

Effect of Filter Coffee and Instant Coffee Consumption on the Fasting Plasma Homocysteine Levels of Healthy Female Volunteers, Who Are Non-Coffee Drinkers

Janaki S

Department of Homescience, Women's Christian College, Chennai – 6, India

Abstract: A high plasma total homocysteine concentration is associated with increased risk of cardiovascular disease. Consumption of unfiltered or filtered coffee raises total homocysteine concentrations in healthy volunteers. Caffeine might be a factor that elevates the total homocysteine concentration, because it may inhibit the conversion of homocysteine to cysteine by acting as a vitamin B6 antagonist. Hence, the present study was designed to investigate the effect of filter coffee and instant coffee consumption on the fasting plasma homocysteine levels of healthy female volunteers, who are non-coffee drinkers. Nine healthy female volunteers between 18-22yrs, who are non-coffee drinkers were selected. Filter coffee and instant coffee powder, which are 100% coffee as per label were selected, prepared and standardized. Supplementation period lasted for five weeks. The volunteers were provided with 400ml(400mg caffeine) of filter coffee per day per volunteer for the first period of two weeks and to the same group, after a week of washout period, 500ml(402mg caffeine) of instant coffee per day per volunteer for the next two weeks. The plasma fasting homocysteine concentration was estimated on the blood collected before and after the supplementation of coffee groups. There was a significant increase in the fasting plasma homocysteine levels of both the groups after two weeks of coffee consumption. This increase was statistically significant at 5% level. But when the increase in the fasting plasma homocysteine levels between two groups was compared, there was no significant difference at 5% level. It could be concluded from the study that consumption of filter coffee and instant coffee for a period of two weeks had an effect on the fasting plasma homocysteine levels of healthy female volunteers. No difference between the two groups could be due to equal amount of caffeine intake. Elevated plasma homocysteine levels are reported to be a positive risk factor in CHD.

Keywords: homocysteine, filter coffee, instant coffee, caffeine, CHD.

1. Introduction

In the general population, there is a gradient of coronary heart disease associated with increasing levels of plasma homocysteine (Verhoef et al., 1996). Grubben et al (2000) showed that a high consumption of unfiltered coffee caused an elevation in total homocysteine concentration. Oshaug et al (1998) has reported a positive association between heavy coffee drinking and plasma concentrations of total homocysteine. Coffee has attracted interest for a long time as a potential health hazard. Christensen et al (2001) describe a randomized trial showing that abstaining from coffee reduces serum cholesterol and plasma homocysteine. Jacques et al (2001) suggested that other dietary and lifestyle factors, including vitamin B6, riboflavin, alcohol and caffeine intakes as well as smoking and hypertension, influence circulating total homocysteine concentrations. Caffeine is a naturally occurring substance found in the leaves, seeds or fruits of more than 60 plant species. The amount of caffeine in food product varies depending on the serving size, type of product and preparation method (Hogan et al., 2002). Hence, the present study was designed to investigate the effect of filter coffee and instant coffee consumption on the fasting plasma homocysteine levels of healthy female volunteers, who are non-coffee drinkers.

2. Review Literature

As a food additive, caffeine is used in various foods for its flavor contribution. The amount of caffeine in food products varies depending on the serving size, type of products and preparation method. The plant variety also affects caffeine content of tea and coffee (Hogan et al., 2002). The caffeine content of a cup of coffee (150ml) is about 100mg (Manay, 2000). Moderate caffeine consumption is generally considered to be about 300mg, which is equivalent to approximately 3 cups of coffee (Hogan et al., 2002).

