Methylenetetrahydrofolate reductase C677T and *A1298C* polymorphisms and colorectal cancer: The Fukuoka Colorectal Cancer Study

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Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme regulating folate metabolism, which affects DNA synthesis and methylation. This study investigated the relation of *MTHFR C677T* and *A1298C* polymorphisms to colorectal cancer in a case-control study in Fukuoka, Japan. The subjects comprised 685 incident cases of histologically confirmed colorectal adenocarcinomas and 778 community controls selected randomly in the study area. The genotype was determined by the PCR-RFLP method using genomic DNA extracted from buffy coat. Alcohol use was ascertained by in-person interview. Statistical adjustment was made for gender, age class, area, and alcohol use. The *MTHFR 677TT* genotype was associated with a statistically significant decrease in the risk with an adjusted odds ratio of 0.69 (95% confidence interval 0.51–0.93) compared with the *677CC* and *677CT* combined, and the decrease was most evident in individuals with no alcohol consumption. While the *A1298C* polymorphism showed no measurable association with the overall risk of colorectal cancer, the *1298CC* genotype was associated with a statistically significant increase in the risk when alcohol consumption was high, and was also associated with an approximately 2-fold increase in the risk of each of proximal and distal colon cancer. The findings add to evidence that individuals with the *MTHFR 677TT* genotype have a decreased risk of colorectal cancer in the absence of folate depletion, suggesting a protective role of folate by ensuring a sufficient thymidylate pool for DNA synthesis. Because very few individuals had the *1298CC* genotype, the findings regarding the *A1298C* polymorphism need careful interpretation and confirmation in larger studies. (Cancer Science 2004; 95: 908–913)

Much attention has recently been drawn to the role of folate metabolism in colorectal carcinogenesis.[1, 2] Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme regulating folate metabolism. It irreversibly converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the major form of folate in blood.[2] The substrate of MTHFR, 5,10-methylenetetrahydrofolate, is required for conversion of deoxyuridylate to thymidylate. Depletion of 5,10-methylenetetrahydrofolate results in uracil misincorporation into DNA, and removal of this abnormal base may lead to single and double strand breaks.[3, 4] Furthermore, insufficient thymidylate can increase DNA misrepair, resulting in overall DNA damage in the cell.[5] On the other hand, 5-methyltetrahydrofolate provides the methyl group for methylation of homocysteine to methionine. Imbalanced DNA methylation, i.e., global genomic hypomethylation and methylation of usually unmethylated CpG sites, has been implicated in colorectal carcinogenesis.[6–8]

Two common functional polymorphisms are known in the *MTHFR* gene; one is the *C677T* polymorphism in exon 4, resulting in an alanine-to-valine substitution at codon 222,[9] and the other is the *A1298C* in exon 7, resulting in a substitution of glutamate with alanine at codon 429.[10] Individuals who are homozygous for the variant allele of the *MTHFR C677T* polymorphism have been shown to have no less than 30% of normal enzyme activity, and heterozygotes (*CT*) have been shown to have 65% of normal enzyme activity.[9] As regards the *MTHFR A1298C* polymorphism, individuals with the *1298CC* genotype have been shown to have 60% of the enzyme activity of those with the *AA* genotype.[10]

Two early studies in the United States showed a decreased risk of colorectal cancer associated with *MTHFR 677TT* genotype, especially in individuals with high folate intake and with low alcohol intake.[11, 12] A consistent, but less evident, association was reported in two subsequent case-control studies in the United States.[13, 14] However, other case-control studies have failed to substantiate a protective association with the *677TT* genotype in various countries, including the United States.[15–21] Few studies have addressed the association between the *MTHFR A1298C* polymorphism and colorectal cancer.[14–16, 22] Of these, only one study showed a decreased risk of colorectal cancer associated with the *1298CC* genotype.[15]

Here, we report the relation of the *MTHFR C677T* and *A1298C* polymorphisms to colorectal cancer in a case-control study. We also examined the interaction of these polymorphisms and alcohol consumption on the risk of colorectal cancer, because alcohol is known to exert adverse effects on folate metabolism.[23] Further, the relation to these polymorphisms was examined by subsite of the colorectum, because previous studies suggested a stronger association of *C677T* with proximal colon cancer.[13, 18]

Materials and Methods

A case-control study was designed to examine the relation of lifestyle factors and genetic susceptibility to the risk of colorectal cancer. Cases were recruited from eight large hospitals in the study area (Fukuoka City and three adjacent areas), and controls were randomly selected in the community by frequency-matching to the distribution of incident cases with respect to sex and 10-year age class. The study protocol was approved by

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the ethical committees of the Faculty of Medical Sciences, Kyushu University and of all but two of the participating hospitals. Those two hospitals had no ethical committee at the time of the survey, and the survey was conducted at those hospitals with permission from the director of each hospital. This procedure conformed to the guidelines of the ethical committee of Kyushu University.

Subjects. Cases comprised a consecutive series of patients with histologically confirmed incident colorectal adenocarcinomas, who were admitted to two university hospitals or six affiliated hospitals for surgical treatment during the period from October 2000 to December 2003. Other eligibility criteria included the following characteristics: age of 20–74 years at the time of diagnosis, residence in the study area, no prior history of partial or total removal of the colorectum, familial adenomatous polyposis or inflammatory bowel disease, and mental competence to give informed consent and to complete the interview. Research nurses visited each hospital weekly, and determined the eligibility of cases by referring to admission logs and medical records. Research nurses contacted each eligible patient with permission from an attending doctor, and interviewed the patient after obtaining written informed consent.

Of 1053 eligible cases, a total of 840 cases (80%) participated in the interview, and 685 out of them gave informed consent to genotyping. Reasons for nonparticipation were patient refusal (n=115), refusal of patient's physician (n=46), and failure to make contact (n=52).

Eligibility criteria for controls were the same as described for cases except for two items, i.e., having no diagnosis of colorectal cancer and age of 20–74 years at the time of selection. A total of 1500 persons were selected as control candidates by two-stage random sampling. Numbers of control candidates by sex and 10-year age class were determined in accordance with sex- and age-specific numbers of incident cases of colorectal cancer in the Osaka Cancer Registry during the period 1988 to 1992.[24] The first step was a random selection of 15 small areas out of 178 in total. The small areas roughly corresponded to primary-school zones, merged with sparse-population zones. Approximately 100 persons were randomly selected in each small area using the municipal resident registry, with allowance for proportions of residents for each small area by sex and 10-year age class.

A letter of invitation was sent to each candidate, and a telephone call was made if the candidate was listed in the telephone directory. At most three additional letters of invitation were mailed to nonrespondents. A total of 833 persons participated in the survey, and 778 gave informed consent to genotyping. Reasons for exclusion and nonparticipation were death (n=7), migration from the study area (n=22), undelivered mail (n=44), mental incompetence (n=19), history of partial or total removal of the colorectum (n=21), diagnosis of colorectal cancer after the survey (n=5), no response (n=158), and refusal (n=391). After exclusion of the first six categories of outcomes (n=118), the net participation rate was calculated as 60% (833/1382).

Neither ethnicity nor nationality was specifically elicited in the survey, but almost all of the eligible cases and control candidates were considered to be Japanese in ethnicity, based on their names.

Interview. Research nurses interviewed cases and controls in person regarding physical activity, smoking, alcohol use, parental history of colorectal cancer, past history of selected diseases, and bowel habit by using a uniform questionnaire. Most of the questions were closed-ended, though some of the quantitative questions were open-ended. Average annual alcohol consumption at the time of 5 years prior to the interview was ascertained. Individuals reported the average number of days per week that alcohol was consumed and the average amount of alcohol per day of drinking alcohol. The amount of alcohol was expressed in the conventional unit; one *go* (180 ml) of *sake*, one large bottle (633 ml) of beer, and half a *go* (90 ml) of *shochu* were each expressed as one unit; and one drink (30 ml) of whisky or brandy and one glass (100 ml) of wine were each converted to half a unit. Reproducibility of the questionnaire was tested on 29 control subjects (14 men and 15 women) with an interval of approximately 1 year, and the reported alcohol intake was highly reproducible (Spearman's r=0.82). The cases were interviewed before or after surgery in the hospital wards, and the interview of controls was done at community halls, clinics, work place, home, or Kyushu University. A sample of venous blood (5 ml) was taken after the interview.

Genotyping. DNA was extracted from the buffy coat by using a commercial kit (Qiagen GmbH, Hilden, Germany). Genotyping was done by one of the authors (GY) using the PCR-RFLP method. The PCR was performed in a reaction mixture of 10 μl containing 0.5 units of *Taq* and 1 μl of template DNA with a concentration of approximately 50–150 ng/μl. The *MTHFR C677T* genotype was determined, as described by Fross *et al.*,[9] by using primers 5′-TGAAG GAGAA GGTGT CTGCG GGA-3′ and 5′-AGGAC GGTGC GGTGA GAGTG-3′. After the initial denaturation at 94°C for 5 min, 30 cycles of PCR were performed for 30 s at 94°C, for 30 s at 62°C, and for 30 s at 72°C, with a final extension at 72°C for 5 min. The PCR product was digested with 12 units of *Hin*fI for 3 h at 37°C in a mixture of 20 μl, which cleaves the 198-bp PCR product into two fragments of 175 and 23 bp when the *C677T* mutation exists. The digested PCR products were separated by electrophoresis on a 3% agarose gel (NuSieve GTG), and visualized with ethidium bromide.

The *MTHFR A1298C* genotype was determined by the method described elsewhere.[10, 14] Primers were 5′-CTTTG GGGAG CTGAA GGACT ACTAC-3′(sense) and 5′-CACTT TGTGA CCATT CCGGT TTG-3′ (antisense). The PCR conditions consisted of an initial denaturation at 94°C for 5 min, 35 cycles of 94°C for 30 s, 62°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 10 min. The PCR product of 163 bp was digested with 10 units of *Mbo*II in a reaction mixture of 20 μl for 3 h at 37°C. The digestion results in fragments of 56, 31, 30, 28, and 18 bp for the *1298A* allele, and fragments of 84, 31, 30, and 18 bp for the *1298C* allele. Electrophoresis was done on 4% agarose gel (MetaPhor FMC), and the genotype was discernible by detection of the 84- and 56-bp fragments.

Statistical analysis. The association of *MTHFR* genotypes with the risk of colorectal cancer was examined by means of multiple logistic regression analysis, including indicator variables for gender, 5-year age class (the lowest class of <40 years), resident area (Fukuoka City or suburban area), and alcohol intake (0, 0.1–0.9, 1.0–1.9, or ≥2.0 units per day) as covariates. Adjusted odds ratio (OR) and 95% confidence interval (CI) were obtained from the logistic regression coefficient and its standard error for the corresponding indicator variable. In the analysis of interaction between genotype and alcohol use, genotypes *677CC* and *677CT* and genotypes *1298AA* and *1298AC* were combined, respectively, and alcohol consumption was categorized into three levels of 0, 0.1–0.9, and ≥1.0 units/day because the number of individuals in the highest alcohol category (≥2.0 units per day) was small. Statistical significance for the interaction was tested by using the likelihood ratio test comparing the logistic models with and without combined terms for the genotypes and alcohol categories. The criterion of statistical significance was a two-sided P value of less than 0.05 or a 95% CI that did not include unity. All statistical analyses were done using the SAS version 8.2 (SAS Institute, Inc., Cary, NC).

Results

Numbers of men in the 685 cases and 778 controls were 426 (62%) and 490 (63%), respectively. Mean age of the cases was 60 years (range 27–74), and that of the controls was 59 years (range 22–75). As for residence, 420 cases (61%) and 501 controls (64%) were residents in Fukuoka City. Cases of cancer of the proximal colon, distal colon, and rectum numbered 150 (22%), 232 (34%), and 290 (42%), respectively; the remaining 13 cases (2%) had cancer at multiple sites.

