Over 50 years ago, it was reported that under acidic conditions, some vinyl ethers can react with organic hydroperoxides to form perketals (16, 17). At around 1990, Porter et al. (18, 19) reported the application of vinyl ether (*trans*-2-phenylcyclohexyl 2-propen-2-yl ether) for the synthesis of optically pure hydroperoxides (e.g., α-phenethyl hydroperoxide, 2-octyl hydroperoxide, and some fatty acid methyl ester hydroperoxides). The procedures include protecting the racemic hydroperoxides as perketals by using vinyl ether, separating the perketal diastereomers by chromatography, and regenerating the optically pure hydroperoxides from the perketal diastereomers. Baba et al. (21) used MxP for the conversion of LAMeOOH into its perketal. The perketal was utilized for preparation of PLPCOOH (21), but the preparation needed several synthetic steps. In this study, we thought that if MxP reacts efficiently with a variety of LOOHs (refer to the beginning of this article), this reaction can be applied for preparation of pure LOOHs (Fig. 1A).

We investigated and optimized the preparation conditions of LOOH (Fig. 2 and Table 1). Under optimal conditions, when the lipid used (PLPC, PLPE, PLPS, ChL, LLL, LA, or LAMe) was subjected to RB-catalyzed photo-, UV photo-, or LOX-1-catalyzed oxidation, the unsaturated fatty acid (linoleoyl) residue was converted to hydroperoxides. In cases of RB-catalyzed photo- and UV photo-oxidation of lipids, the structure of hydroperoxy linoleic residue was characterized as a mixture of 13-hydroperoxy-9*Z*,11*E*-octadecadienoate, 9-hydroperoxy-10*E*,12*Z*-octadecadienoate, 13-hydroperoxy-9*E*,11*E*-octadecadienoate, and 9-hydroperoxy-10*E*,12*E*-octadecadienoate. In LOX-1-catalyzed oxidation, 13-hydroperoxy-9*Z*,11*E*-octadecadienoate was produced predominantly. In addition, we found that RB-catalyzed photo- and UV photo-oxidation of ChL yielded mono-hydroperoxide as well as bis-hydroperoxide. Moreover, these reactions produced mono-, bis-, and tris-hydroperoxides of LLL. These LOOHs were partly decomposed to a wide range of secondary oxidation products, including hydroxides, epoxides, aldehydes, and ketones. These secondary oxidation products were the major obstacle in the isolation of pure LOOH.

For the reaction of LOOH with MxP, one of the important parameters was the reaction solvent. We found that dichloromethane, chloroform, or acetonitrile could be used as a solvent, but other water-containing solvents inhibited the reaction. Reaction temperature is also important since high temperatures (above 30°C) caused the formation of unknown products. Based on these results, we optimized the reaction conditions (Fig. 3 and Table 2). Under optimal conditions, LOOH was almost completely converted (above 90%) to perketal within a short time period of less than 3 h. The lipophilic perketal was eluted in a position apart from that of intact LOOH, and thereby the perketal could be identified and isolated by semipreparative LC (Fig. 4). No decomposition products of perketal were detected, indicating the stability of perketal. In addition, because MxP did not react with the secondary oxidation products, the isolation of perketal could be performed effectively. To reveal this point, we selected RB-photo-oxidized PLPC as an example (the example has an advantage to show that MxP reacts selectively to LOOHs but not to other oxidation secondary products derived from the RB-photo-oxidation).

A possible reaction scheme for the regeneration of intact LOOH from perketal is shown in **Fig. 9**. As shown in the scheme, the kind and the concentration of the acid used were important, and these factors were optimized (Fig. 5 and Table 3). In this study, chloroform/methanol (1:1, v/v) was used as the reaction solvent, and methanol or acetonitrile could also be used. Under optimal conditions, perketal

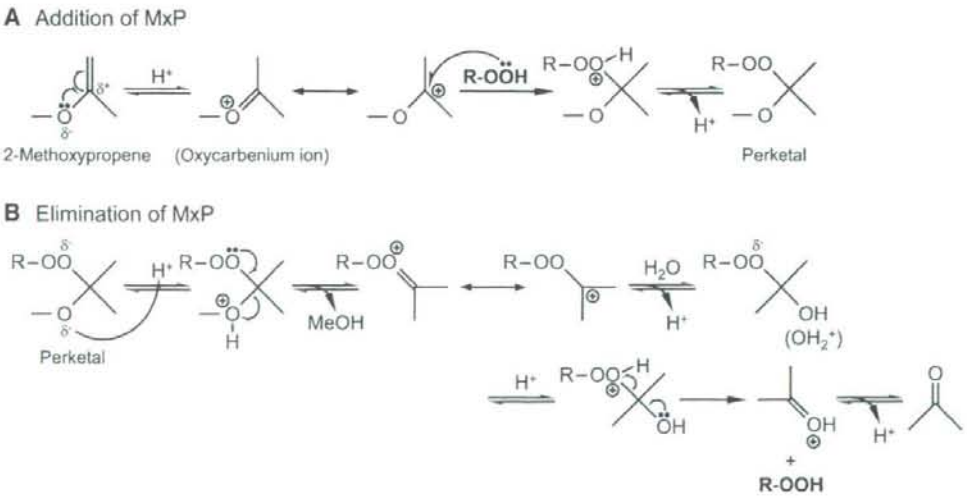

Fig. 9. Presumed mechanism of the reaction between MxP and hydroperoxide. A: Addition of MxP by nucleophilic addition of hydroperoxide to 2-methoxypropene. B: Elimination of MxP and regeneration of hydroperoxide.

was converted to original LOOH with a high yield (above 90%), and the regenerated LOOH was next subjected to final semipreparative LC purification (Fig. 6).

The obtained LOOHs after photo-oxidation were highly pure (Fig. 7, Table 3), but structural isomers were present (e.g., the prepared PLPCOOH contained mainly 13-hydroperoxy-9*Z*,11*E*-octadecadienoate and 9-hydroperoxy-10*E*,12*Z*-octadecadienoate). However, the "structural mixture" would be sufficient for use as a standard in most analytical and quantitative experiments. In contrast, when LOX-1-catalyzed oxidation was carried out, a pure LOOH isomer bearing 13-hydroperoxy-9*Z*,11*E*-octadecadienoate moiety was obtained. On the other hand, by using the present method, we prepared pure bis-hydroperoxide of ChL. Its structure was speculated based on the following findings: *1*) it reacted with two MxP molecules; and *2*) when the MxP adduct was subjected to alkaline hydrolysis, the reaction yielded LAOOMxP and perketal of cholesterol hydroperoxide.

With regard to the stability test, perketals were found to be more stable than LOOHs. Although perketal bearing an acidic carboxyl group (PLPSOOMxP or LAOOMxP) tended to degrade to some extent after 12 months storage at −30°C, such degradation was kept to a minimum by storage at −80°C for 3 months. We therefore recommended the following procedures: researchers should prepare and store perketals at −80°C in advance, regenerate LOOH from perketals before the experiment (e.g., quantitative study), and use the pure LOOH as the reference material.

In conclusion, we developed a convenient method for preparing a wide variety of pure LOOH references. We are now synthesizing and using the pure LOOH (e.g., PLPCOOH) as the standard for quantification of phosphatidylcholine hydroperoxide present in the blood plasma of healthy and nonhealthy humans, as well as a model compound to evaluate its biological functions in cell culture studies. We propose to use the MxP procedures developed by us as gold standard in the preparation method for the LOOH assay.

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Usefulness of Pravastatin in Primary Prevention of Cardiovascular Events in Women

Analysis of the Management of Elevated Cholesterol in the Primary Prevention Group of Adult Japanese (MEGA Study)

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Background—It is well known that statins reduce the risk of cardiovascular disease. However, the effect of statins in women for the primary prevention of cardiovascular disease has not been determined. We conducted an exploratory analysis of the effect of diet plus pravastatin therapy on the primary prevention of cardiovascular events in women with data from a large-scale primary prevention trial with pravastatin.

Methods and Results—Patients with hypercholesterolemia (5.7 to 7.0 mmol/L) and no history of coronary heart disease or stroke were randomized to diet or diet plus pravastatin 10 to 20 mg/d and followed up for ≥5 years. We investigated the effect of diet plus pravastatin treatment on cardiovascular events in 5356 women during the 5-year follow-up. The incidence of cardiovascular events in the women was 2 to 3 times lower than that in men. The occurrence of cardiovascular events was 26% to 37% lower in the diet plus pravastatin treatment group than in the diet alone group. Although these differences did not reach statistical significance, the overall risk reductions were similar to those in men. Notably, women ≥60 years of age treated with diet plus pravastatin had markedly higher risk reductions for coronary heart disease (45%), coronary heart disease plus cerebral infarction (50%), and stroke (64%) than did women treated with diet alone.

