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Coffee as a risk factor for Cardiovascular Diseases

A Literature Study

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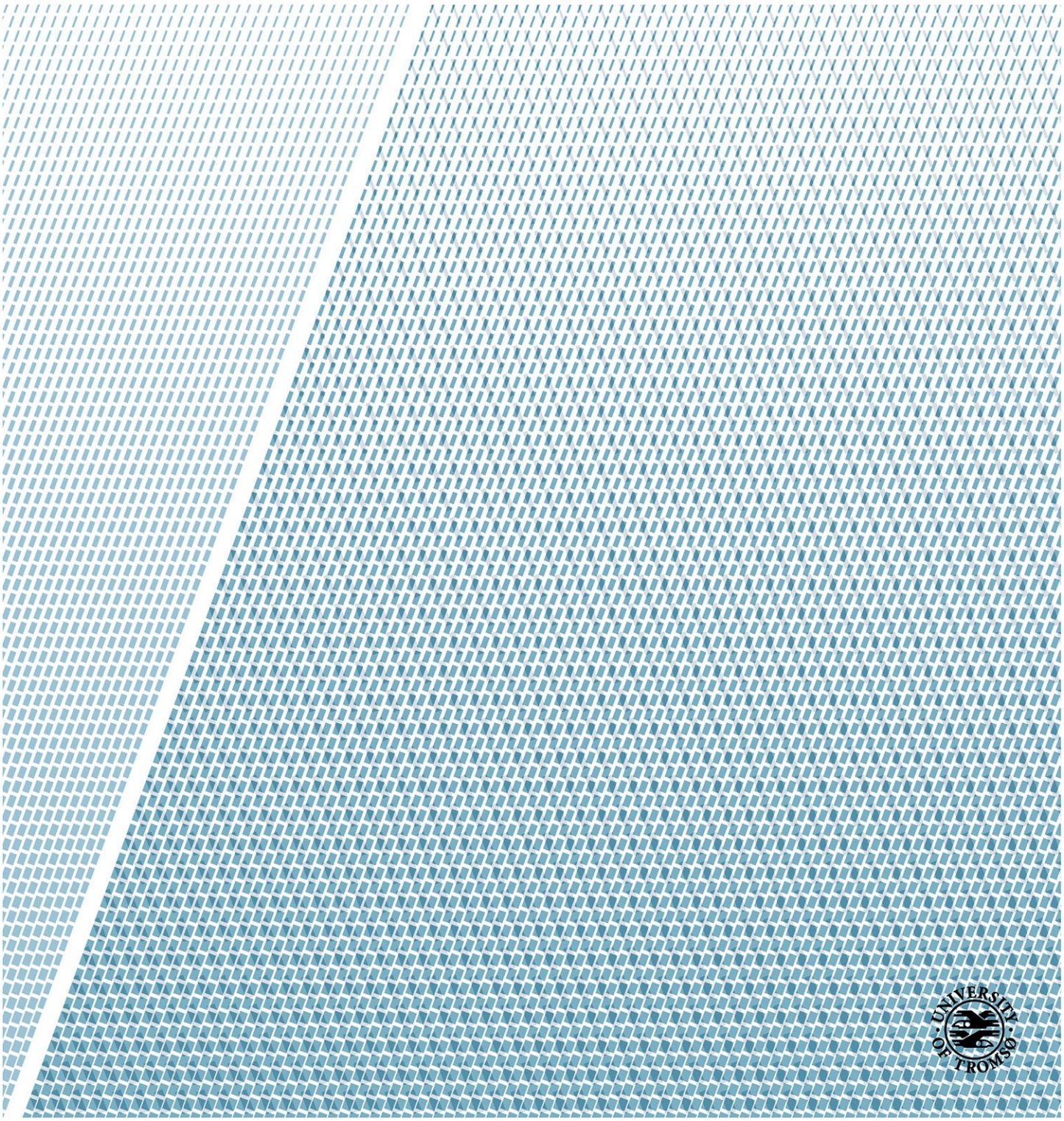

Preface

The chosen topic, coffee consumption and its effects on cardiovascular health can be explained by a personal interest and the desire to understand in more depth, the effects coffee has on cardiovascular health. The reason for this interest is related to the worldwide popularity of coffee as a beverage, along with the conflicting results on the research based evidence done over the years about coffee's effects on health. This interest has gradually grown as a result of the polarity of what coffee's once illustrated effects on health was, to what it is today. Coffee has been gaining popularity as a protective entity in cardiovascular medicine. As a result, consumption of coffee is being encouraged through the media, especially over the internet on health and lifestyle websites.

The thesis focuses and explores the conflicting evidence on the consumption of coffee over three decades, and the role of nicotine on the consumption of caffeine. The thesis also focuses on the incriminating evidences between the researches done in the past from the research done in the present. I think it is important to gain knowledge on whether there are negative side effects to excessive consumption of coffee, as well as to understand why brewing methods play an important role on the effects coffee has on cardiovascular health.

Coffee is the most widely drunk beverage around the world, especially within Scandinavia;[1] according to the statistics portal for data market, 1211 cups of coffee per capita was drank in Sweden alone in 2014, which was highest in the world after Finland at 1252 cups of coffee per capita. Norway came in at 5th place with 916 cups per capita and Denmark at 6th place with 845 cups per capita in 2014.[1]

The importance to explore the full effects of coffee related health problems, including brewing methods, is mainly due to the high consumption rate of coffee around the world. In the context of public health, knowing how a popular beverage such as coffee effects the cardiovascular risks, can eventually influence mortality rates related to cardiovascular problems in the future.

Acknowledgements

I would like to take the opportunity to thank my supervisor Sidsel Graff-Iversen for having taken me as her student, and being patient with me while I worked on the thesis for the past year. I feel very grateful to have Marko as my co-supervisor, and feel my up most gratitude to him, who took his time from vacationing to give me feedback, and helped me throughout the process, whether it being searching papers from the main library to asking me about how the process was going every now and then. Lastly I would like to thank my family who encouraged me to not give up and put up with me during the process. Thank you so much!

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Abstract

The World Health Organization has claimed that non-communicable diseases kill 38 million people each year, and cardiovascular diseases (CVD) related deaths was the number 1 cause.

Coffee is the most drank beverage all over the world. Researches in the past, had suggested that coffee consumption may increase risks of CVD.

A literature study was performed using online search engines, google scholar and pubmed, in order to assess and compare evidence of the effects of coffee on cardiovascular health. Searches were done throughout the writing process. Filters were used to make the search process easier. Searches were performed according to publication dates between 1st January 1974 and 31st December 2015. Specific words like 'coffee', 'risk', 'cardiovascular diseases', 'brewing methods', were combined with 'or' and 'and' during the search process.

Over the decades, researches acknowledged the consistency of coffee's role in increasing the lipid profile in humans. However, they were able to claim that the brewing methods had an important role to the outcome of the results. In more recent times, it has been suggested that presences of antioxidants in coffee may have beneficial effects on over-all health of individuals.

There has been extensive research done on coffee over the past 5 decades, and the effects it may have on health, with conflicting results. However, it seems that the quantity of consumption along with the methods of preparation may lead to different outcomes.

Keywords:

- Coffee
- Cardiovascular diseases
- Risks
- Homocysteine
- Cholesterol
- Lipid profile
- Kahweol
- Cafestol
- Myocardial infraction

Abbreviations:

- CVD- Cardiovascular diseases
- WHO- World Health Organization
- NCD- Non-communicable diseases
- LDL - Low density lipoproteins
- HDL- High density lipoproteins
- MI- Myocardial infractions
- CHD- Coronary heart diseases

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1. Rational

Cardiovascular diseases are among the four major non-communicable diseases (NCD) responsible for leading causes of death worldwide. According to the World Health Organization (W.H.O), 52% of all death in 2012 were NCD related, and 37% of the 52% of the lives claimed were due to Cardiovascular diseases.[9]

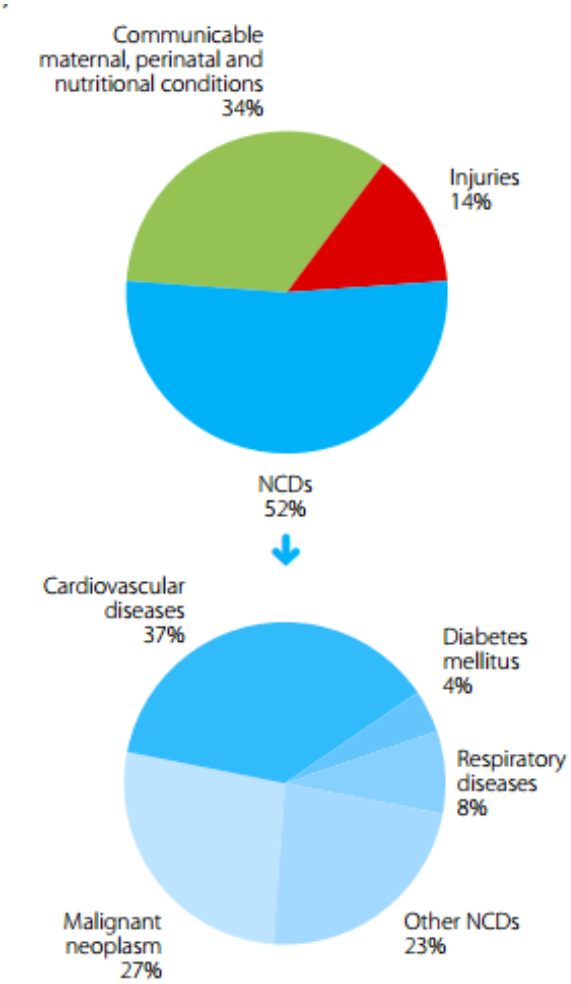


Fig 1: Proportion of global deaths under the age of 70 years, by cause of death. Estimates from 2012. *(WHO. Global status report on noncommunicable diseases. 2014. P10; fig 1.3. Available from: http://apps.who.int/iris/bitstream/10665/148114/1/9789241564854_eng.pdf?ua=1)*

As reported by the W.H.O, Cardiovascular diseases (CVDs):

- are the number 1 cause of the global deaths;
- 17.5 million people were estimated to have died in 2012 due to CVD related causes, of which 7.4 million were due to coronary heart disease, and the remaining 6.7 million were cerebrovascular accidents (CVA).
- Preventable.
- Population with high risk factors can be managed appropriately if they are detected early on.[10]

Through the years, risk factors for cardiovascular diseases have been identified as preventable and non-preventable. Among preventable causes, cigarette smoking, alcohol use, inactivity, and general life style choices were attributable to the significant portion of CVD related deaths.[10]

In previous years, coffee was identified as a potential culprit to a cause of CVD related deaths. However, the results from more recent research favoured coffee as a protective agent against CVD.

The mere contradiction of the earlier research and effects of other factors related to the population of coffee drinkers, is something I wish to explore in this thesis. How coffee drinking is related to smoking is also explored later on in the thesis, as the relation between the two came up as a coincidence while reading papers based on the effects coffee had over CVDs.

2. Objective

The objective of this literature study is to assess whether there are any benefits of drinking coffee on the cardiovascular health and to briefly analyse the relationship of caffeine to that of nicotine.

3. Thesis Structure

The structure of the thesis is gradually built up for the purpose of understanding the thesis topic, and to better understand the controversies attached to the research over the years.

The rational part of the thesis, Chapter 1, gives an overall explanation as to how this thesis topic is of importance to public health. Chapter 2 states the objective, and chapter 4 explains the literature search strategy, including methods and materials. The definitions and clarifications of terms that were stated was also included in this section. In chapter 5, a simplified version of the physiology of the cardiovascular system is included along with the definition of CVD in accordance to WHO's definition is given.

In chapter 6, methods of how the first three Tromsø studies were conducted is explained. The reason why this was introduced separately is because the older Tromsø study was among the first studies to recognize the importance of brewing methods, and the thesis primarily focuses on the evolvement of the idea of the risk factor, being coffee, from the past to the present, and what we know now that we didn't know then.

The discussion of coffees effect on risk of CVD is found in chapter 7 under the title, "The discover of coffee on cardiovascular risks." Chapter 8 is the result section, found under the title, "Recent Research on Coffee and Cardiovascular health."

In chapter 9, the combined effects of coffee and smoking are discussed along with why smokers may drink more coffee than none smokers.

Chapter 10 is the methodological limitations and chapter 11 is the summary and conclusion part of the thesis.

4. Method and Materials

A literature study was performed with the purpose to describe the effects of coffee on cardiovascular health in humans, by discussing and comparing evidence.

