



## RESEARCH ARTICLE

### CLINICAL ANALYSES IN HEALTHY ADULT INDIVIDUALS, CONSUMERS OF CAFFEINATED AND DECAFFEINATED COFFEE

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#### ABSTRACT

In the present study had as an objective to study the effects of the consumption of caffeinated and decaffeinated coffee on human health. Forty-eight healthy adult individuals were selected, who were appraised at the beginning and the the end of the research through clinical exams (glucose, lipidic profile, uric acid levels, complete blood count, thyroid hormones and routine urine) and its characteristics as a functional food. The individuals were separated in twelve groups by age, physical activity (active and sedentary) and the type of drink consumed (caffeinated and decaffeinated coffee) and guided as to how to prepare the drink and the daily amount to be consumed during the six months. After collection of all data, they were submitted to the statistical analysis through the Scott-Knott test and Student t test at the level of 5% of probability, using the SISVAR program. From the results, a significant reduction was observed in the total cholesterol levels HDLc and LDLc, uric acid, platelets and hematocrits and an increase in the leucocytes, independent of coffee type consumed, age group and physical activity. The consumption of caffeinated as well as decaffeinated coffee promoted improvements or did not interfere in the appraised factors, evidencing that the caffeine is not the component responsible for the alterations. Therefore, the results show that coffee presents characteristics that suggests its effect as a functional food, possibly attributed the other components of the drink.

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## INTRODUCTION

In regions under development, as their economies are industrialized, the chronic-degenerative diseases, such as diabetes mellitus, arterial hypertension and atherosclerosis, become prevalent, mainly because of the adoption of the occidentalized lifestyle, characterized by higher sedentariness indices, accompanied by diets rich in lipids and with little fiber (Nobre et al., 2005). The exams laboratoriais constitute an important tool to aid in the diagnosis, attendance and a lot of times, in the prevention of pathologies. There are various pieces of research that indicate coffee as beneficial for human health acting as a stimulator for several parts of the organism. Caffeine for being found in several foods and drinks, is one of the most consumed substances throughout the world.

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Besides caffeine, coffee contains a series of other substances, such as phenolic polymers, chlorogenic acids, lipids and terpenes that in association, possess different biological effects: antioxidant action, antimutagenic, antibiotic, anti hypercholesterolemic and antihypertensive (LIMA, 2014). Coffee is also a principal source of chlorogenic acid, a strong antioxidant that can improve sensitivity to insulin. It also helps to inhibit glucose absorption in the intestine. In another study with decaffeinated coffee it appeared to delayed the absorption of intestinal glucose and increase the concentrations of glucagon-like peptide-1, which is well known for its beneficial effects on insulin secretion and action. The researchers also noticed that their results could be due to the effects of other components, different from the caffeine found in coffee, which could also explain why coffee affects the uric acid levels, but not tea (CHOI et al., 2007). Subsequent works associated coffee with lower C peptide levels (an insulin level marker). The researchers suggest that as there is a strong relationship

between insulin resistance and high levels of uric acid, the decrease in the insulin levels associated with the consumption of coffee can lead to lower levels of uric acid (CHOI *et al.*, 2007). However, in agreement with Martín & Saturnino (2007) a significant relationship exists between the type of the coffee drink preparation and the increase of the serum cholesterol (SC) levels. This tendency was observed in volunteers that did not drink boiled and filtered coffee in comparison with those that drank filtered coffee. Being such, the coffee could also increase the levels of circulating triglycerides. Those responsible for these effects are two diterpenes; cafestol and kahweol present in the coffee bean, and 80% of both compounds are retained by the filter paper used in coffee preparation, and therefore, it substantially reduces the high effect of serum cholesterol (SC). Several mechanisms have been proposed to explain the effect of the cafestol and kahweol on serum cholesterol (SC) and triglycerides (TG): decrease of the LDL receptors in post-transcriptional mechanism regulators, lowering of bile acid synthesis, increase in the proteins activity of the serum responsible for the transfer of HDL SC to LDL and increase of the hepatic synthesis of VLDL. Along those lines, the present research had as an objective to study the effects of caffeinated and decaffeinated coffee consumption, on the health of active and sedentary adult volunteers, appraised through clinical exams (glucose, lipidic profile, uric acid levels, complete blood count, thyroid hormones and routine urine), and also, its probable characteristics as functional food and above all have the opportunity to show the people, through scientific studies, by reputable journals that coffee consumption has beneficial effects and demystify negative relationship with health.

