

Italian Style Brewed Coffee: Effect on Serum Cholesterol in Young Men

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D'Amicis A (National Institute of Nutrition, via Ardeatina 546, 00178 Rome, Italy), Scaccini C, Tomassi G, Anaclerio M, Stornelli R and Bernini A. Italian style brewed coffee: effect on serum cholesterol in young men. *International Journal of Epidemiology* 1996; 25: 513–520.

Background. Increases in blood lipids have been observed in humans when coffee is brewed by the boiling method. The purpose of this study was to evaluate if giving up Italian coffee might reduce blood cholesterol levels.

Methods. Eighty-four normolipidaemic young adult males, after a 3-week baseline (BL), were randomly assigned to three different regimens of coffee consumption: espresso (E), mocha (M), and no coffee, but tea (T). The average coffee consumption during intervention (I) was 3.1 ± 1.2 and 2.8 ± 1.1 cups per day for espresso and mocha group respectively (espresso: 25–35 ml/cup; mocha: 40–50 ml/cup). Total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were measured eight times during the study. Dietary pattern, alcohol consumption, smoking habits, drug use, and anthropometric data were also recorded.

Results. The changes observed in serum cholesterol concentration between baseline and intervention were not statistically different in all groups. The changes were 0.0 mmol/l (T), +0.10 mmol/l (E) and +0.05 mmol/l (M) for total serum cholesterol; 0 mmol/l (T), -0.02 mmol/l (E) and -0.03 mmol/l (M) for HDL-C; -0.13 mmol/l (T), +0.02 mmol/l (E) and -0.05 mmol/l (M) for LDL-C. Serum triglycerides showed a significant increase during intervention ($P < 0.01$ by ANOVA) in all groups with a change of 0.18 mmol/l, 0.18 mmol/l and 0.22 mmol/l, for tea, espresso and mocha group respectively.

Conclusion. The results indicate that coffee brewed in the Italian way does not alter blood levels of total cholesterol, HDL-cholesterol and LDL-cholesterol, since no significant differences were observed in these blood parameters after a 6-week break from coffee consumption.

Keywords: espresso and mocha coffee, brewing methods, serum cholesterol, intervention study

The first experiment on coffee consumption and blood cholesterol in hypercholesterolaemic subjects was described by Egede-Nissen in 1970; although the observation was not adequately controlled the author suggested that subjects abstaining from coffee could reduce their total serum cholesterol levels by 17%.¹

The strongest positive association between coffee consumption and serum cholesterol is reported in the Tromsø Study, from Scandinavia,² where coffee is often boiled.

The hypothesis that the brewing method of coffee might have an effect on this association has been discussed by Bak³ in an extensive meta-analysis of cross-sectional studies. It has been observed that populations who drank boiled coffee show an increase in total cholesterol concentration fourfold higher than populations drinking filtered coffee. Recent experimental studies

confirm Bak's observation, excluding any relationship between filtered coffee consumption and serum cholesterol level.^{4–10} An Italian experimental study on 'espresso' coffee consumption and serum cholesterol, reports no association between the two variables,¹¹ while another Italian population study shows that men drinking five cups of coffee or more per day had a significantly higher level of serum cholesterol than any other category of coffee consumption.¹²

Other studies indicate a positive association between coffee consumption and blood cholesterol; however, the results are confounded by other factors. Fried *et al.*¹³ report a significant positive association between consumption of filtered normal coffee and serum lipids. They observe an increase of total cholesterol, due both to LDL-cholesterol and HDL-cholesterol, in the group consuming 720 ml per day of filtered normal coffee, while no increase is observed in the group drinking 720 ml of filtered decaffeinated coffee, and in the group drinking 320 ml of filtered normal coffee. Superko *et al.*¹⁴ found an increase of LDL-cholesterol and apolipoprotein B in subjects consuming decaffeinated filtered coffee, without any increase among drinkers of

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normal coffee. The authors hypothesize that the difference between caffeinated and decaffeinated coffee could be due to a factor present in the *Robusta* coffee species, usually used for the preparation of decaffeinated coffee, but not present in the *Arabica* species. In the Israeli Cordis study, the consumption of 'mud' coffee (a type of Turkish coffee which is boiled) and of instant coffee (soluble), results in an increase of serum total cholesterol and LDL-cholesterol.¹⁵ The study was not controlled for milk and milk products consumption, but the authors observe that 'mud' coffee is normally drunk without milk and instant coffee is usually with milk.

