

## Chronic consumers of boiled coffee have elevated serum levels of lipoprotein(a)

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**Abstract.** Urgert R, Weusten-van der Wouw MPME, Hovenier R, Lund-Larsen PG, Katan MB (Department of Human Nutrition, Agricultural University, Wageningen, The Netherlands). Chronic consumers of boiled coffee have elevated serum levels of lipoprotein(a). *J Intern Med* 1996; **240**: 367–71.

**Objectives.** Lipoprotein(a) consists of an LDL-particle attached to apolipoprotein(a), which is made by the liver. Diterpenes present in boiled coffee raise serum levels of LDL cholesterol and of the liver enzyme alanine aminotransferase in man. We investigated the association between intake of boiled coffee and serum levels of lipoprotein(a).

**Design, setting and subjects.** Healthy Norwegians 40–42 years of age, who habitually consumed five or more cups of boiled coffee per day ( $n = 150$ ) were compared with matched filter coffee consumers ( $n = 159$ ) in a cross-sectional study, as part of the Norwegian National Health Screening in 1992.

**Results.** The median lipoprotein(a) level was 13.0 mg dL<sup>-1</sup> (10th and 90th percentile: 2.5 and 75.0 mg dL<sup>-1</sup>, respectively) on boiled and 7.9 mg dL<sup>-1</sup> (10th and 90th percentile: 1.9 and 62.5 mg dL<sup>-1</sup>, respectively) on filter coffee ( $P = 0.048$ ). Means  $\pm$  SE were 25.8  $\pm$  2.4 mg dL<sup>-1</sup> and 19.6  $\pm$  2.0 mg dL<sup>-1</sup>, respectively ( $P = 0.04$ ). Although not statistically significant, subjects consuming nine or more cups of coffee per day had higher lipoprotein(a) levels than those drinking five to eight cups per day in both coffee groups.

**Conclusion.** Chronic consumers of unfiltered, boiled coffee have higher serum levels of lipoprotein(a) than filter coffee drinkers.

**Keywords:** boiled coffee, cross-sectional study, lipoprotein(a), Norwegians.

### Introduction

Lipoprotein(a) [Lp(a)] consists of a large glycoprotein, apolipoprotein(a), attached to an LDL molecule [1]. A high serum Lp(a) level is a risk factor for developing coronary atherosclerosis and other vascular diseases [1–5]. Lp(a) levels are largely under genetic control [6], and are unaffected by many factors that modulate LDL-cholesterol levels [1, 7], including diet [8]. An exception is formed by dietary *trans* fatty acids, which raise serum Lp(a) levels [9, 10].

Consumption of unfiltered, boiled coffee is associated with raised serum cholesterol levels in humans in both intervention trials [11–13] and epidemiological studies [14–17]. Recently, it was shown that the diterpenes cafestol and kahweol are

responsible for the hyperlipidaemic effects of boiled coffee [18, 19]. Scandinavian boiled coffee provides 3–4 mg of each diterpene per cup, whereas paper-filtered coffee contains less than 0.1 mg of each diterpene per cup [20].

The liver appears to be the target organ of coffee diterpenes, as cafestol and kahweol elevate the serum activity of alanine aminotransferase [19, 21] and reduce that of  $\gamma$ -glutamyltransferase [19, 22]. The liver is also the main source of apolipoprotein(a) [23], and the serum level of Lp(a) is largely determined by the rate of Lp(a) production [24]. Whether ingestion of coffee diterpenes with boiled coffee results in altered serum levels of Lp(a) is as yet unknown.

We compared serum Lp(a) levels in healthy

Norwegians consuming unfiltered boiled coffee with those in matched filter-coffee drinkers.

## Materials and methods

### Subjects

Subjects were recruited as part of the Norwegian National Health Screening in 1992, a population-based study in southern Norway [17]. Persons aged 40–42 years attending the screening were asked to fill out a questionnaire concerning their coffee usage. Those who reported drinking five or more cups of either boiled or filter coffee per day received an oral explanation about the purpose of our study. The questionnaire did not provide information on duration and history of coffee intake. Subjects were considered eligible if they were healthy, did not take any medication known to affect liver enzymes or serum lipids, and did not consume more than three alcohol-containing beverages per day. Those who were willing to participate gave their written informed consent. Prior approval for the study was obtained from the appropriate Committee on Human Ethics.