## MATERIALS AND METHODS

### Experimental Design

After approval of the project by the of Human Research Ethics Commission of the Academical Center of Lavras, the present research was developed with 48 healthy, sedentary and active adult individuals of both sexes, 20 to 50 years of age from the city of Lavras - MG. Lectures were given to the the volunteers for the presentation of the research project, about the health benefits of coffee consumption. Later, a medical history form was filled out and soon afterwards the individuals were selected, pregnant women and the cardiopaths being excluded. The already registered were mobilized to undergo the biochemical exams, under the supervision of the biochemical pharmacist responsible. For accomplishment of the exams, each individual was instructed to abstain from caffeinated drinks for 72 hours and fast for a period of 12 hours. For the routine urine exam analyzes, the mid-stream of the first urine of the day was collected and for the blood tests, after the collection of the same, the glucose levels, serum lipids, urico acid, complete blood count and thyroid hormones were appraised. The final healthy volunteer grouping into groups classified as active and sedentary was made in according to the volunteers' responses, being considered active those who practiced physical activity three or more times a week and sedentary those who did not practice physical activity or up to twice a week. The results of the individuals' clinical exams were appraised by the cardiologist team member who judged each as capable or not capable, to participate in the research.

The blocks were separated as to age group and physical activity (active or sedentary) and consumption of filtered coffee, caffeinated or descaffeinated (3 to 4 cups of 100 mL coffee/day, for a period of six months). The sample used was of the *Coffea arábica* species, collected from a lot of export type coffee, peeled cherry grains and natural "soft" drink preparation process, obtaining from the roasting, a medium roast. The decaffeinated coffee was of a commercial brand, the whole product also being of a same lot.

The experiment was conducted according to a completely randomized design with four repetitions. The treatment portions were arranged in a factorial outline 3x2x2 (3 age groups, 2 physical activity situations and 2 types of coffee) and subdivided in time (beginning of the treatment and six months after), making a total of 48 volunteers. The age groups, that constituted the blocks were: young (20 to 29); intermediate (30 to 39) and superior (40 to 50 years). 24 volunteers started to ingest coffee with caffeine and the other 24 began to ingest decaffeinated coffee, the same were guided to consume from three to four coffee cups (100mL/day) for a period of six months, besides being alerted as to the consumption of other foods with caffeine, seeking to assure higher precision of the experiment. At the end of the study, the clinical analyses exams were carried out again, to verify possible variations in the parameters studied. After collection of all data, they were submitted to the statistical analysis through the Scott-Knott test and Student t test at the level of 5% of probability, using the SISVAR program.

## RESULTS AND DISCUSSION

The individuals presented normal values in the clinical laboratory exams in agreement with the references, being one of the parameters used to select the healthy individuals.

### Glucose

There was not significant difference ( $P>0.05$ ), in the glucose level for any of the factors (age group, (physical activity level and stage). The average value found was of 90.23 mg/dl, which is considered normal according to the reference values used, according to (WALTERS *et al.*, 1998). Such behavior leaves clear the non interference of the coffee in the levels of blood glucose. In relation to the results of the glucose levels, they are in agreement with the results obtained by Petrie *et al.* (2004), who also verified that the ingestion of coffee or isolated caffeine does not induce significant changes in the levels of blood glucose or insulin concentrations. However, those results do not corroborate with the results of Pizziol *et al.* (1998) who, working with 30 healthy individuals, non smokers, in the age group from 26 to 32, verified that the caffeine caused intolerance to the glucose by inducing an increase of the glycemic levels that is independent of insulin. Van Dam (2006) also relates that the effect of coffee consumption on the glucose metabolism is not similar to that of isolated caffeine, because the caffeine has the effect of increasing the intolerance to the glucose, and the coffee does not have that effect. Actually, the caffeine consumption (substance) has the effect of favoring glucose intolerance. However, the regular and chronic consumption of coffee, especially the decaffeinated, has an effect of improving the

tolerance to glucose and, consequently, to protect the habitual consumers against the diabetes type 2 (BATTRAM, 2006). Johnston *et al.* (2003) in a crossover study, working with nine healthy individuals, verified that the chlorogenic acids and the caffeine present antagonistic effects on the glucose transport, reduce the glucose intestinal absorption rate, which can lead to weight loss in the human diet. They also verified that there were higher plasma glucose concentrations and insulin as to the consumption of caffeinated coffee in relation to the decaffeinated coffee.