According to the studies in which coffee brewing method seems to influence serum cholesterol levels, and considering the hypothesis of Zoch *et al.*¹⁶ on the lipid-rich fraction as a factor responsible for the serum cholesterol increase, we carried out the present study to evaluate whether the Italian way of preparing coffee (espresso or mocha) might influence blood cholesterol levels in men. A group of habitual coffee drinkers were asked to abstain from coffee consumption for 6 weeks, replacing it with tea. Two groups of subjects were used as controls: the first was asked to drink only mocha coffee, the second only espresso coffee. If the Italian methods of brewing coffee were associated with high blood cholesterol (as observed with boiled coffee), we would expect a reduction of blood cholesterol in the group drinking tea.

METHODS

Study Design

The experiment was designed as a randomized trial, and carried out in two phases: 1) a 3-week baseline, during which subjects consumed their normal amounts of coffee; 2) a 6-week intervention period, during which subjects were randomly assigned to three groups: the first consuming coffee brewed as 'espresso'; the second group consuming coffee brewed as 'mocha'; and the third consuming no coffee at all but only regular tea.

During the intervention phase no other limitation was imposed on the subjects, other than to maintain their normal lifestyle and dietary habits. Since the selected sample was composed of habitual coffee consumers, the tea group was considered as the experimental group. Tea contains caffeine but we assumed that caffeine had no effect on serum cholesterol, according to several observations.^{7,17}

Subjects

The sample size was established on the basis of the power of 80% and 95% confidence interval assuming that the test would detect a difference of about 0.2 mmol/l

(8 mg/dl) of serum cholesterol. We also took into consideration intra- and interindividual serum cholesterol variations. On this basis, the size of each group was established in about 30 subjects and blood samples were collected at least three times during each phase.

The subjects, all males, were selected from physicians recruited for one-year obligatory training as Officers in the Health Military Corps at the Army Medical School of Florence (Italy). These subjects were selected for two main reasons: first, as soldiers they are considered to be reasonably healthy, which minimizes the effects of other confounding factors; second, the medical cultural background and their professional ethic, together with their military position, made them more likely to comply with the study protocol. After a seminar, providing information on the study design and scientific background 93 volunteers were screened, 92 were included in the study according to exclusion criteria (not habitual coffee consumer, or suffered from hyperlipidaemia, hypertension, diabetes, or had a history of ischaemic disease). At the beginning of the study four subjects were excluded because of difficulties in drawing blood and four subjects were unable to ensure compliance. The final study sample was therefore composed of 84 subjects. Subjects gave their informed consent and the study protocol was accepted by the ethical committee of the Army Medical School.

During the study the subjects were under military training. They were confined to the school but could go out for about 4 hours every evening. The beginning of the study coincided with the beginning of military training.

Physical Measurements

Body weight and height were measured according to standard techniques and instrumentation. Blood pressure (systolic and diastolic) was measured by sphygmomanometer, according to the standard technique. Basic clinical examination and clinical history were collected for each subject, with particular attention paid to lipidaemic profile and family history of cardiovascular diseases.

A self-administered questionnaire (concerning smoking habits, alcohol and drugs use, and physical activity) was also given to the subjects.

Dietary Profile

On three different occasions a dietary history was taken from the subjects by a well trained dietitian, using an *ad hoc* constructed semi-quantitative food frequency questionnaire. This methodology does not allow the precise quantification of food intake, however, it allows ranking of intake levels and the comparison between groups. Food consumption was transformed into nutrient intake

by using Italian food composition tables.¹⁸ Coffee consumption and added milk were self-recorded daily (on a personal form), during the 6-week intervention period.

Talks and informal meetings reinforced compliance with the protocol. No specific tests have been used to control compliance. Some subjects belonging to the tea group had some discomfort when abstaining from coffee, referring to a mild headache and asthenia for a couple of days (however, they all complied with the agreed procedure).

Biochemical Measurements

Blood was drawn by vacutainer from subjects after a 12-hour fast at the beginning of the week. Blood samples were centrifuged within one hour and sera were stored at -20°C . Eight blood samples were collected for each subject (three in the baseline and five in the intervention period). In order to avoid the inter-day laboratory variation, all the samples of each subject were analysed on the same occasion (control sera were added to the set of analyses). Total serum cholesterol was analysed by the spectrophotometric enzymatic method (Peridochrom CHOD PAP kit test High Performance, Boehringer Mannheim).¹⁹ HDL-cholesterol was separated by precipitation with phosphotungstic acid.²⁰ Triglycerides were analysed by Triglycerides GPO-PAP High Performance enzymatic colorimetric test (Boehringer Mannheim).²¹ LDL-cholesterol was derived by Friedwald formula.²²

The laboratory was enclosed in a standardization circuit for total cholesterol, HDL-cholesterol and triglycerides. The intra-laboratory CV% was 0.94, 3.22 and 1.47, for total cholesterol, HDL-cholesterol and triglycerides, respectively.