Conclusions—Treatment with pravastatin in women with elevated cholesterol but no history of cardiovascular disease provides a benefit similar to that seen in men, and this benefit is more marked in older women. This treatment should be considered routinely for primary cardiovascular protection in women with elevated cholesterol levels. (***Circulation.*** **2008;117:494-502.)**

Key Words: coronary disease ■ hypercholesterolemia ■ prevention ■ stroke ■ women

It is well known that the incidence of and mortality rates from cardiovascular disease (CVD) are lower in women than in men of the same age. However, as in men, the incidence of ischemic heart disease in women rises with age, and the incidence in women reaches the level in men a decade later.[1–3]

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Factors possibly explaining this ≈10-year lag in disease onset between men and women include direct actions of estrogen on blood vessels[4] and women's lifestyle characteristics (related to smoking, alcohol consumption, dietary style, exercise, etc) that are favorable for the prevention of cardiovascular events.[5] However, the estrogen hypothesis has been controversial, and the risk factors of diabetes mellitus or hypertension are more prevalent in women.[5] Thus, there is still some argument about the reasons for the delayed onset of cardiovascular events in women.[6] Indeed, the risk for cardiovascular events is lower for women than for men of corresponding age because the average life expectancy is 10 years longer for women; thus, the overall number of CVD events analyzed for all age groups does not differ between men and women.[1,2] Moreover, reports have shown that the prognosis for CVD is less favorable in women than in men.[7] Therefore, it is important to establish valid means for preventing the onset of CVD in women. Numerous large-scale studies designed to evaluate the usefulness of hydroxymethyl glu-

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taryl coenzyme A reductase inhibitors (statins) in primary and secondary prevention of CVD conducted since the 1990s[8–17] have enrolled primarily men. No study conducted to date has conclusively demonstrated the usefulness of statin therapy as a means of primary prevention of CVD in women. Moreover, the effectiveness of statins in women remains unknown, despite some attempts to determine it through meta-analyses.[18,19]

In Japan, the recently reported Management of Elevated Cholesterol in the Primary Prevention Groups of Adult Japanese (MEGA) study was designed to evaluate the usefulness of pravastatin in the primary prevention of CVD.[20,21] Of the 7832 patients enrolled in this study, 68% were women; >5000 women were followed up for a mean of 5.3 years. The number of women in this study is the largest of any study of statins conducted to date. Here, we summarize the comparison of the results between women and men and report the results of a detailed investigation in women in the MEGA study.

Methods

The MEGA study design and overall findings have been reported previously.[20,21] Briefly, in this prospective, randomized, open, blinded end-point study[22] conducted between 1994 and 2004, men and postmenopausal women (physician diagnosis) 40 to 70 years of age with hypercholesterolemia whose total cholesterol (TC) levels ranged from 5.7 to 7.0 mmol/L with no history of coronary heart disease (CHD) and cerebrovascular disease visiting the outpatient clinic were asked to join the study; all subjects provided written informed consent. The eligibility check was done on individual patients by attending physicians on the basis of their diagnosis. Then, their eligibility was determined by laboratory testing of their serum cholesterol using a standardized procedure on 2 or 3 occasions over 12 weeks. Eligible patients were randomly assigned to the National Cholesterol Education Program (NCEP) step I[23] diet alone group or to the NCEP step I diet plus pravastatin by computerized randomization by the permuted-block method and stratified according to sex, age, and medical institution. Major exclusion criteria included familial hypercholesterolemia, a history of CVD, a current diagnosis of malignancy, and secondary hyperlipidemia. The dose of pravastatin was 10 to 20 mg/d, the approved dose in Japan.

Patients were evaluated during every visit to the hospital/clinic. Each patient received a medical check, including onset of end points, by the attending physician at 1, 3, and 6 months after the start of follow-up and every 6 months thereafter. For each event, the diagnosis was made by the attending physician (including data from ECG and myocardial scintigraphy as needed) and reported in detail. ECGs were performed once a year. Information on individual patients was entered into the case report forms by their attending physicians and reported to the data center. For each event, detailed information was obtained from physicians and evaluated by the End-Point Committee under blinding according to the criteria we reported previously.[21] Throughout the study period, TC, high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and lipoprotein(a) levels were measured centrally at the same laboratory using methods standardized by the Centers for Disease Control and Prevention (Atlanta, Ga). Low-density lipoprotein cholesterol (LDL-C) level was estimated by the Friedewald formula.[24] The primary composite end point was the first occurrence of CHD, comprising fatal and nonfatal myocardial infarction, cardiac and sudden death, coronary revascularization procedure, and angina. Secondary end points included stroke, cerebral infarction, intracranial hemorrhage, CHD plus cerebral infarction, cerebral infarction plus transient ischemic attack, all cardiovascular events, and total mortality. All events were independently verified by the End-Point Committee in the blinded manner. Patients in both groups were counseled to follow the NCEP step I diet throughout the study period. Treatment in the diet plus pravastatin group was initiated at pravastatin 10 mg/d. During follow-up, the dose of pravastatin could be adjusted by the treating physician, with uptitration to 20 mg/d if the TC level did not decrease to ≤5.69 mmol/L in compliance with the approved Japanese dose. Patients in each group exceeding a TC of 6.98 mmol/L, even after enhancement of assigned treatment, could be switched to other aggressive treatments, including statin therapy. Concomitant treatment for complications was not restricted in either group.

The follow-up period was initially scheduled for 5 years; however, on the basis of the recommendation of the Data and Safety Monitoring Committee, the study was continued an additional 5 years to increase the number of events. Thus, patients who provided written consent at 5 years to continue the study were followed up until the end of March 2004. The trial was conducted in compliance with the ethics principles of the Declaration of Helsinki and the Japanese Ministry of Health, Labor, and Welfare ordinance regarding post-marketing surveillance.

Statistical analyses were performed following the intention-to-treat principle. The analysis sets were determined by the Data Review Committee before the end of the study in a blinded manner based on prerandomization patient data to avoid the possibility of introducing bias. The effect of diet plus pravastatin on primary and secondary end points in women was examined mainly using the 5-year data to reduce the effect of the high drop-in rate for statin use in the patients randomized to diet only caused by the additional follow-up period. Baseline characteristics and lipid changes during each year of the 5-year study period were evaluated in women. Time-to-event curves for major events were estimated by the Kaplan–Meier method in women and men and in both treatment groups. The effect of diet plus pravastatin treatment for women was compared with men for the major end points of CHD, CHD plus cerebral infarction, stroke, and total mortality and was evaluated in men and women stratified by age. The classification criteria of each event were reported previously.[22]

The evaluation of safety, including severe adverse events and cancers that occurred after 5 years, was based on the entire study period for each patient to determine the safety of long-term treatment. The log-rank test was used for comparison between groups. Hazard ratios and their confidence intervals were estimated by Cox proportional-hazards model. Adjusted analysis also was conducted by Cox proportional-hazards model with age, hypertension, diabetes mellitus, and HDL-C level as covariates. Smoking was not used as a covariate because of the low proportion of female smokers. TC was not used for the adjusted analysis because eligible patients for this study had a narrow range of TC (5.7 to 7.0 mmol/L). The factors adjusted in this model were determined by risk factor analysis of the present data. Heterogeneity of treatment effect for major end points (CHD, CHD plus cerebral infarction, stroke, and total mortality) between men and women was examined by testing for treatment-sex interaction with the Cox proportional-hazards model, including treatment group, sex, and the interaction term. For the examination of the effects of aging, Cox modeling was adjusted for diabetes, hypertension, and HDL-C level.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Baseline Characteristics and Lipid Parameters

Of the 7832 patients enrolled in MEGA, 5356 (68.4%) were women and 2476 (31.6%) were men. Table 1 summarizes the baseline characteristics by sex.

No difference was found in any baseline characteristic between the diet group and diet plus pravastatin group in women. The mean age in women was 59.7 years, slightly higher than in men. Fewer women (17.8%) than men (27.5%) had diabetes mellitus. However, slightly more women had hypertension (42.6% of women versus 40.2% of men). Baseline TC level did not differ markedly between women (6.3 mmol/L) and men (6.2 mmol/L). However, baseline TG

Table 1. Baseline Characteristics According to Sex

Characteristics	Women			Men, Total (n=2476)
	Diet (n=2718)	Diet+Pravastatin (n=2638)	Total (n=5356)	
Demographics				
Age				
Mean (SD), y	59.8 (6.0)	59.6 (6.2)	59.7 (6.1)	55.2 (8.3)
<55 y, n (%)	592 (21.8)	599 (22.7)	1191 (22.2)	1183 (47.8)
≥55 y, n (%)	2126 (78.2)	2039 (77.3)	4165 (77.8)	1293 (52.2)
BMI, mean (SD), kg/m²	23.7 (3.1)	23.7 (3.3)	23.7 (3.2)	24.2 (2.8)
SBP, mean (SD), mm Hg	132.4 (17.0)	132.2 (17.0)	132.3 (17.0)	132.0 (16.2)
DBP, mean (SD), mm Hg	78.2 (10.1)	77.6 (10.3)	77.9 (10.2)	80.2 (10.3)
Hypertension,* n (%)	1155 (42.5)	1126 (42.7)	2281 (42.6)	996 (40.2)
Diabetes,* n (%)	475 (17.5)	477 (18.1)	952 (17.8)	680 (27.5)
Smoker,† n (%)	171 (6.3)	163 (6.2)	334 (6.2)	1280 (51.7)
Drinking, n (%)	337 (12.4)	329 (12.5)	666 (12.4)	1697 (68.5)
Lipid values, mmol/L				
TC, mean (SD)	6.3 (0.3)	6.3 (0.3)	6.3 (0.3)	6.2 (0.3)
LDL-C, mean (SD)	4.1 (0.4)	4.1 (0.4)	4.1 (0.4)	4.0 (0.5)
HDL-C, mean (SD)	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)	1.4 (0.4)
TG, median (interquartile range)	1.3 (1.0–1.8)	1.3 (1.0–1.8)	1.3 (1.0–1.8)	1.7 (1.3–2.4)
Lp(a), mean (SD)	0.9 (0.9)	0.9 (0.9)	0.9 (0.9)	0.8 (0.9)

BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; and Lp(a), lipoprotein(a). LDL-C was calculated with the Friedewald formula.