### Serum lipids

#### Total cholesterol

In Table 1, the average total cholesterol values are presented, according to age group (20 to 29; 30 to 39; 40 to 50 years), type of coffee (caffeinated and decaffeinated) and stage (before and after the supply of the different types of coffee for six months) for the individuals evaluated in the research. For the results of Table 1, regarding the average total cholesterol values, according to age group and type of coffee consumed during the experiment for the individuals evaluated in the research, it is observed that there was significant difference ( $P < 0.01$ ) for the youngest individuals (20-29 years), who consumed caffeinated coffee, which presented a lower total cholesterol value than the individuals in the of intermediate (30-39 years) and superior (40 to 50 years) age range, at the beginning of the research. That situation repeated after the six months of consumption. It is also observed that just the individuals of the intermediate age group presented reduction of the total cholesterol level after the six months of caffeinated coffee consumption. However, for those that consumed the decaffeinated drink, it was observed that the highest total cholesterol values in the beginning of the research were verified in the intermediate age group individuals. After the six months of decaffeinated coffee ingestion, it was observed that the individuals of the youngest and superior age groups presented a significant reduction ( $P < 0.05$ ), in the levels of total cholesterol. Thus, for the total cholesterol exams carried out before and after the treatments with coffees (caffeinated and decaffeinated), in other words, after six months of consumption, there was an average reduction of 13.5mg/dL in the levels of the total cholesterol in the evaluated individuals. That indicates that the consumption of filtered coffee, caffeinated or decaffeinated, contributes to the decrease of the total cholesterol and it allows to conclude that the caffeine was not the substance responsible for that behavior. In the values used as reference, it is observed that the general average (182.5 mg/dL) obtained of the individuals was inside the range considered borderline (170 to 199 mg/dL) (SANTOS, 2001).

**Table 1. Average total cholesterol values according to age range, coffee type consumed in the six months of the experiment, stage of measurement of the individuals**

Age range <sup>1</sup>	Type of coffee <sup>2</sup>			
	Caffeinated		Decaffeinated	
	Before	After	Before	After
20 to 29 anos	151.0 bA	145.6 bA	183.3 bA	163.1 aB
30 to 39 anos	214.1 aA	191.6 aB	202.5 aA	194.3 bA
40 to 50 anos	190.1 aA	188.8 aA	194.0 aA	171.4 aB
Standard Error	12.5			

Averages followed by the same small letter in the column and capital on the line for each type of coffee do not differ among themselves by the Scott-Knott test (1), and the Student t test (2), with a nominal level of significance of 5%.

#### HDLc

For the variable HDLc there was significant difference ( $P < 0.01$ ) for the stage factor. It is verified, in Table 2, that after the six months of use of different types of coffee (caffeinated and decaffeinated), the average of the levels of HDL cholesterol reduced from 50.85 mg/dl to 46.40 mg/dl. However, the average value was 48.65 mg/dl, which is considered normal in according to the reference values (SANTOS, 2001).

**Table 2. Average HDLc values, according to age range, physical activity levels, type of coffee consumed in the six months of the experiment, stage at which the measurements were taken in the individuals**

Stage	HDLc (mg/dL)	Standard error
Before	50.85 a	0.81
After	46.40 b	

Averages followed by the letter in the column for each factor do not differ among themselves by the Student t test, with a nominal level of significance of 5%.

#### LDLc

In Table 3, the average LDLc values are presented, according to the age group, level of physical activity and type of coffee consumed in the six months of the experiment. It appears that for the example of the total cholesterol, the individuals of the superior age group presented values significantly ( $P < 0.01$ ) higher for the fraction LDL in relation to the individuals of the inferior age group. The youngest individuals (20-29 years), presented a lower value of LDL cholesterol (92.20 mg/dl) in relation to the individuals in the intermediate (30-39 years) and superior (40 to 50 years) age groups, who presented 122.19 mg/dl and 116.41 mg/dl on average, respectively. The average value found for LDL cholesterol was 110.26 mg/dl, which is considered desirable (100 to 129 mg/dl) (SANTOS, 2001).

