance to healthcare professional such as nutritionists and dietitians who could use these to encourage the adoption of a diet with an increased lycopene intake through tomato consumption to reduce BP and further reduce the risk of CVD. These may be useful specially for patients with hypertension or borderline hypertension, the intake of fruit and vegetables should be encouraged and the positive effects of tomato consumption should be highlighted.

9.5 Future Work

To the best of our knowledge, no previous studies have compared the two different varieties of tomato (rich in trans-lycopene and cis-lycopene). The positive impacts of lycopene on inflammatory biomarkers encourage further studies with a larger population to investigate the effects of carotenoid consumption, as well as the influence of other components present in tomato, such as vitamin C, polyphenols or other micronutrients, which could act in a synergistic/antagonistic way regulating the activity of these molecules. Such studies should target the female individual as well as individuals at high CVD risk. It would also be useful to evaluate other foods known to reduce CVD risk, such as beetroots, with lycopene-rich tomatoes. Beetroots have been shown to lower BP, but they have never been tested alongside tomato. This combination might benefit cardiovascular health significantly.

Lastly, future studies could use different -omics methodologies to identify markers of food intake (i.e. tomato), as well as to evaluate the impact of tomato consumption on new markers of CVD (Silva et al., 2015).

Table 9.1 Research questions raised from each chapter.

Chapter	Question	Answer(s)
1	1. What are the links between diet and cardiovascular health?	The links between diet and cardiovascular health were reviewed. Some epidemiological studies showed high intake of fruit and vegetable could reduce the incidence of CVD.
	2. Is there any evidence on specific food particularly fruit and vegetables?	Tomato is one of fruit and vegetable that have beneficial effects on cardiovascular health.
2	1. What is the epidemiological evidence on tomato consumption and its association with CVD risk?	Both high tomato and lycopene consumption play a beneficial role in preventing CVD and early death. High tomato/lycopene intake or status were associated with significant reductions of 26% in stroke (p=0.001), 14% in CVD (p=0.003); while high serum lycopene concentration was associated with significant reduction of 37% in mortality (p>0.001).
	2. What is the epidemiological evidence on lycopene consumption and its association with CVD risk?	
3	1. What is the interventional evidence on the effects of tomato supplementation on CVD risk factors?	Tomato supplementation was associated with significant reduction of LDL-cholesterol by 0.22 mmol/L (p=0.006) and IL-6 by 0.25 pg/mL (p=0.03) and improvement of FMD by 2.53% (p=0.01).
	2. What is the interventional evidence on the effects of lycopene supplementation on CVD risk factors?	Lycopene supplementation was associated with significant reductions in SBP by 5.66 mmHg (p=0.002).
4	1. Does the carotenoid content vary in different varieties of tomato?	The mean trans-lycopene and cis-lycopene content of Piccolo were 8.32±0.14 mg/100 g and 0.42±0.01 mg/100 g respectively, while cis-lycopene content of Oranjstar were 2.64±0.08 mg/100 g. The mean β-carotene content of Piccolo over 10 days were 0.89±0.02 mg/100 g while β-carotene content of Oranjstar were 0.68±0.03 mg/100 g
	2. Does the carotenoid content change during postharvest storage?	The carotenoid content of Piccolo and Oranjstar did not change significantly over the 10 days of storage.

(**Table 9.1** continues on next page)

