COFFEE AND CARDIOVASCULAR RISK;

AN EPIDEMIOLOGICAL STUDY

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Pieter-Jan Kersbergen

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Promotor:	Prof. Dr. A. Hofman
Co-promotor:	Dr. D.E. Grobbee
Overige leden:	Prof. Dr. J.C. Birkenhäger
	Prof. Dr. J.F. Koster
	Prof. D.S. Thelle

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Voor Jeroen

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Chapter 6.3	Bak AAA, Van Vliet HHDM, Grobbee DE. Coffee, caffeine and hemostasis; results from two randomized studies. Atherosclerosis (in press).
Chapter 7.2	Bak AAA, Grobbee DE. Caffeine, serum lipids and blood pressure; results from a double blind study (submitted).

CHAPTER 1

INTRODUCTION

The uniformity that we have achieved with drugs still does not apply to our food and drink. We continue to enjoy poorly characterized infusions of roasted and ground coffee beans, and, indeed, still scrutinize the health implications of this practise (New Engl J Med 1984;310;783).

1. INTRODUCTION

Medicine's accusing finger, habitually wagging at life's pleasures, has not bypassed coffee. Although a comparatively recent arrival on the pleasure scene - it's use became fashionable in the Western world less than three centuries ago - coffee competes successfully with the older pleasures of the table, the bed and the wine cellar as a target of medical attacks (1).

A positive association of coffee consumption with coronary heart disease was first suggested by Paul et al. in 1963, on the basis of a prospective follow-up study (2). In subsequent years, this finding was both affirmed and contradicted by others, as reviewed in chapter 2.

In 1983, Thelle and co-workers reported a positive link between serum cholesterol concentration and coffee intake in 7368 men and 7213 women aged 20-54 years, living in Tromsø. The association remained highly significant after adjustment for several covariates, including degree of adiposity, alcohol intake and cigarette smoking. The effect was substantial, men who drank nine or more cups per day had cholesterol levels that were on average 0.67 mmol/l higher than in non-drinkers (3). This finding stimulated many investigators to reanalyse cross-sectional data to assess the association between coffee and serum lipids. These studies, carried out by different investigators, in different places at different times were re-analysed in a uniform way to enable comparisons, and explore the reasons for differences in results between studies. This meta-analysis on coffee and serum lipids was supplemented by an analysis of data on coffee and blood pressure from cross-sectional studies (chapter 3).

The strong association between coffee and serum cholesterol levels observed in Norway, as compared to other Western-European countries, soon pinpointed the coffee brewing method as a possible explanation for the inconsistent findings in this field. The majority of the Tromsø residents brew their coffee by boiling, without using any type of filter. Some preliminary experiments using boiled coffee, were consistent with the Tromsø cross-sectional findings. In order to assess the effect of coffee consumption on serum lipid levels, and the part played by the brewing method, a large randomized study was conducted. In the same experiment, effects on blood pressure were investigated. The results are described in chapters 4 and 5.

Although literally hundreds of chemicals have been identified in coffee, caffeine is by far the best known and best studied substance (4). Moreover, it is the coffee-constituent with the worst reputation to the extent that, to quote Grady: "... if caffeine isn't bad for you, it should be" (5). Acute studies on blood pressure quite consistently show a temporary pressor effect of caffeine in caffeine-naive subjects. Information on the long-term, chronic effects of caffeine was lacking for blood pressure as well as for

serum lipids. These issues were addressed in a double-blind randomized trial, as described in chapter 6.

Finally, the fascinating field of hemostasis was entered, as a logical next step in the study of coffee and cardiovascular risk. Chapter 7 comprises a review of the available literature on coffee and hemostasis and the findings on selected hemostatic parameters in our own experiments on coffee and caffeine.

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CHAPTER 2

COFFEE AND CARDIOVASCULAR DISEASE

In a 1967 issue of Nature, tea was reported to protect against atherosclerosis. The evidence was based on comparing autopsy data of an overt coffee drinking population (Americans) and an overt tea drinking population (Chinese). The coffee drinkers had a higher degree of atherosclerosis (Nature 1967;216:1015-6).

2. COFFEE AND CARDIOVASCULAR DISEASE

Introduction

The association between coffee and cardiovascular mortality and morbidity has been debated for several decades. In 1963, Paul and coworkers observed a positive link between coffee use and cardiovascular disease in a cohort study (1). This report was followed by many others, providing both confirmation and refutation of the hypothesis that coffee consumption increases the risk of a cardiovascular event (2-26). Tables 2.1 and 2.2 summarize the main characteristics and results of the studies on coffee and fatal and non-fatal cardiovascular disease, including 17 studies with a cohort design and 10 case-control studies. The last column in table 2.1 reflects the overall conclusion of the investigators. The discrepancy between the findings may result from issues such as study design, choice of control group in case-control studies, recall bias and time interval between coffee intake assessment and cardiovascular event. Moreover, coffee consumption has been related to a number of cardiovascular risk indicators, such as smoking, dietary fat intake and low physical activity (27). Inadequate adjustment for these confounding variables may affect the results of a study on coffee and cardiovascular disease. In the following paragraphs the available studies will be discussed, with special emphasis on methodological aspects.

Design and bias

The relationship between coffee and cardiovascular disease has been investigated in both cohort studies and case-control studies with inconsistent results. Most case-control studies show an increased risk of myocardial infarction in heavy coffee consumers (3,4,10,12,21-23). By contrast, most cohort studies strongly suggest the absence of a link between coffee and cardiovascular disease (6,7,9,10,11,13,14,16,19,24,26). To complicate the issue, however, some other case-control studies show no increased risk for coffee consumers (2,5,8) and results of a few cohort studies support a positive association (1,17,20,25).

Recall bias

According to Dawber et al. from the Framingham study, prospective follow-up studies offer the best design to reach valid conclusions regarding the relation of any host or environmental factor to disease development (7). The cohort design reduces the possibility of biased reporting of coffee use or other characteristics. In a case-control study, cases may systematically overreport coffee-consumption because they are aware of a possible link between coffee and myocardial infarction and as a result the effect will be overestimated. Alternatively, cases may underreport smoking relative to controls, possibly caused by feelings of guilt, and again overestimation of the effect will result, since part of the apparent effect of coffee is confounded by heavier smoking in the

cases. Wilhelmsen proposed that the psychological impact of the experience of a myocardial infarction influences the patients dietary reporting (10). The possibility that questions about coffee use in the period prior to hospitalization are subject to recall bias is difficult to exclude. For this reason, positive findings in case-control studies always need careful interpretation. In the case-control study of Hennekens et al. the cases died of coronary heart disease. Wives of cases and controls were asked about coffee consumption habits of their husbands. If one considers coffee drinking unhealthy, wives of patients might have overestimated their husbands' coffee consumption, whereas wives of controls might have underestimated, resulting in overestimation of a fatal effect of coffee use. The investigators, however, reported no increased risk of coffee use on fatal myocardial infarction (8).

Hospital controls

Two early case-control studies performed in Boston showed increased risk of non-fatal myocardial infarction with increased coffee consumption (3,4). The results, however, are often criticised to be distorted by the inclusion of hospitalized patients with chronic conditions among the controls, resulting in overestimation of the effect. Rosenberg and co-workers expressed their concern of inclusion of an excessive number of subjects with gastro-intestinal or other diseases in which coffee drinking has been either abandoned or medically proscribed. Among hospitalized women, those admitted for chronic conditions reported significantly less coffee consumption than those admitted for acute conditions (28). In another study, coffee use in hospitalized subjects was compared with coffee consumption in the general population. Those hospitalized for gastro-intestinal disorders and conditions such as diabetes mellitus, rheumatic heart disease, chronic lung disorders, cardiovascular disease and chronic nephritis, were consuming less coffee than population controls (29). The results indicate that the referent group in hospital based case-control studies on coffee and cardiovascular disease should be restricted to patients hospitalized for conditions not affecting dietary habits. Otherwise, an association observed in a case-control study might be due to decreased coffee consumption among controls rather than to an excessive consumption among the cases. The group of Rosenberg performed several case-control studies, in which controls were hospital admitted for conditions unrelated to coffee consumption such as traumatic injury and non-respiratory infections (12,21,22). Conditions of recent and rapid onset are unlikely to have influenced coffee consumption in the period before admission. By contrast, Jick reanalyzed the Boston results and concluded that coffee drinking habits were not different between acute and chronic patients and thus the results were unaffected by the inclusion of patients with chronic conditions in the control series (30). The choice of hospital controls is an important methodological issue, potentially leading to biased results. The principle issue, however, is the comparability of cases and controls with respect to the accuracy of information of the determinant under study (31).

Table 2.1. Studies on coffee and fatal and nonfatal cardiovascular disease. The last column reflects the opinion of the authors on the association between coffee and cardiovascular risk.

Ref.	*Year publ.*	*First Author*	*Design*	*Size n or c/c*	*Years of follow-up*	*Relative risk estimate*	*Statistical significance*	*Endpoints*	*Authors conclusion*
1	63	Paul	cohort	1162	4	1.3 5 cups/day	< 0.025	all chd	present
2	65	Little	cc	86/84	0	1.0 ?	?	nonfatal mi	absent
3	72	BCDSP	cc	276/1104	0	1.3 1-5 cups/day		nonfatal mi	present
						2.1 6+ cups/day	< 0.003 trend		
4	73	Jick	cc	440/123190	0	1.6 1-5 cups/day	< 0.001	nonfatal mi	present
5	73	Klatsky	cc	464/928	0	1.0 6+ vs. < 6	?	nonfatal mi	absent
6	73	Hrubec	cohort	10744	?	1.3 1-5 cups/day		angina	dubious
						1.5 6+ cups/day	0.06 trend		
7	74	Dawber	cohort	4492	12	1.0 any level	?	mortality	absent
						1.0 any level	?	all chd	absent
8	76	Hennekens	cc	649/649	0	1.2 1-5 cups/day	ns	fatal mi	absent
						1.0 6+ cups/day	?		absent
9	77	Yano	cohort	7705	6	? 5+ cups/day	ns	all chd	absent
10	77	Wilhelmsen	cohort	846	12	?	?	all chd	absent
			cc	220/846	0	?	"significant"	nonfatal mi	present?
11	78	Heyden	cohort	2530	4.5	1.0 5+ vs. < 5	?	mortality	absent
						1.0 5+ vs. < 5	?	fatal chd	absent
						1.0 5+ vs. < 5	?	fatal cva	absent
12	80	Rosenberg	cc	487/980	0	1.4 5+ cups/day	0.05	nonfatal mi	present?
13	81	Murray	cohort	16911	11.5	1.0 any level	?	fatal chd	absent
						0.9 7+ cups/day	0.01 trend	non-chd death	artificial
14	84	Welin	cohort	855	17	? any level	ns	all chd	absent
			cohort	6500	3-7	? any level	ns	all chd	absent
						? any level	ns	mortality	absent

continued...

Table 2.1 (cont.).

Ref.	*Year publ.*	*First Author*	*Design*	*Size n or c/c*	*Years of follow-up*	*Relative risk estimate*	*Statistical significance*	*Endpoints*	*Authors conclusion*
15	84	Kahn	cohort	27,530	21	1.2 3+ vs. < 1	ns	mortality	absent
16	86	Jacobsen	cohort	16,555	11.5	0.9 7+ vs. < 2	ns	all chd	absent
17	86	LaCroix	cohort	1,130	19-35	1.8 5+ cups/day	ns	all chd	absent
					< 5	2.5 5+ cups/day	< 0.05	all chd	present
18	86	Vandenbroucke	cohort	1,583 (m)	25	1.4 5+ cups/day	± 0.10	mortality	present
				1,508 (f)		0.8 5+ cups/day	ns		absent
19	87	Yano	cohort	7,194	15	1.2 no/coffee	ns	all chd	absent
20	87	LeGrady	cohort	1,910	19	1.3 6+ vs. < 6	< 0.05	mortality	dubious
						1.7 6+ vs. < 6	< 0.05	fatal chd	present
21	87	Rosenberg	cc	491/1,119	0	0.8 1-4 cups/day	ns	non-fatal mi	
						2.1 5+ cups/day	0.05		present
						1.0 1-4 c/d: caf	?		
						1.6 5-9 c/d: caf	0.05		
						2.1 10+ c/d: caf	< 0.05		
						1.3 1-4 c/d: decaf	ns		
						1.2 5+ c/d: decaf	ns		
22	88	Rosenberg	cc	1,873/1,161	0	1.4 1-2 cups/day	< 0.001 trend	nonfatal mi	present
						1.6 3-4 cups/day			
						1.8 5-9 cups/day			
						2.9 10+ cups/day			
23	89	LaVecchia	cc	262/519	0	1.7 4+ cups/day	0.02 trend	nonfatal mi	present
24	89	Wilson	cohort	5,209	10-20	1.0 per cup	ns	all cvd	absent
25	89	Tverdal	cohort	38,564	6.4	2.2 9+ vs.< 1: m	< 0.05	fatal chd	present
						5.1 9+ vs.< 1: f	ns		present

chd = coronary heart disease, mi = myocardial infarction, cva = cerebrovascular accident, cvd = cardiovascular disease, cc = case control

Table 2.2. Variables adjusted for in the analysis of 25 studies on coffee and fatal and nonfatal cardiovascular disease.

Study (ref)	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*
Age		+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+
Body mass index								+										+			+	+	+	+	
Sex			+	+	+									+	+						+	+	+		
Smoking			+		+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+
Alcohol								+		+								+				+	+		
Diabetes mellitus		+						+				+			+						+	+	+		
Hypertension		+						+				+			+		+				+	+	+		
Hypercholesterolemia								+				+									+		+		
Physical activity								+														+			
Systolic BP					+					+				+				+						+	+
Diastolic BP					+															+						
Serum cholesterol					+					+				+			+	+		+	+			+	+
ECG abnomalities					+																				
History MI				+				+							+							+			
Oral contraceptives												+											+		
Season								+																	
Residence			+					+				+	+			+					+	+			
Ethnicity					+																				
Religion								+														+			
Coffee additives								+																	
Tea consumption								+																	
Occupation								+																	
Type A personality																						+			

Follow-up time

In a study of Schreiber and co-workers coffee drinking habits were found to vary significantly over time. A random sample of 2,714 US citizens were asked to compare present caffeinated coffee drinking with consumption in the 10 preceding years. 55 % had changed, with 38 % currently drinking less and 17 % drinking more than they had 10 years earlier (32). These results suggest that past coffee and caffeine consumption habits may be poor surrogates for current intake and if it is recent or cumulative use of coffee that affects the risk, a single measure in the distant past is unsatisfactory and could result in underestimation of an effect. This is supported by the relatively consistent absence of coffee effects on cardiovascular disease in studies where the intake was assessed long before the coronary events. The positive finding of LeGrady et al. in a study with a follow-up time of 19 years is an exception (20). The limitation of inadequate consideration of changes in coffee consumption over time was overcome by LaCroix et al. by measuring coffee drinking and smoking repeatedly during a long-term cohort study of medical students (17). The positive association observed, was strongest when the time between reported coffee intake and the coronary event was shortest. The relative risk of coronary heart disease for consumers of 5 cups or more per day compared to non-consumers was observed to be 1.8 (n.s.) with coffee intake assessed 19 to 34 years before the coronary event and 2.5 (95 % CL 1.1, 5.8) with coffee consumption assessed less than 5 years ago (17). By contrast, several authors emphasized the need in follow-up studies on coffee and mortality for exclusion of deaths occurring shortly after the coffee consumption is reported (13,16). In two cohort studies mortality in the first years of follow-up related strongly to coffee drinking habits; those with low coffee consumption having higher than expected mortality (13,16). Apparently, patients with chronic diseases and a higher risk of dying tend to have lower coffee consumption than the general population.

Confounding

In a cross-sectional study of 14,582 men and women, coffee drinking was negatively related to the use of low-fat milk, use of table fat high in poly-unsaturated fatty acids, use of fruits and vegetables, and positively associated with bread consumption. Three persons out of four with high coffee consumption (> 8 cups per day) were daily smokers, in contrast to about a quarter of those with low coffee consumption (<1 cup per day). In women and young men, high coffee consumption was associated with low physical activity at leisure. These data suggest that high coffee consumption may be an indicator of a lifestyle with high risk of coronary heart disease (27). Clustering of risk factors was also found by others (33,34). Hennekens proposed that the Boston investigators reporting a positive association between coffee use and nonfatal myocardial infarction did not control for enough variables. In their own case-control

study they demonstrated a nearly two-fold increase in risk of death from ischemic heart disease in heavy coffee drinkers when controlling only for those variables used in the Boston study, but when they controlled for additional variables, such as physical activity and coffee additives, no increase in risk was found (8). Rosenberg et al. emphasized the need for studies with detailed information on dietary factors that are possibly related to coffee consumption and cholesterol levels (22). An atherogenic diet was indeed shown to be more common among men who drink a great deal of coffee than among other men (35). Other investigators suggested the possibility that coffee consumption is a marker of stress (17,36). Highly stressed subjects may drink considerable amounts of coffee during the day. In a study of 2,714 white US adults, however, of 32 risk factors analysed by linear and logistic regression, only sex and cigarette smoking were found to be important potential confounders of the relationship between coffee intake and disease (37). The significant correlation between coffee consumption and the later development of ischemic heart disease observed by Paul et al. in 1968 was later demonstrated to be entirely accounted for by tobacco use (38). This observation is shared by many other investigators (7,9,11,15,18,22). In the study of LeGrady, however, an association between coffee and fatal coronary heart disease was present in both smokers and non-smokers (20). It seems important to consider changes over time in smoking (17). In populations with growing numbers of subjects who have quit smoking, the measure of smoking at baseline may greatly overestimate actual exposure to cigarettes as follow-up time increases (39). According to LaCroix et al., some investigators did not find an association, because the comparison group in their studies comprised a substantial number of coffee drinkers, e.g. persons consuming less than 5 cups per day (11), rather than the completely unexposed segment of the cohort of non-drinkers alone (17). By contrast, Rosenberg et al. argued that the health related behavior of men who never drink coffee may differ in important ways, that are difficult to measure, from that of men who drink coffee (22). Thus, a comparison of coffee drinkers with never drinkers may well overestimate an adverse effect of coffee on the risk of myocardial infarction because of the inability to control completely for such behavior. Therefore, it is more convincing if a statistically significant trend is observed over the entire range of coffee consumption categories. On the other hand, the possibility of a threshold phenomenon, where no effect is present until a certain level of the exposure is reached, cannot be ruled out. In this view, a very high coffee intake may increase an individual's risk (3,4,21,25).

Biological plausibility

Only a few papers on coffee and cardiovascular disease discuss the mechanism of the potential connection (16,17,23,26,40). The most plausible biological explanation is a positive link between coffee intake and serum cholesterol level or blood pressure.

Alternatively, coffee may induce cardiac arrhythmias (41,42). These variables, potentially in the causal pathway from coffee to cardiovascular disease are frequently included in the multivariate analyses (5,8,10,12,14,17,18,20,21,23-25). This may lead to overadjustment of the risk estimate (22,43,44). From both non-experimental studies and randomized trials, boiled coffee was shown to raise serum cholesterol levels (45-48). The cholesterol increasing effect of coffee is probably mediated by brewing method and seems to be confined to methods where no filter is used, such as boiled coffee, Turkish coffee, espresso and percolated coffee. Therefore, the positive association between coffee and non-fatal myocardial infarction in an Italian study may be explained by the serum cholesterol increasing effect of espresso coffee (23). Likewise, the positive results of some American studies might be explained by the use of percolated coffee (3,4,12,17,20-22). Given the strong association observed between coffee consumption and serum cholesterol level in the Norwegian population, it is remarkable that Jacobsen et al. did not find an association between coffee consumption and ischemic heart disease in a large cohort study (16). By contrast, a positive association between heavy coffee use and cardiovascular death was reported by Tverdal and colleagues (25). The relative risk estimate reached statistical significance in men, but not in women. The evidence for a blood pressure raising effect of coffee is inconsistent and, if present, only small (46,49-51). The study of Martin et al. comprised 10,064 diagnosed hypertensive individuals. During 4 years of follow-up, all-cause mortality and cardiovascular mortality did not change by level of caffeine consumption (40). In a double-blind, placebo-controlled study, a large dose of caffeine (300 mg) did not cause ventricular arrhythmias in 70 patients with a recent myocardial infarction (52).

Conclusions

The study of coffee and cardiovascular disease is complicated by many potential sources of bias. Early case-control studies linking coffee intake to myocardial infarction have not been consistently supported by several relevant case-control and prospective follow-up studies with diverse design features.

The main problem in case-control studies seems to be recall bias. Cases just recovering from a myocardial infarction, or partners of cases who just died of a coronary event, may overestimate coffee consumption and consequently, the risk estimate of coffee use may be too high. A careful choice of control subjects in case-control studies is of paramount importance. If the study is hospital-based, subjects with conditions leading to decreased coffee consumption should preferably be excluded from the control group. If not, the overall risk estimate for coffee use may be determined by diminished coffee consumption among controls rather than increased consumption among cases.

In a prospective cohort study, the interval between the measurement of coffee intake and the manifestations of coronary disease may be too long for an association to be

observed. Alternatively, the interval may be too short, resulting in high event rates among low coffee consumers. This can be explained by the observation that patients suffering from chronic diseases and a higher risk of dying soon, tend to drink less coffee than healthy subjects. Since coronary atherosclerosis is thought to develop gradually over a period of several years before it produces clinical symptoms it seems most accurate to evaluate the cumulative effect of coffee consumption over 5 years (22,53). This, however, would not apply to more acute effects of coffee, e.g. arrhythmias and hemostatic changes.

Coffee consumers may be at increased risk of a number of diseases, not because of coffee intake per se, but because of other aspects of their lives and lifestyle. Coffee consumption appears to be strongly related to smoking habits. Moreover, high dietary fat intake, low physical activity, psychological stress and other riskindicators for coronary heart disease are reported to be associated with heavy coffee use. These variables should be accounted for in the multivariate analysis of studies dealing with coffee and cardiovascular disease. Adjustment for potential intermediate factors such as serum cholesterol and blood pressure should be avoided.

Only four out of seventeen follow-up studies showed a positive association between coffee use and cardiovascular disease (1,17,20,25). The report of Paul et al. was followed several years later by a paper in which the positive findings were contributed to smoking habits among coffee drinkers (1,38). The prospective study of LaCroix et al. included only 51 cases of coronary heart disease and consequently, the confidence interval around the risk estimate was wide (RR 2.5, 95% CL 1.1, 5.8) (17). In the study of LeGrady et al, the relative risk of a fatal coronary event was 1.33 (1.07, 1.65) for men drinking 6 or more cups of coffee per day compared with those drinking less (20). The time interval between coffee intake assessment and coronary event was 19 years. Finally, positive findings were reported from a large Norwegian study (25). For heavy coffee consumers (more than 9 cups a day) relative to subjects drinking less than 1 cup per day, the relative risk was 2.2 (1.1, 4.5) for men and 5.1 (0.4, 60.3) for women.

In summary, most reliable results are to be expected from large, prospective follow-up studies with cumulative data on coffee consumption for about 5 years preceding the cardiovascular event. Other lifestyle features, associated with both coffee consumption pattern and cardiovascular risk, including smoking, dietary fat intake and physical activity, should be adjusted for in the analyses. The available data justify the conclusion that moderate coffee consumption is not likely to be a major risk factor for fatal and nonfatal cardiovascular disease.

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CHAPTER 3

COFFEE, SERUM LIPIDS AND BLOOD PRESSURE: A META-ANALYSIS OF CROSS-SECTIONAL STUDIES

Most researchers realize the need for stating their results in the context of previous studies, as evidenced by the literature review section in almost all scientific articles. Meta-analysis is a further development and refinement of this approach offering a more rigorous and coherent treatment of past research work (J Clin Epidemiol 1989;42:1021-4).

3.1 INTRODUCTION

Is there a link between coffee consumption and serum cholesterol levels and how does coffee use relate to blood pressure? The importance of these questions is obvious. Because coffee is widely used, even a small increase in serum cholesterol or blood pressure would have considerable public health consequences. The relationship between coffee and cardiovascular disease, however, is still subject of debate. Some reports suggest an increase in risk of cardiovascular disease with increasing coffee consumption (1-7). Most findings, however, do not support the hypothesis of a link between coffee use and risk of cardiovascular disease (8-18). The inconsistency in results might be explained by methodological differences between studies as discussed in chapter 2 of this thesis. The incomplete adjustment in some analyses for confounding variables such as smoking and dietary habits, may have resulted in false positive findings. Alternatively, some studies may have been too small, lacking power to detect a small effect of coffee on cardiovascular risk. While the presence or absence of a relationship between coffee use and cardiovascular disease has not been conclusively established, it is important to study the effects of coffee use on serum cholesterol levels and blood pressure.

Over the past two decades, a series of non-experimental studies on coffee and serum lipids has been published (19-44). Papers about coffee and blood pressure are more sparse (23,30,44-52). The present analysis was undertaken to provide a comprehensive assessment of the non-experimental evidence on the effect of coffee on serum cholesterol and blood pressure. Two narrative reviews of the available literature have left the discussion unsettled, both on the effect of coffee on serum lipids and on blood pressure (53,54). As the studies included in these reviews were conducted in different populations, in different countries, with different lifestyles and different ways of brewing coffee, it seemed worthwile to explore the importance of these differences through comparison of the magnitude of the coffee-effect across studies. Therefore, we performed a meta-analysis of cross-sectional studies on coffee, serum lipids and blood pressure.

In contrast to an original study, no new observations are gathered in meta-analytic research. In a meta-analysis, the individual as unit of observation is replaced by a summary result of an original study, which becomes the new unit of subsequent study (55). There are two basic approaches to the analysis of these data. Some global average or "typical" effect estimate can be calculated or, alternatively, the focus may be on the heterogeneity of studies. Though most literature on meta-analysis in medical research relates to randomized clinical trials, issues in favor or against meta-analyses are basically the same for non-experimental studies (56-66). Since a meta-analysis is a retrospective look at data already collected, it is important to try to make the process as unbiased and well defined as possible (57). For the current analysis, the following

protocol, adapted from a flow chart for meta-analysis proposed by L'Abbé et al. was used (67): 1) Specification of hypotheses and objectives, 2) stating inclusion criteria, 3) acquisition of data, 4) description of study characteristics, 5) assessment of study quality, 6) re-analysis of the study results, 7) exploration of sources of variation, 8) statistical pooling, 9) review of bias, and, finally, 10) discussion of the results. Every step will be discussed separately, starting with an outline of general aspects of meta-analyses in section 3.2, and followed by the details of the current study in section 3.3.

3.2 GENERAL ASPECTS OF META-ANALYSIS

3.2.1 Hypotheses and objectives - general aspects

If there are specific hypotheses, they should preferably be specified early on because they can guide the selection of individual studies and the choice of background variables to be examined (68). The next critical step of a meta-analysis is to set the objective (61,67-69). In most epidemiological settings, this translates to the measure of effect to be estimated (69). The variables to be studied, can be specified in categories of outcome, exposure, confounders, intermediates and effect modifiers (69). In particular, outcome and exposure variables form part of the criteria for inclusion and exclusion of studies in a meta-analysis. The outcome of the meta-analysis should cover one or more well-defined endpoints (65). Overviews focussing on all-cause mortality have a distinct advantage, as there can be no ambiguity concerning this endpoint. The degree of similarity in endpoint definition of the various studies influences the biological meaning of pooled data (61). Of equal necessity is an exact specification of the exposure under study. In reviewing clinical trials, all treatment characteristics, such as type of drug, dose, dosing intervals and concomitant treatment should be stated, because it is likely that treatment efficacy is related to these factors (61,65). Once outcome and exposure are specified, potential confounders may be identified. Often, certain confounders are so important that failure to adjust for them will invalidate inferences on the pooled results. An example is the confounding effect of cigarette smoking in the study of coffee and heart disease (69). Intermediate variables, acting along a possible causal pathway from exposure to outcome, are sometimes either neglected or treated as confounders. According to Greenland, investigators frequently adjust for potential intermediates and meaningful associations are masked as a consequence (69). For example, in studies on coffee and cardiovascular disease, adjustments are often made for serum cholesterol despite its likelihood of being an intermediate variable. Finally, an attempt should be made to identify modifiers of the effect under study. In particular, qualitative effect modifiers, i.e., variables for which the exposure has an effect within some, but not all categories, are of interest (69). Overviews may allow for a limited number of pre-specified subgroups to be tested with adequate power (61). Indeed, an analysis of the heterogeneity of studies is likely to bring more important information than some typical or average effect (55,68,69).

