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**Coffee and Cholesterol  
- Impact of Brewing Methods**

*From the seventh survey of the Tromsø Study in 2015-2016*

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## Preface

When time had come to find a subject for my master's thesis, I immediately knew I wanted to ask Maja-Lisa Løchen, consultant cardiologist at The University Hospital of Northern Norway (UNN Tromsø) and a professor of preventative medicine at Department of Community Medicine (ISM) at UiT The Arctic University of Norway. She had been an excellent supervisor for me in 2017, when I wrote about suicide prevention in indigenous adolescents. Løchen asked Tom Wilsgaard, professor in biostatistics at ISM, UiT, to co-supervise a project. Wilsgaard suggested using the newest wave of the Tromsø Study (Tromsø 7, 2015-2016) to look into the brewing method of coffee's impact on serum cholesterol levels. I soon realised that this subject had deep roots in the Tromsø Study and the research community in Tromsø. Both epidemiological studies and randomised controlled trials were done in the seventies and eighties, being the first in the world to discover the association between coffee and serum cholesterol. I feel honoured for the opportunity to bring up this research subject again and hope it will stimulate to further research on coffee and preventative health.

A sincere thank you to my supervisors. Maja-Lisa Løchen has once again been thorough, supportive, and has given valuable advice. Tom Wilsgaard has given irreplaceable guidance with regards to implementation of the statistical analysis. It wouldn't have been possible without them.

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## Abstract

**Objectives and methods:** Coffee, especially boiled/plunger coffee, raises serum cholesterol (S-TC and S-LDL) because of its diterpenes, cafestol and kahweol. Epidemiological research comparing all the different brewing methods' impact on serum cholesterol was still yet to be done. The aim of this study was to quantify the association between serum total cholesterol and serum low-density lipoprotein (S-TC and S-LDL) cholesterol and consumption of variously brewed coffee. By taking a cross-sectional epidemiological approach, using data from the 7<sup>th</sup> survey of the Tromsø Study (Tromsø 7, 2015-2016), we assessed 6,816 women and 7,309 men. Using multivariable linear regression models, the goal was to obtain knowledge on how much brewing method impacted serum cholesterol, and whether there was any difference in the associations between the various brewing methods and serum cholesterol.

**Results:** Boiled coffee consumption is associated with increased S-TC and S-LDL cholesterol for both women and men. Consuming 6-8 cups of boiled/plunger coffee per day increased S-TC with 0.20 mmol/L and S-LDL 0.18 mmol/L for women and S-TC 0.27 mmol/L S-LDL 0.26 mmol/L for men ( $p \leq 0.001$ ), compared to subjects not drinking boiled/plunger coffee. Similarly, consumption of 6-8 cups of filtered coffee per day is associated with increased S-TC in women (0.10 mmol/L, 95%CI=0.01-0.20) but not in men, compared to subjects drinking 0 cups. However, when changing inclusion criteria to include all subjects answering questions regarding filtered coffee, nonsignificant associations were observed. Intake of 3-5 cups of espresso daily is associated with increased S-TC (0.16 mmol/L, 95%CI=0.07-0.25) and S-LDL (0.13 mmol/L, 95%CI=0.05-0.22) in men but not in women, compared with subjects drinking 0 cups of espresso per day. This association becomes stronger with increasing espresso consumption in men. Instant coffee consumption had no clinically significant association with S-TC and S-LDL.

**Conclusion:** Boiled and plunger coffee, espresso, filtered coffee, and instant coffee affected serum cholesterol from the most to the least, respectively. Male espresso consumption's association with serum cholesterol differs from previous studies, and further research is needed to establish whether there is a causal link.

## Abbreviations

Ac-CoA	Acetyl Coenzyme A
AMI	Acute myocardial infarction
$\beta$	Unstandardized regression coefficient
BP	Blood pressure
CI	Confidence interval
CVD	Cardiovascular disease
DPU	Data and publication board, the Tromsø Study
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
mmol/L	Millimole per litre
NCD	Noncommunicable diseases
Ox-LDL	Oxidised low density lipoprotein
Q1/2	Questionnaire 1 or 2 in the Tromsø Study
RCT	Randomised controlled trial
Serum cholesterol	S-TC and S-LDL
S-LDL	Serum low density lipoprotein
S-TC	Serum total cholesterol
TC	Total cholesterol
TG	Triglycerides
VLDL	Very low-density lipoprotein
WHO	World Health Organisation

# 1 Background

The Nordic countries are leading in coffee consumption world-wide. Norway ranks second, with an average consumption of 9.4 kg coffee beans per person per year. Only Finland drinks more, with 11.7 kg (1). Because of the high consumption of coffee, even small health effects from this popular beverage could have considerable public health consequences and is therefore an interesting and important topic of research.

## 1.1 Cholesterol and its role in pathophysiology of atheroma and ischemia

Cholesterol is an important organic lipid. It is a principal structural component of all cellular membranes and is thus essential for life. 75% of the body's cholesterol is synthesised in the liver, and 25% comes from the diet, mainly from eggs, cheese, meat or fish (2).

Cholesterol is synthesised from Acetyl Coenzyme A (Ac-CoA) through many steps, and the rate limiting step is catalysed by the enzyme HMG-CoA reductase. Intracellular cholesterol is a negative feedback inhibitor of HMG-CoA reductase activity (3). This creates a stable intracellular concentration of cholesterol.

Both cholesterol and triglycerides are transported in the blood as a compound in lipoproteins; small packages of lipids with a coat of cholesterol and proteins. Measuring plasma lipoproteins gives the amount of cholesterol present in the blood (2). An apolipoprotein called low-density lipoprotein (LDL) carries 70% of circulating cholesterol in the body. It transports cholesterol from the liver to other tissues. A high concentration of S-LDL (serum low-density lipoprotein) cholesterol is associated with atheromatous disease and is therefore known as "the bad cholesterol". About 75% of LDL is removed from the circulation by uptake into the liver cells via hepatic LDL receptors. The circulating concentration of LDL rises if there either is excess production of LDL or deficient LDL receptors (2).

With a higher concentration of S-LDL, a higher amount of the LDL cholesterol is taken up peripherally, e.g. in non-LDL scavenger receptors on monocytes. Circulating LDL-rich monocytes migrate into the subendothelial space of arteries, where they become macrophages. In the arterial wall, LDL can turn into oxidised LDL (ox-LDL). Macrophages take up ox-LDL by phagocytosis and become foam cells. These are lipid-rich cells with cholesterol droplets in the cytosol. Foam cells will get deposited in the lamina media wall of

blood vessels and initiate fatty streaks that is the precursor of atheromatous plaques. Ox-LDL can turn into a cytotoxic and chemotactic lipid that can further activate the endothelium (3).

Atheroma is a focal thickening of the intima of arteries, produced by a combination of cells, connective tissue, lipids and debris. A collagen-rich cap covers the atheromatous plaque. Plaque destabilisation underlies the development of acute coronary syndromes and many cases of ischaemic stroke (3).

## **1.2 Risks and guidelines on serum cholesterol**

According to the *World Health Organization* (WHO), noncommunicable diseases (NCD) are responsible for 41 million deaths annually, equivalent to 71% of all deaths globally.

Cardiovascular diseases (CVD) account for 44% of NCD deaths (4). *The Norwegian Directorate of Health's* goals are to reduce early death of the four top NCDs (CVD, diabetes, chronic lung diseases and cancer) by 30% before 2030 in the Norwegian population (5).

Serum total cholesterol (S-TC) contains LDL, high-density lipoproteins (HDL), and very low-density proteins (VLDL) (6). Desirable serum concentrations are  $\leq 5.0$  mmol/L for TC and  $\leq 3$  mmol/L for LDL cholesterol (2). The major risk associated with elevated serum cholesterol levels (both S-TC and S-LDL cholesterol alone) is CVD. The relationship is strongest for coronary and peripheral vascular atherosclerosis, and to a lesser extent also cerebrovascular disease and atherothrombotic stroke (3). Elevated S-TC and S-LDL cholesterol is one of the most important modifiable risk factors for CVD together with tobacco, hypertension and diabetes (7).

In 2008, the global prevalence of raised S-TC among adults was 39% (8). Globally, a third of ischaemic heart disease is attributable to elevated cholesterol levels and is estimated to cause 4.4 million deaths per year (9). It is therefore a major attribution to the global burden of disease.

*NORRISK 2* is a Norwegian risk assessment tool to estimate the 10-year risk of CVD. It is an algorithm that includes the variables age, sex, smoking habits, S-TC, blood pressure (BP) and early myocardial infarction in the nearest family. It contains age dependent cut-offs for when CVD preventative treatment is recommended. With a S-TC of  $\geq 7.0$  mmol/L, medical treatment is recommended independent of other risk factors (10).



Statins is the most common cholesterol lowering group of drugs. These drugs are HMG-CoA reductase inhibitors; thus, inhibiting the rate limiting step in the intrinsic cholesterol synthesis. In 2017, 567,398 Norwegians were prescribed a cholesterol lowering drug (11).

Diet and lifestyle impacts serum cholesterol concentrations (12). An intake of saturated fat and trans-fat increases the level of S-TC and S-LDL cholesterol, while unsaturated fat lowers it (13). Elevated serum levels lead to an increased risk of CVD. It is therefore relevant to assess nutritional compounds that affects these serum levels in the population.

### **1.3 Tromsø's history of research on coffee**

The Tromsø Study is Norway's most comprehensive population study. It has been repeated seven times and involves a large proportion of Tromsø Municipality's population (14). The study was initiated in 1974 as an attempt to investigate the high incidence of CVD in males in Northern Norway. In the mid-seventies, one in five Norwegian men died of acute myocardial infarction before the age of 75 (15). There was a north-south gradient, which greatly affected Northern Norway. Founders of the study wanted more information regarding who gets CVD, reasons for the high mortality and prevention strategies. In further waves of the Tromsø Study, women were also included, and the study gradually expanded to include many other diseases (14).

From the first survey of the Tromsø Study (Tromsø 1, 1974) it became apparent that men from Tromsø had higher S-TC concentrations, higher blood pressure and higher prevalence of smokers compared to the southern Norwegian population (16). Using the second survey (Tromsø 2, 1979-1980), Thelle et al. (1983) coincidentally found a strong and significant association between a high consumption of coffee and a high cholesterol level (17). This led to two additional studies:

A crossover experiment by Arnesen et al. (1984) lasting over 9 weeks was performed in 17 healthy volunteers divided into two groups. Group 1 drank  $\geq 6$  cups of boiled coffee per day for four weeks and then drank no coffee for four weeks. Group 2 did the same in inverted order. The last week, subjects returned to their habitual intake of coffee. The results showed that S-TC was significantly lowered by a mean of 8.7% after no coffee for four weeks. The study concluded that boiled coffee significantly increases S-TC concentrations in healthy subjects (18).

A randomised controlled trial (RCT) by Førde et al. (1985) included 33 men with hypercholesterolaemia. Subjects were randomly assigned to either continue their habitual coffee intake, stop drinking coffee altogether, or stop drinking coffee for five weeks, before continuing drinking either boiled or filtered coffee. Concentrations of S-TC fell significantly in all subjects abstaining coffee for the first five weeks. S-TC increased in subjects subsequently returning to boiled coffee, but remained unaffected in subjects returning to filtered coffee (19). This raised the hypothesis that it was boiled coffee, not filtered coffee, that raised S-TC.

Using data from the third survey of the Tromsø Study (Tromsø 3, 1986-1987), Bønaa et al. (1988) explored the association between brewing method and S-TC. They found a dose dependent increase in S-TC for boiled coffee drinkers. Subjects drinking filtered coffee however, had no such association (20). This confirmed the hypothesis that boiled coffee had an impact on S-TC that filtered coffee did not have.

Since the seventies, mean S-TC and S-LDL cholesterol levels and the proportion of the population with elevated levels, has decreased for all ages and the whole Norwegian population (21-23). Hopstock et al. found that the decrease in S-TC from 1979 to 2016 in age group 40-49 years was 1.2 mmol/L for women and 1.0 mmol/L for men (23). A meta-analysis has showed that a decrease in S-TC of 1.0 mmol/L was associated with a half, a third and a sixth lower ischaemic heart disease mortality in both genders at the ages of 40-49, 50-69 and 70-89 years, respectively (24). Moreover, data from the Tromsø Study suggest that two thirds of the decrease in mortality from CVD can be explained by lowered BP, serum cholesterol and smoking in the population. Favourable changes in serum cholesterol contribute to 32% of the decline (25). Boiled coffee raises serum cholesterol, and consumption of boiled coffee decreased in this time frame, thus suggesting change of coffee habits to play a part in the decrease of CVD in Northern Norway from the seventies until today.