As regards *MTHFR C677T*, frequencies of the *CC*, *CT*, and *TT* genotypes among controls were 36%, 47%, and 17%, respectively. The distribution of *C677T* genotype in the controls was in agreement with the Hardy-Weinberg equilibrium (P=0.82). The *677TT* genotype was less frequent in cases than in controls, and the adjusted OR of colorectal cancer for the *677TT* genotype compared with the *677CC* genotype was statistically significantly lower than unity (Table 1). Adjusted OR for *677TT* versus *677CC* and *CT* combined was 0.69 (95% CI 0.51–0.93). On the other hand, there was no material difference in the distribution of *MTHFR A1298C* genotypes between cases and controls (Table 1). Distributions of the *A1298C* genotypes in cases and controls were each compatible with the Hardy-Weinberg equilibrium (P=0.995 in cases and P=0.29 in controls). The adjusted OR for the *1298CC* versus *1298AA* genotype was slightly greater than unity, but the increase was not statistically significant. Even the comparison for *1298CC* versus *1298AA* and *1298AC* combined did not result in a statistically significant increase (adjusted OR 1.67, 95% CI 0.91–3.06).

Men and women showed similar associations with both *C677T* and *A1298C* polymorphisms. In men, adjusted ORs (and 95% CIs) for *677CC*, *677CT*, and *677TT* were 1.00 (referent), 0.95 (0.71–1.26), and 0.67 (0.45–1.01), respectively. The corresponding values for women were 1.00 (referent), 0.80 (95% CI 0.55–1.17), and 0.60 (95% CI 0.34–1.05), respectively. Adjusted ORs (and 95% CIs) for *1298AA*, *1298AC*, and *1298CC* were 1.00 (referent), 1.12 (0.84–1.48), and 1.38 (0.60–3.16), respectively, in men and 1.00 (referent), 0.97 (0.66–1.41), and 1.96 (0.79–4.87), respectively, in women.

Table 2 shows the distribution of combined genotypes with respect to *MTHFR C677T* and *A1298C*. The two polymorphisms were at linkage disequilibrium. No individual with the *677TT* genotype had the *1298C* allele, and only one of those having the *677CT* genotype was a variant homozygote of *1298CC*. Nonetheless, the OR for the *677TT* genotype was significantly decreased compared with the *677CC* genotype in the

Table 1. Adjusted odds ratios (OR) and 95% confidence intervals (CI) of colorectal cancer according to *MTHFR C677T* and *A1298C* polymorphisms

MTHFR genotype	Number (%)		Adjusted OR[1] (95% CI)
	Cases	Controls	
C677T			
CC	270 (39.4)	278 (35.7)	1.00 (referent)
CT	330 (48.2)	367 (47.2)	0.89 (0.71–1.12)
TT	85 (12.4)	133 (17.1)	0.64 (0.47–0.89)
A1298C			
AA	438 (64.0)	515 (66.2)	1.00 (referent)
AC	220 (32.1)	244 (31.4)	1.07 (0.85–1.34)
CC	27 (3.9)	19 (2.4)	1.71 (0.93–3.14)

1) Adjusted for gender, 5-year age class, area, and alcohol use.

Table 2. Adjusted odds ratios (OR) and 95% confidence intervals (CI) of colorectal cancer according to *MTHFR C677T* and *A1298C* genotypes in combination

MTHFR C677T		*MTHFR A1298C*		
		AA	*AC*	*CC*
CC	No.[1]	123/126	120/134	27/18
	OR (95% CI)[2]	1.00 (referent)	0.93 (0.65–1.32)	1.53 (0.80–2.95)
CT	No.	230/256	100/110	0/1
	OR (95% CI)	0.89 (0.66–1.22)	0.90 (0.62–1.31)	—
TT	No.	85/133	0/0	0/0
	OR (95% CI)	0.64 (0.44–0.94)	—	—

1) Numbers of cases/controls.
2) Adjusted for gender, 5-year age class, area, and alcohol use.

Table 3. Adjusted odds ratios (OR) and 95% confidence intervals (CI) of colorectal cancer in relation to the *MTHFR C677T* and *A1298C* genotype and alcohol use in combination

MTHFR genotype		Alcohol intake (unit/day)[1]		
		0	<1	1+
C677T				
CC+CT	No.[2]	241/254	124/158	235/233
	OR (95% CI)[3]	1.00 (referent)	0.91 (0.67–1.23)	1.15 (0.85–1.54)
TT	No.	31/57	20/31	34/45
	OR (95% CI)	0.58 (0.36–0.93)	0.73 (0.40–1.33)	0.89 (0.53–1.47)
A1298C				
AA+AC	No.	260/301	140/184	258/274
	OR (95% CI)	1.00 (referent)	0.96 (0.72–1.29)	1.18 (0.89–1.57)
CC	No.	12/10	4/5	11/4
	OR (95% CI)	1.38 (0.58–3.28)	1.03 (0.27–3.93)	3.69 (1.14–12.0)

1) One unit of alcohol intake corresponds to 1 *go* (180 ml) of *sake*, 0.5 *go* (90 ml) of *shochu*, 1 large bottle (633 ml) of beer, 2 drinks (60 ml) of whiskey, and 2 glasses (200 ml) of wine.
2) Numbers of cases/controls.
3) Adjusted for gender, 5-year age class, and area.

Table 4. Adjusted odds ratios (OR) and 95% confidence intervals (CI) of colorectal cancer according to *MTHFR C677T* and *A1298C* genotypes by subsite[1)]

MTHFR genotype	Proximal colon		Distal colon		Rectum	
	No.	OR (95% CI)	No.	OR (95% CI)	No.	OR (95% CI)
C677T						
CC	59	1.00 (referent)	95	1.00 (referent)	110	1.00 (referent)
CT	75	0.95 (0.65–1.40)	105	0.78 (0.56–1.08)	144	0.97 (0.72–1.31)
TT	16	0.58 (0.32–1.05)	32	0.65 (0.41–1.03)	36	0.67 (0.43–1.04)
A1298C						
AA	96	1.00 (referent)	140	1.00 (referent)	192	1.00 (referent)
AC	47	1.05 (0.71–1.54)	81	1.25 (0.91–1.73)	90	1.02 (0.76–1.38)
CC	7	2.09 (0.84–5.22)	11	2.36 (1.08–5.16)	8	1.15 (0.49–2.70)

1) Adjusted for gender, 5-year age class, area, and alcohol use.

group of the *1298AA* genotype, while the *A1298C* polymorphism was unrelated to colorectal cancer in the group of the *677CC* genotype. Further, individuals heterozygous with respect to both *C677T* and *A1298C* polymorphisms showed no measurable decrease in the OR as compared with wild homozygotes of the two polymorphisms.

A decrease in the OR of colorectal cancer associated with the *677TT* genotype was most evident in those with no consumption of alcohol, and the decrease was less in those with higher consumption of alcohol (Table 3). The interaction between *C677T* and alcohol use on the risk of colorectal cancer was not statistically significant, however (P=0.62). Regarding the *A1298C* polymorphism, individuals with a high alcohol consumption who had the *1298CC* genotype showed a statistically significant increase in the OR as compared with those with no alcohol consumption who had the *1298AA* or *1298AC* genotype, although the interaction was not statistically significant (P=0.41). An increase in the OR for the combination of high alcohol consumption and the *1298CC* genotype was also observed in the analysis of individuals with the *677CC* genotype; adjusted OR was 3.16 (95% CI 0.94–10.6) as compared with those with no alcohol consumption who had the *1298A* allele.

The relation of the *C677T* and *A1298C* polymorphisms to proximal colon cancer, distal colon cancer, and rectal cancer is shown in Table 4. Cases with cancer at multiple sites were excluded in this analysis. A decrease in the OR associated with the *677TT* genotype was observed for each site of cancer, although none of the decreases reached statistical significance. As regards the *A1298C* polymorphism, individuals with the *1298CC* genotype showed an approximately 2-fold increase in the OR of proximal and distal colon cancer. Similar results were also obtained in the subgroup analysis limited to individuals with the *677CC* genotype; adjusted ORs of proximal colon cancer, distal colon cancer, and rectal cancer for the *1298CC* versus *1298AA* genotype were 2.18 (95% CI 0.79–6.02), 2.07 (95% CI 0.87–4.94), and 1.04 (95% CI 0.41–2.59), respectively.

Discussion

We observed a decrease in the risk of colorectal cancer associated with the *MTHFR 677TT* genotype. The finding is in agreement with observations in several studies in the United States and in Hawaii,[11–14)] but at variance with the results from other studies in the United States,[15)] Europe,[16–18)] Australia,[19)] Korea,[20)] and Mexico.[21)] In the present study, as also observed by physicians and health professionals in the United States,[11, 12)] a protective association with the *677TT* genotype was primarily confined to those with no alcohol consumption. Alcohol consumption leads to folate depletion, probably by decreasing intestinal absorption and hepatic uptake,[23)] increasing renal excretion,[23)] and cleaving folate.[25)] The thymidylate synthesis pathway, rather than the process of DNA methylation, seems to be biologically linked with the protective association with the *677TT* genotype.[11, 12)] Under a condition of sufficient folate, low activity of MTHFR leads to buildup of 5,10-methylentetrahydrofolate, which is required for conversion of uridylate to thymidylate. An adequate pool of thymidylate decreases deoxyuridylate-induced DNA damage and ensures efficient DNA synthesis and repair.[3–5)] In this regard, inconsistency in the association with *MTHFR 677TT* genotype among studies may be related to different folate levels in different populations. Folate intake seems fairly high among adults in Japan; the average intake was estimated to be 330 μg per day in the National Nutrition Survey in 2001. This level is higher than the average intake for supplement nonusers (290 μg/day) in the period before fortification with folic acid in the United States,[26)] and is near to the average intake in the mid 1980s (400 μg/day) among health professionals in the United States.[27)]

Two previous studies showed that the *MTHFR 677TT* genotype was more strongly[13)] or exclusively[18)] associated with decreased risk of proximal colon cancer. The site-specific analysis is of interest because different molecular alterations have been implicated in carcinogenesis of the proximal and distal sites of the colorectum.[28)] Genetic alterations such as *K-ras* and *p53* mutations were shown to be more frequent in the distal site, while microsatellite instability (MSI) was almost exclusively associated with proximal colon cancer.[29–32)] Interestingly, the *MTHFR 677TT* genotype was shown to be positively associated with MSI-positive colorectal cancer, but not with MSI-negative cancer.[19)] In the present study, however, a decreased risk associated with the *677TT* genotype was observed for both distal colon cancer and rectal cancer, as well as for proximal colon cancer.

Few studies have previously examined the relation between the *MTHFR A1298C* polymorphism and colorectal cancer. A study in the United States showed a statistically significant decrease in the risk of colon cancer for the *1298CC* genotype compared with the *1298AA* genotype, while showing no measurable decrease in the risk associated with the *MTHFR 677TT* genotype.[15)] Two other studies suggested a slightly decreased risk of colon or colorectal cancer associated with the *1298CC* genotype among physicians in the United States[22)] and in Hawaii,[14)] whereas the *1298CC* genotype was associated with a statistically nonsignificant increase in the risk in Germany.[16)] In the present study, individuals with the *1298CC* genotype showed a small, statistically nonsignificant increase in the overall risk of colorectal cancer and a significant increase in the risk when alcohol consumption was high. Moreover, a 2-fold increase in the risk associated with the *1298CC* genotype was noted for proximal and distal colon cancer. These findings are, however, difficult to interpret because of the small numbers of individuals with the *1298CC* genotype in the subgroup analysis. Further studies are needed to confirm the present findings with

regard to the *A1298C* polymorphism.

As reported in many different populations,[33] the *A1298C* polymorphism was at linkage disequilibrium with the *MTHFR C677T* polymorphism. Frequencies of the *677T* and *1298C* alleles were 41% and 18%, respectively, among the controls in the present study. These frequencies are similar to those reported in random samples of Japanese in Hawaii[14] and Japan,[34] but the frequencies of *677T* and *1298C* alleles in Japanese seem to differ from the frequencies in Caucasians. Frequencies of both the *677T* and *1298C* alleles are generally in the range of 30–35% in Caucasians.[11–19] The relatively lower frequency of the *1298C* allele makes it somewhat difficult to study the relation of *MTFHR A1298C* to colorectal cancer in Japanese.