3.2.2 Inclusion criteria and acquisition of data - general aspects

Inclusion criteria

Studies are selected for meta-analysis based on variables as study design, sample size, or whether or not the study is published (67). Investigators may disagree on the inclusion and exclusion criteria for a particular meta-analysis and for this reason,

criteria and their rationale should be stated and all studies should be listed, both included and excluded (67).

Literature search

In meta-analyses, a literature search is analogous to the collection of data in a single study. Procedures used in the search must be explicated to such a degree that they can be replicated. Obviously, unpublished studies are not found by literature searches. Because it is possible that published studies are systematically different from unpublished studies, meta-analyses based on literature searches alone may lead to biased results (56,58,59,64,66,67,69-71). By contrast, in a recent editorial in the New England Journal of Medicine it has been suggested that a meta-analysis is more likely to be accurate if it is based on published studies than if it nets all studies, published and unpublished (72). More on publication bias will follow in section 3.2.7.

Missing data

There are several options when confronted with missing or insufficient data. One is to try to obtain missing information directly from the authors. When effect sizes are not extractable from certain studies, and when efforts to get this information directly from the authors fail, it makes sense to focus quantitative analyses on the subgroup of studies with adequate information. Basing analyses on data that seems firm can only increase confidence in the review as a whole, even at the expense of the number of studies included (68).

3.2.3 Study characteristics - general aspects

Once studies are collected and chosen on the basis of inclusion and exclusion criteria, they have to be reviewed and summary information abstracted (67).

3.2.4 Study quality assessment - general aspects

In most papers discussing the methodology of meta-analysis, a section is dedicated to quality assessment (55,56,67,70,73). An adequate assessment of the quality of original studies should precede a quantitative meta-analysis (55). For randomized clinical trials, special quality assessment systems were developed (56,67). Chalmers et al. proposed a system to evaluate the design, implementation and analysis of randomized controlled trials, resulting in an index of quality (56). As quality assessment is an inherently subjective process, the potential for error and bias is substantial. Therefore, it has been recommended to form a group of investigators to develop a quality assessment protocol. Quality scores can be used in various ways. A previously defined cut-off point could be used to exclude studies from a meta-analysis. Alternatively, quality scores can be used to weight the contribution of individual studies to the pooling. Finally, study outcomes can be correlated with quality scores to determine the association of study quality with results (67).

No method for quality assessment of non-experimental studies has been published (55). Determinants of quality of cross-sectional studies include sample size, general setting in which data are collected and accuracy of measurement of exposure and outcome variables.

3.2.6 Sources of variation and statistical pooling - general aspects

Heterogeneity

Non-uniformity of study outcomes may reflect the fact that the effect varies according to a particular characteristic. Part of the analysis may investigate the effect of subdividing the studies according to one or more determinants of the outcome in order to determine the impact of these characteristics (67). The study of heterogeneity may actually be the main purpose of a meta-analysis.

Homogeneity

Across studies where the measure of outcome is judged to be relatively homogeneous, or constant, a summary measure of outcome, such as a weighted mean can be derived from pooling the results (67). An underlying assumption in combining individual study results to arrive at a summary measure is that differences between studies are due to chance alone (sampling variation), and therefore all study results are homogeneous. In other words, when results are combined, random error cancels out and "n" results give a more precise estimate than one. This assumption, however, should be questioned when variations do not seem to be due to chance alone. In that case, pooling results may be meaningless and in some cases may in fact be ill-advised. As a first step to explore this issue, a graphic display of the individual results is helpful. More formal statistical approaches, such as regression techniques can be used to investigate if the study outcomes are consistent or vary. However, the power of statistical tests for homogeneity is often low because most meta-analyses pool the results of a limited set of individual studies. Therefore, investigators should resort to informed subjective judgement of study outcomes for homogeneity when the formal statistical tests fail to reject the homogeneity assumption. Several authors have warned against relying on statistics alone with regard to homogeneity testing (67-69).

3.2.7 Review of bias - general aspects

The limitations of any approach to literature review that potentially invalidate the inference, can be summarized as follows: 1) sampling bias due to reporting and publication policies, 2) the absence in published studies of specific data desired for review, 3) biased exclusion of studies by the investigator, 4) the uneven quality of the primary data and 5) biased outcome interpretation (70).

Which studies to include in a meta-analysis depends upon the availability of research reports, how many there are altogether, whether many are published, the

frequency and quality of different research designs, and, of course, the objective of the analysis. There are two main options. First, to use every available study. However, if it is clear that a particular study is fundamentally flawed due to inadequate methodology it is hard to argue for its inclusion. False information is not to be prefered to no information. When studies are excluded, the extent to which the exclusions influence the overall conclusions should be discussed. Attempts to include all available material may be hindered by practical problems such as difficulty to locate studies. The alternative option is to use published studies only and to omit unpublished data (67,68).

Attempts to publish a study in a scientific journal are not always successful. A portion of the body of information remains in the file drawers, in the form of unpublished studies. Two kinds of studies have an acknowledged smaller chance of being published, irrespective of the quality of the study. First, those studies who fail to find associations, so called "negative results". For this reason, a meta-analysis based on published reports only, will yield results biased towards an overestimation of effects. Second, new or unpopular data tend to be underreported in the published literature also. According to Greenland, the only safeguard against such "publication bias" is to detect unpublished results as well as published reports through methods such as direct inquiry among researchers in the area (69). To evaluate the extent to which the medical literature may be misleading as a result of publication bias, a random sample of 291 authors who had previously published randomized clinical trials (RCT) was asked whether they had participated in unpublished RCTs. Of 141 responders, 58 had conducted unpublished RCTs and they reported 196 (21.3 %) RCTs that remained unpublished of a total of 921 trials in which they participated. Unpublished studies were significantly different from published studies, with respect to outcome (unpublished studies were more often negative trials favoring control regimen) and statistical significance (59). Various attempts have been made to assess the impact of the "file drawer threat" by making some adjustment for unpublished studies. Rosenthal, for example, has proposed a procedure which calculates the number of hypothetical no-effect studies required to bring a significant overall p-level to non- significance (74). Methods like this, however, can only provide a rough guide, based as they are on assumptions about the missing data (64).

The peer review system prevents some papers of dubious quality from being published. Consequently, unpublished results may be less reliable, since they have not been found acceptable by peer reviewers and may not be collected with the same rigor or accuracy as published results (75). This prompts a careful interpretation if data are used from studies that investigators or scientific journals chose not to publish. These studies could have serious methodological limitations and this may well apply more often to negative studies than to positive ones. The potential problems inherent to the

use of unpublished results make it unclear whether both sources of data should be given equal weight (57).

3.2.8 Discussion - general aspects

Some 15 years ago, Glass introduced the term meta-analysis in a study of the efficacy of psychotherapy (76). According to Yenicek, meta-analysis in medicine is not well defined and its specific methods are underdeveloped (55).

The benefits of meta-analysis can be summarized as follows. Meta-analysis forces systematic thinking about methods, populations, interventions and outcomes during the process of accumulating evidence. In addition, the combination of data from several studies increases generalizability and potentially increases statistical power, thus permitting more complete assessment of the impact of a procedure or variable. In terms of assessing causality of associations, consistent findings in different populations under different circumstances may strenghthen the case for a causal association (67). Moreover, quantitative measures across studies can give insight into the nature of relationships among variables and provide a mechanism for detecting and exploring apparent contradictions in results (70).

Criticism of meta-analyses includes the contention that it disregards the quality of studies and, if not only good studies are aggregated, a meta-analysis is just "an exercise in mega-silliness" (77). Another criticism is that it is illogical to combine results from studies that used different types of subjects, and different measurement techniques (77-80). This is a problem in any type of literature review. This comment can also be viewed as a major advantage of meta-analysis: the reasons for dissimilarities between studies may offer valuable information. A next problem stems from the potential selection bias in published research. Although meta-analysis is more explicit, it may be no more objective than a narrative review (70,81). In addition to these criticisms, there are other potential limitations to meta-analysis. First, the validity of meta-analysis depends on complete and accurately reported information in published articles. Surveys of the literature have shown that important information is often not reported, making it difficult to answer questions posed in the meta-analysis protocol (56,58,82). This is not a weakness of meta-analysis itself, but points to the need for more standardized and comprehensive reporting in the scientific journals (56,83). Second, although pooling of results increases statistical power compared with that in the original study, the relative validity of the various statistical techniques available for pooling results has not been fully studied and more work is needed in this area (67).

3.3 A META-ANALYSIS OF COFFEE, SERUM LIPIDS AND BLOOD PRESSURE

3.3.1 Hypotheses and objectives

In the present analysis of results of cross-sectional studies on coffee, serum lipids and blood pressure, the following hypotheses were tested:

- There is a positive association between coffee consumption and serum total cholesterol and LDL-cholesterol level. The same applies to apolipoprotein B. This association is dependent on the brewing-method: coffee brewed by boiling and other filter-lacking methods increase serum total cholesterol, while filtered coffee has no effect. The effect is not modified by gender.
- HDL-cholesterol and apolipoprotein A1 are not influenced by coffee consumption.
- Heavy coffee consumption increases blood pressure.

Quantification of effects

The statistical analysis will be described in detail in sections 3.3.5 and 3.3.6. In short, for each study the mean levels of serum cholesterol and blood pressure for each coffee consumption category were tabulated. Using a weighted linear regression model, a ß coefficient was estimated indicating the mean change in serum cholesterol or blood pressure per cup of coffee used.

Outcome

Two outcomes were studied: serum lipids and blood pressure.

Exposure

The exposure under investigation is coffee consumption. A study of caffeine intake was not conducted, since this would demand information on type of coffee (regular or decaffeinated) as well as on tea and cola consumption and intake from caffeine containing drugs. Only a very limited number of large-scale cross-sectional studies supply data on caffeine intake (24,29,36,40,51,52). From other studies, there is little reason to believe that caffeine intake affects serum lipid levels (84,85). Results on blood pressure might be of more interest, since short-term experimental studies suggest a small, transient, blood pressure raising effect of caffeine (86-88). This blood pressure rise on caffeine intake seems to be present only in persons who did not consume any caffeine for at least 10 hours. Two long-term experimental studies, however, showed virtually no change in blood pressure on abstinence from caffeine (89,90).

Confouders

Heavy coffee drinking may be an indicator of an "unhealthy" lifestyle (91). A high smoking frequency (22,23,30,32,43,91,92), a high dietary fat intake with low P/S ratio (32,91-93), a high body mass index (41,92,94), and a limited physical activity level (33,91) are reported to be associated with heavy coffee use. Coffee consumption habits may change with age; some report increased consumption with age (41,47,95,96), others

no change (23,39) or a decreased consumption with age (27). Still other cross-sectional studies have suggested an association with anxiety and type A personality (97-99). As age, smoking, body mass index, dietary fat intake and physical activity may also be related to serum cholesterol concentration and blood pressure level, these factors are the most important confounders of the relationship between coffee and serum cholesterol or blood pressure. Rejecting the unhealthy-lifestyle-view, Hemminki postulated that in the long run only persons with good health and good resistance to coffee's possible harmful effects can drink high amounts of this beverage (100).

Intermediates

The mechanism for a relationship between coffee and serum lipids has not been definitely established. It has been suggested that coffee contains substances which reduce the excretion of bile acids and neutral sterols, thereby increasing the cholesterol level (101,102). Boiled coffee, prepared by boiling ground coffee with water and allowing the grounds to settle, seems to contain more lipid material than filtered coffee. A lipid-enriched fraction from boiled coffee in an amount of 80 g per day (1.3 g of lipids), was shown to elevate serum total cholesterol levels in 10 volunteers (103). Coffee might affect blood pressure via its caffeine content. Several mechanisms have been proposed to explain the pressor effect of caffeine (54). Caffeine appears to exert a positive inotropic effect on the cardiac muscle (104,105). Other investigators reported a caffeine induced rise in vascular resistance (106) or increase in sympathetic activity (86). For the present analysis of cross-sectional studies, data on potential intermediates is lacking, with the exception of caffeine intake. Therefore, the only concern is erroneous adjustment of coffee intake for total caffeine consumption.

Effect modifiers

In a review on coffee and cholesterol, Thelle and co-workers describe three studies with an apparent lack of association between the two variables in men, but a positive association in women (21,29,33). These discrepant findings remain unexplained (53). In a recently published study by Stensvold et al., again a generally stronger association was observed among women (44). The effect of coffee on serum lipids seems to be determined by the brewing method used. Evidence is accumulating from both non-experimental and experimental studies, pointing at a cholesterol raising effect of boiled coffee compared to filtered coffee (42,44,107-109). Boiled coffee, a common brew in part of Norway and Finland, is prepared without use of a filter. It can be speculated that other brewing methods lacking a filter, like (Turkish) mud coffee and espresso coffee, may also raise serum cholesterol levels. In summary, the effect of coffee on serum lipids and blood pressure may be modified by gender and coffee brewing method. In virtually all papers, results for coffee effects are presented for men and women separately. In the present meta-analysis, subgroups based on gender and coffee brewing method have been studied. Details on categories of studies according to

brewing methods are given in section 3.3.5.

3.3.2 Inclusion criteria and acquisition of data

Inclusion criteria

Inclusion of the studies was based on the following criteria:

- Cross-sectional study.
- Availability of data on coffee and serum lipids and/or coffee and blood pressure.
- More than 200 subjects per group of men and women.
- For each coffee-consumption category the mean level of serum cholesterol or blood pressure, as well as the number of subjects should be known.
- More than two coffee consumption categories identified.

The two last criteria were set to permit reanalysis of all studies in the same way. For the regression procedure, at least 3 consumption categories in each study were needed.

Literature search

For the present study we identified all published non-experimental studies on coffee consumption and serum cholesterol and blood pressure by reviewing reference lists in relevant papers, and conducting manual and computer Medline searches of published articles. At December 1, 1989, the literature search resulted in 26 papers reporting on coffee and serum lipids, and 11 reports on coffee and blood pressure. From only 5 studies, results on both serum lipids and blood pressure were obtained (23,29/52,30,31/49,44). The main source for unpublished studies was the Workshop on Coffee and Coronary Heart Disease with special emphasis on the Coffee-blood lipids relationship (Göteborg, 1989), organized by Prof. D.S. Thelle. At this meeting results of several unpublished studies were presented, and the investigators were asked to provide their data to be used in the present meta-analysis.

Missing data

In abstracting data from the published studies an important problem became apparent. Frequently, data relevant to the meta-analysis were not included in the reports, such as the number of subjects in each consumption category, unadjusted data, standard errors or confidence intervals for effect measures. Furthermore, it seemed likely that most investigators reporting on serum lipids would also have data on blood pressure. To obtain the missing data, investigators were contacted. Data abstract forms were developed to record all information needed. We asked for mean levels of serum lipids/blood pressure by each coffee consumption category, 1) unadjusted, 2) adjusted for age and body mass index and 3) adjusted for a maximum set of confounding variables, which had to be specified on the forms. These results were to be reported separately for men and women, and for different coffee brewing methods. Most studies, however, did not include a question on type of brew in their protocol. Data that were

already available from published reports were inserted in the forms. Investigators were asked to check and update the data, and add information on variables not (yet) published. They were also asked to indicate the brew(s) mainly used by the study population.

Table 3.1 summarizes the studies considered for the analysis. Most investigators sent us additional information to the reported results, and some unpublished material was obtained. The data on blood pressure are somewhat disappointing. Probably, some information still remains in files. Information on triglycerides, LDL-cholesterol, VLDL-cholesterol, apolipoprotein A1, A2 and B was available for 8, 6, 1, 1, 1, and 2 studies respectively, and these numbers were considered too small to permit a reliable meta-analysis. Data on effects of tea consumption were available for 6 studies only and, therefore, not included in our analysis. Four studies were too small according to the preset exclusion criteria (22,27,34,46). Four studies had to be excluded because only two coffee consumption categories were specified (22,38,47,48). Unfortunately, eight studies had to be excluded because the presentation of results did not allow extraction of our effect measure (21,23,24,26,34,40,45,46). Some of these studies were conducted several years ago and the data were no longer available for evaluation. The first Tromsø study on coffee and serum lipids was excluded because an update of the data on the same study population has been published (42,96). In summary, 21 studies were included in the present analysis. The general characteristics of the included and excluded studies are presented in section 3.3.3.

Table 3.1. Cross-sectional studies on coffee, serum total cholesterol (Tchol), HDL-cholesterol (HDL) and blood pressure (BP). Availability of data and reasons for exclusion.

Study number	*First author (ref)*	*Country*	*Year of publication*	*Tchol*	*HDL*	*BP*	*Reason for exclusion*
1	Lang (47)	Algiers	1983			++	2 consumption categories
2	Shirlow (29/52)	Australia	1984/88	**		**	
3	Piettinen (41)	Belgium	1988	+	+		
4	Birkett (51)	Canada	1988			++	
5	Tuomilehto (39)	Finland	1987	+	+		
6	Reuanen	Finland					1)
7	Reuanen	Finland					1)
8	Lang (48)	France	1983			++	2 consumption categories
9	Arab (27)	Germany	1983	++			sample size too small, no information on non-significant results
10	Kark (35)	Israel	1985	++	++		
11	Green (37)	Israel	1986	++	++		
12	Kark	Israel		**	**		
13	Periti (50)	Italy	1987			++	
14	Panico	Italy		**		**	
15	Hofman (28)	Netherlands	1983	++		**	
16	Kromhout	Netherlands		**	**		
17	Verschuuren	Netherlands					1)
18	Bonaa (42)	Norway	1988	+			
19	Stensvold (44)	Norway	1989	++	++	++	
20	Solvoll (43)	Norway	1989	++			
21	Lee	Scotland		**	**		
22	Welin (30)	Sweden	1984	++		++	

continued .....

Table 3.1 (cont).

Study number	*First author (ref)*	*Country*	*Year of publication*	*Tchol*	*HDL*	*BP*	*Reason for exclusion*
23	Little (19)	USA	1966	+			case-control study
24	Sacks (20)	USA	1975	+	+		case control study
25	Nichols (21)	USA	1976	+			no category means
26	Bertrand (45)	USA	1978			+	no category means
27	Heyden (22)	USA	1979	+	+		sample size too small, only 2 consumption categories
28	Prineas (23)	USA	1980	+		+	no category means
29	Phillips (24)	USA	1981	++	**		coffee and tea together
30	Medeiros (46)	USA	1982			+	sample size too small, no category means
31	Shekelle (25)	USA	1983	++		**	
32	Kovar (26)	USA	1983	+			no numbers per category
33	Klatsky (31/49)	USA	1985/86	++		++	
34	Haffner (32)	USA	1985	+	+		
35	Mathias (33)	USA	1985	++	++		
36	Williams (34)	USA	1985	+			no category means, sample size too small
37	Curb (36)	USA	1986	+			
38	Donahue (38)	USA	1987	+			only 2 consumption categories
39	Davis (40)	USA	1988	++			men and women together
40	Anderson	USA					1)

\+ only published information available
++ additional information supplied by the investigators
** unpublished data

1) Study known from oral presentation, data not received from author.

Table 3.2. Studies included in the pooled analysis. Serum total cholesterol.

Study	Country	Age	Men	Women	Brewing	Published results	Adjustment
2	Australia (29)	20-70	2724	2033	instant	*> 200 mg caffeine vs. < 200 mg* men 0.03 mmol/l (ns) women 0.29 mmol//l (sign)	age, BMI, occupation
3	Belgium (41)	18-65	15,954	2,116	filtered	*20 or more cups per weeks vs. 0* men 0.10 mmol/l ($p < 0.001$) women 0.01 mmol/l (ns)	age, BMI, smoking, alcohol, intake of fat and cholesterol
5	Finland (39)	25-64	4744	4495	filtered	*4 cups or more vs. 0* men 0.34 mmol/l ($p < 0.001$) women 0.26 mmol/l ($p < 0.05$)	age, BMI, smoking, alcohol, intake of fat, sugar, physical activity, fasting time
10	Israel (37)	20-39 40-69	309 349		mud	*5 cups or more vs. 0* young: 0.34 mmol/l ($p = 0.03$) old: 0.19 mmol/l ($p = 0.56$)	age, BMI, smoking, alcohol, sport in leisure time
11	Israel (35)	35-64	1007	589	mud	*5 cups or more vs. 0* men 0.50 mmol/l ($p < 0.001$) *coffee vs. no coffee* women 0.34 mmol/l ($p < 0.01$)	age, BMI, smoking, intake of fat, carbohydrates, tea, ethnicity, education, season of year
12	Israel	25-74	770	820	mud* instant*		age, ethnicity, season
14	Italy	20-59	2426	2614	espresso		age, BMI, smoking, alcohol, intake of fat

continued....

Table 3.2 (cont.).

Study	Country	Age	Men	Women	Brewing	Published results	Adjustment
15	Netherlands (28)	20-49	2219	2123	filtered	*5-8 cups vs. 0* men 0.05 mmol/l (ns) women-0.27 mmol/l (ns)	age, BMI, smoking, physical activity
16	Netherlands	65-85	825		filtered		age, BMI, smoking, alcohol, intake of fat
18	Norway (42)	20-59				*9 cups or more vs < 1*	age, BMI, smoking, intake of fat, salt, physical activity
			6807	5417	boiled *	men 0.61 mmol/l (0.21,-1.01) women 0.40 mmol/l (0.07,0.73)	
			2228	1620	filtered *	men 0.14 mmol/l (-0.35,0.63) women 0.02 mmol/l (-0.49,0.53)	
			224	168	instant *	men 0.56 mmol/l (-0.89,2.01) women-0.59 mmol/l (-1.99,0.81)	
19	Norway (44)	40-42				*9 or more cups vs. < 1*	BMI, smoking, intake of fat, salt, time since last meal, physical activity
			4658	5132	boiled *	men 0.40 mmol/l women 0.25 mmol/l	
			7659	7865	filtered *	men 0.03 mmol/l (ns) women 0.12 mmol/l	
			850	824	instant *	men -0.08 mmol/l (ns) women 0.38 mmol/l (ns)	
20	Norway (43)	35-49	8169	8925	boiled	*regression, coffee 1 cup/day* men ß = 0.043 mmol/l ($p < 0.001$) women ß = 0.046 mmol/l ($p < 0.001$)	age, BMI, smoking, alcohol, type of table fat and milk, bread, tea and milk consumption, physical activity

continued.....

Table 3.2 (cont.).

Study	Country	Age	Men	Women	Brewing	Published results	Adjustment
21	Scotland	40-59	4627	4497	instant		age, BMI
22	Sweden (30)				filtered	*11 cups or more vs. 1-2 cups*	no adjustment
		50	855			0.05 mmol/l	
		57	175			0.60 mmol/l	
		67	674			-0.10 mmol/l	
31	USA, Chicago (25)	41-57	1873		percolated/ filtered	*regression, coffee 1 cup/day* men ß = 0.005 mmol/l (p = 0.72)	age, BMI, smoking, alcohol, intake of fat
33	USA, California (31)	14-98	22,187	25,424	percolated/ filtered	*6 cups or more vs. 0* men 0.25 mmol/l (p < 0.001) women 0.15 mmol/l (p < 0.001)	age, BMI, smoking, alcohol, marital status, education, birthplace
34	USA, Texas (32)	25-64	1228	923	percolated/ filtered	*8 cups or more vs. < 1* men 0.51 mmol/l (p < 0.01) women 0.46 mmol/l (p = 0.05)	age, BMI, smoking, alcohol, estrogen use
35	USA, San Diego (33)	20-89	320	381	percolated/ filtered	*961 ml coffee or more vs. 0-229 ml* men 0.12 mmol/l (p = 0.6) women 0.53 mmol/l (p = 0.01)	age, BMI, smoking, alcohol, oral contraceptive use, estrogen use, regular excercise, intake of fat, sugar and cream in coffee
37	USA, Hawai (36)	46-65	5858		percolated/ filtered	*regression, coffee 1 cup/day* men ß = 0.02 mmol/l (p < 0.001)	age, smoking, alcohol, intake of fat, diastolic blood pressure

* denotes that a question on brewing method was included in the study protocol.

Table 3.3. Studies excluded from the pooled analysis. Serum total cholesterol.

Study	Country	Age	Men	Women	Brewing	Published results	Adjustment
6	Finland	30-69	6285		?		?
7	Finland	30-69	1935	1843	?		?
9	Germany (27)				filtered	*6 cups or more vs. 0*	not adjusted
		18-24				men 0.33 mmol/l	
		65-74				men ns	
17	Netherlands	20-60	3002	3315	filtered		age, BMI, smoking, alcohol
25	USA, Tecumseh (21)	20-69	2011	2290	percolated/ filtered	*correlation coeff. coffee-serum cholesterol*	
						men 0.08 (ns)	
						women 0.05 ($p < 0.05$)	
27	USA, Evans County (22)	??	166		percolated/ filtered	*coffee vs. no coffee*	age, sex, BMI
						men smoking 0.70 mmol/l	
						men non-smoking 0.03 mmol/l	
29	USA, California (24)				percolated/ filtered	*regression, coffee and tea, 1 cup/day*	BMI, alcohol, smoking, cola intake
		20-34	551			men ß = 0.02 mmol/l (ns)	
		20-44		121		women ß = -0.01 mmol/l (ns)	
		35-64	1624			men ß = 0.02 mmol/l ($p < 0.05$)	
		45-64		159		women ß = -0.04 mmol/l (ns)	

continued....

Table 3.3 (cont.).

Study	Country	Age	Men	Women	Brewing	Published results	Adjustment
32	USA (26)	20-49	2437	2661	percolated/ filtered	*9 or more cups vs. < 1* men -0.05 mmol/l (ns) women 0.16 mmol/l (ns)	age
36	USA, Stanford (34)	30-55	77		percolated/ filtered	*Pearsons's correlation* men 0.008 ($p < 0.05$)	? age, smoking, alcohol, diet, fitness
38	USA, Beaver county (38)	20-24	259	213	percolated/ filtered	*3 cups or more vs. 0* men 0.01 mmol/l (ns) women-0.14 mmol/l (ns)	BMI, smoking, alcohol, adherence to low cholesterol diet
39	USA (40)	30-69	9043		percolated/ filtered	*regression, coffee 1 cup/day* men ß = 0.01 mmol/l ($p < 0.05$)	age, sex, BMI, smoking, DBP, physical activity, stress, education
40	USA	17-70	1743	1878			

Table 3.4. Studies included in the pooled analysis. Systolic blood pressure.

Study	Country	Age	Men	Women	Brewing	Published results	Adjustment
2	Australia (52)	20-70	2921	2226	instant	*caffeine use < 3 hrs before vs. no caffeine use last 9 hrs* men 4 mm Hg ($p < 0.01$) women 4 mm Hg ($p < 0.01$)	age, BMI, smoking, alcohol, first degree relative with hypertension, serum cholesterol
4	Canada (51)	> 17	1015	1148	filtered	*regression, caffeine mg/day* ß = 0.0012 mm Hg	age, sex
13	Italy (50)	18-62	402	98	espresso	*regression, coffee 1 cup/day* ß = -0.80 mm Hg ($p < 0.01$)	age, sex, BMI, smoking, alcohol
14	Italy	20-59	2426	2614	espresso		age, BMI, smoking, alcohol, fat
15	Netherlands (28)	20-49	2219	2123	filtered		age, BMI, smoking, physical activity
19	Norway (44)	40-42				*9 cups or more vs. < 1 cup/day*	BMI, smoking, intake of fat, salt, time since last meal, physical activity
			4658	5132	boiled *	men 1.0 mm Hg (ns) women-2.8 mm Hg (ns)	
			7659	7865	filtered *	men -1.4 mm Hg (ns) women-0.7 mm Hg (ns)	
					instant*		

continued....

Table 3.4 (cont.).

Study	Country	Age	Men	Women	Brewing	Published results	Adjustment
22	Sweden (30)				filtered	*11 cups or more vs. 1-2 cups*	unadjusted
		50	855			men 4.2 mm Hg (ns)	
		57	175			men 16.9 mm Hg (sign)	
		67	644			men 16.4 mm Hg (sign)	
31	USA, Chicago (25)	41-57	1873		percolated/ filtered		age, BMI, smoking, alcohol
33	USA, California (49)	14-98	20,171	23,191	percolated/ filtered	*regression, coffee 1 cup/day* men ß = -0.4 mm Hg ($p < 0.001$) women ß = -0.8 mm Hg ($p < 0.001$)	age, BMI, smoking, alcohol, uric acid, tea consumption, total cholesterol, glucose, potassium, total calcium, creatinine, hemoglobin

* denotes that a question on brewing method was included in the study protocol.

Table 3.5. Studies excluded from the pooled analysis. Systolic blood pressure.