## **1.4 Further research on coffee**

Coffee contains coffee oil. Urgert et al. (1996) found that the main diterpenes in coffee oil, cafestol and kahweol, are the cholesterol raising compounds of coffee (26). Cafestol affects cholesterol more than kahweol (27). This seems to be specific for humans. Boiled coffee contains 1-2 g of oil per litre, whereas the lipid content of filtered coffee is negligible (28). These compounds are retained in the filter in filtered coffee but not in boiled coffee and

provides an explanation why boiled coffee leads to higher S-TC and S-LDL cholesterol concentrations compared to filtered coffee. Table 1 explains brewing methods, levels of cafestol and kahweol in various coffee brews, and their predicted impact on serum cholesterol.

Furthermore, coffee contains more than a thousand diverse phytochemicals. The main active compounds include caffeine, chlorogenic acid, cafestol, and kahweol (29). The intake of each compound depends on the variety of coffee species, roasting degree, type of brewing method and serving size. In addition to the effect of raising serum lipids, *in vitro* and *in vivo* experimental results show that the two diterpenes cafestol and kahweol exert multiple potential pharmacological actions such as anti-inflammation, hepatoprotective, anti-cancer, anti-diabetic, and anti-osteoclastogenesis activities (27).

Large epidemiological studies have observed J- and U-shaped relationships between habitual coffee consumption and incidence of CVD. Lowest incidence of CVD was observed in individuals with medium coffee intake (30). These data support the protective effect of drinking moderate quantities of coffee (1-2 cups daily) (31). A meta-analysis of prospective cohort studies from 2014 showed that subjects drinking 3-5 cups per day had the lowest risk of CVD, and heavy consumption was not associated with elevated CVD risk (32).

This is an interesting paradox, as coffee also raises S-TC and S-LDL cholesterol and is thought to increase atheroma and thereby increase risk of CVD. This leads us back to the question of brewing method and the subsequent raise in S-TC levels, as Bønaa et al. already asked back in 1988 (20); *is it all in the brewing?*

Tverdal et al. (2020) took this question further and studied the association between amount and type of coffee (filtered, unfiltered) and mortality in a large cohort of middle-aged men and women (33). They found that filtered coffee is associated with lower mortality than no coffee or unfiltered coffee only. Among coffee consumers, subjects drinking 1-4 cups of filtered coffee had the lowest mortality. Furthermore, the raised mortality from ischaemic heart disease for unfiltered coffee is mediated partly through its association with total cholesterol.

Different studies still yield different results regarding coffee and cholesterol. For example, Strandhagen et al. (2003) found that filtered coffee raises serum cholesterol (34), whilst Jee et al. (2001) found no such association (35). This may be because several factors influence diterpene contents; the quality of the coffee bean also plays a role. *Coffea arabica* (arabica) and *Coffea canephora* (robusta) are the most important coffee species. Arabica has higher commercial value and, in general, more favourable sensory characteristics. Most commercial roasted and ground coffees are actually blends of the two species (36). The variability of cafestol and kahweol contents is high among species with different genetic backgrounds or origins. Moeenfarid et al. (2020) did a literary review where Arabica coffee was found to have kahweol contents between 182 and 1265 mg/100 g and cafestol contents between 182 and 1308 mg/100g. Robusta coffee concentrations, on the other hand, were 151-363 mg/100 g and 0-20 mg/100 g, respectively (37). This shows that Arabica coffee contains the highest concentrations of cholesterol raising diterpenes. Furthermore, an inverse relationship was found between roasting degree and the cafestol concentration in brews prepared without using a paper filter (38). Similarly, an inverse relationship between particle size of grounded coffee beans and diterpene concentrations in espresso has been demonstrated (39). Taken together, these findings may provide an explanation to why studies yield differing results for both epidemiological cross-sectional studies and RCTs.

Cai et al. (2012) did a meta-analysis of RCTs, looking into the association between serum lipids and coffee consumption, depending on the various brewing methods (40). They found that unfiltered and boiled coffee significantly increased S-TG and S-LDL cholesterol concentrations compared to filtered coffee. The coffee dose was independently associated with a net change in S-TG, even after adjusting for types of coffee and study duration. This means that filtered coffee also exerted an increase in serum lipids, thus not as much as unfiltered coffee. Additionally, hyperlipidaemic subjects were more sensitive to the cholesterol-raising effect of coffee. Cai et al. intended to differentiate between brewing methods used in the various RCTs. They used the groups; decaffeinated coffee, filtered coffee and regular coffee. However, they did not define “regular coffee”, and it seemed to include both espresso, mocha pot, plunger and boiled coffee. Going through data from Cai et al. and the RCTs they based their study on, the various brewing method’s impact remains unclear.

Current advice regarding coffee in the Norwegian Health Directorate’s *National guidelines for prevention of cardiovascular disease* (2018) state: “When it comes to coffee, boiled coffee and

other types of unfiltered coffee from machines that is based on whole coffee beans, is what increases total and LDL cholesterol” [translated from Norwegian] (10). This statement lacks nuance, because research regarding cholesterol-raising effects of espresso and coffee machines using whole beans is inconclusive (37). Although Urgert et al. (1996) theoretically calculated the cholesterol rising effects of the various brews (Table 1) (26), an epidemiological comparison of all the brewing methods of coffee’s impact on serum cholesterol is still yet to be done.

## **2 Aim**

Increased knowledge on how lifestyle affects health status can help improve the individual’s and the population’s health. Increasing knowledge regarding how brewing methods affect serum cholesterol (S-LDL and S-TC) provides a promising avenue for investigation. The aim of this study was therefore to quantify the association between serum cholesterol and consumption of variously brewed coffee in both men and women. Using the 7<sup>th</sup> survey of the Tromsø study (Tromsø 7, 2015-2016), the goal was to obtain knowledge on how much the brewing method impacts serum cholesterol and whether there are differences in the associations between the various brewing methods and serum cholesterol.

## **3 Materials and methods**

### **3.1 Literature search**

PubMed was used in a systematic literature search for articles (Figure 1). Titles, abstracts and subject headings in the database PubMed was searched with the use of the following phrases: (‘coffee’ or ‘cafestol’ or ‘kahweol’) and (‘cholesterol’ or ‘triglycerides’ or lipids’). Inclusion criteria were: 1. Published in PubMed; 2. In English; 3. The last ten years; 4. Had humans as test objects. Articles that seemed irrelevant by reading the title and/or abstract were excluded. References of reviews and papers identified by the search were also examined.

### **3.2 Study population**

The Tromsø Study is Norway’s most comprehensive population study through the last 47 years. The study has been repeated every sixth to seventh year in the time frame 1974-2016. This cross-sectional study utilised data from the 7<sup>th</sup> survey of the Tromsø Study (Tromsø 7, 2015-2016). All women and men aged 40 years and above (n=32,591) living in the

municipality of Tromsø in Northern Norway were invited to participate in the study. They received an invitation with an information pamphlet, a questionnaire of four pages (Q1), and access to a digital platform containing a second questionnaire (Q2). They were also invited to meet up in person for a clinical examination and to get non-fasting blood samples drawn (14).

65 % of the people invited joined the study (21,083 in total, 11,074 women and 10,009 men). For the present study, subjects with missing baseline covariates were excluded (n=1,218). Furthermore, for two out of three statistical models, subjects with missing coffee covariates were excluded (n=5,740). The reason for exclusion was to have the same study population in the statistical models, so that the various models would be directly comparable. The final study population included 14,125 participants from Tromsø 7, 7,309 women and 6,816 men (Figure 2).

### **3.3 Ethics**

Privacy is a central aspect of large population studies like Tromsø 7. The Tromsø Study has concession from both the Data Protection Authority and Regional committee for medical and health research ethics in Northern Norway (REC North). Those with access to the data also have statutory confidentiality. Furthermore, researchers who utilize data cannot get access to information that makes it possible to identify individual participants. Information gathered is used solely for the purpose of approved research projects. All participants in Tromsø 7 have given informed consent to use clinical data for research (14).

### **3.4 Variables**

Variables were selected from Q1 and from blood sample results. Dependent variables in this study are S-LDL and S-TC, both measured in millimole per litre (mmol/L).

Q1 included four questions regarding coffee consumption, asking how many cups of a specific brew of coffee they drank daily. The different brews were defined as: filtered coffee; boiled coffee/French plunger coffee (coarsely ground coffee for brewing); instant coffee; espresso-based coffee (from coffee machines, capsules etc.). For each brew, subjects were divided into five groups based on how many cups of that specific brew they drank per day: 0 cups, 1-2 cups, 3-5 cups, 6-8 cups, and  $\geq 9$  cups daily.

The following independent variables were included and adjusted for as possible confounding factors: age (years), Body Mass Index (BMI), daily smoking, physical activity, and level of

education. BMI was calculated by using the formula  $BMI = \frac{m}{h^2}$ , where  $m$  is the mass in kilograms, and  $h$  is the height in meters. Smoking was defined by the following question: “Do you or did you smoke daily?”, and possible answers were: 1. Yes, now; 2. Yes, previously; 3. Never. Level of physical activity was defined as “Physical exertion in leisure time over the last year” and was divided into four groups: 1. Reading, watching TV/screen or other sedentary activity; 2. Walking, cycling, or other forms of exercise at least 4 hours a week; 3. Participation in recreational sports, heavy gardening, snow shovelling etc. at least 4 hours a week; 4. Participation in hard training or sports competitions, regularly several times a week. Education was defined as “The highest level of education you have completed” and was divided into four groups. 1. Primary/partly secondary education (up to 10 years of schooling); 2. Upper secondary education (a minimum of 3 years); 3. Tertiary education, short: College/university less than 4 years; 4. Tertiary education, long: College/university 4 years or more.

### **3.5 Statistical analysis**

Baseline characteristics of subjects, according to categories of coffee intake, were summarised as means and standard deviations (continuous variables) or percent (categorical variables). To assess possible differences between groups, linear regression was used for continuous variables and chi-square test for categorical variables.

This cross-sectional study examined the association between various coffee brews and S-TC and S-LDL cholesterol, respectively. The analysis was done separately for each coffee brew, and also separately for men and women. Differences in mean levels of plasma lipids between categories of coffee consumption were tested by using multivariable linear regression models.

For each coffee brew, analyses with three models were made. All models adjusted for age, BMI, daily smoking, physical activity, and level of education. Model 2 additionally adjusted for combined coffee habits; the other coffee brews than the one tested for were included as covariates in the multivariable linear regression model. Model 3 included the same covariates as in model 1 but was run without requiring a complete set of values for all coffee type variables, and therefore included subjects with missing answers on other coffee covariates. For example, when filtered coffee was the exposure variable all subjects with non-missing on filtered coffee were included regardless of whether boiled coffee was missing. Furthermore, for practical reasons, the coffee category containing both boiled and plunger coffee is in the analysis defined as “boiled coffee”.

Unstandardized regression coefficients ( $\beta$ ) with 95% confidence intervals (CI) for each category of coffee intake were calculated.  $\beta$  is the estimated difference in mean S-LDL cholesterol or S-TG concentration in mmol/L between each level of coffee intake and the reference level (0 cups per day) if the effect of all other covariates is held constant.

Additional analyses were made to further investigate underlying implications of the results. The analysis group for model 1 and 2 was compared with the group of subjects excluded because of missing answers, hereby called the missing group. Eventual significant differences between groups were tested for by using chi-square tests for categorical variables and linear regression for continuous variables.

Assumptions of normality was ensured by visual inspection of distribution of residuals using histograms. Plots with predicted standardized values and standardized residuals were visually inspected, and no pattern of heteroscedasticity was observed. No collinearity between predictor variables was observed (all variance inflation factors  $<1.2$ ).

A p-value  $<0.05$  was considered statistically significant. The data was analysed with the program IBM SPSS Statistics 25.0 and 26.0 for Macintosh (Statistical Package for Social Sciences, Chicago, IL, USA). The graph was made in GraphPad Prism version 8.0.

## 4 Results

Baseline characteristics according to daily consumption of various coffee brews are presented in Tables 2-5. The mean age was 56.5 years. Subjects who drank more coffee per day were significantly more likely to smoke daily and less likely to have a higher education than those who drank less coffee (Tables 2-5). For example, among women who drank  $\geq 9$  cups of filtered coffee per day, 56.4 % were daily smokers. In comparison, only 7.9 % of women drinking 1-2 cups of filtered coffee were daily smokers ( $p<0.001$ ) (Table 3). Similar trends were seen for both sexes and for all coffee brews ( $p<0.001$ ). Also, 35.3 % of women who drank  $\geq 9$  cups of filtered coffee per day had primary/secondary school as their highest level of education, compared to 17.0 % of women who drank 1-2 cups ( $p<0.001$ ) (Table 3). Similar trends are also here seen in for both sexes and for all coffee brews ( $p<0.001$ ).