**Table 3. Average values of LDL cholesterol according to age range, physical activity levels, type of coffee consumed in the six months of the experiment, stage at which the measurements were taken in the individuals**

Age Range (years) <sup>1</sup>	LDL cholesterol (mg/dL)	Standard error
20 to 29	92.20 b	
30 to 39	122.19 a	6.72
40 to 50	116.41 a	
Stage <sup>2</sup>		
Before	114.73 a	2.28
After	105.80 b	

Averages followed by the same letter in the column for each factor do not differ among themselves by the Scott-Knott test (1), and the Student t test (2), with a nominal level of significance of 5%.

#### VLDLc

There was not significant difference ( $P > 0.05$ ), in the VLDLc levels for any of the factors evaluated; age group, level of

physical activity and stage (before and after the six months of diet with the coffee drink) for the individuals evaluated in the research.

### Triglycerides

There was not significant difference ( $P > 0.05$ ), in the triglyceride levels for any of the factors evaluated; age group, level of physical activity and stage (before and after the six months of diet with the drink coffee) for the individuals evaluated in the research. That indicates that the consumption of coffee did not influence in that variable. For the results obtained in the present work, in relation to the lipid profile, it was observed that the VLDL triglyceride and cholesterol levels maintained stable. There was significant reduction in the total cholesterol levels, HDL cholesterol and LDL cholesterol, independent of the type of coffee consumed after the six month diet. That shows, therefore, that it was not the caffeine that was the responsible element for that reduction, indicating that other components of the coffee exist, promoting such effect. Those results corroborate with those obtained by Natella *et al.* (2007), who when working with 10 healthy individuals and the ingestion of 200 ml of coffee, verified that the isolated caffeine did not present any antioxidant effect on the total cholesterol. There is evidence that the phenols can reduce the level of LDL. In their works, they also verified that the use of the coffee filtered drink did not alter the LDL levels, they also relate that the consumption of coffee increases the antioxidant capacity of the plasma, the metabolites of the coffee phenolic acids being responsible for that action. Also Sotillo and Hadley (2002), working with mice, verified that the chlorogenic acids were capable to significantly reduce the cholesterol and triglyceride levels in the blood. According to Bonita *et al.* (2007), the polyphenols are components present in the filtered as well as the non-filtered coffee drink and they have potential cardiovascular benefits, through antioxidant mechanisms related to the oxidation of LDL (once oxidized, LDL loses the capacity to transport the cholesterol that is deposited inside the arteries leading to obstruction), as well as in the reduction of blood pressure.

However, their benefits are less obvious when consuming the unfiltered drink. According to Greenberg *et al.* (2006), several researchers found association between the consumption of coffee and the elevation of serum lipids. The studies showed that the coffee that was consumed in that period was merely boiled and not filtered, which elevated the serum lipid concentrations. However, the type of coffee more consumed in Brazil and in the United States is the filtered coffee, which does not affect the serum lipid concentrations. Urgert *et al.* (1997) they relate that the alteration of cholesterol related to the coffee consumption is related to the form in the which the drink is prepared. According to those same authors, the non-filtered coffee drinks were capable to increase by 10% the level of total cholesterol with a dosage of five 200 ml cups per day after three months of consumption. Also Bonita *et al.* (2007) verified that just the high consumption ( $> 6$  cups/Day) of boiled, non-filtered coffee is harmful to the heart, as a result of the dose related to an increase of total cholesterol and LDL cholesterol, because of the diterpene oils, while in the individuals that drank the filtered coffee there was a reduction. According to those same authors, individuals that drank the

