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Papers

Cross sectional study of effects of drinking green tea on cardiovascular and liver diseases

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Abstract

Objective: To investigate the association between consumption of green tea and various serum markers in a Japanese population, with special reference to preventive effects of green tea against cardiovascular disease and disorders of the liver.
Design: Cross sectional study.
Setting: Yoshimi, Japan.
Subjects: 1371 men aged over 40 years resident in Yoshimi and surveyed on their living habits including daily consumption of green tea. Their peripheral blood samples were subjected to several biochemical assays.
Results: Increased consumption of green tea was associated with decreased serum concentrations of total cholesterol (P for trend <0.001) and triglyceride (P for trend=0.02) and an increased proportion of high density lipoprotein cholesterol together with a decreased proportion oflow and very low lipoprotein cholesterols (P for trend=0.02), which resulted in a decreased atherogenic index (P for trend=0.02). Moreover, increased consumption of green tea, especially more than 10 cups a day, was related to decreased concentrations of hepatological markers in serum, aspartate aminotransferase (P for trend=0.06), alanine transferase (P for trend=0.07), and ferritin (P for trend=0.02).
Conclusion: The inverse association between consumption of green tea and various serum markers shows that green tea may act protectively against cardiovascular disease and disorders of the liver.

Key messages

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- This cross sectional **s**tudy in Japanese men shows that increased consumption of green tea is associated with decreased serum concentrations of total cholesterol, triglyceride, and atherogenic index
- High consumption of green tea may also protect against disorders of the liver in terms of serum markers

Introduction

Tea has recently received a lot of attention as a protective agent against cancer and cardiovascular disease, two important targets of preventive medicine for adults. Green tea has many advantages over chemical preventive agents--tea is non-toxic and thus readily available to the general population.

Several laboratory studies on crude extract or constituents ofblack and green teas have shown inhibitory effects on oxidation and mutagenicity.[1] [2] [3] [4] Moreover, one of the catechins, (-)--epigallocatechin gallate, (a main constituent of green tea leaves) significantly inhibited the promotion of tumours and carcinogenesis in animal experiments.[5] [6] [7] [8] [9] Animal experiments have also studied the protective effects of green tea against cardiovascular disease.[10] [11] [12] Ingestion of extract of green tea in rats has significantly decreased plasma cholesterol and triglyceride concentrations and the ratio oflow and very low density lipoprotein cholesterol concentrations to high density lipoprotein cholesterol concentrations.[13] [14] [15] No clear evidence for protective effects of tea in humans, however, has been reported. Only a few studies have examined the relation between tea intake and the incidence of cancer or cardiovascular disease, and their results were inconsistent.[16] [17] [18]

With regard to prevention of cardiovascular disease, the association between tea drinking and serum concentrations of lipids and other markers must be confirmed first. Nevertheless, to our knowledge only one study of green tea has been carried out, and this showed an inverse association between consumption of green tea and serum total cholesterol concentration but not between green tea and triglyceride or lipoprotein concentrations.[19] Only a few studies of black tea have been carried out, and they did not find a significant association between consumption of black tea and serum lipid concentration because few "heavy tea drinkers" were included in the study populations.[20] [21] We investigated the association between consumption of green tea and various serum markers in a Japanese population, with special reference to preventive effects of the tea against cardiovascular disease and disorders of the liver.

Methods

In 1986 we began a prospective cohort study in residents aged over 40 years old in the town of Yoshimi in the prefecture of Saitama. To carry out the epidemiological survey we used a self administered questionnaire covering 90 lifestyle factors--such as present and past eating habits and history of consumption of cigarettes and alcohol--and history of disease, present state of health, and medication. Information on consumption of green tea (categorised as </= 3, 4-9, or >/= 10 cups a day) was extracted from the answers on the questionnaire.

The survey covered 8553 individuals (95% of all residents aged over 40), who were invited to participate in yearly health screening checks. Of those who agreed to participate, 3625 self selected individuals (1371 men, 2254 women) gave peripheral blood samples at each health screening check. Samples taken during 1986-90 were subjected to biochemical and immunological assays. All blood samples were collected between 1 pm and 3 pm after strict fasting of > 12 hours before the health checks in July, August, or September. All assays were started within five hours of the blood samples being taken.