Chapter	Question	Answer(s)
5	1. What are the gaps in the literature in relation with RCT supplementing tomato on cardiovascular risk factors?	Systematic reviews and meta-analyses revealed that previous controlled experimental studies on the effects of tomato consumption have focused on red tomato and red tomato products or lycopene supplementation. RCT comparing different varieties is lacking in the literature.
6	1. Do different varieties of cherry tomato have the same extent of beneficial effects on BP and vascular function?	The resting SBP was significantly reduced by 3.3 mmHg (p=0.021) after consumption of Piccolo. The 24-Hour ambulatory DBP was significantly reduced by 2.33 mmHg (p=0.006) and 1.56 mmHg (p=0.049) after the consumption of Piccolo and Oranjstar, respectively. In addition, a substantial decrease in nighttime ambulatory SBP by 4.52 mmHg (p=0.039) and nighttime ambulatory DBP reduced by 3.82 mmHg (p=0.016) and a significant decrease in daytime ambulatory DBP by 1.78 mmHg (p=0.047) following the consumption of Piccolo.
	2. Do these health benefits differ according to age?	In the age group above 30 years of age, consumption of Piccolo was a significant reduction in daytime ambulatory SBP (3.3 mmHg, p=0.043) and DBP (3 mmHg, p=0.03) compared with Oranjstar. In addition, consumption of Piccolo significantly improved FMD by 3.43% (p=0.026) when compared to intake of Oranjstar in age group above 30 years. In the age group above 30 years, consumption of Piccolo significantly improved PWA by 2.48% (p=0.013) from baseline. No significant differences in the younger group, or in any other BP measurements and vascular measurements were observed. Piccolo improved BP and FMD superior to Oranjstar.

(**Table 9.1** continues on next page)

Chapter	Question	Answer(s)
7	1. Do different varieties of cherry tomato have the same extent of beneficial effects on blood-borne biomarkers of vascular function?	Supplementation of Piccolo reduced sICAM-1 by 49.56 ng/mL (p<0.001). Consumption of Oranjstar showed a significant reduction of sICAM-1, sVCAM-1 and E-selectin by 59.14 ng/mL (p<0.001), 67.35 ng/mL (p=0.003) and 6.65 ng/mL (p=0.016) respectively. The reduction of sICAM-1, sVCAM-1 and E-selectin were independently to blood lipid profile. Oranjstar reduced adhesion molecules superior to Piccolo.
	2. Do these health benefits differ according to age?	In the age group above 30 years of age, supplementation of Piccolo significantly reduced IL-6 by 1.22 pg/mL (p=0.035), compared with baseline values; Oranjstar consumption was also significantly lower (by 0.82 pg/mL, p=0.026) than the changes observed with Piccolo.
	3. Are changes in FMD associated with improvements on blood-borne biomarkers of vascular function?	No, only Piccolo was more beneficial to FMD but less beneficial to blood-borne biomarkers of vascular function (sICAM-1, sVCAM-1, E-selectin and P-selectin); while Oranjstar was more beneficial to blood-borne biomarkers, sICAM-1, sVCAM-1, E-selectin, but less beneficial to FMD
8	What is the evidence for nutritional interventions enhancing microvascular vasodilation and arterial stiffness?	Arterial stiffness showed statistically significant improvement from nutritional interventions, however the clinical significance of these is likely to be low. There was a limited number of studies for microvascular vasodilation, and they did show signs of improvement from nutritional intervention.