Study	Country	Age	Men	Women	Brewing	Published results	Adjustment
1	Algiers (47)	15-70	1098	380	filtered/ mud	*regression, coffee 1 cup/day* ß = 0.56 mm Hg (ns)	sex, age, BMI, smoking, physical activity, rural vs. urban residency, tea consumption
8	France (48)	18-60	3704	2617	filtered	*regression, coffee 1 cup/day* ß = 0.23 mm Hg ($p < 0.02$)	sex, age, BMI, smoking, alcohol, sports activity, low social class
26	USA (45)	??	72,101		percolated/ filtered	*9 cups or more vs. 0* SBP > 200 mm Hg -0.1 %	unadjusted
28	USA, Minneapolis (23)	35-57	7009		percolated/ filtered	*correlation coeff. coffeecons-cholesterol* men 0.034 ($p < 0.05$)	
30	USA, Mississippi (46)	17-60	157	49	percolated/ filtered	*regression, caffeine mg/day* ß = 0.0005 mm Hg (ns)	sex, age, BMI, smoking, race

3.3.3 Study characteristics

Some characteristics and the main results of the studies are shown in tables 3.2-3.5. Studies were categorized according to outcome variable (serum total cholesterol and systolic blood pressure) and whether or not they could be included in the present study. The column on results reflects the diversity in presentation, data reduction and statistics in the original reports. Two methods dominate. First, a comparison between highest and lowest coffee consumption group or highest consumption group and non-consumers. Second, a continuous analysis of the effect of coffee on either serum lipids or blood pressure. The set of confounders for which adjustment was possible or considered necessary, differed markedly between the studies. In our view, a direct comparison of these data is not possible. Consequently, a re-analysis of the (in part unpublished) data was performed, to permit a more valid comparison and to investigate the reasons for variability between studies. A question on the method of coffee brewing was included in three studies (12,18 and 19) only. All other studies were categorized according to the main brew used, as indicated by the investigators (section 3.3.2). Studies from the United States are referred to as "percolated/filtered", because both brews are in use and the exact ratio cannot be determined (section 3.3.8).

3.3.4 Study quality assessment

For the present analysis based only on large-scale cross-sectional studies, the following characteristics were evaluated in the process of quality assessment.

Number of study subjects

Sample size was considered to be an important determinant of quality of the results. Studies involving less than 200 subjects per subgroup for gender and brewing method were excluded. Within each study, the regression analysis was weighted according to number of subjects per consumption category.

General setting of the data collection

Virtually all studies were performed in the same survey-like way: A random subset of a population was asked to participate. A clinical examination including measurement of height, weight, blood pressure is performed and a blood sample is taken. The broad similarity of studies with regard to general setting, obviates the use of this characteristic in the quality assessment.

Measurent of exposure

Schreiber et al. compared the number of cups subjects reported they drank and an estimation of their weekly consumption using 3 measurement refinements: a weighted average of weekday and weekend consumption, size of cup used and the amount of each cup or mug of coffee consumed (110). Subjects tended to drink more coffee on weekdays than on weekends. However, when questioned about their usual coffee habits, most subjects will report weekday intake only. Of the two factors affecting volume, the

size of the cup or mug used had a substantial effect on misclassification of coffee intake. Since mug use was more prevalent among heavy drinkers, the degree of misclassification of respondents was greatest among those who reported drinking 4 or more cups of coffee (110). Detailed information on either cup size or intake on different days of the week is lacking in the studies involved in the present analysis. In all studies dietary habits were assessed by a questionnaire. Coffee consumption is usually recorded in categories. The first category is zero or less than 1 cup a day, followed by categories that differ for each study. The categorization is determined by the average coffee consumption in the particular population and preference of the investigators. In Scandinavian countries with high coffee consumption, categories up to 11 cups or more per day are defined, while in Israel the highest consumption is 5 cups or more per day. Some investigators ask for the exact number of cups of coffee consumed per day, but most of them ask in categories, for example 0, 1-2, 3-4, 5-6 or 0, 1-4, 5-8. A common method is to assign category midpoints to categories. This has its justification from linearity but gives no solution for open-ended categories (such as "over six cups per day"). If, however, no frequency distribution for exposure is available, it may be the only choice, along with arbitrary assignments to open-ended categories (69). We added 1.5 cups of coffee to the highest open-ended consumption category of each study. For example, in study 19 the highest consumption category, 9 or more cups per day was classified as 10.5 cups per day. Fortunately, coffee use was not asked with special emphasis, and is not emotionally laden or suspect, as for example smoking and alcohol use.

Measurement of outcome

Blood pressure readings and serum lipid determinations are subject to measurement error. This is to some extent reflected in the standard deviations of the measurements. Yet, because for many studies standard deviations were not available and could not be calculated either, quality assessment of the measurent of outcome was considered not feasible for the present analysis. In summary, only the sample size of the studies and the number of subjects per consumption category could be used as characteristics determining quality.

3.3.5 Re-analysis of study results and sources of variation

The collected data of the included studies were reanalyzed in a uniform way to allow a comparison between studies of the results on coffee and serum total cholesterol, HDL-cholesterol and systolic and diastolic blood pressure (referred to as "variables"). The maximal adjusted mean levels of the variables for each coffee consumption category were used (section 3.3.2).

A multiple regression procedure yielded a regression coefficient (β_1), indicating the mean change in the outcome variable for each cup of coffee used. The effects

were weighted according to a direct function of the sample size, i.e. the number of subjects in the consumption categories. Subjects who never drink coffee may be considered a special group with regard to health related behaviour and, therefore, their serum lipids and blood pressure levels may differ from coffee-consumers for reasons that are difficult to account for (6). Consequently, the "no-coffee-category" has its own coefficient β_2, in the applied regression model denoting the difference between the actual mean value of the no-coffee category and the intercept based on the linear trend with slope β_1. The full procedure is described in appendix I. These weighted regression analyses were performed for all studies included, and within each study for each variable and brewing method available. Men and women were analyzed separately. The results, stratified for gender and brewing method, are shown in tables 3.6-3.13 and figures 3.1-3.8.

Regression coefficients of serum total cholesterol are positively signed, which indicates a rise in serum total cholesterol with increased coffee consumption. Statistical significance, however, is not reached in all studies, and seems to be dependent on the brewing method used. All studies on filtered coffee show, if anything, a small positive effect on serum total cholesterol, statistically significant in only 1 out of 7 studies (men and women). For boiled coffee, the results were quite uniform in both sexes, with linear trend coefficients ranging from 0.022 to 0.044 mmol/l/cup, all statistically significant. The studies from the United States, categorized as percolated/filtered, are less consistent with regression coefficients ranging from -0.016 to 0.105 mmol/l/cup. Moreover, most confidence intervals are wide and include zero. Five studies included subjects consuming instant coffee. Only the (unpublished) study from Scotland yielded a statistically significant result 0.025 (95 % confidence limits 0.015, 0.035) mmol/l/cup for both men and women, adjusted for age and body mass index. Unfortunately, only one study on espresso coffee could be included. This Italian study indicated a positive association between the use of espresso and serum total cholesterol, statistically significant in men. Interestingly, three studies from Israel, where "mud coffee" or Turkish coffee is the common brew, showed highly significant β_1 coefficients for men, with point estimates up to 0.105 mmol/l/cup. One study including both sexes showed the same result for women, while another study yielded a β_1 of -0.002 mmol/l/cup for women. The data on HDL-cholesterol are difficult to interpret due to the small number of studies. Both positive and negative results are observed, most of them small and not statistically significant.

Data on blood pressure show a rather different pattern: β_1 coefficients tend to be negative, indicating a fall in blood pressure with increased coffee consumption. Statistical significance is reached in a subset of studies, including filtered, boiled, percolated/filtered coffee, instant coffee and espresso. Data on Turkish coffee and blood pressure are not available. For all brews the significant results are

counterbalanced by non-significant studies. Strongest evidence for a favourable effect on blood pressure was observed in two Italian studies: in men systolic blood pressure decreased by 0.44 (-0.55, -0.33) mm Hg/cup and 1.33 (-1.76, -0.90) mm Hg/cup, in women by 1.09 (-2.93, 0.75) mm Hg/cup and 0.60 (-0.76, -0.44) mm Hg/cup.

Tables 3.6-3.13 also show the $ß_2$ values which, as explained in appendix I, denote the differences between the actual means of the no-coffee category and the intercept based on the linear trend with slope $ß_1$, for each study. Most studies yield a negatively signed $ß_2$, both for serum lipids and blood pressure. Statistical significance is reached in many studies, which reflects an observed serum lipid concentration or blood pressure level well below the estimated value. For this reason, a separate treatment of the no-coffee category in the analyses is justified.

3.3.6 Statistical pooling

The appropriateness of the weighted mean as a meta-analytic summary of the effect under study depends on a homogeneity assumption. This assumption states that the studies are estimating the same underlying true value for the effect, i.e. after considering the extent of the real effect and bias in each study, the studies should on the average yield the same value, so that differences between the estimates are entirely due to random error (69). In the present study, homogeneity was assessed both by judgment of the graphs by eye and by formal statistical testing, as described in appendix II. In the statistical approach, a non-significant chi-square value supported the hypothesis that the study results were homogeneous. Heterogeneity was assumed at a level of 5 %. The results of the statistical homogeneity test, shown in tables 3.6-3.13, compare favourably with judgment of homogeneity by eye (figures 3.1-3.8). If homogeneity was not rejected, a pooled $ß_1$ was calculated for each subgroup of gender/brewing method (tables 3.6-3.13). The formulas for the calculation of the pooled $ß_1$ ($ß_1$*) and its standard error are given in appendix II. With regard to serum total cholesterol, the common effect indicates a positive relationship with coffee consumption, regardless of the brewing method used. The association seems strongest for boiled coffee and Turkish coffee. For HDL-cholesterol, the pooled $ß_1$ could be calculated for 2 groups only. In women, HDL-levels raised with increased consumption of filtered coffee. As far as homogeneity permitted calculation of an average blood pressure response across studies, a favourable effect of coffee use is observed, i.e. higher coffee consumption is associated with a lower blood pressure level.

Table 3.6. Serum total cholesterol (mmol/l/cup), men. Results for each study separately and, if homogeneity could be assumed, pooled coefficients of linear regression for categories of brewing method.

Brewing method	*Study*	*Coefficients of linear regression* β_1 *(95% CL)*	β_2 *(95% CL)*	*Test for homogeneity* chi^2	*df*	*sign*	*Pooled coefficient of linear regression,* β_1 *(95% CL)*
Filtered	3	0.009 (-0.005, 0.022)	- 0.036 (-0.188, 0.116)	6.45	7	ns	0.008 (0.002, 0.014)
	5	0.008 (-0.017, 0.033)	- 0.245 (-0.605, 0.116)				
	15*	0.024 (0.005, 0.042)	0.016 (-0.270, 0.300)				
	16	0.028 (-0.008, 0.064)	- 0.189 (-0.671, 0.293)				
	18	0.013 (-0.008, 0.034)	- 0.173 (-0.333,-0.012)				
	19	0.002 (-0.008, 0.010)	- 0.234 (-0.326,-0.141)				
	22	0.002 (-0.053, 0.057)	- 0.424 (-1.311, 0.464)				
Percolated	31	0.024 (-0.007, 0.055)	0.053 (-0.318, 0.425)	2.08	5	ns	0.019 (0.012, 0.027)
	33	0.019 (0.009, 0.030)	- 0.109 (-0.169,-0.049)				
	34	0.052 (-0.010, 0.114)					
	35	-0.016 (-0.085, 0.055)					
	37	0.019 (0.006, 0.031)	- 0.129 (-0.225,-0.032)				
Instant	2*	0.008 (-0.018, 0.034)	0.015 (-0.160, 0.189)	12.83	5	$p < 0.025$	
	12	0.012 (-0.013, 0.036)					
	18	0.017 (-0.034, 0.069)	- 0.386 (-0.670,-0.102)				
	19	-0.002 (-0.011, 0.009)	- 0.187 (-0.250,-0.125)				
	21*	0.025 (0.015, 0.035)	0.006 (-0.051, 0.063)				
Boiled	18	0.044 (0.024, 0.064)	- 0.249 (-0.475,-0.023)	2.28	3	ns	0.038 (0.027, 0.049)
	19	0.022 (-0.002, 0.046)	- 0.296 (-0.510,-0.081)				
	20	0.041 (0.024, 0.059)					

continued....

Table 3.6 (cont.).

Brewing method	*Study*	*Coefficients of linear regression* β_1 *(95% CL)*	β_2 *(95% CL)*	*Test for homogeneity* chi^2	*df*	*sign*	*Pooled coefficient of linear regression,* β_1 *(95% CL)*
Espresso	14*	0.046 (0.003, 0.090)	0.034 (-0.176, 0.244)				
Turkish	10	0.069 (0.045, 0.092)	-0.117 (-0.235, 0.002)	1.72	3	ns	0.072 (0.054, 0.090)
	11	0.105 (0.052, 0.157)	0.277 (-0.016, 0.570)				
	12*	0.065 (0.032, 0.099)					

* denotes unpublished study

Table 3.7. Serum total cholesterol (mmol/l/cup), women. Results for each study separately and, if homogeneity could be assumed, pooled coefficients of linear regression for categories of brewing method.

Brewing method	*Study*	*Coefficients of linear regression* β_1 *(95% CL)*	β_2 *(95% CL)*	*Test for homogeneity* chi^2	*df*	*sign*	*Pooled coefficient of linear regression,* β_1 *(95% CL)*
Filtered	3	0.005 (-0.007, 0.018)	-0.010 (0.122, 0.102)	11.1	5	$p < 0.05$	
	5	0.002 (-0.009, 0.013)	-0.246 (-0.375,-0.116)				
	15	0.005 (-0.016, 0.025)	0.028 (-0.221, 0.278)				
	18	0.017 (-0.001, 0.035)	-0.068 (-0.184, 0.048)				
	19	0.021 (0.014, 0.027)	-0.053 (-0.119, 0.015)				
Percolated	33	0.008 (0.002, 0.014)	-0.093 (-0.124,-0.062)	643.53	3	$p < 0.005$	
	34	0.029 (-0.006, 0.063)					
	35	0.105 (0.100, 0.109)					
Instant	2*	0.018 (-0.039, 0.074)	0.295 (-0.107, 0.696)	3.38	5	ns	0.022 (0.020, 0.024)
	12	-0.006 (-0.076, 0.065)					
	18	-0.027 (-0.093, 0.038)	-0.442 (-0.746,-0.138)				
	19	0.009 (-0.031, 0.050)	-0.113 (-0.344, 0.119)				
	21*	0.025 (0.015, 0.035)	0.039 (-0.022, 0.100)				
Boiled	18	0.033 (0.026, 0.039)	-0.161 (-0.223,-0.100)	2.42	3	ns	0.032 (0.028, 0.035)
	19	0.030 (0.026, 0.035)	-0.240 (-0.278,-0.201)				
	20	0.044 (0.026, 0.062)					
Espresso	14*	0.016 (-0.021, 0.053)	-0.011 (-0.173, 0.150)				
Turkish	11	-0.002 (-0.039, 0.035)	-0.352 (-0.564,-0.140)	5.83	2	ns	0.035 (0.014, 0.057)
	12*	0.054 (0.028, 0.081)					

* denotes unpublished study

Table 3.8. HDL-cholesterol (mmol/l/cup), men. Results for each study separately and, if homogeneity could be assumed, pooled coefficients of linear regression for categories of brewing method.

Brewing method	*Study*	*Coefficients of linear regression* β_1 *(95% CL)*	β_2 *(95% CL)*	*Test for homogeneity* chi^2	*df*	*sign*	*Pooled coefficient of linear regression,* β_1 *(95% CL)*
Filtered	3	-0.001 (-0.002,-0.000)	-0.023 (-0.032,-0.014)	10.81	4	P < 0.05	
	5	0.003 (0.001, 0.005)	0.020 (-0.012, 0.053)				
	16	-0.004 (-0.013, 0.005)	-0.084 (-0.210, 0.042)				
	19	-0.001 (-0.004, 0.002)	-0.068 (-0.101,-0.036)				
Percolated	34	-0.003 (-0.009, 0.004)					
Instant	21*	-0.009 (-0.017,-0.002)	-0.043 (-0.086,-0.001)				
Boiled	19	-0.002 (-0.008, 0.005)	-0.026 (-0.086, 0.033)				
Turkish	10	-0.002 (-0.011, 0.007)	0.023 (-0.022, 0.069)	0.75	2	ns	-0.001 (-0.010, 0.007)
	11	0.011 (-0.019, 0.040)	0.068 (-0.094, 0.229)				

* denotes unpublished study

Table 3.9. HDL-cholesterol (mmol/l/cup), women. Results for each study separately and, if homogeneity could be assumed, pooled coefficients of linear regression for categories of brewing method.

Brewing method	*Study*	*Coefficients of linear regression* β_1 *(95% CL)*	β_2 *(95% CL)*	*Test for homogeneity* chi^2	*df*	*sign*	*Pooled coefficient of linear regression,* β_1 *(95% CL)*
Filtered	3	0.004 (0.001, 0.007)	-0.024 (-0.051, 0.004)	3.68	3	ns	0.003 (0.001, 0.006)
	5	0.005 (0.000, 0.009)	-0.001 (-0.058, 0.056)				
	19	-0.003 (-0.010, 0.004)	-0.051 (-0.120, 0.018)				
Percolated	34	0.010 (0.008, 0.011)					
Instant	21*	-0.002 (-0.009, 0.005)	-0.050 (-0.090,-0.010)				
Boiled	19	-0.001 (-0.008, 0.007)	-0.019 (-0.087, 0.048)				
Turkish	11	0.020 (0.003, 0.038)	0.032 (-0.071, 0.135)				

* denotes unpublished study

Table 3.10. Systolic blood pressure (mm Hg/cup), men. For each study separately and, if homogeneity could be assumed, pooled coefficients of linear regression for categories of brewing method.

Brewing method	*Study*	*Coefficients of linear regression* β_1 *(95% CL)*	β_2 *(95% CL)*	*Test for homogeneity* chi^2	*df*	*sign*	*Pooled coefficient of linear regression,* β_1 *(95% CL)*
Filtered	4	0.22 (-0.47, 0.91)		2.96	4	ns	-0.30 (-0.38, -0.22)
	15*	-0.32 (-0.42, -0.22)	-5.72 (- 7.22, -4.21)				
	19	-0.30 (-0.50, -0.10)	-2.76 (- 4.90, -0.62)				
	22	-0.11 (-0.59, 0.37)	-0.34 (- 8.03, 7.34)				
Percolated	31*	-0.29 (-0.72, 0.14)	-5.59 (-10.68, -0.51)	0.26	2	ns	-0.18 (-0.26, -0.10)
	33	-0.18 (-0.27, -0.09)	0.19 (- 0.30, 0.67)				
Instant	2*	-0.60 (-1.35, 0.15)	-1.77 (- 6.74, 3.21)	1.99	2	ns	-0.07 (-0.21, 0.07)
	19	-0.05 (-0.19, 0.09)	-2.07 (- 2.96, -1.18)				
Boiled	19	-0.09 (-0.30, 0.12)	-2.36 (- 4.27, -0.45)				
Espresso	13	-1.33 (-1.76, -0.90)	-1.52 (- 4.38, 1.35)	15.12	2	$p < 0.005$	
	14*	-0.44 (-0.55, -0.33)	-0.80 (- 1.34, -0.27)				

* denotes unpublished study

Table 3.11. Systolic blood pressure (mm Hg/cup), women. Results for each study separately and, if homogeneity could be assumed, pooled coefficients of linear regression for categories of brewing method.

Brewing method	*Study*	*Coefficients of linear regression* β_1 *(95% CL)*	β_2 *(95% CL)*	*Test for homogeneity* chi^2	*df*	*sign*	*Pooled coefficient of linear regression,* β_1 *(95% CL)*
Filtered	4	-0.10 (-0.79, 0.59)		7.60	3	ns	-0.29 (-0.39, -0.19)
	15*	-0.45 (-0.60, -0.30)	1.55 (- 0.29, 3.40)				
	19	-0.19 (-0.31, -0.07)	-2.53 (- 3.73, -1.34)				
Percolated	33	-0.38 (-0.67, -0.09)	0.29 (- 1.23, 1.81)				
Instant	2*	-1.12 (-2.00, -0.24)	-5.41 (-11.63, 0.82)	2.83	2	ns	-0.49 (-0.98, 0.00)
	19	-0.21 (-0.80, 0.38)	-3.29 (- 6.67, 0.09)				
Boiled	19	-0.11 (-0.36, 0.14)	-2.44 (- 4.60, -0.29)				
Espresso	13	-0.67 (-1.88, 0.53)	-1.22 (- 8.44, 6.00)	0.01	2	ns	-0.60 (-0.76, -0.44)
	14*	-0.60 (-0.76, -0.44)	1.05 (0.34, 1.76)				

* denotes unpublished study

Table 3.12. Diastolic blood pressure (mm Hg/cup), men. Results for each study separately and, if homogeneity could be assumed, pooled coefficients of linear regression for categories of brewing method.

Brewing method	*Study*	*Coefficients of linear regression* β_1 *(95% CL)*	β_2 *(95% CL)*	*Test for homogeneity* chi^2	*df*	*sign*	*Pooled coefficient of linear regression,* β_1 *(95% CL)*
Filtered	4	0.20 (-0.64, 1.04)		1.77	4	ns	-0.25 (-0.33, -0.17)
	15*	-0.26 (-0.49, -0.03)	-2.07 (-5.60, 1.46)				
	19	-0.26 (-0.35, -0.17)	-2.26 (-3.20, -1.31)				
	22	-0.13 (-0.43, 0.17)	-0.49 (-5.29, 4.30)				
Percolated	31*	-0.37 (-0.66, -0.08)	-3.97 (-7.39, -0.56)	5.91	2	ns	-0.04 (-0.12, 0.04)
	33	-0.00 (-0.09, 0.09)	-0.11 (-0.63, 0.40)				
Instant	2*	-0.38 (-0.68, -0.08)	-2.96 (-4.94, -0.97)	5.63	2	ns	-0.03 (-0.11, 0.05)
	19	-0.01 (-0.08, 0.06)	-1.48 (-1.93, -1.04)				
Boiled	19	-0.12 (-0.18, -0.06)	-1.20 (-1.77, -0.63)				
Espresso	13	-0.57 (-1.02, -0.12)	-0.66 (-3.66, 2.34)	6.99	2	$p < 0.05$	
	14*	0.05 (-0.03, 0.13)	-0.05 (-0.42, 0.32)				

* denotes unpublished study

Table 3.13. Diastolic blood pressure (mm Hg/cup), women. Results for each study separately and, if homogeneity could be assumed, pooled coefficients of linear regression for categories of brewing method.

Brewing method	*Study*	*Coefficients of linear regression* β_1 *(95% CL)*	β_2 *(95% CL)*	*Test for homogeneity* chi^2	*df*	*sign*	*Pooled coefficient of linear regression,* β_1 *(95% CL)*
Filtered	4	0.22 (-0.86, 1.30)		1.67	3	ns	-0.22 (-0.30, -0.14)
	15*	-0.27 (-0.38, -0.16)	0.76 (-0.62, 2.15)				
	19	-0.19 (-0.28, -0.10)	-1.71 (2.58, -0.83)				
Percolated	33	-0.03 (-0.13, 0.07)	-0.14 (0.68, 0.39)				
Instant	2*	-0.36 (-1.03, 0.31)	-3.69 (-8.44, 1.06)	0.11	2	ns	-0.27 (-0.62, 0.08)
	19	-0.23 (-0.64, 0.18)	-2.04 (-4.41, 0.33)				
Boiled	19	-0.08 (-0.13, -0.03)	-0.97 (-1.43, -0.50)				
Espresso	13	-1.01 (-1.61, -0.42)	-3.96 (-7.55, -0.38)	4.80	2	ns	-0.37 (-0.55, -0.19)
	14*	-0.32 (-0.50, -0.14)	0.07 (-0.71, 0.85)				

* denotes unpublished study

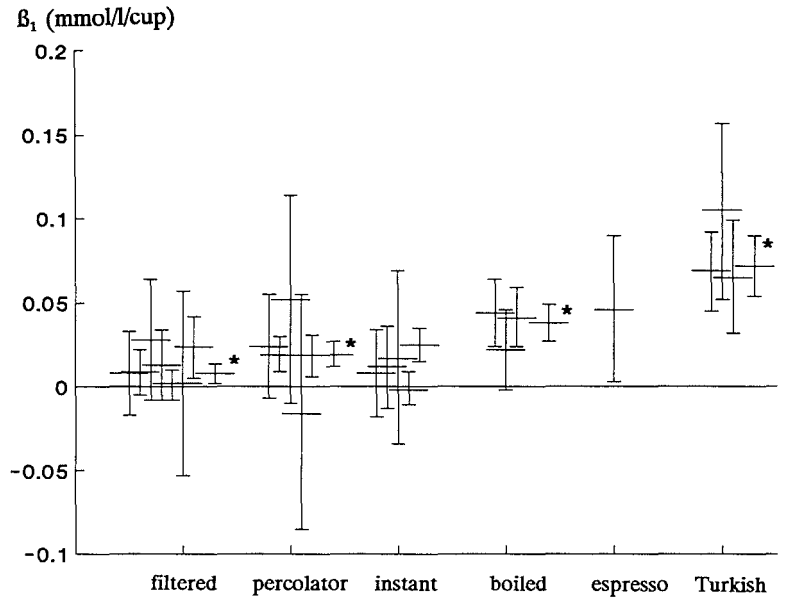

Figure 3.1. Serum total cholesterol, men.
*Coefficient of linear regression β_1 (95 % CL) for each included study. * indicates the pooled coefficient for a category of brewing method.*

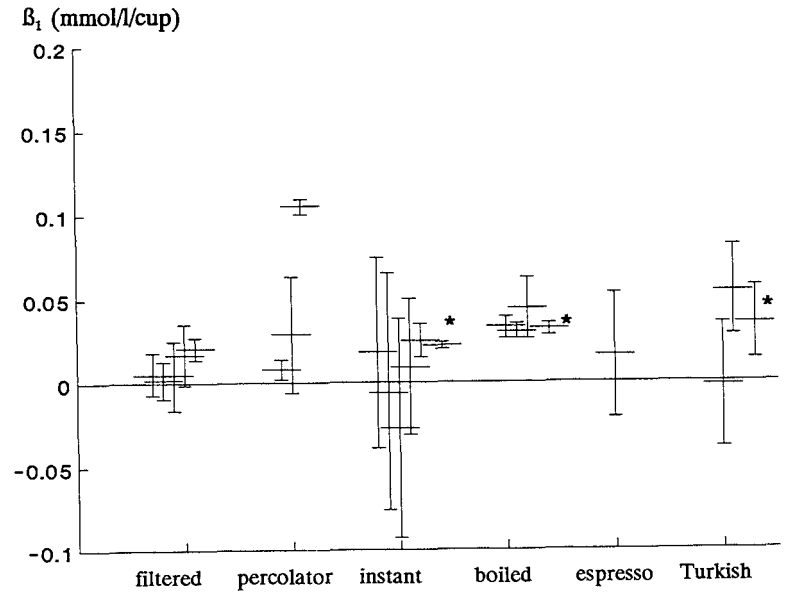

Figure 3.2. Serum total cholesterol, women.

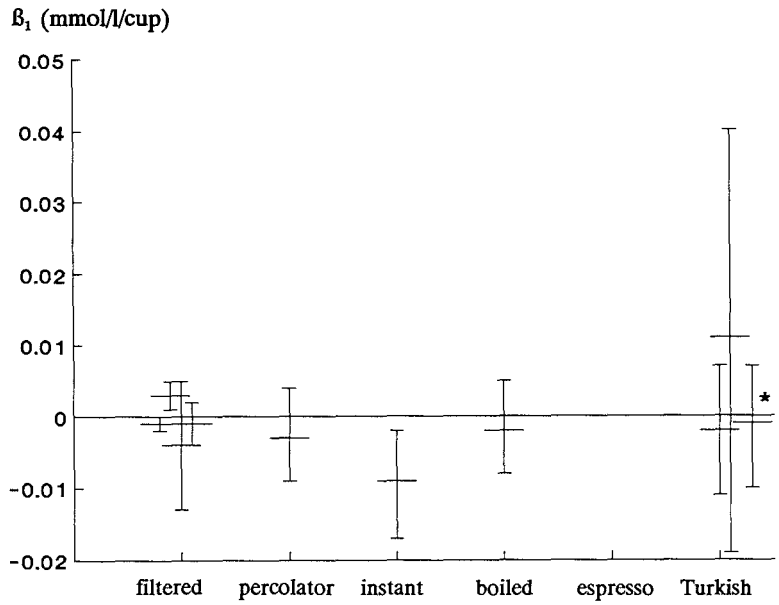

Figure 3.3. HDL-cholesterol, men.