The value of the baseline period represents the mean of the first three blood measurements (1st and 2nd baseline + 1st sample collected at the beginning of the first week of the intervention period). The intervention value is the mean of the five blood samples during the intervention period (2nd, ..., 6th).

Coffee Preparation

During the intervention period subjects consumed their breakfast, coffee or tea breaks, after-meal coffee and late coffee, in an official cafeteria which was always open. Subjects belonging to the two coffee groups were also asked to drink their usual number of cups of coffee.

Espresso coffee was prepared using a professional machine by extraction with water at a pressure of about 9 bar and at a temperature of $90\text{--}94^{\circ}\text{C}$ with the ground coffee contained in a metal filter. About 6 g of finely ground medium to dark roasted coffee were used per cup; the volume of one cup of espresso coffee was 20–35 ml.

TABLE 1 Description of the three study groups

	Tea	Espresso	Mocha
Number of subjects	28	28	28
Age (yr)	27.0 \pm 1.5	27.3 \pm 0.9	26.9 \pm 1.2
Weight (kg)	76.8 \pm 10.8	74.4 \pm 9.0	74.3 \pm 9.2
Height (cm)	176.9 \pm 7.5	176.2 \pm 6.7	177.0 \pm 6.5
BMI (kg/m^2)	24.5 \pm 2.6	24.0 \pm 2.3	23.9 \pm 2.7
SBP (mmHg)	113.8 \pm 6.5	117.9 \pm 8.0	117.3 \pm 6.3
DBP (mmHg)	69.8 \pm 6.3	76.3 \pm 8.1	74.8 \pm 6.9
Alcohol consumers (%)	73	68	82
Smokers (%)	23	39	39
Physical activity			
never (%)	8	4	14
minutes/day	21	21	18
Drug users (%)	12	14	4

Differences among the three experimental groups were not statistically significant by χ^2 test or one-factor ANOVA.

SBP: systolic blood pressure, DBP: diastolic blood pressure.

Values are expressed as per cent of the group or as mean \pm standard deviation (SD).

Mocha coffee was brewed in an aluminium mocha coffee pot, which consists of three parts. Water is placed in the lower portion and ground coffee is placed above this in a metal filter; the boiling water, under the slight pressure created by the steam, rises through the bed of ground coffee in the filter and passes through a tube into the upper portion of the coffee pot. The resulting coffee is poured into cups and drunk while still hot. Usually 6 g of finely ground (medium to dark roasted) coffee were used per cup; the volume of one cup was 40–50 ml.

All coffee used was a mixture of *Arabica* species, available on the Italian market. Tea was a brand of regular tea, prepared by infusion in hot water. All subjects were invited to add milk and sugar to coffee or tea according to their normal habit.

Statistics

All calculations were performed by using StatView package. The differences between and within groups were tested by one-factor and two-factor ANOVA, and by ANOVA for repeated measurements.

RESULTS

Compliance with the protocol was good and during the intervention only one subject of the tea group declared that he had consumed one cup of mocha coffee. Habitual alcohol use, smoking habits, physical activity and physical characteristics of subjects are shown in Table 1.

TABLE 2 Daily dietary pattern of the three experimental groups during baseline and intervention periods. Results are expressed as mean \pm SD