Figure 3 gives an overview over main results from model 1. In the multivariable models (Tables 6-13) it was found that the association between coffee and cholesterol varied with



various brews. Results are from model 1 unless stated otherwise, which means they are adjusted for age, BMI, daily smoking, physical activity, and level of education.

Boiled and plunger coffee consumption (hereby called boiled coffee) is associated with increased S-TC and S-LDL in both women and men (Tables 6-7). From the lowest (0 cups) to the highest ( $\geq 9$  cups) boiled coffee consumption category, mean S-TC concentrations increased by 0.40 mmol/L in women ( $p < 0.001$ ) and 0.27 mmol/L in men ( $p < 0.001$ ) compared to subjects drinking 0 cups of boiled coffee per day. For model 2, S-TC increased somewhat more, with 0.48 mmol/L and 0.33 mmol/L ( $p < 0.001$ ), respectively. For model 3, S-TC increased somewhat less, with 0.31 mmol/L and 0.23 mmol/L, respectively ( $p < 0.001$ ). Thus, the association was maintained through models 1 to 3

For the association between consumption of filtered coffee and S-TC, there was a significant linear trend in women ( $p = 0.013$ ), but not for men ( $p = 0.949$ ) (Table 8). S-TC concentrations in women who drank 6-8 cups of filtered coffee a day was 0.10 mmol/L higher (95%CI=0.01-0.20) than for those who drank 0 cups of filtered coffee per day. In model 2, adjusting for combined coffee habits, S-TC concentrations in the same group was 0.17 mmol/L higher than for those who drank 0 cups (95%CI=0.08-0.27). Furthermore, the p-value was lower both for women ( $p < 0.001$ ) and for men ( $p = 0.006$ ), thus making male results significant. However, in model 3, including those with missing answers on other coffee covariates, the association between S-TC and filtered coffee became weaker and less significant than in model 1. Women who drank 6-8 cups had 0.07 mmol/L higher cholesterol than those who drank 0 cups. There was no significant linear trend for men. For LDL cholesterol, changes in serum concentrations were not significant for neither women or men, except for women in model 2 ( $p = 0.007$ ) (Table 9).

Espresso consumption was associated with increased S-TC and S-LDL in men through models 1-3 (Table 10-11). Serum cholesterol concentrations increased for each increasing level of consumption up to 6-8 cups daily, and there was a significant linear trend ( $p = 0.001$  for S-TC and S-LDL). Drinking 3-5 cups of espresso per day was associated with S-TC (0.16 mmol/L, 95%CI=0.07-0.25) and S-LDL (0.13 mmol/L, 95%CI=0.05-0.22) in men compared to subjects drinking 0 cups per day. The corresponding figure in men who drank 6-8 cups was 0.24 mmol/ for both S-TC and S-LDL (95%CI =0.04-0.43 and 0.05-0.42, respectively). For women, the only significant result was that a consumption of 3-5 cups of espresso per day

was associated with an increase in S-TC of 0.11 mmol/L (95%CI=0.02-0.10). There was no significant linear trend.

When adjusting for combined coffee habits in model 2, the association between espresso consumption and S-TC and S-LDL cholesterol concentration became stronger, whilst p-values remained low ( $p < 0.001$ ) in men. The rise of S-TC concentrations in men who drank 3-5 cups was raised from 0.16 mmol/L to 0.19 mmol/L. The corresponding figure in men who drank 6-8 cups was raised from 0.24 mmol/L to 0.28 mmol/L. The same trends were seen with S-LDL cholesterol concentrations. For women, the linear trend became significant (S-TC:  $p = 0.002$ , S-LDL:  $p = 0.035$ ) and drinking 3-5 cups of espresso a day was associated with an increase in 0.15 mmol/L S-TC (95%CI=0.60-0.25).

In model 3, including subjects with missing answers on other coffee covariates, the association between S-TC and espresso only had slight changes to the original model, e.g. men who drank 6-8 cups of espresso had an increase of 0.21 mmol/L S-TC versus 0.24 mmol/L in the original model 1. The corresponding figure for women was 0.02 mmol/L and was not significant. The association was still strong for men ( $p < 0.001$ ). For women there was barely a significant linear trend ( $p = 0.043$ )

Men who drank  $\geq 9$  cups of espresso per day had a decrease in S-TC concentrations of -0.27 mmol/L compared to men who drank 0 cups (Table 10). This group had a small sample size ( $n = 41$ ) and results were not statistically significant. All other espresso groups in men had a statistically significant increase in S-TC and S-LDL (Tables 10-11).

For instant coffee, no significant trend was observed in either women or men, except for S-TC in model 2 ( $p = 0.022$  for women and  $p = 0.025$  for men). Women drinking  $\geq 9$  cups of instant coffee daily had an increase in S-TC of 0.10 mmol/L compared to women drinking 0 cups in model 2 (Tables 12-13).

## **5 Discussion**

This epidemiological cross-sectional study has investigated the association between various coffee brews and S-TC and S-LDL, respectively.

### **5.1 Most important findings**

Overall, boiled/plunger coffee, espresso, filtered coffee and instant coffee affected serum cholesterol from the most to the least, respectively.

For boiled/plunger coffee there was a highly statistically significant positive association with S-TC and S-LDL cholesterol concentrations for both women and men. For espresso, the association was significant for men but not for women. It was vice versa with filtered coffee, showing a small significant association for women and not for men. Instant coffee, on the other hand, had a significant linear trend solely in model 2. However, this seemed like a negative association between S-TC and instant coffee consumption for men. Through all brews, the association was strengthened when additionally adjusting for other coffee brews (model 2).

## **5.2 Findings in relationship with other literature**

### **5.2.1 Boiled/plunger coffee and instant coffee**

Results regarding boiled/plunger coffee coincide with previous studies (19, 35, 40). Also, previous studies regarding instant coffee found no association with increased serum cholesterol (40), and the inconsistent findings in the present study are in line with this.

### **5.2.2 Espresso**

Interestingly, men who drank espresso daily had significantly higher S-TC and S-LDL cholesterol concentrations than those who did not. There was a significant positive linear trend. Contrary to those findings however, the randomised intervention study of D'Amicis et al. (1996) and the cross-sectional study of Grioni et al. (2015) showed no significant association between consumption of espresso coffee and increase of serum cholesterol concentrations (15, 41). Results from these two Italian studies differ from results in the present study. There are several possible explanations for this. One could think that Norwegian men who drank espresso also drank other coffee brews which caused the rise in serum cholesterol. However, when adjusted for combined coffee habits, the association between espresso and serum cholesterol was strengthened, suggesting that espresso itself rises serum cholesterol.

Furthermore, the questionnaire regarding coffee in Tromsø 7 had a wide definition of espresso, including coffee machines, capsules and mocha pots. Moeenfarid et al. (2016) looked into the variability of diterpenes in different types of espresso (39). They tested a variety of capsule- and pod-based brews, mocha pots, vending machines, and espresso machines, in addition to the other classical types of coffee. They found that cafestol concentrations were 36 mg/L for mocha and 54 mg/L for espresso machine. For capsules, pods and vending machines, cafestol concentrations varied between 10 and 43 mg/L. Wuerges

et al. (2016) found similar results when testing different commercial capsule coffee in Brazil (42). In comparison, boiled coffee and filtered coffee contained 232 mg/L and 5 mg/L of cafestol, respectively. This suggests that espresso brews may have an intermediate contribution to the intake of cafestol and kahweol compared to other types of coffee.

Diterpene levels, however, varies within the various types of espresso.

Additionally, Italians drink small cups of espresso, and one cup is defined as a 30 mL serving (41). However, the volume of coffee brew per cup varies from person to person, depending on the cultural and personal preferences. Norwegians are used to large cups of filtered coffee, and this habit is likely to lead to large cups of espresso as well. If one cup of “Norwegian” espresso is four times the size of a cup of Italian espresso, more diterpenes will be ingested per cup of coffee. Variability in cup size might be a reason why Italian and Norwegian studies yield different results.

The association between espresso and serum cholesterol was stronger for men than for women. Non-physiological explanations for this could hypothetically be: 1. A smaller number of women drinking larger quantities of coffee yielding non-significant results; 2. Smaller cups of espresso for women than for men; 3. Other brewing methods of espresso dominating (capsule, espresso machine or mocha pot) in women compared to men; 4. Sex differences regarding subjective views on coffee intake leading to different reporting in the questionnaire.

### **5.2.3 Filtered coffee**

Results regarding filtered coffee’s effect on serum cholesterol vary in the literature, but consensus is that it is more beneficial than unfiltered coffee (20, 34, 40). Strandhagen et al. (2003) found that filtered coffee did raise serum cholesterol and warranted a study on the paper filter quality and physical properties of the filters (34). Two recent studies did just that, and tested various types of commercially available filters, to assess whether the filter’s diterpene-retaining function varied (43, 44). They found that cafestol and kahweol concentrations in the brews varied from 1.62 to 2.98 mg/L and 0.73 to 1.95 mg/L, respectively. Additionally, they found that the highest concentrations were obtained using filters with micro perforations. The filters showed high fat permeability. The porosity of the paper filter and the particle size of the ground roasted coffee were determinant factors in obtaining filter coffee brews with lower cafestol contents. It is though unclear whether the variance in diterpene-retaining function is large enough to have clinical relevance. Therefore,

the small but significant raise in S-TC in women who drink filtered coffee could be because of the diterpene content that pass through the filter.

#### **5.2.4 Sex differences in response to diterpenes**

Men have previously been found to have larger responses of S-TC and S-LDL cholesterol concentrations to saturated fat and cafestol than women do. Weggermans et al. found in 1999 a discrepancy between men and women in the response of serum cholesterol when given concentrates of diterpenes cafestol and kahweol (45). This was done by comparing responses in nine controlled trials with healthy subjects. The adjusted response of both S-TC and S-LDL cholesterol to cafestol was 0.22 mmol/L larger in men than in women. Age, BMI, change in weight during the trial and baseline cholesterol level were adjusted for. Lack of compliance, the menstrual cycle, contraceptives and a smaller total intake of energy could not explain the sex discrepant. The authors suggested that the physiological explanation could involve sex hormones. Most of the women in their study were premenopausal. Another study found that female hamsters started having as large serum cholesterol responses to change in diet as male hamsters after being castrated (46). Furthermore, a meta-analysis from 2019 showed that all serum lipoproteins except for HDL-cholesterol were significantly higher in postmenopausal women compared to premenopausal women (47). However, this is difficult to measure, as cholesterol also increases with increasing age. The mean age for menopause is 52.9 years, with a range from 40.3-58.9 (48). As subjects in the present study have a mean age of 56.5 years, a major part, but not all, of these women would have been postmenopausal when the study was conducted. This could have affected the results for women, and the results are not necessarily directly applicable to groups of solely pre- or postmenopausal women.

### **5.3 Strong and weak aspects**

The Tromsø Study has a high participation rate, a large number and an equal distribution between women and men. Also, because all inhabitants of a specific area are invited, participants will be quite representative with regards to the distribution of socioeconomic and health factors in the population. It also captures Northern Norway's specific coffee habits, which will be different from e.g. Italian coffee habits. The questionnaire, clinical examinations and blood tests are done with standardised methods and with experienced leaders and personnel. The well-known potential confounders are registered and taken account for in the statistical analysis.

A possible weakness in this study is that we had to exclude subjects with missing covariates (n=6,958) from the original population (n=21,083). A baseline table comparing these two groups was made. Most of the excluded participants had missing answers on questions regarding coffee (n=5,740, Figure 2). Subjects in the missing group are older, have higher serum cholesterol levels, smoke less, have fewer years of education, and have a larger proportion of women. Model 3 did in part compensate for this by not excluding subjects with missing answers on other coffee covariates; thus, making the study population larger. It did not change the main resulting trends for espresso but did show a weaker association between filtered coffee and S-TC in women.

The highest-consuming coffee group ( $\geq 9$  cups per day) was quite small, and results in those groups were often not significantly different from the other groups. Some subjects in those groups wrote a number so high that it is plausible that they misread the question. For example, 10 women and 10 men wrote that they drank between 20 and 46 cups of filtered coffee daily. 4 women said they drank 20-40 cups of filtered coffee daily. To explore this, the analysis was also run with 20 cups as a cut-off. This did not affect the results.

## **5.4 Implications**

The results from this study was, as predicted, that various coffee brews would affect serum cholesterol in various ways. Boiled/plunger coffee, espresso, filtered coffee and instant coffee affected serum cholesterol from the most to the least, respectively. This coincides with the theoretical dose-response curve based on diterpene content made by Urgert et al. (1996) (49) (Table 1).