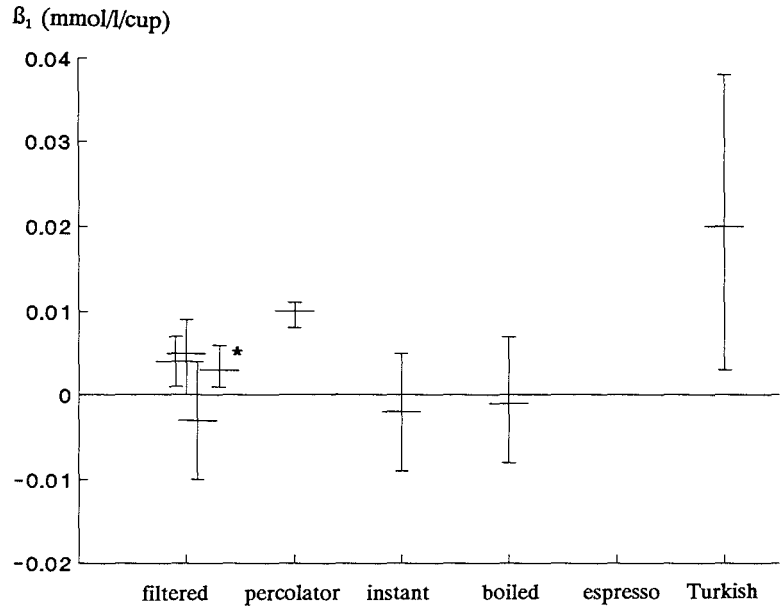

Figure 3.4. HDL-cholesterol, women.

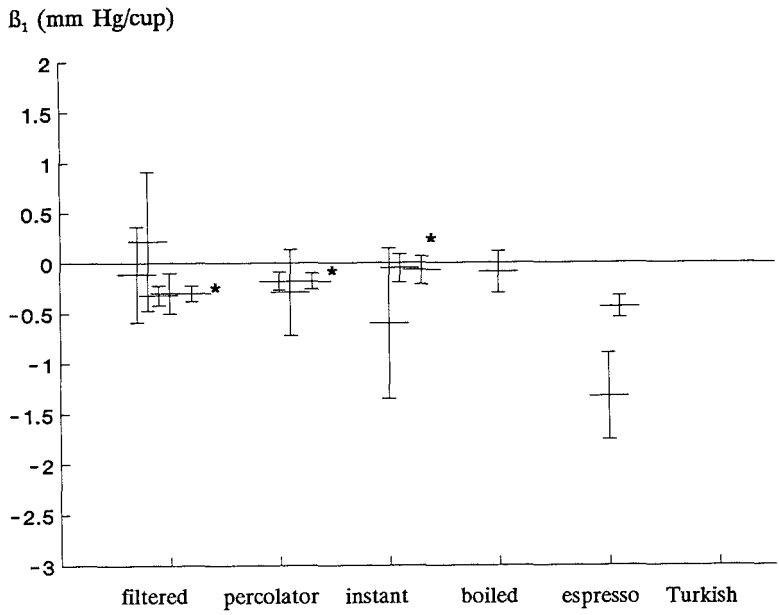

Figure 3.5. Systolic blood pressure, men.

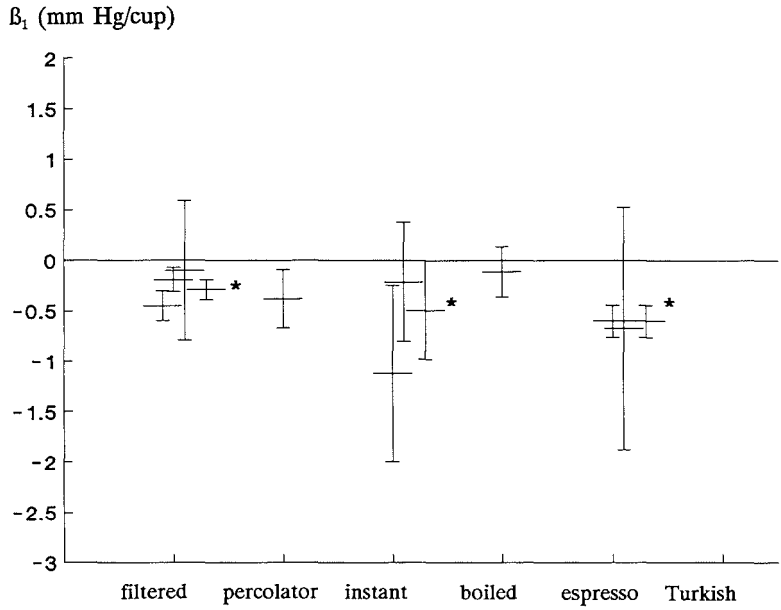

Figure 3.6. Systolic blood pressure, women.

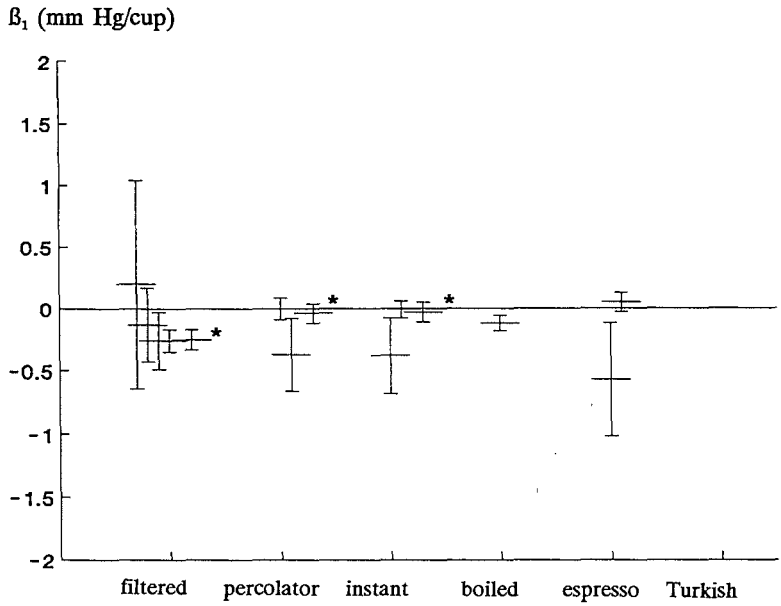

Figure 3.7. Diastolic blood pressure, men.

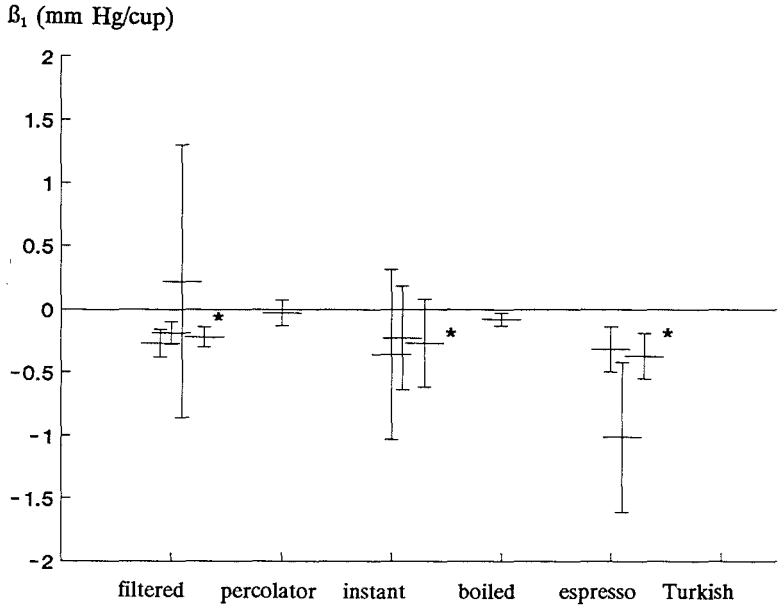

Figure 3.8. Diastolic blood pressure, women.

3.3.7 Bias

Problems of bias in the present study will be considered following the summary of bias in meta-analysis provided by Thacker (70).

Sampling bias due to reporting and publication policies

In a cross-sectional study on cardiovascular risk indicators the question on coffee consumption pattern is one small item on an extensive list of topics addressed. Moreover, the information on coffee is easily obtained without additional costs. Therefore, and in particular if no association between coffee and serum lipids or blood pressure is observed, the urge to publicize the results may be small. In favor of this suggestion is the upsurge of publications on coffee and serum lipids induced by the strong positive findings in Tromsø (96). Investigators were clearly stimulated by the findings of Thelle and co-workers to reanalyze their own data on the subject and to report the results irrespective of the outcome (25-30). Since the quality of cross-sectional studies concerning the effect of coffee on serum lipids and blood pressure shows limited variation, the option to include every available study seems most appropriate. The practical problem, however, to obtain data of unpublished studies for a meta-analysis is considerable. As indicated in table 3.1, we were not very successful in obtaining data from unpublished studies reported at the Workshop on Coffee and Coronary Heart Disease (Göteborg, 1989) and the same applies to blood pressure data, even from researchers who willingly supplied us with missing information on published results on coffee and serum lipids. This experience discouraged further inquirements for unpublished data. The unattractiveness of putting the data at the disposal of other investigators and the effort to carry out the actual additional extraction of files and calculations may be the main reasons for refusal. The impact of publication bias and the subsequent possibility of overestimation of the effect size can be estimated in tables 3.6-3.13. With regard to coffee and blood pressure, new data were obtained from four studies: The Western Electric Study, USA (25), the EPOZ study from the Netherlands (28), an Australian population study, from which data were published on caffeine and blood pressure (29), and finally an Italian study (Panico). The results compare fairly well with the available published data, and even yield pronounced negative point estimates (ranging from -0.29 to -0.60 mm Hg/cup for systolic blood pressure). Unpublished results on coffee and serum cholesterol were obtained from 5 studies: an Australian population study reporting on caffeine (52), the Scottish MONICA study (Lee), a community health study from Israel (Kark), the Dutch Zutphen study (Kromhout) and an Italian population study (Panico). Compared to the published data, they show no extreme values and, if anything, point estimates tend to be positive. The limited set of data from unpublished studies prevents a balanced evaluation of the impact of publication bias. The available results, however, indicate no major discrepancy between published and unpublished results and a tendency towards more

non-significant findings among unpublished data could not be detected.

Absence in published studies of specific data desired for review

The specific hypotheses regarding the effects of coffee on LDL-cholesterol and apolipoproteins could not be tested due to insufficient availability of data. To permit reanalysis of the studies, at least mean values of blood pressure or serum cholesterol for three or more coffee consumption categories were needed. Furthermore, the number of subjects in each consumption category was to be reported. For 10 studies these data were not available and, therefore, these studies were excluded from the analysis (21-24,26,34,45-48). Table 3.2 provides the published results of included and excluded studies. As far as comparison is possible, the excluded studies are not different from the included studies with respect to direction and magnitude of the effect.

Biased exclusion of studies by the investigator

The inclusion criteria for the present meta-analysis, as stated in section 3.3.2, are unequivocal and objective. No study was excluded for subjective reasons and, therefore, evaluation of studies by more than one informed investigator was considered unnecessary.

Uneven quality of the primary data

As discussed in the section on quality assessment, quality of the used cross-sectional studies is considered to be mainly determined by sample size. In the re-analysis, regression analysis was weighted according to number of subjects. To correct possible errors in the published data, investigators were asked to check the data of the completed forms. Two investigators actually discovered small errors in their databases during reanalysis, which slightly altered the results.

Biased outcome interpretation

In order to compare the results of the studies used, data were reanalyzed in a uniform way, thereby limiting the chance of biased outcome interpretation. Readers can draw their own conclusions by scrutinizing the tables and figures.

3.3.8 Discussion

The findings of this meta-analysis of 21 cross-sectional studies on coffee can be summarized as follows. There is a small, albeit significant positive association between coffee use and serum total cholesterol. As hypothesized in section 3.3.1, the positive link depends on the brewing method. Consumption of boiled coffee, a common brew in parts of Scandinavia, increases serum total cholesterol, as already confirmed by experimental studies (107-109). Turkish coffee, and, at least in men, espresso coffee seem to exert the same effect. These observations from cross-sectional studies are, as yet, not supported by experimental evidence. It should be noted that the Italian studies are classified as espresso. To quote Dr. S. Panico, however, "many Italians proudly

consume mocca coffee". As explained in the Glossary, the underlying principle is similar for these brews. A paper filter is not used during preparation of the coffee. All American studies were categorized as filtered/percolated. A considerable shift from the habit of drinking percolated coffee to filtered coffee took place between 1975 and 1985. The percentages percolated and filtered coffee were 75 %, 20 % in 1975, and 25 %, 66 % in 1985 (111). Unfortunately, more detailed data on brewing method are lacking. Some studies included in the meta-analysis were conducted in a period that percolated coffee was the most common brew in the USA (25,33,36). Data of more recent studies were collected in a period of increasing popularity of filtered coffee. Since filtered coffee has only a very small effect on serum total cholesterol, the potential cholesterol increasing effect of percolated coffee is diluted. In women, the small increase in serum total cholesterol on filtered coffee is accompanied by an increase in HDL-cholesterol. A possible explanation might be a higher use of oral contraceptives among heavy female coffee consumers. Unfortunately, LDL-cholesterol and apolipoproteins could not be included in the meta-analysis, due to insufficient data. Results from experimental studies support the hypothesis that the cholesterol increasing effect of boiled coffee is accompanied by a rise in both LDL-cholesterol and apolipoprotein B (108,109).

The apparent negative link between coffee and blood pressure observed in this meta-analysis is difficult to interpret. A possible explanation might involve alleviation of anxiety with a consequent decrease in blood pressure. Experimental studies do not support such a favourable effect of coffee consumption on blood pressure. Short-term studies invariably show a blood pressure increasing effect of coffee and caffeine (86-88). In longer term studies, abstinence from coffee or caffeine was observed to decrease blood pressure (89,90).

The principle advantage of the present meta-analysis of studies on coffee and serum lipids and blood pressure is obvious. All included studies were reanalyzed in a similar way which permitted a strict comparison between studies. Moreover, for some subgroups an average "overall" effect could be calculated. Brewing method was considered an important modifier of the effect of coffee on serum lipids, and therefore our main focus of interest. All studies were categorized according to the coffee brew mainly used.

The major problem in a meta-analysis of cross-sectional studies on coffee and cardiovascular risk indicators, is publication bias. The results from the unpublished studies did not deviate much from the published data. The number of unpublished studies included, however, was very small, precluding a reliable estimation of the impact of publication bias. The easy, inexpensive way to obtain data on coffee use in a large cross-sectional study, combined with a tendency to ignore non-significant associations both by authors and editors of scientific journals, may have resulted in a lot of unpublished information remaining in file drawers. Our difficulties in obtaining data

from unpublished studies on coffee use and serum lipids and blood pressure, rendered us pessimistic about the possibility to perform a reliable meta-analysis of cross-sectional studies on these subjects. Therefore, the presented results should be interpreted with care and, preferably, only used as an indicator for further experimental research. Future studies should focus on a potential cholesterol raising effect of espresso coffee, percolated and Turkish coffee. For all brews, experimental evidence for a long-term change in blood pressure induced by coffee use is lacking.

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Appendix I Model for estimating effects per study (112).

The explanatory variable y_i (e.g. blood pressure) observed in individual i in a certain study is assumed to be explained as follows:

$$y_i = \beta_0 + \beta_1 X_i + \beta_2 Z_i + \epsilon_i$$

which is a simple form of a piecewise linear model with explanatory variables X_i and Z_i, where X_i is the number of cups of coffee consumed per day and Z_i a dummy variable which equals 1 if $X_i = 0$ and 0 elsewhere. The term ϵ_i is a normally distributed error term with mean zero and variance σ_ϵ^2 for all i in the study. The model specifies that there is a trend β_1 in blood pressure with the consumption of coffee and that there is a deviation β_2 from this trend when consumption is nil. However, the individual observations are not available, but only averages of y_i per X_i - category. The model for these averages in an $X_i = X$ category with n_X observations is

$$(1/n_X) \sum_{i=1}^{n_X} y_i = \beta_0 + \beta_1 X + \beta_2 Z_X + (1/n_X) \sum_{i=1}^{n_X} \epsilon_i$$

which can also be written as

$$\bar{y}_X = \beta_0 + \beta_1 X + \beta_2 Z_X + \bar{\epsilon}_X$$

where $\bar{\epsilon}_X$ is a normally distributed error term with mean zero and variance σ_ϵ^2 / n_X and therefore dependent on X. For efficient estimation of the ß coefficients, a weighted regression analysis is appropriate. In order to make the error homoskedastic and independent of X, all terms are weighted by $\sqrt{n_X}$ resulting in the following linear equation

$$y_X \sqrt{n_X} = \beta_0 \sqrt{n_X} + \beta_1 (X \sqrt{n_X}) + \beta_2 (Z_X \sqrt{n_X}) + u$$

The error term $u = \epsilon_X \sqrt{n_X}$ is normally distributed with mean zero and variance σ_ϵ^2 for all X and therefore meets the basic requirements for linear regression analysis. The ß coefficients can now be estimated in the last model by linear regression through the origin, provided that at least four (including X = 0) or three (excluding X = 0) X-categories are available in a study.

Appendix II Meta-analysis of effects across studies (113).

In each study j an estimate for the trend effect β_{1j} of coffee consumption on an explanatory variable (e.g. blood pressure) is b_{1j} with estimated standard error SE (b_{1j}) as calculated from a weighted linear regression analysis as described above.
Across k studies j = 1,...,k one may test whether the b_{1j} are estimators for the same common effect β_1*. It is assumed here that we have assembled k mutually homogeneous studies, i.e., studies with the same characteristics such as sex of the population studied and brewing method. If there is homogeneity across studies an estimator for the common effect β_1* is b_1* which is a weighted average of the estimators b_{1j} with the reciprocal of their estimated variances SE^2 (b_{ij}) as weights:

$$b_1^* = \sum_{j=1}^{k} \{b_{1j} / SE^2(b_{1j})\} \; / \; \sum_{j=1}^{k} \{1 / SE^2(b_{1j})\}$$

with standard error

$$SE(b_1^*) = \sqrt{\left[\sum_{j=1}^{k} \{1/SE^2(b_{1j})\}\right]^{-1}}$$

If there is a common effect across the k studies, the fluctuation in the b_{1j} is only due to sampling variation and an appropriate test statistic is:

$$chi^2(k) = \sum_{j=1}^{k} (b_{1j} - b_1^{\cdot})^2 / SE^2(b_{1j}),$$

which under the null hypothesis of homogeneity (i.e. there is a common effect) is asymptotically chi-squared distributed with k degrees of freedom.

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CHAPTER 4

COFFEE AND SERUM LIPIDS

4.1 COFFEE AND SERUM CHOLESTEROL IN EXPERIMENTAL STUDIES

The key-reference on the relationship between coffee and serum lipids is the Tromsø paper by Thelle, Arnesen and Førde (1). They reported a strong association between coffee and serum total cholesterol. As a matter of course, these findings were followed by several experiments, two of which were performed by the same group of investigators in Tromsø. Only one long-term experiment on coffee and serum lipids was performed in the "pre-Tromsø period". The experimental studies on coffee and serum cholesterol are summarized in table 4.1.

Naismith and co-workers showed that serum cholesterol levels increased by 0.6 mmol/l during a period of 14 days on 8-12 cups of decaffeinated coffee per day, in 20 men and women. In the subsequent period of 20 days on regular instant coffee, serum cholesterol decreased again by 0.3 mmol/l, not reaching baseline levels (2). In the first Norwegian study, the effects on serum lipids of consumption of boiled coffee and abstinence from coffee were compared in a cross-over design. On abstinence from boiled coffee, serum total cholesterol decreased by 0.4 mmol/l and increased again when returning to boiled coffee (3). One year later, an intervention study was reported in hypercholesterolemic men (4). The 33 participants were assigned to 4 parallel intervention groups, continuing on habitual coffee use for 10 weeks, drinking no coffee for 10 weeks, drinking no coffee for 5 weeks followed by 5 weeks on boiled coffee, and drinking no coffee for 5 weeks followed by 5 weeks on filtered coffee, respectively. A fall in serum total cholesterol on abstinence from boiled coffee was observed by 1.2 mmol/l (95 % confidence interval 0.7, 1.7). Changing to boiled coffee raised cholesterol concentrations again, while changing to filtered coffee did not affect serum cholesterol levels. Aro and co-workers from Finland published two cross-over experiments, one focussing on instant coffee and tea, the other on filtered coffee and boiled coffee (5,7). The latter, examining the effects of different brewing methods, similar to the methods used in the Norwegian studies, confirmed the previous findings, showing an increase of 0.6 mmol/l after 4 weeks on boiled coffee and a decrease after filtered coffee or tea. LDL-cholesterol and apo lipoprotein B were also observed to increase significantly on boiled coffee. Again, the participants in this study were hypercholesterolemic (7). In the other study by this group of investigators, in 12 normocholesterolemic subjects, no cholesterol raising effect of instant coffee could be observed (5). This finding is in accordance with two British trials which also examined the effect of instant coffee (6,13). Finally, the results from 5 experiments, that have been presented at recent scientific meetings, have only been published in abstract form (8-12). Aro and co-workers performed a cross-over study and showed a dose-dependent increase in serum cholesterol on boiled coffee, compared to filtered coffee (8). Van Dusseldorp et al. did not find a difference in serum cholesterol between two 6-week periods on regular

coffee and decaffeinated coffee (9). In a very small Japanese study, filtered coffee was observed to lead to a non-significant increase in serum cholesterol and to a statistically significant increase in HDL-cholesterol (10). Fried et al. reported a small decrease in total cholesterol, HDL-cholesterol and apolipoprotein A1 on abstinence from coffee and caffeine for a period of 2 months. Marked changes in LDL-cholesterol and apolipoprotein B were not observed (11). In the study by Superko and co-workers, a statistically significant, but puzzling, increase in serum apolipoprotein B and LDL-cholesterol was reported in a group on decaffeinated coffee, compared to a group on regular coffee or a group abstaining from coffee (12).

These results, albeit interesting, should be interpreted with some caution. First, the small numbers of subjects in these trials affect the precision of the effect estimates. Second, the open character of some of the studies makes their interpretation difficult. Third, the relatively short duration of the intervention periods may not exclude an effect in a longer study. Next, two of the most convincing studies were performed in hypercholesterolemic subjects, who may respond differently from the population at large (5,8). Finally, all studies on boiled coffee were conducted in Norway and Finland, were people traditionally consumed this kind of brew. Determinants of the serum lipid profile, as yet unknown, particularly operating in Scandinavian people, might explain the association between boiled coffee and serum lipids.

We conducted a randomized trial, which had some advantages over the previous studies on coffee and serum lipids. In a parallel design, 107 participants were randomly allocated to 3 intervention groups, respectively drinking filtered coffee, boiled coffee or no coffee at all for a period of nine weeks. The two coffee drinking groups were to follow strict instructions on the preparation of their coffee. All subjects had normal serum cholesterol values at baseline. Before the study, all participants consumed filtered coffee, permitting the evaluation of the effect of boiled coffee in a population not used to it.

Table 4.1. A summary of published experimental studies on coffee and serum lipids.

First author (ref)	*Number men*	*women*	*Participants*	*Study design*	*Protocol/intervention*	*Net change from baseline in serum cholesterol (mmol/l) (mean and 95 % CL)*
Naismith (2)	14	6	normocholesterolemic aged 21-49	open	10 d habitual 14 d decaf 20 d instant	- + 0.6 + 0.3
Arnesen (3)	17		normocholesterolemic aged ?	cross-over	1) 4 w boiled 5 w no coffee 2) 4 w no coffee 5 w boiled	- - - 0.4 - 0.3 + 0.6
Førde (4)	33		hypercholesterolemic aged 35-54	parallel	1) 10 w habitual 2) 10 w no coffee 3) 5 w no coffee 5 w boiled 4) 5 w no coffee 5 w filtered	+ 0.4 (-0.5, 1.3) - 1.2 (-1.7, -0.7) - 0.9 (-1.4, -0.4) - 0.4 (-0.7, -0.1) - 0.9 (-1.2, -0.6) - 0.9 (-1.7, -0.1)
Aro (5)	6	6	normocholesterolemic aged 33-45	cross-over	3 w instant 3 w tea 3 w rosehip tea	+ 0.1 (-0.4, 0.6) - 0.1 (-0.6, 0.4) 0.0 (-0.5, 0.5)
Hill (6)	15		normocholesterolemic aged ?	cross-over	4 w instant 4 w no coffee 4 w tea	no consistent findings
Aro (7)	21	21	hypercholesterolemic aged 31-60	cross-over	4 w boiled 4 w filtered	+ 0.6 (0.1, 1.1) - 0.2 (-0.8, 0.4)

Table 4.1 (cont).

First author (ref)	*Number men*	*women*	*Participants*	*Study design*	*Protocol/intervention*	*Net change from baseline in serum cholesterol (mmol/l) (mean and 95 % CL)*
Aro (8)	13	28	normocholesterolemic aged ?	cross-over	4 w boiled 4 w filtered (2-14 cups/day)	boiled vs. filtered - 0.7 (-1.1,-0.3) + 0.3 (-0.1, 0.7)
v Dusseldorp (9)	22	23	normocholesterolemic aged 25-45	cross-over	6 w regular 6 w decaffeinated	regular vs. decaf 0.0 (-0.1, 0.1)
Ishikawa (10)	3	8	normocholesterolemic aged 20-43	open	3 w no caffeine 4 w filtered	 + 0.2 (ns)
Fried (11)	74		normocholesterolemic aged ?	open	2 m no coffee and caffeine	- 0.1 (-0.2, 0.0)
Superko (12)	181		? ?	parallel	2 m regular 1) 2m regular 2) 2m decaffeinated 3) 2m no coffee	not available
Burr (13)	35	19	? aged 18-58	cross-over	4 w regular instant 4 w decaff. instant 4 w no coffee	regular vs. no coffee 0.04 (-0.15, 0.24) decaf. vs. no coffee 0.05 (-0.14, 0.25)
Rosmarin (14)	24		normocholesterolemic aged 22-45	cross-over	2 m filtered regular 2 m no coffee	regular vs. no coffee - 0.01 (ns)

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4.2 THE EFFECT ON SERUM CHOLESTEROL LEVELS OF COFFEE BREWED BY FILTERING OR BOILING[1]

Introduction

The possibility that the consumption of coffee may increase the risk of coronary heart disease has been suspected for many years (1). Early reports showed conflicting results, but more recent studies support the view that the consumption of coffee is positively linked to cardiovascular morbidity (2-4). The observed increased risk may be explained in part by an effect of coffee consumption on known cardiovascular risk factors, in particular serum cholesterol levels (5-30). Several nonexperimental studies have investigated the potential effect of coffee in raising cholesterol levels. Thelle et al. reviewed 22 cross-sectional studies and concluded that most, but not all, showed an increase in serum cholesterol levels with higher coffee consumption (31). The inconsistency may result from methodologic discrepancies among studies, but there is also increasing evidence that differences in brewing methods are important. The strongest evidence for a direct association between coffee consumption and serum cholesterol levels comes from Scandinavia, where in some areas coffee is brewed predominantly by boiling. When the brewing method is taken into account, the consumption of filtered coffee has a considerably weaker association with serum cholesterol levels than the consumption of coffee prepared by boiling (29). Findings in three experimental studies from Norway and Finland have suggested that serum cholesterol levels fall when boiled coffee is no longer consumed (32-34). These intervention studies involved small numbers of participants, however, and were of relatively short duration (four or five weeks). In addition, two of these studies were performed in subjects with hypercholesterolemia, who may respond differently from the population at large.

To reassess the effect of coffee consumption on serum lipid levels and the part played by brewing methods, we conducted a randomized trial among 107 young, healthy adults in three parallel groups. For a period of nine weeks, the subjects were randomly assigned to groups drinking filtered coffee, boiled coffee, or no coffee at all.

Methods

Subjects

The study subjects were selected from a cohort of 596 young adults participating in a follow-up study of cardiovascular-risk indicators. This cohort is a random sample from a population-based study that was initiated in 1975 and included 4649 young subjects

[1]N Engl J Med 1989;321:1432-7.

from the Dutch town of Zoetermeer (35). The members of the cohort have been examined annually since the start of the study. Those who were 18 years of age or older and who habitually drank coffee were invited to participate in the present study, and 107 agreed to take part. All the participants gave informed consent. The mean baseline coffee consumption of the subjects was 5.6 cups per day. All drank filtered coffee.