	Experimental group	Baseline	Intervention	<i>P</i> by repeated measures
No. of subjects	28	28	28	
Energy (MJ)	Tea	12.29 \pm 3.33	10.09 \pm 2.53	0.0001
	Espresso	11.47 \pm 3.05	9.23 \pm 2.20	0.0001
	Mocha	12.14 \pm 2.88	9.92 \pm 2.20	0.0005
	1-factor ANOVA	0.58	0.46	
	2-factor ANOVA			0.999
Coffee (cups)	Tea	2.5 \pm 1.9 (2–8)		
	Espresso	3.4 \pm 1.9 (2–6)	3.1 \pm 1.2 (2–5)	0.23
	Mocha	3.1 \pm 2.0 (2–4)	2.8 \pm 1.1 (2–3)	0.38
	1-factor ANOVA	0.31	0.0001	
	2-factor ANOVA			0.0001
SFA (% energy)	Tea	6.6 \pm 1.9	6.0 \pm 3.2	0.37
	Espresso	6.3 \pm 2.2	5.5 \pm 2.5	0.06
	Mocha	6.1 \pm 2.0	4.9 \pm 1.5	0.02
	1-factor ANOVA	0.67	0.28	
	2-factor ANOVA			0.75
PUFA (% energy)	Tea	2.2 \pm 1.0	1.3 \pm 0.6	0.0007
	Espresso	1.9 \pm 1.0	1.6 \pm 1.1	0.11
	Mocha	1.8 \pm 0.7	1.3 \pm 0.6	0.007
	1-factor ANOVA	0.35	0.34	
	2-factor ANOVA			0.20
MUFA (% energy)	Tea	7.2 \pm 1.6	6.2 \pm 3.6	0.17
	Espresso	7.3 \pm 2.7	6.6 \pm 2.4	0.19
	Mocha	6.7 \pm 2.0	5.6 \pm 2.3	0.07
	1-factor ANOVA	0.53	0.42	
	2-factor ANOVA			0.89

SFA = saturated fatty acids; PUFA = polyunsaturated fatty acids; MUFA = monounsaturated fatty acids.

No differences among experimental groups by one-factor ANOVA were observed. About 74% of subjects drank alcoholic beverages, mostly wine and beer (corresponding to about 40 g of alcohol per day; this consumption was steady during the study period). Thirty-four per cent of subjects smoked, on average 10–20 cigarettes per day and more than 90% took regular physical exercise for about 20 min/day besides regular military training.

Analgesics were the drugs mainly used, and no hypocholesterolaemic or antihypertensive substances (such as beta blockers) were used. The mocha group showed the highest percentage of alcohol drinkers, the lowest proportion of subjects performing physical activity, and lowest use of drugs. Body Mass Index (BMI) was generally slightly higher than normal in all groups²³ and blood pressure was within the normal range.

Body weight was also measured at the end of the intervention (data not shown), and a non-significant change of -1.2 kg on average was observed compared to the baseline.

The intakes of energy, coffee and fatty acids (expressed as % of energy) of the three study samples during baseline and intervention periods are reported in Table 2.

The mean coffee consumption was 3.4 ± 1.9 (range 2–6) cups/day and 3.1 ± 2.0 (range 2–4) during baseline and 3.1 ± 1.2 (range 2–5) cups/day and 2.8 ± 1.1 (range 2–4) cups/day during intervention for espresso and mocha group, respectively. As required by the study protocol, subjects belonging to the tea group did not consume coffee [2.5 ± 1.9 (range 2–8) cups/day during baseline] during the intervention. No significant differences among groups in the two phases of the study were observed by one-factor ANOVA for energy and fatty acid intake.

During intervention all the selected dietary parameters tended to decline, compared to the baseline period. The within-group differences between baseline and intervention (by repeated-measure ANOVA) were highly statistically significant for energy in the three groups ($P < 0.001$) and for PUFA in mocha and tea group ($P < 0.007$ and $P < 0.0007$). Nevertheless two-factor ANOVA did not reveal any significant interaction between treatment and time for dietary intake of energy and fatty acids. Dietary cholesterol (data not shown) was not significantly different among groups, ranging from 353 to 371 mg/day during baseline, and from 318 to 329 mg/day during intervention.

TABLE 3 Levels of serum lipids (mmol/l) during baseline and intervention. Results are expressed as mean \pm SD

	Experimental group	Baseline	Intervention	P by repeated measures
No. of subjects	28	28	28	
Total cholesterol	Tea	4.78 \pm 0.75	4.78 \pm 0.64	0.58
	Espresso	4.73 \pm 0.59	4.83 \pm 0.70	0.26
	Mocha	4.60 \pm 0.59	4.65 \pm 0.57	0.32
	1-factor ANOVA	0.43	0.57	
	2-factor ANOVA			0.39
HDL-cholesterol	Tea	1.22 \pm 0.18	1.22 \pm 0.18	0.96
	Espresso	1.24 \pm 0.23	1.22 \pm 0.23	0.53
	Mocha	1.19 \pm 0.16	1.16 \pm 0.16	0.57
	1-factor ANOVA	0.31	0.36	
	2-factor ANOVA			0.40
LDL-cholesterol	Tea	3.05 \pm 0.67	2.92 \pm 0.65	0.08
	Espresso	2.95 \pm 0.54	2.97 \pm 0.65	0.60
	Mocha	2.87 \pm 0.59	2.82 \pm 0.54	0.27
	1-factor ANOVA	0.58	0.65	
	2-factor ANOVA			0.17
Triglycerides	Tea	1.12 \pm 0.38	1.30 \pm 0.44	0.016
	Espresso	1.10 \pm 0.26	1.28 \pm 0.36	0.0003
	Mocha	1.12 \pm 0.29	1.34 \pm 0.37	0.007
	1-factor ANOVA	0.96	0.83	
	2-factor ANOVA			0.78