Another point of interest was whether heterozygotes for both *MTHFR C677T* and *A1298C* polymorphisms had lower risk of colorectal cancer as compared with wild homozygotes of the two polymorphisms. Individuals with combined heterozygosity for *MTHFR 677CT* and *1298AC* showed reduced enzyme activity, elevated plasma homocysteine, and decreased plasma folate, similar to those with the *677TT* genotype.[10] However, there was no evidence that the combined genotypes of *677CT* and *1298AC* conferred a decreased risk of colorectal cancer in the present study.

Several methodological advantages of the present study deserve discussion. This is probably the second largest study that has ever been reported regarding the MTHFR genotype and colorectal cancer. Among the largest studies are a multicenter study including 1467 colon cancer cases and 1821 controls in the United States,[13] a study of 548 cases and 656 community controls in Hawaii,[14] and a study of 555 cases and 875 controls in North Carolina.[15] The size of study is particularly important in investigating the role of rare genotypes in the gene-environment, or gene-gene interaction. Also notable are the fairly high participation rates in both cases (80%) and controls (60%). It is generally argued that bias related to selection or confounding is unlikely to occur in studies of genotypes and disease because of the so-called Mendelian randomization,[35] but selection as regards environmental factors modifying the association with a specific genotype could distort the true association with the genotype. We used alcohol consumption five years prior to the interview. We have no data as to how valid the recalled alcohol consumption in the past was, although it was found to be highly reproducible. The lack of information as to folate intake was another weakness in the present study. Knowledge of the interaction between folate intake and the *MTHFR* polymorphisms would be useful in elucidating the role of the *MTHFR* polymorphisms in colorectal carcinogenesis.

In summary, a large case-control study in Japan showed a decreased risk of colorectal cancer associated with the *MTHFR 677TT* genotype, especially among individuals with no alcohol consumption. A decreased risk associated with the *MTHFR 677TT* genotype was observed for cancers of the proximal colon, distal colon, and rectum. The *MTHFR 1298CC* genotype was associated with an increased risk when alcohol consumption was high, and was also associated with increased risks of proximal and distal cancer. The latter findings need careful interpretation and confirmation in larger studies, because very few individuals had the *1298CC* genotype.

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1. Lucock M. Folic acid: nutritional biochemistry, molecular biology and role in disease processes. *Mol Genet Metab* 2000; **71**: 121–38.
2. Sharp L, Little J. Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. *Am J Epidemiol* 2004; **159**: 423–43.
3. Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, Wickramasinghe SN, Everson RB, Ames BN. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. *Proc Natl Acad Sci USA* 1997; **94**: 3290–5.
4. Duthie SJ. Folic acid deficiency and cancer: mechanisms of DNA instability. *Br Med Bull* 1999; **55**: 578–92.
5. Duthie SJ, Narayanan S, Blum S, Piric L, Brand G. Folate deficiency *in vitro* induces uracil misincorporation and DNA hypomethylation and inhibits DNA excision repair in immortalized normal colon epithelial cells. *Nutr Cancer* 2000; **37**: 245–51.
6. Laird PW, Jaenisch R. The role of DNA methylation in cancer genetic and epigenetics. *Annu Rev Genet* 1996; **30**: 441–64.
7. Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa JP. CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci USA* 1999; **96**: 8681–6.
8. van Rijnsoever M, Grieu F, Elsaleh H, Joseph D, Iacopetta B. Characterisation of colorectal cancers showing hypermethylation at multiple CpG islands. *Gut* 2002; **51**: 797–802.
9. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP *et al.* A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995; **10**: 111–3.
10. van der Put NM, Gabreels F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, van den Heuvel LP, Blom HJ. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? *Am J Hum Genet* 1998; **62**: 1044–51.
11. Chen J, Giovannucci E, Kelsey K, Rimm EB, Stampfer MJ, Colditz GA, Spiegelman D, Willett WC, Hunter DJ. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res* 1996; **56**: 4862–4.
12. Ma J, Stampfer MJ, Giovannucci E, Artigas C, Hunter DJ, Fuchs C, Willett WC, Selhub J, Hennekens CH, Rozen R. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. *Cancer Res* 1997; **57**: 1098–102.
13. Slattery ML, Potter JD, Samowitz W, Schaffer D, Leppert M. Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. *Cancer Epidemiol Biomarkers Prev* 1999; **8**: 513–8.
14. Le Marchand L, Donlon T, Hankin JH, Kolonel LN, Wilkens LR, Seifried A. B-Vitamin intake, metabolic genes, and colorectal cancer risk (United States). *Cancer Causes Control* 2002; **13**: 239–48.
15. Keku T, Millikan R, Worley K, Winkel S, Eaton A, Biscocho L, Martin C, Sandler R. 5,10-Methylenetetrahydrofolate reductase, codon 677 and 1298 polymorphisms and colon cancer in African Americans and whites. *Cancer Epidemiol Biomarkers Prev* 2002; **11**: 1611–20.
16. Plaschke J, Schwanebeck U, Pistorius S, Saeger HD, Schackert HK. Methylenetetrahydrofolate reductase polymorphisms and risk of sporadic and hereditary colorectal cancer with or without microsatellite instability. *Cancer Lett* 2003; **191**: 179–85.
17. Sachse C, Smith G, Wilkie MJ, Barrett JH, Waxman R, Sullivan F, Forman D, Bishop DT, Wolf CR. A pharmacogenetic study to investigate the role of dietary carcinogens in the etiology of colorectal cancer. *Carcinogenesis* 2002; **23**: 1839–49.
18. Toffoli G, Gafa R, Russo A, Lanza G, Dolcetti R, Sartor F, Libra M, Viel A, Boiocchi M. Methylenetetrahydrofolate reductase 677C→T polymorphism and risk of proximal colon cancer in north Italy. *Clin Cancer Res* 2003; **9**: 743–8.
19. Shannon B, Gnanasampanthan S, Beilby J, Iacopetta B. A polymorphism in the methylenetetrahydrofolate reductase gene predisposes to colorectal cancers with microsatellite instability. *Gut* 2002; **50**: 520–4.
20. Park KS, Mok JW, Kim JC. The 677C>T mutation in 5,10-methylenetet-

rahydrofolate reductase and colorectal cancer risk. *Genet Test* 1999; **3**: 233–6.
21. Delgado-Enciso I, Martinez-Garza SG, Rojas-Martinez A, Ortiz-Lopez R, Bosques-Padilla F, Calderon-Garciduenas AL, Zarate-Gomez M, Barrera-Saldana HA. 677T mutation of the MTHFR gene in adenomas and colorectal cancer in a population sample from the Northeastern Mexico. Preliminary results. *Rev Gastroenterol Mex* 2001; **66**: 32–7.
22. Chen J, Ma J, Stampfer MJ, Palomeque C, Selhub J, Hunter DJ. Linkage disequilibrium between the 677C>T and 1298A>C polymorphisms in human methylenetetrahydrofolate reductase gene and their contributions to risk of colorectal cancer. *Pharmacogenetics* 2002; **12**: 339–42.
23. Halsted CH, Villanueva JA, Devlin AM, Chandler CJ. Metabolic interactions of alcohol and folate. *J Nutr* 2002; **132**: 2367S–72S.
24. Parkin DM, Whelan SL, Ferlay J, Raymond L, Young J (eds). Cancer incidence in five continents. vol VII. Lyon: IARC; 1998.
25. Shaw S, Jayatilleke E, Herbert V, Colman N. Cleavage of folates during ethanol metabolism. Role of acetaldehyde/xanthine oxidase-generated superoxide. *Biochem J* 1989; **257**: 277–80.
26. Bailey LB. Folate, methyl-related nutrients, alcohol, and the MTHFR 677C→T polymorphism affect cancer risk: intake recommendations. *J Nutr* 2003; **133**: 3748S–58S.
27. Giovannucci E, Chen J, Smith-Warner SA, Rim EB, Fuchs CS, Palomeque C, Willett W, Hunter DJ. Methylenetetrahydrofolate reductase, alcohol dehydrogenase, diet, and risk of colorectal adenomas. *Cancer Epidemiol Biomarkers Prev* 2003; **12**: 970–9.
28. Richman S, Adlard J. Left and right sided large bowel cancer. *BMJ* 2002; **324**: 931–2.
29. Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science* 1993; **260**: 816–9.
30. Breivik J, Lothe RA, Meling GI, Rognum TO, Borresen-Dale AL, Gaudernack G. Different genetic pathways to proximal and distal colorectal cancer influenced by sex-related factors. *Int J Cancer* 1997; **74**: 664–9.
31. Soong R, Powell B, Elsaleh H, Gnanasampanthan G, Smith DR, Goh HS, Joseph D, Iacopetta B. Prognostic significance of TP53 gene mutation in 995 cases of colorectal carcinoma. Influence of tumour site, stage, adjuvant chemotherapy and type of mutation. *Eur J Cancer* 2000; **36**: 2053–60.
32. Elsaleh H, Joseph D, Grieu F, Zeps N, Spry N, Iacopetta B. Association of tumour site and sex with survival benefit from adjuvant chemotherapy in colorectal cancer. *Lancet* 2000; **355**: 1745–50.
33. Ogino S, Wilson RB. Genotype and haplotype distributions of MTHFR677C>T and 1298A>C single nucleotide polymorphisms: a meta-analysis. *J Hum Genet* 2003; **48**: 1–7.
34. Yoshimura K, Hanaoka T, Ohnami S, Ohnami S, Kohno T, Liu Y, Yoshida T, Sakamoto H, Tsugane S. Allele frequencies of single nucleotide polymorphisms (SNPs) in 40 candidate genes for gene-environment studies on cancer: data from population-based Japanese random samples. *J Hum Genet* 2003; **48**: 654–8.
35. Davey Smith G, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol* 2003; **32**: 1–22.

Diabetologia

Coffee consumption and glucose tolerance status in middle-aged Japanese men

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Abstract

Aims/hypothesis. Several studies have reported that coffee has a protective effect against the development of type 2 diabetes. However, few of these studies used the standard glucose tolerance test to diagnose type 2 diabetes. The aim of this study was to investigate the relationship between coffee and green tea consumption and glucose tolerance status as determined using a 75-g OGTT.

Methods. We performed a cross-sectional study of 3224 male officials of the self-defence forces. Glucose tolerance status was determined in accordance with the 1998 World Health Organization criteria, and average intakes of coffee and green tea over the previous year were assessed by a self-administered questionnaire. The figures obtained were adjusted for BMI, physical activity and other factors.

Results. A total of 1130 men were identified as having glucose intolerance (IFG, IGT or type 2 diabetes). Compared with those who did not consume coffee on a daily basis, fasting and 2-h post-load plasma glucose levels were 1.5% and 4.3% lower in those who drank 5 cups of coffee or more per day respectively. The adjusted odds ratios of glucose intolerance for categories of <1, 1–2, 3–4 and ≥5 cups of coffee per day were 1.0 (referent), 0.8 (95% CI 0.6–1.0), 0.7 (95% CI 0.6–0.9) and 0.7 (95% CI 0.5–0.9) respectively (p=0.0001 for trend). No clear association was observed between green tea drinking and glucose tolerance status.

Conclusions/interpretation. Coffee consumption may inhibit postprandial hyperglycaemia and thereby protect against the development of type 2 diabetes mellitus.

Keywords Type 2 diabetes · Coffee · Cross-sectional study · Middle-aged · Japanese · Men.

Introduction

Type 2 diabetes has become a global health burden; worldwide, the number of people with type 2 diabetes was approximately 135 million in 1995, and this figure is predicted to rise above 300 million by 2025 [1]. In Japan, it is estimated that nearly 7 million individuals suffer from type 2 diabetes, and that another 7 million have a pre-diabetic condition [2]. Of the lifestyle factors associated with the risk of type 2 diabetes, obesity and physical inactivity are the two most important factors involved in the development of the disease [3]. Additionally, it has been suggested that Japanese individuals may have a higher genetic susceptibility to type 2 diabetes [4, 5].