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Appendix A

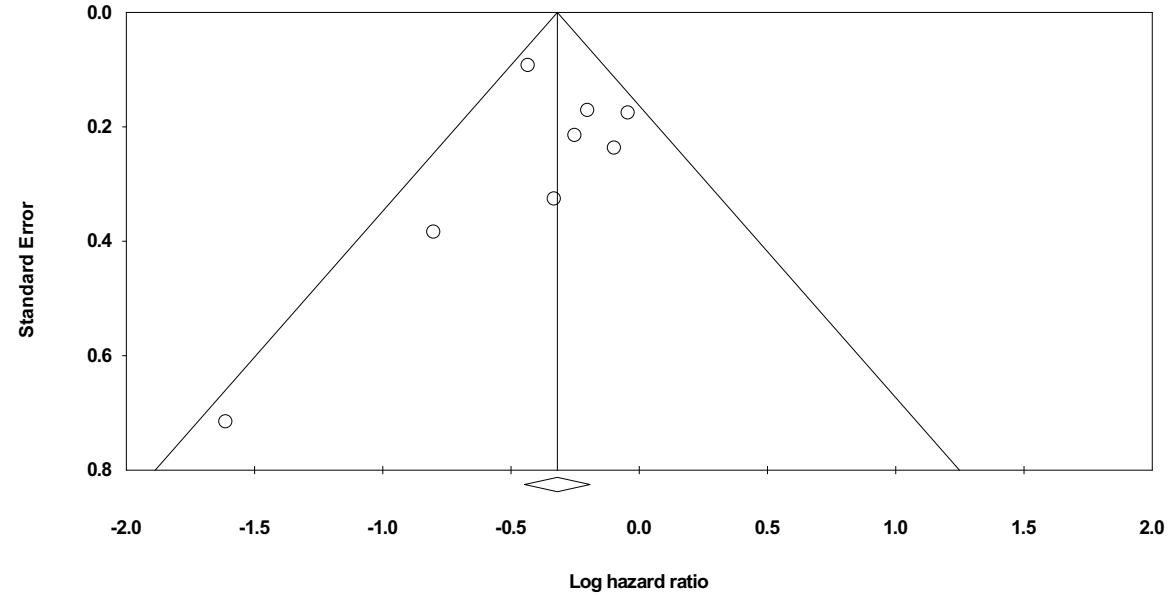
Supplementary information for Chapter 2

Appendix A1 PRISMA checklist.

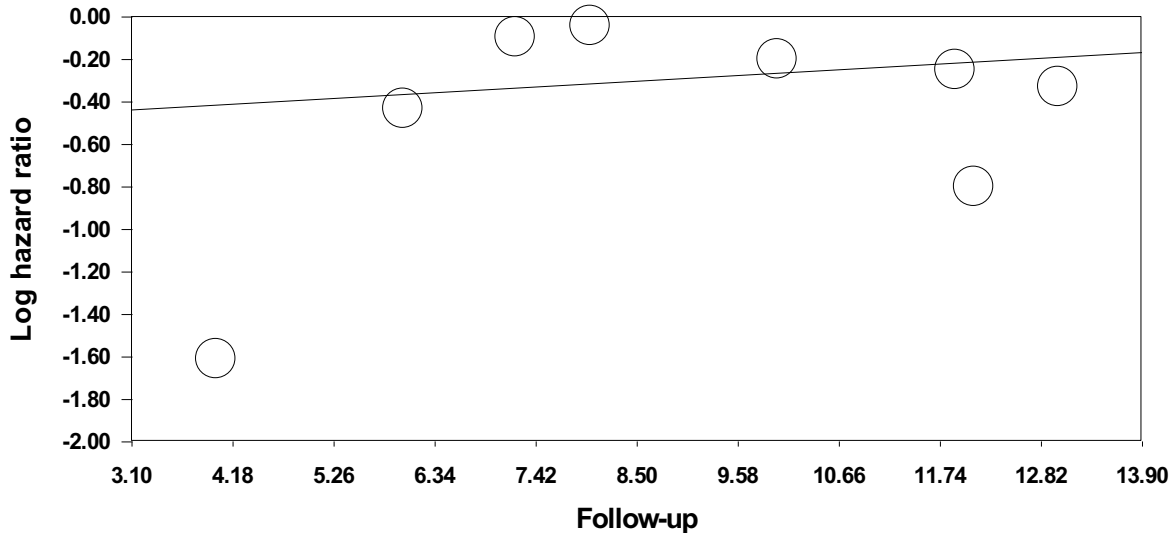
Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	37
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	N/A
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	37-38
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	37-38
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	38-39
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	38
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	38
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	39
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	39
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	38-39

(**Appendix A1** continues on next page)