The components of the serum were determined for each blood sample: total protein, albumin, globulin, protein fractionation, total cholesterol, triglyceride, lipoprotein fractions (agarose gel

electrophoresis), alanine aminotransferase, aspartate aminotransferase, haemoglobin, packed cell volume, chloride, potassium, sodium, vitamin A, and lipid peroxides (thiobarbituric acid assay). Immunological assays determined the cytotoxic activity of natural killer cells, subsets of T cells, and reactivity of peripheral blood lymphocytes in the presence of phytohaemagglutinin. Blood pressure was measured when the blood samples were taken. Of the 3625 participants, 2255 (780 men, 1475 women) had a yearly health check more than twice during the study period. At these participants' second visit, their blood samples were subjected to assay serum concentrations of selenium, (alpha) fetoprotein, carcinoembryonic antigen, ferritin (radioimmunoassays), and isolation of DNA from lymphocytes. Immunological and genetic characteristics of this cohort population have been reported previously.[22] [23] [24] The present study dealt with only 1371 men as the effects of green tea were not clearly observed among the women, whose serum lipid concentrations and hepatological markers remained low because so few of them were drinkers and smokers.

Multivariate analysis of variance was used to control for the effects of confounding factors with the software package SPSS. Potential covariates considered in this analysis were age, cigarette smoking, alcohol consumption, and relative body weight, which is the ratio of observed weight to the standard one calculated for individual height, sex, and age in the Japanese population. Age was categorised as <40, 40-49, 50-59, 60-69, or >/= 70 years. Cigarette smoking was categorised according to whether subjects had never smoked, were past smokers, or were current smokers and according to the number of cigarettes they smoked daily times the number of years they had been smokers (<500, 500-999, or >/=1000). Alcohol consumption was categorised according to whether subjects had never drunk alcohol, were past drinkers, occasional drinkers, or daily drinkers and according to the amount of alcohol (ml ethanol) they drank daily times the number of years they had been drinking (<500, 500-999, 1000-1499, or >/=1500). Relative body weight was categorised as </=0.79, 0.80-0.89, 0.90-1.09, 1.10-1.19, or >/=1.20.

Results

CONSUMPTION OF GREEN TEA AND CHARACTERISTICS OF SUBJECTS

Table I shows the baseline characteristics of the subjects by category of consumption of green tea. Increased consumption was associated with increased age. It is interesting that consumption of green tea was closely associated with cigarette smoking status; it showed no relation, however, to alcohol consumption or relative body weight.

TABLE I--Baseline characteristics of 1371 men by consumption of green tea (cups/day)

	</=3	4-9	>/=10	Total
No of men	383	672	316	1371
Mean (SE) age (years)	51.9 (0.7)	55.5 (0.5)	59.2 (0.6)	55.4 (0.3)
No (%) of current smokers	224 (58.5)	407 (60.6)	219 (69.3)	850 (62.0)
No (%) of regular and occasional drinkers	201 (52.5)	339 (50.4)	163 (51.6)	703 (51.3)
Mean (SE) relative body weight	0.99 (0.006)	0.99 (0.005)	0.99 (0.007)	0.99 (0.003)

CONSUMPTION OF GREEN TEA AND SERUMLIPID AND LIPOPROTEIN CONCENTRATIONS

Consumption of green tea was significantly associated with lower serum concentrations of lipids and lipoproteins. An increase in consumption substantially decreased serum total cholesterol and triglyceride concentrations, and this strong association remained almost unaltered even after age, cigarette smoking, alcohol consumption, and relative body weight were controlled for (table II). The significant reduction of serum total cholesterol and triglyceride concentrations found in the group that consumed the most green tea shows that >/= 10 cups a day of green tea consumption is effective in modulating serum lipids.