Recent epidemiological studies have suggested a possible protective effect of coffee against type 2 diabetes. A prospective study performed in the Netherlands reported that coffee drinking was associated with a decreased risk of type 2 diabetes [6]. This finding has since been replicated in several follow-up and cross-sectional studies [7, 8, 9, 10, 11]. However, a

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Abbreviations: MET, metabolic equivalents · SDF, self-defence forces

health examination survey of the Finnish population [12] and a population-based study of Pima Indians [13] failed to observe a protective effect of coffee. Only two of these studies used the standard glucose tolerance test to diagnose type 2 diabetes [11, 13]. The aim of the present study was to investigate the relationship between daily intakes of coffee and green tea and glucose tolerance status as determined by a 75-g OGTT in middle-aged Japanese men.

Subjects and methods

Study population. Subjects were male officials in the self-defence forces (SDF) who underwent a pre-retirement health examination between January 1997 and March 2002 at the Self-Defense Forces Fukuoka Hospital and the Self-Defense Forces Kumamoto Hospital; these two hospitals cover the Kyushu district. All officials retiring from the SDF receive a pre-retirement health examination as part of a nationwide programme which offers a comprehensive medical examination. Details of the health examination have been described elsewhere [14, 15, 16, 17].

We consecutively recruited 3413 men aged 46–59 years, 3224 of whom were included in the present study. Of the remainder, five men refused to participate in the study and 184 men were excluded for the following reasons: endocrine diseases (n=15), chronic pancreatitis (n=9), chronic hepatitis or liver cirrhosis (n=79), use of steroids (n=9), past history of gastrectomy (n=57) and missing information as regards covariates under study (n=20). Some of the men met two or more of the exclusion criteria. There were 17 men whose glucose tolerance status was not determined.

The study was approved by the Ethics Committee of the Kyushu University Faculty of Medical Sciences. All study subjects gave written informed consent prior to their participation in the study.

Glucose and other measurements. Over a 5-day admission period, routine medical tests and examinations included a 75-g OGTT. After an overnight fast, venous blood was drawn for measurement of plasma glucose before and 2 h after the oral glucose load. Plasma glucose levels were determined by the glucose oxidase method using commercial reagents (Shino Test, Tokyo, Japan). Subjects were classified as having normal glucose tolerance, IFG, IGT, or type 2 diabetes in accordance with the World Health Organization (WHO) diagnostic criteria, as revised in 1998 [18]. Men with a history of dietary or drug treatment for type 2 diabetes were regarded as having known type 2 diabetes, irrespective of their glucose levels. Body weight and height were recorded, and BMI (kg/m^2) was calculated as a measure of obesity.

Assessment of coffee and green tea consumption and lifestyle characteristics. A self-administered questionnaire was used to ascertain coffee and green tea consumptions, smoking habits, alcohol use, leisure-time physical activity and other lifestyle characteristics.

Average weekly frequencies of coffee and green tea drinking over the previous year were obtained. Those drinking coffee and green tea on a daily basis reported the number of cups of each consumed per day. In a validation study based on the 28-day diet record over the past year [19], the estimated intake of each beverage showed good agreement between the two methods. The Spearman rank correlation coefficients for coffee and green tea were 0.75 and 0.64 respectively. Caffeine ingestion was estimated using the published caffeine concentrations in coffee (0.04%) and green tea (0.02%) [20]. An average serving of one cup of coffee was estimated to be equal to 150 ml, while an average serving of one cup of green tea was estimated to be equal to 100 ml. Coffee accounted for the majority (66%) of caffeine intake. Ever-smokers were defined as individuals who had ever smoked one cigarette or more per day for at least 1 year or longer. The cumulative exposure of ever-smokers to cigarette smoking was expressed as cigarette-years, the average number of cigarettes smoked per day multiplied by years of smoking. Alcohol drinkers were defined as those who had consumed alcohol once or more per week over a period of 1 year or longer. Past-drinkers were distinguished from lifelong non-drinkers. Alcohol consumption was calculated for current drinkers based on the reported frequencies of consumption of five different alcoholic beverages (sake, shochu, beer, whisky [including brandy] and wine) and amount consumed per occasion over the previous year. Questions on leisure-time physical activity were slightly changed in April 1999. Prior to the revision, the subjects were first asked about their average frequency of regular participation in recreational exercise and sport over the previous year using a closed-ended question (none, 1–2, 3–4, 5–6 times per week or daily). If the subjects participated in recreational physical activity once per week or more frequently, they reported the type of regular exercise or sport and the time spent per occasion. Up to three types of regular exercises were recorded. In the revised questionnaire, the subjects were first asked whether they had regularly participated in recreational activity (one or more times per week) during the previous year. Those who had regularly participated reported up to three types of physical activities, together with the frequency per week and time spent per occasion on each activity. The reported type of exercise was classified as light, moderate, heavy or very heavy activity in accordance with the published energy expenditure requirements in terms of metabolic equivalents (MET) for different physical activities [21]. The time spent on recreational exercise was multiplied by the corresponding MET value (light 2, moderate 4, heavy 6 or very heavy 8) to yield a MET-hour score.

Statistical analysis. Differences in means, medians and proportions of confounding factors across the coffee and green tea consumption categories were statistically tested by one-way ANOVA, the Kruskal–Wallis test and the chi square test respectively. Analysis of covariance was used to calculate the mean concentrations of fasting and 2-h post-load plasma glucose according to the consumption of each beverage in subjects without known type 2 diabetes, with adjustment for possible confounding effects of hospital, rank in the SDF, parental history of diabetes, BMI, cigarette smoking, alcohol intake and leisure time physical activity. In addition, as coffee and green tea drinking were inversely correlated (Spearman rank correlation −0.15), we mutually adjusted for coffee and green tea consumption. Odds ratios and 95% confidence intervals of IFG, IGT, newly diagnosed type 2 diabetes and known type 2 diabetes in relation to levels of coffee and green tea consumption, were obtained from multiple logistic regression analysis while adjusting for the above-mentioned confounding variables, using normal glucose tolerance as the referent group. Trends of the association were assessed by the Wald statistic in logistic regression analysis, in which ordinal values were assigned to categories of each factor.

Categories of coffee and green tea consumption were both defined as <1, 1–2, 3–4 and ≥5 cups per day. BMI was divided into quartiles using the 25th, 50th and 75th percentiles in the distribution as cut-off points. Use of BMI as a continuous vari-

Table 1. Potential confounding variables according to daily coffee consumption

Variable	Coffee consumption (cups per day)								p value[a]
	<1		1–2		3–4		≥5		
	(n=1145)		(n=717)		(n=805)		(n=557)		
Age (years)	52.4	(0.9)	52.4	(1.0)	52.4	(0.9)	52.4	(0.9)	0.73
Examination at Fukuoka Hospital	54		62		66		73		0.001
Low ranks in the SDF	67		64		61		54		0.001
Parental history of diabetes	10		8		10		11		0.44
BMI (kg/m^2)	23.9	(2.6)	24.0	(2.7)	23.6	(2.5)	24.0	(2.7)	0.009
Ever-smoking	68		72		77		84		0.001
Cigarette-years	510	(300–640)	600	(400–720)	600	(400–750)	640	(500–900)	0.0001
Current alcohol use	86		83		82		77		0.003
Alcohol (ml/day)	48	(25–74)	46	(25–72)	45	(26–71)	44	(22–71)	0.13
MET-hours per week	16	(5–30)	16	(6–27)	14	(4–27)	13	(1–27)	0.002

Data are percentages, means (SD) or medians (interquartile ranges). [a] One-way ANOVA for mean difference, the Kruskal–Wallis test for median difference and the chi-square test for percentage difference

Table 2. Potential confounding variables according to daily green tea consumption

Variable	Green tea consumption (cups per day)								p value[a]
	<1		1–2		3–4		≥5		
	(n=453)		(n=622)		(n=1083)		(n=1066)		
Age (years)	52.4	(0.9)	52.4	(0.9)	52.4	(1.0)	52.4	(0.9)	0.68
Examination at Fukuoka Hospital	66		63		64		58		0.002
Low ranks in the SDF	65		60		62		63		0.45
Parental history of diabetes	10		10		10		9		0.83
BMI (kg/m^2)	24.1	(2.8)	23.8	(2.5)	23.8	(2.5)	23.9	(2.6)	0.25
Ever-smoking	74		75		75		72		0.31
Cigarette-years	600	(400–800)	600	(400–700)	600	(400–720)	600	(400–720)	0.27
Current alcohol use	85		86		84		79		0.02
Alcohol (ml/day)	45	(23–73)	47	(25–73)	45	(25–71)	45	(24–72)	0.78
MET-hours per week	12	(0–27)	15	(4–27)	16	(6–28)	16	(4–28)	0.12

Data are percentages, means (SD) or medians (interquartile ranges). [a] One-way ANOVA for mean difference, the Kruskal–Wallis test for median difference and the chi-square test for percentage difference

able did not change the results. Rank in the SDF was divided into two categories (low or high); cigarette smoking was divided into four categories (never-smokers and tertiles of cigarette-years in ever-smokers); alcohol intake was stratified into five categories (never-drinkers, past-drinkers and tertiles of alcohol consumed per day in current drinkers); and leisure time physical activity was divided into four levels (no regular exercise and tertiles of MET-hours per week in regular participants). Age was within a limited range from 46–59 years, and 98% of the subjects were aged 50–54 years. Age was thus not adjusted for in analysis. Repeated analyses controlling for age as a continuous variable produced essentially the same results as reported below. Indicator variables representing categories of the above-mentioned confounding factors were included in the models as independent variables.

Two-sided p values less than 0.05 were regarded as statistically significant. All analyses were performed using Statistical Analysis System (SAS), Version 6.12 (SAS Institute, Cary, N.C., USA).

Results

Among the 3224 male officials, there were 204 (6%) prevalent cases of IFG, 568 (18%) of IGT, 171 (5%) of newly diagnosed type 2 diabetes, and 187 (6%) of known type 2 diabetes.

The characteristics of the study sample varied according to the level of coffee consumption (Table 1). Men in the higher coffee consumption categories were more likely to be examinees at the Fukuoka Hospital and were more likely to have achieved a higher rank in the SDF. The frequency of ever-smokers and the cumulative amount of cigarettes smoked increased with increased coffee consumption. Furthermore, subjects with a higher intake of coffee were less likely to be current drinkers of alcohol and were less likely to participate in physical exercise during leisure time. BMI varied with coffee consumption; however, this associa-

Table 3. Plasma glucose concentrations according to daily consumption of coffee or green tea

	Daily consumption (cups per day)								*p* value for trend
	<1		1–2		3–4		≥5		
	Crude mean (SEM)								
Coffee									
Fasting (mmol/l)	5.63	(0.02)	5.52	(0.03)[a]	5.51	(0.03)[a]	5.49	(0.03)[a]	0.0003
2-h post-load (mmol/l)	6.92	(0.06)	6.74	(0.08)	6.60	(0.07)[a]	6.66	(0.09)[a]	0.001
Green tea									
Fasting (mmol/l)	5.60	(0.04)	5.57	(0.03)	5.53	(0.02)	5.55	(0.02)	0.18
2-h post-load (mmol/l)	6.76	(0.10)	6.71	(0.08)	6.68	(0.06)	6.86	(0.06)	0.23
	Adjusted mean (SEM)[b]								
Coffee									
Fasting (mmol/l)	5.60	(0.02)	5.52	(0.03)[a]	5.53	(0.03)[a]	5.52	(0.03)[a]	0.02
2-h post-load (mmol/l)	6.92	(0.06)	6.74	(0.07)	6.63	(0.07)[a]	6.63	(0.09)[a]	0.001
Green tea									
Fasting (mmol/l)	5.59	(0.04)	5.57	(0.03)	5.53	(0.02)	5.54	(0.02)	0.16
2-h post-load (mmol/l)	6.71	(0.09)	6.72	(0.08)	6.69	(0.06)	6.86	(0.06)	0.15

Men with known type 2 diabetes were excluded from the analysis. [a] $p<0.05$ vs drinking <1 cup per day; [b] adjusted for hospital, rank, parental history of diabetes, BMI, smoking, alcohol intake, leisure time physical activity, and either green tea or coffee intake