*Hypertension and diabetes were based on physician diagnosis.

†Smoker includes current and past smokers.

level was lower in women (1.3 mmol/L) than men (1.7 mmol/L), and HDL-C level was higher in women (1.5 mmol/L) than men (1.4 mmol/L) (Table 1).

In women, TC levels decreased by 1.1%, 2.5%, and 2.7% in the diet group and by 12.4%, 12.6%, and 11.8% in the diet plus pravastatin group at 1, 3, and 5 years, respectively, relative to baseline. LDL-C level decreased by 1.6%, 4.6%, and 4.9% and by 19.1%, 20.2%, and 19.1% in the 2 groups at 1, 3, and 5 years, respectively. On average, TC and LDL-C reductions after diet therapy were higher by ≈0.7% and

Table 2. Lipid Profiles During the Study Periods in Women

	Baseline	Year 1	Year 3	Year 5	Average	Average Change in Men
Patients, n						
Diet	2718	2296	2006	1362	2718	1248
Diet+pravastatin	2638	2185	1951	1330	2638	1228
TC, mean±SD, mmol/L (% change)						
Diet	6.3±0.3	6.2±0.6 (−1.1)	6.1±0.7 (−2.5)	6.1±0.7 (−2.7)	6.2±0.4 (−2.0)	−2.7
Diet+pravastatin	6.3±0.3	5.5±0.6 (−12.4)	5.5±0.6 (−12.6)	5.5±0.7 (−11.8)	5.5±0.5 (−11.9)	−11.0
LDL-C, mean±SD, mmol/L (% change)						
Diet	4.1±0.4	4.0±0.6 (−1.6)	3.9±0.7 (−4.6)	3.9±0.7 (−4.9)	3.9±0.5 (−3.3)	−4.6
Diet+pravastatin	4.1±0.4	3.3±0.6 (−19.1)	3.2±0.6 (−20.2)	3.3±0.6 (−19.1)	3.3±0.5 (−18.7)	−17.6
HDL-C, mean±SD, mmol/L (% change)						
Diet	1.5±0.4	1.6±0.4 (1.9)	1.6±0.4 (4.8)	1.6±0.4 (5.0)	1.6±0.4 (2.3)	1.7
Diet+pravastatin	1.5±0.4	1.6±0.4 (4.4)	1.6±0.4 (6.3)	1.6±0.4 (6.2)	1.6±0.4 (4.3)	5.5
TG, median [interquartile], mmol/L (% change)						
Diet	1.3 [1.0–1.8]	1.2 [0.9–1.8] (−6.9)	1.2 [0.9–1.7] (−7.8)	1.2 [0.9–1.7] (−6.9)	1.3 [1.0–1.7] (−1.2)	−3.3
Diet+pravastatin	1.3 [1.0–1.8]	1.2 [0.9–1.6] (−13.1)	1.2 [0.9–1.6] (−11.4)	1.2 [0.9–1.6] (−12.2)	1.2 [1.0–1.6] (−7.2)	−7.7

LDL-C was calculated with the Friedewald formula. All lipids measurements within ±3 months from measurement points at 1, 3, and 5 years after initiation of treatment were averaged. All lipid values were measured at the central laboratory. Percent changes were averaged in each individual versus baseline.

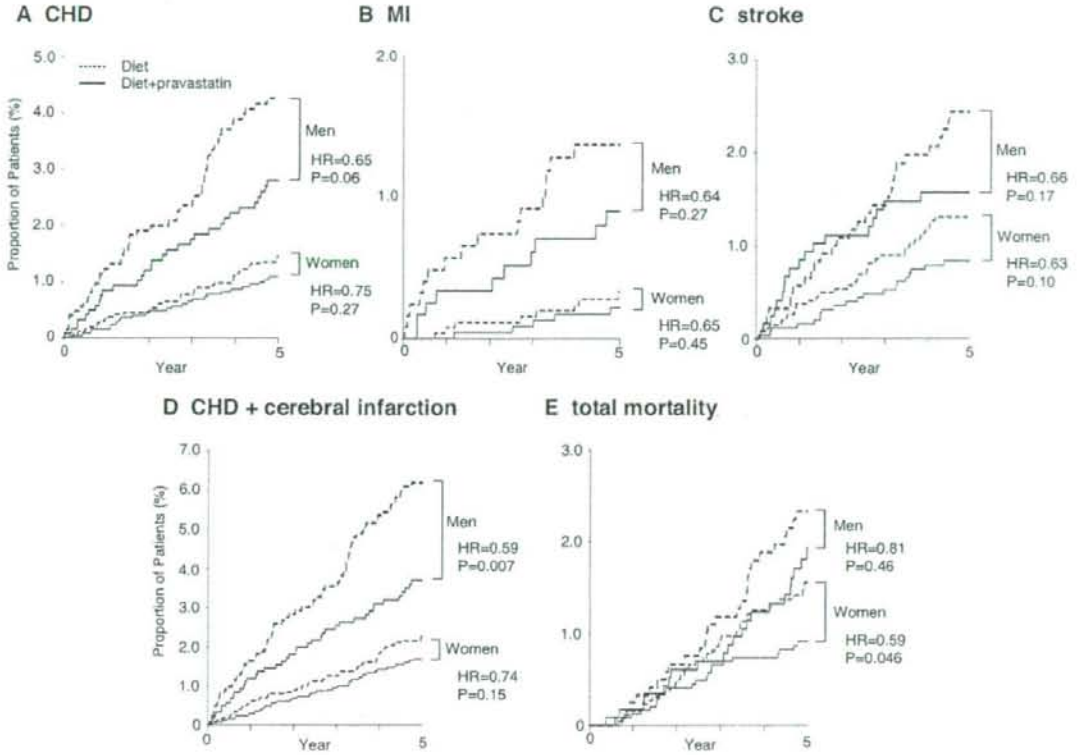

Figure 1. Kaplan–Meier curves for major end points in men and women. MI indicates myocardial infarction.

1.3%, respectively, in women than in men, and these 2 parameters after diet plus pravastatin therapy were lower by 0.9% and 1.1%, respectively, in women than in men.

HDL-C levels rose by 2.3% on average after diet therapy and by 4.3% on average after diet plus pravastatin therapy. TG levels decreased by 1.2% on average after diet therapy and by 7.2% on average after diet plus pravastatin therapy (Table 2).

Incidence of Outcomes

The incidence of events was compared between women and men by Kaplan–Meier methods for fatal/nonfatal CHD, myocardial infarction, stroke, CVD, and total mortality during the 5-year follow-up (Figure 1). The incidence of all major events was consistently increased during the follow-up periods in both women and men, and the difference in the incidence rate between women and men did not change throughout the study period. The incidence ratios of the major end points during the 5-year follow-up in women and men by randomized group are shown in Figure 2. In women in the diet group, the incidence of CHD and CHD plus cerebral infarction was 2.9 and 4.6 per 1000 person-years, respectively, which was one-third that in men (8.9 and 12.9 per 1000 person-years, respectively), and the incidence of stroke and all-cause death was 2.7 and 3.1 per 1000 person-years, respectively, which was about one-half that seen in men (5.0 and 4.7 per 1000 person-years, respectively). In the diet plus pravastatin group, the difference between women and men for the incidence rates of these events was similar to that in the diet group.