4.1 Definitions and clarifications

Cardiovascular diseases as stated by the World Health Organization[2] are;

"a group of disorders of the heart and blood vessels and include:

- *coronary heart disease: disease of the blood vessels supplying the heart muscle;*
- *cerebrovascular disease: disease of the blood vessels supplying the brain;*
- *peripheral arterial disease: disease of blood vessels supplying the arms and legs;*
- *rheumatic heart disease: damage to the heart muscle and heart valves from rheumatic fever, caused by streptococcal bacteria;*
- *congenital heart disease: malformations of heart structure existing at birth;*
- *deep vein thrombosis and pulmonary embolism: blood clots in the leg veins, which can dislodge and move to the heart and lungs.[2]"*

Myocardial infractions are more commonly known as heart attacks and cerebrovascular incidents are called strokes in layman terms. These are acute events caused by a build-up of fatty deposits, calcium and blood clots that block or narrow the arteries, preventing blood from flowing to the heart or brain. Strokes can also be caused by bleeding from within the brain.[2]

In this thesis, cardiovascular diseases do not include the whole array of the definition given by the WHO, instead for the purpose of this thesis only, cardiovascular diseases include coronary heart diseases, mainly myocardial infractions and coffee drinking as a potential risk modifier.

What is Cholesterol? According to the National Heart, Lung, and Blood institute, cholesterol is a substance that is produced by the liver, and is required for the production of some hormones, vitamin D and for some substances that help digest food.[3]

In the bloodstream, cholesterol is transported in small packages made of lipids (on the inside) and proteins (on the outside). These packages are called lipoproteins. The lipoproteins are responsible for carrying cholesterol throughout the body. There are mainly two types of cholesterols, low density lipoproteins (LDL), and high density lipoproteins (HDL). High levels of LDL cholesterol is responsible for the build up of cholesterol in the arteries, and is therefore sometimes referred to as “bad cholesterol”. HDL cholesterol is responsible for the transport of cholesterol from other parts of your body back to your liver, where it is than excreted.[3]

The condition in which there is presence of too much cholesterol in the blood is called “high blood cholesterol”. Risk of coronary heart disease is greater in individuals with higher levels of blood cholesterol, mainly in those individuals with high LDL levels, whereas high levels of

HDL lower the chances of coronary heart diseases. High blood cholesterol is problem because it usually manifests without any signs or symptoms and is only detected through blood testing.[3]

Coronary heart disease is the disease of the arteries suppling the heart muscles. According to the British heart Foundation, Coronary heart disease is a condition in which there is a build up of fatty materials (plaque) within the coronary arteries thereby causing insufficient supply of oxygenated blood to the coronary muscles.[5] Atherosclerosis is the condition caused due to the build-up of plaque within the arteries. Plaque can consist of cholesterol, fat, calcium, and other substances found in blood.[4]

According to the National Institute of Health (NIH), measuring cholesterol levels are as follows;

- *"**Total cholesterol**–a measure of the total amount of cholesterol in your blood, including low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol.*
- ***LDL (bad) cholesterol**– Low Density lipoproteins, the main source of cholesterol build up and blockage in the arteries*
- ***HDL (good) cholesterol**–High Density lipoproteins helps remove cholesterol from your arteries*
- ***Triglycerides**–another form of fat in your blood that can raise your risk for heart disease."*[4]

Total Cholesterol Level	Category
Less than 200mg/dL	Desirable
200-239 mg/dL	Borderline high
240mg/dL and above	High

LDL (Bad) Cholesterol Level	LDL Cholesterol Category
Less than 100mg/dL	Optimal
100-129mg/dL	Near optimal/above optimal
130-159 mg/dL	Borderline high
160-189 mg/dL	High
190 mg/dL and above	Very High

HDL (Good) Cholesterol Level	HDL Cholesterol Category
Less than 40 mg/dL	A major risk factor for heart disease
40—59 mg/dL	The higher, the better
60 mg/dL and higher	Considered protective against heart disease

Table 1: Value of cholesterol levels defined as optimal, near optimal, borderline high, high or very high, as a risk factor for cardiovascular disease. *(Source: National Heart, Lung and Blood institute. Cholesterol Levels: What You Need to Know. 2012 (Cited on 2016 Feb 8). 7(2):6-7. Available from: https://www.nlm.nih.gov/medlineplus/magazine/issues/summer12/articles/summer12pg6-7.html).*

What is Coffee?

When we refer to coffee, we mean the beverage made of roasted coffee beans. The method of roasting and production is not explored in the thesis. However, the method of brewing is taken into account, along with additional substances such as sugar and cream added to the coffee.

The coffee beverage is produced by roasting coffee beans before the brewing process. The coffee bean is extracted from the fruit of the coffee tree which is referred to as a "coffee cherry".[6]

According to the National Coffee Association (NCA), Coffee originates from a plant called Coffea, and has 500 genera and 6000 species. However, in the commercial coffee industry, only 2 coffee species out of the 6000 are used; Coffee Arabica and Coffee Canephora, also known as Robusta.[7]

Over 2,000 substances have been found in coffee. Coffee mainly consists of carbohydrates, making up for 38–42% of the toasted coffee bean. Lipids and amino acids are the other main components found in coffee, making about 20% and 10% in remaining consistency respectively. Melanoidins give coffee its brown colour, and make up 23% of the weight of the coffee bean. Minerals contained in coffee include, aliphatic and chlorogenic acids, trigonellines, and volatile aromas. Caffeine is the most studied alkaloid to be present in the coffee bean, making up 1.3 to 2.4% of its weight. Other alkaloids present in coffee, include purinic alkaloids- theobromine and theophylline; and pyridine alkaloids- trigonelline.[19]

Cafestol and **kahweol** are the two diterpene molecules found in coffee, which manifest as oily droplets in the coffee brew.[8] These two diterpenes will be looked at more closely in the discussion part of the thesis.

Homocysteine: Homocysteine is an amino acid that is produced by the body during the methionine cycle, when adenosine is comically broken down into homosysteine. It was first discovered in 1932 by Butz and du Vigneaud by chance during their study on insulin. When methionine was heated in sulfuric acid, the methionine transformed into a new amino acid that had similar properties to that of cysteine.[48,49] Homocysteine levels in the blood, like cholesterol levels, have been claimed to be a possible predictor in the out comes in risk of CVDs' by scientists in the past.[69] However, it is no longer being considered as an independent risk factor.[66] More about homocysteine is discussed in a later chapter.

4.2 Literature search strategy:

Systematic searches were performed to find relevant background theory that were available to identify relevant studies to discuss in the paper. Searches were performed on observational studies, mainly prospective cohorts were preferred, using the online search engines PubMed, google scholar, and from reference lists of papers that were searched during the research process. Filters were used to make the search easier. The following words were searched multiple times during the thesis: 'coffee', 'cardiovascular diseases and coffee', 'homocysteine', 'metabolism of coffee', 'cholesterol and coffee', 'cafestol and kaweol', 'Tromsø study', 'Scandinavia type boiled coffee'.

Human studies were mainly used to build up the theory, however animal studies were only included when human studies were limited.

Different sources were used to obtain evidences to support or to disprove the theory and claims. Background theory was obtained from reports, text books, and web pages. Reviews

were used as research support on background theory when other materials were lacking No unpublished literature was used.

Searches were done several times during the writing process to find theory, and evidence showing an effect or no effect. The search criteria were as follows:

- research papers had to be published between 1 January 1974 and 31 December 2015. Primary studies done within Scandinavia was preferred since the thesis was inspired by the 1st Tromsø studies, it would be easier to compare the older studies with newer studies from within the same region, where otherwise cultural and lifestyle differences may have altering consequences on the results. Other studies and reviews were used to back up claims when necessary;
- articles describing the brewing methods;
- both single studies and reviews were used;
- the primary objective had to concern coffee intake as an exposure and CVD as an outcome.

Search criteria were intended to find articles specific and relevant to the topic. All studies discussed in chapters 6 and 7 were published before 31 December 2015. The Tromsø heart study from previous decades was the primary study of choice, because the aim of the Tromsø Study when it was first initiated in 1974, was primarily to determine the reasons for the high mortality of cardiovascular diseases, and to develop ways of preventing CVDs. While more recent studies were chosen between 1st January 2000 to 31st December 2015, as long as they could be compared to the older Tromsø studies. Differences in the initial

research were compared and history of the relationship between cardiovascular diseases and coffee was explored.

Studies preferences were ranked according to geographical locations from most preferred to the least preferred in the following order: - Scandinavia; Nordic regions; Western Europe; others. The reason for this preference was to minimise bias regarding diet and lifestyle associated with different geographical locations, and to make it easier to compare the older Tromsø studies with the latest studies on how we used to perceive coffee to what we perceive it to now in regards to cardiovascular health and medicine. Only studies published in English were used. For the 'latest research', papers between 1st January 2000 and 31st December 2015 were used.

The literature sources according to preference in study designs: prospective cohort studies; randomized control trials; case control studies; and literature reviews. Although RCTs would have been preferable due to the lower chances of bias, for this thesis topic however, cohort study designs seemed more appropriate.

4.3 Materials

Materials chosen for the purpose of the thesis were older studies primarily from within the population based studies within the Tromsø cohort, between 1970's and 1999's. I chose these studies because the Tromsø study was among the first cohort studies to recognize the effects of the brewing methods involved in the outcome of CVD among coffee drinkers. These studies were used as the 'build up' material in the thesis, and helped to explore

whether coffee was a risk to the outcome, CVD. Newer studies from 2000 January 1 till 2015 December 31, were used for the purpose of understanding the evolvement of coffee and the reported consequences on cardiovascular health, and to compare the outcomes.

Summary of the materials used:

Country of Origin of the studies used: Norway, Sweden, United States of America, Australia, Netherlands, Italy.

Study design: Cohort studies, Randomized control trials, Case control studies, cross sectional studies, and literature reviews.

Exposure: Coffee.

Outcomes: acute myocardial infractions, raised serum lipid profiles.

Adjusting: smoking.

Limitations: possible biases present in the study designs used, therefore increasing chances for less accurate results.

The materials used for the thesis is given in more detail in the supplementary part of the thesis.

(Over view of the literature search strategy and characteristics of the studies used are included in the “Supplementary materials” in the end of the thesis).

5. Cardiovascular Diseases & Health

The heart is a muscular organ responsible for pumping blood to different parts of the body. It is located in the thoracic cavity and consists of 4 chambers, but acts like 2 separate pumps. The right side of the heart is responsible for pumping blood from the heart to the lungs, and the left side is responsible for pumping blood from the heart to the rest of the body.[11] Deoxygenated blood from the rest of the body is collected in the veins, which transports the blood to the right side of the heart. From there, the blood is transported into the lungs for excretion of carbon dioxide and is then re-oxygenated. The oxygenated blood is then transported to the left side of the heart, where it is then pumped to the rest of the body. The heart beats about 60 to 70 times per minute at rest, and increases gradually with physical activity.[12] Coronary arteries are responsible for supplying the heart muscles with oxygenated blood and nutrients and for the excretion of deoxygenated blood from the heart muscles. The inner system within the heart that is responsible for supplying the heart muscles, is called the coronary circulation.[11]

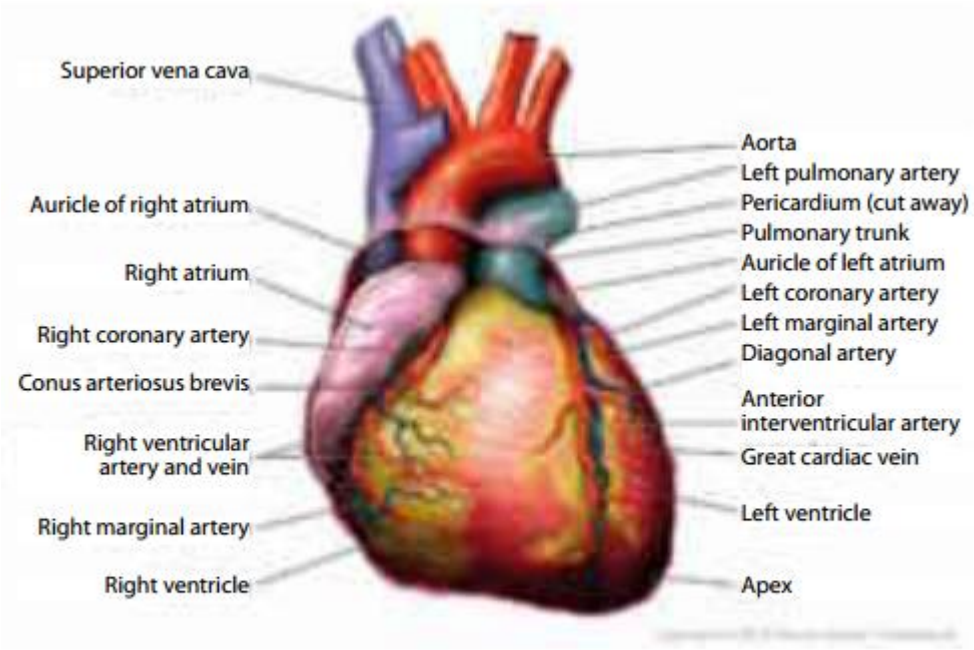


Fig 2: Anatomy of the Heart. *(Image source: Biology for kids (online). Updated 2016 Jun (Cited on 2016 Jun 15). Available from: http://www.ducksters.com/science/blood_and_the_heart.php).*