Table 4. Odds ratio for each glucose tolerance status according to level of coffee consumption

Glucose tolerance status		Coffee consumption (cups per day)				*p* value for trend
		<1	1–2	3–4	≥5	
Normal	No.	680	475	557	382	
IFG	No.	81	42	49	32	
	Crude OR	1.0 (referent)	0.7 (0.5–1.1)	0.7 (0.5–1.1)	0.7 (0.5–1.1)	0.07
	Adjusted OR[a]	1.0 (referent)	0.8 (0.5–1.2)	0.9 (0.6–1.3)	0.9 (0.6–1.4)	0.53
IGT	No.	234	138	116	80	
	Crude OR	1.0 (referent)	0.8 (0.7–1.1)	0.6 (0.5–0.8)	0.6 (0.5–0.8)	<0.0001
	Adjusted OR[a]	1.0 (referent)	0.8 (0.6–1.1)	0.6 (0.5–0.8)	0.6 (0.4–0.8)	<0.0001
Newly diagnosed type 2 diabetes	No.	70	30	40	31	
	Crude OR	1.0 (referent)	0.6 (0.4–1.0)	0.7 (0.5–1.0)	0.8 (0.5–1.2)	0.18
	Adjusted OR[a]	1.0 (referent)	0.6 (0.4–1.0)	0.8 (0.5–1.2)	0.8 (0.5–1.3)	0.37
Known type 2 diabetes	No.	80	32	43	32	
	Crude OR	1.0 (referent)	0.6 (0.4–0.9)	0.7 (0.4–1.0)	0.7 (0.5–1.1)	0.06
	Adjusted OR[a]	1.0 (referent)	0.6 (0.4–1.0)	0.7 (0.4–1.0)	0.7 (0.4–1.1)	0.09

Data are odds ratios (95% CI). [a] Adjusted for hospital, rank, parental history of diabetes, BMI, smoking, alcohol intake, leisure time physical activity and green tea intake. OR, odds ratio

tion was not simple. Few variables were associated with green tea consumption (Table 2). Subjects with a higher intake of green tea were less likely to have undergone their health examination at the Fukuoka Hospital and were less likely to be current alcohol users.

Table 3 summarises the crude and adjusted mean concentrations of fasting and 2-h post-load plasma glucose according to coffee and green tea consumption, excluding those with known type 2 diabetes. Subjects who drank coffee on a daily basis had lower fasting and post-load plasma glucose levels. Surprisingly, the reduction in post-load plasma glucose concentrations with increasing levels of coffee intake was more pronounced than the corresponding decrease in fasting plasma glucose concentrations. Compared with those who did not consume coffee on a daily basis, fasting and post-load glucose concentrations were 1.5% and 4.3% lower in men who drank 5 cups of coffee or more per day respectively. Green tea consumption was not inversely associated with either fasting or

Table 5. Odds ratio for each glucose tolerance status according to level of green tea consumption

Glucose tolerance status		Green tea consumption (cups per day)				*p* value for trend
		<1	1–2	3–4	≥5	
Normal	No.	277	414	735	668	
IFG	No.	35	48	64	57	
	Crude OR	1.0 (referent)	0.9 (0.6–1.5)	0.7 (0.4–1.1)	0.7 (0.4–1.1)	0.04
	Adjusted OR [a]	1.0 (referent)	0.9 (0.6–1.5)	0.7 (0.4–1.1)	0.6 (0.4–1.0)	0.02
IGT	No.	86	103	180	199	
	Crude OR	1.0 (referent)	0.8 (0.6–1.1)	0.8 (0.6–1.1)	1.0 (0.7–1.3)	0.85
	Adjusted OR[a]	1.0 (referent)	0.8 (0.6–1.2)	0.8 (0.6–1.1)	1.0 (0.7–1.3)	0.85
Newly diagnosed type 2 diabetes	No.	25	33	49	64	
	Crude OR	1.0 (referent)	0.9 (0.5–1.5)	0.7 (0.4–1.2)	1.1 (0.7–1.7)	0.69
	Adjusted OR[a]	1.0 (referent)	1.0 (0.6–1.7)	0.8 (0.5–1.3)	1.1 (0.7–1.9)	0.56
Known type 2 diabetes	No.	30	24	55	78	
	Crude OR	1.0 (referent)	0.5 (0.3–0.9)	0.7 (0.4–1.1)	1.1 (0.7–1.7)	0.15
	Adjusted OR[a]	1.0 (referent)	0.6 (0.3–1.0)	0.7 (0.4–1.2)	1.1 (0.7–1.7)	0.25

Data are odds ratios (95% CI). [a] Adjusted for hospital, rank, parental history of diabetes, BMI, smoking, alcohol intake, leisure time physical activity and coffee intake. OR, odds ratio

post-load plasma glucose concentrations. Those with the highest green tea intake had higher post-load plasma glucose concentrations; however, this trend was not statistically significant.

Tables 4 and 5 show the crude and adjusted odds ratios of IFG, IGT, newly diagnosed type 2 diabetes and known type 2 diabetes according to levels of coffee and green tea consumption, with normal glucose tolerance as the referent group. Coffee intake was not independently associated with IFG. Odds ratios of IGT, newly diagnosed type 2 diabetes and known type 2 diabetes were generally lower than unity among coffee drinkers, although not all of the decreases in odds ratios were statistically significant. The inverse association was particularly evident for IGT ($p<0.0001$ for trend). No clear association was observed between green tea drinking and glucose tolerance status, although green tea drinkers had a reduced odds ratio for IFG.

The adjusted odds ratios of glucose intolerance (IFG, IGT and type 2 diabetes) for categories of <1, 1–2, 3–4 and ≥5 cups of coffee per day were 1.0 (referent), 0.8 (95% CI 0.6–1.0), 0.7 (95% CI 0.6–0.9) and 0.7 (95% CI 0.5–0.9) respectively ($p=0.0001$ for trend). The corresponding values for green tea were 1.0 (referent), 0.8 (95% CI 0.6–1.1), 0.8 (95% CI 0.6–1.0) and 1.0 (95% CI 0.8–1.2) respectively ($p=0.95$ for trend).

We also performed an analysis of caffeine intake, with adjustment for hospital, rank in the SDF, parental history of diabetes, BMI, cigarette smoking, alcohol intake and leisure time physical activity. As caffeine ingestion was strongly correlated with coffee consumption (Spearman correlation coefficient 0.89), coffee and caffeine were not simultaneously included in the analysis. The adjusted mean concentrations of fasting and post-load plasma glucose according to quartile of daily caffeine intake were 5.63, 5.53, 5.55 and 5.50 mmol/l ($p=0.004$ for trend) and 6.88, 6.78, 6.75 and 6.62 mmol/l ($p=0.01$ for trend) respectively. Compared with those in the lowest quartile of daily caffeine intake, fasting and post-load plasma glucose concentrations were 2.2% and 3.8% lower in men in the highest quartile. The adjusted odds ratios of glucose intolerance according to quartile of daily caffeine consumption were 1.0 (referent), 0.8 (95% CI 0.6–1.0), 0.8 (95% CI 0.6–1.0) and 0.7 (95% CI 0.5–0.8) respectively ($p=0.0005$ for trend).

Discussion

In the present study, we have demonstrated an inverse relationship between coffee consumption and glucose intolerance, particularly IGT, by using the standard glucose tolerance test. Our study adds to increasing evidence that coffee affords protection against the development of type 2 diabetes. The present findings are consistent with those of recent prospective studies [6, 8, 9, 10] and cross-sectional studies [7, 11], but are in disagreement with the observations reported by population-based studies in the Finnish population [12] and among Pima Indians [13]. With regard to the assessment of type 2 diabetes, the majority of studies used self-reported questionnaires and/or registers of diabetic patients receiving treatment, whereas few studies adopted the standard glucose tolerance test [11, 13].

Although coffee contains many compounds which mediate a variety of physiological functions, the main biological effects of coffee drinking have been attributed to caffeine [22]. A prospective study in the

US reported that total caffeine estimated from coffee and other sources was associated with a statistically significant lower risk of type 2 diabetes, and that this association remained significant after adjustment for coffee consumption [8]. A cross-sectional study in Japan also demonstrated that total caffeine estimated from coffee and three types of tea was associated with lower fasting glucose concentrations, and that the inverse association between caffeine from coffee alone and fasting plasma glucose was stronger than that for total caffeine [7]. In our study, total caffeine estimated from coffee and green tea was inversely related to fasting and post-load plasma glucose and to the risk of type 2 diabetes. The magnitude of the inverse relationship between caffeine and glucose intolerance was similar to that observed for coffee. We were not able to address the question of whether or not coffee is related to glucose intolerance independently of caffeine, because coffee consumption and caffeine intake were strongly correlated with each other.

Of particular interest were the findings that coffee consumption was more strongly associated with decreased concentrations of post-load plasma glucose than fasting plasma glucose, and that coffee consumption was almost unrelated to IFG. These results suggest that coffee consumption may inhibit postprandial hyperglycaemia and thereby afford protection against the development of type 2 diabetes mellitus.

The present study had several associated strengths in addition to the use of a 75-g OGTT. Almost all SDF officials in the Kyushu district participated in the health examination programme at two SDF hospitals prior to their retirement. Thus, the study population was almost unselected. In addition, the study population was relatively large. The subjects were relatively homogeneous in terms of social background as well as age range.

One of the limitations of the present study was its cross-sectional nature. An association observed in a cross-sectional study does not necessarily indicate a causal relationship. As diabetes may have affected coffee consumption levels, we treated men with a history of diabetes separately in the analysis. Another limitation was that the observed relationship between coffee drinking and glucose intolerance may be attributed in part to undetermined characteristics of coffee drinkers, although important factors associated with type 2 diabetes were statistically adjusted for. Caffeine ingestion was estimated based on only coffee and green tea consumption; thus, caffeine intake may have been misclassified to some extent. However, other caffeine-containing beverages, such as black tea and cola, are probably consumed to a far lesser extent by middle-aged men in Japan, as suggested by the survey on beverage preference [23]. Finally, the study subjects were men who served in the SDF up to retirement, and may therefore differ from the general population with respect to various lifestyle characteristics. Consequently, our findings may not be directly applied to the general population.

In conclusion, using a 75-g OGTT to diagnose diabetes, the present study of middle-aged Japanese men provides further evidence for the protective role of coffee or caffeine in the pathogenesis of type 2 diabetes. The biological effects of caffeine and other constituents of coffee deserve further investigation.