The changes in serum total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides during baseline and intervention are reported in Table 3.

As shown by one-factor ANOVA, the among-group differences for all parameters were not statistically significant either at baseline or after intervention. Also, the repeated measure analysis demonstrates no significant between-period differences in blood lipids for any groups, except in the case of triglycerides.

Figures 1 and 2 show the point by point values of total cholesterol and LDL-cholesterol during the study. Trends were fairly comparable, the values during the baseline and intervention periods being similar.

DISCUSSION

In our group of young and healthy subjects the restriction of the consumption of Italian coffee (tea group) did not result in changes in blood lipids and lipoproteins. Our findings on young men are in agreement with the studies in which coffee brewed by filtration did not affect the serum cholesterol.^{5,7-10} The boiling method requires 10 or more minutes of direct contact between ground coffee and boiling water. However, the methods based on filtration require briefer contact between coffee and hot water (20-100 seconds), in addition to the presence of the filter between the ground coffee and beverage.

As hypothesized by Zoch *et al.*¹⁵ the factor in coffee responsible for the increase of blood cholesterol should be sought in the lipid-rich soluble fraction of the boiled coffee. Yet, as reported by Van Dusseldorp *et al.*²⁴ this factor is retained by the paper filter. The authors in fact indicate that more than 80% of the lipid-soluble substances present in boiled coffee are removed by filtering.

Weusten-Van der Wouw *et al.*²⁵ reported that cafestol and kahweol (non-triglyceride lipids present in coffee) significantly raised serum cholesterol levels in three volunteers, confirming that these components are responsible for the cholesterol-raising effects of boiled coffee and coffee lipids. Most of the rise was associated with the LDL-cholesterol fraction.

The fact that in our study there was no significant difference in serum cholesterol profile among the three experimental groups indicates that the 'increasing cholesterol' factor does not pass through the 'obstacle' created by the coffee machine or by the bed of ground coffee. Also, the very short contact-time between coffee and hot water in the espresso and mocha preparations might contribute to reducing the efficiency of the extraction of the 'increasing cholesterol' factor in the beverage. Furthermore, at the level of coffee consumption in our groups (about 3 cups/day), the quantities of cafestol and kahweol ingested during the day would not be enough to affect serum cholesterol.