3. Physiological effects of caffeine

Caffeine, the most widely ingested methylxanthine, is readily absorbed by the gut and does not accumulate in the body because it is rapidly metabolized by the liver and eliminated. It has an average half-life of approximately four hours, although individual metabolism rates vary between two and ten hours (Gray, 1998). Caffeine raises slightly the blood pressure, slows and strengthens the heart, stimulates renal activity and prevents fatigue and depression. The most noticeable effect of tea and coffee is a mild cerebral stimulation; they clear away the "cobwebs". It is doubtful, however, if in the long run, the mental labor ultimately accomplished can be increased by coffee or tea (Hogan et al., 2002). The comfort and cheer obtained from a cup of coffee or tea cannot be attributed solely to its caffeine. Coffee contains certain aromatic oils, which are pleasing to the taste and mildly stimulating to the stomach. By retarding

slightly, the emptying of the stomach, it also increases the satiety value of the meal. Taken hot these beverages stimulate gastrointestinal motility and when taken cold in hot weather is refreshing (Hogan et al., 2002).

4. Materials and Method

The experimental design selected for this study was a “pre-test post-test, single blind type”. The volunteers were briefed about the importance of the study, after which the interview schedule was administered to them. Each volunteer was interviewed individually by the investigator regarding her name, age, economic status, family history of heart disease and dietary habits and the responses were recorded. A purposive sampling technique was employed to select the volunteers for the study. The criteria for selection of the volunteers are age between 18-22yrs, non-coffee drinkers, willingness to avoid other caffeine containing foods items and B-complex supplementation, if any, throughout the study period. The sample comprised of nine healthy female volunteers who are non-coffee drinkers selected from Women's Christian College(WCC) hostel, Chennai. The coffee powder was selected using convenient sampling technique. NESCAFE CLASSIC instant coffee powder and LEO filter coffee powder were selected. Both samples contained 100% coffee as per the label information. The caffeine content of coffee powder were estimated (Pearson, 1973).

4.1 Filter coffee (drip filtration method)

Each subject had to be given 21gms of filter coffee per day. Weighed 189gms of filter coffee powder (supplying 400mg of caffeine per subject per day) for the preparation of coffee for nine girls. In the filter vessel, the coffee powder was put and 1800ml of boiling water was poured to make decoction. The filtered decoction collected was 1500ml, which was diluted to 3600ml with water and 36tbsp of sugar added and mixed well. The coffee was filtered and divided into nine portions of 400ml each and bottled. The bottles were labeled with the name of the volunteers and refrigerated for consumption throughout the day.

4.2 Instant coffee

Each subject had to be given 8.5gms of instant coffee powder per day. Weighed 76.5gms of instant coffee powder (supplying 402mg of caffeine per subject per day) and dissolved with 4400ml of water and 45tbsp of sugar added and mixed well. The coffee was filtered and divided into nine portions of 500ml each and bottled. The bottles were labelled with the name of volunteers and refrigerated for consumption throughout the day.

The study period lasted for five weeks. On the first day, the volunteers were requested to report at Homescience Laboratory, WCC, Chennai at 8.00 am in a ten to twelve hours post-absorptive state for blood collection. After blood collection, the volunteers were instructed to report at Homescience department every morning at 8.00 am. They were instructed about the importance of compliance. The investigator explained to the volunteers that the bottles containing 400ml of filter coffee with their names were left

in the refrigerator from which they should consume the coffee at their convenient timing. They continued this procedure for a period of two weeks. On the day 15, volunteers were instructed to report at 8.00 am in Homescience Laboratory, WCC, Chennai in a ten to twelve hours post-absorptive state for blood collection. This marked the end of supplementation phase I with filter coffee. There was washout period of 7 days from day 15 to day 21. During this period the volunteers were instructed to refrain from consumption of coffee and other food source of caffeine. On the day 22, the volunteers reported at 8.00 am at Homescience Laboratory, WCC, Chennai in a ten to twelve hours post-absorptive state for blood collection. After blood collection, the volunteers were instructed to report at the Homescience department every morning at 8.00 am. The investigator explained to the volunteers that the bottles containing 500ml of instant coffee with their names were left in the refrigerator from which they had to consume the coffee at their convenient timing. This lasted for a period of two weeks. On the day 36 which was the end of supplementation phase II, the volunteers were instructed to report at 8.00 am in Homescience Laboratory, WCC, Chennai in a ten to twelve hours post-absorptive state for blood collection. The analyses of the fasting plasma homocysteine were carried out at Apollo laboratory, Chennai. Plasma homocysteine level was estimated using FPIA method (Abbott Diagnostics Division, 2002).