Earlier findings regarding boiled coffee and instant coffee (40) are strengthened by the present findings. A dose-response curve was seen for boiled coffee and S-TC and S-LDL cholesterol for both women and men (Figure 3), and no clinically relevant association between consumption and serum cholesterol was seen for instant coffee (Table 12-13). Previous health advice regarding avoiding boiled and plunger coffee (10) should therefore remain as standing after presenting these results.

Less clear is what should be advised for filtered coffee and espresso, as previous studies show conflicting results. In the present study, serum cholesterol was significantly raised in women who consumed filtered coffee. Serum cholesterol was less raised in model 3, when including all subjects who answered the specific question regarding filtered coffee, thus questioning the

present association. However, there is little clinical significance for women drinking 6-8 cups of filtered coffee per day, since S-TC increases with 0.10 mmol/L compared to women not drinking filtered coffee daily, which is a relatively low raise. Therefore, filtered coffee should remain as the coffee brew to recommend for the population. For espresso coffee, the raise in S-TC in male consumers was higher (0.24 mmol/L for 6-8 cups) than for filtered coffee and is cause for recommendation to limit excess use. Also, as explained in the introduction, coffee seems to have beneficial aspects, and a consumption of 1-5 cups daily is overall associated with better health outcomes including CVD (30-32).

Several aspects should be examined more closely. The mystery of why filtered coffee yield differing results in different studies should be solved. A clarification of what “espresso” is could help to further analyse whether it raises serum cholesterol, and at which volumes. It would also be interesting to clarify sex hormones’ roles in the cholesterol raising response to ingesting diterpenes. In order to clarify these conflicting and unclear findings, an RCT would be required, including standardised brewing methods, coffee beans, roasting degree, coffee particle- and cup sizes, particularly for espresso coffee. The goal should be to explore whether there is a reliable dose-response curve to diterpene intake and raise in serum cholesterol.

## **6 Conclusion**

This study quantified the association between serum cholesterol and consumption of variously brewed coffee in men and women in the 7<sup>th</sup> survey of the Tromsø study (Tromsø 7, 2015-2016). From this data, knowledge was obtained on how much the brewing methods impacted serum cholesterol and differences between brewing methods was observed.

Boiled/plunger coffee, espresso, filtered coffee and instant coffee affected serum cholesterol from the most to the least, respectively. Findings regarding boiled/plunger coffee and instant coffee confirms previous studies on this matter. Previous health advice regarding avoiding boiled and plunger coffee should therefore be maintained after presenting these findings.

Espresso did significantly raise serum cholesterol in men in this study, but further research on this should be conducted.

## Tables and Figures

*Table 1: Preparation techniques of various coffee brews, levels of cafestol and kahweol in coffee brews, and predicted effects on serum cholesterol levels with chronic consumption of five cups per day. Based on Urgert et al. (1995) (49). Estimates are based on the observation of Weusten-van der Wouw et al. (50) that every 10 mg of cafestol plus a similar amount of kahweol raises serum cholesterol by 0.13 mmol/L.*

Type of coffee	Preparation technique	Diterpenes per cup		Predicted rise in serum cholesterol levels with consumption of five cups/day (mmol/L)
		Cafestol (mg)	Kahweol (mg)	
<b>Plunger/French press</b>	Hot water is poured onto coarse grounds, and after 2-3 min the metal screen strainer is pushed down to separate the grounds from the fluid	3.5	4.4	0.23
<b>Boiled</b>	Coarse grounds are boiled with water for $\geq 10$ min, or infused with hot water, and the liquid is decanted without the use of a filter	3.0	3.9	0.19
<b>Filtered</b>	Boiled water is poured over finely ground roasted coffee beans in a paper filter, either by hand or by using an electric coffee maker	0.1	0.1	<0.01
<b>Espresso</b>	Hot water is forced under high pressure through a bed of finely ground, usually dark roasted, coffee beans	1.5	1.8	0.10
<b>Mocha</b>	Just overheated water is forced through a bed of finely ground, usually dark roasted, coffee beans	1.1	1.4	0.07
<b>Instant</b>	2-3 g of soluble coffee granules are dissolved into 150-190 mL of hot water	0.2	0.2	0.01



Table 2: Baseline characteristics according to consumption of boiled coffee in women and men. The Tromsø Study 2015-2016.

Characteristics	Cups of Boiled Coffee per Day					p
	0	1-2	3-5	6-8	≥9	
<b>Women</b>						
<i>n</i>	6100	746	369	69	25	
Age, years	56.1 (11.0)	54.5 (10.2)	59.2 (11.5)	59.4 (10.3)	59.2 (9.5)	<0.001
LDL cholesterol, mmol/L	3.53 (1.0)	3.55 (1.0)	3.71 (1.0)	3.84 (1.0)	3.91 (0.9)	<0.001
Total cholesterol, mmol/L	5.50 (1.1)	5.54 (1.0)	5.72 (1.1)	5.82 (1.1)	6.01 (0.9)	<0.001
BMI, kg/m <sup>2</sup>	27.0 (5.0)	26.3 (4.8)	27.5 (5.1)	28.0 (5.1)	27.4 (5.5)	<0.001
Daily smoking, %						
Yes, now	13.4 %	9.8 %	14.4 %	40.6 %	35.0 %	<0.001
Yes, previously	42.0 %	45.4 %	46.3 %	37.7 %	48.0 %	0.177
Never	44.6 %	44.8 %	39.3 %	21.7 %	16.0 %	<0.001
Physical activity, %						
Low	14.1 %	10.1 %	12.2 %	23.2 %	16.0 %	0.004
Moderate	65.1 %	64.2 %	67.2 %	62.3 %	76.0 %	0.640
High	18.5 %	22.0 %	18.4 %	14.5 %	4.0 %	0.042
Very high	2.4 %	3.8 %	2.2 %	0.0 %	4.0 %	0.111
Highest level of education, %						
Primary/secondary ≤ 10 years	21.5 %	14.9 %	30.1 %	46.4 %	56.0 %	<0.001
Upper secondary 13 years	25.6 %	20.8 %	23.8 %	24.6 %	20.0 %	0.069
College/university < 4 years	18.7 %	17.2 %	15.7 %	13.0 %	16.0 %	0.377
College/university ≥ 4 years	34.3 %	42.2 %	30.4 %	15.9 %	8.0 %	<0.001
<b>Men</b>						
<i>n</i>	5639	670	346	103	58	
Age, years	56.9 (11.2)	54.7 (10.5)	59.1 (11.4)	61.9 (11.1)	60.3 (11.7)	<0.001
LDL cholesterol, mmol/L	3.59 (1.0)	3.68 (1.0)	3.73 (1.0)	3.77 (1.1)	3.77 (1.0)	0.004
Total cholesterol, mmol/L	5.3 (1.1)	5.47 (1.1)	5.54 (1.0)	5.55 (1.16)	5.59 (1.1)	<0.001
BMI, kg/m <sup>2</sup>	27.8 (4.0)	28.6 (3.7)	27.7 (4.0)	27.5 (3.8)	27.9 (4.9)	<0.001
Daily smoking, %						
Yes, now	12.1 %	11.0 %	15.6 %	25.2 %	46.6 %	<0.001
Yes, previously	43.4 %	41.0 %	48.8 %	48.5 %	39.7 %	0.130
Never	44.5 %	47.9 %	35.5 %	26.2 %	13.8 %	<0.001
Physical activity, %						
Low	14.9 %	14.6 %	15.0 %	14.6 %	25.9 %	0.240
Moderate	51.8 %	47.0 %	52.0 %	46.6 %	48.3 %	0.160
High	29.7 %	33.6 %	30.3 %	37.9 %	24.1 %	0.082
Very high	3.7 %	4.8 %	2.6 %	1.0 %	1.7 %	0.184
Highest level of education, %						
Primary/secondary ≤ 10 years	20.0 %	18.5 %	26.9 %	46.6 %	53.4 %	<0.001
Upper secondary 13 years	30.4 %	26.9 %	32.7 %	25.2 %	32.8 %	0.196
College/university < 4 years	22.1 %	21.6 %	18.2 %	14.6 %	8.6 %	0.017
College/university ≥ 4 years	27.4 %	33.0 %	22.3 %	13.6 %	5.2 %	<0.001

Values are means with standard deviation in parenthesis (continuous variables) or percentage (categorical variables). LDL: low-density lipoprotein; mmol/L: millimole per litre; BMI: body mass index

Table 3: Baseline characteristics according to consumption of filtered coffee in women and men. The Tromsø Study 2015-2016.

Characteristics	Cups of Filtered Coffee per Day					p
	0	1-2	3-5	6-8	≥9	
<b>Women</b>						
<i>n</i>	2589	1566	2451	570	133	
Age, years	54.5 (11.4)	56.1 (11.1)	57.4 (10.6)	58.3 (9.8)	56.6 (9.6)	<0.001
LDL cholesterol, mmol/L	3.49 (1.0)	3.51 (1.0)	3.58 (1.0)	3.66 (0.97)	3.67 (0.9)	<0.001
Total cholesterol, mmol/L	5.44 (1.1)	5.50 (1.1)	5.59 (1.0)	5.62 (1.0)	5.62 (1.0)	<0.001
BMI, kg/m <sup>2</sup>	27.1 (5.4)	26.5 (4.7)	26.9 (4.8)	27.2 (4.8)	27.9 (5.5)	<0.001
Daily smoking, %						
Yes, now	10.6 %	7.9 %	13.6 %	30.0 %	56.4 %	<0.001
Yes, previously	37.2 %	41.0 %	48.9 %	47.5 %	29.3 %	<0.001
Never	52.3 %	51.1 %	37.5 %	22.5 %	14.3 %	<0.001
Physical activity, %						
Low	15.4 %	11.7 %	12.8 %	13.9 %	19.5 %	0.002
Moderate	62.1 %	66.2 %	67.1 %	68.4 %	80.5 %	0.001
High	19.9 %	19.3 %	17.7 %	16.1 %	19.5 %	0.159
Very high	2.7 %	2.8 %	2.4 %	1.6 %	0 %	0.169
Highest level of education, %						
Primary/secondary ≤ 10 years	20.0 %	17.0 %	23.0 %	32.3 %	35.3 %	<0.001
Upper secondary 13 years	23.7 %	21.3 %	27.2 %	30.0 %	30.1 %	<0.001
College/university < 4 years	18.7 %	19.3 %	17.7 %	16.5 %	16.5 %	0.467
College/university ≥ 4 years	37.5 %	42.4 %	32.1 %	21.2 %	18.0 %	<0.001
<b>Men</b>						
<i>n</i>	1975	1201	2455	842	343	
Age, years	55.6 (11.4)	57.3 (11.6)	57.6 (11.2)	57.9 (10.5)	55.83 (9.7)	<0.001
LDL cholesterol, mmol/L	3.6 (1.0)	3.59 (1.0)	3.6 (1.0)	3.63 (1.0)	3.61 (1.0)	0.446
Total cholesterol, mmol/L	5.38 (1.1)	5.35 (1.1)	5.36 (1.1)	5.41 (1.0)	5.37 (1.1)	0.712
BMI, kg/m <sup>2</sup>	27.9 (4.2)	27.6 (3.7)	27.7 (3.9)	27.9 (4.0)	28.5 (4.1)	0.001
Daily smoking, %						
Yes, now	11.6 %	7.2 %	10.0 %	21.3 %	36.2 %	<0.001
Yes, previously	40.7 %	40.5 %	46.0 %	46.9 %	43.1 %	<0.001
Never	47.7 %	52.2 %	44.0 %	31.8 %	20.7 %	<0.001
Physical activity, %						
Low	16.4 %	14.2 %	12.9 %	14.8 %	24.8 %	<0.001
Moderate	50.0 %	51.9 %	52 %	53.3 %	45.2 %	0.076
High	29.2 %	29.8 %	31.7 %	29.2 %	28.3 %	0.308
Very high	4.5 %	4.2 %	3.4 %	2.6 %	1.7 %	0.025
Highest level of education, %						
Primary/secondary ≤ 10 years	20.6 %	17.0 %	20.0 %	23.9 %	35.3 %	<0.001
Upper secondary 13 years	27.8 %	30.6 %	29.2 %	34.8 %	37.3 %	<0.001
College/university < 4 years	22.3 %	21.1 %	22.4 %	20.8 %	16.9 %	0.169
College/university ≥ 4 years	29.3 %	31.4 %	28.4 %	20.5 %	10.5 %	<0.001

Values are means with standard deviation in parenthesis (continuous variables) or percentage (categorical variables). LDL: low-density lipoprotein; mmol/L: millimole per litre; BMI: body mass index

Table 4: Baseline characteristics according to consumption of espresso in women and men. The Tromsø Study 2015-2016.