TABLE II--Serum total cholesterol and triglyceride concentrations in 1330 men by consumption of green tea (cups/day)

	</=3	4-9	>/=10	P for trend
No of men*	376	651	303	
Mean (SE) total cholesterol (mmol/l):				
Crude	4.85 (0.05)	4.76 (0.03)	4.58 (0.05)	<0.001
Adjusted+	4.85 (0.05)	4.76 (0.04)	4.58 (0.05)	<0.001
95% Confidence interval	4.76 to 4.94	4.69 to 4.83	4.49 to 4.69	
Mean (SE) triglyceride (nmol/l):				
Crude	1.66 (0.07)	1.59 (0.04)	1.45 (0.05)	0.02
Adjusted+	1.65 (0.06)	1.60 (0.05)	1.45 (0.07)	0.02
95% Confidence interval	1.53 to 1.77	1.50 to 1.69	1.32 to 1.58	

*Number of subjects for whom all assays were completed.
+Adjusted for age, cigarette smoking, alcohol consumption, and relative body weight.

Mean serum lipoprotein concentrations show that increased consumption of green tea was associated with an increased serum concentration of high density lipoprotein cholesterol and a decreased concentration of serum low density and very low density lipoprotein cholesterols (table III). Serum concentrations of lipoproteins were measured by an electrophoretic separation in agarose, and the proportions of lipoprotein fractions in total lipoprotein were estimated as percentages. The association was found only in the group that drank the most green tea. A ratio of low density lipoprotein cholesterol to high density lipoprotein cholesterol, which is sometimes referred to as the atherogenic index, may also be an important index for arteriosclerosis (table III). A significant decrease in the ratio of low density lipoprotein to high density lipoprotein cholesterol was observed in the group that drank the most green tea.

TABLE III--Serum concentrations of high density lipoprotein and low and very low density lipoprotein cholesterols and atherogenic index in 1330 men, by consumption of green tea (cups/day)

	</=3	4-9	>/=10	P for trend
No of men	376	651	303	
Mean (SE) high density lipoprotein cholesterol as % of total lipoproteins:				
Crude	36.4 (0.4)	36.5 (0.3)	37.4 (0.4)	0.02
Adjusted*	36.4 (0.3)	36.4 (0.3)	37.4 (0.4)	0.02
95% Confidence interval	35.8 to 37.1	35.9 to 36.9	36.7 to 38.1	
Mean (SE) low density lipoprotein + very low density lipoprotein % in total lipoproteins				
Crude	62.6 (0.3)	62.6 (0.3)	61.7 (0.4)	0.02
Adjusted*	62.5 (0.3)	62.6 (0.3)	61.7 (0.3)	0.02
95% Confidence interval	61.9 to 63.1	62.1 to 63.1	61.0 to 62.3	
Mean (SE) atherogenic index+				
Crude	1.19 (0.02)	1.20 (0.01)	1.16 (0.02)	0.07
Adjusted* mean (SE)	1.20 (0.02)	1.20 (0.01)	1.16 (0.02)	0.02
95% Confidence interval	1.17 to 1.23	1.17 to 1.22	1.13 to 1.19	

*Adjusted for age, cigarette smoking, alcohol consumption, and relative body weight.
+Low density lipoprotein cholesterol/high density lipoprotein cholesterol.

We also studied serum concentrations of lipid peroxides in relation to consumption of green tea. It has been suggested that lipid peroxidation was involved in the process of oxidative DNA damage and cytotoxicity as well as in that of arteriosclerosis.[25] [26] Our data on the same cohort population of men showed that serum concentrations of lipid peroxides among current smokers (n=850) were higher (9.0 (SE 0.1) nmol/ml) than those among non-smokers (n=226) (8.4 (0.2) nmol/ml; P<0.05). We found that serum concentrations of lipid peroxides among heavy smokers inversely changed with the amount of green tea consumed, while those among non-smokers did not. The crude mean serum concentration of lipid peroxides among heavy smokers with a smoking index of >/= 500 was decreased with increased consumption of green tea (8.3 (0.3) nmol/ml among 171 smokers who consumed daily >/= 10 cups; 9.0 (0.2) nmol/ml among 262 smokers who consumed daily 4-9 cups; 9.4 (0.3) nmol/ml among 122 smokers who consumed daily </= 3 cups (P < 0.05 for trend)). It is worth noting that high serum concentrations of lipid peroxides among heavy smokers were reduced to the concentrations among non-smokers when they consumed >/= 10 cups of green tea a day. Adjustments for age, alcohol consumption, and relative body weight did not affect the association.