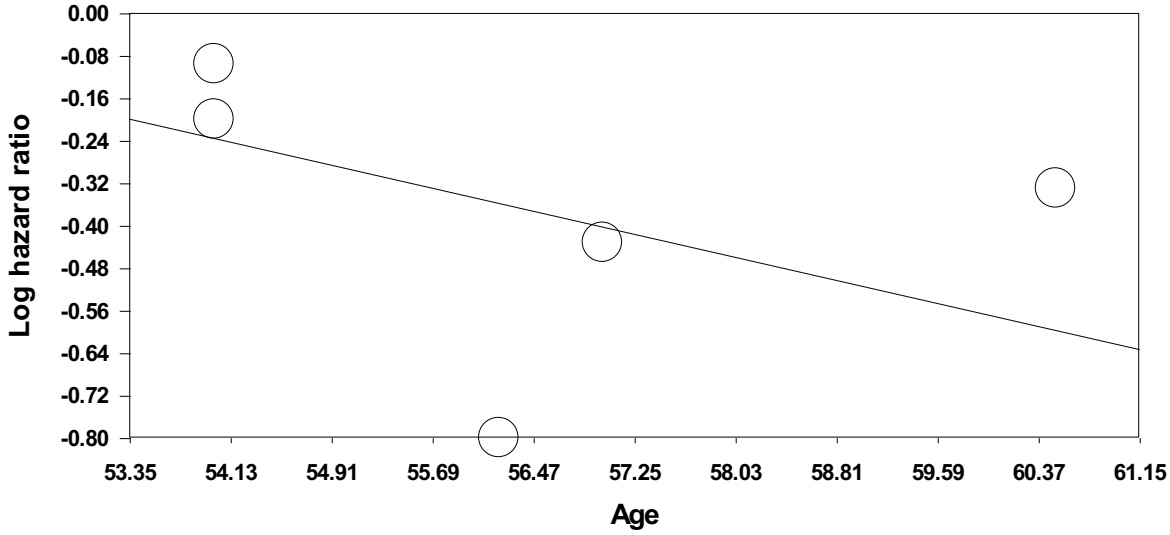
Section/topic	#	Checklist item	Reported on page #
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	38
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	40
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	40
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	39-40



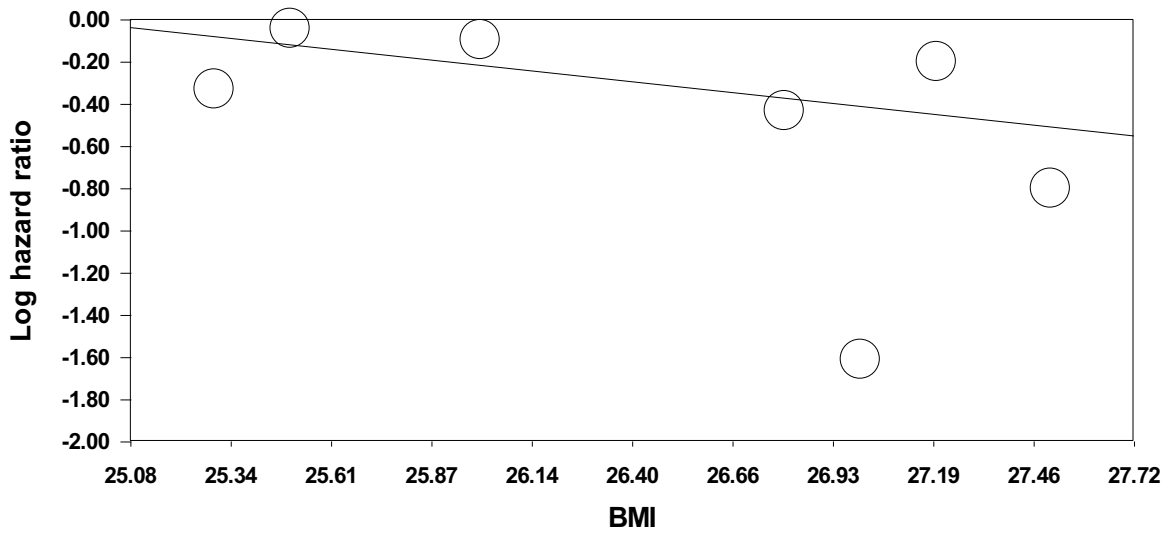
Appendix A2 Funnel Plot of standard error for the association between stroke and serum level/intake of lycopene.