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References

1. King H, Aubert RE, Herman WH (1998) Global burden of diabetes, 1995–2025: prevalence, numerical estimates, and projections. Diabetes Care 21:1414–1431
2. Akazawa Y, Akazawa S (2002) Diabetes in Japan and the global burden of diabetes [article in Japanese]. Nippon Rinsho 60 [Suppl 8]:93–102
3. Hu FB, Manson JE, Stampfer MJ et al. (2001) Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. N Engl J Med 345:790–797
4. Fujimoto WY, Leonetti DL, Kinyoun JL et al. (1987) Prevalence of diabetes mellitus and impaired glucose tolerance among second-generation Japanese-American men. Diabetes 36:721–729
5. Gimeno SG, Ferreira SR, Franco LJ, Hirai AT, Matsumura L, Moises RS (2002) Prevalence and 7-year incidence of Type II diabetes mellitus in a Japanese-Brazilian population: an alarming public health problem. Diabetologia 45:1635–1638
6. van Dam RM, Feskens EJ (2002) Coffee consumption and risk of type 2 diabetes mellitus. Lancet 360:1477–1478
7. Isogawa A, Noda M, Takahashi Y, Kadowaki T, Tsugane S (2003) Coffee consumption and risk of type 2 diabetes mellitus. Lancet 361:703–704 (Letter)
8. Salazar-Martinez E, Willett WC, Ascherio A et al. (2004) Coffee consumption and risk for type 2 diabetes mellitus. Ann Intern Med 140:1–8
9. Rosengren A, Dotevall A, Wilhelmsen L, Thelle D, Johansson S (2004) Coffee and incidence of diabetes in Swedish women: a prospective 18-year follow-up study. J Intern Med 255:89–95
10. Tuomilehto J, Hu G, Bidel S, Lindstrom J, Jousilahti P (2004) Coffee consumption and risk of type 2 diabetes mellitus among middle-aged Finnish men and women. JAMA 291:1213–1219
11. Agardh EE, Carlsson S, Ahlbom A et al. (2004) Coffee consumption, type 2 diabetes and impaired glucose tolerance in Swedish men and women. J Intern Med 255: 645–652
12. Reunanen A, Heliovaara M, Aho K (2003) Coffee consumption and risk of type 2 diabetes mellitus. Lancet 361: 702–703 (Letter)
13. Saremi A, Tulloch-Reid M, Knowler WC (2003) Coffee consumption and the incidence of type 2 diabetes. Diabetes Care 26:2211–2212 (Letter)
14. Kono S, Shinchi K, Ikeda N, Yanai F, Imanishi K (1991) Physical activity, dietary habits and adenomatous polyps of

the sigmoid colon: a study of self-defense officials in Japan. J Clin Epidemiol 44:1255–1261
15. Kono S, Handa K, Hayabuchi H et al. (1999) Obesity, weight gain and risk of colon adenomas in Japanese men. Jpn J Cancer Res 90:805–811
16. Sasazuki S, Kono S, Todoroki I et al. (1999) Impaired glucose tolerance, diabetes mellitus, and gallstone disease: an extended study of male self-defense officials in Japan. Eur J Epidemiol 15:245–251
17. Miyake Y, Eguchi H, Shinchi K, Oda T, Sasazuki S, Kono S (2003) Glucose intolerance and serum aminotransferase activities in Japanese men. J Hepatol 38:18–23
18. Alberti KG, Zimmet PZ (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 15:539–553
19. Lee KY, Uchida K, Shirota T, Kono S (2002) Validity of a self-administered food frequency questionnaire against 7-day dietary records in four seasons. J Nutr Sci Vitaminol (Tokyo) 48:467–476
20. The Resources Council of the Science and Technology Agency of Japan (2000) Standard Tables of Food Composition in Japan, 5th revised edn. The Ministry of Finance Printing Office, Tokyo
21. Ainsworth BE, Haskell WL, Leon AS et al. (1993) Compendium of physical activities: classification of energy costs of human physical activities. Med Sci Sports Exerc 25:71–80
22. International Agency for Research on Cancer (1991) Coffee, tea, mate, methylxanthines and methylglyoxal. In: IARC monographs on the evaluation of carcinogenic risks to humans, vol 51. IARC, Lyon, France, pp 41–206
23. All Japan Coffee Association (1997) The 8th Survey on the Trend of Coffee Demand in Japan. All Japan Coffee Association, Tokyo

Volume–Outcome Relation for Hospitals Performing Angioplasty for Acute Myocardial Infarction

— Results From the Nationwide Japanese Registry —

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Background The purpose of this study was to use a contemporary database to examine the relationship between annual hospital volume and the outcomes of percutaneous coronary interventions (PCIs) for acute myocardial infarction (AMI), given the wide spread use of coronary stents. An inverse relation exists between the number of PCIs and short-term outcome, but PCI practice has been changing with the availability of new devices such as stents.

Methods and Results Data from the 1997 Japanese nationwide registry were analyzed to determine the relation between the annual hospital volume of PCI procedures for patients with AMI and in-hospital mortality, as well as the need for coronary artery bypass graft (CABG) surgery. A total of 129 hospitals (2,491 patients) were divided into terciles according to the annual volume. Of the procedures, 39% involved coronary stents. Median annual PCI volumes varied across terciles from low=10, middle=33, and high=89. After adjusting for patient characteristics, there was no significant relationship between volume and in-hospital mortality (trend P=0.66) and CABG (trend P=0.35). Among patients who received stents (n=958), there was no significant association between volume and either mortality or CABG.

Conclusions Using the contemporary database, there was no significant relationship between hospital volume and in-hospital outcome among AMI patients undergoing PCIs. (*Circ J* 2004; **68:** 887–891)

Key Words: Angioplasty bypass surgery; Mortality; Myocardial infarction; Risk factors

Several studies have demonstrated better outcomes for patients undergoing percutaneous coronary interventions (PCIs) at hospitals with a high annual volume of procedures![1–6] This result has been also documented in patients with acute myocardial infarction (AMI); that is, patients treated with PCI at high-volume centers have a lower mortality![7–9]

Recent advances in PCI technology, specifically the advent of coronary stents;[10,11] have yielded significantly better results for the treatment of AMI[12,13] and reduced complications following PCI, such as the risk of undergoing subsequent coronary artery bypass grafting (CABG)![14] In addition, there have been other changes in practice patterns that might be expected to improve outcomes following PCI, such as the use of lower profile balloons, better guiding catheters, and new antiplatelet agents. Given all these advances, it is necessary to re-evaluate the relationship between hospital volume and the outcomes of PCIs using more current data. In fact, Ho et al have reported that the disparity in outcome between low- and high-volume hospitals has narrowed over time![15]

To this end, we used the 1997 data from the Japanese Coronary Intervention Study (JCIS), an extensive nationwide survey of PCI practice in Japan;[11,16–19] to investigate whether hospital volume is related to the in-hospital outcomes for AMI patients in the current era of interventional cardiology. The data set is representative for the entire nation and includes patients with AMI who were treated at hospitals with a wide range of experience.

Methods

Patient Population

Patient selection and data collection have been described previously![17,18] Briefly, JCIS surveyed 109,788 PCI procedures performed at 1,023 institutions in Japan during 1997. The patient characteristics and outcomes were evaluated in 10,642 randomly selected PCIs, which represented approximately 10% of all PCIs registered in the JCIS. All patients with AMI (n=2,606) who had undergone PCI were identified. Inclusion criteria were patients with AMI who presented within 6 h of symptom onset, or between 6 and 24 h if they had persistent symptoms with evidence of ongoing ischemia, including chest pain and ST-segment elevation in the infarct region. Patients with an incomplete data set regarding the infarct-related artery (n=112) and in-hospital complications (n=3) were excluded. Thus, a total of 2,491 patients remained in the main analysis of the present study.

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Table 1 Hospitals Ranked According to Tercile of Annual Volume of AMI Patients Undergoing PCI

Tercile	Range of volume	Median of volume	Hospitals n	Patients n
Low	1–16	10	44	323
Middle	17–55	33	42	1,025
High	56–370	89	43	1,143
Total			129	2,491

Data Collection

The patients' demographic information, cardiovascular history, their risk factors (eg, hypercholesterolemia, smoking, hypertension, and diabetes mellitus) were recorded. Hypercholesterolemia was defined as total cholesterol ≥220 mg/dl; hypertension was defined as systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg; diabetes mellitus was defined as fasting blood sugar ≥140 mg/dl or blood sugar during a 75-g oral glucose tolerance test ≥200 mg/dl; renal failure was defined as serum creatinine ≥2.5 mg/dl; single-vessel disease was defined as ≥51% stenosis in any of the major coronary arteries or their major branches; and multivessel disease was defined as ≥51% stenosis in either 2 or all 3 major epicardial coronary arteries. Further, left ventricular ejection fraction (LVEF) was assessed by any method, including left ventricular angiography, echocardiography and radionuclide angiography, and categorized into 2 groups: LVEF ≥50% and LVEF <50% or unknown.

Outcome Measures

The outcomes were in-hospital mortality, in-hospital bypass surgery following PCI, and the combined endpoint of in-hospital mortality or CABG.

Statistical Analysis

Hospitals were classified into 3 categories at terciles of the volume of procedures performed during 1997. Differences in demographic, medical, angiographic, and procedural variables were statistically assessed by chi-square test (categorical variables) and Student's t-test (continuous variables). The relationship between hospital volume and in-hospital outcomes was examined in terms of odds ratio (OR) using multiple logistic regression analysis. Adjustment was made for age, gender, previous myocardial infarction, hypercholesterolemia, smoking, hypertension, diabetes mellitus, renal failure, cerebrovascular disease, prior PCI, prior CABG, number of diseased vessels, attempted lesion, LVEF, types of devices, and backup cardiac surgery. Subgroup analyses were also performed with stratification as regards age, sex, number of diseased vessels, LVEF, devices, and backup cardiac surgery.

Significance of a trend with increasing hospital volume categories was assessed with the Cochran–Armitage test and reported as a trend 'P'. All probability values were 2-tailed. Statistical significance was defined as $p<0.05$ or 95% confidence intervals (CIs) that did not include 1.0. All analysis was performed with the SAS 6 statistical programs (SAS Institute, Cary, NC, USA).

Results

Study Population

Table 1 shows the range and median of 2,491 patients with AMI undergoing PCI at 129 hospitals ranked according to annual volume. Baseline demographic, medical, angiographic, and procedural characteristics of the patients according to hospital volume are shown in Table 2. In general, there were few significant differences among patients

Table 2 Demographic, Medical, Angiographic, and Procedural Characteristics of Patients According to Tercile of Annual Volume

	Tercile			Trend P
	Low (n=323)	Middle (n=1,025)	High (n=1,143)	
Age (years, mean±SD)	65.0±11.2	65.2±11.3	65.4±11.5	0.80
>75 (%)	22.9	21.8	22.4	0.88
Male (%)	74.3	74.9	73.9	0.88
Prior myocardial infarction (%)	1.6	2.6	2.0	0.42
Hypercholesterolemia (%)	41.8	36.0	37.3	0.17
Smoking (%)	55.1	51.8	51.4	0.48
Hypertension (%)	48.9	47.5	52.3	0.07
Diabetes mellitus (%)	32.2	28.7	28.5	0.41
Renal failure (%)	2.8	1.3	2.8	0.30
Cerebrovascular disease (%)	8.1	10.5	7.5	0.20
Prior PCI (%)	9.0	7.6	8.1	0.85
Prior CABG (%)	1.9	1.4	1.8	0.73
No. of diseased vessels (%)				
Single	58.3	58.2	59.0	0.93
Multivessel	39.0	38.3	36.4	0.54
Left main trunk	1.2	2.6	3.9	0.03
Attempted coronary artery (%)				
Right	35.6	34.2	36.2	0.60
Left anterior descending	53.3	51.4	48.4	0.07
Left circumflex	10.5	12.4	12.3	0.54
Left main	0.3	1.6	2.5	<0.01
Ejection fraction (mean±SD)	55.1±12.7	55.0±14.2	54.1±14.4	0.38
<50% or unknown (%)	55.1	53.5	56.8	0.30
Device (%)				
Balloon angioplasty	65.6	63.8	55.1	<0.01
Stent	32.2	34.0	44.3	<0.01
Surgical backup (%)	46.8	72.2	87.5	<0.01

PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting.

Table 3 Rates of In-Hospital Outcomes According to Tercile of Annual Volume

Outcome	Tercile			Trend P
	Low (n=323)	Middle (n=1,025)	High (n=1,143)	
Mortality (%)	8.4	7.2	7.4	0.66
CABG (%)	1.9	0.8	1.0	0.35
Mortality or CABG (%)	9.9	7.8	8.1	0.45

CABG, coronary artery bypass grafting.