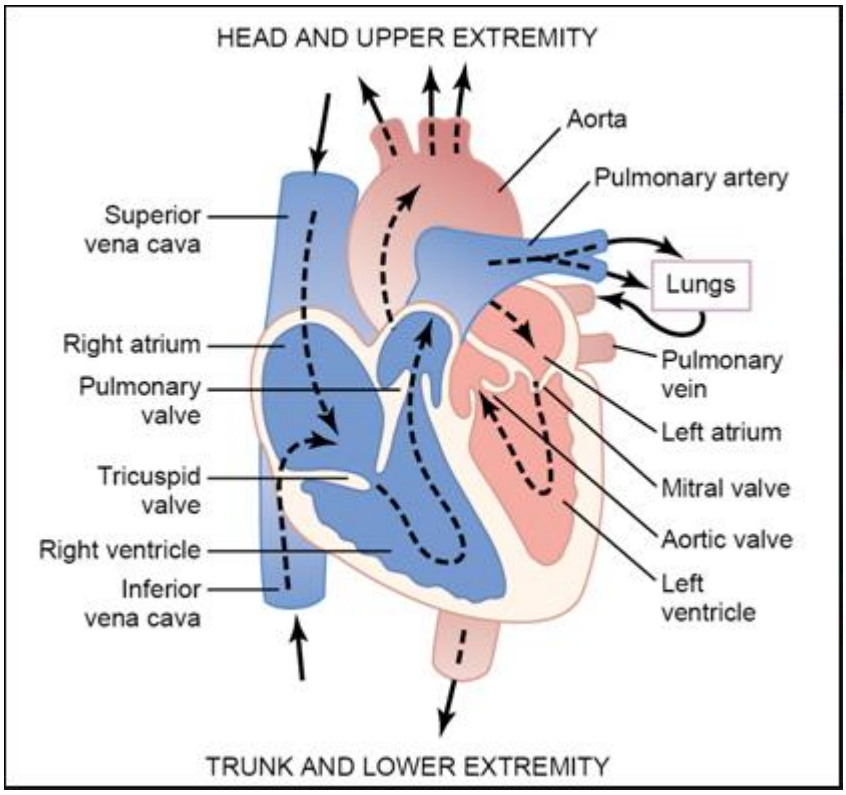


Fig 3: The heart as a two way pump. *(Heart muscles, as pump and funtions of valves. Cited on 2016 Feb 8. Available from: http://medicinembbs.blogspot.se/2011/02/heart-musclesas-pump-and-functions-of.html).*

The World Health Organisation (W.H.O), describes cardiovascular diseases as a group of disorders of the heart and blood vessels. These include:

- coronary heart disease;
- cerebrovascular disease (CVI- cerebrovascular incidents and CVA- cerebrovascular accidents);
- peripheral arterial disease;
- rheumatic heart disease (caused by streptococcal bacteria);
- congenital heart disease;

- deep vein thrombosis and pulmonary embolisms.[10]

Myocardial infractions(MIs) and CVAs are acute events and are caused by a blockage preventing blood from flowing into the heart or brain. Atherosclerosis is a condition that is responsible for the occurrences of MIs and CVAs. It is characterized by a build-up of plaque (fatty deposits) in the inner walls of the blood vessels that supply the heart or brain. CVA's can also be caused by a haemorrhage within the blood vessels in the brain or from blood clots. MIs and CVAs are presentes result as acute conditions, associated with a combination of risk factors. These risk factors include smoking, excessive alcohol intake, unhealthy diet, obesity and physical inactivity, hypertension, diabetes and hyperlipidaemia.[10]

For the purpose of this thesis, incidence of CVA/CVI was not included in the investigative process. Occurrence of MI among coffee drinkers was the primary objective.

6. Introduction to the Tromsø Study

This thesis is primarily based on the findings from the older Tromsø studies done between 1974 and 1987. The reason why these studies were primarily chosen was because the Tromsø study was one of the first studies to recognise the effects of brewing methods on the serum cholesterol levels in the study participants. University of Tromsø initially funded the project, but over the years, other contributions have been made to the project from organizations

such as the National Screening Services, the Research Council of Norway, Northern Norway Regional Health Authority, Norwegian Council on Cardiovascular Diseases and Norwegian Foundation for Health and Rehabilitation.[13]

The Tromsø study was initiated in 1974 in an attempt to help combat the high mortality rate of cardiovascular diseases in Norway. Mortality rates due to cardiovascular causes was highest amongst the middle-aged population, primarily among the Norwegian male population. Norwegian men in the mid 1970's, were at a higher risk of dying due to a cardiovascular disease before the age of 75 years. Tromsø study was initiated with the goal to determine the cause of the high cardiovascular mortality rates, and also to develop ways of preventing CVDs.[13]

Table 2: The Tromsø study.[72]

Study year	Study's name	Number of participants	Age group
1974	Tromsø 1	6595 men	20-49
1979-80	Tromsø 2	16621 men and women	20-54
1986-87	Tromsø 3	21826 men and women	12-67
1994-95	Tromsø 4	27158 men and women	25-97
2001-02	Tromsø 5	8130 men and women	30-89
2007-8	Tromsø 6	12984 men and women	30-87
2015-16	Tromsø 7 (ongoing)	33423 men and women (eligible)	40+

The Tromsø Study consists of six surveys that have been conducted in the municipality of Tromsø from 1974, and has been an active project since then. The thesis uses the first three of the Tromsø Study surveys dating from 1974-1987, to understand how coffee consumption was related to high cholesterol levels, thereby increasing the risk of CVD.

The Tromsø 1, 1974: 8866 men were invited to participate in the first survey, between the ages of 20-49. The participation rate was 83%. Questionnaires were sent out with the invitation. Questionnaires were pharased into 'yes' or 'no' questions, and the participants were asked about their medical history, on known diagnosis of cardiovascular diseases and diabetes, or whether they had symptoms of cardiovascular diseases, smoking habits and physical activity during work and leisure time. The participants were interviewed, and measurements of height, weight and blood pressure, along with blood samples for measurents of serum total cholesterol, triglycerides and haemoglobin, were taken.[73] In the first study they discovered the effects of high density lipoproteins in the prevention of myocardial infractions.[73]

Table 3: The Tromsø 1 study[73]:

Age	Invited	Participants	%
20-24	1662	1014	61,0
25-29	1996	1374	68,8
30-34	1741	1321	75,9
35-39	1249	1022	81,8
40-44	1095	929	84,8
45-49	1123	935	83,2
TOTAL	8867	6595	74,4

The Tromsø 2 study, 1979-1980: Tromsø Study 2 took place between 1979 to 1980. A total of 16 620 people took part in the second survey, which included 8477 men between the ages of 20-54 years, and 8143 women between the ages of 20-49 years. The men who were invited to participate in the first Tromsø Study were reinvited to participate in the second survey. Participation rate was 74%. A questionnaire similar to the first survey about diagnosed cardiovascular diseases and diabetes, current symptoms, and life style habits, was sent out, along with an invitation to measure height, weight, blood pressure, serum total cholesterol, HDL cholesterol, triglycerides and blood sugar level. People that attended the study received a second set of questionnaire regarding their diet, medical histories including family medical histories, and social conditions. Based on the results from the Tromsø 2 study, it was discovered that the consumption of coffee increased serum cholesterol levels.[74]

Table 4: The Tromsø 2 study[74]:

Age	Men invited	Women invited	Male participants	Female participants	% men	% women
20-24	1782	2000	1027	1383	57,6	69,1
25-29	2261	2286	1477	1734	65,3	75,9
30-34	2278	2087	1713	1784	75,2	85,5
35-39	1786	1539	1422	1376	79,6	89,4
40-44	1212	1088	1002	998	82,7	91,7
45-49	1077	958	912	868	84,7	90,6
50-54	1085		934		85,2	
TOTAL	11481	9958	8477	8143	73,8	81,8

The Tromsø 3 study, 1986-1987: The third survey of theTromsø Study was taken between 1986-1987. 21 826 people participated in the third survey, among which 10 963 were men between the ages of 20-61, and 10 863 women between the ages of 20-56. This time an additional 10 persent of the population between the ages of 12-19 were randomly selected, and those who had previously participated in the family intervention study, which was a study that had been carried out shortly after the second Tromsø, were also included. Similar questionnaires as the first two studies were sent out, with additional questions on coffee consumption habits, salty food intake and fat preference in the diet, along with an invitation to measure height, weight, blood pressure, serum total cholesterol, HDL cholesterol, triglycerides and gamma-glutamyltransferase (gamma-GT), and a simple electrocardiogram was carried out. Those that attended the study got a second set of questionnaires to fill out, including general health, medical history including the family medical history, medications, diet, lifestyle and social conditions. The Tromsø 3 study, confirmed that unfiltered coffee increased serum cholesterol. It was also found that people who developed myocardial infarction had higher levels of homocysteine than healthy people.[75]

Table 5: The Tromsø 3 study, 1986-1987[75]:

Age	Men invited	Women invited	Male participants	Female participants	% men	% women
12-14	219	242	181	195	82,6	80,6
15-19	522	524	368	390	70,5	74,4
20-24	2076	2102	1160	1303	55,9	62,0
25-29	2211	2194	1318	1572	59,6	71,6
30-34	2327	2367	1583	1848	68,0	78,1
35-39	2261	1987	1710	1681	75,6	84,6
40-44	1893	1722	1485	1526	78,4	88,6
45-49	1318	1177	1076	1059	81,6	90,0
50-54	1048	961	892	874	85,1	90,9
55-59	990	427	836	381	84,4	89,2
60-64	416	36	354	30	85,1	83,3
65-67	0	6	0	4	0	66,7
TOTAL	15281	13745	10963	10863	71,8	79,0

7. The discovery of the effects of coffee as a cardiovascular disease risk factor

From an earlier study based on the Tromsø study 1, Dag S. Thelle and his colleagues, noticed the presence of a positive correlation between mortality due to cardiovascular diseases, high serum cholesterol and smoking,[14] which were risk factors that were already recognized long before the Tromsø study. At the time, it was believed that the reason for higher mortality due to CVD among the

population in Northern Norway was due to high serum cholesterol levels, and higher percentage of smokers compared to other regions in Norway. Smoking was singled out as the cause for high serum cholesterol levels leading to CHD related deaths.[14]

Førde OH et al. conducted a 10week experiment after the second Tromsø study survey. The objective of Førde's experiment was to assess the effects of coffee consumption and coffee brewing methods on serum cholesterol levels.[15] The trial consisted of 33 men with hypercholesterolaemia, who were randomly assigned to 1) continue drinking boiled coffee as per usual; 2) stop drinking coffee for the whole duration of the experiment (10 weeks); 3) stop drinking coffee for the first 5 weeks, after then start drinking boiled coffee again for another 5 weeks; and 4) stop drinking coffee for the first 5 weeks, then start drinking coffee again, but only filtered coffee.