Efficacy and Safety of Pravastatin Treatment

The incidence of CHD in women was proportionally lower by 25% (hazard ratio [HR], 0.75; 95% CI, 0.45 to 1.25) in the diet plus pravastatin group compared with the diet group, with no significant interaction ($P=0.67$ for interaction) between women and men (HR, 0.65; 95% CI, 0.41 to 1.02; Figures 1 and 2). Furthermore, the incidence of stroke, CHD plus cerebral infarction, cerebral infarction plus transient ischemic attack, and all cardiovascular events for women was proportionally lower by 37% (HR, 0.63; 95% CI, 0.36 to 1.10), 26% (HR, 0.74; 95% CI, 0.50 to 1.12), 23% (HR, 0.77; 95% CI, 0.41 to 1.44), and 28% (HR, 0.72; 95% CI, 0.50 to 1.02), respectively, in the diet plus pravastatin versus diet group. Total mortality in women was lower by 41% (HR, 0.59; 95% CI, 0.35 to 0.997) in the diet plus pravastatin group compared with the diet group (Table 3 and Figure 1). The risk of major events was decreased by 41%, 34%, and 19% for CHD plus cerebral infarction, stroke, and total mortality, respectively, in men; no significant interaction was found between women and men (Figure 2).

In women, risk of events such as CHD rose with age and tended to rise particularly markedly in women ≥55 years of age at enrollment (Figure 3).

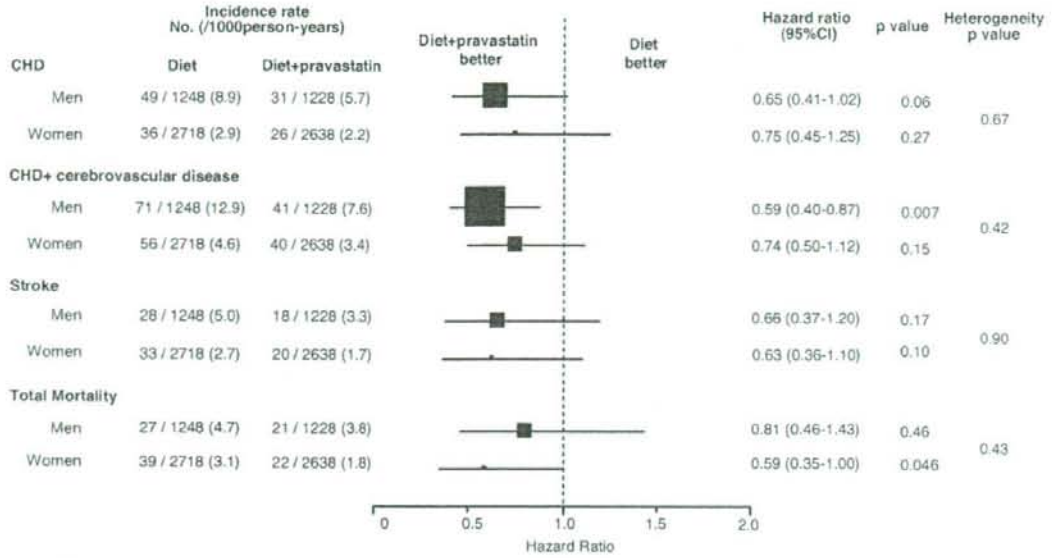

Figure 2. Heterogeneity for major end points between men and women. Square size indicates the event incidence rate.

In the analysis according to age, diet plus pravastatin treatment reduced the incidence of CHD, CHD plus cerebral infarction, and stroke more markedly as age increased. The magnitude of risk reduction for CHD, CHD plus cerebral infarction, and stroke was greater by ≥30% in patients ≥55 years of age. No difference was observed between age and total mortality (Figure 4).

No difference was observed in the incidence of severe adverse events in women in the diet group (8.9%; n=242) and diet plus pravastatin group (9.6%; n=252). Tissue site

Table 3. End Points in Women

	No. (per 1000 Person-Years)		Crude		Adjust*	
	Diet (n=2718)	Diet+Pravastatin (n=2638)	HR (95% CI)	P	HR (95% CI)	P
Primary point						
CHD	36 (2.91)	26 (2.20)	0.75 (0.45–1.25)	0.27	0.74 (0.45–1.23)	0.25
Secondary end points						
Stroke	33 (2.67)	20 (1.69)	0.63 (0.36–1.10)	0.10	0.63 (0.36–1.09)	0.10
Cerebral infarction	20 (1.62)	14 (1.18)	0.73 (0.37–1.45)	0.36	0.72 (0.37–1.43)	0.35
Intracranial hemorrhage	12 (0.97)	6 (0.50)	. . .	. . .	. . .	. . .
Not classifiable	1 (0.08)	0 (0.00)	. . .	. . .	. . .	. . .
CHD + cerebral infarction	56 (4.55)	40 (3.39)	0.74 (0.50–1.12)	0.15	0.73 (0.49–1.10)	0.14
Cerebral infarction+TIA	23 (1.86)	17 (1.43)	0.77 (0.41–1.44)	0.42	0.77 (0.41–1.43)	0.41
All cardiovascular events	74 (6.04)	51 (4.33)	0.72 (0.50–1.02)	0.07	0.71 (0.50–1.01)	0.06
Total mortality	39 (3.10)	22 (1.83)	0.59 (0.35–0.997)	0.046	0.59 (0.35–0.998)	0.049
Cardiovascular death	4 (0.32)	4 (0.33)	. . .	. . .	. . .	. . .
Non-cardiovascular death	35 (2.78)	18 (1.50)	0.54 (0.31–0.95)	0.03	0.54 (0.31–0.95)	0.03
Cancer	19 (1.51)	10 (0.83)	0.55 (0.26–1.19)	0.12	0.55 (0.26–1.19)	0.13
Accident	5 (0.40)	3 (0.25)	. . .	. . .	. . .	. . .
Other	6 (0.48)	3 (0.25)	. . .	. . .	. . .	. . .
Unknown	5 (0.40)	2 (0.17)	. . .	. . .	. . .	. . .

TIA indicates transient ischemic attack. HR and 95% CI were estimated for the end points having >20 events in the combined treatment groups. CHD included fatal and nonfatal myocardial infarction, angina, cardiac and sudden death, and revascularization.

*Adjusted by age, HDL-C, hypertension, and diabetes.

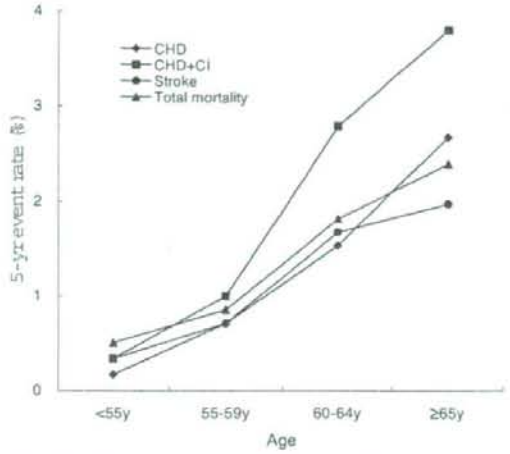

Figure 3. Incidence of major end points according to age group in women during the 5-year follow-up.

and total incidence rate of cancer did not differ significantly between the 2 groups (n=78, 5.55/1000 person-years; and n=74, 5.46/1000 person-years, respectively; Table 4).

Table 4. Cancers in Women

	No. (per 1000 Person-Years)			
	Diet (n=2718)	Diet+Pravastatin (n=2638)	HR (95% CI)	*P*
All cancers	78 (5.55)	74 (5.46)	0.98 (0.71–1.35)	0.91
Gastrointestinal	38 (2.69)	31 (2.27)	0.84 (0.52–1.35)	0.47
Respiratory	6 (0.42)	4 (0.29)	0.69 (0.20–2.46)	0.57
Breast	15 (1.06)	10 (0.73)	0.69 (0.31–1.53)	0.36
Genitourinary	10 (0.70)	14 (1.02)	1.45 (0.64–3.27)	0.37
Other	12 (0.85)	17 (1.24)	1.48 (0.70–3.09)	0.30

First occurrence of cancers was counted.