5. Results And Discussion

The data recorded by interview schedule were processed, tabulated and subjected to descriptive analysis. The results of the analysis of plasma homocysteine levels were subjected to inferential statistical analysis.

All the nine female volunteers in the study belonged to the age group between 18-22 years. According to Brattstrom et al (1994) the average plasma total homocysteine concentration are markedly higher in men than in women. The difference between the sexes could be due to larger muscle mass in men, since the formation of muscles is associated with the simultaneous formation of homocysteine in connection with creatinine synthesis (Norlund et al., 1998). In the observation of Gartler et al (1981), high homocysteine concentrations in the elderly may be related to declining activity of cystathionine β -synthase and possibly other enzymes involved in homocysteine metabolism. Boers et al (1983) reported elevated homocysteine concentration in women after menopause. It could be due to the influence of sex hormones (Andersson et al., 1992). The height of the volunteers ranged between 150-163cms and weight ranged from 42-55kgs. The Body Mass Index (BMI) of the volunteers ranges from 20 -23. Among the volunteers 33% were postgraduate and 67% undergraduate students. All the volunteers belonged to high income group.

Information on the familial incidence revealed that 33% of the volunteers did not have family history of heart disease whereas 67% had family history of heart disease. The C>T polymorphism is the gene that encodes MTHFR (methylenetetrahydrofolate reductase) has been investigated most extensively in relation to its effect on the total homocysteine concentration. The higher total homocysteine

concentrations are most pronounced in 677 C>T subjects with a marginal folate status (McQuillan et al., 1999). Patients with inherited defects of homocysteine metabolism, like MTHFR deficiency (Rosenblatt, 1989) and defects in cobalamin metabolism (McCully, 1969) suffer from vascular diseases at a very young age.

All the volunteers were non-vegetarians. Among them, 89% consume tea daily. Milo, bournvita or complan were never consumed by a majority of volunteers. Chocolate milk shake and chocolate icecream were consumed monthly. Pepsi and Cola were consumed weekly by a major percentage. Brown chocolate consumption was found to be twice a week or monthly. Cocoa beverages were consumed only once a month. Information regarding the dietary pattern of the volunteers revealed that wheat was consumed by 78% weekly. Most of the volunteers did not consume ragi. Among the pulses and legumes, bengal gram and black gram dhal were consumed weekly. Red gram dhal was consumed by majority of the volunteers every day. The other pulse as green gram, cowpea and soyabean was either consumed occasionally or never. Consumption of carrot was found to be either twice a week or weekly. Yam consumption was either occasional or never. Onion was consumed daily by the volunteers.

In a meta-analysis of randomized intervention trials that assessed the effects of folate, vitamin B12, vitamin B6 in lowering homocysteine concentrations, dietary supplementation with folate showed the strongest effect, reducing blood homocysteine concentration by 25%. Vitamin B12 produced an additional 7% reduction in blood homocysteine concentrations, whereas vitamin B6 did not

produce a significant additional effect (Saw et al., 2001). As dietary supplementation of folate has an effect on blood homocysteine level, the questionnaire formulated for the present study included only the vegetables rich in folate based on ICMR, Nutritive value of Indian foods, 2001. In the present study, vegetables like brinjal, cucumber, pumpkin, ková, french bean were consumed either occasionally or never. Tomato was consumed daily and snake gourd, ladies finger were consumed weekly. Majority of volunteers consumed eggs twice weekly. Consumption of liver was found to be either occasional or never.