Protocol

The total study period was 12 weeks. During a three-week run-in period, all the participants continued to consume filtered coffee. After this period, they were randomly assigned to one of three groups receiving four to six cups of boiled coffee a day, four to six cups of filtered coffee a day, or no coffee, for a period of nine weeks. Randomization was performed after stratification for sex by assigning a computer-generated random number to each subject. All the participants had to drink three cups of tea per day, in order to make the groups similar with respect to their intake of caffeine from this source. Blood samples were obtained twice during the run-in period and after three, six, and nine weeks of intervention. An additional blood sample was obtained 12 weeks after the trial had ended. On these occasions body weight and blood pressure were also measured.

Brewing Methods

The filtered coffee was prepared in a commercially available electric coffee maker by dripping hot water on coffee and filtering it through paper. The subjects using boiled coffee prepared it by pouring 0.5 liter of boiling water on 20 g of coarsely ground coffee in a thermos bottle and waiting for at least 10 minutes before drinking it. Just before consumption, the liquid was decanted without the use of a filter. This protocol is like those used in previous studies involving boiled coffee (33). Coffee and the brewing devices were given to the participants free of charge. The coffee was drunk from 140-ml cups. Similar blends of coffee were used to prepare both the boiled and the filtered coffee, and the degree of roasting was also similar. The coffee for boiling was ground more coarsely than the coffee for filtering. The caffeine content of the filtered coffee and the boiled coffee (determined after 30 minutes in the thermos bottle) was 670 mg per liter and 630 mg per liter, respectively.

Measurements

Blood samples were obtained from nonfasting subjects, and samples frozen at -20 °C were analyzed in a blinded fashion. All determinations were carried out in our department's laboratory, which participates in the Lipid Standardization Program of the World Health Organization's Regional Lipid Reference Center in Prague, Czechoslovakia. An automated enzymatic procedure was used to determine serum total cholesterol levels (36). High-density lipoprotein and low-density lipoprotein cholesterol levels were measured by the same method after precipitation. The phosphotungstate

method of Burstein and coworkers (37) with a minor modification as described by Grove (38) was used to precipitate high-density lipoprotein cholesterol. The precipitation of low-density lipoprotein cholesterol was carried out with polyvinyl sulfate (Boehringer Mannheim, Mannheim, West Germany). Apolipoproteins A-I and B were assayed by an automated immunoturbidimetric method (Kone Diagnostics, Espoo, Finland). Apolipoprotein A-II was assayed by radial immunodiffusion against specific antiserum (Boehringer Mannheim) with use of the method of Cheung and Albers, with slight modifications (39). The caffeine concentration in serum and saliva samples was determined by reversed-phase high-performance liquid chromatography (40).

Height and weight were measured with the subjects wearing indoor clothing without shoes. Questionnaires on the intake of dietary fat that recorded how often and in what amount the subjects ate 81 food items were completed at baseline and at the end of the study period. From these forms, we calculated the subjects' mean daily intake of total fat and animal fat using a computerized food-composition table (41). We assessed the use or nondairy creamers in coffee as part of the questionnaire.

Compliance

Adherence to the study protocol was assessed in three ways. First, each participant kept a diary on the amount of coffee, tea, cola, and chocolate consumed. In the coffee groups, the empty packages of coffee were returned and counted. Participants were asked during all examinations and home visits to describe the steps involved in their assigned brewing method. Moreover, serum caffeine levels were measured in blood samples obtained during the scheduled visits to the examination center. Finally, each participant was visited twice, announced, during the study period. During these visits, saliva samples were obtained to determine caffeine levels.

Statistical Analysis

The effects of boiled or filtered coffee were analyzed by comparing the mean changes in serum lipid levels from baseline in the three study groups. The second series of measurements taken during the run-in period were used as baseline values in the comparison. Because all the participants drank filtered coffee before the trial, the group that continued to consume filtered coffee was used as the reference group. Therefore, the results for the groups drinking boiled coffee and no coffee are expressed as the change from baseline minus concomitant changes in the filtered-coffee group, after three, six, and nine weeks of intervention. Differences between groups are expressed as means and 95 percent confidence limits. In an additional analysis, adjustments were made for unintended changes in body weight during the intervention period with use of a multiple linear-regression model. Separate analyses were performed for men and women and for smokers and nonsmokers. Also, any interaction between sex or smoking habits and the effects of filtered or boiled coffee was tested

Table 4.2. Baseline characteristics in the three study groups.

	No coffee (n = 34)	*Filtered coffee (n = 34)*	*Boiled coffee (n = 33)*
Sex (M/F)	18/16	18/16	18/15
Age (yr)	26.5 (3.8)	25.0 (4.3)	25.5 (3.6)
Weight (kg)	71.5 (13.1)	68.9 (13.0)	67.8 (12.3)
Height (cm)	175.1 (11.5)	175.1 (8.5)	174.6 (9.6)
Total cholesterol (mmol/l)	5.22 (0.93)	5.03 (1.23)	5.09 (1.02)
HDL-cholesterol (mmol/l)	1.23 (0.25)	1.18 (0.31)	1.30 (0.26)
LDL-cholertorol (mmol/l)	3.36 (0.95)	3.06 (1.31)	3.20 (1.03)
Apolipoprotein A-I (mg/dl)	125.1 (22.3)	119.5 (14.2)	123.6 (16.5)
Apolipoprotein A-II (mg/dl)	48.1 (6.6)	46.6 (7.0)	49.1 (10.1)
Apolipoprotein B (mg/dl)	82.3 (21.3)	80.2 (25.2)	80.9 (23.9)
Serum caffeine (umol/l)	14.4 (9.3)	12.8 (10.8)	13.9 (7.7)
Coffee (cups/day)	5.9 (3.0)	5.1 (3.4)	5.8 (3.1)
Cream in coffee (yes/no)	16/18	18/16	18/15
Tea (cups/day)	2.6 (2.7)	1.9 (1.9)	2.1 (2.1)
Caffeine intake (mg/day)	543 (213)	458 (251)	499 (212)
Dietary fat intake (g/day)	88 (40)	90 (31)	106 (44)
Smoking (yes/no)	16/18	15/19	17/16
No. of heavy smokers (>15 cigarettes/day)	11	10	10

Values are means (SD). To convert cholesterol values to milligrams per deciliter, multiply by 38.67. To convert caffeine values to micrograms per milliliter, multiply by 0.19.

by entering interaction terms for these characteristics in the multivariate model (42). The analyses presented below exclude six subjects who did not complete the study. All of them withdrew immediately after randomization. Four were in the group drinking boiled coffee, one in the filtered-coffee group, and one in the group that drank no coffee. The baseline characteristics of these six subjects were similar to those of the other participants.

Results

The baseline characteristics of the three study groups are summarized in Table 4.2. The data indicate that the groups were well balanced in all characteristics. Table 4.3 shows the changes in serum lipid levels in each of the three groups separately. Figures 1 and 2 show the net changes in serum total cholesterol and high-density lipoprotein cholesterol levels from baseline, with the filtered-coffee group as the reference group.

Table 4.3. Mean changes from baseline in serum cholesterol (mmol/l) and apolipoprotein levels (mg/dl) during intervention.

	No coffee (n = 34)	*Filtered coffee (n = 34)*	*Boiled coffee (n = 33)*
Total cholesterol			
3 weeks	-0.06 (-0.22, 0.10)	-0.02 (-0.29, 0.25)	0.27 (0.07, 0.47)
6 weeks	0.01 (-0.21, 0.23)	0.14 (-0.11, 0.39)	0.42 (0.22, 0.62)
9 weeks	-0.03 (-0.21, 0.15)	0.04 (-0.21, 0.29)	0.52 (0.27, 0.77)
HDL-cholesterol			
3 weeks	-0.02 (-0.08, 0.04)	0.00 (-0.06, 0.06)	-0.04 (-0.10,0.02)
6 weeks	-0.03 (-0.07, 0.01)	-0.02 (-0.06, 0.02)	-0.05 (-0.11,0.01)
9 weeks	0.00 (-0.06, 0.06)	0.02 (-0.02, 0.06)	0.01 (-0.03,0.05)
LDL-cholesterol			
3 weeks	-0.06 (-0.26, 0.14)	0.10 (-0.21, 0.41)	0.26 (-0.02,0.54)
6 weeks	0.00 (-0.24, 0.24)	-0.18 (-0.15, 0.51)	0.54 (0.26,0.82)
9 weeks	0.18 (-0.13, 0.49)	-0.03 (-0.27, 0.21)	0.36 (-0.01,0.73)
Apolipoprotein A-I			
3 weeks	0.4 (- 8.0, 8.8)	5.2 (-1.1, 11.5)	9.9 (2.6, 17.2)
6 weeks	-2.8 (- 9.3, 3.7)	6.4 (-1.2, 14.0)	4.2 (-2.1, 10.5)
9 weeks	-5.5 (-12.0, 1.0)	4.9 (-4.5, 14.3)	4.8 (-1.5, 11.1)
Apolipoprotein A-II			
3 weeks	0.0 (- 1.6, 1.6)	-0.5 (-2.3, 1.3)	0.0 (-2.0, 2.0)
6 weeks	-0.1 (- 1.9, 1.7)	0.1 (-1.7, 1.9)	1.9 (-0.1, 3.9)
9 weeks	-0.3 (- 1.9, 1.3)	2.1 (0.5, 3.7)	2.8 (1.0, 4.6)
Apolipoprotein B			
3 weeks	-1.0 (- 6.5, 4.5)	0.3 (- 6.2, 6.8)	1.1 (-6.5, 8.7)
6 weeks	-2.4 (- 7.3, 2.5)	-0.9 (- 6.0, 4.2)	2.9 (-4.4,10.2)
9 weeks	-4.1 (- 8.8, 0.6)	-4.9 (-10.6, 0.8)	3.8 (-4.0,11.6)

Values in parentheses are 95 % CL.

As compared with the changes in the filtered-coffee group, serum total cholesterol levels increased after three, six, and nine weeks of drinking boiled coffee by 0.29 mmolper liter (95 percent confidence limits, -0.04 and 0.62), 0.29 mmol per liter (-0.04 and 0.62), and 0.48 mmol per liter (0.13 and 0.83), respectively. Serum total cholesterol levels in the filtered-coffee group and the group that drank no coffee remained virtually unchanged during the intervention period. Levels of high-density lipoprotein cholesterol were not affected by drinking filtered or boiled coffee. Serum low-density lipoprotein cholesterol levels increased in the group drinking boiled coffee, but as compared with the change in the filtered-coffee group the net increase (0.39 mmol per liter [95

Table 4.4. Mean changes from baseline in body weight and intake of dietary fat during intervention.

	No Coffee (n = 34)	*Filtered coffee (n = 34)*	*Boiled coffee (n = 33)*
Body weight (kg)			
3 weeks	-0.4 (-0.8, 0.0)	-0.1 (-0.5, 0.3)	0.0 (-0.4, 0.4)
6 weeks	-0.6 (-1.0,-0.2)	0.0 (-0.6, 0.6)	-0.4 (-1.0, 0.2)
9 weeks	-0.6 (-1.2, 0.0)	0.1 (-0.3, 0.5)	0.0 (-0.6, 0.6)
Dietary fat (g/day)			
9 weeks	-2.3 (-9.6, 5.0)	-0.5 (-6.8, 5.8)	-5.4 (-17.9,7.1)

Values in parentheses are 95 % CL.

percent confidence limits, -0.04 and 0.82]) did not reach statistical significance. Levels of apolipoproteins A-I, A-II, and B did not change significantly from baseline levels in the three groups.

Table 4.4 shows the findings on intake of dietary fat and body weight during the intervention period. According to the questionnaires completed at baseline and at the end of the trial, the mean intake of dietary fat remained unchanged. The subjects in the two coffee groups who used cream in their coffee did not change that habit during the intervention period. Body weight, however, fell slightly, almost reaching significance in the group that did not drink coffee. The results were therefore adjusted for differences in weight change between the groups, and this did not materially affect the results (figures 4.1 and 4.2).

Separate analyses for men and women and for smokers and nonsmokers revealed no significant interaction between sex or smoking habits an the effect of boiled coffee on serum total cholesterol levels.

At the end of the trial, all the participants resumed their habitual consumption of filtered coffee. The changes in serum total cholesterol levels at 21 weeks from those measured after 9 weeks of intervention were 0.09 mmol per liter (95 percent confidence limits, -0.22 and 0.40) for the group that drank no coffee, 0.05 mmol per liter (-0.26 and 0.36) for the filtered-coffee group, and -0.54 mmol per liter (-0.87 and -0.21) for the group drinking boiled coffee (figure 4.1).

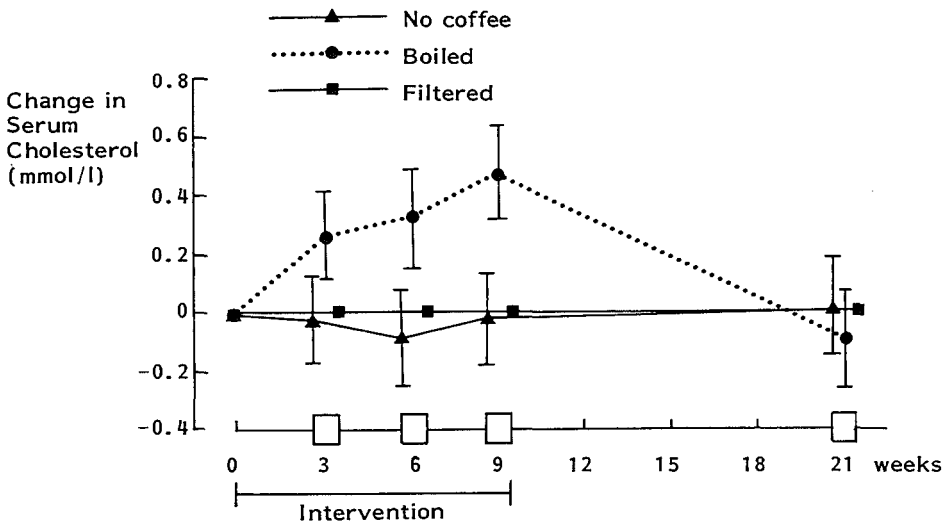

Figure 4.1. Net changes in serum total cholesterol level (mmol/l) from baseline. The filtered-coffee group was the reference group. Results are shown after 3, 6 and 9 weeks of intervention and 12 weeks after the trial ended. Values (mean ± SEM) are adjusted for different changes in body weight in the groups.

The daily records indicated good adherence to the study protocol. Consequently, in the groups drinking filtered and boiled coffee the concentrations of serum caffeine during the intervention period were not statistically different from the baseline levels, a finding compatible with the continuation of their previous intake of caffeine. In the group that drank no coffee, a substantial decline in levels of serum caffeine was observed (figure 4.3). The values in these subjects did not drop to zero, because they ingested caffeine in tea. Measurements of caffeine concentrations in saliva showed similar results (figure 4.4).

Discussion

The main finding of this randomized trial in young adult subjects with normal levels of cholesterol was that the consumption of boiled coffee, as compared with filtered coffee or no coffee, leads to a significant increase in the serum total cholesterol level. After nine weeks, the net increase amounted to almost 10 percent of the baseline level. These observations support the hypothesis that coffee prepared by boiling increases serum total cholesterol levels. The magnitude of the effect is quite similar to that found

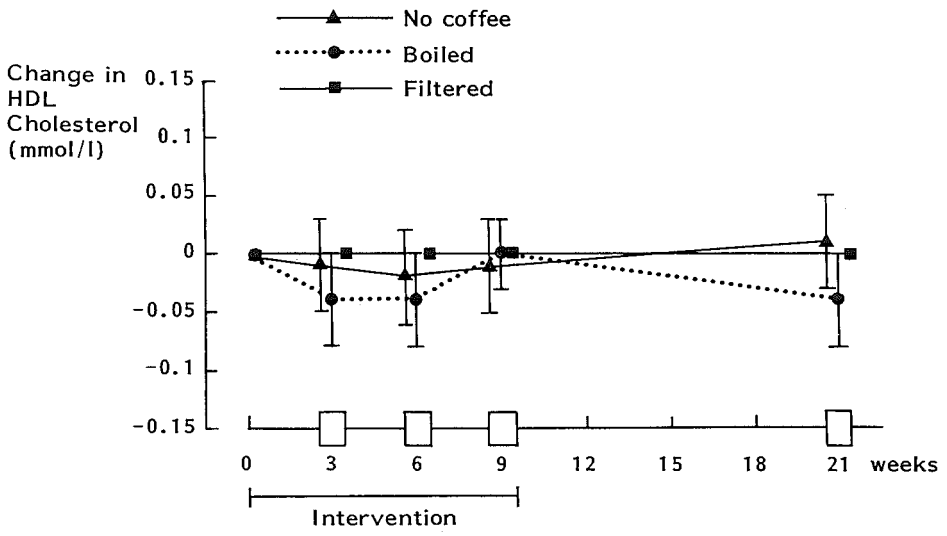

Figure 4.2. Net changes in serum HDL-cholesterol level (mmol/l) from baseline. The filtered coffee group was the reference group. Values (mean ± SEM) are adjusted for different changes in body weight in the groups.

brewed by boiling (11,25,29). On the basis of our study, however, we cannot be sure whether a plateau is reached within nine weeks, because we lack measurementsbetween six and nine weeks. The possibility cannot be excluded that cholesterol levels will rise even further when the consumption of boiled coffee continues after a nine-week period. There was no significant difference between the group consuming filtered coffee and the group that drank no coffee with regard to serum total cholesterol level. Both groups had essentially stable lipid levels during the trial. The increase in the serum total cholesterol level when boiled coffee is consumed is completely reversible if this brewing method is avoided. High-density lipoprotein cholesterol levels were unaffected by intervention. The increase in low-density lipoprotein cholesterol levels did not reach statistical significance, although the magnitude of the increase was similar to that for total cholesterol. This may be explained by the indirect assay method used to estimate concentrations of serum low-density lipoprotein cholesterol, which resulted in a larger measuring error. Moreover, low-density lipoprotein cholesterol levels are known to show relatively high variations in and between subjects. In this study the levels of in nonexperimental studies conducted in Scandinavia, where coffee is predominantly apolipoproteins were almost unchanged during intervention except for a slight

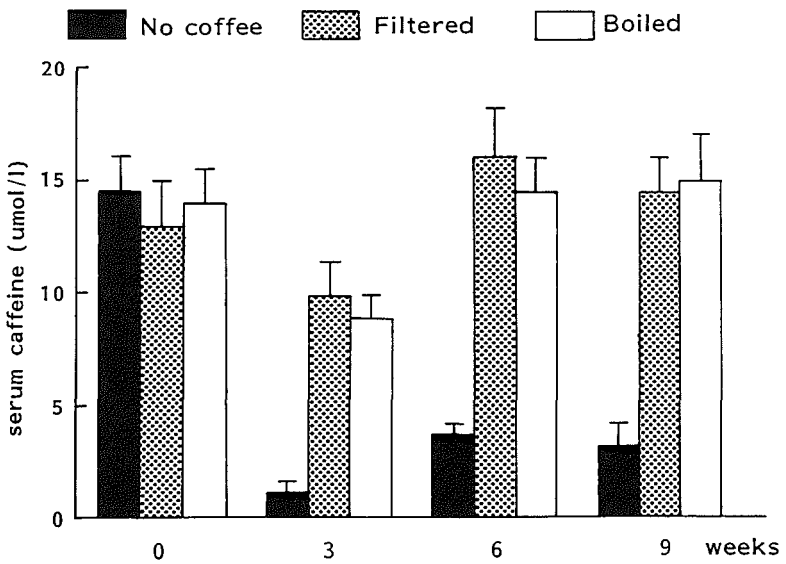

Figure 4.3. Serum caffeine levels (umol/l) at baseline and after 3, 6 and 9 weeks of intervention. Values are means + SEM.

increase in apolipoprotein B levels in the group drinking boiled coffee, which was compatible with previous findings by Aro et al (34).

Before these findings can be accepted, some methodologic aspects must be considered. The intervention may have affected several factors that can influence the serum lipid profile, and this could partly or completely explain the effect of changes in coffee-drinking habits on serum cholesterol levels. In particular, concomitant changes in the intake of fat and body weight during the trial are important. In this study, however, no changes were observed in the intake of total fat or the components of dietary fat. Body weight changed slightly, and our results were adjusted for different changes in body weight from one group to another, but this did not materially change the observations in the study. Also, the study was not blinded. Obviously, blinding is difficult to achieve when the emphasis is not only on coffee consumption but also on brewing methods. However, in order to limit the bias induced by the participants' knowledge of their intervention status, they were not informed about the exact hypothesis under study. Furthermore, laboratory analyses were blinded.

The question of the mechanism by which differences in brewing methods can lead to different effects on serum lipid levels cannot be answered by this study. It can

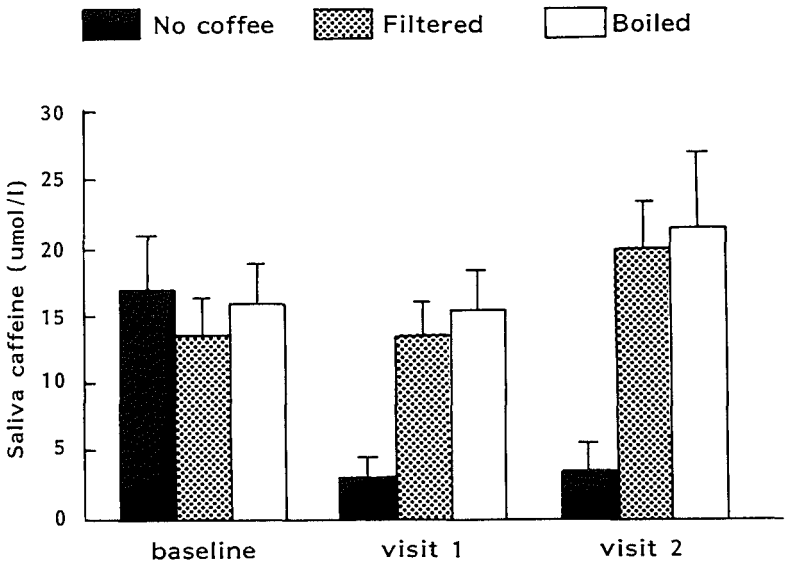

Figure 4.4. Saliva caffeine levels (umol/l) at baseline and at 2 unannounced home visits during the intervention period. Values are means + SEM.

be speculated that the effect is due to the amounts of active substances formed or extracted during preparation. This could result, for example, from the difference in temperature in the brewing procedures. Just after the boiling water was poured overthe coffee in the thermos bottle, the temperature was 92.7 °C, and it decreased by 1.7 °C per hour. The water dripping onto the coffee in the electric coffee maker averaged 90 to 95 °C. The temperature of the sediment was 91.6 °C for a few seconds and decreased to 87.5 °C after three minutes, when all the water had dripped through the paper filter. The temperature of the coffee fluid in the device's glass container was about 85 °C. In general, therefore, the temperature of boiled coffee is higher, and the coffee grounds are exposed to hot water for a longer period. It is also possible that in the process of filtering the active substance is adsorbed to the filter paper or is left behind by selective filtering because of the size of the particles.

In conclusion, our findings in this randomized trial suggest that the consumption of filtered coffee does not affect serum lipid levels. The consumption of boiled coffee, however, has a considerable effect in raising cholesterol levels, amounting to a mean net increase of 0.5 mmol per liter after nine weeks. The boiling of coffee as described in this paper is found predominantly in Scandinavian countries. Yet, because the

mechanism through which this brewing method interacts with the cholesterol-raising effect of coffee is not established, the results may be relevant for the products of similar methods, such as percolated coffee, camp coffee, and Turkish coffee.

Acknowledgement

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CHAPTER 5

COFFEE AND BLOOD PRESSURE

5.1 INTRODUCTION

The effect of coffee on the heart and blood vessels has attracted the attention of investigators for many years (1). Both early reports and recent papers focus mainly on the action of caffeine on the cardiovascular system (2). Coffee, however, has been shown to contain many chemical constituents, all with potential pharmacological effects and it would be remarkable if caffeine proved to be the only substance of significance (3). Unfortunately, the limited knowledge about the vast majority of these constituents prevents drawing firm conclusions about their contribution to the effect of coffee ingestion on cardiovascular risk indicators.

The impact of coffee on blood pressure was assessed in a randomized trial in young subjects consuming filtered, regular coffee at baseline. The blood pressure pattern in the group refraining from coffee was compared with a group continuing on filtered coffee. For all participants in this study, caffeine intake was fixed on three cups of (weak) tea per day.

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5.2 A RANDOMIZED STUDY ON COFFEE AND BLOOD PRESSURE[1]

Introduction

Coffee consumption has been associated with cardiovascular disease (1-3), although this relationship is still subject to debate (4-7). As a major cardiovascular risk indicator, increased blood pressure has been suggested to be responsible for a positive link between coffee consumption and coronary heart disease. So far, attention in research on coffee and blood pressure has been mainly on a potential hypertensive action of caffeine (8). Many short-term studies on the effect of caffeine or coffee on blood pressure were conducted (9-23). In both normotensive (9-20) and hypertensive (21-23) subjects, an immediate rise in blood pressure with coffee or caffeine has been shown, after abstinence from caffeine for at least ten hours. The rise in blood pressure is of short duration, about 4 hours, and is usually accompanied by a small decline in heart rate (11,16). With continued caffeine intake, however, the cardiovascular response is not sustained (10,13). Even caffeine non-users rapidly develop tolerance to its effect, in general after 2 or 3 days of caffeine use. According to Smits et al., subjects showing a rapid clearance of plasma caffeine will reach low plasma caffeine levels after a normal overnight fast and, therefore, the first cups of coffee in the morning will elicit a pressor response in this particular group (18).

From non-experimental studies there is little evidence to support a link between coffee use and increased blood pressure (3,4,24-29). On the contrary, in various studies a negative association between coffee consumption and blood pressure has been reported (30-32).

An experimental study on the effects on blood pressure of coffee use and abstinence from coffee lasting several weeks has, to our knowledge, not been conducted. Furthermore, the influence of the brewing method on the blood pressure response to coffee has not been studied, although several reports on the differential effects of various brewing methods on serum lipids were published recently (33-35). We conducted a randomized trial in 107 young, normotensive adults. The aim of the study was to assess the effect of using filtered coffee, boiled coffee or abstaining from coffee on serum lipids. In addition blood pressure was measured as part of the study. The results on serum lipids have been published separately (36). In this paper findings on blood pressure will be presented.

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[1]J Human Hypert 1990;4:259-64.

Methods

Subjects

The participants in the present study were selected from a cohort of 596 young adults who take part in a follow-up study involving annual exams on cardiovascular risk indicators. This cohort is a random sample from a population-based study that was initiated in 1975 including 4,649 subjects from the Dutch town of Zoetermeer (37). Those members of the cohort who habitually drank coffee and who were over 18 years of age were invited to participate and 107 subjects, aged 18 to 33 years, agreed to take part in this study. All participants gave informed consent. The mean baseline coffee consumption of the subjects was 5.6 cups of filtered coffee per day.

Protocol

The study lasted 12 weeks. In the three week run-in period, during which all participants continued on filtered coffee, blood pressure was measured twice. After stratification for gender, the subjects were randomly assigned to one of three groups receiving either (1) 4-6 cups boiled coffee per day, (2) 4-6 cups filtered coffee per day, (3) no coffee for a period of 9 weeks. Coffee cups of 140 ml were used. All participants were obliged to drink 3 cups of tea per day, in order to make the groups similar with respect to caffeine intake from this source. The subjects in the abstinence group were advised to drink water as an alternative for coffee. During the intervention period blood pressure and body weight were measured at 3, 6 and 9 weeks. Furthermore, blood samples were obtained at these occasions.

Brewing methods

The filtered coffee was prepared in a commercially available electric coffee maker by dripping hot water on coffee and filtering it through paper. The boiled coffee was prepared by pouring boiling water on coarsely ground coffee in a thermosjar and waiting for at least 10 minutes before drinking it (34). The caffeine content was 670 mg/l and 630 mg/l for filtered and boiled coffee respectively.