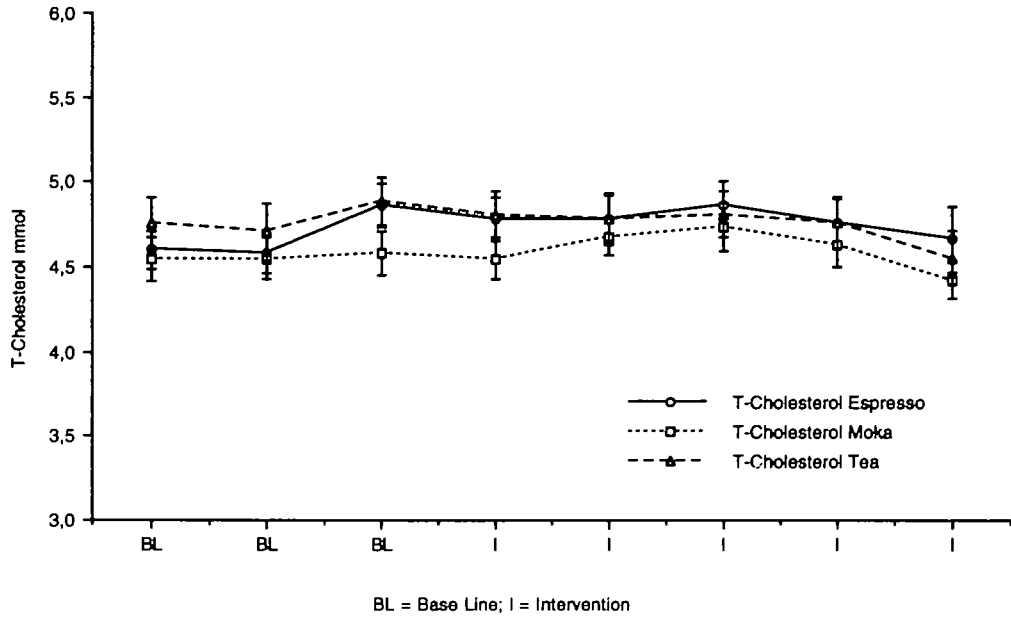


FIGURE 1 Point by point serum total cholesterol levels (mmol/l) in the three experimental groups (mean \pm SE)

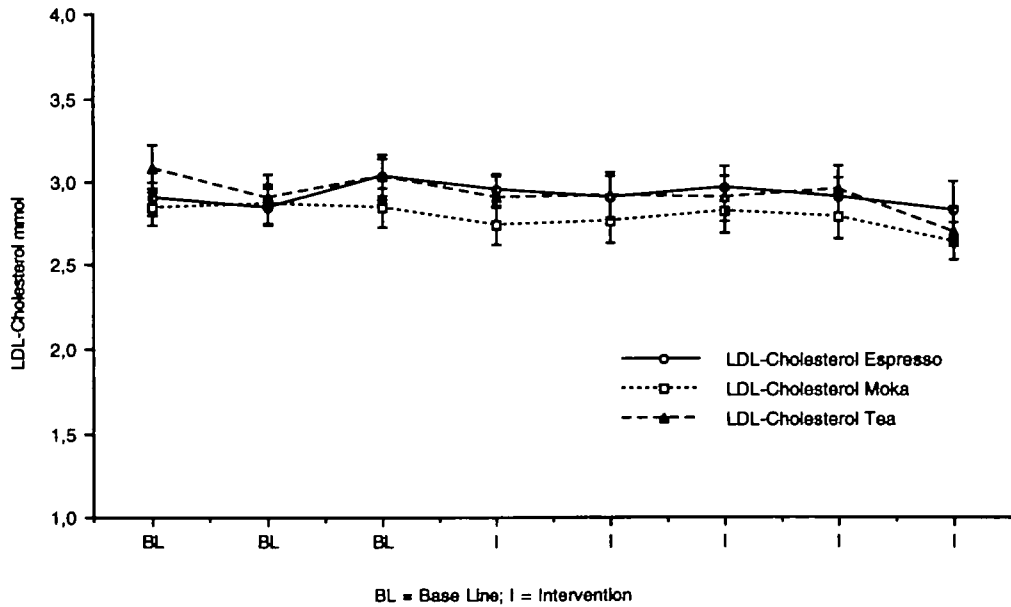


FIGURE 2 Point by point serum LDL-cholesterol levels (mmol/l) in the three experimental groups (mean \pm SE)

With regard to dietary pattern, subjects reduced their energy and fat intake during the intervention compared to the baseline period. Also, the reductions in body weight, although not statistically significant, are probably attributable to the military training and the new and homogeneous diet served by the canteen, compared to the preferred habitual food consumption at home. It could be hypothesized that the homogeneity of the military training tended to reduce the differences of the modifiable variables (anthropometric, nutritional and lifestyle) among subjects. However, in our study, the dietary change did not appear to affect total cholesterol, HDL-cholesterol, and LDL-cholesterol level. Only triglycerides increased during intervention in all three groups, but this particular result is not attributable to coffee consumption because the tea group showed the same increase.

Our results, observed on the same subjects during a random trial, contrast with those observed in an Italian cross-sectional study on 9000 subjects.²⁶ This study shows a positive association between coffee consumption and cholesterol levels, and this relationship seems to exist independently of smoking habits. In an American study, Hyden *et al.*²⁷ found a positive association between cholesterol and coffee only in smokers. All these contrasting results confirm the necessity for prudence in large cross-sectional studies in interpreting the association between cholesterol and coffee, which could be just a marker for a life-style which contains risks for hypercholesterolaemia (clinical status determined by several primary and secondary factors).

In conclusion, the results of our study indicate that coffee prepared by Italian methods (espresso and mocha) does not alter the cholesterolaemic and lipoproteinaemic profile in young men, reinforcing the hypothesis that the brewing method is a determinant factor in the association between coffee consumption and blood cholesterol increase.

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