PREVALENCE OF HEART DISEASE

Our disease prevalence survey, carried out at the baseline, supports our findings on normalisation of serum lipid and lipoprotein concentrations. We calculated the prevalence of self reported heart disease by using the categories of consumption of green tea: the age adjusted rates were 26.0, 29.4, and 39.8 per 1000 in populations with a daily consumption of >/= 10, 4-9, </= 3 cups a day respectively.

CONSUMPTION OF GREEN TEA AND HEPATOLOGICAL MARKERS

Our cross sectional study showed that consumption of green tea also reduced cell damage of the liver in terms of serum markers (table IV). The highest showed an inverse association with serum concentrations of aspartate aminotransferase and alanine aminotransferase, which was of borderline significance.

TABLE IV--Concentrations of aspartate aminotransferase and alanine transferase and serum ferritin concentration in 1330 men, by consumption of green tea (cups/day)

	</=3	4-9	>/=10	P for trend
No of men	376	651	303	
Mean (SE) aspartate aminotransferase (U/l):				
Crude	25.2 (1.0)	25.7 (0.9)	23.2 (0.7)	0.11
Adjusted*	25.4 (1.1)	25.7 (0.8)	23.0 (1.2)	0.06
95% Confidence interval	23.2 to 27.5	24.0 to 27.3	20.7 to 25.3	
Mean (SE) alanine aminotransferase (U/l):				
Crude	24.1 (1.0)	23.0 (0.7)	19.9 (1.1)	0.003
Adjusted*	23.3 (1.0)	23.0 (0.8)	20.8 (1.1)	0.07
95% Confidence interval	21.3 to 25.3	21.5 to 24.6	18.7 to 23.0	
Mean (SE) serum ferritin+ (mg/l):				
No of men	185	395	200	0.02
Crude	111.7 (7.8)	102.1 (5.3)	88.5 (5.3)	0.02
Adjusted*	112.6 (7.4)	102.2 (5.1)	87.5	
95% Confidence interval	98.0 to 127.1	92.1 to 112.2	73.9 to 101.1	

*Adjusted for age, cigarette smoking, and alcohol consumption.
+Determined only in men who had a yearly health check more than twice.

A significant reduction in the crude and adjusted mean serum ferritin concentrations was associated with increased consumption of green tea (table IV). The reduction did not change even after adjustment for haemoglobin concentration. The results suggest that high consumption of green tea prevents cell damage of the liver, resulting in decreased serum concentrations of aspartate aminotransferase and alanine aminotransferase as well as of ferritin.

CONSUMPTION OF GREEN TEA AND HAEMOGLOBIN CONCENTRATION

Increased consumption of green tea was associated with a decreased haemoglobin concentration. The crude mean concentrations of haemoglobin were 151 (SE 0.7) g/l, 148 (0.6) g/l, and 145 (0.8) g/l among subjects with daily consumption of tea of </= 3 cups (n=363), 4-9 cups (n=633), and >/=10 cups (n=292) respectively (P<0.01 for trend). Adjustments for age, cigarette smoking, and alcohol consumption did not alter the results. The proportion of subjects with abnormally low haemoglobin concentrations (<120 g/l) was not related, however, to consumption of green tea: 1.4% (5/363), 3.2% (20/633), and 2.1% (6/292) in subjects who consumed </= 3, 4-9, and >/=10 cups a day respectively. Accordingly, a slight reduction in haemoglobin concentration with increased consumption of green tea does not seem to increase the occurrence of anaemia.