Nygard et al (1997) found that cigarette smoking, coffee consumption and physical inactivity were each positively associated with plasma homocysteine, these associations remained significant after adjustment for dietary intakes of fruit and vegetables and vitamin supplements. A higher dietary folate intake is associated with a lower total homocysteine level in adults, independent of other dietary and lifestyle factors (Rasmussen et al., 2000). Shimakawa et al (1997) observed inverse relations between vitamin B6 and total homocysteine concentration. Ubbink et al (1998) observed in study samples with elderly and middle-aged subjects, a lower total homocysteine concentrations at higher B12 intake. Haulrik et al (2002) found no association between high protein and high methionine diet on the plasma homocysteine concentration. The hypothesis that increased protein intake and decreased coffee consumption may reduce total homocysteine level and potentially prevent atherosclerotic cardiovascular disease and other disease outcome is supported by Stolzenberg-Solomon et al (1999).

Table 1: Quantity of caffeine consumed by the volunteers

Coffee Powder	Amt/day /voulnteer (g)	Caffeine content mg/100g (Pearson, 1973)	Caffeine/ day (mg)	Caffeine for total two weeks(mg)	't' value	Level of significant at 5%
Filter coffee (Narasus)	21	1900	400	5600	1.21	Not significant
Instant coffee (Nescafe)	8.5	4730	402	5628		

Table 2: Mean fasting plasma homocysteine levels before and after two weeks of consumption

Coffee groups	Mean fasting plasma homocysteine levels		Increment	't' value	Level of significant at 5%
	Before	After			
Filter coffee	8.73 ± 1.23	9.61 ± 1.56	0.88 ± 0.8	2.990	Significant
Instant	9.39 ± 1.48	9.95 ± 1.34	0.56 ± 0.53	3.078	Significant

Table 3: Comparison of the mean fasting plasma homocysteine levels between two coffee groups

Period	Mean fasting plasma homocysteine levels		Difference	't' value	Level of significance at 5%
	Filter coffee (μmol/L)	Instant coffee (μmol/L)			
Before	8.73 ± 1.23	9.39 ± 1.48	0.66	1.02	Not significant
After	9.61 ± 1.56	9.95 ± 1.34	0.34	0.49	

It can be seen from **Table 1** that the caffeine supplied to the volunteers from Instant coffee was 28mg more than the quantity supplied from Filter coffee. But this difference was not statistically significant at 5% level.

At the commencement of the study, before consumption of filter coffee the mean fasting plasma homocysteine levels was 8.73 ± 1.23 (μmol/L) and after two weeks of consumption the levels was 9.61 ± 1.56 (μmol/L). There was an increase of 0.88 ± 0.8 (μmol/L), which was statistically significant at 5% level (**Table 2**). Before consumption of instant coffee the mean fasting plasma homocysteine level was 9.39 ± 1.48 (μmol/L) and after two weeks of consumption the level was 9.95 ± 1.34 (μmol/L). There was an increase of 0.56 ± 0.53 (μmol/L), which was statistically significant at 5% level (**Table 2**). In a study by Verhoef et al (2002), the mean fasting plasma homocysteine levels increased by 0.4(μmol/L) and 0.9 (μmol/L) respectively after caffeine and coffee treatment compared with placebo.

It can be seen from **Table 3**, that there was no significant (at 5% level) difference in the plasma homocysteine levels between the two groups after two weeks of consumption of filter coffee and instant coffee. Urgert et al (1996) ruled out the diterpenes cafestol and Kahweol, which are known for their cholesterol raising effects, they are retained by a paper filter. Chlorogenic acid could be partly responsible for the

higher homocysteine concentrations observed in coffee drinkers or of black tea increases plasma homocysteine concentration (Olthof et al., 2001). Unfiltered coffee increases plasma homocysteine concentrations in volunteers with normal initial concentrations. It is unclear whether the effect is caused by the cholesterol raising diterpenes present exclusively or factors that are also present in filtered coffee. Both unfiltered and filtered coffee appears to contain a factor that raises plasma homocysteine. This factor is potentially also present in soluble (instant) coffee, espresso and other types of coffee (Grubben et al., 2000).