Table 4 Unadjusted and Adjusted Odds Ratios of In-Hospital Outcomes by Tercile of Annual Volume

Outcome/model	Odds ratio (95% CI)		Trend P
	Middle (n=1,025)	High (n=1,143)	
In-hospital mortality			
Unadjusted	0.85 (0.54–1.35)	0.87 (0.55–1.37)	0.67
Adjusted for demographic variables	0.87 (0.54–1.38)	0.87 (0.55–1.38)	0.66
Adjusted for demographic and medical variables	0.98 (0.56–1.71)	1.04 (0.60–1.80)	0.80
Adjusted for demographic medical, angiographic and procedural variables	0.91 (0.50–1.67)	0.84 (0.46–1.56)	0.57
CABG			
Unadjusted	0.42 (0.14–1.21)	0.51 (0.19–1.40)	0.36
Adjusted for demographic variables	0.42 (0.14–1.21)	0.51 (0.19–1.40)	0.35
Adjusted for demographic and medical variables	0.43 (0.14–1.32)	0.55 (0.19–1.55)	0.44
Adjusted for demographic, medical, angiographic and procedural variables	0.31 (0.09–1.05)	0.32 (0.10–1.06)	0.14
In-hospital death or CABG			
Unadjusted	0.77 (0.50–1.18)	0.80 (0.52–1.22)	0.45
Adjusted for demographic variables	0.78 (0.50–1.20)	0.80 (0.52–1.22)	0.44
Adjusted for demographic and medical variables	0.84 (0.50–1.40)	0.90 (0.55–1.48)	0.86
Adjusted for demographic, medical, angiographic and procedural variables	0.76 (0.44–1.31)	0.70 (0.40–1.23)	0.26

Adjustment was made for demographic (age, gender), medical (previous myocardial infarction, hypercholesterolemia, smoking, hypertension, diabetes mellitus, renal failure, cerebrovascular disease, prior PCI, prior CABG), angiographic (number of diseased vessels, attempted lesion, ejection fraction), and procedural (types of devices, cardiac surgery as a backup) variables.

Table 5 Subgroup Analysis of In-Hospital Mortality by Tercile of Annual Volume

Subgroup	Odds ratio (95% CI)		Trend P
	Middle (n=1,025)	High (n=1,143)	
Age			
<75 years old (n=1,938)	1.19 (0.50–2.81)	1.15 (0.48–2.74)	0.85
≥75 years old (n=553)	0.52 (0.21–1.32)	0.45 (0.17–1.17)	0.15
Sex			
Male (n=1,853)	1.11 (0.47–2.65)	1.15 (0.48–2.77)	0.78
Female (n=638)	0.70 (0.27–1.81)	0.55 (0.21–1.45)	0.23
No. of diseased vessels			
Single (n=1,461)	0.62 (0.24–1.60)	0.76 (0.30–1.92)	0.81
Multivessel (n=935)	0.87 (0.36–2.06)	0.81 (0.34–1.94)	0.64
Ejection fraction			
≥50% (n=1,116)	2.31 (0.16–33.24)	0.78 (0.05–12.42)	0.51
<50% (n=1,375)	0.89 (0.48–1.62)	0.86 (0.47–1.60)	0.68
Devices			
Balloon angioplasty (n=1,496)	0.75 (0.35–1.63)	0.78 (0.35–1.72)	0.66
Stent (n=958)	1.06 (0.37–3.04)	0.89 (0.32–2.52)	0.70
Surgical backup			
Yes (n=1,891)	0.68 (0.31–1.49)	0.81 (0.38–1.70)	0.84
No (n=600)	1.61 (0.48–5.41)	0.33 (0.06–1.83)	0.26

Adjustment was made for demographic (age, gender), medical (previous myocardial infarction, hypercholesterolemia, smoking, hypertension, diabetes mellitus, renal failure, cerebrovascular disease, prior PCI, prior CABG), angiographic (number of diseased vessels, attempted lesion, ejection fraction), and procedural (types of devices, cardiac surgery as a backup) variables.

according to the tercile of volume. Notable differences included significantly higher proportions of left main disease patients in high-volume hospitals (trend P=0.03). High-volume hospitals were more likely to use stents instead of balloon angioplasty than lower volume hospitals (trend P<0.01). There was a trend toward greater availability of cardiac surgery as a backup in high-volume hospitals (trend P<0.01).

In-Hospital Outcomes

For the study population overall, the unadjusted rates of outcomes during hospitalization are shown in Table 3. The unadjusted in-hospital mortality rate was 8.4% in the lowest quartile and 7.4% in the highest quartile. There was no significant difference in the rates of in-hospital mortality across the terciles of hospital volume (trend P=0.66). Similarly, the unadjusted rates of CABG during the same hospitalization and the combined endpoint of mortality or CABG

demonstrated no significant relationship with hospital volume.

Table 4 shows the relationship between hospital volume and outcomes after stepwise adjustment for various patient characteristics. There was no significant association between hospital procedure volume and in-hospital mortality rate even after the adjustment of variables. Highest volume hospitals tended to be associated with a lower likelihood of CABG (OR 0.32, 95% CI 0.10–1.06, P=0.14); however, the trend did not reach statistical significance. Adjusted rates of the combined in-hospital mortality and CABG also did not differ with the categories of hospital volume.

The results of subgroup analysis for the in-hospital mortality stratified by age, sex, number of diseased vessels, LVEF, types of devices used, and backup cardiac surgery are shown in Table 5; they are similar to those found in the primary analysis. Even in the subgroup who received coronary stent placement, there was no measurable association between the hospital volume and in-hospital mortality (trend P=0.70).

Discussion

The major finding of the present study is that, using the contemporary PCI database, the rates of adverse in-hospital outcomes including mortality and subsequent bypass surgery in patients undergoing PCI for AMI were comparable across categories of hospital volume. Adjusting for differences in demographic, medical, angiographic, and procedural variables did not alter these findings. No relationship between volume and outcomes was found after stratification by age, sex, single- or multivessel disease, LVEF and the subset of patients treated with coronary stent placement.

Comparison With Previous Studies

The results of the present study are consistent with those from 2 prior studies, which found no relationship between volume and outcomes for primary angioplasty[20,21] In a subgroup analysis of patients in the Myocardial Infarction Triage Intervention study, Every et al noted no difference in outcome for 995 patients treated at high-volume centers compared with 1,394 patients treated at low-volume centers[20] Danchin et al found no difference in relative mortality between high- and low-volume hospitals in a cohort of 721 patients during 1995[21] However, these results differ from those reported in 3 previous studies based on registry or multicenter data[7–9] Using the 1994–1998 National Registry of Myocardial Infarction (NRMI) registry data, Canto et al demonstrated an inverse relationship between post-PCI mortality rates and hospital volume[7] Magid et al showed that in-hospital death was inversely related to the number of cases each hospital performed annually using 1994–1999 NRMI registry data[8] Vakili et al, based on the 1995 New York State Coronary Angioplasty System Registry (CARS) data, found an inverse relationship between hospital volume and the outcome of both in-hospital death and CABG[9]

The difference between these studies and our study may be a consequence of our use of more recent data that reflect the widespread use of coronary stents. According to the JCIS registry, the use of stents has increased dramatically and the lower volume facilities were more likely to use them than the higher volume centers;[11] in fact, the prevalence of stent use was as high as 30–50%. Even though we found that the higher volume tercile was more likely to use stents than the lower volume tercile, the lowest volume hospitals performed 32.2% of stent placements, which was even higher than the values at high volume centers in the study of Vakili et al during 1995 (20%)[9] The use of stents may be expected to reduce the incidence of re-infarction and recurrent ischemia, so the increasing use of stents by low volume facilities until they were nearly comparable with the rates in high volume facilities might account for the absence of a volume–outcome relation in the present study. In addition, the increased cumulative experience for all interventionists in stabilizing AMI patients using pharmacological and mechanical therapies might have contributed to the improvement in outcome. Alternatively, our finding might be unique to the PCI practice patterns in Japan![16]

Study Limitations

First, important clinical variables concerning the severity of the patient's illness, such as cardiogenic shock, presence of heart failure on admission, antecedent thrombolytic therapy with salvage PCI, and the use of intra-aortic balloon pumps and transvenous pacing, were not included in the present study. Nor did we include angiographic variable such as the prevalence of final TIMI-3 flow in the infarct vessel and the presence of no-reflow and other embolic complications. Second, the JCIS database did not record procedure volume per physician, so there might be a physician volume–outcome relation in our study patients. However, as recommended by the American College of Cardiology (ACC) and American Heart Association (AHA)[22] it may be acceptable for operators with small annual volumes to perform PCI if they work at high-volume centers and are cautious in their case selection. Therefore, we consider that hospital volume–outcome data may persist after accounting for procedure volume per cardiologist. However, we need to be cautious about extending our findings to operators with low volumes until the outcome for this group is assessed. Third, given that the JCIS registry contains a higher concentration of low-volume hospitals[16] further studies are necessary to determine whether the findings of this study are consistent. Specifically, it is important to continually assess the outcome in low-volume facilities. Finally, the practice of interventional cardiology continues to change. The use of stents is still growing and new antiplatelet agents are being used with increased frequency. What effect this will have on practice and outcomes and their relationship between volume and outcome is not yet known. Despite these shortcomings, administrative data are the only source of information by which the volume–outcome relation may be examined for a large sample of hospitals.

Clinical Implications

In-hospital mortality and CABG rates were similar across hospital volumes in the present study, which has important implications for decisions regarding minimum volume standards and regionalization of innovative technologies[15,23] The relative performance advantage of high- versus low-volume hospitals might have decreased over time[15] and therefore, regionalization might have ensured better outcomes in the early stages of a new intervention. However, if access to care is affected by timing and distance, such as PCI for the patients with AMI, less centralization may be preferred as technologies improve[24,25]

Conclusions

In the modern era of interventional cardiology, after adjusting for confounding variables between patients, outcomes in patients undergoing PCI for AMI are comparable for hospitals across a spectrum of annual volumes. Continual assessment of progress in the evolution of PCI is essential for the determination of definitive guidelines.