Discussion

Our cross sectional study showed a close association between high consumption of green tea and normalisation of serum components, which reflects cardiovascular and liver diseases. Increased consumption of green tea, especially more than 10 cups a day, was associated with decreased serum total cholesterol and triglyceride concentrations; increased high density lipoprotein cholesterol concentrations and decreased low density and very low density lipoprotein cholesterol concentrations, resulting in a reduced atherogenic index; decreased serum concentrations of lipid peroxides among smokers; and decreased aspartate aminotransferase and alanine aminotransferase concentrations, and serum ferritin concentration. The associations between green tea and serum total cholesterol and triglyceride concentrations and between green tea and high and low density lipoprotein cholesterol

concentrations are well correlated with experimental observations and the prevalence of heart disease in our cohort, implying that green tea may act preventively against cardiovascular disease. Moreover, this is the first time that an inverse association between consumption of green tea and cell damage of the liver, based on serum markers in humans has been reported.

Advancing age was associated with increased consumption of green tea. This could imply that consumption was also associated with being part of a healthier surviving cohort. If this is the case statistical adjustment for age used in this study could be insufficient to eliminate this effect. A follow up study is needed to clarify this problem.

Increased generation of oxygen radicals and subsequent formation of lipid peroxides were suggested to be cytotoxic and sometimes carcinogenic. Various forms of iron participated in radical reaction in vivo.[26] [27] Thus high stores of body iron were reported to be associated with increased risk of cancers such as lung, liver, and colon but not stomach.[28] [29] [30] With regard to stores of body iron and lipid peroxidation, our cohort data showed a significant association between serum ferritin concentrations, a stable marker for stores of body iron, and serum concentrations of lipid peroxides (correlation coefficient 0.25, $P<0.001$). The inverse association between the serum concentrations of ferritin and lipid peroxides and consumption of green tea suggests that the tea has protective effects on the development of cancer.

Given the potential implication of our findings, a follow up study of the cohort population is warranted to assess the association between consumption of green tea and various diseases including cancer. A follow up study and intervention study by green tea extract are under way.

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1. Osawa T, Namiki M, Kawakishi S. Role of dietary antioxidants in protection against oxidative damage. *Basic Life Sci* 1990;52:139-53. [Medline]

2. Zhao B, Li X, He R, Cheng S, Wenjuan X. Scavenging effect of extracts of green tea and natural antioxidants on active oxygen radicals. *Cell Biophys* 1989;14:175-85. [Medline]

3. Husain SR, Cillard J, Cillard P. Hydroxyl radical scavenging activity of flavonoids. *Phytochemistry* 1987;26:2489-91.

4. Nakayama T. Suppression of hydroperoxide-induced cytotoxicity by polyphenols. Cancer Res 1994;54(suppl):1991-3S.

5. Wang ZY, Cheng SJ, Zhou ZC, Athar M, Khan WA, Bickers DR, et al. Antimutagenic activity of green tea polyphenols. Mutat Res 1989;223: 273-85.

6. Yoshizawa S, Horiuchi T, Fujiki H, Yoshida T, Okuda T, Sugimura T. Antitumor promoting activity of (-)-epigallocatechin gallate, the main constituent of "tannin" in green tea. *Phytotherapy Research* 1987;1:44-7.

7. Conney AH, Wang ZY, Huang MT, Ho CT, Yang CS. Inhibitory effect of green tea on tumorigenesis by chemicals and ultraviolet light. *Prev Med* 1992;21:361-9. [Medline]

8. Fujiki H, Yoshizawa S, Horiuchi T, Suganuma M, Yatsunami J, Nishiwaki S, et al. Anticarcinogenic effects of (-)-epigallocatechin gallate. *Prev Med* 1992;21:503-9. [Medline]

9. Wang ZY, Huang MT, Ferraro T, Wong CQ, Lou YR, Reuhl K, et al. Inhibitory effect of green tea in the drinking water on tumorigenesis by ultraviolet light and 12-O-tetradecanoylphorbol-13-acetate in the skin of SKH-1 mice. *Cancer Res* 1992;52:1162-70. [Medline]