Discussion

Of the patients enrolled in this study, ≈70% (5356 patients) were women, more than any previous study of statins and prevention. The results of the present analysis are based on detailed analyses of the women in the MEGA study. In the women in the MEGA study, the incidence of events was lower for women than for men, and the risk for women tended to rise with age, similar to the findings in previous reports.[1–3] The generally low incidence of events in women requires a large sample size to make a valid comparison, which may have contributed to the lack of studies of women to date. The Air

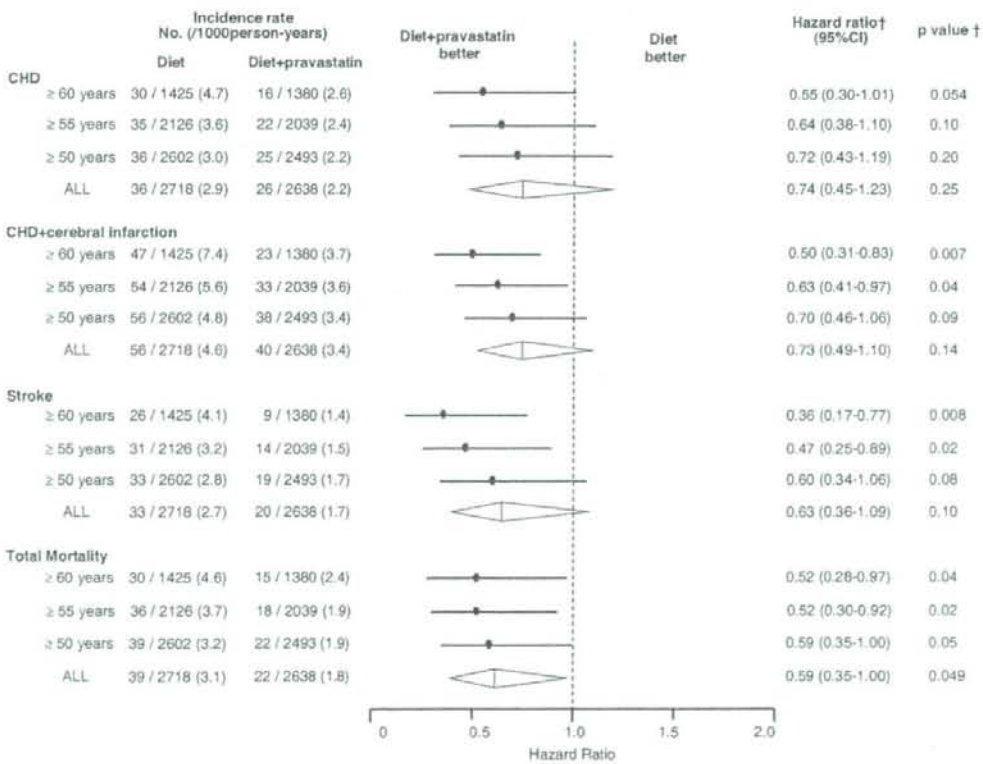

Figure 4. Relationship between major end points and aging for women and the effect of pravastatin. †HR was calculated with the Cox proportional-hazards model adjusted by age, HDL-C level, hypertension, and diabetes.

Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS),[11] Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial–Lipid Lowering Trial (ALLHAT-LLT),[14] Anglo-Scandinavian Cardiac Outcomes Trial–Lipid Lowering Arm (ASCOT-LLA),[15] and Heart Protection Study (HPS)[16] studied primary prevention, and Scandinavian Simvastatin Survival Study (4S),[8] Long-Term Intervention With Pravastatin in Ischaemic Disease (LIPID),[12] Cholesterol and Recurrent Events (CARE),[10] HPS,[16] and Prospective Study of Pravastatin in the Elderly at Risk (PROSPER)[13] studied secondary prevention and conducted only subgroup analyses in women. Some studies demonstrated a 4% to 46% reduction in risk for relapse of CHD after treatment with statins in secondary prevention, but the reduction was statistically significant only in CARE. Among the primary prevention studies, the risk of developing CHD while on statins was significantly reduced by 24% in HPS and by 45% in AFCAPS/TexCAPS (P=NS), whereas in ALLHAT-LLT and ASCOT-LLA, no risk reduction associated with statin therapy was observed. In HPS, a significant reduction in the incidence of CHD was observed, but in AFCAPS/TexCAPS and ASCOT-LLA, there were not enough events to allow valid evaluation of the efficacy of statins in women. Moreover, ALLHAT-LLT was difficult to evaluate because a risk reduction was not observed in the main analysis.

In the present study, the incidence of cardiovascular events was lower in women than in men. Among background variables possibly associated with the risk for cardiovascular events, the percentage of smokers was lower in women. This may have contributed to the lower incidence of cardiovascular events in women than in men. Furthermore, the percentage of diabetic patients was much lower in women than in men. Body mass index and TG level were slightly lower in women. Thus, the lower percentage of women with risk factors is probably associated with the difference in the incidence of events between men and women. The influence of alcohol was not clear in the present study because alcohol intake was not measured. Thus, even with a low incidence of cardiovascular events, there was a 25% reduction in the risk of CHD with diet plus pravastatin therapy versus diet alone in the MEGA study. It is likely that the small number of events in MEGA did not allow detection of a statistical difference because the number of events experienced in present study was very low. In the comparison of risk reduction by diet plus pravastatin between men and women, the observed risk reduction for women compared with men was 25% versus 35% for CHD, 26% versus 41% for CHD plus cerebral infarction, 37% versus 34% for stroke, and 41% versus 19% for total mortality. Thus, although the incidence of CVD is 3 times lower in women than in men, women had a very similar risk reduction for overall CVD events compared with men. A significant interaction was not found between men and women for the major cardiovascular events, and these findings indicate that the best overall estimation of the effect of diet plus pravastatin treatment is based on the total population.

An epidemiological study showed that the incidence of CHD in women begins to rise sharply starting from 55 to 60 years of age, ie, at the onset of menopause.[1] We observed that the incidence of CHD events rose with age in the present study (Figure 3). Because of reports that the pattern of the age-related increase of CHD in women tends to lag ≈10 years behind that in men,[1,2,18] we evaluated the effect of pravastatin in relation to age and found that diet plus pravastatin treatment was more effective with increasing age in women and that the incidence of cardiovascular events was halved in older patients by diet plus pravastatin treatment in the present study. A similar observation has been reported with low-dose aspirin therapy[25]; no overall risk reduction for CVD events was seen in the total population, but there was a significant risk reduction in the patients ≥65 years of age (no data were reported for women 60 to 64 years). Although the exact distribution of age among women in past studies is not clear from the published reports, with the exception of PROSPER in which the mean age of women was 75 years,[13] the mean age of women was ≈60 years in most previous studies and did not differ markedly from that in the present study, suggesting that these studies included relatively young patients in whom risk for cardiovascular events was low, probably leading to lack of evident difference in the magnitude of risk reduction between statin-treated and control groups. In the 2-year Canadian Coronary Atherosclerosis Intervention Trial (CCAIT) study with lovastatin,[26] LDL-C level was decreased by ≈30% in both men and women, whereas progression of coronary artery stenosis was suppressed more significantly in women than in men. The estimated number needed to treat to prevent 1 event was the same in men and women in the meta-analysis of the secondary prevention trials,[19] so there is no evidence for the view that the failure of statins to decrease the risk of CHD in women is associated with the mechanism of onset of CHD. The lack of evident reduction in CHD in women by statin therapy in past studies seems more likely attributable to the lack of statistical power because of the inclusion of a high percentage of relatively young patients whose risk for CHD events was very low.

In the present study, the effectiveness of pravastatin in younger women was not demonstrated because of an insufficient number of younger women enrolled to detect a difference. As recommended in the NCEP guidelines,[27] coexisting risk factors should be taken into consideration when younger women are treated. In view of reports that the age-related incidence of hyperlipidemia and CHD rises in women ≈10 years later than in men, it is likely that the risk for cardiovascular events rises after menopause. It is an interesting question whether the onset of CHD events could be reduced further if cholesterol-lowering therapy were started at an earlier stage of hypercholesterolemia.

Similar to CHD, the risks for onset of stroke, CHD plus cerebral infarction, and all CVD were reduced in women in the pravastatin group by 37%, 26%, and 28%, respectively. The finding that pravastatin reduced the incidence of stroke is valuable. Calculated from absolute risk reduction in the present study, the number needed to treat to prevent 1 CHD event is 176 for women ≥55 years of age and 106 for women ≥60 years of age. For the combination of CHD plus cerebral infarction, the number needed to treat to prevent 1 event is 109 for women ≥55 years of age and 61 for women ≥60 years of age. This suggests that pravastatin may be expected to exert a particularly high efficacy in older women.

A significant reduction in total mortality was seen in the diet plus pravastatin group. Surprisingly, death from atherosclerotic

causes was not reduced in women, and the reduction in total mortality was due primarily to a reduction in death from nonatherosclerotic diseases (primarily cancer). However, because the incidence of cancer was not different between the 2 treatment groups, the exact reason for this reduction in total mortality in the pravastatin plus diet group is unknown.

Severe adverse events (including cancers) did not significantly differ between the diet plus pravastatin and diet groups in women; notably, no increase in the incidence of cancers specific to women (ie, breast cancer, gynecologic genital cancer) was seen. Among previous studies, the CARE study reported that the incidence of breast cancer was significantly higher in a pravastatin group compared with a control group.[10] However, considering that only 576 women were enrolled in the CARE study and that the present study included 10 times as many women and revealed a lower incidence of breast cancer in the diet plus pravastatin therapy group than in the diet alone group, it seems unlikely that taking pravastatin elevates the risk of developing breast cancer in women. These findings do not contradict the results of a meta-analysis of cancers that was recently reported.[28]

A limitation to consider when the results from this analysis are interpreted is that the proportional-hazards model for men regarding stroke did not appear to satisfy the proportional-hazards assumption (Figure 1C). The reason is that the occurrence of intracranial hemorrhage in men was higher in the diet plus pravastatin group than in the diet group during the first 12 months of follow-up (6 versus 1 event). However, judging from the Kaplan–Meier curves, this likely does not affect the overall conclusions. Another limitation is that the analyses in the subgroups of women according to age are exploratory because of the small numbers of events and multiple statistical testing. Thus, we need to interpret the results for these analyses with caution.