non-filtered coffee drink had 65% higher values in relation to those who consumed the filtered drink. Yoshihiro *et al.* (1999), evaluating the effect of coffee on the levels of serum cholesterol and lipoproteins in 4,587 Japanese, in the age group from 48 to 56 years, verified that the HDL level was not altered, LDL was increased and there was a triglyceride reduction on a diet of instant coffee. The same was observed by Onuegbu and Agbedana (2001), evaluating the effect of the coffee (Nescafe), on the lipids and lipoproteins in healthy individuals, verified that there was an increase of the concentration of total cholesterol and LDL cholesterol, however; for HDL and triglycerides there were no significant differences. Comparing the results of the present research with those found in the literature, it is verified that the preparation method used (filtered coffee) and the consumption dose (4 cups/day), considered normal for the tested population, did not lead to elevation of the total cholesterol and of the fractions considered as risk variables for cardiovascular diseases and even promoted the reduction in some of them, independent of the drink consumed. However, in other research, with the preparation of the non-filtered coffee and in doses superior to 6 cups/day, some negative effects were observed in those factors that nullify the advantages of the coffee use as a supplier of a large variety of beneficial bioactive principles. Therefore, results the obtained indicated that the levels of total cholesterol, LDL and HDL cholesterol reduced after the consumption of coffee, while the triglyceride levels and VLDL cholesterol were not altered. A solid relationship was not observed between physical activity and cholesterol levels (total and fractions) and triglycerides.

### Uric acid

It is observed in Table 4, in relation to the stage factor, that there was significance ( $P < 0.01$ ) in the levels of uric acid that was reduced from 4.43 mg/dl to 4.10 mg/dl, after six months of the coffee drink consumption. The average value found was 4.27 mg/dl, which is considered normal, because in according to the values used as a reference they are 1.5 to 6.0 mg/dl, for women and 2.5 to 7.0 mg/dl, for men (PARDINI, 2007; WALTERS *et al.*, 1998). The reduction of the uric acid is beneficial mainly for individuals with a propensity to develop diseases associated to the accumulation of uric acid.

**Table 4. Average uric acid values (mg/dL) according to the stage factor before and after six months of the experiment**

Stage	Uric acid (mg/dL)	Standard error
Before	4.43 a	
After	4.10 b	0.08

Averages followed by the same letter do not differ among themselves by the Student t test, with a nominal level of significance of 5%.

However, in relation to the uric acid levels, it can be concluded that the levels reduced with the consumption of caffeinated and decaffeinated coffee. Those results are in agreement with those obtained by Choi & Curhan (2007), who, working with 2,240 Japanese men, also verified that with the consumption of coffee, it was possible to reduce the levels of uric acid, but they did not attribute that effect to the caffeine, because the

**Table 5. Average values EC (units/mm<sup>3</sup>), HM (g/dl), HT (%), LF (mil/mm<sup>3</sup>), LC (mil/mm<sup>3</sup>) and PT (mil/mm<sup>3</sup>), according to age range, physical activity, type of coffee consumed before and after the six months of the experiment for the individuals evaluated in the research**

Factors evaluated	EC	HM	HT	LF	LC	PT
Age Range (years) <sup>1</sup>	SE*0,9	SE 0,37	SE 0,10	SE 2,52	SE 0,31	SE 12,7
20 to29	5.6 a	14.57 a	42.56 a	36.16a	6.76a	242.84a
30 to 39	4.8 a	14.32 a	41.97 a	38.68a	6.11a	259.63a
40 to 50	4.1 a	14.29 a	41.93 a	34.54a	6.63a	246.72a
Physical Activity <sup>2</sup>	SE 0,6	SE 0,30	SE 0,81	SE 1,45	SE 0,27	SE10,59
Active	4.90 a	14.86 a	42.31 a	37.55a	5.78b	229.27b
Sedentary	4.73 a	14.30 a	42.00 a	35.67a	7.21a	270.19a
Type of coffee <sup>2</sup>	SE 0,16	SE 0,30	SE 0,81	SE 1,45	SE 0,27	SE10,59
Caffeinated	4.84 a	14.59 a	42.79 a	36.98a	6.17b	245.50a
Decaffeinated	4.80 a	14.19 a	41.52 a	36.24a	6.83a	253.96a
Stage <sup>2</sup>	SE 0,14	SE 0,09	SE 0,25	SE 0,74	SE 0,11	SE 3,13
Before	4.94 a	14.42 a	42.73 a	36.62 a	6.28b	261.77a
After	4.70 a	14.36 a	41.58 b	36.61 a	6.72a	237.69b

Averages followed by the same small letter in the column do not differ among themselves by the Scott-Knott test (1), and the Student t test (2), with a nominal level of significance of 5%. \* Standard error

**Table 6. Average values of urine density (UD), thyroid hormones: Ultra sensitive TSH (uUI/mL) and free T4 (ng/dL), according to age range, physical activity, type of coffee consumed before and after the six months of the experiment**