Table 6: Coffee consumption and serum lipid concentrations in men with hypercholesterolaemia[15]:

Mean (SD) cholesterol concentrations (mmol/l) at screening and follow up, baseline concentrations, and mean (SD) changes (mmol/l) from baseline values in subjects drinking coffee as usual for 10 weeks (group 1), subjects abstaining from coffee for 10 weeks (group 2), and subjects abstaining for five weeks and then drinking boiled (group 3) or filter (group 4) coffee

	Group 1 (n = 8)	Group 2 (n = 9)	Group 3 (n = 8)	Group 4 (n = 8)
Total cholesterol:				
At screening	8·64 (0·46)	8·87 (0·87)	8·58 (1·06)	8·94 (0·41)
At follow up	8·89 (0·25)	8·97 (0·42)	8·76 (0·19)	8·93 (0·45)
Baseline	8·48 (0·78)	8·72 (0·75)	8·45 (1·17)	9·10 (0·71)
Change from baseline:				
After 2·5 weeks	0·32 (1·04)	−0·36 (0·60)	−0·52 (0·35)	−0·51 (0·89)
After 5 weeks	0·67 (1·43)	−0·90 (0·85)	−0·88 (0·68)	−0·91 (0·47)
After 7·5 weeks	0·34 (1·25)	−1·01 (0·95)	−0·55 (0·97)	−0·70 (0·95)
After 10 weeks	0·35 (1·35)	−1·16 (0·73)	−0·36 (0·45)*	−0·91 (1·14)

*n = 7.
Conversion: SI to traditional units—Cholesterol: 1 mmol/l ≈ 39 mg/100 ml.

A positive correlation was found between coffee consumption and high serum lipid concentrations.[15] They observed fluctuations of serum cholesterol levels with different levels of coffee consumption. It was suggested that the brewing methods could be an important contributing factor to the observed increase in the serum total cholesterol levels in the study participants. Cholesterol concentrations dropped in all subjects who were told to stop drinking coffee for the first of the five weeks. Those that abstained from coffee drinking for a full duration of the study (which was 10 weeks long), noticed a continuous decline in serum lipid concentrations while the subjects that went back to drinking boiled coffee after 5week duration, an incline in the cholesterol concentrations was observed. However, in those that drank filtered coffee, the serum cholesterol levels remained the unchanged after recommencing to the consumption of coffee.[15] During the experimental period, the participants were encouraged to eat their normal diets. Participants were chosen specifically because they showed no improvements in their cholesterol levels for 3 years since the 1st Tromsø study survey, and could not reduce their cholesterol levels through diet. However, the experiment failed to give a clear deduction of to what extent coffee consumption may have been responsible for the increase in cholesterol levels related to the brewing methods.[15]

Here after, studies aimed to take a closer look at the effect coffee consumption had on cardiovascular disease risks. It was observed that coffee consumption

was positively associated with higher total cholesterol and triglycerides in both genders. In the study by Dag S. Thelle et al, "Doses Coffee raise serum cholesterol?", based on the Tromsø study 2, it was found that the consumption of coffee was positively associated with higher levels of total cholesterol in both gender groups, and an inverse relation to HDL levels was observed in women.[16] The relation between coffee and cholesterol levels in the blood remained statistically significant ($P<0.0001$ in a covariance analysis),even after adjusting for age, body mass index (BMI), physical activity, smoking, and alcohol consumption. The total cholesterol level was 5.56±0.05 mmol per liter in men drinking less than one cup of coffee per day, and 6.23±0.03 mmol per liter in participants who consumed more than nine cups per day. For women total cholesterol level was 5.32±0.05 mmol per liter in those that drank less than one cup of coffee per day, and 5.92±0.04 mmol per liter in those that drank more than nice cups of coffee per day. The conclusion made from this study was that coffee consumption was the major contributing factor for the variations in the levels of total cholesterol.[16] However, in this study, they failed to investigate the brewing methods used for coffee among the study participants.

Due to the popularity of coffee in all of Scandinavia, the effect of coffee on cardiovascular disease risk began to be questioned in more frequency. It was not long after the 2nd Tromsø study, researchers considered the effects of coffee in relation to the brewing methods. This took effect in Tromsø study 3, where brewing methods of coffee was investigated. Kaare Bønaa, Egil Arnesen, Dag Steinar Thelle, and Olav Helge Førde, in their study "Coffee and Cholesterol: Is it

all in the brewing," found that boiled coffee consumption was as important as age of the participants, in determining the serum cholesterol concentrations. Boiled coffee consumption was associated with high cholesterol levels.[17]

It wouldn't be long until researchers would come up with the question of whether coffee drinkers in general had an unhealthy life style to begin with. In one such study, it was found that coffee drinkers (mainly women and younger men) were less likely to exercise, and 75% of the population that drank more than 8 cups of coffee were also smokers. It was indicated that among the smokers, high coffee consumption was associated with a higher cigarette consumption, and a negative correlation between fruit and vegetable consumption with coffee consumption was visible.[18]

In the Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) study, conducted in Northern Sweden, B.Lindahl et al., reported that participants who drank boiled coffee had significantly higher serum cholesterol levels than those who drank filtered coffee. It was further reported that those who consumed filtered coffee also consumed more fat in their diet. However, after adjusting for those who consumed more fat in the filtered coffee group, using statistical analysis, a linear correlation between boiled coffee consumption and serum cholesterol levels was observed. They ultimately concluded that boiled coffee was responsible for increased serum cholesterol levels.[37]

Based on the evidences of from the Tromsø and the MONICA studies, regarding coffee consumption and cardiovascular health, it was reasonable to question the association between coffee intake and the risk of CVD, and lifestyle in general. However, it has been evident from some of these studies, that the brewing methods were an important influence to the outcomes, and therefore needed to be investigated upon. When investigating the effects of coffee on the cardiovascular disease risk, it has been indicated that coffee drinkers are also heavy smokers and probably have an unhealthy life style, and coffee alone may not be the contributing factor. There may also be a correlation between the preference of brewing methods used, daily lifestyle choices, and socioeconomic conditions that contributes to these risks.

7.1 Mechanism of action of caffeine in the human body

Caffeine is also known as 1,3,7-Trimethyl-2,6-dioxopurine or 1,3,7-trimethylxanthine[23]. It is the most widely consumed central-nervous-system stimulant and is naturally found in coffee, tea, cocoa among others.[20, 21,22] Coffee contains 71-220mg/150ml of caffeine.[24] As mentioned before coffee arabica (*Coffea arabica*) and robusta (*Coffea canephora*) are the two main commercial coffee types drank around the world. Caffeine content in coffee arabica ranges from 71 to 120 mg per cup (in a standard 150ml cup), and robusta the caffeine contant is a little higher between 131 to 220 mg/150 ml.[23]

Caffeine acts as a central nervous system stimulant, relaxes smooth muscle, stimulates cardiac muscle, and stimulates diuresis. On a cellular action level, caffeine has been observed to inhibit cyclic nucleotide phosphodiesterases, antagonise adenosine receptors, and modulate intracellular calcium handling.[24]

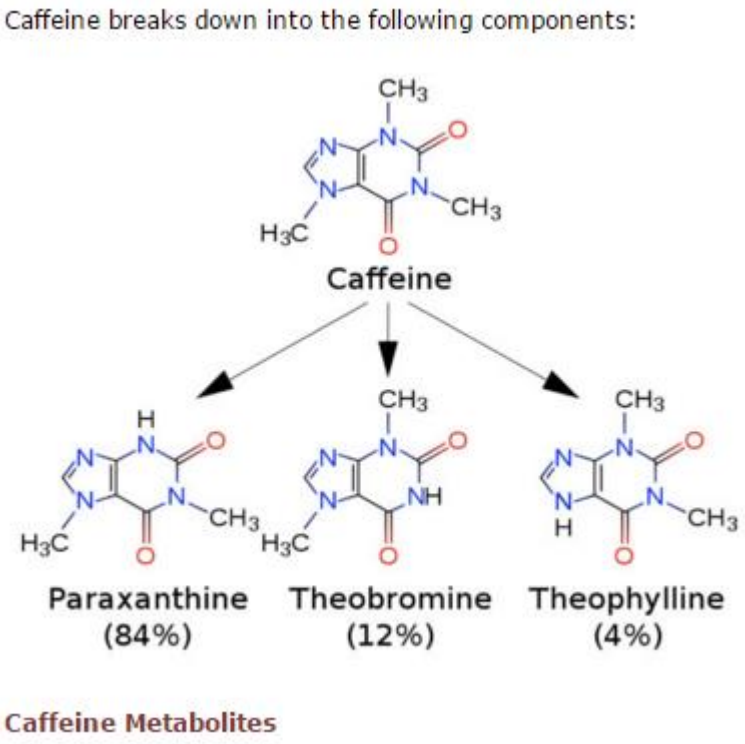


Fig 4: Caffeine breaks down into paraxantine, theobromine and theophylline during metabolism. *(Effects of caffeine. Cited on 2016 Mar 11. Available from: http://udel.edu/~danikoll/metabolism.html).*

Within the human body, caffeine is metabolized into Paraxanthine, Theobromine, and Theophylline. There are more than 25 metabolites of coffee, three main already mentioned above.[25] In humans approximately 3% of

ingested caffeine is excreted, while the remaining 97% is completely metabolized.[26] The main route of metabolism in humans takes place in the liver, and is initiated by the enzyme CYP1A2. The final product of caffeine metabolism yields paraxanthine as the main product and makes up for approximately 80% of the total by-product.

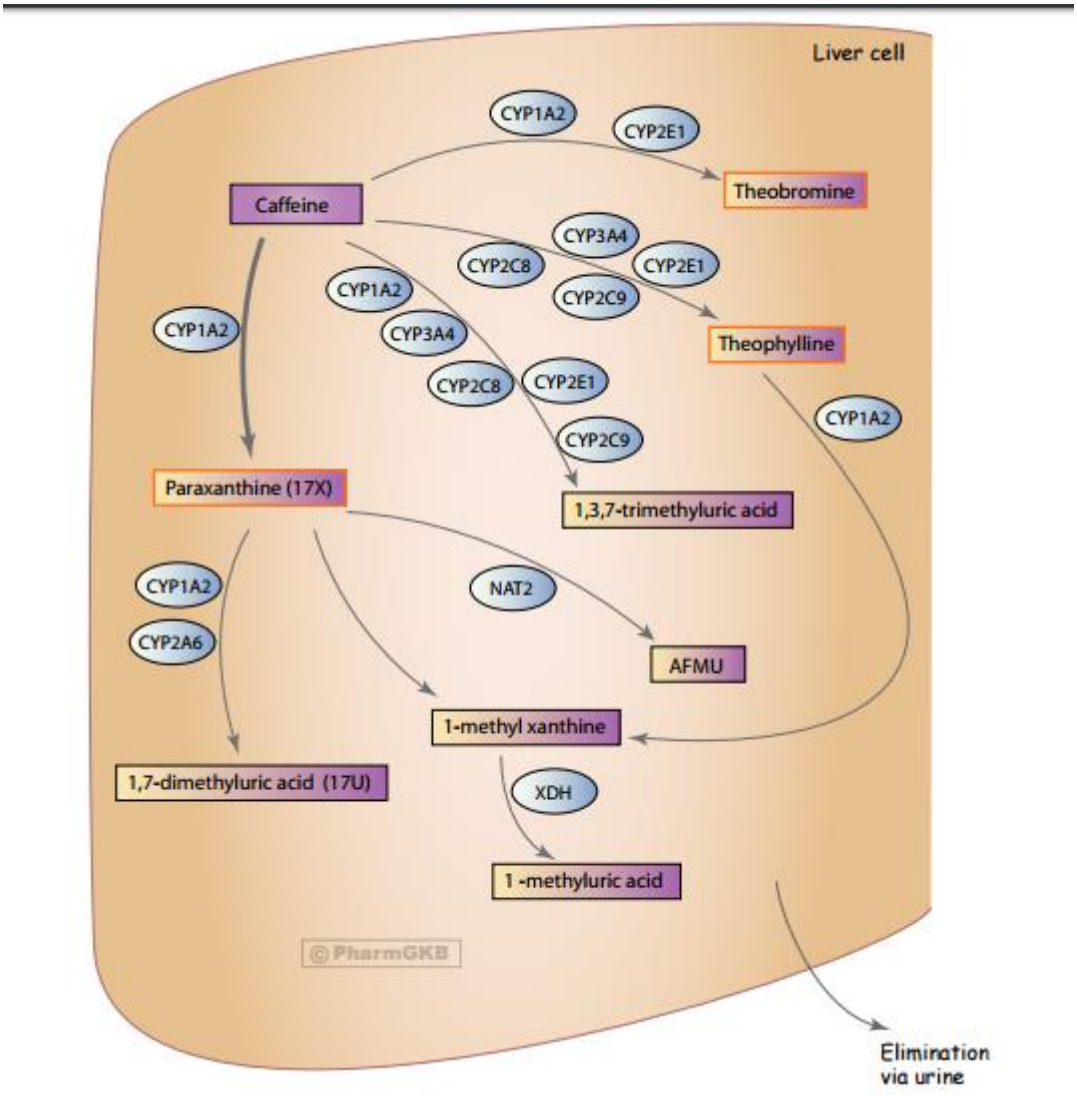


Fig 5: Caffeine pathway during metabolism. *(Thorn Caroline F, Aklillu Eleni, McDonagh Ellen M, Klein Teri E, Altman Russ B. "PharmGKB summary: caffeine pathway"Pharmacogenetics and genomics (2012). Cited on 2016 Apr 14. Copy rights by PharmGKB 2001-2016. All rights received and granted to use from the*

purpose of the thesis by PharmGKB and Stanford University, 2016 Jun 14. Available from: https://www.pharmgkb.org/pathway/PA165884757).