Measurements

Blood pressure was measured after 10 minutes of rest with a random-zero sphygmomanometer by one trained paramedical assistant. All measurements were performed late in the afternoon or early in the evening. Most participants visited the research centre at the same time for each session. Subjects were asked to refrain from caffeine for at least one hour before the measurements. Smoking and alcohol consumption were not restricted. The mean of two readings, in sitting position, was used in the analysis. The readings were separated in time by a count of the heart rate. Following blood pressure measurements, non-fasting venous blood samples were obtained. Body height and weight were measured with the subjects wearing indoor clothes without shoes. Caffeine concentrations in serum and saliva samples were

determined by reversed-phase high-performance liquid chromatography (14).

Data-analysis

The effects of intervention were examined by comparing the mean changes in blood pressure from baseline levels between the three groups after 3, 6 and 9 weeks of intervention. The second baseline measurements during the run-in period were taken as baseline levels. Because all participants consumed filtered coffee before the trial, the group continuing on filtered coffee was considered as the reference group. Therefore, the results for the group using boiled coffee and the abstinence group are expressed as the change from baseline minus concomitant changes in the filter group. In an additional analysis, adjustments were made for potential confounding variables, using a multiple linear regression model. Finally, in an attempt to take baseline differences in blood pressure between the groups into account, the multivariate analysis was repeated entering baseline blood pressure level as a separate covariate in the model. Separate analyses were performed for men and women, and smokers and non-smokers. Also, interaction between gender, smoking habits and intervention effects was tested by entering interaction-terms for these characteristics in the multivariate model (38). The analyses presented below exclude 6 subjects who did not complete the study. All of them ended their participation just after randomization. Four of them were in the group on boiled coffee, one in the group on filtered coffee and one in the abstinence group.

Compliance

Adherence to the study protocol was assessed in three ways. First, a daily diary was kept on the amount of coffee, tea, cola and chocolate consumed. Second, serum caffeine levels were measured in blood samples obtained at the scheduled visits at the examination center. Finally, each participant was visited twice during the study without prior announcement. At these visits, a saliva sample was obtained for determination of caffeine levels.

Results

Baseline characteristics for the three study groups are summarized in table 5.1. The groups appear to be well matched, although randomization did not result in similar baseline blood pressure levels in the three groups.

The patterns for the systolic and diastolic blood pressure were remarkably similar in the groups using either filtered coffee or boiled coffee (table 5.2). After 9 weeks on boiled coffee, mean changes from baseline for SBP and DBP were 0.4 mm Hg (95 % confidence limits -3.7, 4.5) and -0.1 mm Hg (-3.4, 3.2), compared to the filter group. By contrast, both SBP and DBP decreased in the abstinence group. Compared to the filter group only the fall in SBP after 9 weeks was statistically significant, -6.1

Table 5.1. Baseline characteristics in the three study groups.

	No coffee	*Filtered coffee*	*Boiled coffee*
Number	34	34	33
Gender (M/F)	18/16	18/16	18/15
Age (yrs)	26.5 (3.8)	25.0 (4.3)	25.5 (3.6)
Body weight (kg)	71.5 (13.1)	68.9 (13.0)	67.8 (12.3)
Height (cm)	175.1 (11.5)	175.1 (8.5)	174.6 (9.6)
SBP (mm Hg)	124.3 (13.9)	119.8 (12.0)	116.6 (13.1)
DBP (mm Hg)	72.7 (8.5)	70.0 (8.7)	68.6 (10.0)
Heart rate (bts/min)	78.1 (12.0)	74.2 (12.0)	70.7 (10.2)
Serum caffeine (umol/l)	14.4 (9.3)	12.8 (10.8)	13.9 (7.7)
Coffee (cups/day)	5.9 (3.0)	5.1 (3.4)	5.8 (3.1)
Tea (cups/day)	2.6 (2.7)	1.9 (1.9)	2.1 (2.1)
Caffeine intake (mg/day)	543 (213)	458 (251)	499 (212)
Smoking (yes/no)	16/18	15/19	17/16
Alcohol (consumptions/day)	0.5 (0.8)	0.5 (0.6)	0.4 (0.3)

Values are means, standard deviations between parentheses.

mm Hg (-10.8,-1.4). Body weight changed slightly during the intervention period. During 9 weeks, body weight fell by a mean of 0.6 kg in the abstinence group, compared to 0.1 kg weight loss in the filter group. Therefore, the final results are adjusted for different changes in body weight between the study groups. After adjustment for differences in weight change, the observed reduction in SBP was still significant, -4.9 mm Hg (-9.0, -0.8). With additional adjustment for baseline levels of SBP, the effect remained similar, though smaller, -3.4 mm Hg (-7.1, 0.3) (figure 5.1). Separate analyses for men and women, and smokers and non-smokers revealed no significant interaction by gender or smoking habits and the effect of coffee use on blood pressure. There was no significant difference in heart rate change between the groups, although again the abstinence group showed a decreasing trend, compared to the coffee groups (table 5.2).

We combined the two coffee groups and compared the abstinence group with this "pooled" coffee group. This analysis showed a decrease of SBP, DBP and heart rate in the abstinence group compared to the coffee group. In the abstinence group, levels of SBP after 3, 6 and 9 weeks were -4.0 mm Hg (-7.5, -0.5), -2.9 mm Hg (-6.6, 0.8) and -5.1 mm Hg (-8.6, -1.6). For DBP the results were -1.0 mm Hg (-4.1, 2.1), -0.8 mm Hg (-3.7, 2.1) and -2.8 mm Hg (-5.7, 0.1) respectively. These values are adjusted for different changes in body weight between the study groups. Compared to

Table 5.2. Changes from baseline in blood pressure (mm Hg) and heart rate (beats/min) during intervention.

	No coffee (n=34)	*Filtered coffee (n=34)*	*Boiled coffee (n=33)*
Systolic blood pressure			
3 weeks	-2.4 (-5.5, 0.7)	1.5 (-1.1, 4.1)	2.0 (-0.9, 4.9)
6 weeks	-1.7 (-5.2, 1.8)	0.8 (-2.2, 3.7)	2.1 (-0.5, 4.7)
9 weeks	-3.1 (-6.6, 0.4)	3.0 (0.1, 5.9)	2.9 (0.4, 5.5)
Diastolic blood pressure			
3 weeks	0.9 (-1.7, 3.5)	0.4 (-2.2, 3.0)	3.6 (1.0, 6.2)
6 weeks	2.4 (-0.3, 5.1)	2.6 (0.4, 4.8)	3.3 (0.9, 5.7)
9 weeks	-0.4 (-2.8, 2.0)	2.6 (0.6, 4.6)	2.4 (-0.2, 5.0)
Heart rate			
3 weeks	-2.8 (-5.7, 0.1)	-0.8 (-3.9, 2.3)	0.6 (-2.5, 3.7)
6 weeks	-2.6 (-5.3, 0.1)	-2.0 (-5.1, 1.1)	1.8 (-1.3, 4.9)
9 weeks	-4.5 (-8.6,-0.4)	-0.5 (-3.2, 2.2)	1.2 (-2.9, 5.3)

Values are means, 95 % confidence limits between parentheses.

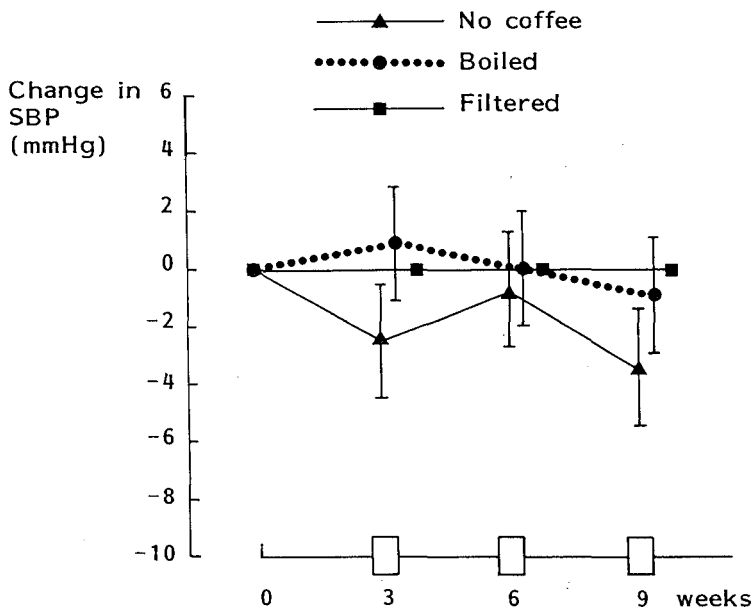

Figure 5.1. Net changes in systolic blood pressure (mm Hg) from baseline with the filter group as reference group. Results after 3, 6 and 9 weeks of intervention. Values (means ± SEM) are adjusted for SBP at baseline and for different changes in body weight between the groups.

Table 5.3. Caffeine levels in serum and saliva (umol/l) at baseline and during intervention.

	No coffee (n = 34)	*Filtered coffee (n=34)*	*Boiled coffee (n=33)*
Serum caffeine			
baseline	14.4 (1.5)	12.9 (2.1)	13.9 (1.5)
3 weeks	1.0 (0.5)	9.8 (1.5)	8.8 (1.0)
6 weeks	3.6 (0.5)	16.0 (2.1)	14.4 (1.5)
9 weeks	3.1 (1.0)	14.4 (1.5)	14.9 (2.1)
Saliva caffeine			
baseline	17.5 (4.1)	13.9 (3.1)	16.5 (3.1)
homevisit 1	3.1 (1.5)	13.9 (2.6)	16.0 (3.1)
homevisit 2	3.6 (2.1)	20.6 (3.6)	22.1 (5.7)

Values are means, standard errors between parentheses.

the coffee group, the heart rate decreased with 2.7 (-6.4, 1.0), 2.5 (-6.0, 1.0) and 4.9 (-9.8, 0.0) beats per minute in the abstinence group. The daily records indicated good adherence to the study protocol. In the groups drinking filtered and boiled coffee the concentrations of serum caffeine during intervention did not change as was to be expected when continuing with a similar caffeine intake. In the abstinence group a substantial fall in serum caffeine was observed, but values did not reach zero due to caffeine intake from tea. Caffeine concentrations in saliva showed similar results (table 5.3).

Discussion

The main finding of this randomized trial in young, normotensive subjects is that systolic blood pressure falls when refraining from coffee use, during a period of nine weeks. Although the fall in blood pressure was already apparent at the third week, it was only significant at the end of the study period. This observation cannot be accepted before some methodological issues are considered.

First, randomization was not entirely successful as indicated by discrepancies in baseline blood pressure levels between the groups. The SBP of the subjects drinking filtered coffee and boiled coffee was lower than that of the subjects in the abstinence group (table 5.1). It is, therefore, important to exclude regression to the mean as an explanation for part of our findings. Since the participants had been studied already for a period of over 10 years, blood pressure readings from preceding years could be reviewed and these were similar to the baseline values in this study. In 1984, SBP in

the abstinence group, and the groups on filtered coffee and boiled coffee were 122.8 (11.7), 121.4 (10.6) and 115.9 (11.2) mm Hg. The corresponding DBP readings averaged 71.8 (6.4), 69.3 (9.3) and 68.0 (8.6) mm Hg. This agreement of the baseline readings with previous data implies that the observed differences between the groups are not due to random variation. Consequently, regression to the mean is unlikely to be responsible for differential changes in blood pressure between the groups. Moreover, adjustment for the blood pressure levels at baseline, did not change the direction of the effect. Second, it was impossible to conduct the study blind and, consequently, our findings on blood pressure must be interpreted with some caution. However, the participants were not informed about the hypotheses under study until after the last measurements, to limit the potential for bias. Finally, the slight differences between the groups regarding body weight change during the intervention period and in baseline blood pressure levels could confound the results. Adjustment for these variables resulted in a smaller difference in systolic blood pressure between the group drinking filtered coffee and the abstinence group.

There are no previous reports on the blood pressure response to abstinence from coffee for a period of this length. Robertson et al. observed only a transient fall in systolic blood pressure in the first two days on abstinence from caffeine for 2 weeks (23). Recently, Van Dusseldorp et al. reported a statistical significant fall in SBP of 1.5 mm Hg and in DBP of 1.0 mm Hg on changing from regular coffee to decaffeinated coffee (39).

Previous studies suggest that caffeine intake might be responsible for the effect of coffee on blood pressure. As indicated before, experimental studies have shown that the acute effects of caffeine on blood pressure are only observed in subjects who refrained from caffeine for at least 10 hours (12,16,19). In our study, blood pressure measurements were always performed at the end of the day when the participants in the coffee groups had consumed several cups of coffee. Therefore, in the present study, there is no need for concern about short-term effects of caffeine. Whether the fall in blood pressure is the result of abstinence from coffee or from caffeine is difficult to answer on the basis of this study. In a double-blind randomized study on caffeine, however, we did not observe an effect on blood pressure during abstinence from caffeine for a period of nine weeks, suggesting that some other compound in coffee may influence blood pressure (40). Short-term and long-term effects on blood pressure may be phenomena due to different modes of action of caffeine or other components of coffee on the cardiovascular system. From our data however, evidence for a causal mechanism cannot be derived. The pulse rate showed a slight decrease during abstinence from coffee. This change, however, was not significantly different from zero and is at variance with previous findings suggesting that short-term blood pressure elevation related to coffee intake is generally accompanied by a small decrease in heart

rate. Previous data on the relationship between boiled coffee and blood pressure are not available, but in this study the two brewing methods showed a similar blood pressure response.

In conclusion, our findings suggest that abstinence from coffee for a period of nine weeks may reduce systolic blood pressure by 3-4 mm Hg. Considering the widespread use of coffee, this small effect is of potential importance in strategies to prevent and treat elevated blood pressure. Future studies are needed to confirm our findings and shed light on the responsible factor in coffee.

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CHAPTER 6

COFFEE AND HEMOSTASIS

6.1 INTRODUCTION

In view of the potential relationship between coffee and cardiovascular morbidity and mortality, considerable evidence has accumulated on the effects that coffee use may have on serum lipids, and to a lesser extent, on the association of coffee and blood pressure. A major role in the process of atherosclerosis, however, is played by the hemostatic system (1). Therefore, it is of interest to explore the relationship between coffee use and hemostasis. In reviewing the literature on this subject, as discussed in section 6.2, it became clear that the data concerning the effect of coffee on hemostatic parameters is limited. This lack of knowledge stimulated us to investigate the impact of coffee and caffeine use on some selected hemostatic factors. The explorative findings in two experimental studies are reported in section 6.3.

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6.2 COFFEE, CAFFEINE AND HEMOSTASIS: A REVIEW[1]

Introduction

Adverse effects of coffee consumption on cardiovascular risk have been reported (1-3) and the potential relationship between coffee use and cardiovascular morbidity and mortality has fostered the interest in the association between coffee and various cardiovascular risk indicators. Apart from a direct promotion of atherosclerotic vessel disease, coffee may induce unfavourable changes in levels of risk factors for cardiovascular disease, most notably the serum lipid profile and the blood pressure. Several studies on coffee and serum lipids and coffee and blood pressure were reported, as recently reviewed (4,5). In addition, coffee may trigger progression of the occlusion of a vessel already affected by atherosclerotic plaque formation through enhanced thrombogenesis. This would explain the observation that coffee use is most clearly related to the incidence of coronary heart disease, when the intake was assessed shortly before the event (1). Only limited information is available on the potential thrombogenic capacity of coffee use.

The hemostatic factors may be categorized in various ways. Factors discriminated are (a) involved in the clotting system, (b) responsible for fibrinolysis, and (c) associated with platelet activation (figure 6.1). Disturbances in either of those systems may lead to enhancement of thrombotic tendencies or an inadequate fibrinolytic response and thereby to aggrevation of atherosclerotic lesions or rapid progression of vessel stenosis (6-14).

Only limited data is available on the hemostatic effects of coffee and caffeine. Moreover, the information is mainly derived from in vitro experiments or short-term non-randomized studies. The reported studies will be discussed according to the subdivision of the hemostatic process given before.

Clotting factors

Several factors involved in activation of the clotting system have been linked to cardiovascular disease but the association is best documented for factor VII, VIII and fibrinogen. In prospective studies, elevated levels of these factors were shown to be independent predictors of cardiovascular events (10,11,13,14).

A weak positive association between coffee consumption and fibrinogen was observed in a large Finnish cross-sectional study (15). The plasma fibrinogen concentration raised with increasing coffee consumption in this study of 1,166 54-year old Finnish men. In an older experimental study on 20 volunteers, abstinence from

[1]Netherl J Med (in press).

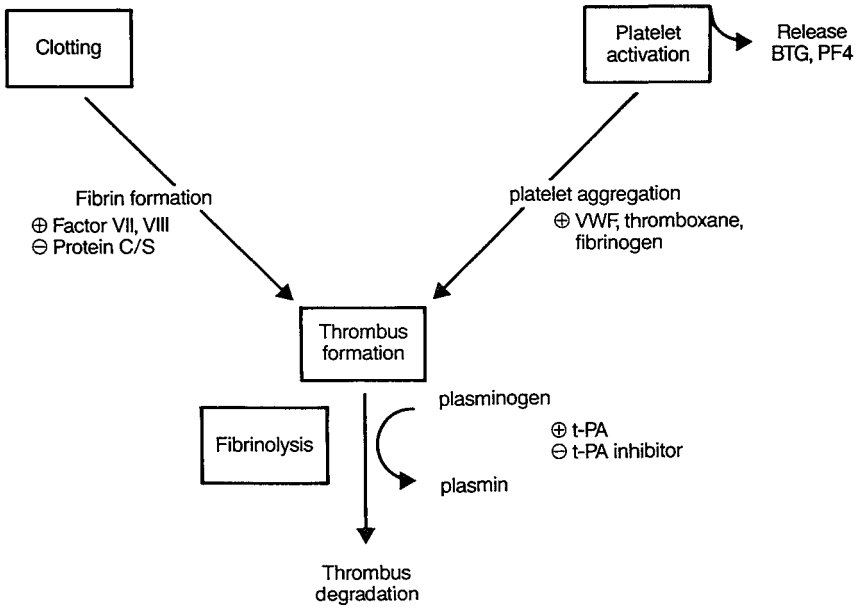

Figure 6.1. Components of the hemostatic system studied in association with coffee or caffeine.

caffeine did not change the level of fibrinogen (16). The habitual caffeine intake of the participants in this study averaged 560 mg/day, corresponding to approximately 6 cups of regular coffee. During the course of the experiment, including 14 days on decaffeinated coffee (12 mg caffeine/day), followed by 20 days on instant coffee (875 mg caffeine/day), fibrinogen levels were not affected.

The clotting factors and the complex system of interacting factors that counteract the coagulation cascade have not yet fully been studied in connection to coffee. Considering the association with cardiovascular disease, it would be of particular interest to study the effect of coffee use on fibrinogen, factor VII and the protein C - protein S system (13,14,17,18). These parameters were included in two 12-week randomized studies we recently conducted, as reported separately (19). Findings of the first study, on 107 young healthy adults, indicated no effect of coffee use on fibrinogen, clotting factor VII activity, factor VIII antigen, protein C and protein S. In the second study, the impact of caffeine consumption on fibrinogen and factor VII activity was assessed and again no changes in these variables were observed during 9 weeks of intervention.

Fibrinolysis

The fibrinolytic system forms one of the anti-thrombotic forces (20). The central component of this system is the proenzyme plasminogen, which can be converted to the fibrinolytically active enzyme plasmin. The activation of plasminogen can be initiated by tissue plasminogen-activator (t-PA), a physiologic activator of fibrinolysis (21). Reduced fibrinolytic activity was reported to be linked to coronary artery disease as early as 1966 (22). Recently, the topic has regained interest due to the development of new laboratory methods to study the fibrinolytic process. Increased plasma levels of a rapid inhibitor of t-PA, resulting in a reduced fibrinolytic capacity, were observed in patients with previous myocardial infarction (23).

Contradictory effects of coffee on the fibrinolytic system have been reported (24-26). Al Samarrae and Truswell found that whole blood fibrinolysis time was shortened 30 and 60 minutes after consumption of coffee in ten out of twelve subjects (25). This effect appeared to have been due to caffeine, because the effect of regular coffee was significantly greater than when decaffeinated coffee was tested. In the second experiment, on 9 healthy volunteers, a fibrinolytic potential of caffeine was observed; levels of circulating plasminogen activator inhibitor were reduced and of tissue plasminogen activator increased after 1 week of ample caffeine ingestion, compared to levels after 1 week of caffeine abstinence (26). The participants received caffeine in regular coffee ad libidum, supplemented with 3 tablets of 100 mg caffeine every day. Earlier, Naismith did not find an effect of abstinence from caffeine on blood clot lysis time (16). Yet, the fibrinolytic system is very dynamic and, therefore, these results should be regarded with caution.

Platelet activation

The third category of platelet-related factors, may also play an important role in the atherosclerotic process, as recently reviewed by Ross (27). The major events are platelet activation, release of platelet constituents (thromboxane, platelet factor 4 and beta-thromboglobulin) and, finally, platelet aggregation. Platelet factor 4 and beta-thromboglobulin are indices of platelet activation in vivo that may be measured in plasma (28). Thromboxane is a potent platelet aggregator and vasoconstrictor and consequently it may stimulate atherogenesis (29,30). The platelet capacitiy for thromboxane release can be evaluated by measurement of thromboxane B2, a stable metabolite of thromboxane.

In relation to coffee and caffeine, several in vitro and in vivo studies have been reported on this component of the hemostatic process (15,16,31-39). In an experimental setting, abstinence from regular coffee did not affect platelet adhesiveness (16). Considering the effect of coffee drinking on platelet activation and release, Ammaturo and associates conducted a study on 12 healthy subjects (31). The plasma beta-

thromboglobulin concentration was determined before and 1 hour after administration of 100 mg of caffeine. The observed increase in beta-thromboglobulin was statistically significant, and the authors conclude that caffeine in quantities corresponding to one cup of coffee stimulates platelet activation and release in vivo. In an alternative approach, the effect of coffee on platelet activation was investigated by measuring thromboxane B2 production by platelets, either in vitro after aggregation induced by collagen (32), or in vivo (33). Neither short-term (80 minutes), nor long-term (3 weeks) effects could be detected. In one in vitro experiment with human platelet enriched plasma, it was shown that coffee extracts contain compounds that actively inhibit platelet aggregation (34). By contrast, similar in vitro studies seemed to indicate that caffeine and related methylxanthines at high concentrations might have an anti-aggregatory action (35-37). In an experiment by Monti et al., caffeine reduced platelet metabolism in vitro as measured by a microcalorimetric method (38). The dose of caffeine and coffee in these in vitro studies was substantially above levels of normal consumption, up to 100 times more, and extrapolation of these in vitro observations to the more physiologic situation in vivo, is therefore questionable. Happonen and co-workers observed an increased platelet aggregability in vitro with increased coffee consumption in 1,166 middle-aged Finnish men (15). In a study in 4 volunteers, caffeine reduced maximal aggregation of platelets in vitro induced by adrenaline and collagen as aggregating agents (39). However, direct measurement of platelet aggregability is difficult and affected by many extraneous factors, leading to a large measurement error and high intra-individual variability.

Conclusion

In view of the apparent association between coffee and cardiovascular disease, the study of coffee and hemostasis is of potential interest. The presently available evidence in favor or against such an association, however, is not very convincing and sometimes even conflicting. The findings on fibrinogen level and platelet in vivo activation as measured by plasma beta-thromboglobulin, seem to be the most reliable. If anything, these reports indicate an unfavorable effect of coffee, i.e. coffee may enhance thrombotic tendencies. Most of the discussed, explorative studies on coffee, caffeine and hemostasis were performed in small numbers of subjects. Moreover, the unblinded, open nature of the majority of research in this area impedes the interpretation of the results. Therefore, more definite conclusions on the interference of coffee use with the hemostatic system can only be made if more data are available. In particular, randomized studies of coffee and caffeine intake in humans are indicated to assess the impact and the potential significance of coffee in quantities as commonly used by millions of people.

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6.3 COFFEE, CAFFEINE AND HEMOSTASIS; RESULTS FROM TWO RANDOMIZED STUDIES[1]

Introduction

Only a limited number of reports on the effect of coffee consumption on hemostatic factors is available (1-12). In a review of the literature we concluded that the evidence in favor or against an association between coffee consumption and hemostasis is not very convincing yet and sometimes even conflicting (13). The reported link between coffee consumption and cardiovascular disease warrants the study of the influence of coffee on hemostatic factors.

We recently conducted two randomized trials on the effects of consumption of coffee and caffeine on cardiovascular risk indicators. The primary objective was to study the influence on serum lipids and blood pressure of which the results are reported separately (14,15). In addition to serum lipids, selected hemostatic parameters were determined in the obtained blood samples: fibrinogen, clotting factor VII activity, factor VIII antigen, protein C and protein S. Previous studies have indicated that these hemostatic parameters may be involved in the pathogenesis of atherothrombotic cardiovascular disease (16-21). The first study focussed on the effect of coffee and the putative influence of two common brewing methods. In the second study the role of caffeine was investigated. This paper presents the findings in both trials on changes in hemostatic variables.

Methods

Subjects

For both studies, subjects were recruited from a cohort of 596 young adults who take part in a follow-up study with annual exams on cardiovascular risk indicators conducted in Zoetermeer, the Netherlands. This cohort is a random sample from a population-based study that was initiated in 1975 (22). Those members of the cohort who used coffee habitually and were between 18 and 32 years of age, were invited to participate in the coffee studies. All participants gave informed consent.

Study design

In study A, 107 subjects agreed to participate and these were randomly allocated to one of three intervention groups using either (1) 4-6 cups filtered coffee per day, (2) 4-6 cups boiled coffee per day, or (3) no coffee at all for a period of nine weeks. In study B, 69 subjects were randomly assigned to one of two groups using either (1) 4-6 cups filtered decaffeinated coffee per day and for each cup of coffee a tablet

[1]Atherosclerosis (in press, slightly modified).

containing 75 mg caffeine or (2) 4-6 cups filtered decaffeinated coffee per day and an equal number of placebo tablets, for a period of nine weeks. In this double-blind study caffeine intake from other sources was not allowed. The duration of both studies was 12 weeks with an intervention period of 9 weeks. At the end of the 3-week run-in period, randomization took place after stratification for gender.

Measurements

Analyses of the hemostatic variables were performed in blood samples obtained at baseline and after 9 weeks of intervention. All samples were analysed in one batch. Plasma was collected from citrate-anticoagulated blood after centrifugation for 10 minutes at 1750 g at room temperature and stored at - 80 °C. Fibrinogen was determined by the method of Clauss according to the instructions of the manufacturer (Merz and Dade). Factor VII activity was measured on the ACL (Instrumentation Laboratory) using factor VII deficient plasma (Ortho Diagnostic Systems) and Thromborel-S (Behring) as reagens. The plasma concentration of factor VIII (Von Willebrand factor), protein C and S was determined by a double antibody sandwich Elisa on microtitre plates (antisera, Dakopatts). Free protein S was measured after treatment of the plasma with PEG 8000 (23). Caffeine concentrations in serum and saliva samples were determined by reversed-phase HPLC (24).

Compliance

Adherence to the study protocol was assessed in three ways. First, individual daily records were kept on the amount of coffee, tea, cola and chocolate consumed. In addition, unused packages of coffee and, in study B, unused tablets were returned and counted. Second, serum caffeine levels were measured in blood samples obtained at the scheduled visits at the examination center. Finally, each participant was visited twice during the study without prior announcement. At these visits, a saliva sample was obtained for determination of caffeine levels.

Data-analysis

The effects of intervention were assessed by comparing the mean changes from baseline between the groups. Because all participants consumed filtered, regular coffee before the intervention, the group on filtered coffee in study A and the group on caffeine tablets in study B were considered as the reference groups. Changes in the other groups are expressed as changes from baseline minus concomitant changes in the reference group to assess the net effect of the interventions on the hemostatic variables. Findings are expressed as mean differences between groups and 95 % confidence limits. Subjects who did not complete the study (6 in study A and 7 in study B) were excluded from the analysis.

Table 6.1. Study A: a randomized trial of coffee and coffee brewing method. Baseline characteristics in the three intervention groups.

	No coffee	*Filtered coffee*	*Boiled coffee*
Number	34	34	33
Gender (M/F)	18/16	18/16	18/15
Age (yrs)	26.5 (3.8)	25.0 (4.3)	25.5 (3.6)
Body weight (kg)	71.5 (13.1)	68.9 (13.0)	67.8 (12.3)
Height (cm)	175.1 (11.5)	175.1 (8.5)	174.6 (9.6)
Serum caffeine (umol/l)	14.4 (9.3)	12.8 (10.8)	13.9 (7.7)
Coffee (cups/day)	5.9 (3.0)	5.1 (3.4)	5.8 (3.1)
Tea (cups/day)	2.6 (2.7)	1.9 (1.9)	2.1 (2.1)
Caffeine intake (mg/day)	543 (213)	458 (251)	499 (212)
Current smoking (yes/no)	16/18	15/19	17/16

Values are means, standard deviations between parentheses.

