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

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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%).

2.3. Statistical analysis

All data were reported on standardized forms, which were then entered into a database. Categorical variables among the groups were assessed by the chi-square test or Fisher's exact test. The mean levels of variables between two groups were compared by unpaired *t*-test or Mann–Whitney *U*-test. Comparison between more than two groups was done by Kruskal–Wallis test. Differences in serum lipid levels and IMT between baseline and after 24 months in each group were compared using paired *t*-test or Wilcoxon's signed-ranks test. Between group, mean percentage change from baseline to 24 months was compared using multiple regression analysis with the Bonferroni correction. Percentage change was calculated by the following formula: % change = (value at 24 months − baseline value)/baseline value × 100. $P < 0.05$ were considered statistically significant in all analyses.

3. Results

3.1. Baseline characteristics of the three groups by sex

In men, no significant differences in age, BMI, blood pressure, smoking, history, or IMT were found between the three groups. The TC, LDL-C and TG levels were significantly higher in groups A and B than in group C ($P < 0.01$), respectively. No significant difference in TC, LDL-C, or HDL-C level was found between groups A and B. HDL-C was significantly higher in group A than group C ($P < 0.01$) (Table 1).

In women, age, BMI, blood pressure, history of hypertension, serum cholesterol, TG and IMT were significantly higher in groups A and B than in group C ($P < 0.01$), respectively; whereas, smoking was significantly higher in group C than in groups A and B ($P < 0.01$), respectively. The TC, LDL-C, and IMT levels were significantly higher in group B than group A ($P < 0.01$).

3.2. Changes in variables at baseline and 24 months (Tables 2 and 3)

In men, no significant change in BMI was found in the A, B, or C group; however, a significant increase of BMI was found in the TC turning abnormal group ($P = 0.046$) (Table 2). The systolic blood pressure level significantly increased in groups A and C ($P = 0.019$ and $P < 0.001$), respectively, but were not changed in group B. No significant change in diastolic blood pressure level was found in any group over 24 months.

The TC and LDL-C levels significantly decreased over 24 months in groups A and B ($P < 0.001$), but in group A the TC level continued over 220 mg/dl throughout the 24 months. No significant change in the TC or LDL-C level was found in group C; however, a significant decrease in the TC and LDL-C level was found in the continuous TC normal group ($P < 0.001$) and a significant increase in TC level was found in the TC turning to abnormal group ($P < 0.001$). The HDL-C level significantly decreased over 24 months in group B ($P = 0.019$). In groups A and C, no significant change in HDL-C was found over 24 months. The TG level significantly decreased over 24 months in group B ($P = 0.012$). No significant change in TG was found in group A or C over 24 months.

The IMT level was significantly reduced after 24 months in groups A, B, and C ($P = 0.002$, $P = 0.002$, and $P < 0.001$), respectively. In group C, a significant decrease in IMT was found in the continuous TC normal group ($P < 0.001$), whereas no significant change was found in the TC turning abnormal group ($P = 0.481$).

In women, BMI was significantly decreased in groups A and B ($P < 0.001$ and $P = 0.033$), respectively, but was not changed in group C (Table 3). The systolic blood pressure level significantly increased in groups A and C ($P = 0.029$ and $P < 0.001$), respectively, but was not changed in group B. No significant change in diastolic blood pressure level was found in any group over 24 months.

The TC and LDL-C levels significantly decreased over 24 months in groups A and B ($P < 0.001$), but in group A the TC level over 24 months was continuously higher than 220 mg/dl. In group C, the TC and LDL-C levels were significantly increased ($P = 0.004$ and 0.036), respectively. Significant decreases in TC and LDL-C were found in the continuous TC normal group ($P = 0.047$ and 0.022), whereas significant increases in TC and LDL-C were found in the TC turning to abnormal group ($P < 0.001$). The HDL-C level significantly decreased over 24 months in groups A and B ($P = 0.041$ and $P < 0.001$), respectively. In group C, a significant increase in the HDL-C level was found in the TC turning abnormal group ($P = 0.006$). The TG level significantly increased over 24 months in group C ($P = 0.014$); however, this was only true for the continuous TC normal group ($P = 0.025$). No significant change in TG was found in groups A and B over 24 months of follow-up.

IMT was significantly reduced after 24 months of follow-up in groups A and B ($P < 0.001$). In group C, no significant change in IMT was found; however, a significant decrease in IMT was found in the continuous TC normal group ($P = 0.006$), whereas no significant change in IMT was found in the TC turning abnormal group ($P = 0.358$).

3.3. Mean percentage change of lipid and IMT levels between the baseline and 24 month values (Figs. 1 and 2)

In men, the TC and LDL-C change was significantly higher in group B (−21.4 and −23.3%) than in groups A (−6.8 and −10.1%) and C (−0.9 and −1.3%) ($P < 0.01$), respectively (Fig. 1A). The TC and LDL-C change was significantly higher in group A than in group C ($P < 0.01$).