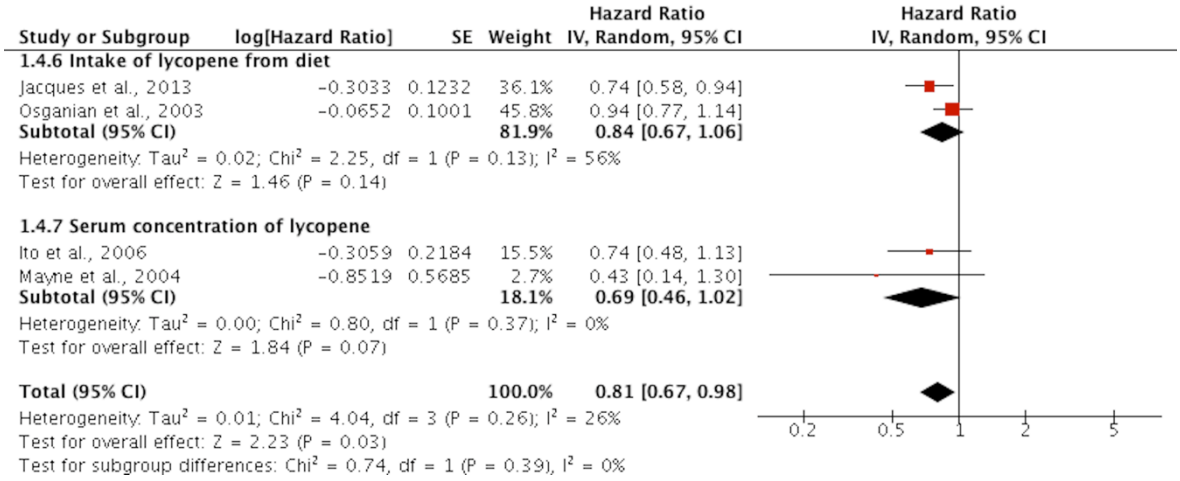
Appendix A3 Meta-regression on the effect of follow-up duration on the association between stroke and serum level/intake of lycopene.



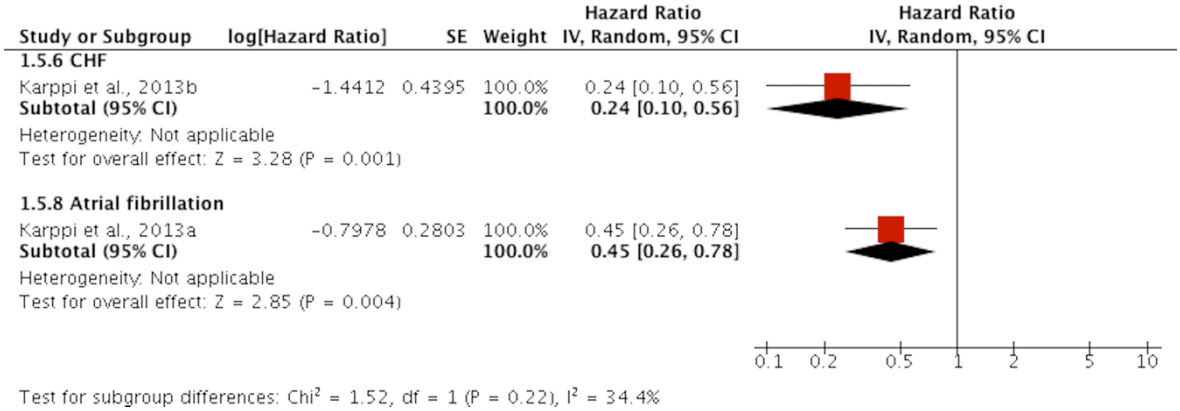
Appendix A4 Meta-regression on the effect of age on the association between stroke and serum level/intake of lycopene.