Characteristics	Cups of Espresso per Day					p
	0	1-2	3-5	6-8	≥9	
<b>Women</b>						
<i>n</i>	5529	1212	502	57	9	
Age, years	57.6 (11.2)	51.7 (8.6)	51.8 (8.7)	51.7 (7.0)	53.9 (7.4)	<0.001
LDL cholesterol, mmol/L	3.57 (1.0)	3.43 (0.9)	3.54 (0.9)	3.46 (0.7)	3.58 (0.6)	0.001
Total cholesterol, mmol/L	5.55 (1.1)	5.39 (1.0)	5.52 (1.0)	5.49 (0.80)	5.51 (0.7)	<0.001
BMI, kg/m <sup>2</sup>	27.0 (5.0)	26.4 (4.8)	26.8 (5.1)	26.8 (3.9)	29.0 (4.4)	0.003
Daily smoking, %						
Yes, now	14.1 %	8.9 %	15.7 %	19.3 %	22.2 %	<0.001
Yes, previously	42.3 %	41.1 %	46.4 %	63.2 %	55.6 %	0.005
Never	43.6 %	50.0 %	37.8 %	17.5 %	22.2 %	<0.001
Physical activity, %						
Low	14.2 %	11.3 %	13.1 %	12.3 %	22.2 %	0.091
Moderate	66.1 %	62.0 %	62.0 %	63.2 %	77.8 %	0.031
High	17.5 %	32.2 %	21.3 %	24.6 %	0 %	<0.001
Very high	2.2 %	3.5 %	3.6 %	0 %	0 %	0.016
Highest level of education, %						
Primary/secondary ≤ 10 years	24.7 %	11.1 %	14.3 %	12.3 %	22.2 %	<0.001
Upper secondary 13 years	25.8 %	20.8 %	25.1 %	26.3 %	22.2 %	0.008
College/university < 4 years	17.7 %	20.5 %	18.5 %	24.6 %	44.4 %	0.030
College/university ≥ 4 years	31.8 %	47.7 %	42.0 %	36.8 %	11.1 %	<0.001
<b>Men</b>						
<i>n</i>	4976	1060	626	113	41	
Age, years	58.6 (11.5)	52.8 (9.4)	51.8 (8.6)	51.2 (8.1)	49.4 (7.6)	<0.001
LDL cholesterol, mmol/L	3.56 (1.0)	3.70 (1.0)	3.78 (1.0)	3.92 (0.85)	3.49 (0.79)	<0.001
Total cholesterol, mmol/L	5.33 (1.1)	5.46 (1.0)	5.56 (1.1)	5.66 (0.9)	5.19 (0.89)	<0.001
BMI, kg/m <sup>2</sup>	27.8 (4.0)	27.8 (4.0)	27.8 (3.7)	28.3 (3.8)	28.2 (2.9)	0.619
Daily smoking, %						
Yes, now	13.2 %	9.7 %	10.4 %	22.1 %	34.1 %	<0.001
Yes, previously	44.4 %	38.8 %	42.5 %	50.4 %	46.3 %	0.008
Never	42.4 %	51.5 %	47.1 %	27.4 %	19.5 %	<0.001
Physical activity, %						
Low	15.2 %	13.4 %	13.9 %	20.4 %	29.3 %	0.017
Moderate	52.3 %	47.6 %	50.0 %	44.2 %	48.8 %	0.034
High	29.5 %	33.8 %	30.0 %	30.1 %	19.5 %	0.044
Very high	3.0 %	5.2 %	6.1 %	5.3 %	2.4 %	<0.001
Highest level of education, %						
Primary/secondary ≤ 10 years	23.5 %	14.1 %	13.3 %	13.3 %	19.5 %	<0.001
Upper secondary 13 years	30.9 %	26.9 %	27.5 %	41.6 %	29.3 %	0.003
College/university < 4 years	20.8 %	22.9 %	24.9 %	28.3 %	26.8 %	0.029
College/university ≥ 4 years	24.8 %	36.1 %	34.3 %	16.8 %	24.4 %	<0.001

Values are means with standard deviation in parenthesis (continuous variables) or percentage (categorical variables). LDL: low-density lipoprotein; mmol/L: millimole per litre; BMI: body mass index

Table 5: Baseline characteristics according to consumption of instant coffee in women and men. The Tromsø Study 2015-2016.

Characteristics	Cups of Instant Coffee per Day					p
	0	1-2	3-5	6-8	≥9	
<b>Women</b>						
<i>n</i>	5849	1017	366	62	15	
Age, years	55.4 (10.8)	58.6 (11.1)	60.0 (11.4)	62.3 (10.9)	59.8 (13.3)	<0.001
LDL cholesterol, mmol/L	3.52 (1.0)	3.61 (1.0)	3.68 (1.1)	3.6 (1.1)	3.73 (0.8)	0.004
Total cholesterol, mmol/L	5.50 (1.0)	6.61 (1.1)	5.67 (1.2)	5.66 (1.2)	5.70 (0.9)	0.001
BMI, kg/m <sup>2</sup>	26.9 (5.1)	26.9 (4.9)	27.3 (5.0)	26.7 (5.2)	28.0 (6.1)	0.565
Daily smoking, %						
Yes, now	12.5 %	14.4 %	19.9 %	37.1 %	26.7 %	<0.001
Yes, previously	42.1 %	44.3 %	45.9 %	45.2 %	33.3 %	0.373
Never	45.4 %	41.3 %	34.2 %	17.7 %	40.0 %	<0.001
Physical activity, %						
Low	13.4 %	13.7 %	18.0 %	11.3 %	20.0 %	0.113
Moderate	65.0 %	66.4 %	62.0 %	72.6 %	66.7 %	0.432
High	19.0 %	18.1 %	16.9 %	16.1 %	13.3 %	0.759
Very high	2.6 %	1.9 %	3.0 %	0 %	0 %	0.379
Highest level of education, %						
Primary/secondary ≤ 10 years	20.4 %	25.3 %	27.9 %	33.9 %	40.0 %	<0.001
Upper secondary 13 years	24.4 %	26.2 %	28.4 %	37.1 %	33.3 %	0.048
College/university < 4 years	18.4 %	18.3 %	17.5 %	11.3 %	13.3 %	0.639
College/university ≥ 4 years	36.8 %	30.3 %	26.2 %	17.7 %	13.3 %	<0.001
<b>Men</b>						
<i>n</i>	5521	813	374	85	23	
Age, years	56.4 (11.2)	58.5 (11.1)	59.9 (10.8)	59.7 (10.2)	57.7 (9.3)	<0.001
LDL cholesterol, mmol/L	3.56 (1.0)	3.39 (1.0)	3.55 (1.0)	3.55 (1.1)	3.44 (1.1)	0.061
Total cholesterol, mmol/L	5.36 (1.1)	5.47 (1.1)	5.31 (1.1)	5.35 (1.2)	5.31 (1.3)	0.077
BMI, kg/m <sup>2</sup>	27.8 (4.0)	27.8 (3.9)	28.2 (4.2)	28.1 (3.9)	27.8 (2.7)	0.290
Daily smoking, %						
Yes, now	12.2 %	12.3 %	16.3 %	21.2 %	39.1 %	<0.001
Yes, previously	42.0 %	46.4 %	56.1 %	49.4 %	52.2 %	<0.001
Never	45.7 %	41.3 %	27.5 %	29.4 %	8.7 %	<0.001
Physical activity, %						
Low	14.7 %	15.7 %	15.5 %	24.7 %	17.4 %	0.122
Moderate	50.8 %	53.0 %	53.5 %	50.6 %	56.5 %	0.640
High	30.8 %	27.7 %	28.6 %	23.5 %	21.7 %	0.175
Very high	3.8 %	3.6 %	2.4 %	1.2 %	4.3 %	0.485
Highest level of education, %						
Primary/secondary ≤ 10 years	20.0 %	22.6 %	25.9 %	38.8 %	30.4 %	<0.001
Upper secondary 13 years	28.7 %	32.0 %	32.9 %	24.7 %	43.5 %	0.179
College/university < 4 years	22.0 %	20.7 %	19.5 %	18.8 %	21.7 %	0.692
College/university ≥ 4 years	28.3 %	24.7 %	21.7 %	17.6 %	4.3 %	<0.001

Values are means with standard deviation in parenthesis (continuous variables) or percentage (categorical variables). LDL: low-density lipoprotein; mmol/L: millimole per litre; BMI: body mass index

Table 6: Linear regression coefficients for the association between serum total cholesterol and consumption of boiled coffee, by sex. The Tromsø Study 2015-2016.

Boiled coffee — Total Cholesterol mmol/L					
Cups of coffee per day	<i>n</i>	Model 1* β (95%CI)	Model 2** β (95%CI)	<i>n</i> ***	Model 3*** β (95%CI)
<i>Women</i>					
0	6100	0(reference)	0(reference)	6198	0(reference)
1-2	746	0.08(0.00, 0.16)	0.10(0.02, 0.18)	1099	0.09(0.03, 0.16)
3-5	369	0.13(0.03, 0.24)	0.20(0.09, 0.31)	590	0.17(0.08, 0.25)
6-8	69	0.20(-0.04, 0.44)	0.28(0.03, 0.52)	109	0.24(0.04, 0.43)
≥9	25	0.40(0.00, 0.80)	0.48(0.08, 0.89)	46	0.31(0.01, 0.60)
<i>p</i> linear trend		<0.001	<0.001		<0.001
<i>Men</i>					
0	5639	0(reference)	0(reference)	5732	0(reference)
1-2	670	0.09(0.01, 0.18)	0.11(0.02, 0.19)	919	0.14(0.06, 0.21)
3-5	346	0.22(0.10, 0.33)	0.27(0.15, 0.39)	510	0.26(0.17, 0.36)
6-8	103	0.27(0.06, 0.48)	0.33(0.12, 0.54)	146	0.22(0.05, 0.40)
≥9	58	0.27(-0.01, 0.54)	0.33(0.05, 0.61)	84	0.23(-0.00, 0.46)
<i>p</i> linear trend		<0.001	<0.001		<0.001

\*Model 1: Adjusted for age, BMI, daily smoking, physical activity, and education

\*\* Model 2: Model 1 + adjusted for filtered coffee, espresso, and instant coffee

\*\*\*Model 3: Model 1 + including subjects with missing answers on other coffee covariates

β: regression coefficient. Difference in total cholesterol (mmol/L) compared to the reference group of 0 cups per day. CI: Confidence Interval

Table 7: Linear regression coefficients for the association between serum LDL cholesterol and consumption of boiled coffee, by sex. The Tromsø Study 2015-2016.

Boiled coffee — LDL Cholesterol mmol/L					
Cups of coffee per day	<i>n</i>	Model 1* β (95%CI)	Model 2** β (95%CI)	<i>n</i> ***	Model 3*** β (95%CI)
<i>Women</i>					
0	6100	0(reference)	0(reference)	6198	0(reference)
1-2	746	0.07(0.00, 0.15)	0.08(0.01, 0.16)	1099	0.07(0.01, 0.13)
3-5	369	0.11(0.01, 0.21)	0.15(0.05, 0.25)	590	0.13(0.05, 0.21)
6-8	69	0.18(-0.04, 0.41)	0.23(0.00, 0.46)	109	0.21(0.03, 0.39)
≥9	25	0.27(-0.10, 0.65)	0.32(-0.05, 0.69)	46	0.28(0.00, 0.55)
<i>p</i> linear trend		0.001	<0.001		<0.001
<i>Men</i>					
0	5639	0(reference)	0(reference)	5732	0(reference)
1-2	670	0.07(-0.01, 0.15)	0.08(-0.00, 0.16)	919	0.11(0.04, 0.18)
3-5	346	0.18(0.07, 0.28)	0.21(0.10, 0.32)	510	0.22(0.13, 0.31)
6-8	103	0.26(0.07, 0.45)	0.31(0.11, 0.50)	146	0.21(0.05, 0.37)
≥9	58	0.21(-0.05, 0.46)	0.25(-0.00, 0.51)	84	0.17(-0.05, 0.38)
<i>p</i> linear trend		<0.001	<0.001		<0.001

\*Model 1: Adjusted for age, BMI, daily smoking, physical activity, and education

\*\* Model 2: Model 1 + adjusted for filtered coffee, espresso, and instant coffee

\*\*\*Model 3: Model 1 + including subjects with missing answers on other coffee covariates

β: regression coefficient. Difference in LDL cholesterol (mmol/L) compared to the reference group of 0 cups per day. CI: Confidence Interval

Table 8: Linear regression coefficients for the association between serum total cholesterol and consumption of filtered coffee, by sex. The Tromsø Study 2015-2016.