There are 5 main metabolic pathways for the metabolism of caffeine in humans. Out of the 5, 3 of them involve demethylization of N-3 to form Paraxanthine, N-1 to form Theobromine, and N-7 to form Theophylline again. In the liver, the hepatic cytochrome P-450 (CYP), an isoenzyme, metabolizes the caffeine through the process of demethylization into paraxanthine (85%), theobromine (12%), and theophylline (4%). The 4th pathway involves C-8 hydroxylation to form 1,3,7-trimethyluric acid. The remaining caffeine that is not degraded, is eliminated via the renal system.[18,25]

The xanthine derivatives act as an adenosine receptor antagonist,inhibit cyclic nucleotide phosphodiesterase activity, mobilize calcium, and inhibit monoamine oxidase activity.[25,27,28] Caffeine is responsible for antagonising the adenosine receptor in the brain, kidney, cardiovascular system, respiratory system, gastrointestinal system, and the adipose tissues.[25,29]

7.2 How Cafestol and kahweol raise serum cholesterol

Cafestol and Kahweol are fat-soluble compounds called diterpenes found in coffee, responsible for raising serum cholesterol levels in humans.[30,31,32,33,34,36]

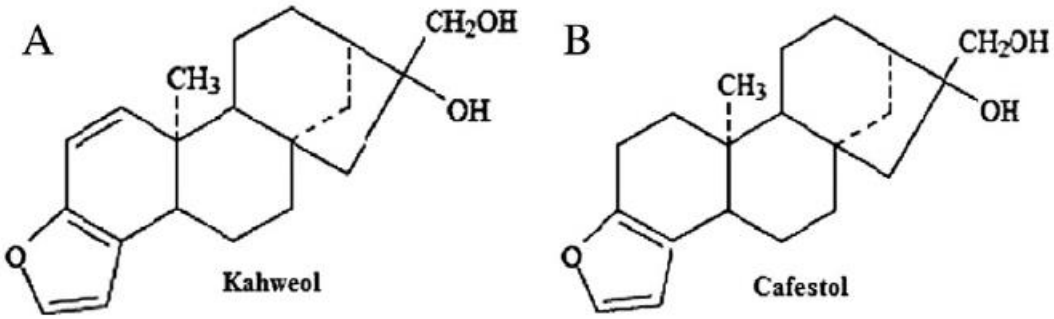


Fig6: Molecular structure of Kaweol and Cafestol. (*Validation of near-infrared spectroscopy for the quantification of cafestol and kahweol in green coffee - Scientific Figure on ResearchGate. (cited on 2016 Mar 18). Available from: https://www.researchgate.net/273544323_fig1_Fig-1-Structural-formulas-of-cafestol-A-and-kahweol-B).*

Naturally, the concentration of cafestol and kahweol vary depending on the coffee species. Coffee Arabica contains more of both the diterpenes than Robusta. Coffee Robusta contains only half of the content of cafestol as coffee Arabica, and almost no kahweol.[30]

Experiments with the two diterpens have proved that cafestol is responsible for raising serum cholesterol levels more potently, while kahweol does not raise serum cholesterol levels as much.[30,31,32,33] Experiments involving brewing methods have provided evidence that cafestol and kahweol do not pass through the paper filter.[17,35,36] This explains why Scandinavian boiled coffee, Turkish coffee, espresso coffee, and French press (cafetière) coffee, contain higher

levels of cafestol and kahweol compared to filtered coffee, percolated coffee or instant coffee.

Arild C. Rustan et al. concluded based on their findings, that coffee diterpenes cafestol and kahweol decreased the binding, uptake, and degradation of LDL in human hepatoma cells. Their findings suggested that the cholesterol-raising effects of cafestol and kahweol involved the downregulation of the hepatic LDL receptors by posttranscriptional mechanisms.[31]

Sabine M. Post et al., in their study using rat hepatocytes, were able to determine how cafestol and kahweol increased serum cholesterol levels in humans.[32] It was found that cafestol suppressed bile acid synthesis by a direct inhibitory effect on cholesterol 7α-hydroxylase activity and by downregulation of mRNA of cholesterol 7α-hydroxylase and sterol 27-hydroxylase. LDL-receptor, HMG-CoA reductase, and HMG-CoA synthase mRNA levels were downregulated simultaneously with the decrease in bile synthesis. Cafestol reduced bile acid synthesis more potently than the mixture of cafestol and kahweol at the same concentration. The down regulation of LDL-receptors in hepatocytes and the decrease of bile acid synthesis after the consumption of boiled coffee, was presumed to have led to the increase of serum cholesterol levels in the blood.

The study by Marie-Louise Ricketts and colleagues, was an in vitro experimentation, and an in vivo study on APOE3Leiden transgenic mice, which led to the conclusion that mechanism by which cafestol rose serum cholesterol

in humans involved a gene called fibroblast growth factor 15 or FGF 15. Cafestol activated farnesoid X receptor (FXR), induced FGF15, which reduced the effects of three liver genes that regulate cholesterol levels.[33] The mechanism of how cafestol raises serum cholesterol in humans is illustrated in figure 7:

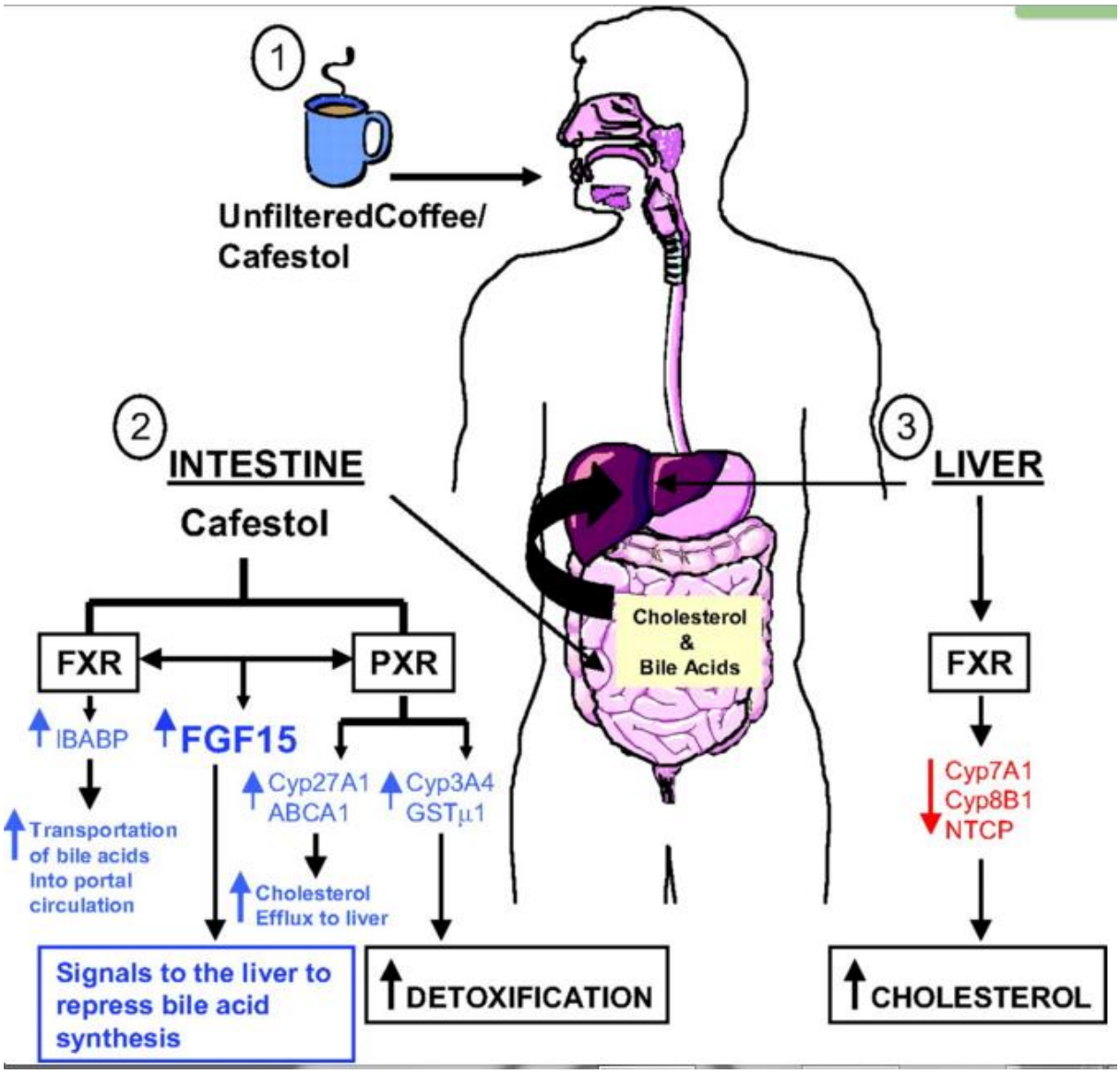


Fig 7: Metabolic involvement of coffee's cholesterol in increasing cholesterol levels after ingestion in the human body. (Cited on 2016 Mar 18. Available from: http://press.endocrine.org/na101/home/literatum/publisher/endo/journals/co

ntent/mend/2007/mend.2007.21.issue-7/me.2007-0133/production/images/large/zmg0070740480009.jpeg).

"1) Coffee containing cafestol is ingested and passed along into the stomach and small intestine. 2) In the small intestine cafestol activates the nuclear hormone receptors, farnesoid X receptor (FXR) and pregnane X receptor (PXR). IBABP is induced by cafestol in a FXR-dependent manner, further increasing the transportation of bile acids into the portal circulation. Upon activation of PXR, cafestol induces the expression of Cyp27A1 and ABCA1, resulting in an increase in the efflux of cholesterol into the portal circulation. Cafestol also induces Cyp3A11 and GSTμ1 gene expression via PXR, leading to an increase in detoxification. Cafestol acts via both FXR and PXR to induce FGF15, which signals to the liver to repress bile acid synthesis. 3) In the liver, Cyp7A1, Cyp8B1, and NTCP expression is repressed via FXR, thereby reducing the synthesis of bile acids. The direct regulation of such FXR and PXR target genes in the intestine combines with indirect effects in the liver to contribute to the cholesterol-raising effect of cafestol in humans."[33]

In another experiment, Bente Halvorsen et al, explored the effects of a coffee lipid (cafestol) on cholesterol metabolism in human skin fibroblasts. They concluded that cafestol decreased the binding, uptake, and degradation of LDL in human skin fibroblasts (HSF), decreased the LDL receptor protein level, reduced cholesterol synthesis and increased the cholesterol esterification. Their findings suggested that the mechanisms responsible for the cholesterol-raising

effects of cafestol involve a post-transcriptional down-regulation of the LDL receptor.[34]

7.3 Homocysteine; what is it; how is it involved in the metabolic process; and how is it related to cardiovascular diseases and coffee?

Homocysteine is an amino acid in the body which is formed as an intermediate product during the metabolism of methionine into cysteine.[48,49,50]

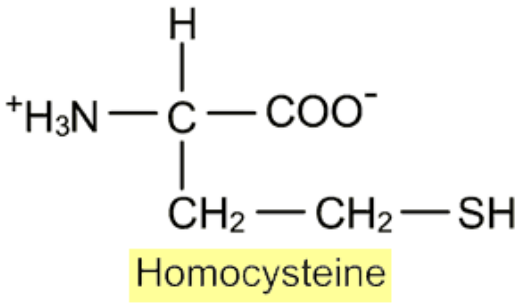


Fig8: Molecular structure of homocysteine. (Internet source. Cited on 2016 May 14. Available from: http://www.wiley.com/legacy/college/boyer/0470003790/cutting_edge/homocysteine/homocysteine.htm).