### Blood sampling and assays

Non-fasting blood was obtained by venepuncture, allowed to clot, and centrifuged. Sera were randomly coded, shipped to Wageningen, and stored at  $-80^{\circ}\text{C}$ . Lp(a) was assayed blindly within 12 months with an enzyme-linked immunosorbent assay (Immunozytm Lp(a), Immuno GMBH, Heidelberg, Germany). The lower limit of detection of Lp(a) was  $1\text{ mg dL}^{-1}$ , which according to the manufacturer is higher than the maximum cross-reactivity of the Lp(a)-antibodies with plasminogen and apolipoprotein B. For each subject, Lp(a) was analysed in duplicate in separate runs. The intra- and interassay coefficients of variation for a control pool (target  $14\text{--}21\text{ mg dL}^{-1}$ ) were 14.8 and 9.6%, respectively, over a period of 3 months. Serum lipids and serum activities of liver enzymes were assayed as described [12].

### Statistical analyses

Unpaired, two-sided *t*-tests were used to compare the means of serum variables between the two groups.

As the distributions of Lp(a) levels were positively skewed, we also compared the two groups with Mann–Whitney *U* tests.

## Results

Consumers of boiled ( $n = 150$ ) and filter coffee ( $n = 159$ ) were well matched with regard to sex, age, body mass index, use of medicines, smoking habits and alcohol consumption (Table 1). The use of boiled coffee was associated with higher serum levels of total cholesterol and lower serum activities of  $\gamma$ -glutamyltransferase, in agreement with earlier findings [14–17, 25].

The median level of Lp(a) was  $13.0\text{ mg dL}^{-1}$  (range  $0\text{--}130\text{ mg dL}^{-1}$ ) among consumers of boiled coffee and  $7.9\text{ mg dL}^{-1}$  (range  $0\text{--}144\text{ mg dL}^{-1}$ ) among consumers of filter coffee ( $P = 0.048$ ) (Fig. 1); means  $\pm$  SE were  $25.8 \pm 2.4\text{ mg dL}^{-1}$  and  $19.6 \pm 2.0\text{ mg dL}^{-1}$ , respectively ( $P = 0.047$ ).

The median Lp(a) levels were for men  $14.1\text{ mg dL}^{-1}$  for boiled and  $7.8\text{ mg dL}^{-1}$  for filter coffee, and for women  $10.7\text{ mg dL}^{-1}$  for boiled and  $8.3\text{ mg dL}^{-1}$  for filter coffee. The median Lp(a) level was  $13.6\text{ mg dL}^{-1}$  in the subjects drinking more than nine cups of boiled coffee per day, and  $11.7\text{ mg dL}^{-1}$  in those drinking less than nine cups. For filter coffee, the

**Table 1** Characteristics of 309 healthy Norwegians aged 40–42 years chronically drinking five or more cups of boiled or filter coffee per day<sup>a</sup>

	Filter coffee <i>n</i> = 159	Boiled coffee <i>n</i> = 150
Number of men/women	88/71	79/71
Age (years)	$41 \pm 1$	$41 \pm 1$
Body Mass Index ( $\text{kg m}^{-2}$ )	$25 \pm 3$	$25 \pm 4$
Women taking contraceptive steroids	2	2
Coffee consumption		
Five to eight cups per day	133	117
Nine or more cups per day	26	33
Smokers	89	87
Alcohol users	146	135
Medication users <sup>b</sup>	20	21
Total cholesterol ( $\text{mmol L}^{-1}$ )	$5.67 \pm 1.05$	$5.98 \pm 1.08^{\text{c}}$
Triglycerides ( $\text{mmol L}^{-1}$ )	$1.92 \pm 1.24$	$2.14 \pm 1.55$
Alanine aminotransferase ( $\text{U L}^{-1}$ )	$19 \pm 10$	$20 \pm 10$
Aspartate aminotransferase ( $\text{U L}^{-1}$ )	$22 \pm 7$	$22 \pm 7$
$\gamma$ -Glutamyltransferase ( $\text{U L}^{-1}$ )	$26 \pm 24$	$20 \pm 17^{\text{c}}$

<sup>a</sup> Values are numbers, or means  $\pm$  SD.

<sup>b</sup> Any medications used in the month previous to the screening. None of the subjects used medicines on a daily basis.

<sup>c</sup> Significantly different from filter coffee group:  $P < 0.05$ .

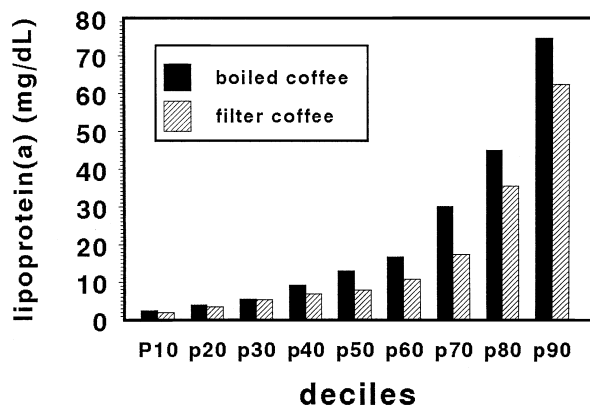


Fig. 1 Serum lipoprotein(a) levels among 309 healthy Norwegians aged 40–42 years, chronically consuming five or more cups of boiled ( $n = 150$ ) or filter coffee ( $n = 159$ ) per day. Values represent the serum Lp(a) level of the 15th, 30th, 45th etc. consumer of boiled coffee, and the 16th, 32nd, 48th, etc. consumer of filter coffee when subjects were ranked according to lipoprotein (a) level within coffee usage groups.