Table 6.2. Study B: a double blind randomized trial of caffeine intake. Baseline characteristics in the two intervention groups.

	Caffeine	*No caffeine*
Number	32	30
Gender (M/F)	18/14	16/14
Age (yrs)	24.6 (4.1)	25.8 (4.1)
Body weight (kg)	73.1 (10.9)	72.6 (13.1)
Height (cm)	177.6 (9.1)	177.2 (9.6)
Serum caffeine (umol/l)	20.1 (12.4)	21.6 (11.8)
Coffee (cups/day)	5.8 (3.3)	5.9 (3.3)
Tea (cups/day)	1.4 (1.7)	1.5 (1.3)
Caffeine intake (mg/day)	505 (242)	508 (242)
Current smoking (yes/no)	16/16	19/11

Values are means, standard deviations between parentheses.

Table 6.3. Study A: a randomized trial of coffee and coffee brewing methods. Hemostatic values at baseline and after 9 weeks of intervention. Changes from baseline are calculated with the filter group as reference.

	Group	*Baseline (mean (SD))*	*Intervention (mean (SD))*	*Change from baseline (mean, 95 %CL)*
Fibrinogen (g/l)	filtered	2.2 (0.5)	2.5 (0.6)	
	no coffee	2.3 (0.6)	2.4 (0.5)	-0.2 (-0.6, 0.2)
	boiled	2.3 (0.6)	2.4 (0.5)	-0.1 (-0.5, 0.3)
Factor VII activity (%)*	filtered	86.8 (19.4)	84.8 (18.0)	
	no coffee	85.3 (13.5)	86.3 (17.8)	3.0 (-4.7,10.7)
	boiled	90.8 (22.6)	88.0 (24.7)	-0.7 (-9.5, 8.1)
Factor VIII antigen (%)*	filtered	91.3 (44.2)	85.6 (37.5)	
	no coffee	85.2 (36.9)	75.1 (36.7)	-4.4 (-31.3,22.5)
	boiled	82.6 (41.7)	85.0 (33.9)	8.1 (-17.2,33.4)
Protein C (U/ml)	filtered	1.02 (0.27)	0.87 (0.22)	
	no coffee	1.04 (0.26)	0.89 (0.19)	0.00 (-0.14,0.14)
	boiled	0.92 (0.22)	0.91 (0.22)	0.14 (-0.30,0.02)
Protein S free (U/ml)	filtered	0.33 (0.10)	0.26 (0.09)	
	no coffee	0.31 (0.08)	0.26 (0.07)	0.02 (-0.02,0.06)
	boiled	0.31 (0.09)	0.27 (0.12)	0.03 (-0.02,0.08)
Protein S total (U/ml)	filtered	0.80 (0.17)	0.70 (0.14)	
	no coffee	0.84 (0.18)	0.70 (0.14)	-0.04 (-0.12,0.04)
	boiled	0.81 (0.20)	0.70 (0.18)	-0.01 (-0.09,0.07)

* Factor VII activity and Factor VIII antigen expressed as percentage of normal pool plasma.

Table 6.4. Study B: a double blind randomized trial of caffeine intake. Hemostatic values at baseline and after 9 weeks of intervention. Changes from baseline are calculated with the caffeine group as reference.

	Group	*Baseline (mean (SD))*	*Intervention (mean (SD))*	*Change from baseline (mean, 95 % CL)*
Fibrinogen (g/l)	caffeine	2.6 (0.4)	2.6 (0.6)	
	no caffeine	2.6 (0.6)	2.5 (0.5)	-0.1 (-0.3, 0.1)
Factor VII activity (%)*	caffeine	91.2 (17.0)	85.3 (18.3)	
	no caffeine	92.0 (20.5)	93.3 (24.7)	7.2 (-1.8,16.2)

* Factor VII activity expressed as percentage of normal pool plasma.

Results

In both studies, the baseline characteristics were similar for the intervention groups, as shown in tables 6.1 and 6.2. The levels of the measured hemostatic variables, at baseline and after 9 weeks of intervention, are shown in tables 6.3 and 6.4.

In study A fibrinogen, factor VII activity, factor VIII antigen, protein C and protein S (free and total) were measured. The group abstaining from coffee for a period of nine weeks showed no statistically significant changes from baseline, compared to the group on filtered coffee. The same applies to the group on boiled coffee (table 6.3). In an additional analysis, we combined the two coffee groups and compared the abstinence group with this pooled coffee group. Again, no significant differences were observed between these groups.

In study B only fibrinogen and factor VII activity were measured. The changes from baseline in the no-caffeine group were -0.1 g/l (95 % confidence limits -0.3, 0.1) for fibrinogen, and +7.2 % (-1.8, 16.2) for factor VII activity, with reference to the caffeine group. These changes did not reach statistical significance (table 6.4).
In addition, using multiple linear regression analysis, adjustments were made for baseline levels of the hemostatic variables, without materially affecting the results.

Compliance as assessed by the diaries of the participants and counting unused coffee packages and tablets was very good. The results on the measurements of caffeine in serum and saliva samples are presented in tables 6.5 and 6.6. The caffeine levels in the abstinence group in study A and in the no caffeine group in study B show

Table 6.5. Study A: a randomized trial of coffee and coffee brewing methods. Caffeine levels in serum and saliva (umol/l) at baseline and during intervention.

	No coffee	*Filtered coffee*	*Boiled coffee*
Serum caffeine			
baseline	14.4 (1.5)	12.9 (2.1)	13.9 (1.5)
3 weeks	1.0 (0.5)	9.8 (1.5)	8.8 (1.0)
6 weeks	3.6 (0.5)	16.0 (2.1)	14.4 (1.5)
9 weeks	3.1 (1.0)	14.4 (1.5)	14.9 (2.1)
Saliva caffeine			
baseline	17.5 (4.1)	13.9 (3.1)	16.5 (3.1)
homevisit 1	3.1 (1.5)	13.9 (2.6)	16.0 (3.1)
homevisit 2	3.6 (2.1)	20.6 (3.6)	22.1 (5.7)

Values are means, standard errors between parentheses.

Table 6.6. Study B: a double-blind randomized trial of caffeine intake. Caffeine levels in serum and saliva (umol/l) at baseline and during intervention.

	Caffeine	*No caffeine*
Serum caffeine		
baseline	20.1 (2.1)	21.6 (2.1)
3 weeks	24.2 (2.6)	4.1 (2.1)
6 weeks	25.7 (3.1)	4.1 (2.1)
9 weeks	36.6 (3.6)	0.0 (0.0)
Saliva caffeine		
baseline	18.0 (3.1)	21.6 (4.1)
homevisit 1	26.3 (3.6)	2.1 (2.1)
homevisit 2	21.6 (3.6)	2.1 (1.5)

Values are means, standard errors between parentheses.

a clear and highly significant drop during the intervention period. In the subjects on coffee in study A and on caffeine tablets in study B, caffeine levels did not significantly change during the trial.

Discussion

The primary objective of our studies was to investigate the influence of coffee on serum lipids (14). However, the design of the studies permits the assessment of concomitant changes in other cardiovascular risk indicators such as blood pressure (15). Moreover, these trials of long duration, 9 weeks, and with large intervention groups provided an excellent opportunity to explore the relationship between coffee, caffeine and some hemostatic variables.

Our results, obtained in 2 randomized trials over periods of 9 weeks, indicate no effects of consumption of either coffee or caffeine on hemostatic variables, such as fibrinogen, factor VII activity, factor VIII antigen, protein C and protein S. On abstinence from filtered coffee, the plasma levels of these variables do not seem to be affected. Moreover, the method of brewing does not influence the levels of the hemostatic factors. In contrast, serum cholesterol increases on consumption of boiled coffee (14). From our double-blind randomized study on the effect of caffeine it appears that fibrinogen and factor VII activity are not changed following abstinence from caffeine. These "negative" results cannot be explained by poor adherence to the study protocol; the compliance of the participants, as measured in several ways, was very good.

Previous data are only available on the relationship between coffee and fibrinogen. In one large cross-sectional study, increased coffee consumption was associated with increased plasma fibrinogen levels (9). In an experimental study, however, no change in fibrinogen concentration was observed on abstinence from caffeine (2). The last observation is in agreement with our findings on caffeine.

In summary, our experimental results suggest that coffee and caffeine have no effect on factors involved in the clotting system. This, however, does not rule out the possibility that coffee use induces unfavourable changes in other components of the hemostatic process. Further studies, in particular on the effects of coffee consumption on platelet activity are required.

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CHAPTER 7

CAFFEINE, SERUM LIPIDS AND BLOOD PRESSURE

7.1 INTRODUCTION

Caffeine from natural sources has been consumed and enjoyed by people throughout the world for many centuries (1). According to historical records, the oldest caffeine-containing beverage is tea. The first reference to its use is attributed to the legendary Chinese emperor Shen Nung in 2737 B.C. Coffee may have been cultivated in Ethiopia as early as the sixth century and was first used as a food, the berries being eaten as a whole or crushed. Coffee as a hot beverage came into use about 1000 A.D., but did only first reach Europe in the seventeenth century. The use of caffeine as a flavor component in cola-type softdrinks originates from around the turn of the century (2). Since its original chemical isolation in 1820, caffeine has been used therapeutically in infant apnea, as a bronchial and cardiac stimulant and in migraine headache. Furthermore, caffeine is a constituent of certain analgesics, weight control aids and alertness compounds (1).

The health consequences of caffeine have been discussed ever since its isolation. Myocardial infarction, hypertension, arrhythmias, anxiety, fibrocystic breast disease, infertility, malignancy and birth defects have all been suspected to be related to ingestion of caffeine (3). Concern for potential adverse effects of caffeine, made many coffee consumers to switch from regular to decaffeinated coffee. In the Netherlands, 6.2 % of the population drinks decaffeinated coffee (4), and in the United States this percentage amounted to 16.7 % in 1989 (5). With regard to the cardiovascular system, however, there is little evidence for a beneficial effect of abstinence from caffeine and the use of decaffeinated coffee (6).

From short-term studies on caffeine and blood pressure, contradictory findings are reported. In 1934, Horst et al. investigated the effect of regular coffee and decaffeinated coffee on blood pressure and reported that caffeine raised blood pressure and lowered heart rate (7). Other researchers reported that caffeine had no effect on either blood pressure or heart rate (8,9). A major reason for these discrepancies appears to be the failure in many of the early studies to distinguish between non-consumers and habitual consumers of caffeine. Evidence for tolerance to the cardiovascular effects of caffeine has now been clearly demonstrated. The acute blood pressure increasing effect of caffeine is only observed in subjects who abstained from all methylxanthine-containing beverages for at least 10 hours (10). Essentially complete tolerance develops to this effect after caffeine consumption for 1-4 days (11). More on the short-term effects of caffeine and coffee on blood pressure can be found in section 5.2.

Acute studies on caffeine use and serum lipids consistently show a rise in plasma free fatty acids (12-19). Patwardhan et al. observed doubling of the free fatty acids level 1 hour after ingestion of 250 mg caffeine by 16 healthy volunteers. The elevation

remained for about 4 hours (17). By contrast, serum total cholesterol and triglycerides are unaffected by caffeine use (12-16,18,19). All studies were performed in caffeine-naive subjects. The caffeine induced increase in FFA seems to be mediated by antagonism of endogenous adenosine, resulting in enhanced production of cyclic AMP, which in turn promotes lipolysis (20).

Evidence for long-term effects of caffeine on serum lipids and blood pressure is virtually lacking. We therefore conducted a 12-week double blind intervention study, in which participants were randomly assigned to caffeine tablets or placebo tablets. In both intervention groups, caffeine intake from any other source was not allowed, permitting evaluation of the isolated effect of caffeine by comparison of the two groups.

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7.2 EFFECTS OF CAFFEINE ON BLOOD PRESSURE AND SERUM LIPIDS; RESULTS FROM A DOUBLE BLIND STUDY[1]

Introduction

Since many years, the widespread use of coffee has led some to suspect that the habit might have detrimental effects on health. Already almost a decade ago an analysis of available data in favour or against coffee use concluded that at that time no strong arguments would support an advice to abandon drinking coffee (1). In recent years, however, the possibility that coffee use is associated with an increased incidence of cardiovascular disease has gained renewed interest. The most prominent compound of coffee is caffeine, and it has been suggested that some of the risks related to coffee result from the effects of this substance. Indeed, short-term in vivo and in vitro studies have shown that caffeine has various pharmacological properties which include effects on the cardiovascular system (2,3). Given the half-life of plasma caffeine, many coffee drinkers have plasma caffeine levels that never fall below biochemical detection limits. Because of the large number of people consuming coffee, even relatively small effects of caffeine on blood pressure or serum lipids might eventually have large public health consequences.

Results from non-experimental studies on coffee and blood pressure are conflicting (4-6). In most short-term experimental studies, a transient blood pressure rise on caffeine is observed, lasting for about 4 hours (7). This blood pressure elevation is usually accompanied by a decrease in heart rate. Recently, we observed a significant decrease in systolic blood pressure on abstinence from coffee for 9 weeks (8).

In most cross-sectional studies on serum lipids, only caffeine intake from coffee is positively associated with serum cholesterol (9-11). Shirlow and coworkers reported a significant association between serum cholesterol and total caffeine consumption irrespective of source, for females only (12). In two small experimental studies, caffeine had no detectable effect on serum lipids (13-14).

To derive more conclusive evidence for the effect of caffeine on serum lipids and blood pressure, we conducted a double blind, randomized trial with two parallel groups in 69 young, healthy adults. All participants were regular coffee users. For a period of nine weeks, subjects were randomly allocated to groups receiving either caffeine tablets or placebo tablets while using decaffeinated coffee. In both groups, caffeine intake from other sources was not allowed.

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[1]Submitted for publication.

Methods

Subjects

The participants in the present study were selected from a cohort of 596 young adults who take part in a follow-up study of cardiovascular risk indicators. This cohort is a random sample from a population-based study that was initiated in 1975 and included 4,649 young subjects from the Dutch town of Zoetermeer (15). The members of the cohort have been examined annually since the start of the study. Those from the cohort with habitual coffee use and aged 18 years or over, were invited for the present study and 69 subjects agreed to take part. All participants gave informed consent after detailed explanation of objective and nature of the study. In The Netherlands, coffee is brewed almost exclusively by filtering through paper. The mean baseline coffee consumption of the subjects was 5.9 cups of filtered, regular coffee per day.

Protocol

The total study period was 12 weeks with 9 weeks of intervention. The run-in period, during which subjects continued on regular coffee, lasted 3 weeks. After stratification for gender, the participants were randomly assigned to one of two groups. Group 1 received 4-6 cups of filtered decaffeinated coffee per day and an equal number of tablets containing 75 mg caffeine, i.e. the amount similar to an average Dutch cup of coffee. Group 2 received 4-6 cups of filtered decaffeinated coffee per day and an equal number of placebo tablets. The caffeine and placebo tablets were similar, except for the caffeine content. To mimic the taste of caffeine, 5 mg quinine was added to each placebo tablet. The participants in both study groups were not allowed to use any caffeinated beverages or caffeine containing medication during the intervention period. Decaffeinated coffee and cola were given free of charge. The study was performed double blind. Baseline measurements were performed at the start and at the end of the run-in period and effect measurements after 3, 6 and 9 weeks of intervention.

Brewing method

The filtered coffee was prepared in a commercially available electric coffee maker by dripping hot water on coffee and filtering it through paper.

Measurements

Blood samples were obtained from non-fasting subjects and analyzed blinded in the laboratory of our department. The laboratory participates in the Lipid Standardization Programme of the WHO Regional Lipid Reference Centre in Prague. An automated enzymatic procedure was used for determination of serum total cholesterol (CHDD-PAP High Performance, Boehringer Mannheim, FRG). HDL- and LDL-cholesterol were measured by the same method after precipitation. The phosphotungstate method according to Burstein and coworkers (16), with a minor modification as described by Grove (17) was used for precipitation of HDL. For LDL-cholesterol precipitation was carried out with polyvinyl sulphate (Boehringer Mannheim, FRG). Apolipoproteins A1

and B were assayed by an automated immunoturbidimetric method (Kone, Espoo, Finland). Caffeine concentrations in serum and saliva samples were determined by reversed-phase HPLC (18). At each occasion, blood pressure was measured twice after 10 minutes of rest with a random-zero sphygmomanometer. The two readings were separated in time by a count of the heartrate. The mean of the two measurements was used in the analyses. Body height and weight were measured with the subjects wearing indoor clothes without shoes. An 81-item quantitative food frequency questionnaire on dietary fat intake was completed at baseline and at the end of the study period. From these forms, mean daily total fat and animal fat intake were calculated using a computerized food composition table (19).

Compliance

Adherence to the study protocol was assessed in three ways. First, a diary was kept on the amount of coffee, tea, cola and chocolate consumed. In addition, unused tablets were returned and counted. Second, serum caffeine levels were measured in blood samples obtained at the scheduled visits at the examination centre. Finally, each participant was visited twice during the study without prior announcement. At these visits, a saliva sample was obtained for determination of caffeine levels. Caffeine concentrations in plasma and saliva are closely correlated (20). Moreover, collection of saliva is convenient and non-invasive and, therefore, saliva sampling can serve as an excellent tool for drug monitoring (21).

Data-analysis

The effect of caffeine on serum lipids and blood pressure was assessed by comparing the change from baseline levels between the groups after 3, 6 and 9 weeks of intervention. The second series of measurements during the run-in period were taken as baseline values for comparison of intervention effects. Changes from baseline are presented with 95 % confidence limits (95 % C.L.). Because all participants consumed caffeine before the trial, the group continuing on caffeine was considered as the reference group. Therefore, changes in the group on placebo tablets are expressed as the changes from baseline minus the concomitant changes in the group on caffeine. In separate analyses, changes in serum lipids and blood pressure were adjusted for body weight changes at 3, 6 and 9 weeks by linear regression analysis, in order that unintended (and unbalanced) changes in body weight during the intervention are taken into account. Moreover, interaction between gender, smoking habits and intervention effects was tested both by stratified univariate analysis and by entering interaction-terms for these characteristics in the multivariate model (22). The analyses presented below exclude 7 subjects who did not complete the study. Three of them were in the caffeine group (2 women, 1 man) and four of them were in the placebo group (2 women, 2 men). Baseline characteristics of these subjects were similar to those who completed the study.

Table 7.1. Baseline characteristics in the two intervention groups (mean (SD)).

	Caffeine	*No caffeine*
Number	32	30
Gender (M/F)	18/14	16/14
Age (yrs)	24.6 (4.1)	25.8 (4.1)
Body weight (kg)	73.1 (10.9)	72.6 (13.1)
Height (cm)	177.6 (9.1)	177.2 (9.6)
SBP (mm Hg)	125.2 (13.0)	122.5 (13.0)
DBP (mm Hg)	74.3 (8.9)	73.6 (8.5)
Heart rate (beats/min)	71.0 (9.8)	74.2 (9.7)
Total cholesterol (mmol/l)	5.39 (0.78)	5.39 (1.18)
HDL-cholesterol (mmol/l)	1.23 (0.29)	1.27 (0.28)
LDL-cholesterol (mmol/l)	3.50 (0.82)	3.52 (1.20)
Apolipoprotein A1 (mg/dl)	136.1 (19.6)	143.1 (23.0)
Apolipoprotein B (mg/dl)	92.3 (19.9)	93.5 (27.0)
Serum caffeine (umol/l)	20.1 (12.4)	21.6 (11.8)
Coffee (cups/day)	5.8 (3.3)	5.9 (3.3)
Tea (cups/day)	1.4 (1.7)	1.5 (1.3)
Caffeine intake (mg/day)	505 (242)	508 (242)
Dietary fat intake (g/day)	94 (17)	90 (17)
Smoking (yes/no)	16/16	19/11

Results

Baseline characteristics for the two study groups are summarized in table 7.1. The data indicate that the groups were well-balanced for all characteristics.

Table 7.2 shows the changes in serum lipids for the two study groups. Levels of serum total cholesterol, HDL-cholesterol and LDL-cholesterol were not affected by abstinence from caffeine. Moreover, changes in apolipoproteins A1 and B were not significantly different between the groups either. The changes in systolic and diastolic blood pressure and heart rate were not significantly different between the two groups, as shown in table 7.3.

Changes in dietary fat intake were similar in the 2 groups. The mean changes in dietary fat intake were for the caffeine and no caffeine group -3.1 g/day (95 % confidence limits -4.5,-1.7) and -4.4 (-5.8,-3.0) respectively. Body weight increased by 0.7 (0.1,1.3) kg and 0.9 (0.3,1.5) kg in the caffeine group and no caffeine group respectively. Adjustment for the non-significant differences in body weight change between the groups, did not affect the results. Separate analyses for men and women and smokers and non-smokers revealed no significant interaction by gender or smoking

Table 7.2. Mean levels (SE) of serum cholesterol (mmol/l) and apolipoproteins (mg/dl) during intervention. The last column reflects the net changes from baseline with the caffeine group as reference group (mean (95% CL)).

	Caffeine (n = 32)	*No caffeine (n = 30)*	*Net change from baseline*
Total cholesterol			
3 weeks	5.45 (0.11)	5.51 (0.22)	0.06 (-0.19,0.31)
6 weeks	5.53 (0.15)	5.28 (0.23)	-0.23 (-0.54,0.08)
9 weeks	5.48 (0.13)	5.44 (0.23)	-0.03 (-0.30,0.24)
HDL-cholesterol			
3 weeks	1.23 (0.05)	1.26 (0.05)	-0.01 (-0.07,0.05)
6 weeks	1.25 (0.05)	1.27 (0.05)	-0.04 (-0.12,0.04)
9 weeks	1.29 (0.05)	1.30 (0.06)	-0.06 (-0.16,0.04)
LDL-cholesterol			
3 weeks	3.71 (0.13)	3.64 (0.23)	-0.08 (-0.47,0.31)
6 weeks	3.75 (0.15)	3.55 (0.23)	-0.22 (-0.57,0.13)
9 weeks	3.78 (0.16)	3.69 (0.25)	-0.11 (-0.52,0.30)
Apolipoprotein A1			
3 weeks	139.1 (4.1)	142.0 (3.8)	-4.1 (-11.6, 3.4)
6 weeks	134.2 (2.7)	134.7 (4.3)	-6.5 (-16.5, 3.5)
9 weeks	138.9 (3.7)	140.5 (4.0)	-5.3 (-14.9, 4.3)
Apolipoprotein B			
3 weeks	98.0 (3.7)	97.1 (4.8)	-2.2 (-9.8, 5.4)
6 weeks	98.5 (4.4)	93.5 (5.0)	-6.3 (-15.1, 2.5)
9 weeks	95.2 (3.3)	99.1 (5.4)	2.7 (-4.4, 9.8)

habits and the effect of caffeine on either serum lipids or blood pressure.

Compliance as assessed by the diaries of the participants was good. Consumption of caffeine exceeding 200 mg per 3-week period was reported 8 times in the caffeine group and 6 times in the no-caffeine group. The caffeine content of regular coffee, tea, chocolat and cola was assumed to be 75, 30, 5 and 40 mg per cup or glass. Mean caffeine intake after 3, 6 and 9 weeks of intervention was 144 (SE 58) mg, 68 (25) mg and 96 (40) mg in the caffeine group and 48 (13) mg, 54 (20) mg and 123 (98) mg in the placebo group. From the returned unused tablets the number of used tablets could be calculated. Less than 76 used tablets indicated more than 10% non-compliance, and was observed in 2, 1 and 3 subjects after 3, 6, and 9 weeks of intervention in the caffeine group and in 0, 2 and 3 subjects in the placebo group. The results on the measurements of caffeine in serum and saliva samples are represented in figures 7.1-7.4. The caffeine levels in the no caffeine group show a clear and highly significant drop during the intervention period. The mean caffeine levels went not completely

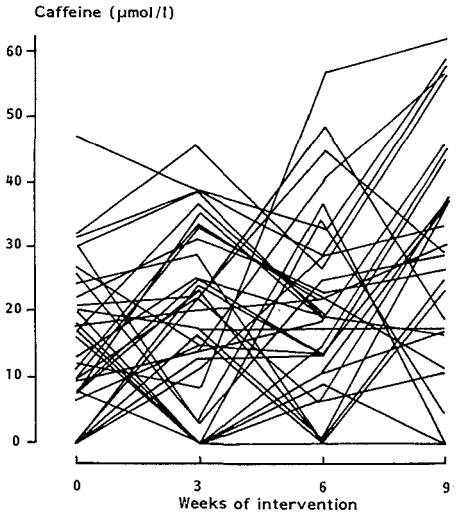
Table 7.3. Mean levels (SE) of blood pressure (mm Hg) and heart rate (beats/min) during intervention. The last column refects the net changes from baseline with the caffeine group with the caffeine group as reference group (mean (95% CL)).

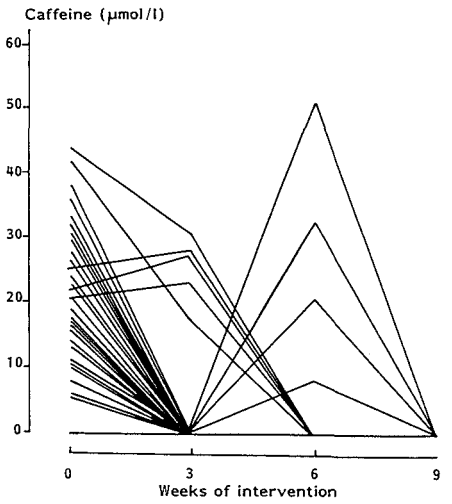
	Caffeine (n=32)	*No caffeine (n=30)*	*Net change from baseline*
Systolic blood pressure			
3 weeks	126.6 (2.2)	124.3 (2.4)	0.3 (-3.8, 4.4)
6 weeks	127.2 (2.4)	126.0 (2.1)	2.2 (-2.3, 6.7)
9 weeks	123.7 (2.2)	120.9 (2.1)	0.6 (-4.7, 5.9)
Diastolic blood pressure			
3 weeks	74.7 (1.7)	74.7 (1.4)	0.6 (-2.7, 3.9)
6 weeks	76.3 (1.6)	73.3 (1.7)	-1.9 (-4.8, 1.0)
9 weeks	74.3 (1.4)	72.5 (1.3)	-0.6 (-4.1, 2.9)
Heart rate			
3 weeks	74.4 (2.0)	76.6 (1.8)	-1.1 (-5.4, 3.2)
6 weeks	73.4 (2.2)	76.0 (1.8)	-0.9 (-5.2, 3.4)
9 weeks	75.3 (2.2)	77.3 (2.1)	-1.4 (-5.9, 3.1)

down to zero in the no caffeine group due to a few non-compliers. For these individuals, results were not different compared to the rest of the group.

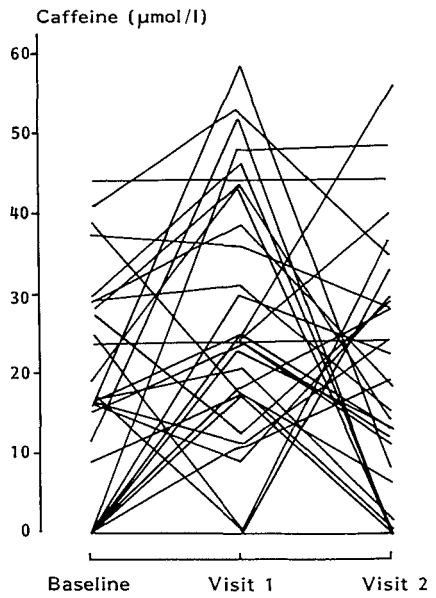
Discussion

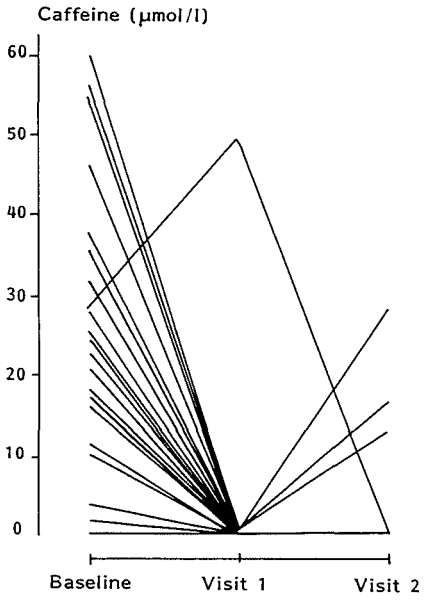
The main finding of this double blind, randomized trial in young subjects is that abstinence from caffeine for a period of nine weeks has no effect on either serum lipids or blood pressure. The design of the present study, its size and duration and the resulting confidence limits of the estimated effect of caffeine on two major cardiovascular risk indicators, make an effect of coffee use mediated by caffeine very unlikely. The observation that abstinence from caffeine does not influence serum lipids, is in agreement with our previous findings in a randomized trial on coffee, serum lipids and blood pressure. This trial was not blinded and focused on brewing method rather than caffeine content of the coffee. In that study, however, no statistically significant difference was present in serum lipids between the group consuming filtered coffee and the group abstaining from coffee for a period of nine weeks (23). In the same experimental study, we observed a reduction in blood pressure on abstinence from





Figures 7.1 and 7.2. Individual caffeine levels in serum in the caffeine group (left) and the no-caffeine group (right).