Table 1
Baseline characteristics of the study groups by sex

	Men ($n = 398$)				Women ($n = 992$)			
	Group A ($n = 107$)	Group B ($n = 33$)	Group C ($n = 258$)	P-value	Group A ($n = 330$)	Group B ($n = 126$)	Group C ($n = 536$)	P-value
Age (years)	59.1 ± 9.7	60.6 ± 8.1	59.9 ± 12.4	0.264	59.0 ± 10.7[b]	62.2 ± 8.7[b]	52.6 ± 13.1	<0.001
Body mass index (kg/m^2)	23.7 ± 3.1	23.2 ± 3.5	23.2 ± 2.8	0.397	22.9 ± 3.4[b]	23.3 ± 3.4[b]	21.9 ± 2.9	<0.001
Blood pressure (mmHg)								
Systolic	131.8 ± 16.3	132.4 ± 17.2	132.3 ± 18.3	0.988	130.3 ± 19.6[b]	133.1 ± 19.6[b]	121.9 ± 19.2	<0.001
Diastolic	78.8 ± 10.3	77.3 ± 11.5	79.4 ± 10.4	0.550	77.4 ± 10.6[b]	78.3 ± 10.0[b]	73.0 ± 10.7	<0.001
Smoking (%)	80 (74.8)	25 (75.8)	207 (80.2)	0.477	35 (10.6)[b]	7 (5.6)[b]	81 (15.1)	<0.01
History (%)								
Hypertension	37 (34.6)	10 (30.3)	103 (39.9)	0.416	97 (29.4)[b]	40 (31.7)[b]	101 (18.8)	<0.001
Diabetes mellitus	15 (14.0)	4 (12.1)	21 (8.1)	0.216	18 (5.5)	4 (3.2)	20 (3.7)	0.388
Cardiovascular disease	4 (3.7)	0 (0)	16 (6.2)	0.24	9 (2.7)	3 (2.4)	6 (1.1)	0.200
Cerebrovascular disease	3 (2.8)	0 (0)	7 (2.7)	0.628	4 (1.2)	2 (1.6)	3 (0.6)	0.425
Serum cholesterol (mg/dl)								
Total	243.2 ± 19.4[b]	250.8 ± 23.7[b]	187.2 ± 21.7	<0.001	246.3 ± 23.2[a,b]	257.7 ± 31.4[b]	190.3 ± 20.2	<0.001
Low-density lipoprotein	157.1 ± 21.3[b]	159.5 ± 25.8[b]	109.0 ± 21.9	<0.001	157.6 ± 24.2[a,b]	168.8 ± 27.3[b]	110.8 ± 19.5	<0.001
High-density lipoprotein	58.7 ± 14.9[b]	58.5 ± 18.5	55.1 ± 12.6	0.125	65.4 ± 15.4[b]	67.8 ± 14.9	62.8 ± 13.2	<0.01
Serum triglycerides (mg/dl)	137.1 ± 68.8[b]	163.8 ± 94.1[b]	115.2 ± 62.9	<0.001	105.2 ± 47.8[b]	117.1 ± 59.7[b]	83.4 ± 48.6	<0.001
Intima-media thickness (mm)	0.88 ± 0.26	0.96 ± 0.30	0.88 ± 0.21	0.352	0.83 ± 0.18[a,b]	0.88 ± 0.19[b]	0.75 ± 0.16	<0.001

Data represents the mean value ± S.D. or number (%) of subjects. Group A, lifestyle modification; group B, lifestyle modification with lipid-lowering drug; group C, normal TC.

[a] $P < 0.01$, compared to group B.

[b] $P < 0.01$, compared to group C.

Table 2
Changes in variables from baseline to 24 months of follow-up in men

	Men (n = 398)			
	Baseline, mean ± S.D.	24 months, mean ± S.D.	Percentage change	P-value
Body mass index (kg/m^2)				
Group A	23.7 ± 3.1	23.8 ± 3.4	−0.4	0.523
Group B	23.2 ± 3.5	22.9 ± 3.4	−1.3	0.056
Group C	23.2 ± 2.8	23.2 ± 3.0	0.0	0.393
Continuous TC normal group	23.2 ± 2.9	23.2 ± 3.0	0.0	0.822
TC turning abnormal group	22.9 ± 2.7	23.4 ± 3.1	2.2	0.046
Systolic blood pressure (mmHg)				
Group A	131.8 ± 16.3	134.8 ± 17.7	2.3	0.019
Group B	132.4 ± 17.2	132.8 ± 17.0	0.3	0.957
Group C	132.3 ± 18.3	135.5 ± 20.1	2.4	<0.001
Continuous TC normal group	132.4 ± 18.2	135.6 ± 19.8	2.4	<0.001
TC turning abnormal group	131.6 ± 20.2	134.3 ± 24.3	2.1	0.355
Diastolic blood pressure (mmHg)				
Group A	78.9 ± 10.3	80.7 ± 11.6	2.3	0.069
Group B	77.4 ± 11.6	79.0 ± 11.9	2.1	0.513
Group C	79.4 ± 10.4	79.4 ± 12.0	0.0	0.982
Continuous TC normal group	79.3 ± 10.3	79.4 ± 12.2	0.1	0.926
TC turning abnormal group	79.6 ± 11.1	79.0 ± 9.5	−0.8	0.815
TC (mg/dl)				
Group A	243.2 ± 19.4	226.6 ± 24.9	−6.8	<0.001
Group B	250.8 ± 23.7	197.2 ± 25.5	−21.4	<0.001
Group C	187.2 ± 21.7	185.6 ± 25.6	−0.9	0.218
Continuous TC normal group	185.9 ± 21.7	181.5 ± 22.5	−2.4	<0.001
TC turning abnormal group	201.8 ± 14.6	231.0 ± 9.6	14.5	<0.001
LDL-C (mg/dl)				
Group A	157.1 ± 21.3	141.2 ± 24.2	−10.1	<0.001
Group B	159.5 ± 25.8	122.4 ± 26.2	−23.3	<0.001
Group C	109.0 ± 21.9	107.6 ± 23.6	−1.3	0.255
Continuous TC normal group	108.2 ± 23.7	104.1 ± 20.8	−3.8	<0.001
TC turning abnormal group	117.9 ± 21.0	146.5 ± 18.7	24.3	<0.001
HDL-C (mg/dl)				
Group A	58.7 ± 14.9	57.0 ± 14.0	−2.9	0.014
Group B	58.5 ± 18.5	50.4 ± 12.6	−13.8	0.019
Group C	55.1 ± 12.6	54.6 ± 13.3	−0.9	0.266
Continuous TC normal group	54.9 ± 12.5	54.0 ± 13.1	−1.4	0.734
TC turning abnormal group	57.6 ± 14.7	61.0 ± 13.7	5.9	0.136
TG (mg/dl)				
Group A	137.1 ± 68.8	142.4 ± 74.4	3.9	0.313
Group B	163.8 ± 94.1	122.3 ± 54.6	−25.3	0.012
Group C	115.2 ± 62.9	117.2 ± 67.9	1.7	0.608
Continuous TC normal group	113.8 ± 62.8	117.1 ± 69.1	2.9	0.384
TC turning abnormal group	131.7 ± 62.0	117.4 ± 53.3	−10.9	0.351
IMT (mm)				
Group A	0.88 ± 0.26	0.82 ± 0.16	−6.8	0.002
Group B	0.96 ± 0.30	0.80 ± 0.19	−16.7	0.002
Group C	0.88 ± 0.21	0.84 ± 0.16	−4.5	<0.001
Continuous TC normal group	0.89 ± 0.21	0.82 ± 0.17	−7.9	<0.001
TC turning abnormal group	0.87 ± 0.26	0.86 ± 0.12	−1.1	0.481

TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; IMT, intima-media thickness. Group A, lifestyle modification, n = 107; group B, lifestyle modification with lipid-lowering drug, n = 33; group C, normal TC, n = 258; continuous TC normal group, n = 237; TC turning abnormal group, n = 21.