The fact that the data are from Japanese people whose risk for CVD is low compared with other countries[1] is also a limitation for this analysis. The incidence of CHD is quite low for Japanese people, and the incidence of stroke differs markedly between Japanese and Western people. Although the reason for this is not entirely clear, it has been speculated that the Japanese lifestyle plays an important role because a higher incidence of CHD was observed in the Honolulu Heart Program in Japanese who migrated to Hawaii or California than in Japanese living in Japan.[29] The lack of an evidently significant difference for CHD in women in the present study also seems to be attributable to the low incidence of CHD among Japanese people. However because a risk reduction with statins was observed regardless of risk factors in different populations in a large meta-analysis,[30] the results of the present study may be applicable to non-Japanese populations. Because the present study was an open-label study, we took great care to avoid bias in the conduct of this study. To that end, adjudication of end points was done by the End Points Committee, which was blinded to treatment. Furthermore, detailed information about the end points for each group was collected by a contract research organization that was independent of the sponsor. In addition, physicians could at their discretion prescribe statin therapy, either the study drug or another statin, even to patients in the diet only group to limit dropouts. Because the study subjects were followed up as outpatients and are insured by a single-payer national health insurance system, it seems unlikely that treatment differed much between patients other than the allocation to study group. Yet, because we cannot rule out unpredictable bias, it is desirable to carry out a double-blind study of the effect of statins in preventing ischemic heart disease with a target set for women, especially the middle-aged and elderly population.

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CLINICAL PERSPECTIVE

The beneficial effect of statin treatment in reducing the risk of cardiovascular disease is well known from the findings of many large-scale randomized clinical trials. However, there has been some debate about the use of statins in women because of their lower cardiovascular risk compared with men. Notably, a similar reduction in cardiovascular end points was demonstrated with statin use in women and men in the present analysis of the Management of Elevated Cholesterol in the Primary Prevention Groups of Adult Japanese (MEGA) study. Specifically, pravastatin reduced coronary heart disease by 25% and 35%, coronary heart disease and cerebral infarction by 26% and 41%, stroke by 37% and 34%, and total mortality by 41% and 19% in women and men, respectively. Moreover, the beneficial primary prevention effect was more marked in older women. Thus, these findings indicate that it is appropriate and beneficial to consider statin treatment in women with elevated lipids but without a history of cardiovascular disease to reduce their future risk, especially in older women. Long-term use of statin therapy was shown to be safe in the MEGA study, without any increase in serious adverse problems, including female specific cancer.

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Estimation of treatment effect adjusting for treatment changes using the intensity score method: Application to a large primary prevention study for coronary events (MEGA study)

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SUMMARY

The MEGA study was a prospective, randomized, open-labeled, blinded-endpoints study conducted in Japan to evaluate the primary preventive effect of pravastatin against coronary heart disease (CHD), in which 8214 subjects were randomized to diet or diet plus pravastatin. The intention-to-treat (ITT) analysis showed that pravastatin reduced the incidence of CHD (hazard ratio = 0.67; 95 per cent confidence interval (CI): 0.49–0.91) and of stroke events, which was the secondary endpoint in the MEGA study (hazard ratio = 0.83; 95 per cent CI: 0.57–1.21). Owing to considerable treatment changes, it is also of interest to estimate the causal effect of treatment that would have been observed had all patients complied with the treatment to which they were assigned. In this paper, we present an intensity score method developed for clinical trials with time-to-event outcomes that correct for treatment changes during follow-up. The proposed method can be easily extended to the estimation of time-dependent treatment effects, where the technique of g-estimation has been difficult to apply in practice. We compared the performances of the proposed method with other methods (as-treated, ITT, and g-estimation analysis) through simulation studies, which showed that the intensity score estimator was unbiased and more efficient. Applying the proposed method to the MEGA study data, several prognostic factors were associated with the process of treatment changes, and after adjusting for these treatment changes, larger treatment effects for pravastatin were observed for both CHD and stroke events. The proposed method provides a valuable and flexible approach for estimating treatment effect adjusting for non-random non-compliance. Copyright © 2007 John Wiley & Sons, Ltd.

KEY WORDS: non-compliance; time-dependent confounding; causal inference; failure time data; propensity score; structural nested mean model

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1. INTRODUCTION

In a typical clinical trial, patients are randomized to one of the treatment groups and each patient is expected to receive that treatment throughout the follow-up to assess the effect of the treatment on some outcome. However, most clinical trials are not ideal; hence, patients often fail to adhere to their assigned treatment and switch to another trial treatment. Such non-compliance with assigned treatment is a common feature of clinical trials. Recently there has been much interest in methods for analyzing randomized clinical trials of treatments to which the subject are not compliant [1–3].

One approach for analyzing data for non-compliance is the as-treated (AT) analysis, which compares outcomes based on the treatment that patients actually received. When non-compliance is completely at random, that is, independent of both (observed and unobserved) baseline and time-dependent factors, the AT analysis can give a valid test for the null hypothesis of no treatment effect and can also give an unbiased estimate of treatment effect. In most clinical trials, however, patients who comply with their assigned treatment are not comparable with those who do not with respect to some important prognostic factors. In this case, both the decision to comply and the outcome may well depend on underlying possibly unmeasured health status. Thus, when non-compliance is non-random, the AT analysis will not be valid even under the null hypothesis because of the comparison of selected groups [3, 4].

The more commonly used analytic approach is an intention-to-treat (ITT) analysis, which compares outcomes based on the treatment groups randomized by design regardless of whether the patients complied with their assigned treatment. Because the comparability of the treatment groups is guaranteed by randomization, the null hypothesis of no treatment effect for all patients (sharp causal null hypothesis) is preserved in the ITT analysis. That is, successful randomization insures that the ITT comparison provides a valid test for the sharp causal null hypothesis of no treatment effect even in the presence of non-random non-compliance. Moreover, p-values have a randomization interpretation when design-based (randomization-based) analyses are used [5]. Furthermore, the ITT estimate would correspond to the overall treatment effect that would be realized if the treatment were actually adopted and practiced in the community, provided the rate of non-compliance and the factors influencing non-compliance that are observed in the trial are identical to those that would occur in the community. A point against the ITT analysis is that the ITT parameter does not measure the true biological effect of treatment, but rather a mixture of the effect on the compliers with the absence of effect on the non-compliers, because the ITT estimate is the average effect of treatment assignment. Hence, the ITT analysis gives estimates that are biased toward the null when treatment crossover is present, and the ITT measure of treatment effect will diminish as non-compliance increases. Moreover, the rate of non-compliance in the community, once the treatment is adopted, may not be the same as the rate in the original clinical trial.

Therefore, in the analysis of non-compliance data, it is important to estimate the causal effect of treatment, that is, the effect that would be realized if all patients complied with the treatment to which they were assigned. Robins [6–8] has proposed a structural nested mean model (SNMM) to estimate such causal effect in the presence of non-random non-compliance. Under the assumption that non-compliance at each time is at random, given the observed histories that influence a patient's decision to comply, that is, the assumption of no unmeasured confounders, the causal parameter in a SNMM can be estimated by the technique of g-estimation.

Recently, Brumback *et al.* [9] proposed the intensity score approach for the analysis of time-varying treatments in the presence of time-dependent confounding. They provided conditions

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under which the intensity score approach consistently estimates a treatment effect in a SNMM. The intensity score is cumulative differences over time between treatment actually received and treatment predicted by prior observed medical history. The SNMM treatment effect can be obtained by regressing outcomes on the intensity score. Thus, the intensity score approach can provide an easy implementation of g-estimation for the analysis of non-random non-compliance. Since the intensity score approach was originally proposed for continuous outcomes, we extend its use to time-to-event outcomes with censoring. This extension is useful, because censoring due to end of scheduled follow-up requires special care when using g-estimation based on the structural accelerated failure time (SAFT) model [10–16], while the intensity score approach can treat the censoring within the framework of standard regression models. Furthermore, the intensity score approach has the advantage of providing estimates of parameters in a SNMM that allows the treatment effects to vary across time, while it has been difficult to apply such a model in practice using the technique of g-estimation [9].

This article is organized as follows. In Section 2, we describe the motivating study from a large randomized primary prevention study for coronary events, the Management of Elevated Cholesterol in the Primary Prevention Group of Adult Japanese (MEGA) study [17, 18]. In Section 3, we develop the intensity score approach for event times. In Section 4, simulation studies are conducted to compare the performances of the proposed intensity score method with those of the AT, ITT, and g-estimation (semi-parametric randomization-based) analysis [10, 12]. Section 5 presents the analysis results of the MEGA study data. Finally, Section 6 provides some discussions.