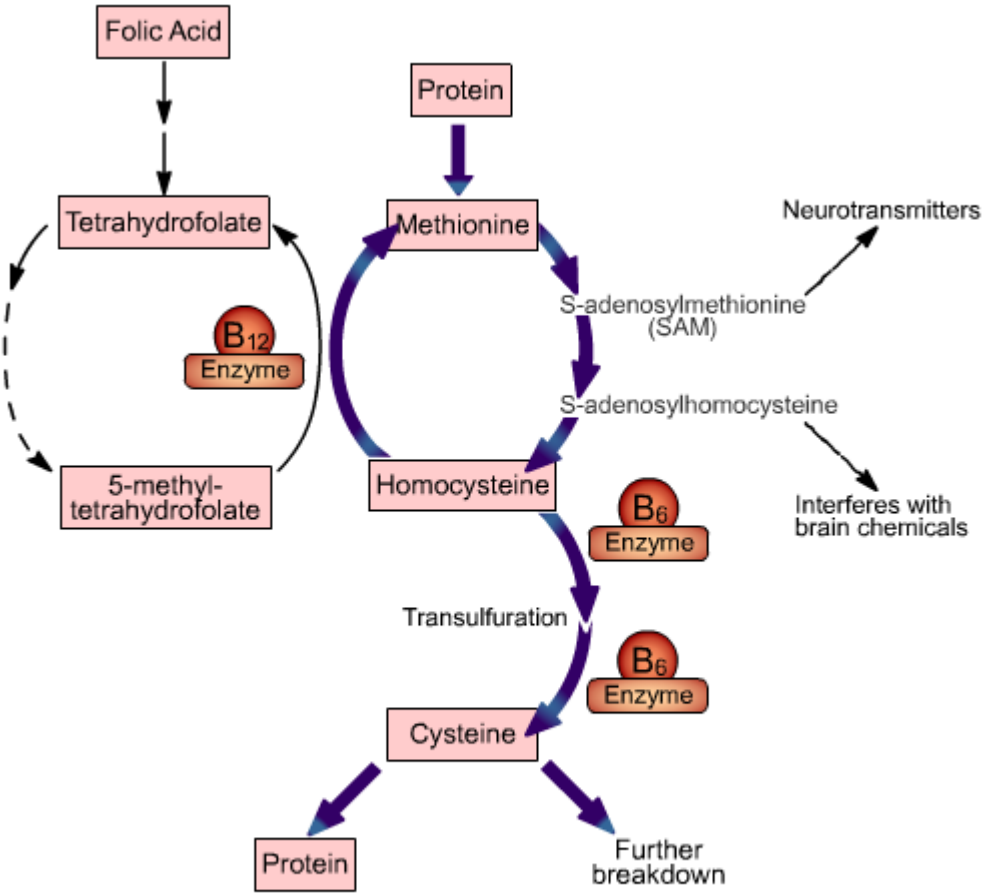
Homocysteine was first discovered by Butz and du Vigneaud in 1932. They heated methionine, an amino acid, in sulfuric acid, which changed the methionine into a new amino acid with similar chemical properties to that of cysteine.[48]

Homocysteine is not found in the diet, but instead is produced in the body during the metabolic process of methionine. In recent years, they have discovered that the B group of vitamins, vitamin B_6, B_{12} and B_8 (folic acid), play a part in the homocysteine production cycle.[48,50]

It was discovered that there were inborn errors in some individuals, perhaps a gene with an abnormal mutation during the metabolism of methionine leading to the accumulation of homocysteine within the body which lead to a rare disease called homocystinuria. This was believed to have led to some people being at a at greater risk of CVD than others, regardless of age or cholesterol level as a factor.[51] During the metabolism of methionine, homocysteine was identified as an intermediate within the cycle, and the enzymes within the cycle required the group B vitamins to act as cofactors to enable proper functionality.[50,51] However the genetic defects in vitamin cofactors, which include vitamin B6, B12, and folate, and nutritional deficiency of B12 and folate, was discovered to have led to abnormal homocysteine accumulation within the body.[51]

During the metabolism of proteins, the protein molecule is broken down into two amino acids. One of these two acids is methionine which breaks down to form homocysteine. Once formed homocysteine is removed by a process called remethylation, which involves folic acid and vitamin B12 to act as cofactors in the reaction. Another way homocysteine can be removed from the body is

through the transformation of homocysteine to cysteine through transulfuration.[50,52]



▲ Homocysteine's place in metabolism

Fig 9: Homosysteine place during metabolism of proteins. *(Internet source. Cited on 2016 Jun 6. Available from: http://www.wiley.com/college/boyer/0470003790/cutting_edge/homocysteine/homocysteine.htm).*

Caffeine has been suggested to be partly responsible as the component in coffee, and not coffee itself, as the factor that influences the rise in homocysteine levels in the blood after consumption.[71] Chlorogenic acid is the

other component found in coffee, an ester of caffeic acid, that has been suggested in raising homocysteine levels after consumption.[56]

The research on the relation between coffee's role in elevation of homocysteine levels in the blood, is still sparse. There is more research being done on homocysteine independent of coffee.

Previously, high levels of homocysteine in the blood had been linked to cardiovascular diseases. It was believed that elevated total homocysteine increment elevated the coronary artery disease (CAD) risk, and folic acid intake, as a preventive measure, was believed to help in the reduction of total homocysteine levels.[69]

In Norway, the Hordaland Homocysteine Study was one of the first to find a rather strong association of homocysteine in serum with established CVD risk factors, suggesting that homocysteine could perhaps have a causal role in the development of CVD.[47] The finding was regarded as very important, particularly in light of new interventions for the prevention of CVD.

Arnesen E et al. examined the association between homocysteine and coronary heart disease (CHD) among the participants in the third Tromsø health study.[68] They found that serum total homocysteine level was an independent predictor of the risk of myocardial infarction in the general population, but the predictive value of homocysteine was lower than that for total cholesterol, smoking and

HDL cholesterol. Threshold levels above which serum homocysteine was associated with CHD events was not found in the study.

In a small clinical trial Esposito F et al., tried to determined the effects of Italian-style coffee consumption on the plasma concentration of glutathione and homocysteine. They were unable to detect any significant rise in homocysteine levels in the blood, from drinking five cups of coffee per day for one week.[70] Although the trial was short and small, the amount of coffee consumed seemed to not have affect on the homocysteine levels in the blood of the participants. In some of these studies, the casual relationship of the quantity of coffee consumed to that of the increase of the level of homocysteine, when observed, seem not to have been taken into consideration about whether drinking coffee only temporary increases homocysteine for a duration, or whether it effects levles in a continuous consistent manner. After these studies, question still remains as to whether healthy subjects with increased homocyteine levels affects the cardiovasucular system in the same way as it would in prediagnosed patients with an underlying health condition or oxidative diseases.

Homocysteine is not a product that can be consumed to increase its levels in the body, unlike sugar or fat. Since homocysteine is produced by the body during the metabolism of the amino acid methionine, it could be related to other factors that increase its presence in the blood such as during oxidative stress, which may act as a secondary cause to the risks of CVD.[76,77]

Homocyteine is not regarded as an 'independent risk' factor for CVD. In more recent years it has been acknowledged as one among many of the biomarkers, which are consequences of other risk factors and early stage of CVD.[66] In theory, homocysteine could affect the cardiovascular system through its atherosclerotic properties on the coronary blood vessels, but it has not been found to have the role as a cardiovascular disease risk factor on its own.[66] Furthermore, there is no support for the hypothesis that interventions to lower blood levels of homocysteine, have any protective effect on cardiovascular health, or reduce risk of cardiovascular disease.[67]

8. Results: Recent Research on Coffee and cardiovascular health

Analysis of the adverse effects of coffee on cardiovascular health from epidemiological studies conducted in the 70's and 80's, researchers were able to claim that method of brewing coffee was important to these risks. Boiled coffee, led to the increase in serum cholesterol, however filtered coffee or instant coffee seemed not to be harmful in the same way. In fact, the opposite seemed to have led to be true. Coffee consumers seemed to benefit from drinking filtered coffee,[45] however some have argued that boiled coffee despite its cholesterol raising properties may still be beneficial to diseases causing oxidative injuries too.[63] This is because coffee contains other components that

that exhibit antioxidant activities like heterocyclic compounds that seems beneficial to cardiovascular health .[63]

By the 90's, some researchers conducted studies that led to the conclusion that coffee may have affected mortality due to coronary heart diseases 'over and above' its effect on raising cholesterol concentrations.[41] However in such studies, brewing methods were not taken into account. Other researchers specifically studying the effects of Scandinavian type boiled coffee were able to conclude that boiled coffee contained lipids that were able to raise the blood cholesterol levels in the body.[42,44]

Boiled coffee is a popular beverage in rural areas especially in the north, both in Norway as well as in Sweden, and has since been recognised to raise blood lipids, which is a risk factor for acute myocardial infractions (MI).

In a more recent nested case-referent study led by a team of Swedish researchers in northern Sweden, L.M Nilsson et al investigated the relation between consumption of filtered and boiled coffee to the risk of first MI. They were able to find a positive association between risk of first myocardial infraction (MI) and consumption of filtered coffee among the male population, and similar risks for women who consumed boiled coffee.[38] The study subjects included 303 men and 72 women. They took 1293 matched referents from the population-based Northern Sweden Health and Disease Study. A food frequency questionnaire was used to ask about coffee consumption, and risk estimates

were calculated by conditional logistic regression. They found a positive association between consumption of filtered coffee and MI risk in men [odds ratio for consumption ≥4 times/day versus ≤1 time/day 1.73 (95% CI 1.05–2.84)]. Similar associations was observed in women who consumed boiled coffee instead of filtered coffee, [odds ratio 2.51 (95% CI 1.08–5.86)]. However these results were no longer statistically significant for women after they had adjusted for smoking, postsecondary education, hypertension, and sedentary lifestyle. They were able to conclude that filtered coffee was positively associated with risks of first MI in men, but was statistically insignificant for women who drank boiled coffee.[38]

In another similar study from Sweden, N.Hammar et al evaluated how much of an influence filtered and boiled coffee consumption had on the incidence of first nonfatal myocardial infarctions. The study was obtained from two population-based case–control studies, one from Stockholm and the other from Västernorrland. The study subjects consisted of 45-70 year olds without a previous myocardial infraction, and were living in Stockholm between the years 1992-93 men, or women between 1992-94 (the Stockholm Heart Epidemiology Programme - SHEEP), or 45-65 year olds living in Västernorrland between 1993-1994 (Västernorrland Heart Epidemiology Programme - VHEEP). New cases of first myocardial infarction in SHEEP and VHEEP were identified from coronary and intensive care units at the department of internal medicine at the emergency hospitals within the two counties, hospital discharge registers, and death certificates from the Swedish National Cause of Death Register. The

diagnostic criteria for myocardial infarction were based on typical symptoms, changes in blood levels of the enzymes CK and/or LD, typical ECG changes and autopsy findings stating signs of recent myocardial necrosis. Each case was matched with a control selected from the study base, together with disease incidence, age, sex and place of resisdency of the case. Cases of first nonfatal myocardial infractions were identified as those who survived more than 28 days after disease onset. Depending on the type and quntity of coffee consumed, the participants were divided into 13 groups. Statistical analysis was performed, and the odds ratios were estimated separately for men and women through logistic regression with adjustment for age and hospital catchment area. There were 1643 nonfatal cases of first myocardial infarctions, 1171 were men and 472 women. Most of the participants consumed filtered coffee (95%). The male participants' who reported coffee consumption of >= 7–9 dL day^{-1} showed an increased incidence of first myocardial infarction, independent of the brewing methods used. Adjustments were made for smoking since smokers tended to drink more coffee overall. Interestingly enough, "men who did not drink coffee showed a tendency towards a slightly increased incidence of myocardial infarction compared with consumers of 3 dL day^{-1} of filtered coffee or less."[39] There were no clear association between consumption of filtered coffee and myocardial infraction among women, however consumption of boiled coffee in women tended to give odds ratios suggesting a possibly increased incidence (RR was 4.97 at 95% CI in those that drank >9 cups of boiled coffee per day). They were able to conclude that consumption of boiled coffee may result in an increased incidence of first nonfatal myocardial infarction, however

consumption of more than 7 dL of filtered coffee per day would still increase the incidence in men.[39]

Table 7: SHEEP VHEEP Study: Relative risk of first nonfatal myocardial infarction for subjects with consumption of boiled coffee compared with subjects with consumption of filtered coffee. (cited on 2016 June 24).