6. Conclusion

It could be concluded from the study that consumption of filter coffee and instant coffee for a period of two weeks had an effect on the fasting plasma homocysteine levels of healthy female volunteers. But, no significant difference in fasting plasma homocysteine levels between two groups could be due to equal amount of caffeine intake. Elevated plasma homocysteine levels are reported to be a positive risk factor in CHD. Therefore, in nutrition counselling for lifestyle modification and primary prevention in delaying the onset of CHD, the deleterious effect of coffee consumption could be advocated.

7. Acknowledgment

I dedicate this work to my guide, honorable (late) Mrs.S.Shivakumar, HOD, Women's Christian College, Chennai-6.

8. Future Scope:

Effect of different sources of caffeine can also be investigated. A placebo comparative study can be designed.

References

- Andersson, A., Brattstrom, L., Israelsson, A., Hamfelt, A and Hultberg, B. (1992) Plasma homocysteine before and after methionine loading with regard to age, gender and menopausal status. *Eur J Clin Invest*, 22: 79-87.
- Boers, G.H., Smals, A.G., Trijbels, F.J., Leermakers, A.I., Kloppenborg, P.W. (1983) Unique efficiency of methionine metabolism in premenopausal women may protect against vascular disease in the reproductive years. *J Clin Invest*, 72: 1971-1976.
- Brattstrom, L., Lindgren, A., Israelsson, B., Andersson, A and Hultberg, B. (1994) Homocysteine and cysteine: determinants of plasma levels in middle-aged and elderly subjects. *J Intern Med*, 236: 633-641.
- Christensen, B., Mosdol, A., Retterstol, L., Landaas, S and Thelle, D.S. (2001) Abstinence from filtered coffee reduced the concentrations of plasma homocysteine and serum cholesterol – a randomized controlled trial. *Am J Clin Nutr*, 74:302-307.
- Gartler, S.M., Hornung, S.K., Motulsky, A.G. (1981) Effect of chronologic age on induction of cystathionine synthase, uroporphyrinogen I synthase, and glucose-6-phosphate dehydrogenase activities in lymphocytes. *Proc Natl Acad Sci U S A*, 78: 1916-1919.
- Gray, J. (1998). Caffeine, coffee and health. *Nutrition and Food science*, 6: 314-319.
- Grubben, M.J., Boers, G.H., Blom, H.J., Broekhuizen, R., de Jong R., van Rijt, J., de Ruijter, E., Swinkels, D.W., Nagengast, F.M and Katan, M.B. (2000) Unfiltered coffee increases plasma homocysteine concentrations in healthy volunteers: a randomized trial. *Am J Clin Nutr*, 71: 480-484.
- Haulrik, N., Soren, T., Dyerberg, J., Stender, S., Annebeth, R.S and Arne, A. (2002) Effect of protein and methionine intakes on plasma homocysteine concentrations: a 6-mo randomized controlled trial in overweight subjects. *Am J Clin Nutr*, 76: 1202-1206.
- Hogan, E., Howard, H., Betsy, A. (2002) Communicating the message: Clarifying controversies about caffeine. *Nutrition Today*, 37: 28-35.
- Jacques, P.F., Bostom, A.G., Wilson, P.W., Rich, S., Rosenberg, I.H and Selhub, J. (2001) Determinants of plasma total homocysteine concentration in the Framingham Offspring Cohort. *Am J Clin Nutr*, 73: 613-621.
- Manay, Shakuntala, N., Shadaksharaswamy, M. (2000) Foods, facts and principles, New age international (p) Ltd Publications, New Delhi. Pg. 244-247
- McCully, K.S. (1969) Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. *Am J Pathol*, 56: 111-128.
- McQuillan, B.M., Beilby, J.P., Nidorf, M., Thompson, P.L and Hung, J. (1999) Hyperhomocysteinemia but not the C677T mutation of methylenetetrahydrofolate reductase is an independent risk determinant of carotid wall thickening – The perth Carotid Ultrasound disease Assessment Study (CUDAS). *Circulation*, 99: 2383-2388.
- Norlund, L., Grubb, A., Fex, G., Leksell, H., Nilsson, J.E., Schenck, H and Hultberg, B. (1998) The increase of plasma homocysteine concentrations with age is partly due to the deterioration of renal function as determined by plasma cystatin C. *Clin Chem Lab Med*, 36: 175-178.
- Nygard, O., Refsum, H., Ueland, P.M., Stensvold, I., Nordrehaug, J.E., Kvale, G and Vollset, S.E. (1997) Coffee consumption and plasma total homocysteine: the Hordaland Homocysteine study. *Am J Clin Nutr*, 65: 136-141.
- Olthof, M.R., Hollman, P.C., Zock, P.L and Katan, M.B. (2001) Consumption of high doses of chlorogenic acid, present in coffee or of black tea increases plasma total homocysteine concentrations in humans. *Am J Clin Nutr*, 73:532-538.
- Oshaug, A., Bugge, K.H., Refsum, H. (1998) Diet, an independent determinant for plasma total homocysteine. A cross sectional study of Norwegian workers on platforms in the North Sea. *Eur J Clin Nutr*, 52: 7-11.
- Rasmussen, L.B., Ovesen, L., Bulow, I., Knudsen, N., Laurberg, P and Perrild, H. (2000) Folate intake, lifestyle factors and homocysteine concentrations in younger and older women. *Am J Clin Nutr*, 72: 1156-1163.
- Rosenblatt, D.S. (1989) Inherited disorders of folate transport and metabolism, in *The Metabolic Basis of Inherited Disease* (Scriver, C.R., Beaudet, A.L., Sly,