2. THE MEGA STUDY

We will briefly describe the motivating study and the data (MEGA study). Full details on the design, conduct, and main clinical results have been reported [17, 18]. The MEGA study is a randomized controlled trial conducted in Japan to evaluate the primary preventive effect of a statin against coronary heart disease (CHD) in daily clinical practice. In this prospective, randomized, open-labeled, blinded-endpoints design study, men and postmenopausal women aged 40–70 years with hypercholesterolemia (total cholesterol (TC) level: 220–270 (mg/dL)) and no history of CHD or stroke were randomized to diet (diet group) or diet plus pravastatin 10–20 mg daily (pravastatin group).

Between February 1994 and March 1999, a total of 15 210 persons visiting outpatient clinics were registered throughout Japan. Of the 15 210 subjects who met the inclusion criteria regardless of their TC levels and who provided signed informed consent, 8214 who met the TC criterion were randomized to either diet or diet plus pravastatin treatment using the permuted block method with stratification according to gender, age, and medical institution. After the exclusion of 382 patients (94 withdrew consent, 224 exclusion criteria violation, and 64 no recorded data after randomization), the remaining 7832 patients were analyzed (3966 diet group; 3866 pravastatin group).

Table I shows the baseline characteristics of the analyzed patients. There was no clinical difference between the two groups in baseline characteristics. Women accounted for 68.4 per cent (5356 patients) of the study population. Mean body mass index (BMI) was 23.8 (kg/m^2). Mean TC, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) levels were 242.6, 156.6, and 57.5 (mg/dL), respectively. Median triglyceride (TG) level was 127.5 (mg/dL). Of the study patients, 41.8 and 20.8 per cent had hypertension and diabetes mellitus based on physician diagnosis, respectively.

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Table I. Baseline characteristics of analyzed 7832 patients.

Characteristics	Diet group ($N=3966$)		Diet+pravastatin group ($N=3866$)	
Age (years), mean (SD)	58.4	(7.2)	58.2	(7.3)
Women, no. (per cent)	2718	(68.5)	2638	(68.2)
BMI (kg/m^2), mean (SD)	23.8	(3.0)	23.8	(3.1)
Current smoker, no. (per cent)	572	(14.4)	612	(15.8)
Current drinking, no. (per cent)	1183	(29.8)	1180	(30.5)
Hypercholesterolemia medication history, no. (per cent)	621	(15.7)	586	(15.2)
Hypertension, no. (per cent)	1664	(42.0)	1613	(41.7)
Diabetes, no. (per cent)	828	(20.9)	804	(20.8)
TC (mg/dL), mean (SD)	242.6	(12.1)	242.6	(12.0)
TG (mg/dL), median (inter-quartile range)	127.5	(95.0–179.0)	127.4	(95.7–176.5)
HDL-C (mg/dL), mean (SD)	57.5	(15.1)	57.5	(14.8)
LDL-C (mg/dL), mean (SD)	156.5	(17.3)	156.7	(17.6)

SD, standard deviation; BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

After randomization, patients were followed at months 1, 3, and 6 and thereafter every 6 months. At each visit, data on treatment compliance, use of concomitant drugs, onset of events, occurrence of adverse events, and laboratory tests including serum lipids were collected by the investigators. Additionally, an ECG (electrocardiogram) was obtained and evaluated annually. The follow-up period was initially scheduled for 5 years; however, on the basis of the recommendation of the Data and Safety Monitoring Committee, the study was continued for an additional 5 years to increase the number of events, and thus, patients who provided written consent at 5 years to continue the study were followed until the end of March 2004 [17, 18].

The primary endpoint was the first occurrence of CHD, comprised of fatal and non-fatal myocardial infarction, angina, cardiac and sudden death, and a coronary revascularization procedure. One of the secondary endpoints was the first occurrence of stroke events. All endpoints were reviewed strictly by the blinded Endpoint Committee and additional information obtained from the physician as needed [17]. A total of 7832 patients were followed by 2658 physicians in 1320 hospitals. The follow-up period was 41 195 person-years (mean follow-up period 5.3 years). CHD events occurred in 101 of 3966 patients in the diet group (2.55 per cent) and 66 of 3866 patients in the pravastatin group (1.71 per cent). Figure 1 shows the Kaplan–Meier curves for CHD events. The ITT analysis indicated that the incidence of CHD was significantly lower by 33 per cent in the pravastatin group than in the diet group (The ITT hazard ratio $=0.67$; 95 per cent confidence interval (CI): 0.49–0.91; $p=0.01$ for the log-rank test) [18].

However, many patients changed to the other trial treatment frequently during the study period (treatment crossover). This was because the protocol in the MEGA study stated that patients in the diet group could be switched to pravastatin treatment when a reduction of TC level was not observed, while patients in the pravastatin group could discontinue pravastatin treatment when the reduction of TC level was observed. The treatment decisions for changing the treatment or increasing the dose of paravastatin were determined by each treating physician. Patients who changed to another trial treatment even once in the first 5 years were 19.9 per cent ($n=790$) in the diet group and 53.4 per cent ($n=2064$) in the pravastatin group. These numbers for the whole

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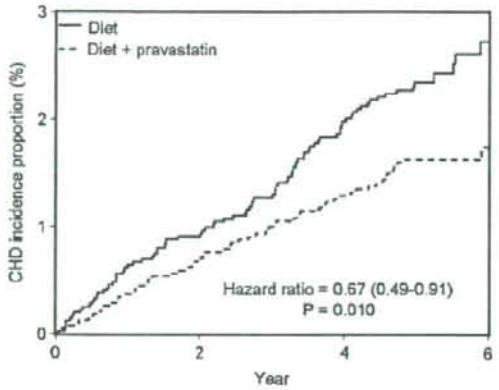

Figure 1. Incidence proportion for CHD events.

10 years were 21.3 per cent ($n=844$) and 63.1 per cent ($n=2441$), respectively. The effect of patients from one treatment to the other is to make the treatment profiles of the two randomized groups more similar than they otherwise would have been, and therefore to move the ITT hazard ratio toward the null.

3. INTENSITY SCORE METHOD

3.1. The multiplicative structural nested mean model

We consider a randomized clinical trial in which two groups (test and control treatment) are compared with respect to time-to-event outcomes and each patient i ($i=1,\ldots,N$) receives one of the treatments at the start of each time t ($t=0,\ldots,M-1$; time zero is the randomization time and the start of the first treatment). However, some patients fail to comply with their assigned treatment and cross over to the other treatment at each time t.

Suppose we have repeated measures on treatment $S_i(t)$ ($S_i(t)=1$ if test treatment, $S_i(t)=0$ if control treatment) and covariates $L_i(t)$ at time t. Let $H_i(t)$ be the observed history of treatment and the covariates prior to treatment at time t, i.e. $H_i(t)=(L_i(0), S_i(0), \ldots, L_i(t-1), S_i(t-1), L_i(t))$, with $H_i(0)=(L_i(0))$. Let $T_i(\overline{S}_i(t), 0)$ denote the potential event times in response to the hypothetical treatments $(S_i(0),\ldots,S_i(t), S_i(t+1)=0,\ldots,S_i(M-1)=0)$. That is, $T_i(\overline{S}_i(t), 0)$ represents the event time we would have observed if, possibly contrary to fact, the patient had his/her actual treatment history up to time t but was then switched to control treatment at time $t+1$ and remained at that treatment until the event occurred. Our notation for the potential outcomes implicitly assumes Rubin's stable unit treatment value assumption, which implies that potential outcomes of patient i do not depend on the treatment received by any other patient [19]. We will also assume that the potential outcomes satisfy the consistency assumption [7] that serves to link the potential outcomes with the observed outcomes T_i. This assumption states that $T_i=T_i(\overline{S}_i(t), 0)$ for all t when actually $S_i(t+1)=\cdots=S_i(M-1)=0$ occurred.

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We introduce a simple multiplicative SNMM [6–8]

$$\log E[T_i(\overline{S}_i(t),0)|H_i(t),S_i(t)]-\log E[T_i(\overline{S}_i(t-1),0)|H_i(t),S_i(t)]=\beta_0 S_i(t) \quad (1)$$

where β_0 is the constant (across t) incremental causal effect of a final treatment $S_i(t)$ at time t on the potential outcome $T_i(\overline{S}_i(t-1),0)$ following a patient's actual treatment through times $0,\ldots,t-1$ and control treatment after $t-1$. Under this constant treatment effect model (1), β_0 multiplied by M (number of visit time) can be interpreted as the average causal treatment effect that would be realized if all patients had continued to comply with the treatment to which they were assigned. Robins [6–8] proposed the estimation method of β_0, the so-called, g-estimation method, under the assumption of sequential conditional independence for any t and k with $k\leqslant t-1$

$$T_i(\overline{S}_i(k),0)\coprod S_i(t)|H_i(t) \quad (2)$$

which states that, when $k\leqslant t-1$, treatment $S_i(t)$ is independent of the potential outcomes $T_i(\overline{S}_i(k),0)$, given the observed history up to time t, $H_i(t)$. In practice, we would not expect this assumption to be precisely true, but given a rich collection of prognostic factors that influence a patient's decision to comply at time t recorded in $H_i(t)$, it may well be approximately true. Robins [6–8] has referred to (2) as the assumption of no unmeasured confounders.