The HDL-C change was significantly higher in group B (−13.8%) than in groups A (−2.9%) and C (−0.9%) (P < 0.05), respectively. The TG change was significantly higher in group B (−25.3%) than in groups A (3.9%) and C (1.7%) (P < 0.05), respectively.

The IMT change was significantly higher in group B (−16.7%) than in group C (−4.5%) (P < 0.05) (Fig. 2A). No significant difference in the IMT change was found between groups A and B or between groups A and C over 24 months.

Table 3
Changes in variables from baseline to 24 months of follow-up in women

	Women ($n = 992$)			
	Baseline, mean ± S.D.	24 months, mean ± S.D.	Percentage change	*P*-value
Body mass index (kg/m^2)				
Group A	22.9 ± 3.4	22.7 ± 3.3	−0.9	<0.001
Group B	23.3 ± 3.4	23.1 ± 3.5	−0.9	0.033
Group C	21.9 ± 2.9	21.9 ± 3.0	0.0	0.198
Continuous TC normal group	21.8 ± 3.0	21.9 ± 3.0	0.5	0.236
TC turning abnormal group	22.3 ± 2.8	22.3 ± 2.8	0.0	0.616
Systolic blood pressure (mmHg)				
Group A	130.3 ± 19.6	131.9 ± 21.3	1.2	0.029
Group B	133.1 ± 19.6	131.8 ± 16.5	−1.0	0.247
Group C	121.9 ± 19.2	124.0 ± 19.8	1.7	<0.001
Continuous TC normal group	121.8 ± 19.3	123.6 ± 19.8	1.5	0.005
TC turning abnormal group	122.2 ± 18.4	127.0 ± 19.4	3.9	0.004
Diastolic blood pressure (mmHg)				
Group A	77.4 ± 10.6	77.8 ± 11.4	0.5	0.318
Group B	78.3 ± 10.0	76.8 ± 9.3	−1.9	0.076
Group C	73.0 ± 10.7	73.8 ± 10.9	1.1	0.063
Continuous TC normal group	72.8 ± 10.8	73.6 ± 10.8	−1.1	0.126
TC turning abnormal group	73.9 ± 10.1	75.2 ± 11.3	1.8	0.223
TC (mg/dl)				
Group A	246.3 ± 23.2	232.3 ± 27.6	−5.7	<0.001
Group B	257.7 ± 31.4	216.4 ± 31.0	−16.0	<0.001
Group C	190.3 ± 20.2	192.7 ± 26.4	1.3	0.004
Continuous TC normal group	187.4 ± 20.2	185.8 ± 21.0	−0.9	0.047
TC turning abnormal group	207.6 ± 9.1	234.6 ± 14.4	13.0	<0.001
LDL-C (mg/dl)				
Group A	157.6 ± 24.2	145.6 ± 28.2	−7.6	<0.001
Group B	168.8 ± 27.3	133.3 ± 32.4	−21.0	<0.001
Group C	110.8 ± 19.5	112.5 ± 24.5	1.5	0.036
Continuous TC normal group	108.7 ± 19.7	106.9 ± 20.5	−1.7	0.022
TC turning abnormal group	123.3 ± 12.4	146.3 ± 18.5	18.7	<0.001
HDL-C (mg/dl)				
Group A	65.4 ± 15.4	64.4 ± 15.4	−1.5	0.041
Group B	67.8 ± 14.9	62.6 ± 14.6	−7.7	<0.001
Group C	62.8 ± 13.2	62.7 ± 13.7	−0.2	0.618
Continuous TC normal group	62.3 ± 13.1	61.7 ± 13.4	−1.0	0.074
TC turning abnormal group	65.9 ± 13.6	68.7 ± 14.1	4.2	0.006
TG (mg/dl)				
Group A	105.2 ± 47.8	100.1 ± 49.7	−4.8	0.087
Group B	117.1 ± 59.7	111.9 ± 59.0	−4.4	0.208
Group C	83.4 ± 48.6	87.9 ± 55.2	5.4	0.014
Continuous TC normal group	82.0 ± 47.8	86.3 ± 53.6	5.2	0.025
TC turning abnormal group	91.7 ± 52.9	97.7 ± 63.3	6.5	0.319
IMT (mm)				
Group A	0.83 ± 0.18	0.79 ± 0.16	−4.8	<0.001
Group B	0.88 ± 0.19	0.81 ± 0.16	−8.0	<0.001
Group C	0.75 ± 0.16	0.74 ± 0.14	−1.3	0.055
Continuous TC normal group	0.75 ± 0.16	0.73 ± 0.14	−2.7	0.006
TC turning abnormal group	0.80 ± 0.15	0.80 ± 0.13	0.0	0.358

TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; IMT, intima-media thickness. Group A, lifestyle modification, $n = 330$; group B, lifestyle modification with lipid-lowering drug, $n = 126$; group C, normal TC, $n = 536$; continuous TC normal group, $n = 460$; TC turning abnormal group, $n = 76$.

In women, the TC and LDL-C change was significantly higher in group B (−16.0% and −21.0%) than in groups A (−5.7% and −7.6%) and C (1.3 and 1.5%) ($P < 0.01$), respectively (Fig. 1B). The TC and LDL-C change was significantly higher in group A than in group C ($P < 0.01$). The HDL-C change was significantly higher in group B (−7.7%) than in groups A (−1.5%) and C (−0.2%) ($P < 0.05$), respectively. TG was significantly increased in

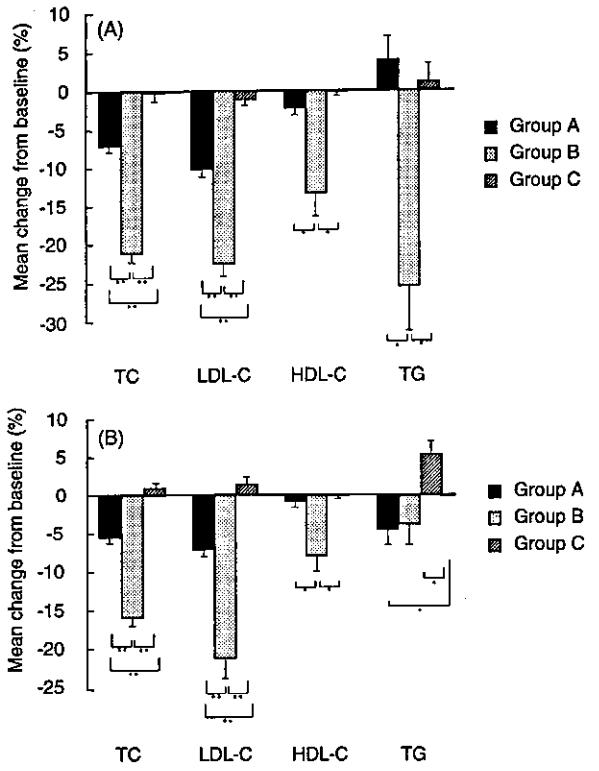

Fig. 1. (A) Mean percentage change in the serum lipid level of men over 24 months. Group A, lifestyle modification; group B, lifestyle modification with lipid-lowering drug; group C, normal TC: $^{**}P < 0.01$, $^{*}P < 0.05$. (B) Mean percentage change in the serum lipid level of women over 24 months. Group A, lifestyle modification; group B, lifestyle modification with lipid-lowering drug; group C, normal TC: $^{**}P < 0.01$, $^{*}P < 0.05$.