- W.S and Valle, D. eds) pg 2049-2064, McGraw-Hill, New York.
- [20] Saw, S.M., Yuan, J.M., Ong, C.N., Arakawa, K., Lee, H.P., Coetzee, G.A and Yu, M.C. (2001) Genetic, dietary and other lifestyle determinants of plasma homocysteine concentrations in middle-aged and older Chinese men and women in Singapore. *Am J Clin Nutr*, 73: 232-239.
- [21] Shimakawa, T., Nieto, F.J., Malinow, M.R., Chambless, L.E., Schreiner, P.J and Szklo, M. (1997) Vitamin intake: a possible determinants of plasma homocyst(e)ine among middle aged adults. *Am Epidemiol*, 7: 285 - 293.
- [22] Stolzenberg Solomon, R.Z., Miller, E.R., Maguire, M.G., Selhub, J and Appel, L.J. (1999) Association of dietary protein intake and coffee consumption with serum homocysteine concentrations in an older population. *Am J Clin Nutr*, 69: 467-475.
- [23] Ubbink, J.B., Fehily, A.M., Pickering, J., Elwood, P.C and Vermaak, W.J.H. (1998) Homocysteine and ischaemic heart disease in the Caerphilly cohort. *Atherosclerosis*, 140: 349-356.
- [24] Urgert, R., Meyboom, S., Kuilman, M., et al. (1996) Comparison of effect of cafetiere and filtered coffee on serum concentrations of liver aminotransferases and lipids: six month randomized controlled trial. *BMJ*, 313: 1362-1366.
- [25] Verhoef, P., Pasman, W.J., Vliet, T.V., Urgert, R., and Katan, M.B. (2002) Contribution of caffeine to the homocysteine-raising effect of coffee: a randomized controlled trial in humans. *Am J Clin Nutr*, 76: 1244-1248.
- [26] Verhoef, P., Stampfer, M.J., Buring, J.E., et al (1996) Homocysteine metabolism and risk of myocardial infarction: relation with vitamin B6, B12 and folate. *Am J Epidemiol*, 143: 845-859.

Author Profile



Janaki s did M.sc., NET, M.phil. She is pursuing M.Phil foods and nutrition, mother Theresa university, Chennai, India. Her area of specialization includes foods and nutrition. Her research interest includes in clinical nutrition and food science