Figures 7.3. and 7.4. Individual caffeine levels in saliva in the caffeine group (left) and the no-caffeine group (right).

coffee for a period of 9 weeks (8). The cross-over study of Burr et al. in 54 subjects revealed a statistically significant higher systolic blood pressure during coffee consumption compared to abstinence from coffee. The difference between the blood pressure response on regular coffee and decaffeinated coffee was, however, not statistically significant. Changes in serum lipids were not significant (24). Another recent study reported by Rosmarin and coworkers likewise showed no change in serum lipids on abstinence from regular coffee (25). If a long-term effect of coffee on blood pressure is real, the results from the present study support the hypothesis that this effect is not mediated by caffeine. Our findings on blood pressure disagree with a double blind trial on the effects of decaffeinated and regular coffee, where a small decrease in both systolic and diastolic blood pressure on decaffeinated coffee wasobserved (26). An explanation for the discrepancy may be that there are more differences between caffeinated and decaffeinated coffee than the caffeine content. Our placebo-controlled design with caffeine tablets, however, enabled us to study the actual effects of caffeine. We did not observe significant interaction by gender or smoking habits and the effect on either serum lipids or blood pressure. Three, non-experimental, cross-sectional studies showed opposite results (12,27,28). First, Heyden et al. reported on a positive interaction between smoking and coffee drinking (27). The study comprised only 361 subjects, and men and women were analysed together. Shirlow et al. likewise observed a significant positive interaction between smoking and caffeine consumption in their association with serum cholesterol, for females, but not for males (12). Finally, Salonen and coworkers showed a positive link between coffee consumption and serum HDL-cholesterol among non-smokers only. This interaction was less consistent for non-HDL-cholesterol (28). In our view it is difficult to obtain conclusive evidence for interaction between caffeine consumption and smoking habits from cross-sectional studies in which residual confounding, for example from differences in dietary habits, might play an important role.

In summary, from this study it appears that caffeine has no adverse effect on cardiovascular risk by inducing unfavourable changes in blood pressure or serum lipids in young healthy adults.

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CHAPTER 8

GENERAL CONCLUSIONS: GROUNDS FOR REASSURANCE?

8. GENERAL CONCLUSIONS - GROUNDS FOR REASSURANCE?

The widespread use of coffee and caffeine and the appearance of unfavorable, yet equivocal reports on coffee and cardiovascular risk justify a thorough and detailed study of these issues. This thesis focusses on long-term, chronic effects of coffee and caffeine use. A review of the available data on coffee and cardiovascular morbidity and mortality showed that most of the evidence does not support a causal role for coffee in cardiovascular disease. As discussed in chapter 2, large, prospective follow-up studies are preferable to case-control studies to obtain valid results. Moreover, careful consideration of lifestyle features, associated with both coffee consumption and cardiovascular risk, such as smoking, dietary fat intake and physical activity, is a prerequisite in the study of coffee and cardiovascular disease.

With regard to coffee and serum lipids, the results of our 12-week randomized trial strongly support the hypothesis that the use of boiled coffee increases serum total cholesterol and low-density lipoprotein cholesterol, the atherogenic subfraction of cholesterol. In this experiment, "boiled" coffee was prepared by pouring boiling water on coarsely ground coffee. While keeping the coffee hot in a thermosjar, at least 10 minutes was waited before the coffee was consumed. This brew is similar to the boiled coffee still prepared by many Norwegian and Finnish people. The mechanism by which boiled coffee exerts a serum cholesterol raising effect is, as yet, largely unclear. Speculations include the higher temperature of the water during preparation of boiled coffee compared to filtered coffee, the longer exposure time to hot water, and lack of the protective, i.e. filtering, effect of the filter paper. Following our study, a small part of the mystery appears to be solved by Zock and coworkers, who obtained a lipid-enriched fraction from boiled coffee. Ten volunteers consumed this substance and after 6 weeks their cholesterol levels were clearly increased (1). The authors state that about 90 % of the lipid material could be hydrolysed by boiling in ethanolic KOH, and must thus have consisted of triglycerides, phospholipids, and other esters of fatty acids. The remaining 10 % (120 mg) is formed by unsaponifiable lipid matter. Since triglycerides and phospholipids do not increase serum cholesterol when given in amounts of 1 g per day, the research group of Katan speculates that the cholesterol raising substance must be located in the unsaponifiable lipids (1). The search for the responsible factor will be time-consuming, but extremely interesting for several reasons. First, because this cholesterol increasing substance may be present in other food-items besides coffee. Second, as it seems to interfere with a powerful pathway, it may clarify another aspect of the complicated cholesterol metabolism, which, finally, may lead to therapeutical consequences. The meta-analysis of cross-sectional data on coffee and serum lipids, presented in chapter 3, focussed primarily on brewing methods. Studies including subjects who drank boiled coffee, showed the expected elevated cholesterol levels with

increasing coffee consumption. Moreover, data from Italy and Israel, where espresso coffee and Turkish coffee are the most common brews, also showed a positive relationship between coffee and serum total cholesterol. These findings call for experimental confirmation. Filtered coffee does not seem to affect serum lipid concentrations, as evidenced by both experimental and non-experimental results. A separate study of the effect of caffeine on serum lipids was necessary because coffee is not the only beverage containing caffeine. In a double-blind randomized trial, the isolated effect of caffeine could be assessed. We observed no change in the serum lipid profile on abstinence from caffeine.

Findings on blood pressure are less consistent. Cross-sectional data indicate a small, non-significant favorable effect of coffee use on blood pressure. By contrast, our experimental study showed a decrease in blood pressure during abstinence from filtered coffee. In the subsequent double blind study of caffeine versus placebo, a fall in blood pressure following abstinence from caffeine was not observed. Therefore, it can be postulated that short-term effects, through caffeine, and long-term effects, by some other compound of coffee, are phenomena due to different modes of action on the cardiovascular system. Further long-term experimental studies on coffee and blood pressure are needed to explain the present discrepancies between experimental and non-experimental observations.

Similar uncertainty remains in the field of coffee and hemostasis. Our experimental studies showed no effect of coffee use on the serum concentrations of fibrinogen, clotting factor VII activity, factor VIII antigen, protein C and protein S and also no effect of caffeine intake on fibrinogen and factor VII activity. Some previous, small-scale experiments, however, both suggested and denied a thrombogenic capacity of coffee and caffeine. Again, future research will shed light on the potential relationship between coffee and hemostasis.

The link between coffee and cardiovascular morbidity and mortality is, if present at all, very weak. Therefore, favorable and unfavorable effects of coffee on serum lipids, blood pressure and hemostatic variables will be either too small to exert an effect on the incidence of cardiovascular disease or, alternatively, will be roughly in balance. Interestingly, in a recently published study from Norway, coffee was observed to increase mortality from coronary heart disease (2). In part, this finding might be explained by the use of boiled coffee among the participants in this cross-sectional study. Serum cholesterol levels, however, were considered in the analysis.

When it is accepted that, under certain conditions, coffee use may influence serum lipid profile and blood pressure, preventive activities may be considered. In principle, there are two intervention strategies that could be followed (3). The first is to identify and treat individuals at risk of developing the disease. The second involves the entire population in order to achieve a beneficial effect on the general distribution

of risk indicators. The whole population approach will be useful in parts of Norway and Finland, where people drink boiled coffee in large quantities. Introduction of paper filter bags in these areas seems sensible and feasible. Following the high-risk approach, individuals with an elevated blood pressure should be advised to try a coffee-free period of an arbitrary 2 months, to allow the evaluation of a potential beneficial effect of this intervention. Moreover, a switch to filtered coffee may be favourable in subjects with a high serum cholesterol concentration, who usually drink boiled coffee, espresso coffee or Turkish coffee (4).

Authors of editorials on coffee and health consequences appear to be enthusiastic coffee consumers, as evidenced by the optimistic bottom line of their papers, whatever depressing news is communicated (5-12). A coffee-lover myself, I am glad to follow this tradition and conclude from this thesis that the use of filtered coffee in moderation is not harmful for the cardiovascular system.

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CHAPTER 9

BREWING METHODS: GLOSSARY

9. BREWING METHODS: GLOSSARY

Boiled coffee
This Northern Scandinavian type of coffee is prepared by boiling coarsely ground light roasted coffee (50 - 70 g/l) in water for 10 or more minutes. It is not unusual that the pot is kept on the stove for prolonged time. The brew is consumed without full separation of the grounds (1 cup = 150 - 190 ml). The participants in the experimental studies described in this thesis prepared this brew by pouring coarsely ground coffee (40 g/l) and freshly boiling water into a thermosjar. They were allowed to drink the coffee after waiting for at least 10 minutes.

Espresso
Brew prepared by extracting 6 - 8 g of finely ground medium to dark roasted coffee with water at 8 - 12 bar and 92 - 95 °C for 15-25 seconds (Italy 25 - 50 ml cup) or longer (France and Switzerland up to 150 ml cup).

Filtered or drip coffee
Brew prepared by pouring boiled water over ground roasted coffee in a paper filter. The degree of roast ranges from light in North America and Scandinavia, medium in Westeuropean countries to dark in France and Italy. Dosage of coffee varies considerably (from about 25 to 75 g/l) depending on national and personal brewing habits. Cupsizes range from 110 - 190 ml.
The participants in the experimental studies described in this thesis prepared this type of coffee with a dosage of 40 g/l using an electric coffee maker.

Instant coffee
Soluble coffee is prepared by dissolving 1.5 - 3.0 g of instant coffee powder into 150 - 190 ml of hot water (worldwide).

Mocca coffee
Brew prepared in the "Neapolitan" coffee maker forcing just overheated water through a bed of finely ground medium to very dark roasted coffee. The Neapolitan coffee maker can be separated by unscrewing into a lower and upper part. Water is poured into the lower part. A metal tunnel containing the ground coffee is inserted into the

lower part and the coffee maker is closed by screwing the upper part on top of the lower part. At heating, water is forced upward through the coffee bed and collected in the upper part.

Percolated coffee

Brew prepared by extracting coarsely ground light roasted (North America 28 - 40 g/l) or medium roasted (United Kingdom about 60 g/l) coffee with recirculating boiling water until the desired brew strength is reached (1 cup = 150 - 190 ml).

Turkish/Greek coffee

This brew is prepared by bringing very finely ground medium to dark roasted coffee (ca. 5 g) in water (ca. 60 ml) to a gentle boil until a foam is formed. Usually sugar (5 - 10 g) is added. The ceremony of gentle boiling till foaming can be repeated a couple of times. (Middle East, 1 cup = 40 - 60 ml).

CHAPTER 10

SUMMARY

10. SUMMARY

This thesis comprises several studies on the effect of coffee and caffeine on cardiovascular risk in general, and the effect on serum lipids, blood pressure and selected hemostatic variables in particular.

The association between coffee use and cardiovascular morbidity and mortality was evaluated by a review of the published results of 17 cohort studies and 10 case-control studies. In the interpretation of the results of these studies, it is important to take into account the strong relationship of coffee consumption and other habits associated with cardiovascular disease, such as smoking and a high dietary fat intake. When these confounders are not considered in the analysis, the risk of coffee use for cardiovascular disease may be overestimated. Most case-control studies yielded positive results, i.e. coffee use was observed to increase the risk of a cardiovascular event. Case-control studies, however, are subject to several forms of bias which could lead to overestimation of the coffee-cardiovascular disease association. The majority of cohort studies, which are more reliable, support the hypothesis that coffee use does not affect the risk of cardiovascular disease (chapter 2).

The non-experimental evidence for the effect of coffee on serum cholesterol and blood pressure was assessed by a meta-analysis of cross-sectional studies. A meta-analysis offers the opportunity to combine data of several studies with the advantage of increased statistical power to calculate a "typical" effect estimate. Alternatively, apparent contradictions between studies may be explored which provides more insight in physiological characteristics and plausibility of the association under study. Authors of papers on coffee and serum lipids or blood pressure were asked to supply us with missing data and additional information on their studies. The crude results of the included studies were re-analyzed in a weighted linear regression model. The results indicate a positive relationship between coffee and serum total cholesterol with statistical significant findings for boiled coffee, espresso coffee and Turkish coffee. The common feature of these brewing methods is that no paper filter is used during preparation of the coffee. High-density lipoprotein cholesterol was not affected by coffee consumption. Data on blood pressure tended towards an inverse relationship with coffee consumption, i.e. decreasing blood pressure with increasing coffee consumption. However, the regression coefficients were small and did not reach statistical significance. With respect to blood pressure, the brewing method is not affecting the results. A potential problem with a meta-analysis of cross-sectional studies on coffee and serum cholesterol and blood pressure is publication bias, leading to overestimation of effects. The inclusion of available unpublished data in the present analysis, however, did not support the effect of this bias (chapter 3).

Further, direct, evidence for a cholesterol-raising effect of boiled coffee was

obtained in an experimental study. Hundred and seven young adults were randomly assigned to one of three intervention groups, receiving four to six cups of boiled coffee a day, four to six cups of filtered coffee a day, or no coffee for a period of nine weeks. As compared with the change from baseline in the filtered coffee group, the serum total cholesterol level increased on the consumption of boiled coffee by 0.48 mmol/l (95 % confidence limits 0.13, 0.83) and low-density lipoprotein cholesterol level increased by 0.39 mmol/l (-0.04, 0.82). The net increase in serum total cholesterol amounted to 10 % of the baseline level after 9 weeks of boiled coffee. The levels of high-density lipoprotein cholesterol and apolipoproteins were not affected by boiled or filtered coffee. There was no significant difference in the change in serum total or low-density lipoprotein cholesterol levels between the filtered-coffee group and the group that drank no coffee (chapter 4).

In the same experimental study, the effects of coffee on blood pressure and heart rate were evaluated. Moreover, the mediating effect of two brewing methods on these variables could be assessed. Both systolic and diastolic blood pressure decreased in the group abstaining from coffee compared to the filtered coffee group, but only the fall in systolic blood pressure after 9 weeks was statistically significant, -6.1 mm Hg (-10.8, -1.4). After adjustment for systolic blood pressure levels at baseline and for body weight change during the study, the observed reduction was -3.4 mm Hg (-7.1, 0.3). The patterns for systolic and diastolic blood pressure were remarkably similar in the groups using either filtered coffee or boiled coffee. After 9 weeks of boiled coffee, mean changes from baseline for systolic and diastolic blood pressure were 0.4 mm Hg (-3.7, 4.5) and - 0.1 mm Hg (-3.4, 3.2) compared to the filter group. The heart rate showed a slight, non-significant decrease in the abstinence group. These findings suggest that abstinence from coffee for a period of several weeks may lead to a slight reduction of blood pressure in young normotensive adults. Our observations on blood pressure need careful interpretation, in particular because the participants were not blinded to their intervention status. Moreover, we cannot compare the results with previous reports on the blood pressure response to abstinence from coffee for a period of this length (chapter 5).

Coffee and hemostasis was the next subject of interest. A review of the presently available evidence in favor or against an association between coffee and hemostatic variables showed inconsistent findings. Data on fibrinogen level and platelet in vivo activation as measured by plasma beta-thromboglobulin indicate, if anything, an unfavorable effect of coffee, i.e. coffee may enhance thrombotic tendencies. In the experimental study described earlier, we explored the impact of coffee consumption on the levels of fibrinogen, clotting factor VII activity, factor VIII antigen, protein C and S. These hemostatic factors were not affected by either consumption of boiled coffee or abstinence from coffee as compared to the use of filtered coffee for 9 weeks.

Likewise they were not affected by abstinence from caffeine (chapter 6).

To study the effects of caffeineon blood pressure and serum lipids, we conducted a double blind, randomized trial with two parallel groups in 69 young, healthy subjects. After a three week run-in period, subjects were randomly assigned to one of two groups receiving either 4-6 cups of filtered decaffeinated coffee per day and an equal number of tablets containing 75 mg caffeine each, or 4-6 cups of filtered decaffeinated coffee per day and an equal number of placebo tablets, for a period of nine weeks. In both groups, caffeine intake from other sources was not allowed. Abstinence from caffeine for a period of nine weeks had no effect on either serum lipids or blood pressure. The compliance of the participants to the intervention, as measured by serum and saliva caffeine concentrations, was very good and could not explain these negative results (chapter 7).

In chapter 8, the findings described in the previous chapters are discussed. Since there is little evidence for a detrimental effect of coffee on cardiovascular disease, favorable and unfavorable effects of coffee on cardiovascular risk indicators are either small or roughly in balance. Recommendations for preventive activities concerning coffee include encouragement of the use of filtered coffee in boiled-coffee-areas in Scandinavia and a eight-week trial period of abstinence from coffee for subjects with a high blood pressure. Subjects with a high serum cholesterol level, who consume boiled coffee, espresso coffee or Turkish coffee may benefit from a switch to filtered coffee. Future cardiovascular studies on coffee are needed, with special emphasis on the long-term effects of coffee on blood pressure and hemostatic parameters. Moreover, experimental evidence for a cholesterol altering effect of espresso coffee and Turkish coffee is lacking and, finally, the underlying mechanism of the low-density lipoprotein cholesterol raising effect of boiled coffee needs further clarification.

SAMENVATTING

Dit proefschrift beschrijft onderzoek naar het effect van koffie en caffeïne op het risico op hart- en vaatziekten in het algemeen en de invloed op serum lipiden, bloeddruk en enkele hemostatische factoren in het bijzonder.

Het verband tussen koffie gebruik en de morbiditeit en mortaliteit van hart- en vaatziekten werd nagegaan in de gepubliceerde resultaten van 17 cohort studies en 10 patiënt-controle studies. Bij de interpretatie is het belangrijk rekening te houden met het sterke verband tussen koffie consumptie en andere gewoonten die samenhangen met hart- en vaatziekten, zoals roken en een hoge voedingsvetinname. Wanneer deze verstorende variabelen niet in de analyse worden betrokken, wordt het risico van koffiegebruik op hart- en vaatziekten wellicht overschat. De meerderheid van de cohort studies, die in dit verband betrouwbaarder zijn, steunt de hypothese dat koffie gebruik geen effect heeft op het risico op hart- en vaatziekten (hoofdstuk 2).

Het niet-experimentele bewijs voor een effect van koffie op het serum cholesterolgehalte en de bloeddruk werd bestudeerd in een meta-analyse van cross-sectioneel onderzoek. Een meta-analyse biedt de mogelijkheid gegevens van verschillende onderzoekingen te combineren, met het voordeel dat het onderscheidingsvermogen om een "typisch" effect te berekenen, toeneemt. Bovendien kunnen schijnbare tegenstrijdigheden tussen studies onderzocht worden, waardoor meer inzicht in de fysiologische achtergrond van het bestudeerde verband ontstaat. Auteurs van artikelen over koffie en serum lipiden of bloeddruk werd gevraagd ons te helpen met ontbrekende gegevens en extra informatie over hun onderzoek. De ruwe resultaten van de studies die voldeden aan de toelatings-criteria van de meta-analyse werden opnieuw geanalyseerd met een gewogen lineair regressie model. De resultaten wijzen op een positief verband tussen koffie en serum totaal cholesterol met statistisch significante bevindingen voor gekookte koffie, espresso koffie en Turkse koffie. Het gemeenschappelijke kenmerk van deze zetmethoden is dat er geen papieren filter wordt gebruikt tijdens de bereiding van de koffie. Het HDL-cholesterol verandert niet door koffieconsumptie. De bloeddruk lijkt een negatief verband te hebben met koffie, d.w.z. de bloeddruk daalt met toenemend koffie gebruik. De regressiecoëfficiënten zijn echter klein en bereiken geen statistische significantie. De koffie-zetmethode beïnvloedt de resultaten van de bloeddruk niet. Een mogelijk probleem bij een meta-analyse van cross-sectionele onderzoekingen over koffie en serum cholesterol of bloeddruk is "publicatie bias", die hier leidt tot een overschatting van effecten. De resultaten van de beschikbare niet-gepubliceerde gegevens in de analyse geven echter geen steun aan het bestaan van dit type bias (hoofdstuk 3).

Voorts werd direct bewijs voor een cholesterol verhogend effect van gekookte koffie verkregen in een experimenteel onderzoek. Honderd en zeven jong-volwassenen

werden random verdeeld over drie interventiegroepen die vier tot zes koppen gekookte koffie per dag dronken, vier tot zes koppen gefilterde koffie, of helemaal geen koffie gedurende negen weken. Vergeleken met de verandering in de filter-koffie groep, nam het serum totaal cholesterolgehalte in de kook-koffie groep toe met 0,48 mmol/l (95% betrouwbaarheidsgrenzen 0,13, 0,83) en het LDL-cholesterol met 0,39 mmol/l (-0,04, 0,82). Na 9 weken kook-koffie bedroeg de netto toename in het serum totaal cholesterol 10% van het beginniveau. De concentraties van HDL-cholesterol en apolipoproteïnen werden niet beinvloed door gekookte of gefilterde koffie. Er was geen significant verschil tussen de verandering in serum totaal cholesterol of LDL-cholesterol van de filter-koffie groep en de groep die geen koffie dronk (hoofdstuk 4).

In hetzelfde experiment werd het effect van koffie op de bloeddruk en de hartfrequentie bestudeerd. Bovendien kon de invloed van twee koffiezetmethoden op deze variabelen nagegaan worden. Vergeleken met de filter-koffie groep nam zowel de systolische als de diastolische bloeddruk af in de groep die geen koffie dronk. Alleen de afname in de systolische bloeddruk na 9 weken was statistisch significant, -6,1 mm Hg (-10,8, -1,4). Na correctie voor het beginniveau van de systolische bloeddruk en voor verandering van het lichaamsgewicht tijdens het onderzoek, was deze afname -3,4 mm Hg (-7,1, 0,3). Het verloop van de systolische en diastolische bloeddruk was opmerkelijk gelijk in de groepen die gefilterde koffie en gekookte koffie dronken. Na negen weken kook-koffie waren de gemiddelde veranderingen in de systolische en diastolische bloeddruk 0,4 mm Hg (-3,7, 4,5) en -0,1 mm Hg (-3,4, 3,2) vergeleken met de filtergroep. De hartfrequentie vertoonde een lichte, niet-significante daling in de abstinentie-groep. Deze bevindingen suggereren dat abstinentie van koffie gedurende enkele weken kan leiden tot een lichte daling van de bloeddruk bij jonge, normotensieve volwassenen. Onze waarnemingen op het gebied van de bloeddruk vereisen voorzichtige interpretatie, in het bijzonder omdat de deelnemers niet blind waren voor hun interventie-status. Bovendien kunnen we de resultaten niet vergelijken met voorgaande studies naar het effect van abstinentie van koffie op de bloeddruk gedurende een periode van deze lengte (hoofdstuk 5).

Koffie en hemostase is het volgende onderwerp dat bestudeerd werd. Een overzicht van de momenteel beschikbare bewijsvoering vóór of tegen een verband tussen koffie en hemostatische variabelen toont uiteenlopende bevindingen. Als de resultaten betreffende fibrinogeen concentraties en in-vivo activering van bloedplaatjes, gemeten via het plasma beta-thromboglobuline, al iets uitwijzen, is het een ongunstig effect van koffie, d.w.z. koffie zou een thrombotische neiging kunnen vergroten. In het eerder beschreven experimentele onderzoek bestudeerden we de invloed van koffie consumptie op de concentratie van fibrinogeen, stollingsfactor VII activiteit, factor VIII antigeen, proteïne C en S. Deze hemostatische factoren werden niet beinvloed door het drinken van gekookte koffie of abstinentie van koffie, vergeleken met het gebruik van

gefilterde koffie gedurende 9 weken. Evenmin waren er veranderingen door abstinentie van caffeïne (hoofdstuk 6).

De effecten van caffeïne op de bloeddruk en serum lipiden werden bestudeerd in een dubbel blind, gerandomiseerd onderzoek met twee parallelle groepen bij 69 jonge, gezonde mensen. Na een drie-weekse inloopperiode werden de deelnemers willekeurig verdeeld in twee groepen die òf 4-6 koppen gefilterde, gedecaffeïniseerde koffie per dag dronken en een zelfde aantal tabletten met 75 mg caffeïne, òf 4-6 koppen gefilterde, gedecaffeïniseerde koffie per dag met een zelfde aantal placebo tabletten, gedurende 9 weken. In beide groepen was caffeïne-inname uit andere bronnen niet toegestaan. Abstinentie van caffeïne gedurende 9 weken had geen effect op de serum lipiden of de bloeddruk. De deelnemers volgden de voorschriften goed op, zoals bleek uit de caffeïneconcentraties in serum en speeksel. Onvoldoende therapietrouw kan de negatieve resultaten dus niet verklaren (hoofdstuk 7).

In hoofdstuk 8 worden de bevindingen uit de voorgaande hoofdstukken besproken. Omdat er weinig bewijs is voor een negatieve invloed van koffie op hart- en vaatziekten, zullen gunstige en ongunstige effecten van koffie op de cardiovasculaire risico indicatoren òf klein zijn, òf ruwweg in evenwicht. Aanbevelingen voor preventieve activiteiten op het gebied van koffie omvatten het gebruik van gefilterde koffie stimuleren in die gebieden in Scandinavië waar veel gekookte koffie wordt gedronken. Verder zouden personen met een verhoogde bloeddruk geadviseerd kunnen worden geen koffie te drinken gedurende een 8-weekse proefperiode. Mensen met een hoog serum cholesterolgehalte die gekookte koffie, espresso koffie of Turkse koffie drinken, kunnen mogelijk profiteren van een omschakeling naar gefilterde koffie. Meer onderzoekingen op het gebied van koffie en hart- en vaatziekten zijn gewenst, met vooral aandacht voor de lange-termijn effecten van koffie op de bloeddruk en op hemostatische factoren. Bovendien ontbreekt het experimentele bewijs voor een cholesterol verhogend effect van espresso koffie en Turkse koffie en tenslotte dient het onderliggende mechanisme van het LDL-cholesterol verhogende effect van gekookte koffie verder onderzocht te worden.

EPILOOG

Op de laatste pagina's van dit boek wil ik graag iedereen die heeft bijgedragen aan het koffie-onderzoek bedanken.

Lieve Jeroen, ik begin gewoon met jou, want hoewel alle anderen óók essentieel waren, was jij dat toch wel het allermeest!

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Verder overigens niets dan lof voor de deelnemers die echt geweldig waren. Vrijwel zonder uitzondering deden zij enthousiast, trouw, fanatiek of braaf aan het onderzoek mee, 43 van hen zelfs aan beide experimenten!

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ABOUT THE AUTHOR

Annette A.A. Bak was born on July 30th, 1961, in Dordrecht. She passed her secondary school exam (Atheneum B) in 1979 at the "Christelijk Lyceum" in Dordrecht. From 1979 to 1986 she studied Medicine at the Erasmus University in Rotterdam. As a medical student she had several jobs in the Sophia Children's Hospital in Rotterdam, varying from nursing to research at the Department of Endocrinology (head: Dr. S.L.S. Drop) and the Research Laboratory (head: Prof. Dr. H.J. Degenhart). In November 1986 she obtained her medical degree and started with a temporary clinical appointment in the same hospital. Since October 1987, she is research fellow at the Erasmus University Rotterdam at the Department of Epidemiology and Biostatistics (head: Prof. Dr. H.A. Valkenburg, in 1988 succeeded by Prof. Dr. A. Hofman). After completing a literature study on cardiovascular risk indicators in children, the "coffee-project" started in February 1988 with the two experimental studies, which form the heart of this thesis. The investigations were performed under supervision of Dr. D.E. Grobbee. Since January 1990, she is coordinator of the Rotterdam Cardiovascular Risk Intervention trial (ROCARI).