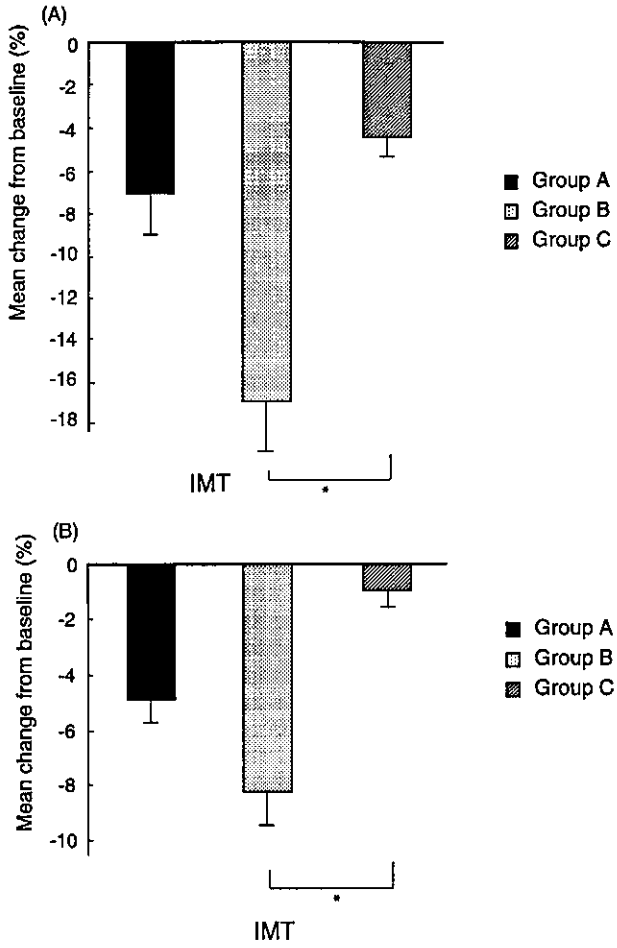

Fig. 2. (A) Mean percentage change in the IMT of men over 24 months. Group A, lifestyle modification; group B, lifestyle modification with lipid-lowering drug; group C, normal TC: $^{*}P < 0.05$. (B) Mean percentage change in the IMT of women over 24 months. Group A, lifestyle modification; group B, lifestyle modification with lipid-lowering drug; group C, normal TC: $^{*}P < 0.05$.

group C (5.4%), but decreased in groups A (−4.8%) and B (−4.4%) (group A or B versus C, $P < 0.05$).

The IMT change was significantly higher in group B (−8.0%) than in group C (−1.3%) ($P < 0.05$) (Fig. 2B). No significant difference in the IMT change was found between groups A and B or between groups A and C over 24 months.

4. Discussion

This large prospective study was designed to evaluate the effectiveness of various treatment regimens in subjects divided into three groups according to their cholesterol level at baseline and assessed by carotid IMT: (A) a lifestyle modification alone group; (B) a lifestyle modification with lipid-lowering drug therapy group; and (C) a control group. To our knowledge, this is the first evidence from a large-scale study to show that lifestyle modification alone can promote a significant reduction of carotid IMT over a 2 year period in men and women with hypercholesterolemia. Based on previous reports [2–4], a 'healthy lifestyle' (e.g., a well-balanced diet, regular physical exercise, smoking stoppage, and moderate alcohol intake) has become the backbone of consensus statements for primary prevention of cardiovascular disease. However, whether lifestyle changes, especially in diet and physical activity, can mediate a protective effect through a favorable influence on carotid atherosclerosis is still controversial.

Hodis et al. [15] reported that patients receiving lovastatin with dietary therapy had consistent reduction of carotid IMT as early as 1 year, whereas patients receiving only dietary therapy had a consistent increase of IMT at 2 and 4 years. The Fukuoka Atherosclerosis Trial [16] also showed that a diet therapy group had a significant reduction of LDL-C, but that IMT progression continued. On the other hand, Markus et al. [17] demonstrated that lifestyle modification, such as BMI reduction and dietary cholesterol intake reduction and quitting smoking, could reduce the annual rate of IMT progression. The results of studies of the effect of physical activity on IMT have been mixed, with a report of a protective

association with workplace activity [21], reduction in men but not women [22], and no relation [23]. Fields et al. [18] found that a multimodality traditional approach involving dietary, exercise, herbal food supplement, and stress reduction approaches could attenuate carotid atherosclerosis, particularly in those with marked cardiovascular risk. The differences in these results may be explained, at least in part, by differences in the populations or the study design, including lifestyle changes or the follow-up period. Our results indicate that comprehensive lifestyle modification can inhibit or reduce carotid IMT progression. It seems reasonable to suppose that a combination of an optimal dietary therapy and increased use of energy from fat through aerobic physical exercise might create a physiologic state that would be beneficial to the carotid arterial wall. If previous studies [15,16] had included more comprehensive lifestyle modification programs (e.g., diet, physical exercise, smoking stoppage, and weight control), the progression of carotid atherosclerosis may have been retarded even without the use of lipid-lowering drugs.

In our study, changes in the serum cholesterol level appeared to be correlated more closely with carotid IMT than did the changes in other lifestyle-related risk factors. Interestingly, the lifestyle modification alone group showed a significant regression of carotid IMT, even though the cholesterol level remained above the recommended level [24]. Schuler et al. [4] also found that patients engaging in regular physical exercise and consuming a low-fat diet over 1 year had a 10% reduction of the mean TC level, and that coronary atherosclerosis progressed in 23% of their patients, but was not changed in 45% and regressed in 32%, even though the TC level continued over 220 mg/dl, as in our lifestyle modification alone group. That a high serum cholesterol level is a major risk factor for the initiation and the development of atherosclerosis is beyond any doubt. However, our results showed that carotid IMT can be reduced by the reduction of TC, even though the TC level may remain above a level at which we would normally expect IMT to increase. Lifestyle changes should be part of any comprehensive treatment program for subjects with hypercholesterolemia, especially for low-risk subjects as were tested in our study, although the underlying mechanisms remain to be completely elucidated.