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References

1. Ellis SG, Omoigui N, Bittl JA, Lincoff M, Wolfe MW, Howell G, et al. Analysis and comparison of operator-specific outcomes in interventional cardiology: From a multicenter database of 4860 quality-controlled procedures. *Circulation* 1996; **93:** 431–439.
2. Hannan EL, Racz M, Ryan TJ, McCallister BD, Johnson LW, Arani DT, et al. Coronary angioplasty volume-outcome relationships for hospitals and cardiologists. *JAMA* 1997; **277:** 892–898.
3. Jollis JG, Peterson ED, Nelson CL, Stafford JA, DeLong ER, Muhlbaier LH, et al. Relationship between physician and hospital coronary angioplasty volume and outcome in elderly patients. *Circulation* 1997; **95:** 2485–2491.
4. Ritchie JL, Maynard C, Chapko MK, Every NR, Martin DC. Association between percutaneous transluminal coronary angioplasty volumes and outcomes in the Healthcare Cost and Utilization Project 1993–1994. *Am J Cardiol* 1999; **83:** 493–497.
5. Lindsay J Jr, Pinnow EE, Pichard AD. Frequency of major adverse cardiac events within one month of coronary angioplasty: A useful measure of operator performance. *J Am Coll Cardiol* 1999; **34:** 1916–1923.
6. Malenka DJ, McGrath PD, Wennberg DE, Ryan TJ Jr, Kellett MA Jr, Shubrooks SJ Jr, et al. The relationship between operator volume and outcomes after percutaneous coronary interventions in high volume hospitals in 1994–1996: The northern New England experience (Northern New England Cardiovascular Disease Study Group). *J Am Coll Cardiol* 1999; **34:** 1471–1480.
7. Canto JG, Every NR, Magid DJ, Rogers WJ, Malmgren JA, Frederick PD, et al. The volume of primary angioplasty procedures and survival after acute myocardial infarction: National Registry of Myocardial Infarction 2 Investigators. *N Engl J Med* 2000; **342:** 1573–1580.
8. Magid DJ, Calonge BN, Rumsfeld JS, Canto JG, Frederick PD, Every NR, et al. Relation between hospital primary angioplasty volume and mortality for patients with acute MI treated with primary angioplasty vs thrombolytic therapy. *JAMA* 2000; **284:** 3131–3138.
9. Vakili BA, Kaplan R, Brown DL. Volume–outcome relation for physicians and hospitals performing angioplasty for acute myocardial infarction in New York state. *Circulation* 2001; **104:** 2171–2176.
10. McGrath PD, Malenka DJ, Wennberg DE, Shubrooks SJ Jr, Bradley WA, Robb JF, et al. Changing outcomes in percutaneous coronary interventions: A study of 34,752 procedures in northern New England, 1990 to 1997 (Northern New England Cardiovascular Disease Study Group). *J Am Coll Cardiol* 1999; **34:** 674–680.
11. Tsuchihashi M, Tsutsui H, Shihara M, Shigematsu H, Yamamoto S, Koike G, et al. Coronary revascularization in Japan. Part 2: Comparison of facilities between 1997 and 1999. *Jpn Circ J* 2001; **65:** 1011–1016.
12. Stone GW, Brodie BR, Griffin JJ, Morice MC, Costantini C, St Goar FG, et al. Prospective, multicenter study of the safety and feasibility of primary stenting in acute myocardial infarction: In-hospital and 30-day results of the PAMI stent pilot trial (Primary Angioplasty in Myocardial Infarction Stent Pilot Trial Investigators). *J Am Coll Cardiol* 1998; **31:** 23–30.
13. Antoniucci D, Santoro GM, Bolognese L, Valenti R, Trapani M, Fazzini PF. A clinical trial comparing primary stenting of the infarct-related artery with optimal primary angioplasty for acute myocardial infarction: Results from the Florence Randomized Elective Stenting in Acute Coronary Occlusions (FRESCO) trial. *J Am Coll Cardiol* 1998; **31:** 1234–1239.
14. Ritchie JL, Maynard C, Every NR, Chapko MK. Coronary artery stent outcomes in a Medicare population: Less emergency bypass surgery and lower mortality rates in patients with stents. *Am Heart J* 1999; **138:** 437–440.
15. Ho V. Evolution of the volume–outcome relation for hospitals performing coronary angioplasty. *Circulation* 2000; **101:** 1806–1811.
16. Shihara M, Tsutsui H, Tsuchihashi M, Shigematsu H, Yamamoto S, Koike G, et al. Coronary revascularization in Japan. Part 1: Survey of facilities during 1997. *Jpn Circ J* 2001; **65:** 1005–1010.
17. Shihara M, Tsutsui H, Tsuchihashi M, Shigematsu H, Yamamoto S, Koike G, et al. Coronary revascularization in Japan. Part 3: Percutaneous coronary intervention during 1997. *Circ J* 2002; **66:** 10–19.
18. Shihara M, Tsutsui H, Tsuchihashi M, Tada H, Kono S, Takeshita A. In-hospital and one-year outcomes for patients undergoing percutaneous coronary intervention for acute myocardial infarction. *Am J Cardiol* 2002; **90:** 932–936.
19. Tsuchihashi M, Tsutsui H, Shihara M, Tada H, Kono S, Takeshita A. Comparison of outcomes for patients undergoing balloon angioplasty vs coronary stenting for acute myocardial infarction. *Circ J* 2003; **67:** 369–374.
20. Every NR, Parsons LS, Hlatky M, Martin JS, Weaver WD. A comparison of thrombolytic therapy with primary coronary angioplasty for acute myocardial infarction: Myocardial Infarction Triage and Intervention Investigators. *N Engl J Med* 1996; **335:** 1253–1260.
21. Danchin N, Vaur L, Genes N, Etienne S, Angioi M, Ferrieres J, et al. Treatment of acute myocardial infarction by primary coronary angioplasty or intravenous thrombolysis in the 'real world': One-year results from a nationwide French survey. *Circulation* 1999; **99:** 2639–2644.
22. Ryan TJ, Antman EM, Brooks NH, Califf RM, Hillis LD, Hiratzka LF, et al. 1999 update: ACC/AHA Guidelines for the Management of Patients With Acute Myocardial Infarction: Executive Summary and Recommendations: A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Management of Acute Myocardial Infarction). *Circulation* 1999; **100:** 1016–1030.
23. Hlatky MA, Dudley RA. Operator volume and clinical outcomes of primary coronary angioplasty for patients with acute myocardial infarction. *Circulation* 2001; **104:** 2155–2157.
24. Nishigaki K, Yamazaki T, Fukunishi M, Tanihata S, Fujiwara H. Assessment of acute myocardial infarction in Japan by the Japanese Coronary Intervention Study (JCIS) Group. *Circ J* 2004; **68:** 515–519.
25. Sakurai K, Watanabe J, Iwabuchi K, Koseki Y, Kon-no Y, Fukuchi M, et al. Comparison of the efficacy of reperfusion therapies for early mortality from acute myocardial infarction in Japan: Registry of Miyagi Study Group for AMI (MsAMI). *Circ J* 2003; **67:** 209–214.

Appendix

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The influence of lifestyle modification on carotid artery intima-media thickness in a suburban Japanese population

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Abstract

To evaluate the influence of lifestyle modification with and without lipid-lowering drug therapy on the carotid arterial wall, we did a 2 year prospective ultrasound study of carotid intima-media thickness (IMT) in 1390 male and female residents of a suburban Japanese town. According to total cholesterol (TC) level at baseline, subjects were classified into a lifestyle modification alone group (TC $\geq$ 220 mg/dl, n = 437), a lifestyle modification with lipid-lowering drug group (TC $\geq$ 220 mg/dl, n = 159), and a control group (TC $<$ 220 mg/dl, n = 794). After 2 years of follow-up, both sexes of both treatment groups showed significant reductions of TC, low-density lipoprotein cholesterol (LDL-C), and IMT, although TC continued over 220 mg/dl in some subjects in the lifestyle modification group. The reduction of TC and LDL-C was significantly higher in the lifestyle modification with lipid-lowering drug group than in the lifestyle modification alone group. Although the IMT reduction was not statistically different between the treatment groups of either sex, the reduction of IMT was greater in the lifestyle modification with lipid-lowering drug group than in the lifestyle modification alone group. Our results indicate that comprehensive lifestyle modification can reduce carotid IMT in the general population, with or without the use of lipid-lowering drugs and that cholesterol reduction is of benefit even when TC level remain above the recommended level.

Keywords: Lifestyle modification; Lipid-lowering therapy; Cholesterol; Carotid arteries; Intima-media thickness

1. Introduction

Lifestyle has a major influence on the development and progression of atherosclerosis. The Leiden Intervention Trial provided the first direct evidence that dietary modification can influence the natural course of coronary artery atherosclerosis [1], showing that a vegetarian diet reduced the ratio of low-density lipoprotein cholesterol (LDL-C) to high-density lipoprotein cholesterol (HDL-C) and showing an association with a reduction in atherosclerosis progression in the coronary artery. Several subsequent clinical trials also showed that lifestyle modification, including dietary changes, increased physical activity, smoking stoppage, and weight control can slow the progression of coronary atherosclerosis [2–4]. Based on these findings, a 'healthy lifestyle' has become the backbone of consensus statements for prevention of cardiovascular disease.

High-resolution B-mode ultrasound is well established as a noninvasive method for assessing arterial intima-media thickness (IMT). Ultrasound measurement of IMT is now widely used in clinical studies as a surrogate marker for atherosclerotic disease. Epidemiological studies indicated an association between carotid IMT and atherosclerotic risk factors and the prevalence of cardiovascular disease [5–9]. In prospective studies, increased carotid IMT was predictive of cardiovascular events [8,10,11].

Lipid-lowering drug therapy, including 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, not only has reduced the incidence of primary and secondary cardiovascular events but also slowed or reversed IMT progression [12–16]. However, few studies have reported that lifestyle modification can reduce IMT progression, even without lipid-lowering drug therapy [17,18]. Moreover, to our knowledge, no previous reports have compared the effect

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of lifestyle modification alone to lifestyle modification with lipid-lowering drug therapy in terms of IMT progression in a large-scale population.

The purpose of this study was to evaluate the effectiveness of lifestyle modification with and without lipid-lowering drug therapy in retarding the progression of carotid atherosclerosis, as assessed by changes in IMT.

2. Methods

2.1. Study design and subjects

This study was designed as a 2 year, prospective clinical trial to evaluate the influence of lifestyle modification with and without lipid-lowering drug therapy on common carotid artery IMT in hypercholesterolemic subjects with a total cholesterol (TC) level of at least 220 mg/dl. A comparison of these groups with normocholesterolemic controls was also done.

Between June and December 1999, potential subjects were recruited from 2410 participants in public health examinations given by the local government for residents aged 20 or older at the Public Health Center in K town, Fukuoka prefecture, Japan [9]. The examination consisted of a general physical examination, a questionnaire, carotid ultrasound, and blood tests. TC, HDL-C, and triglycerides (TG) were measured at a commercial laboratory (SRL Inc., Fukuoka, Japan). LDL-C was calculated according to the Friedewald formula. Exclusion criteria included a serum TG $\geq$ 400 mg/dl, familial hypercholesterolemia, secondary hypercholesterolemia, and complications such as severe liver disease or nephropathy. Written informed consent was obtained from all subjects before examination. However, 1020 of the potential subjects were omitted because of withdrawal of consent or ineligibility, leaving 1390 subjects (398 men and 992 women; mean $\pm$ S.D. age, 56.9 $\pm$ 12.1 years; range 21–86 years) who were enrolled and followed up for 2 years. Of these, 596 hypercholesterolemic subjects were gathered and taught the following lifestyle modifications in a lecture of approximately 1 h by physicians using the same teaching materials. Dietary recommendations were based on the goals of the National Cholesterol Education Program Expert Panel step 2 diet (less than 30% total fat, less than 7% saturated fat, and less than 200 mg of cholesterol per day) [19]. Subjects with a body mass index (BMI) over 25 were instructed to lose weight based on BMI goals, with BMI 22 the target. Physical exercise, such as aerobic walking three times a week (more than 30 min each time), was recommended. All hypercholesterolemic subjects were encouraged to quit negative lifestyle habits such as excessive alcohol consumption and cigarette smoking. After this lesson, all hypercholesterolemic subjects were recommended lipid-lowering drug therapy in addition to lifestyle modification. The hypercholesterolemic subjects were divided into two groups: (1) a lifestyle modification alone group composed of subjects who rejected the use of drugs (group A, $n = 437$) and (2) a lifestyle modification with lipid-lowering drug group (group B, $n = 159$). Lipid-lowering drug therapy with simvastatin at 5 mg per day was begun for those subjects who chose lipid-lowering drug therapy. For subjects already on lipid-lowering drug therapy, there was a washout period of at least 4 weeks before start of the new medication. Monitoring visits were scheduled 4 weeks after the baseline data was gathered and every 2 months thereafter. At each visit, a brief physical examination was done, and the number of tablets was counted to assess compliance. Subjects with a normal TC level were placed in a control group (group C, $n = 794$). Group C was further divided into two groups after 24 months of follow-up, one in which TC was under 220 mg/dl both at baseline and after 24 months (continuous TC normal group, $n = 697$) and the other in which TC was normal at baseline, but had increased to above 220 mg/dl after 24 months (TC turning abnormal group, $n = 97$).

A self-reported questionnaire including questions about personal and family medical histories and lifestyle habits affecting health was given. Hypertension was defined as either systolic blood pressure $\geq$ 140 mmHg, diastolic pressure $\geq$ 90 mmHg, or treatment with antihypertensive medications. Diabetes mellitus was defined as a self-reported history of diabetes, a fasting plasma glucose level $\geq$ 126 mg/dl, or the use of anti-diabetic drugs or insulin.

2.2. Carotid ultrasound measurements

Bilateral carotid artery scanning with high-resolution B-mode ultrasound using a 7.5 MHz mechanical sector transducer on the Aloka SSD-2000 (Aloka Co. Ltd., Tokyo, Japan) was done by four trained physicians as described previously [9,16,20]. The IMT was measured at points 2, 2.5 and 3 cm proximal to the flow divider on the far wall of the right and left common carotid arteries at the end of the diastolic phase, provided that these points were free of plaque, defined as a clearly identified area of focal increased IMT (>1.1 mm). From this, mean IMT was determined for each individual. All assessment of carotid arteries was done blinded to knowledge of clinical history or risk factor profile.

Analysis of within- and between-reader (reading of a given duplicate set of 25 scans) and within-observer (duplicate mean IMT measurements in five subjects) variability was done [20]. The Spearman correlation coefficients for intra-observer and intra-reader measurements were >0.95, respectively, and the mean differences ($\pm$2 S.D.) were <1% (10%). The Spearman correlation coefficients for between-observer and between-reader variability were >0.95 and >0.95, respectively, and the mean differences ($\pm$2 S.D.) were <5% (15%).