Filtered coffee — Total Cholesterol mmol/L					
Cups of coffee per day	<i>n</i>	Model 1* β (95%CI)	Model 2** β (95%CI)	<i>n</i> ***	Model 3*** β (95%CI)
<i>Women</i>					
0	2589	0(reference)	0(reference)	2638	0(reference)
1-2	1566	0.03(-0.04, 0.09)	0.06(-0.01, 0.12)	2210	0.03(-0.03, 0.09)
3-5	2451	0.06(0.00, 0.12)	0.12(0.06, 0.18)	3346	0.06(0.01, 0.12)
6-8	570	0.10(0.01, 0.20)	0.17(0.08, 0.27)	780	0.07(-0.01, 0.15)
≥9	133	0.06(-0.12, 0.24)	0.13(-0.05, 0.31)	188	0.07(-0.83, 0.22)
<i>p</i> linear trend		0.013	<0.001		0.015
<i>Men</i>					
0	1975	0(reference)	0(reference)	2015	0(reference)
1-2	1201	-0.01(-0.09, 0.07)	0.03 (-0.05, 0.10)	1650	0.03(-0.04, 0.10)
3-5	2455	0.00(-0.07, 0.06)	0.06 (-0.00, 0.13)	3335	0.01(-0.05, 0.07)
6-8	842	0.03(-0.06, 0.12)	0.11(0.02, 0.20)	1160	0.02(-0.06, 0.10)
≥9	343	-0.04(-0.16, 0.08)	0.05(-0.08, 0.17)	488	0.01(-0.10, 0.11)
<i>p</i> linear trend		0.949	0.006		0.779

\*Model 1: Adjusted for age, BMI, daily smoking, physical activity, and education

\*\* Model 2: Model 1 + adjusted for boiled coffee, espresso, and instant coffee

\*\*\*Model 3: Model 1 + including subjects with missing answers on other coffee covariates

β: regression coefficient. Difference in total cholesterol (mmol/L) compared to the reference group of 0 cups per day. CI: Confidence Interval

Table 9: Linear regression coefficients for the association between serum LDL cholesterol and consumption of filtered coffee, by sex. The Tromsø Study 2015-2016

Filtered coffee — LDL Cholesterol mmol/L					
Cups of coffee per day	<i>n</i>	Model 1* β (95%CI)	Model 2** β (95%CI)	<i>n</i> ***	Model 3*** β (95%CI)
<i>Women</i>					
0	2589	0(reference)	0(reference)	2638	0(reference)
1-2	1566	0.01(-0.05, 0.07)	0.03(-0.03, 0.09)	2210	0.01(-0.04, 0.07)
3-5	2451	0.03(-0.03, 0.08)	0.07(0.01, 0.13)	3346	0.03(-0.02, 0.08)
6-8	570	0.04(-0.05, 0.13)	0.10(0.01, 0.19)	780	0.02(-0.06, 0.09)
≥9	133	0.03(-0.14, 0.20)	0.09(-0.08, 0.26)	188	0.06(-0.08, 0.20)
<i>p</i> linear trend		0.243	0.007		0.224
<i>Men</i>					
0	1975	0(reference)	0(reference)	2015	0(reference)
1-2	1201	-0.1(-0.08, 0.06)	0.02(-0.05, 0.09)	1650	0.02(-0.04, 0.09)
3-5	2455	-0.02(-0.08, 0.04)	0.05(-0.02, 0.11)	3335	-0.01(-0.06, 0.05)
6-8	842	0.02(-0.07, 0.10)	0.10(0.01, 0.18)	1160	0.01(-0.06, 0.08)
≥9	343	-0.05(-0.17, 0.06)	0.04(-0.08, 0.16)	488	-0.02(-0.12, 0.08)
<i>p</i> linear trend		0.622	0.053		0.745

\*Model 1: Adjusted for age, BMI, daily smoking, physical activity, and education

\*\* Model 2: Model 1 + adjusted for boiled coffee, espresso, and instant coffee

\*\*\*Model 3: Model 1 + including subjects with missing answers on other coffee covariates

β: regression coefficient. Difference in LDL cholesterol (mmol/L) compared to the reference group of 0 cups per day. CI: Confidence Interval

Table 10: Linear regression coefficients for the association between serum total cholesterol and consumption of espresso, by sex. The Tromsø Study 2015-2016.

Espresso — Total Cholesterol mmol/L					
Cups of coffee per day	<i>n</i>	Model 1* β (95%CI)	Model 2** β (95%CI)	<i>n</i> ***	Model 3*** β (95%CI)
<i>Women</i>					
0	5529	0(reference)	0(reference)	5603	0(reference)
1-2	1212	0.00(-0.07, 0.06)	0.017(-0.05, 0.08)	1649	0.02(-0.04, 0.08)
3-5	502	0.11(0.02, 0.10)	0.15(0.60, 0.25)	793	0.08(0.01, 0.16)
6-8	57	0.06(-0.21, 0.33)	0.11(-0.16, 0.38)	82	0.02(-0.21, 0.24)
≥9	9	0.00(-0.66, 0.67)	0.05(-0.61, 0.72)	18	0.22(-0.25, 0.69)
<i>p linear trend</i>		0.084	0.002		0.039
<i>Men</i>					
0	4976	0(reference)	0(reference)	5041	0(reference)
1-2	1060	0.07(0.00, 0.14)	0.08(0.01, 0.15)	1417	0.08(0.02, 0.15)
3-5	626	0.16(0.07, 0.25)	0.19(0.10, 0.28)	947	0.16(0.09, 0.23)
6-8	113	0.24(0.04, 0.43)	0.28(0.80, 0.48)	181	0.21(0.05, 0.37)
≥9	41	-0.27(-0.59, 0.06)	-0.25(-0.58, 0.08)	71	-0.07(-0.32, 0.18)
<i>p linear trend</i>		0.001	<0.001		<0.001

\*Model 1: Adjusted for age, BMI, daily smoking, physical activity, and education

\*\* Model 2: Model 1 + adjusted for boiled coffee, filtered coffee, and instant coffee

\*\*\*Model 3: Model 1 + including subjects with missing answers on other coffee covariates

β: regression coefficient. Difference in total cholesterol (mmol/L) compared to the reference group of 0 cups per day. CI: Confidence Interval

Table 11: Linear regression coefficients for the association between serum LDL cholesterol and consumption of espresso, by sex. The Tromsø Study 2015-2016.

Espresso — LDL cholesterol mmol/L					
Cups of coffee per day	<i>n</i>	Model 1* β (95%CI)	Model 2** β (95%CI)	<i>n</i> ***	Model 3*** β (95%CI)
<i>Women</i>					
0	5529	0(reference)	0(reference)	5603	0(reference)
1-2	1212	0.00(-0.06, 0.06)	0.013(-0.05, 0.07)	1649	0.02(-0.04, 0.07)
3-5	502	0.08(-0.01, 0.17)	0.11(0.021, 0.20)	793	0.04(-0.03, 0.12)
6-8	57	-0.02(-0.27, 0.23)	0.01(-0.23, 0.26)	82	-0.04(-0.5, 0.16)
≥9	9	-0.01(-0.63, 0.61)	0.03(-0.59, 0.65)	18	0.17(-0.27, 0.60)
<i>p linear trend</i>		0.238	0.035		0.269
<i>Men</i>					
0	4976	0(reference)	0(reference)	5041	0(reference)
1-2	1060	0.06(-0.01, 0.13)	0.07(0.00, 0.13)	1417	0.07(0.01, 0.13)
3-5	626	0.13(0.05, 0.22)	0.16(0.07, 0.24)	947	0.13(0.06, 0.20)
6-8	113	0.24(0.05, 0.42)	0.27(0.08, 0.45)	181	0.21(0.07, 0.36)
≥9	41	-0.22(-0.52, 0.08)	-0.22 (-0.52, 0.09)	71	-0.06(-0.29, 0.17)
<i>p linear trend</i>		0.001	<0.001		<0.001

\*Model 1: Adjusted for age, BMI, daily smoking, physical activity, and education

\*\* Model 2: Model 1 + adjusted for boiled coffee, filtered coffee, and instant coffee

\*\*\*Model 3: Model 1 + including subjects with missing answers on other coffee covariates

β: regression coefficient. Difference in LDL cholesterol (mmol/L) compared to the reference group of 0 cups per day. CI: Confidence Interval

Table 12: Linear regression coefficients for the association between serum total cholesterol and consumption of instant coffee, by sex. The Tromsø Study 2015-2016.

Instant coffee — Total Cholesterol mmol/L					
Cups of coffee per day	<i>n</i>	Model 1* β (95%CI)	Model 2** β (95%CI)	<i>n</i> ***	Model 3*** β (95%CI)
<i>Women</i>					
0	5849	0(reference)	0(reference)	5932	0(reference)
1-2	1017	-0.02(-0.09, 0.05)	0.05(-0.02, 0.12)	1451	0.02(-0.04, 0.08)
3-5	366	0.06(-0.05, 0.17)	0.13(0.02, 0.24)	625	0.06(-0.03, 0.14)
6-8	62	-0.06(-0.32, 0.19)	0.03(-0.023, 0.29)	103	-0.06(-0.26, 0.14)
≥9	15	0.07(-0.45, 0.59)	0.10(-0.42, 0.61)	23	0.04(-0.38, 0.46)
<i>p linear trend</i>		0.355	0.022		0.374
<i>Men</i>					
0	5521	0(reference)	0(reference)	5589	0(reference)
1-2	813	0.13(0.05, 0.21)	0.14(0.07, 0.22)	1103	0.121(0.05, 0.19)
3-5	374	-0.02(-0.13, 0.09)	0.04(-0.08, 0.15)	574	0.04(-0.05, 0.13)
6-8	85	0.03(-0.20, 0.26)	0.09(-0.14, 0.32)	130	0.06(-0.13, 0.25)
≥9	23	-0.07(-0.51, 0.36)	0.03(0.31, 0.46)	39	-0.15(-0.48, 0.19)
<i>p linear trend</i>		0.275	0.025		0.079

\*Model 1: Adjusted for age, BMI, daily smoking, physical activity, and education

\*\* Model 2: Model 1 + adjusted for boiled coffee, filtered coffee, and espresso

\*\*\*Model 3: Model 1 + including subjects with missing answers on other coffee covariates

β: regression coefficient. Difference in total cholesterol (mmol/L) compared to the reference group of 0 cups per day. CI: Confidence Interval

Table 13: Linear regression coefficients for the association between serum LDL cholesterol and consumption of instant coffee, by sex. The Tromsø Study 2015-2016.

Instant coffee — LDL Cholesterol mmol/L					
Cups of coffee per day	<i>n</i>	Model 1* β (95%CI)	Model 2** β (95%CI)	<i>n</i> ***	Model 3*** β (95%CI)
<i>Women</i>					
0	5849	0(reference)	0(reference)	5932	0(reference)
1-2	1017	0.02(-0.39, 0.09)	0.04(-0.02, 0.10)	1451	0.02(-0.04, 0.07)
3-5	366	0.06(-0.04, 0.16)	0.10(-0.01, 0.20)	625	0.05(-0.03, 0.13)
6-8	62	-0.09(-0.33, 0.15)	-0.03(-0.27)	103	-0.09(-0.28, 0.10)
≥9	15	0.08(-0.40, 0.56)	0.09(-0.39, 0.57)	23	0.09(-0.31, 0.48)
<i>p linear trend</i>		0.392	0.079		0.402
<i>Men</i>					
0	5521	0(reference)	0(reference)	5589	0(reference)
1-2	813	0.13(0.05, 0.20)	0.14(0.06, 0.21)	1103	0.12(0.06, 0.19)
3-5	374	-0.01(-0.11, 0.09)	0.03(-0.07, 0.14)	574	0.05(-0.03, 0.14)
6-8	85	-0.01(-0.22, 0.20)	0.04(-0.17, 0.25)	130	0.05(-0.12, 0.22)
≥9	23	-0.16(-0.56, 0.24)	-0.09(-0.49, 0.32)	39	-0.14(-0.45, 0.17)
<i>p linear trend</i>		0.334	0.060		0.039

\*Model 1: Adjusted for age, BMI, daily smoking, physical activity, and education

\*\* Model 2: Model 1 + adjusted for boiled coffee, filtered coffee, and espresso

\*\*\*Model 3: Model 1 + including subjects with missing answers on other coffee covariates

β: regression coefficient. Difference in LDL cholesterol (mmol/L) compared to the reference group of 0 cups per day. CI: Confidence Interval





Figure 1: Flow chart showing literature search in PubMed for the subject “Coffee and Serum Cholesterol – the Impact of Brewing Methods”. Inclusion criteria were that the articles should be a. Published in PubMed; b. In English; c. The last ten years; d. Had humans as test objects.