The reduction of carotid IMT was greater in the lifestyle modification with lipid-lowering drug group than in the lifestyle modification alone group in this study. Our results also showed that comprehensive lifestyle modification can reduce carotid IMT, even without the use of lipid-lowering drugs. From a medical cost standpoint, a comprehensive lifestyle modification program would obviously be the best first line of treatment for carotid atherosclerosis. Lifestyle intervention is safe and compatible with concurrent treatment for other conditions with atherosclerotic vascular risk, such as hypertension, diabetes, and obesity [18]. Moreover, atherosclerotic changes in carotid IMT have also become widely accepted as a marker of generalized atherosclerosis and have been associated with future cardiovascular and cerebrovascular events [8,10,11]. Therefore, the lifestyle modification of potential behavior-dependent factors may be a cost-effective way of preventing future vascular events.

In conclusion, our results suggested that comprehensive lifestyle modification can reduce carotid IMT and serum cholesterol levels and that cholesterol reduction provides benefit even when the TC level remains above that usually recommended.

Acknowledgements

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References

[1] Arntzenius AC, Kromhout D, Barth JD, Reiber JH, Bruschke AV, Buis B, et al. Diet, lipoproteins, and the progression of coronary atherosclerosis. The Leiden Intervention Trial. N Engl J Med 1985;312:805–11.
[2] Ornish D, Brown SE, Scherwitz LW, Billings JH, Armstrong WT, Ports TA, et al. Can lifestyle changes reverse coronary heart disease? Lifestyle Heart Trial Lancet 1990;336:129–33.
[3] Watts GF, Lewis B, Brunt JN, Lewis ES, Coltart DJ, Smith LD, et al. Effects on coronary artery disease of lipid-lowering diet, or diet plus cholestyramine, in the St. Thomas' Atherosclerosis Regression Study (STARS). Lancet 1992;339:563–9.
[4] Schuler G, Hambrecht R, Schlierf G, Niebauer J, Hauer K, Neumann J, et al. Regular physical exercise and low-fat diet. Effects on progression of coronary artery disease. Circulation 1992;86:1–11.
[5] Salonen JT, Salonen R. Ultrasound B-mode imaging in observational studies of atherosclerotic progression. Circulation 1993;87:56–65.
[6] Burke GL, Evans GW, Riley WA, Sharrett AR, Howard G, Barnes RW, et al. Arterial wall thickness is associated with prevalent cardiovascular disease in middle aged adults: the Atherosclerotic Risk in Communities (ARIC) Study. Stroke 1995;26:386–91.
[7] Mannami T, Konishi M, Baba S, Nishi N, Terao A. Prevalence of asymptomatic carotid atherosclerotic lesions detected by high-resolution ultrasonography and its relation to cardiovascular high risk factors in the general population of a Japanese city: the Suita Study. Stroke 1997;28:518–25.
[8] Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction; the Rotterdam Study. Circulation 1997;96:1432–7.
[9] Maeda N, Sawayama Y, Tatsukawa M, Shimizu C, Kashiwagi S, Hayashi J. *Chlamydia pneumoniae* seropositivity and early carotid atherosclerosis in a suburban Japanese population. Atherosclerosis 2002;164:313–9.
[10] Chambless LE, Heiss G, Folsom AR, Rosamond W, Szklo M, Sharrett AR, et al. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerotic Risk in Communities (ARIC) Study, 1987–1993. Am J Epidemiol 1997;146:483–94.
[11] Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu CR, Liu CH, et al. The role of carotid arterial intima media thickness in predicting clinical coronary event. Ann Intern Med 1998;128:262–9.

[12] Furberg CD, Adams Jr HP, Applegate WB, Byington RP, Espeland MA, Hartwell T, et al. Effect of lovastatin on early carotid atherosclerosis and cardiovascular events. Asymptomatic carotid artery progression. Circulation 1994;90:1679–87.
[13] Crouse III JR, Byington RP, Bond MG, Espeland MA, Craven TE, Sprinkle JW, et al. Pravastatin, lipids, and atherosclerosis in the carotid arteries (PLAC-II). Am J Cardiol 1995;75:455–9.
[14] Salonen R, Nyyssonen K, Porkkala E, Rummukainen J, Belder R, Park JS, et al. Kuopio Atherosclerosis Prevention Study (KAPS). A population-based primary preventive trial of the effect of LDL lowering on atherosclerotic progression in carotid and femoral arteries. Circulation 1995;92:1758–64.
[15] Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu C, Liu C, et al. Reduction in carotid arterial wall thickness using lovastatin and dietary therapy: a randomized controlled clinical trial. Ann Intern Med 1996;124:548–56.
[16] Sawayama Y, Shimizu C, Maeda N, Tatsukawa M, Kinukawa N, Koyanagi S, et al. Effects of probucol and pravastatin on common carotid atherosclerosis in patients with asymptomatic hypercholesterolemia. Fukuoka atherosclerosis trial (FAST). J Am Coll Cardiol 2002;39:610–6.
[17] Markus RA, Mack WJ, Azen SP, Hodis HN. Influence of lifestyle modification on atherosclerotic progression determined by ultrasonographic change in the common carotid intima-media thickness. Am J Clin Nutr 1997;65:1000–4.
[18] Fields JZ, Walton KG, Schneider RH, Nidich S, Pomerantz R, Suchdev P, et al. Effect of a multimodality natural medicine program on carotid atherosclerosis in older subjects: a pilot trial of Maharishi Vedic Medicine. Am J Cardiol 2002;89:952–8.
[19] Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. Summary of second report of the National Cholesterol Education Program (NCEP), Expert panel on detection, evaluation and treatment of high blood cholesterol in adults (adult treatment panel II). J Am Med Assoc 1993;269:3015–23.
[20] Maeda N, Sawayama Y, Tatsukawa M, Okada K, Furusyo N, Shigematsu M, et al. Carotid artery lesions and atherosclerotic risk factors in Japanese hemodialysis patients. Atherosclerosis 2003; 169:183–92.
[21] Folsom AR, Eckfeldt JH, Weitzman S, Ma J, Chambless LE, Barnes RW, et al. Relation of carotid artery wall thickness to diabetes mellitus, fasting glucose and insulin, body size, and physical activity. Stroke 1994;25:66–73.
[22] Stensland-Bugge E, Bonaa KH, Joakimsen O. Age and sex differences in the relationship between inherited and lifestyle risk factors and subclinical carotid atherosclerosis: the Tromso Study. Atherosclerosis 2001;154:437–48.
[23] Ebrahim S, Papacosta O, Whincup P, Wannamethee G, Walker M, Nicolaides AN, et al. Carotid plaque, intima media thickness, cardiovascular risk factors, and prevalent cardiovascular disease in men and women: the British Regional Heart Study. Stroke 1999;30:841–50.
[24] Hata Y, Mabuchi H, Saito Y, Itakura H, Egusa G, Ito H, et al. Report of the Japan Atherosclerosis Society (JAS) guideline for diagnosis and treatment of hyperlipidemia in Japanese adults. J Atheroscler Thromb 2002;9:1–27.