corresponding values were  $8.0 \text{ mg dL}^{-1}$ , and  $6.9 \text{ mg dL}^{-1}$ , respectively (all differences  $P > 0.05$ ).

## Discussion

We found that the median level of serum Lp(a) in Norwegians habitually consuming unfiltered, boiled coffee was significantly higher than in their peers drinking filter coffee. Our data also suggests that the elevation of Lp(a) may depend on the amount of coffee consumed, and that the effect of boiled coffee is stronger in men than in women. These results did not reach statistical significance, however, which may be attributable to reduced power due to limited sample sizes.

The insensitivity of Lp(a) levels to environmental influences [7, 8] renders it unlikely that our finding was biased. In addition, the two groups were well matched with respect to age, sex, body mass index, smoking habits, and alcohol use. The use of drugs or contraceptive pills during the previous month in the two groups was infrequent and similarly distributed (Table 1). Dietary factors that influence Lp(a) levels are limited to *trans* fatty acids [9, 10] and possibly fish oils [8]. It is unlikely, however, that consumers of boiled and filter coffee differed enough in their intake of fatty acids to explain the effect seen here.

The Lp(a) particle contains apolipoprotein(a) [apo(a)], which is remarkably polymorphic [26]. Serum Lp(a) levels correlate inversely with the molecular weight of the apo(a) isoform and the size

of the corresponding gene [27]. We have no information on apo(a) phenotype distribution of the two groups. Although all subjects were living in southern Norway, more people consuming boiled coffee may have descended from ancestors that originated from northern Norway—where consumption of boiled coffee is more common [17]. Therefore, confounding by genetic differences cannot be fully excluded.

The higher level of Lp(a) among boiled-coffee consumers was an unexpected finding. In two short-term controlled trials with a large number of Dutch volunteers, coffee oil rich in diterpenes significantly lowered serum Lp(a) (Urgert *et al.*, 1996, unpublished observations). In the present study, serum levels of cholesterol were higher in the boiled-coffee group, and  $\gamma$ -glutamyltransferase activities were lower, which is consistent with both epidemiological [14–17, 25] and experimental studies [19]. These variables, as well as the Lp(a) concentration of each subject were determined blindly in the same set of serum aliquots. In addition, all sera of subjects consuming boiled or filter coffee had been stored under the same conditions, and analysed for Lp(a) in random order, except for one analysis run which after breaking of the code turned out to have comprised sera of 36 boiled-coffee drinkers and no filter-coffee drinkers, and one run that had contained sera of 42 filter-coffee drinkers and only nine boiled-coffee drinkers. However, the median level of Lp(a) in the first run was  $11.5 \text{ mg dL}^{-1}$  ( $n = 36$ ) against  $13.0 \text{ mg dL}^{-1}$  for the entire boiled-coffee group ( $n = 150$ ), and in the second run  $8.6 \text{ mg dL}^{-1}$  ( $n = 42$ ) against  $7.9 \text{ mg dL}^{-1}$  for the entire filter-coffee group ( $n = 159$ ). Therefore, if any systematic bias was introduced at this stage, it should have weakened the association between coffee type and Lp(a) levels instead of having strengthened it.

Therefore, there seems to be a discrepancy between effects on Lp(a) levels with short-term and chronic intake of coffee diterpenes. In short-term trials, it was observed that coffee diterpenes increased serum activities of alanine aminotransferase [19, 22]. This suggests that the liver is the target organ for coffee diterpenes. Disturbed hepatocyte integrity may reduce circulating Lp(a) levels, as seen in patients with biliary cirrhosis or other liver disease [28, 29]. In the present study, however, the boiled-coffee drinkers had no elevated serum activities of alanine aminotransferase ([19], see also Table 1). It is therefore

possible that the liver adapts to the effects of cafestol and kahweol with chronic consumption of boiled coffee. Therefore, intervention trials of longer duration are needed to reveal the time course of the effect of coffee diterpenes on serum Lp(a).

Tverdal *et al.* [30] found an effect of boiled-coffee consumption on mortality from coronary heart disease, over and above its effect on serum cholesterol. Higher serum levels of Lp(a) in those drinking boiled coffee, as observed in this study, might partly account for this additional risk.

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