	Men				Women			
Coffee (dL day^{-1})	RR[a]	95% CI	RR[b]	95% CI	RR[a]	95% CI	RR[b]	95% CI
≤3	1.48	0.85–2.55	1.45	0.83–2.52	1.07	0.45–2.53	1.01	0.42–2.45
4–6	1.29	0.84–1.98	1.21	0.78–1.87	2.00	1.07–3.73	1.95	1.02–3.73
7–9	2.21	1.28–3.83	2.07	1.18–3.61	1.56	0.68–3.59	1.66	0.69–3.98
>9	1.26	0.67–2.38	1.14	0.60–2.16	3.68	0.40–33.71	3.47	0.37–32.47
All[c]			1.41	1.07–1.85			1.63	1.04–2.56

a Relative risk adjusted for age and hospital catchment area.
b Relative risk adjusted for age, hospital catchment area and smoking.
c Adjusted for age, hospital catchment area, smoking and amount coffee consumed.

Trine Ranheim and Bente Halvorsen in their review[63], mention that moderate daily filtered coffee consumption was not associated with any adverse effects on cardiovascular outcomes. They were able to provide sufficient data to support their theory on anioxidant activities, which seemed to have an inverse assosiation with the risk of type 2 diabetes mellitus as well. Ranheim and Halvorsen used Chuyen NV's work on 'Maillard reaction and food processing', as a source to indicate that melanoidins exhibited strong antioxidant activity, while they mentioned that Borrelli RC et al, Shizuuchi S and Hayase F were able to show that melanoids also inhibited lipid oxidation. Ranheim and Halvorsen mention Azam S et al. work on anioxidents present in coffee in their paper 'antioxidant and prooxidant properties of caffeine, theobromine and xanthine,'

to acknowledge that, “When coffee drinks were examined for antioxidant properties, measured as DNA-protection through quenching of hydroxyl radical generating systems, caffeine and its metabolites, theobromine and xanthine, appeared to possess strong DNA-protective effects.” They were able to put forth several other studies that were able to make a connection to coffees antioxidant properties to make the argument that coffee could possibly prevent diseases by antioxidative effects. One such study they put forth was a study by Yukawa et al. on healthy men, who were asked to drink 24g of coffee per day for one week, to observe how coffee affected LDL oxidative susceptibility. They mention that Yukawa et al. observed that coffee consumption had significantly reduced the susceptibility of LDL oxidation, which was similar to what another researcher, Richelle et al., had also studied on the relative antioxidant activity of coffee, tea, and cocoa using an in vitro LDL oxidation model. They were able to claim that the beverages contained polyphenols with high antioxidant activities, especially in coffee. Ranheim and Halvorsen claimed that from “these studies it could be indicated that regular coffee ingestion may favourably affect cardiovascular risk status by modestly reducing LDL oxidation susceptibility.” They were able to conclude that coffee, depending on the brewing methods, raised cholesterol levels due to reduced clearance of cholesterol-rich lipoproteins together with increased secretion of triacylglycerol-enriched lipoproteins (VLDL), but its antioxidant properties were still beneficial to the health of individuals in preventing diseases that could cause oxidative injuries.[63]

In eastern Finland, P Kleemola et al. studied the the relation of coffee drinking with fatal and nonfatal coronary heart disease (CHD) and all-cause mortality.[40] The prospective cohort study included 20 179 participents that were selected randomly. The participants included both men and women between the ages of 30-59 years. The baseline examination included habitual coffee drinking, behavior, risk factors of CHD, and medical history. The study participents were followed for 10 years using the national hospital discharge and death registers. By using the analyses of variance, they were able to test the differences in the mean values of cardiovascular risk factors, and the Cox proportional hazards model was used to preform the multivariate analyses. They used the same model to calculate the estimate of the relative risks and their 95% confidence intervals. Coffee drinking was used as a dummy variable at 4 drinking levels. Moderate drinking level was used as the baseline level and was defined as, 1 to 3 cups of coffee intake daily. The model also included the known cardiovascular risk factors such as smoking, serum cholesterol level, BP, and history of MI. Adjustments for age was made and the SAS statistical program was used to perform the statistical analyses. It was discovered that men drank heavier amounts of coffee than women, and older participents were more likely to drink more coffee than their younger counterparts. They found that men who drank more coffee were more likely to be smokers and have higher serum cholesterol levels aswell. Nondrinkers were found to have slightly lower BMI and systolic blood pressure than coffee drinkers. Light coffee drinking, defined as 1-3 cups of coffee per day, had slightly higher diastolic bloodpressure than noncoffee drinkers, moderate coffee drinkers (4-7 cups daily), or even heavy coffee

drinkers (>7 cups daily). The prevalance of coffee drinking to that of higher serum cholesterol levels and smoking in women, were directly propotional. Women who were heavrier coffee drinkers were also more likely to be smokers and have higher serum cholesterol levels. A tendency for increase in blood pressure increased also with increase in coffee intake until >7 cups. The BMI in women was lowest in people who were moderate coffee drinkers, but increased in the higher coumption group of coffee drinkers. They found that the risk of nonfatal MI in men was not associated with coffee drinking. Risk ratios of CHD and total mortality was higher among noncoffee drinkers than coffee drinkers after adjusting for smoking, serum cholesterol , BP, and previous MI. In women, no significant association between coffee drinking and CHD mortality was found. In their multivariate model, they found that the risk ratio of nonfatal MI in nondrinkers was higher than in coffee drinkers. With increase in coffee intake, a decrease in total mortality was observed with women, and was statistically significant (P=.07, multivariate model). The authors claimed that no association was found between coffee drinking and the risk of nonfatal MI. Noncoffee drinkers were more likely to die due to a coronary heart event than those who drank coffee. The association between coffee drinking and total mortality in men, was the lowest in the group who drank moderate amounts of coffee. In women, coffee drinking was inversely propotional to total mortality, that is, with increasing coffee drinking, incidence in total mortality derceased. They were able to prove their theory that heavy coffee drinking was not a risk factor for CHD.[40]

Australian researchers OB Tofler et al. mention that depending on the study design, the role of coffee consumption in the onset of myocardial infarction varies.[43] Him and others[40] have reported that reviews of published reports show that cohort data suggests very little risk of coronary heart disease among habitual coffee drinkers, but case-control data suggests that risks for CVD increases in people that drink more than five cups of coffee per day. To test the theory, Tofler et al. conducted an experiment in the coronary care unit at the Royal Perth Hospital in Australia. They tired to obain information on the lifestyle and habits of the study participants, including coffee consumption, of those who had been admitted with chest pain. Questionnaires was given to the patients and details were self reported. A similar questionnaire was given to people at the cardiology out patient clinic. They found that among the 182 patients with myocardial infarction or unstable angina, and 185 patients with chronic stable coronary heart disease, from the Cardiology Outpatient Clinic, was that people with acute coronary syndromes drank more than five cups of coffee per day (18 vs 7.5%; *P* = 0.003). After adjusting for age, smoking status, hypertension, dyslipidaemia and diabetes, the odds ratio was found to be 2.51 (95% CI 1.43,4.43; P = 0.021). They came to the conclusion that according to their case-control study, there was evidence of an increase in the risk of myocardial infraction or unstable angina among those who drank more than 5 cups of coffee per day.[43]

The reason why the results from case control studies might differ to that of cohort studies could be due to poor control for confounding among case control

studies.[40] It has been suggested that the discrepancy between the findings from cohort and case-control studies may be explained if coffee was to act as a triggering event in people with chronic CHD which led to MI, instead of it being a risk factor for CHD.[40]

In one RCT, Urgert et al., experimented on twenty-six healthy volunteers (10 men and 16 women) between the ages of 18–53 years. They were asked to consume 1 L/d of paper-filtered coffee brewed with 70 g regular ground beans or no coffee for 4 weeks, each in a randomized crossover design.[46] The aim of the experiment was to find out whether heavy filtered coffee drinking is related to high homocysteine concentrations. They reported that duing the coffee drinking period of the experiment, heavy coffee drinking raised the fasting plasma concentration of total homocysteine in 24 of 26 subjects. The mean homocysteine level rose by 1.5 ± 1.5 mol/L (95% CI: 0.9, 2.1 mol/L) after 3–4 wk of coffee drinking. The outcome was not affected by the treatment order. The mean increase was 1.8 ± 2.2 mol/L in subjects who were switched from no coffee to coffee (n = 15) and 1.3 ± 0.8 mol/L in those switched from coffee to no coffee (n = 11). Coffee drinking increased plasma total homocysteine by 22 ± 23% after 2 weeks and by 18 ± 16% after 3–4 weeks (range: 2% to 67%). They noticed that there was no significant difference in the circulationg B vitamins during the treatment period. From this study, drinking large quantities of paper-filtered coffee raised fasting plasma concentrations of total homocysteine in healthy individuals over a period of time.[46] In an older population based study conducted in Norway, Nygård et al. were able to claim that elevated plasma

total homocysteine level was associated with major components of the cardiovascular risk profile, including gender, age, smoking habits, cholesterol level, and level of physical activity.[47]

Homocysteine has not been classified as a cardiovascular disease risk factor under stratification guidelines, this is because homocyteine is not regarded as an independent risk factor for CVD.[66] Ganguly and Alam, from their review, mention that from the work of other authors on homocysteine research, have indicated that there is some relationship towards moderately elevated homocysteine levels and the risk of CVD, but this could be due to a secondary consequence leading to atherosclerosis, which could increase risks of a cardiovascular events in patients.[66] However, there is still not enough evidence on homocysteine to label it as an 'independent risk factor'. Homocysteine lowering interventions do not seem to be effective in preventing CVDs, as it was thought to have been in theory.[67] This fact could probably be treated as 'evidence' that homocysteine is in fact not responsible for increasing risks of CVD, but could be a factor that is present as a 'produce' from other metabolic disorders, that could could cause heart disease as a secondary condition.

9. Combined effect of smoking and coffee on the cardiovascular system.

Studies have shown that caffeine intake in smokers is greater than that of none smokers.[57,58] It has been suggested that the reason behind heavy coffee drinking in smokers is because nicotine present in tobacco allows the body to eliminate caffeine at a greater pace. Therefore, in order to get the effects of caffeine in the presence of nicotine in the body, more coffee or any caffeinated beverage needs to be consumed.[59]

Smoking increases platelet aggregation resulting in a hypercoagulable state, raised fibrinogen levels and polycythaemia. Smoking also disrupts the inner vascular lining, causing a chroming inflammatory state resulting in dyslipidaemia.[65]

According to some researches' the combined effect of habitual coffee drinking and smoking could significantly place an individual at a greater risk of CVD.[60] In an earlier study they found that smokers that drank more than 5 cups of coffee a day had higher levels of LDL cholesterol, and lower levels of HDL than those that did not smoke or drank little to no coffee. However, they were unable to firmly conclude that coffee consumption and smoking resulted in high LDL levels and lower HDL levels in the body. Instead they concluded that "there may be an interactive effect of smoking and consumption of coffee on lipids and lipoproteins, at least within that study population."[61]

On the bases of the separate effects of consumption of coffee and smoking, researches have put forward as to a combination of smoking and coffee drinking poses a greater risk for CVDs. The reason is that smoking and caffeine separately increase arterial stiffness, and there by lead to systemic hypertension. A combination of both could result in the impact of the two stimuli being greater than the sum of the separate impacts of the two stimuli alone. That is smoking alone can result in stiffness of arteries, and coffee alone without the combination of smoking could have similar effects. However, the combination of both is perhaps worse than the effects of one of the single stimuli.[62]

10. Methodological limitations

Limitations on the research of the effects coffee may have on the cardiovascular system resulting in a cardiovascular incident in coffee drinkers, exists in the limitations of the study designs used during the research, and my own ability to interpret the studies' discussions and findings.

The following could be classified as the general limitations of the studies used in the thesis:

- Low number of RCTs;
- Small sample sizes;
- Differences among study populations;

- Measure used to collect data;
- Introduction of researchers personal biases in literature reviews.

1. Low number of RCTs:

   Randomized control trials, are a type of study design that tries to minimize bias through strong experimental controls. It is considered as the 'gold standard' in study designs. During the literature searches, the number of RCTs were fewer, compared to cohort and case control study designs. Due to the fewer number of RCTs available, the thesis mainly uses observational studies, therefore introducing more chances for biases.

   Table 8: Study designs used in section 7:

Førde OH et al. (1985) (15)	Randomised intervention study
Thelle DS et al. (1976) (1983) (14) (16)	Prospective cohort study
Bønaa K et al. (1988) (17)	Prospective cohort study
Lindahl B et al. (1991) (37)	Prospective cohort study
Jacobsen BK et al. (1987) (18)	Cross sectional study

Observational studies were selected mainly due to the lack of availability of RCTs. However, cohort study design can provide information about the causation of disease from the time of exposure to the occurrence of the disease. With the data

from cohort studies, it is possible to calculate cumulative incidences, which are the most direct measurement of the risk of developing disease. However, the risk of bias is greater.