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HEPATOLOGY

Double point mutation in the core promoter region of hepatitis B virus (HBV) genotype C may be related to liver deterioration in patients with chronic HBV infection

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Abstract

Background and Aim: Hepatitis B virus (HBV) genotype C has a more severe pathogenesis than genotype B in Japan. We retrospectively investigated the relationship between HBV genotype and the core promoter (CP) (nt 1762 and 1764) and precore (PreC) (nt 1896) mutations of the HBV genome.

Methods: A total of 129 Japanese patients (42 genotype B and 87 genotype C) with chronic HBV infection, living in two different geographical areas in Japan, were evaluated (mean follow-up period 10.1 ± 3.8 years). In 2000, CP and PreC HBV mutations were analyzed by direct sequencing from sera. Hepatitis B e antigen (HBeAg), HBV DNA and serial alanine aminotransferase (ALT) changes were followed and determined using serological methods.

Results: Genotype C patients had significantly higher rates of HBeAg (40.2% *vs* 2.4%), HBV DNA positivity (75.9% *vs* 7.1%) and ALT abnormality (71.3% *vs* 11.9%) than genotype B patients (all $P < 0.05$). Among genotype B patients, CP wild type (92.9%) was predominant and PreC mutation (88.1%) was predominant. However, among genotype C patients, CP mutation (75.9%) was predominant and PreC mutation (66.7%) was predominant. The CP mutation was found significantly more in genotype C than in genotype B ($P < 0.05$). Of the 67 patients with ALT abnormality, five (7.5%) genotype B and 62 (92.5%) genotype C patients (31 HBeAg positive and 31 negative) were found. Among the 31 genotype C patients who were HBeAg positive, the combination of CP mutation and PreC wild (54.8%) was predominant, while among the remaining 31 genotype C patients who were HBeAg negative, the combination of CP mutation and PreC mutant (71.0%) was predominant.

Conclusion: Genotype C might be one of the worse prognostic markers in patients with chronic HBV infection, possibly because of mutation in the CP region.

Key words: chronic hepatitis B, core promoter mutant, genotype, precore mutant.

INTRODUCTION

Hepatitis B virus (HBV) infection is a major public health problem, with more than 350 million HBV carriers estimated worldwide.[1] During the course of the disease, seroconversion from hepatitis B e antigen (HBeAg) to antibody to HBeAg (anti-HBe) generally indicates a favorable outcome, with the cessation of active viral replication and benign non-progressive liver disease.[2] However, some HBV carriers have persistent viremia and liver damage after seroconversion from HBeAg to anti-HBe.[3–5]

We previously reported that there was a significant epidemiological difference in the clinical course of chronic HBV infection, even within one country such as Japan. Patients in Fukuoka had higher rates of HBeAg positivity and liver damage than those in Okinawa, and the former less frequently had HBeAg negativity than

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the latter.[6,7] This has been recognized as reflecting hepatitis B surface antigen (HBsAg) subtype, because HBsAg subtypes adr and adw are most common in Fukuoka and Okinawa, respectively. Also, the rates and ages of seroconversion from HBeAg to anti-HBe are higher and occur at a younger age in patients with HBsAg subtype adw than in those with adr. Recently, in place of this HBsAg subtype, the HBV genotype has become well used in the analysis of the relationship between heterogeneity of HBV genome and clinical features.[8-10] The HBV genotypes B (mainly HBsAg subtype adw) and C (mainly subtype adr) are commonly observed in Japan and other Asian countries.[5,11-14]

The core promoter region (CP) of HBV directs the transcription of both species of 3.5 kb messenger RNA (mRNA): pregenomic RNA (pgRNA) and precore mRNA (PreC mRNA). The pgRNA is translated into core protein and polymerase (reverse transcriptase) protein, and serves as a template for reverse transcription of HBV after being packed into core particles.[15] A pair of point mutations of the CP, an adenine (A) to thymine (T) transversion at nucleotide (nt) 1762 together with a guanine (G) to A transition at nt 1764 (1762T and 1764 A), were first described in the CP of Japanese patients infected with HBV.[16] These 1762T and 1764 A double mutations are frequently found in patients with chronic hepatitis or fulminant hepatitis,[17,18] and less often in asymptomatic healthy carriers.[19]

The PreC mRNA is translated into a PreC/core fusion protein that is post-translationally modified to yield HBeAg.[20] The most common PreC mutation that prevents HBeAg production is a G to A change at nt 1896, which creates a novel translational stop codon (at PreC codon 28) leading to premature termination of the translation of the PreC protein.[21,22] PreC mutations (1896 A) were reported to be possibly associated with more active liver disease and HBV viremia.[21]

These CP and PreC mutations of the HBV genome have been associated with loss of HBeAg and liver deterioration.[21-24] We have already demonstrated that Japanese genotype C patients have more severe liver deterioration than genotype B patients because of the delay of HBeAg disappearance and continued HBV replication after HBeAg disappearance.[5] The reason behind the pathogenesis of HBV genotype C compared to genotype B is unclear. Few research has been carried out to clarify the relationship between these mutations and HBV genotypes in Japanese patients with chronic HBV infection. To clarify this issue, we investigated if genotypic different pathogenesis exists in the HBV genome, especially in CP and PreC regions in Japanese patients with chronic HBV infection from two geographical areas of Japan.