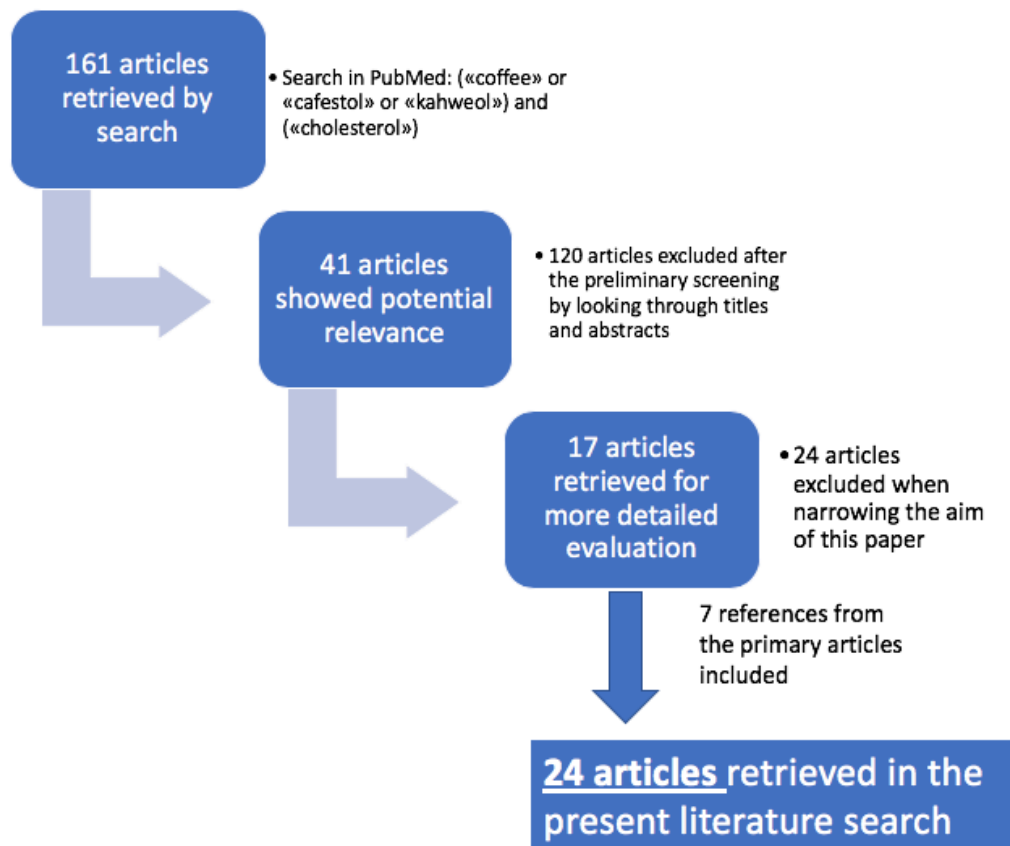


Figure 2: Study flow chart showing included subjects for analyses regarding the association between coffee consumption and serum cholesterol. The Tromsø Study 2015-2016.

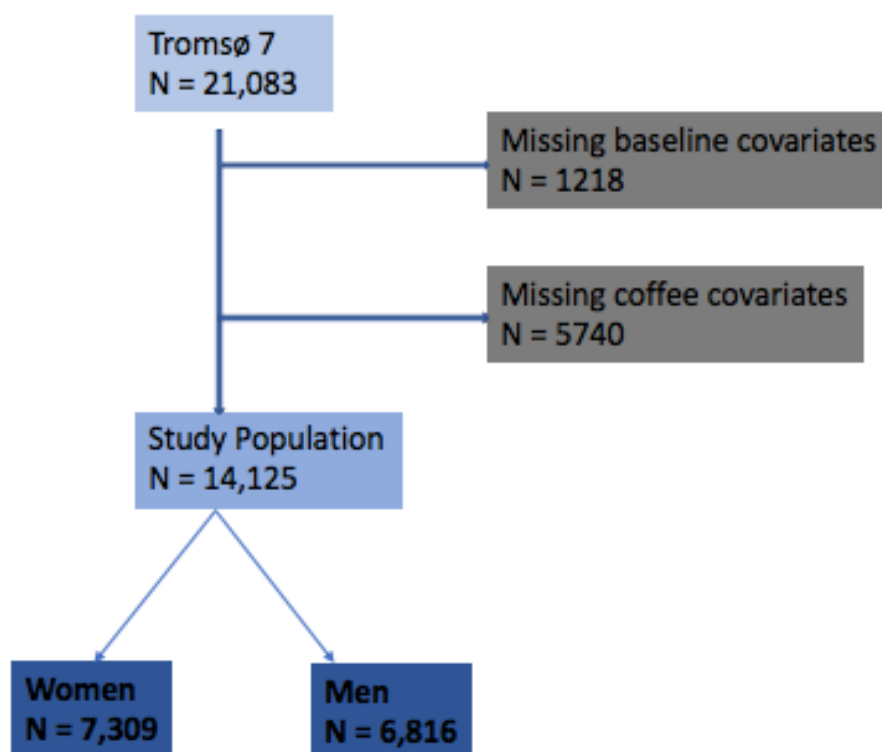
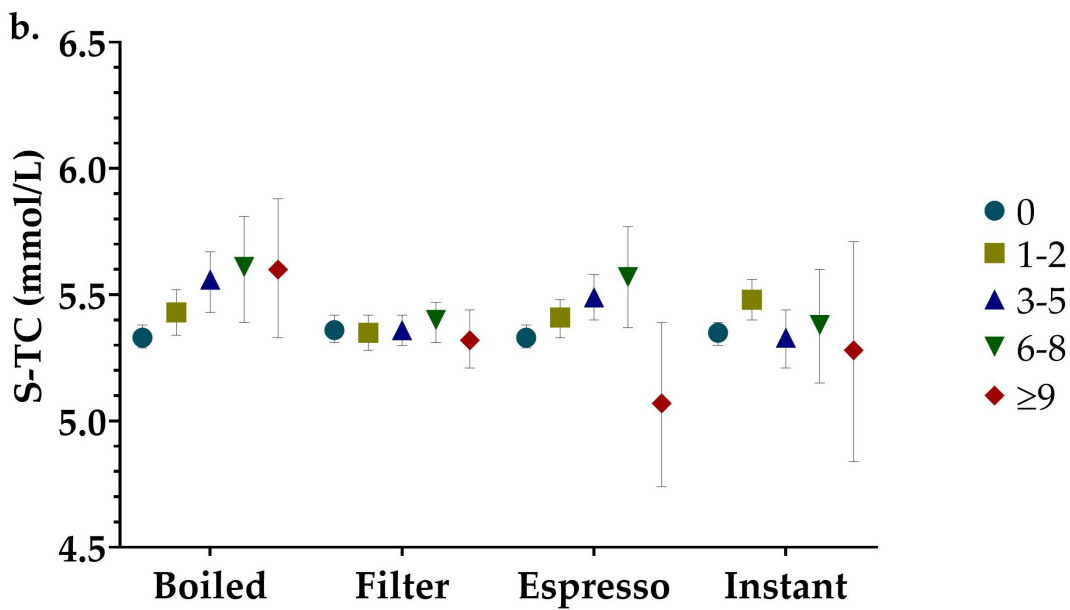
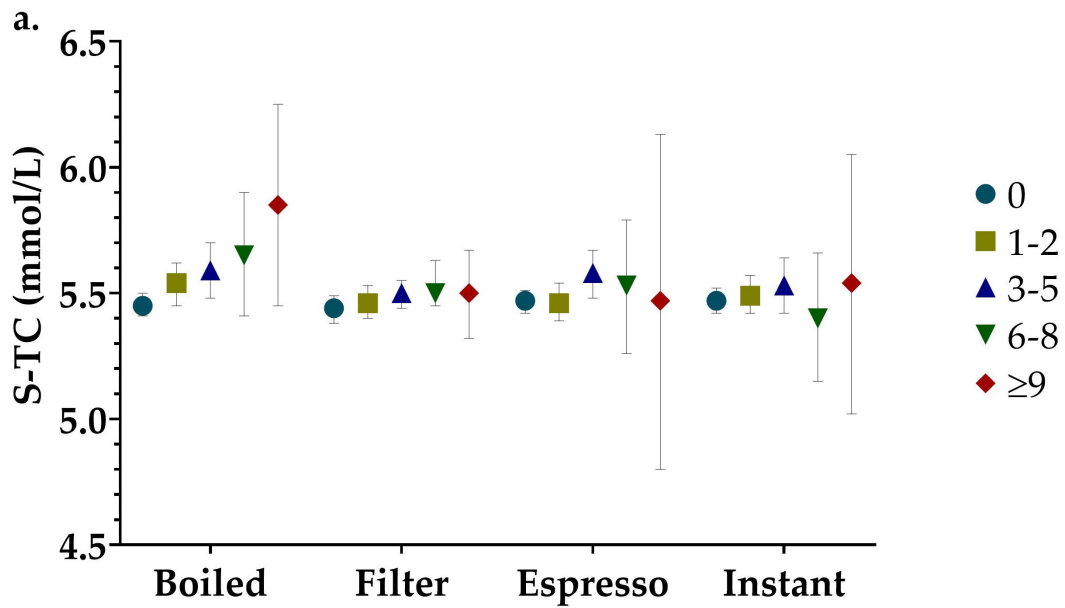


Figure 3: Association between coffee consumption and serum total cholesterol levels (S-TC) in various coffee brews. Adjusted for age, BMI, level of education, and level of physical activity. a. Women; b. Men. The Tromsø Study 2015-2016.



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## Appendix

<p><b>Reference:</b> Grioni S, Agnoli C, Sieri S, Pala V, Ricceri F, Masala G, et al. (2015) Espresso Coffee Consumption and Risk of Coronary Heart Disease in a Large Italian Cohort. PLoS ONE 10(5): e0126550. doi:10.1371/journal.pone.0126550</p>			<p><b>Study design:</b> Cohort study</p>	
			<p><b>Grade - quality</b> <span style="background-color: #90EE90;">Moderate</span></p>	
Objective	Material and methods	Results	Discussion/comments	
<p>The objective of this study was to examine the association between Italian-style (espresso and mocha) coffee consumption and CHD risk.</p>	<p>In the period of 1993-1998, 47,021 volunteers from all regions of Italy was recruited EPICOR, a prospective cohort study to investigate the risk of CVD. Exclusion criteria were; a prior history of stroke or MI, not completing the questionnaires, missing values of covariates, drinking &gt;15 cups per day, and those with an energy intake-BMR ratio in the first and last half percentiles. A total of 43,249 volunteers age 35-75 were included, and the mean follow up time was 10.9 years. 1472 people were randomly selected for a case-cohort study nested in the EPICOR population. Participants completed a validated semi-quantitative food frequency questionnaire (FFQ). Information about medical history and socioeconomic variables was collected using a standardized questionnaire. Measurements and a fasting blood sample was taken. Electronic hospital discharge records and mortality files were linked to the study database to identify incident and fatal cases of CHD using the ICD 9<sup>th</sup> edition codes. Coffee consumption was divided into four groups, from &lt;1 cup/day to &gt;4 cups/day. ANOVA was used for continuous variables and X<sup>2</sup>-test for categorical variables. In 1472 volunteers, the association of coffee intake with cholesterol levels was also examined.</p>	<p>Those who drank &gt;4 cups of coffee per day were younger, more likely to smoke, have high energy intake, have high fruit and vegetable intake, have low tea intake and be less likely to have a BMI &lt;25 and hypertension. CHD was significantly higher than reference (&lt;1 cup per day) for coffee consumption &gt;2 cups/day, with HRs of 1.37 (95% CI 1.03-1.82) for &gt;2-4 cups/day and 1.52 (95% CI 1.11-2.07) for &gt;4 cups/day. In a randomly selected sub-cohort, coffee consumption was not significantly associated with plasma levels of total, LDL or HDL cholesterol, or triglycerides.</p>	<p><i>Were the groups comparable with regards to important background factors?</i> Yes.  <i>Are the groups recruited from the same population?</i> Yes.  <i>Were the exposed individuals representative for a defined population?</i> Unknown, as they were recruited from blood donor centres and women's screening centres.  <i>Was the study prospective?</i> Yes, but they only looked at baseline characteristics and the endpoint CVD.  <i>Was the exposition and the results measured equally and reliably in the two groups?</i> Yes.  <i>Was the study population big enough? Was an analysis of the fall out number conducted?</i> Yes. Analysis of fall-out number not needed because they obtained data from the hospitals.  <i>Was the follow up period for a long enough time to show positive and/or negative results?</i> Yes, 10 years.  <i>Are important confounding factors taken care of in the study design and/or conduction of the study?</i> Yes, they had adjusted for both physical and socioeconomic factors.  <i>Is the one who made judgements of the results (end points) blinded with regards to groups?</i> Unknown.  <b>Strengths:</b>                      Its prospective design. The Italians almost exclusively drink Italian-style coffee, thereby reducing confounding due to variation in preparation method.  <b>Weaknesses:</b>                      The recruited population might not be fully representative of the general population. Plasma lipid profile was available only for a random subsample which may not be representative of the cohort as a whole.  <i>Is there other literature that strengthens/weakens the results?</i> Both yes and no, but this study is unique regarding to Italian-style coffee.  <i>Do the results have plausible biological explanations?</i> Yes, perhaps the lower volume of coffee per cup, and the short time frame the beans and water is in contact.</p>	
<b>Conclusion</b>				<p>Consumption of over 2 cups/day of Italian-style coffee is associated with increased CHD risk but was not associated with plasma lipid changes. The adverse effect of consumption appears unrelated to lipid profile.</p>
<b>Country</b>				Italy
<b>Year of collection of data</b>				1993-2008