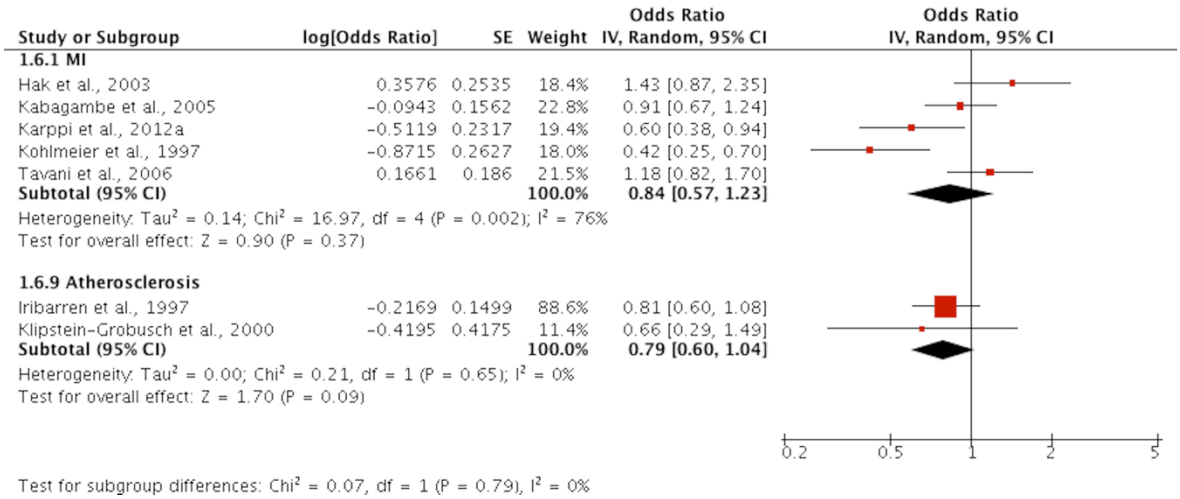
Appendix A5 Meta-regression on the effect of BMI on the association between stroke and serum level/intake of lycopene.



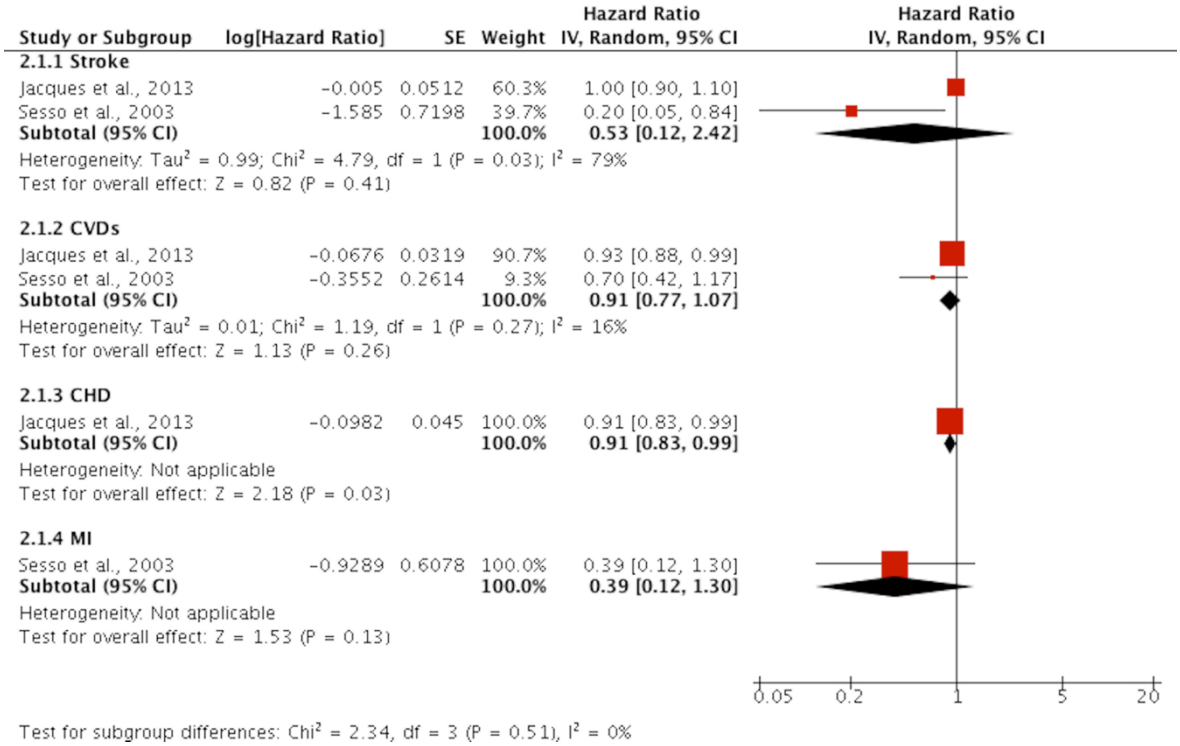
Appendix A6 Forest plot of the association between high serum level/intake of lycopene and risk of CHD.