10. Young W, Hotovec RL, Romero AG. Tea and atherosclerosis. *Nature* 1967;216:1015-6. [Medline]

11. Sano M, Takenaka Y, Kojima R, Saito S-I, Tomita I, Katou M, et al. Effects of pu-erh tea on lipid metabolism in rats. *Chem Pharm Bull* 1986;34:221-8. [Medline]

12. De Whalley CV, Rankin SM, Hoult JRS, Jessup W, Leake DS. Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. *Biochem Pharmacol* 1990;39:1743-50. [Medline]

13. Muramatsu K, Fukuyo M, Hara Y. Effect of green tea catechins on plasma cholesterol level in cholesterol-fed rats. J Nutr Sci Vitaminol 1986;32: 613-22.

14. Chisaka T, Matsuda H, Kubomura Y, Mochizuki M, Yamahara J, Fujimura H. The effect of crude drugs on experimental hypercholesteremia: mode of action of (-)-epigallocatechin gallate in tea leaves. *Chem Pharm Bull* 1988;36:227-33. [Medline]

15. Ikeda I, Imasato Y, Sasaki E, Nakayama M, Nagao H, Takeo T, et al. Tea catechins decrease micellar solubility and intestinal absorption of cholesterol in rats. *Biochim Biophys Acta* 1992;1127:141-6. [Medline]

16. Yang CS, Wang ZY. Tea and cancer. *J Natl Cancer Inst* 1993;85:1038-9. [Abstract/Free Full Text]

17. Brown CA, Bolton-Smith C, Woodward M, Tunstall-Pedoe H. Coffee and tea consumption and the prevalence of coronary heart disease in men and women: results from the Scottish Heart Health Study. *J Epidemiol Community Health* 1993;47:171-5. [Abstract]

18. Hertog MGL, Feskens EMM, Hollman PCH, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* 1993;342:1007-11. [Medline]

19. Kono S, Shinchi K, Ikeda N, Yanai F, Imanishi K. Green tea consumption and serum lipid profiles: a **cross-sectional** study in Northem Kyushu, Japan. *Prev Med* 1992;21:526-31. [Medline]

20. Green MS, Harari G. Association of serum lipoproteins and health-related habits with coffee and tea consumption in free-living subjects examined in the Israeli CORDIS study. *Prev Med* 1992;21:532-45. [Medline]

21. Stensvold I, Tverdal A, Solvoll K, Foss OP. Tea consumption. Relationship to cholesterol, blood pressure, and coronary and total mortality. *Prev Med* 1992;21:546-53. [Medline]

22. Nakachi K, Imai K, Hayashi S-I, Watanabe J, Kawajiri K. Genetic susceptibility to squamous cell carcinoma of the lung in relation to cigarette smoking dose. *Cancer Res* 1991;51:5177-80. [Medline]

23. Nakachi K, Imai K. Environmental and physiological influences on human natural killer cell activity in relation to good health practices. *Jpn J Cancer Res* 1992;83:798-805. [Medline]

24. Nakachi K, Imai K, Hayashi S-I, Kawajiri K. Polymorphisms of the CYP1A1 and glutathione S-transferase genes associated with susceptibility to lung cancer in relation to cigarette dose in a Japanese population. *Cancer Res* 1993;53:2994-9. [Medline]

25. Steinberg D, Parthasarathy S, Carew Te, Khoo JC, Witztum JL. Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 1989;320:915-24. [Medline]

26. Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol* 1990;186:1-85. [Medline]

27. Halliwell B, Gutteridge JMC. Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. *Arch Biochem Biophys* 1986;246:501-14. [Medline]

28. Stevens RG, Beasley RP, Blumberg BS. Iron-binding proteins and risk of cancer in Taiwan. *J Natl Cancer Inst* 1986;76:605-10. [Medline]

29. Stevens RG, Jones DY, Micozzi MS, Taylor PR. Body iron stores and the risk of cancer. *N Engl J Med* 1988;319:1047-52. [Abstract]

30. Selby JV, Friedman GD. Epidemiologic evidence of an association between body iron stores and risk of cancer. *Int J Cancer* 1988;41:677-82. [Medline]