Possible biases introduced within the thesis due to the observational study designs involved include:

- **Selection bias**: the studies used in the thesis could be subjected to selection bias. The study populations involved in most of the cohort, case control and cross sectional studies in the thesis, come from population registries within certain municipalities, or from hospital registries in certain municipalities. The study participants were mainly chosen for the studies based on whether or not they drank coffee. Although other factors were considered, such as lifestyle, quantity of coffee consumption, some of the studies did not explain brewing methods used, type of coffee consumed, or additional substances added to the coffee such as milk/cream or sugar. While some studies used in the thesis, did not consider biological factors, such a genetics, and did not include family history of heart disease in their questionnaires.
- **Information bias**: There is no direct way of knowing how much information bias exists in the thesis. This is because information bias results from inexact reporting of individual factors. Therefore, it should be considered as a factor that could affect the overall outcome of the results used in the thesis.
- **Measurement errors**: Although there is no way of knowing the extent of measurement errors in the studies used in the thesis, this again should be considered as one of the factors that could lead to different measurement

inaccuracies among the different studies, but which result in similar outcomes. This is because even if measurement error occurs, the overall outcome from the effect may not change greatly.

- **Confounding**: Confounding variables make it harder to analyse the study cohort, because the variables first need to be recognized before adjusting for them. For example, in the newer cohort studies, adjustments for possible confounders such as smoking had been made, and coffee was considered as either possible or not possible risk factor depending on the brewing method utilized. However, in the older studies confounders such as smoking was only partially adjusted for, which may have led to inaccurate conclusions.
- **Hawthorne effect**: The Hawthorne effect is an unavoidable observation bias. This is when the study population modifies its behaviour because they know they are being observed and therefore may change their lifestyle choices, such as eating habits, during the study period.
- **Attrition bias**: Cohort study designs require long follow up times, and therefore study participants could be lost to follow up.
- **Recall bias**: In retrospective case control studies, the participants may report inaccurate information which leads to bias within the study.

2. Small sample sizes:

   While some of the studies, such as the Tromsø heart study and the SHEEP and VHEEP study, were relatively larger prosective population based cohorts, the other studies used in the thesis had smaller sample sizes, and maynot have represented the general population.

3. Differences among study populations:

   The study population used in the thesis differ in age, gender, lifestyle, and origin. Studies from within the Scandic regions were preferred, where the participants had similar cultural and traditional backgrounds, but these studies were not exclusively used. While this increases generalization, it also increases chances for biases if the possible confounders were not recognized and adjusted for among the different study population groups.

4. Measure used to collect data:

   Most of the studies used in the thesis involved questionnaires that were self reported. In observational study designs, while there may be no another alternative to obtain information about the participants, potential problems, such as response bias, where the participants tend to responde differently regardless of the actual evidence to suggest otherwise, may not be avoidable. Other problems include how differently people interpret rating scales, understand the questions, and their introspective ability during the answering phase.

5. Introduction of researchers personal biases in literature reviews:

   The thesis includes literature reviews. The research quality depends on the skills of the author to analyse and interpret the available data, and

there is a possiblity that the research may be have been influenced by the author's personal biases during the writing process.

11. Summary and Conclusion:

Originally, the claims of coffee's effects on cardiovascular health was based on the studies that had either not adjusted or partially adjusted for smoking. It was believed that coffee drinkers were at a higher risk of dying from a cardiovascular related cause than those who did not drink coffee. It was not until researchers started to completely adjust for smoking, did they discovered that those who smoked were also heavy coffee drinkers. Smokers were believed to be at a higher risk of dying from a cardiovascular related incident than nonsmokers.

Researchers in Northern Norway however, had different results to that of the rest of the world including results from Southern Norway. While smokers still consumed more coffee than nonsmokers, the population in Northern Norway as a whole were still at a higher risk of dying due to a cardiovascular related incident than those from Southern Norway, especially among those that consumed coffee.[13] It was not until the mid-1980's, researches decided to look at the brewing methods used, and the type of coffee being consumed, that they discovered that consumption of boiled coffee (back in the days) was more common in the north than in the south. It was only than that they realised that the consumption of boiled coffee may have resulted in the elevated lipid levels in the blood, which could have increased the risks of a cardiovascular event in the northern population, when compared to the south. Soon after this discovery, they were successfully able to identify the components in boiled

coffee that was responsible for elevating the total cholesterol levels in the blood as cafestol and kahweol, the two cholesterol components of coffee. Paper filters have been credited to successfully filter cafestol and kahweol.

Over the years, the concept of coffee changed as researchers noticed that drinking filtered coffee did not increase the risks of CVD. They proposed that the antioxidants present in coffee could be of beneficial value to health in general.

However, researchers noticed that some coffee drinkers were more susceptible to CVDs than others, even after adjusting for smoking. They proposed that perhaps the quantity of coffee being consumed resulted in the outcome of the patients' health. It was discovered that drinking coffee in larger quantities led to an increase in levels of homocysteine in the blood. This was when homocysteine was finally suspected as a possible component responsible for increasing risks of CVD. Although this has been debated in length among the research community over recent years, homocysteine is no longer regarded as an independent risk factor, and homocysteine lowering interventions seem to not be beneficial to the outcome in CVD related cases. However, it can still be regarded as one among many of the secondary biomarkers to the risks of CVD's. On the contrary to the believe that increased coffee intake may increase homocysteine, many researchers during cohort study designs, noticed that those that drank no or little coffee (< 3 cups a day), were more likely to experience a CVD related incident than those that were heavy coffee drinkers, after adjusting for smoking. This could possibly be because homocysteine is not a real risk factor for CVD.

It can be argued that the type of study design, could delegate the results of the studies. Some researchers have suggested that cohort study designs show very little risk of coronary heart disease among habitual coffee drinkers, but case-control studies have a tendency to suggest that risks for CVD increases among the heavier coffee drinkers (> 5 cups per day). This is because of possible biases present in both the study designs. Case control studies may also have poorer control for confoundings than in cohort studies, resulting in slighlty different outcomes. It has been suggested that the discrepancy between the findings from cohort and case-control studies may be explained if coffee was to act as a triggering event in people with chronic CHD which led to MI, instead of it being a risk factor for CHD.

It is clear that the effects of coffee on the cardiovascular system depends on the brewing methods as well as the quantity of consumption. Other factors such as lifestyle, genetics, economical background and education still play a major role in the outcome of cardiovascular related deaths all over the world, and coffee in itself should not be used as a means of prevention.

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Supplementary material

Supplementary table 1: Database and search strategy.

Cardiovascular Diseases	Google scholar	“Cardiovascular diseases” AND “Coffee” OR “Effects of coffee” AND “ Coffee as a risk factor for CVD” OR “Risk factors of Coffee drinking” AND “The Tromso heart study and coffee” AND “coffee and cardiovascular health research from scandinavia” AND “Coffee consumption on the heart”.
Cardiovascular Diseases	Pubmed	(("coffee"[MeSH Terms] OR "coffee"[All Fields]) AND ("drinking"[MeSH Terms] OR "drinking"[All Fields] OR "alcohol drinking"[MeSH Terms] OR ("alcohol"[All Fields] AND "drinking"[All Fields]) OR "alcohol drinking"[All Fields]) AND ("risk factors"[MeSH Terms] OR ("risk"[All Fields] AND "factors"[All Fields]) OR "risk factors"[All Fields] OR ("risk"[All Fields] AND "factor"[All Fields]) OR "risk factor"[All Fields]) AND ("cardiovascular diseases"[MeSH Terms] OR ("cardiovascular"[All Fields] AND "diseases"[All Fields]) OR "cardiovascular diseases"[All Fields])) AND (("loattrfree full text"[sb] AND hasabstract[text]) AND ("1974/01/01"[PDAT] : "2015/12/31"[PDAT]) AND "humans"[MeSH Terms])

Homocysteine	Google scholar	“Homocysteine and coffee drinking” OR “Effects of homocysteine on the cardiovascular system” AND “About homocysteine” OR “Relation of coffee drinking with serum homocysteine levels”
Homocysteine	Pubmed	(("homocysteine"[MeSH Terms] OR "homocysteine"[All Fields]) AND ("coffee"[MeSH Terms] OR "coffee"[All Fields]) AND ("drinking"[MeSH Terms] OR "drinking"[All Fields] OR "alcohol drinking"[MeSH Terms] OR ("alcohol"[All Fields] AND "drinking"[All Fields]) OR "alcohol drinking"[All Fields])) AND "loattrfree full text"[sb]

Supplementary Table 2: Characteristics of studies included

First author, year and Country of Origin	Study Design	Exposure	Outcomes	Adjusting	Limitations
Jacobsen BK 2011 Norway	Cohort Profile for Tromso Study	Coffee	CVD		
Thelle DS 1976 Norway	Cohort	Serum cholesterol levels, and smoking	CVD	Age, work schedule, health condition, physical activity, and ethnic background	Did not adjust for habitual coffee drinking
Førde OH 1985 Norway	Clinical Trial	Coffee	Increased serum cholesterol	Concomitant use of sugar and cream, smoking, tea drinking	Size of cups was not standardized

Thelle DS 1983 Norway	Cohort	Coffee	Raised serum cholesterol levels	Age, BMI, physical activity in leisure time, cigarette smoking, and alcohol consumption.	Did not adjust for brewing methods of coffee
Bønaa K 1988 Norway	Cohort	Boiled coffee	Raised serum cholesterol	Age, smoking	
Jacobsen BK 1987 Norway	Cross sesctional	Habitual coffee drinking and lifestyle	Total serum cholesterol		Type of coffee consumed was not considered
Van Dusseldorp M 1991 Netherlands	RCT	Boiled and filtered coffee	Raised serum total cholesterol	Changes in body weight during the trial	Small sample size
Nilsson LM 2010 Sweden	Cohort	Filtered and boiled coffee	Risk of first MI	Smoking, postsecondary education, hypertension, and sedentary lifestyle	Small sample size

Hammar N 2003 Sweden	Population based case control study	Boiled and filtered coffee	Incidence of first MI	Quantity of boiled and filtered coffee consumption was adjusted and compared	Retrospective study design may lead to recall bias among the study participants, and poor control over confounding factors
Kleemola P 2000 Finland	Cohort	Coffee	Fatal and non fatal CHD risks and increased total serum cholesterol	Age	Type of coffee consumed and brewing methods were not investigated
Tverdal A 1990 Norway	Cohort	Coffee		Age, total serum and high density lipoprotein cholesterol concentrations, systolic blood pressure, and number of cigarettes per day	Brewing methods of the coffee consumed was not included in the questionnaires handed to the participants
Zock PL 1990 Netherlands	Clinical trial	Coffee	Raised serum cholesterol		Small sample size
Tofler OB 2001 Australia	Case control	Coffee	Episode of first MI or unstable angina	Age, smoking status (past and current), hypertension, dyslipidaemia and diabetes	Small sample size, retrospective study design susceptible to more biases, type of coffee consumed and brewing methods were not investigated

Stensvold I 1996 Norway	Cohort	Coffee	Death resulting from coronary heart disease	Cholesterol concentration	Information on type of coffee was not given in the study
Urgert R 2000	RCT	Coffee	Increased plasma homocysteine		Small sample size
Nygård O 1995 Norway	Cross sectional	Components of the cardiovascular risk profile, ie, male sex, old age, smoking, high blood pressure, elevated cholesterol level, and lack of exercise	Increase in plasma total homocysteine level	Intake of vitamin supplements, fruits, and vegetables	Coffee consumption was not investigated for in the study
Grubben MJ 2000 Netherlands	RCT	Unfiltered coffee	Increased plasma homocysteine levels		Small sample size
Christensen B 2001 Norway	RCT	Abstention from filtered coffee	Lower plasma homocysteine levels	Adjustments for the intake of tea and juice, and brewing methods for coffee	The study was an unblinded
Olthof MR 2001 Netherlands	RCT	Chlorogenic acid	Increased plasma total homocysteine		Small sample size
Heyden S 1979 USA	Cohort	Smoking and coffee drinking	Increase in LDL cholesterol	Sex, age and Quetelet's index (W/H2) of body mass	Alcohol consumption was not considered as a possible confounder

					among the participants, and therefore was not adjusted for
Esposito F 2003 Italy	Clinical trial	Italian style coffee	Change in concentration levels of plasma homocysteine	Vegetable and fruit intake was monitored for.	Small sample size, no blinding
Verhoef P Netherlands 2002	RCT	Caffeine	Raised plasma homocysteine		