<b>Reference:</b> Bønaa K, Arnesen E, Thelle DS, Førde OH. Coffee and cholesterol: is it all in the brewing? The Tromsø Study. <i>BMJ</i> (Clinical research ed). 1988;297(6656):1103-4.			<b>Study design: Cross-sectional study</b>
			<b>Grade - quality</b> <span style="background-color: #90EE90;">High</span>
<b>Objective</b>	<b>Material and methods</b>	<b>Results</b>	<b>Discussion/comments</b>
The objective of this study was to explore the possibility that the method of brewing might be important to the relation between coffee and serum cholesterol concentrations.	Data from the 3 <sup>rd</sup> survey of the Tromsø Study (1986-1987) was used. 21,826 people in the municipality of Tromsø (81.3% of the eligible population) were screened. The analysis was restricted to men and menstruating women aged 20-59 years (n=18012).	Boiled coffee was the most commonly consumed brew of coffee (68% of both sexes). Filtered coffee was the next most popular (23% of the men and 20% of the women). The concentration in the men who drank nine or more cups of coffee a day (all types of brew) was 0.52 mmol/L (10%) higher than that in the men who did not drink coffee at all. The corresponding figure in women was 0.40 mmol/L (8%). For those who drank mainly boiled coffee the increase between those who drank less than one and those who drank nine or more cups was 0.61 mmol/L (11%) in men and 0.40 mmol/L in women. No statistical trend was observed for the other methods of brewing. There was also no correlation between consumption of coffee and serum concentrations of HDL cholesterol and triglycerides. Except for age, coffee was the most important determinant of serum cholesterol concentration in this study. These findings support the suggestion that coffee causes the cholesterol concentrations to increase.	<p><i>Is the aim of the study clearly formulated?</i> Yes.</p> <p><i>Who is included/excluded?</i> Included: Participants in the municipality of Tromsø 20-59 years. Excluded: non-menstruating women.</p> <p><i>What are the results?</i></p> <p><i>Are the results comparable to the actual population?</i> Yes, that is likely. Because of the high attendance rate.</p> <p><b>Strengths:</b> A high attendance rate and a high number of participants. Gathering of new information.</p> <p><b>Weaknesses:</b> Can be hard to draw conclusions from cross sectional studies.</p> <p><i>Is there other literature that strengthens/weakens the results?</i> Yes, definitely.</p> <p><i>Do the results have plausible biological explanations?</i> Yes. We now know that coffee beans contain the diterpenes cafestol and kahweol. These are filtered out in filter coffee, but not in boiled coffee. Therefore, drinking boiled coffee increases serum LDL cholesterol.</p>
<b>Conclusion</b>	They answered questions on how many cups of coffee and what type of coffee they usually drank each day. Total cholesterol concentration was measured. The analysis consisted of the mean serum cholesterol concentrations for each sex according to consumption of coffee and method of brewing adjusted for the influence of age, BMI, cigarette smoking, physical activity in leisure time, and salt and fat intakes.		
<b>Country</b>	Norway		
<b>Year of collection of data</b>	1986-1987		



<b>Reference:</b> Rendon MY, Dos Santos Scholz MB, Bragagnolo N. Physical characteristics of the paper filter and low cafestol content filter coffee brews. Food research international (Ottawa, Ont). 2018;108:280-5.			<b>Study design:</b> Cross sectional study
			<b>Grade - quality</b> High
Objective	Material and methods	Results	Discussion/comments
The objective of this study was to evaluate the diterpene concentrations in filter coffee brews prepared with paper filters of different sizes, colours and origins.	A single coffee sample was used, as the objective was to compare the efficiency of the commercially available paper filters. The coffee beans were selected, roasted and grounded carefully and is well described in the method. The coffee brew was prepared using a 1:10 proportion of ground roasted coffee to water. After the filtering process, the brews were frozen, freeze dried to constant weight and stored at 5 degrees Celsius until analysed. The diterpene concentration in a non-filtered brew, which served as the standard to compare with the filter coffees, was found by preparing a brew using a French press under the same preparation conditions. Five brews were prepared with each type of filter. The diterpenes were isolated and separated. Various brands of filters from various countries were evaluated. Fat permeation rates, air permeance and grammage was determined in the various filtered. Results were analysed by one-way ANOVA.	Cafestol concentrations in the raw and roasted coffees showed no significant difference. The diterpene concentrations of the roasted coffee were higher than in previous studies. The difference can be justified by the environmental and agronomic conditions in the production zones, as well as the harvest and roasting process. The cafestol and kahweol concentrations in the brews varied from 1.62 to 2.98 mg/L and 0.73 to 1.95 mg/L, respectively. The highest concentrations were obtained using filters with micro perforations. The filters showed high fat permeability. The diterpene retention capacities of the filters produces in the different countries were similar. The porosity of the paper filter and the particle size of the ground roasted coffee were determinant factors in obtaining filter coffee brews with lower cafestol contents.	<p><i>Was the exposition and the results measured equally and reliably in the groups? Yes.</i></p> <p><i>Are the methods used when the results were put together clearly described? Yes.</i></p> <p><i>Were the results from the studies put together in a reasonable way? Yes.</i></p> <p><i>Are the authors conclusions supported by data and/or the analyse that is reported in the overview? Yes.</i></p> <p><i>How would you range the scientific quality in this study? High scientific quality.</i></p> <p><b>Strengths:</b> Detailed explanation of methods, including choice of coffee bean, roasting, grounding, extraction. Good use of other literature and comparing results to what others have done.</p> <p><b>Weaknesses:</b> The nature of various coffee beans having various diterpene concentrations makes it harder to compare results. Could be clearer of the practical meaning of their results.</p> <p><i>Is there other literature that strengthens/weakens the results? Yes, mostly strengthens.</i></p> <p><i>Do the results have plausible biological explanations? Yes, or at least plausible physical explanations.</i></p>
<b>Conclusion</b>			
The porosity of the paper filter and the particle size of the ground roasted coffee were determinant factors in obtaining filter coffee brews with lower cafestol contents.			
<b>Country</b>			
Brazil			
<b>Year of collection of data</b>			
2018			

<b>Reference:</b> D'Amicis A, Scaccini C, Tomassi G, Anaclerio M, Stornelli R, Bernini A. Italian style brewed coffee: effect on serum cholesterol in young men. <i>Int J Epidemiol.</i> 1996;25(3):513-20.			<b>Study design:</b> RCT
			<b>Grade - quality</b> High
<b>Objective</b>	<b>Material and methods</b>	<b>Results</b>	<b>Discussion/comments</b>
The objective of this study was to assess whether there is an association between Italian style brewed coffee (espresso and mocha) and serum cholesterol levels in men.	84 normolipidemic habitual male coffee drinkers were recruited from the army. They were divided into three groups: for 6 weeks 1) replacing coffee for tea; 2) drinking only espresso, 3) drinking only mocha. Before this they had a 3-week baseline where they consumed their normal amounts of coffee. Exclusion criteria were hyperlipidaemia, hypertension, diabetes or ischaemic disease. Serum cholesterol was measured eight times. The two coffee groups were asked to drink their habitual amount of coffee. Statistical analysis was done by one-factor and two-factor ANOVA.	Changes observed in serum cholesterol concentrations between baseline and intervention were not statistically different in all groups. The changes were 0.00 mmol/L (tea), +0.10 mmol/L (espresso) and +0.05 mmol/L (mocha) for total serum cholesterol; 0 mmol/L (tea), -0.02 mmol/L (espresso) and -0.03 mmol/L (mocha) for HDL-C; -0.13 mmol/L (T), +0.02 mmol/L (espresso) and -0.05 mmol/L (mocha) for LDL-C. Serum triglycerides showed a significant increase during intervention ( $P < 0.01$ by ANOVA) in all groups with a change of 0.18 mmol/L, 0.18 mmol/L and 0.22 mmol/L, for tea, espresso and mocha group respectively	<p><i>Is the aim of the study clearly formulated?</i> Yes.</p> <p><i>Who is included/excluded?</i> Normolipidemic young men from the Italian medical school army was recruited. Those with ischaemic heart disease, hypertension or diabetes was excluded.</p> <p><i>What are the results?</i> There is no association between Italian brewed espresso and serum cholesterol levels.</p> <p><i>Are the results comparable to the actual population?</i> Maybe. It depends if women, older or comorbid people have a different response to diterpenes than young male.</p> <p><b>Strengths:</b> Randomisation and keeping the other factors constant. Using participants from a military school creates a controlled environment.</p> <p><b>Weaknesses:</b> Only using young men. Letting them use their habitual coffee consumption makes it unclear whether a heavy drinking pattern would raise cholesterol</p> <p><i>Is there other literature that strengthens/weakens the results?</i> Both yes and no. A large Italian cohort showed no association. The present study shows an association in women. Level of consumption probably has a lot to say.</p> <p><i>Do the results have plausible biological explanations?</i> We now know that coffee beans contain the diterpenes cafestol and kahweol, and that espresso has a medium level of diterpenes. It is plausible that a moderate consumption leads to non-significant changes in serum cholesterol.</p>
<b>Conclusion</b>			
Coffee brewed the Italian way does not alter serum cholesterol levels in men.			
<b>Country</b>			
Italy			
<b>Year of collection of data</b>			
1996			

<b>Reference:</b> Strandhagen E, Thelle DS. Filtered coffee raises serum cholesterol: results from a controlled study. European journal of clinical nutrition. 2003;57(9):1164-8.			<b>Study design: Prospective controlled study</b>
			<b>Grade - quality</b> High
<b>Objective</b>	<b>Material and methods</b>	<b>Results</b>	<b>Discussion/comments</b>
The objective of this study was to assess the effects of the intake and abstention of filtered coffee on blood lipids.	The study design was organised as a prospective, controlled study with four consecutive trial periods. The first and third periods were 3 weeks of total coffee abstention. The second and fourth periods consisted of 4 weeks with the subjects consuming 600 mL filter brewed coffee/day.	Coffee abstention for 3 weeks decreased total serum cholesterol by 0.22-0.36 mmol/L. A volume of 600 mL (four cups) of filtered coffee per day for 4 weeks raised total serum cholesterol by 0.15-0.25 mmol/L.	<p><i>Is the aim of the study clearly formulated?</i> Yes.</p> <p><i>Who is included/excluded?</i> Inclusion criteria: age 30-65 y, free of clinically recognised chronic diseases, not on antiepileptic or cholesterol-lowering drugs, had been using coffee on a regular basis for at least 5 y and were currently non-smokers.</p> <p><i>What are the results?</i> Intake of filtered coffee increases serum cholesterol.</p> <p><i>Are the results comparable to the actual population?</i> Yes, probably. In this study they consumed four cups of coffee per day.</p> <p><b>Strengths:</b> Randomisation. Giving participants the same type of coffee grounds, filter and amount of consumption per day.</p> <p><b>Weaknesses:</b> None, but it would have been interesting to have direct comparison with other brews of coffee.</p> <p><i>Is there other literature that strengthens/weakens the results?</i> Yes, both literature that strengthens and weakens the results.</p> <p><i>Do the results have plausible biological explanations?</i> Yes, but as other literature shows that most diterpenes are retained in the filter, it is fascinating how some literature show the association between filtered coffee and serum cholesterol.</p>
<b>Conclusion</b>	Inclusion criteria: age 30-65 y, free of clinically recognised chronic diseases, not on antiepileptic or cholesterol-lowering drugs, had been using coffee on a regular basis for at least 5 y and were currently non-smokers. All participants got the same filter and coffee grounds. 116 participants completed the study.		
Filtered coffee raises total serum cholesterol levels.			
<b>Country</b>			
Sweden			
<b>Year of collection of data</b>			
2003			