Appendix A7 Forest plot of the association between high serum level/intake of lycopene and risk of CHF and AF.



Appendix A8 Forest plot of the association between high serum level/intake of lycopene and risk of MI and atherosclerosis.



Appendix A9 Forest plot of the association between high intake of tomato and risk of CVD events.

Appendix B

Supplementary information for Chapter 3

Appendix B1 PRISMA checklist.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	71
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	N/A
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	71-72
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	71-72
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	72
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	72-73
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	72
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	72
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	73
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	73

(**Appendix B1** continues on next page)

Section/topic	#	Checklist item	Reported on page #
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	72-73
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	73-74
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	74
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	73-74

Appendix C

Supplementary information for Chapter 4

Appendix C1 Red cherry tomatoes (Piccolo) purchased from Marks & Spencer growth by Thanet Earth.

Appendix C2 Red cherry tomatoes (Piccolo) purchased from Marks & Spencer growth by Andy Roe.

Appendix C3 Red cherry tomatoes (Piccolo) purchased from Sainsbury's growth by Thanet Earth.

Appendix C4 Red cherry tomatoes (Red Comet) purchased from Tesco growth in Spain.

Appendix D

Supplementary information for Chapter 5

Appendix D1 CONSORT 2010 checklist of information to include when reporting a randomised trial.

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	113-114
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	114-118
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	113-114
	2b	Specific objectives or hypotheses	113-114
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	114-118
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	N/A
Participants	4a	Eligibility criteria for participants	114
	4b	Settings and locations where the data were collected	117
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	114-118
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	118-129
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	114-115
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	116
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	116
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	116

(**Appendix D1** continues on next page)

Section/Topic	Item No	Checklist item	Reported on page No
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	116
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	116
	11b	If relevant, description of the similarity of interventions	N/A
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	129
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	129
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	118
	13b	For each group, losses and exclusions after randomisation, together with reasons	118
Recruitment	14a	Dates defining the periods of recruitment and follow-up	114, 130
	14b	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	133, 154
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	118
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	135-142, 153-161
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	N/A
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	135-142, 153-161
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	N/A
Discussion			

(**Appendix D1** continues on next page)

Section/Topic	Item No	Checklist item	Reported on page No
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	150, 161
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	N/A
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	143-151, 159-162
Other information			
Registration	23	Registration number and name of trial registry	114
Protocol	24	Where the full trial protocol can be accessed, if available	N/A
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	N/A

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Human VCAM-1 Conjugate to each well.
4. Add 100 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 1.5 hours at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Immediately add 100 µL of Substrate Solution to each well. Cover with a new adhesive strip. Incubate for 20 minutes at room temperature. **Protect from light.**
7. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Appendix D2 Manufacturer's instructions for Human sVCAM-1 ELISA kit.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Human ICAM-1 Conjugate to each well.
4. Add 100 µL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 1.5 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the bench top. Protect from light.**
7. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Appendix D3 Manufacturer's instructions for Human sICAM-1 ELISA kit.