Factors evaluated	UD	TSH	Free T4
Age Range (years) <sup>1</sup>	SE* 0,80	SE 2,36	SE 0,05
20 to29	1021.13 a	2.12 a	1.37 a
30 to 39	1019.00 a	1.61 b	1.33 a
40 to 50	1019.19 a	2.71 a	1.35 a
Physical Activity <sup>2</sup>	SE 0,65	SE 0,19	SE 0,04
Active	1020.19 a	2.27 a	1.37 a
Sedentary	1019.35 a	2.03 a	1.34 a
Type of coffee <sup>2</sup>	SE 0,65	SE 0,19	SE 0,04
Caffeinated	1020.25 a	1.97 a	1.36 a
Decaffeinated	1019.29 a	2.33 a	1.34 a
Stage <sup>2</sup>	SE 0,42	SE 0,11	SE 0,02
Before	1019.92 a	2.20 a	1.35 a
After	1019.63 a	2.09 a	1.35 a

Averages followed by the same small letter in the column do not differ among themselves by the Scott-Knott test (1), and the Student t test (2), with a nominal level of significance of 5%. \* Standard error

decaffeinated coffee also promoted reduction. Kiyohara *et al.* (1999), evaluating the effect of coffee in the reduction of uric acid levels in a population of 2,000 Polish men and women, also verified that there was a uric acid reduction in the individuals that consumed coffee.

### Complete blood count

In Table 5 are found the average values of erythrocytes - EC (units/mm<sup>3</sup>), hemoglobin - HM (g/dl), hematocrits - HT (%), lymphocytes-LF (mil/mm<sup>3</sup>), leukocytes - LC (mil/mm<sup>3</sup>) and platelets - PT (mil/mm<sup>3</sup>), according to the age group, physical activity, type of coffee consumed before and after the duration of the experiment for the individuals evaluated in the research. It is observed that there were no significant differences (P>0.05), for the variables erythrocytes, hemoglobins and lymphocytes, independent of the evaluated factors age group, physical activity and type of coffee consumed during the experiment. However, for the variables hematocrits, leukocytes and plaque, there was a significant reduction (P < 0.01) for the factor stage, after the six months, independent of the coffee type consumption. It is also observed that in the factors leukocytes and plaque, the active individuals presented a lower value in relation to the sedentary. That shows that caffeine was not responsible for that reduction.

### Urine density and thyroid hormones

In Table 6 are the average values of the urine density, thyroid hormones: Ultra sensitive TSH (uUI/mL) and free T4 (ng/dL), according to the age group, physical activity, type of coffee consumed before and after the duration of the experiment. It is observed that there were no significant differences (P>0.05), for the variables: urine density and free T4. However, for the intermediate age group (30 to 39 years), presented an Ultra sensitive TSH value significantly (P < 0.01) lower in relation to the other age groups. The average value found for the urine density was 1,019.77, this being within normal range (reference values-1,010 to 1,030); for Ultra sensitive TSH, the average found was 2.15 uUI/mL, the references values used being 0.400 to 4,000 uUI/mL and for free T4 the average value found was 1.35 ng/dL, the references values used: 0.80 to 1.90 ng/dL for Euthyroidism, ND to 1.0 ng/dL for Hypothyroidism and 1.20 to 6.0 ng/dL for Hyperthyroidism. The results regarding the urine density and thyroid hormones exams are normal according to the references values (PARDINI, 2007; WALTERS *et al.*, 1998).

All the results regarding the complete blood count, urine density and thyroid hormones are normal, according to the

references values (PARDINI, 2007; WALTERS *et al.*, 1998). The analyses of those factors were used with the intention of verifying the conditions of the individuals' health, since exams showing values outside the "normal" range would totally compromise the intended results of the present research. The obligatory requirement for the inclusion in the same, was precisely, active and sedentary healthy adult individuals. Therefore, through the obtained results, it was also observed that the coffee, caffeinated as well as decaffeinated, did not exercise any malicious effect on the appraised factors.

## Conclusion

There was reduction in the levels of total cholesterol, HDLc and LDLc, uric acid, platelets and hematocrits, and increase in the leukocytes, independent of the coffee consumed, age group, level of physical activity, however; in the other variables there were no alterations. Coffee presents characteristics that suggests its effect as a functional food.

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