3.2. *Estimation of β_0 via the intensity score method*

Brumback *et al.* [9] proved that the SNMM treatment effect, that is, g-estimator of β_0, can be obtained by the intensity score method, in which outcomes are regressed on the cumulative intensity score. We utilize their results and consider the accelerated failure time (AFT) model to obtain the consistent estimator of β_0 in the multiplicative SNMM (1). Here, we assume that the observed event time T_i is subject to independent random censoring such as an end-of-study censoring, where T_i for censored subjects is either the time until dropout or the time until end of study.

We assume the following exponential regression model (log-linear model) for T_i [20]:

$$\log T_i=\mu+\beta_I\sum_{t=0}^{M-1}\hat{I}_i(t)+\varepsilon_i \quad (3)$$

where μ is the intercept parameter, $I_i(t)=S_i(t)-E[S_i(t)|H_i(t)]$ is the intensity score at time t, and ε_i follows the extreme value distribution. For binary treatment $S_i(t)$, the time-dependent intensity score $I_i(t)$ measures departures of actual treatment from the propensity score $\Pr[S_i(t)|H_i(t)]$ of Rosenbaum and Rubin [21]. Since the propensity score is usually unknown, it must be estimated from the data. If we assume a parametric model for $\Pr[S_i(t)|H_i(t)]$ such as

$$\operatorname{logit}\Pr[S_i(t)=1|H_i(t)]=\theta^{\mathrm{T}}H_i(t) \quad (4)$$

then the intensity score at time t can be estimated by $\hat{I}_i(t)=S_i(t)-E[S_i(t)|H_i(t);\hat{\theta}]$, where $\hat{\theta}$ is the maximum likelihood estimator of θ under model (4). Here we assume that the intensity score at time t is not equal to zero with probability 1 for each patient, that is, $\hat{I}_i(t)\neq 0$ for any t. This assumption will be satisfied unless there is a covariate level $H_i(t)$ such that all patients with that level of the covariate are certain to receive the treatment.

The estimate for β_I in model (3) can be obtained *via* the ordinary-weighted least-squares (WLS) method. However, the cumulative intensity score is generally uncorrelated with the cumulative propensity score, although $E[I_i(t)E(S_i(t)|H_i(t))]=0$ for any t. Therefore, as Brumback *et al.* [9]

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have pointed out in the case of linear model, to obtain a consistent estimator of β_0 *via* the WLS method for model (3), the correction term $N\beta_I C$ must be subtracted from the WLS estimating function, where $C=(1/N)(\sum_{t=0}^{M-1}\hat{I}_i(t))\omega_i(\sum_{t=0}^{M-1}E(S_i(t)|H_i(t);\hat{\theta}))$ and $\omega_i=\exp(-\mu)\cdot T_i\cdot\exp(-\beta_I\sum\hat{I}_i(t))$. The corrected estimating function for model (3) is

$$U(\mu,\beta_I)\equiv\sum_i^N(-d_i\sum\hat{I}_i(t)+\sum\hat{I}_i(t)\exp[\log t_i-\mu-\beta_I\sum\hat{I}_i(t)])-N\beta_I C=0 \tag{5}$$

where d_i is the event indicator that takes the value of one if the subject failed and zero if the subject is censored. In Appendix A, we show the proof that the correction term must be subtracted from the WLS estimating function to obtain a consistent estimator.

Our estimating function has the form $\sum_i^N U_i(\gamma)=0$, where $\gamma=(\mu,\beta_I,\theta)$ represents the intercept, coefficient of the intensity score, and parameter used to model the propensity score. To correct for having the estimated θ, the asymptotic variance of $\hat{\gamma}$ was obtained by using a sandwich estimator, which was computed as

$$[\hat{E}(\partial U_i/\partial\gamma)]^{-1}[\hat{E}(U_iU_i^{\mathrm{T}})\hat{E}(\partial U_i/\partial\gamma)]^{-1,\mathrm{T}}/N$$

where the estimated expectations were computed using the empirical distribution of the sample.

3.3. Time-dependent treatment effects

An extension of the multiplicative SNMM (1) is to allow the treatment effects to vary across time,

$$\log E[T_i(\overline{S}_i(t),0)|H_i(t),S_i(t)]-\log E[T_i(\overline{S}_i(t-1),0)|H_i(t),S_i(t)]=\beta_0(t)S_i(t) \tag{6}$$

where $\beta_0(t)$ is the causal parameter at each time t. Since $\beta_0(t)$ is the incremental causal effect of a final treatment $S_i(t)$ at time t, the cumulative effect $\sum_{k=0}^{t}\beta_0(k)$ is the average causal treatment effect that would be realized if all patients had continued to comply with the treatment to which they were assigned until time t. Assuming the consistency assumption and the sequential conditional independence (2), the time-dependent causal parameters in model (6) are consistently estimated by fitting the following model [9]

$$\log T_i=\mu+\sum_{t=0}^{M-1}\beta_I(t)\hat{I}_i(t)+\varepsilon_i \tag{7}$$

where the correction term $\sum_i[\hat{I}_i(t)\cdot\omega_i\cdot\sum_{t=0}^{M-1}\{\beta_I(t)E(S_i(t)|H_i(t);\hat{\theta})\}]$ must be subtracted from the WLS estimating function for model (7).

4. SIMULATION STUDY

4.1. Simulation design

To evaluate the performance of the AT, ITT, g-estimation (see Section 4.2) and intensity score methods, we carried out simulation studies under non-random non-compliance. We simulated data from two treatment groups, coded as $R=0$ (control treatment) or $R=1$ (test treatment). About equal sample size of 1000 for each group was randomly generated (total sample size was 2000).

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The simulations were based on 1000 replications so that the estimated coverage probability of a true 95 per cent CI would have a simulation accuracy of approximately 1.35 per cent.

For each subject i ($i=1,\ldots,2000$), a baseline covariate L_i was generated from the normal distribution with mean of 2 and variance of 1. The potential baseline failure time U_i was generated from the following exponential model:

$$U_i = U_0 \exp(\alpha_0 + \alpha_1 L_i) \tag{8}$$

where U_0 was an exponential random number with mean of 1 and $(\alpha_0, \alpha_1) = (3.2, -0.5)$ so that the larger the value of L_i, the shorter the baseline failure time U_i. We evaluated the treatment actually received $S_i(t)$ at three time points $t=0$, 2, and 4, where all subjects were assumed to take the assigned treatment at $t=0$ ($S_i(0)=R_i$) and the treatment crossover occurred at $t=2$ and 4 according to the following model:

$$\text{logit Pr}[S_i(t)] = \gamma_0 + \gamma_1 L_i + \gamma_2 S_i(t-2) \tag{9}$$

where $(\gamma_1, \gamma_2) = (1.2, 4.5)$ so that patients with poor prognosis and taking the test treatment at previous time point tended to receive the test treatment. The non-compliance rate was adjusted by the value of the intercept parameter γ_0, where two settings were considered: 45 per cent ($R=0$) *versus* 15 per cent ($R=1$) and 30 per cent ($R=0$) *versus* 10 per cent ($R=1$). In this non-compliance rate, the subject was considered as a non-complier when the subject received another treatment even once during the study period.

The observed failure time T_i was calculated from the SAFT model

$$U_i = \int_0^{T_i} \exp[-\psi_0 S_i(t)]\,dt \tag{10}$$

where ψ_0 is the causal treatment effect, which was set to $\psi_0 = 0.5$. The observed failure time T_i was censored at the fixed censoring time C, where $C=5$, 70, and ∞, so that the overall censoring proportion was nearly 90, 30, and 0 per cent, respectively.

Simulations were evaluated in terms of the bias, mean-squared error (MSE), mean length of the 95 per cent CI (length), 95 per cent coverage probability (CP), power for rejecting the null hypothesis, and α-error.

4.2. *g-estimation (semi-parametric randomization-based analysis)*

A semi-parametric randomization-based approach to estimate the causal effect has been developed by Robins and coworkers [10, 12]. For time-to-event outcomes, their approach is based on the causal AFT model (10), which relates a patient's observed event time T_i to the potential baseline event time U_i, that would have been observed if no treatment had been given, and the treatment actually received $S_i(t)$ *via* a causal parameter ψ_0. Note that if $S_i(t) \equiv 0$, then equation (10) gives $T_i = U_i$ as expected, while if $S_i(t) \equiv 1$, (10) gives $T_i = U_i \exp(\psi_0)$. Therefore, equation (10) implies that for continuous treatment the potential event time U_i is prolonged by the factor $\exp(\psi_0)$. A positive value of ψ_0 represents a beneficial treatment effect.

To estimate the causal parameter ψ_0, they choose to avoid all assumptions about both observed and unobserved factors that influence an individual's decision to comply such as (2), while comparing outcomes based only on the treatment groups randomized by design, that is, their analyses are randomization-based analysis. The key to understanding their estimation method

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