SIGMA-ALDRICH®
sigma-aldrich.com
3050 Spruce Street, Saint Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com

Product Information

Sigma's Sandwich ELISA kits are in vitro enzyme-linked immunosorbent assays for the quantitative measurement of soluble proteins in a variety of species. Our extensive ELISA selection includes cytokines, growth factors, proteases, soluble receptors, apoptosis effectors, and many other soluble proteins.

Sandwich Assay Procedure

1. Bring all reagents and samples to room temperature (18–25 °C) before use. It is recommended that all standards and samples be run at least in duplicate.

2. Add 100 µL of each standard and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature or overnight at 4 °C with gentle shaking.

3. Discard the solution and wash 4 times with 1× Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100 µL of 1× prepared Detection Antibody to each well. Cover wells and incubate for 1 hour at room temperature with gentle shaking.

5. Discard the solution. Repeat the wash procedure as in step 3.

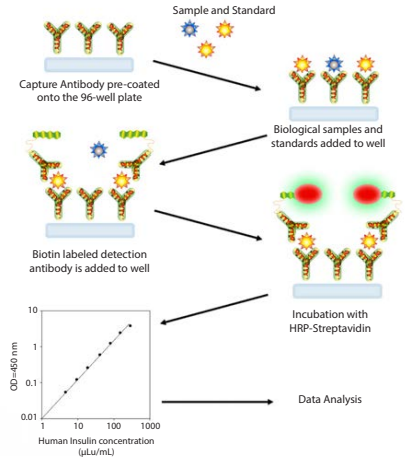
6. Add 100 µL of prepared Streptavidin solution to each well. Cover wells and incubate for 45 minutes at room temperature with gentle shaking.

7. Discard the solution. Repeat the wash as in step 3.

8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Cover wells and incubate for 30 minutes at room temperature in the dark with gentle shaking.

9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.

How It Works



Appendix D4 Manufacturer's instructions for Human E-selectin and P-selectin ELISA kit.

Perform ELISA (Total assay time: 3 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1	Bind antigen 	a. Add 100 µL of standards or samples to the appropriate wells. b. Add 50 µL Hu IL-6 Biotin Conjugate solution into each well except the chromogen blanks. c. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate for 2 hours at room temperature . d. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.
2	Add Streptavidin-HRP 	a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks. b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature. c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.
3	Add Stabilized Chromogen 	a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue. b. Incubate for 30 minutes at room temperature in the dark. **Note:** TMB should not touch aluminum foil or other metals.
4	Add Stop Solution 	Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

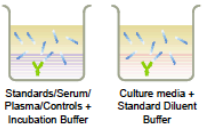
Appendix D5 Manufacturer's instructions for Human IL-6 ELISA kit.

Perform ELISA (Total assay time: 4 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen



a. Add 50 µL of **Incubation Buffer** to wells for serum or plasma samples, standards, or controls; or 50 µL of **Standard Diluent Buffer** to the wells for cell culture samples. Leave the wells for chromogen blanks empty.
b. Add 100 µL of standards, controls, or samples (see "Pre-dilute samples" on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
c. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate for 2 hours at room temperature.
d. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Biotin Conjugate

a. Add 100 µL Hu TNF-α Biotin Conjugate solution into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 1 hour at room temperature .
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 Add Streptavidin-HRP

a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

4 Add Stabilized Chromogen

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

5 Add Stop Solution

Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer (serum/plasma) or with the corresponding cell culture medium (cell culture supernatant) and reanalyze. Multiply the concentration by the appropriate dilution factor.

Appendix D6 Manufacturer's instructions for Human TNF-α ELISA kit.

Appendix E

Supplementary information for Chapter 8

Appendix E1 PRISMA checklist.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	163
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	163-164
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	163-164
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	163-164
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	164
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	165
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	164
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	164
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	164
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	165

(**Appendix E1** continues on next page)

Section/topic	#	Checklist item	Reported on page #
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	165
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	165
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	165

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