METHODS

Study population

A total of 129 Japanese patients with chronic HBV infection, living in two different geographical areas in Japan, were retrospectively studied (mean follow-up period 10.1 ± 3.8 years). Of these, 87 were followed as outpatients at the Department of General Medicine, Kyushu University Hospital in Fukuoka Prefecture from 1988 (mean follow-up period 8.3 ± 1.7 years). The remaining 42 patients were from the Yaeyama District in the Okinawa Prefecture and were followed from 1978 in our study in which free health examinations were given to all residents and announced by distributing written notices to all households and as outpatients at Yaeyama Hospital (mean follow-up period 13.6 ± 4.4 years) (Table 1, Fig. 1).[5,6,25] All patients were able to be followed until 2000, the end of the follow-up period. From our previous Japanese study,[5] genotypes B and C were predominantly found in Okinawa and in Fukuoka, respectively. There was significant clinical difference in HBeAg positivity and liver deterioration between the genotypes, but there was no geographical difference by HBV genotype between Okinawa and Fukuoka.

To investigate the relationship between these mutations and HBV genotypes in Japanese patients with chronic HBV infection, we entered these genotype patients who were matched for sex and age into the present case-control study. The following criteria were fulfilled by all patients: positive for HBsAg for at least 6

Table 1 Characteristics of 129 patients with chronic hepatitis B virus (HBV) infection classified by HBV genotype

Characteristic	HBV genotype			
	B (n = 42)	C (n = 87)	Total (n = 129)	P-value
No. Okinawa patients (%)	38 (90.5)	4 (9.5)	42 (100)	<0.0001
No. Fukuoka patients (%)	4 (4.6)	83 (95.4)	87 (100)	<0.0001
Male : female ratio	24:18	56:31	80:49	NS
Age (mean ± SD years)	49.5 ± 15.7	44.3 ± 14.0	40.4 ± 14.7†	NS
No. HBeAg positive at start of follow-up (%)	14 (33.3)	47 (54.0)	61 (47.3)	<0.05
No. HBeAg positive at end of follow-up (%)	1 (2.4)	35 (40.2)	36 (27.9)	<0.0001
No. with HBV DNA level ≥0.7 Meq/mL (%)‡	3 (7.1)	66 (75.9)	69 (53.5)	<0.0001
No. with ALT abnormality (%)	5 (11.9)	62 (71.3)	67 (51.9)	<0.0001

†Age range at entry 14–72 years; ‡at the end of follow-up observation period. Alanine aminotransferase (ALT) abnormality was defined when an ALT level above 36 IU/L was observed for at least half of each patient's observation period. HBeAg, hepatitis B e antigen; Meq, million genome equivalents.

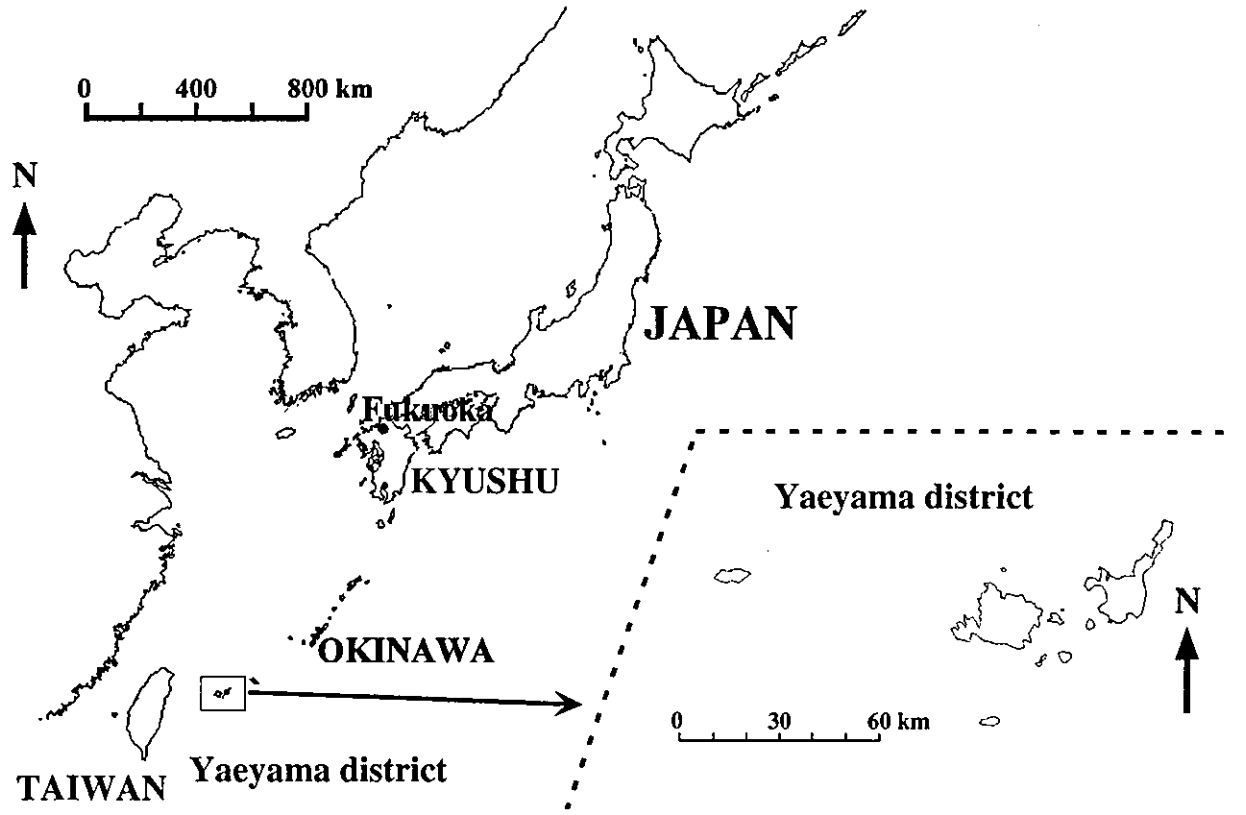

Figure 1 Map of the surveyed areas (Fukuoka and the Yaeyama district of Okinawa) in Japan. The map also shows the surveyed areas in relation to the main islands of Japan and Taiwan.

months; and to establish chronic HBV infection, exclusion of other concomitant causes of liver disease (hepatitis C and alcohol consumption >80 g/day) and relatively rare liver disease (autoimmune hepatitis and metabolic liver disease). All patients were negative for human immunodeficiency virus antibody. All patients were selected based on the availability of two or more serum samples from each year of the follow-up period. Genotyping and analysis of CP and PreC mutations were performed in each patient's sera at the end of follow-up. Informed consent to participate in the study was obtained from all adult participants and from the parents or legal guardians of minors. In genotype C patients, 44 (50.6%) received interferon treatment during the follow-up period but did not receive it within 2 years of this last observation period. None of the genotype B patients received antiviral treatment. In addition, none of the genotype B and C patients received corticosteroid treatment.

Alanine aminotransferase abnormality and classification of clinical status

Using a multiple autoanalyzer, serum was tested for alanine aminotransferase (ALT) levels at least every 6 months during the follow-up period. An ALT abnormality was defined when an ALT level above 36 IU/L was observed for at least half of each patient's observational period. We defined the clinical status of patients by liver histology or laboratory data as follows: asymptomatic healthy carrier (ASC), constantly had normal ALT levels (< 36 IU/L) for more than 3 years during the follow-up period; chronic hepatitis (CH), had ALT abnormality or histologically chronic hepatitis; liver cirrhosis (LC) was diagnosed by liver biopsy or by monitoring conventional laboratory tests and ultrasonography; hepatocellular carcinoma (HCC) was diagnosed by angiography, biopsy or surgical specimen and by monitoring conventional laboratory tests. A liver biopsy was undertaken for 43 genotype C patients and analyzed by a single pathologist.

Serological assay methods

All serum samples were separated soon after collection and stored at –20°C until testing for HBsAg, HBeAg, HBV genotype and HBV DNA. Presence of HBsAg was determined by passive hemagglutination (Mycell, Institute of Immunology, Tokyo, Japan) and HBeAg was determined by RIA (HBeAg RIA; Abbott Laboratories, North Chicago, IL, USA). The mutations of the CP and PreC regions were amplified and analyzed by direct sequencing.

Hepatitis B virus genotyping by ELISA

An enzyme-linked immunosorbent assay (ELISA) was developed for serological determination of the six HBV genotypes designated A, B, C, D, E and F. Monoclonal antibodies were raised against genotype-specific epitopes in the preS2-region product of HBV and labeled with horseradish peroxidase using commercial kits (HBV Genotype EIA, Institute of Immunology, Tokyo, Japan).[26,27] The HBsAg in sera was captured in wells of a microtiter plate coated with monoclonal antibodies 3207 and 5124 A, both directed to the common determinant epitope 'a', and tested for binding with genotype-specific monoclonal antibodies labeled with horseradish peroxidase (monoclonal antibodies 5520, epitope 'b'; T2741, 'm'; K0610A, 'k'; 4408, 's'; 3465, 'u'; 5142 A, 'f'; and 5156, 'g'). From the amino acid sequence found by reaction with monoclonal antibodies, genotypes A to F were determined: b, s and u for genotype A; b and m for B; b, k and s for C; b, k, s and u for D; b, k, s, u, f and g for E; and b, k and f for F. Genotype G was determined by the combination of the above preS2-based ELISA genotype kits for genotype D and HBsAg subtype adw.[28]

Quantitative detection of hepatitis B virus DNA

Serum HBV DNA was assayed by the Quantiplex branched DNA solid-phase assay using a series of nucleic acid probe hybridizations (Chiron Diagnostics, Emeryville, CA, USA), with a lower detection limit of 0.7 million genome equivalents per mL (Meq/mL), approximately 70000 viral genome copies/mL. Hepatitis B virus DNA positivity was defined as an HBV DNA level greater than 0.7 Meq/mL at the end of follow-up. The HBV DNA was quantified at the same time as the HBV genotype and CP/PreC mutations were determined in sera of the end of follow-up.

Hepatitis B virus DNA amplification and direct sequencing

Nucleic acids of HBV DNA were extracted from 100 μL of serum using a commercial kit (Smitest EX-R & D Genome Science, Tokyo, Japan). The extracted DNA was dissolved in 50 μL of H_2O and amplified by nested polymerase chain reaction (PCR). The HBV DNA sequences were amplified using the following nested primers. The outer primers were 5′-GTCTGT GCCTTCTCATCTGC-3′ at nt position 1551–1570 and 5′-AGCTGGAGGAGTGCGAATCC-3′ at nt position 2274–2293. The inner primers were 5′-TCG CATGGAGACCACCGTGA-3′ at nt position 1604–1623 and 5′-AGAATAGCTTGCCTGAGTGC-3′ at nt position 2059–78. The PCR products were subjected to a direct sequencing reaction using an ABI 310 automated sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA, USA). A CP region mutation was designated when regions nt 1762 and 1764 were T and A, and the wild type was designated when the same region was A and G by direct sequencing. A PreC region mutation was designated when nt 1896 was G and the wild type was designated when the same region was A by direct sequencing.

Statistical analysis

Age and follow-up period were expressed as the mean ± standard deviation (SD). The HBV DNA level was expressed as the mean ± standard error (SE). The chi-squared test or Fisher's exact test was used for categorical variables for comparisons between the two groups. For all tests, a P-value under 0.05 was considered to have statistical significance.

RESULTS

Relationship between hepatitis B virus (HBV) genotypes, clinical status and HBV markers

The 42 genotype B patients were classified as 35 (83.3%) ASC, 7 (16.7%) CH and none LC and/or HCC, whereas 87 genotype C patients comprised 3 (3.4%) ASC, 48 (55.2%) CH and 36 (41.4%) LC and/or HCC (Table 2). The classification of ASC was significantly more often observed in genotype B patients than in genotype C patients ($P < 0.0001$), whereas CH and LC and/or HCC were more often observed in genotype C patients than in genotype B patients (both $P < 0.0001$). Progressive liver deterioration was more common in genotype C than B patients. At the start of follow-up, 14 (33.3%) cases of HBeAg positivity were found in 42 genotype B patients, while 47 (54.0%) cases of HBeAg positivity were found in 87 genotype C patients. At the end of follow-up, one (2.4%) case of HBeAg positivity, three (7.1%) of HBV DNA positivity, and five (11.9%) cases ALT abnormality were found in 42 genotype B patients, while 35 (40.2%) cases of HBeAg positivity, 66 (75.9%) of HBV DNA positivity and 62 (71.3%) cases of ALT abnormality were found in 87 genotype C patients. Genotype C patients had significantly higher rates of HBeAg and HBV DNA positivity and ALT abnormality than genotype B patients (all $P < 0.0001$).

Core promoter and precore mutations classified by hepatitis B virus genotype

Table 2 shows the CP and PreC mutations classified by HBV genotype in 129 patients at the end of follow-up. Among 42 genotype B patients, the wild type (92.9%) was predominant in the CP region and the mutation (88.1%) was predominant in the PreC region. However, among 87 genotype C patients, the mutation (75.9%) was predominant in the CP region and the mutation (66.7%) was predominant in the PreC region. The mutation in the CP region was found significantly