Original Article

Association of Platelet Aggregation with Lipid Levels in the Japanese Population: the Suita Study

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***Aim*: Platelets play a pivotal role in atherothrombotic diseases. Platelet aggregability induced by agonists has great interindividual variability; however, the factors influencing platelet aggregability variation have not been characterized in Asia.**

***Methods*: To examine the confounding factors influencing platelet counts and responsiveness to agonists, we measured the platelet counts and platelet aggregability induced by 1.7 μM adenosine diphosphate (ADP) or 1.7 μg/mL collagen using a light transmittance aggregometer in the Japanese general population without medication or cardiovascular disease (387 men and 550 women) in the Suita Study.**

***Results*: Platelet counts were negatively correlated with age in both men and women (Spearman's rank correlation coefficient: $r_s = -0.230$ and -0.227; $p < 0.01$, respectively). In women, platelet counts were correlated negatively with the high-density lipoprotein (HDL) cholesterol level and positively with the low-density lipoprotein (LDL) cholesterol/HDL cholesterol (L/H) ratio ($r_s = -0.135$ and 0.119; $p < 0.01$, respectively). In women, platelet aggregabilities by ADP and collagen were correlated with age ($r_s = 0.118$ and 0.143; $p < 0.01$, respectively), and collagen-induced platelet aggregability was correlated with the LDL cholesterol level, the L/H ratio, and the non-HDL cholesterol level ($r_s = 0.167$, 0.172, and 0.185; $p < 0.01$, respectively). Even after adjustment for age, systolic blood pressure, body mass index, and current smoking and drinking, the association of platelet counts with the L/H ratio in women and associations of collagen-induced platelet aggregability with the L/H ratio and the non-HDL cholesterol level remained.**

***Conclusion*: Examination of platelet counts and platelet aggregability induced by ADP and collagen revealed gender, age and lipid levels as factors influencing inter-individual variability.**

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Key words; LDL cholesterol, Lipid, Platelet aggregation, Platelet count

Introduction

Platelet thrombi form at the site of vascular injury or the site of a ruptured atherosclerotic plaque. Platelets contribute pivotally to atherothrombotic disease such as myocardial infarction and stroke; therefore, the suppression of platelet aggregability using anti-platelet drugs is widely recognized as a therapeutic means to prevent cardiovascular events, and these drugs show evidence of event prevention[1)].

It is generally accepted that the response of platelets to agonists has large inter-individual variability within the population[2-7)]. This interindividual responsiveness has a high degree of heritability[2, 4-6, 8-13)]. In addition, increased platelet aggregability has been shown in women[14, 15)]. Specifically, women showed higher platelet aggregability in response to collagen, adenosine diphosphate (ADP), arachidonic acid, and

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epinephrine in whole blood and platelet-rich plasma than in men[16]. Smoking is a common environmental factor that increases platelet function[17, 18]. In the context of this research, population-based research is helpful in providing information on the confounding factors for platelet responsiveness to agonists; however, these studies are very limited due to the difficulty of platelet aggregability measurement in a community setting. In the large population-based sample of the Framingham Heart Study, platelet responsiveness to agonists was associated with age, body mass index, triglyceride level, high-density lipoprotein (HDL) cholesterol, and diabetes[2]. In this study, higher fibrinogen levels were associated with increased epinephrine-induced aggregation and a tendency to word ADP-induced aggregation[8]. Evidence suggests that increased platelet reactivity could identify individuals at risk for atherothrombotic diseases; however, large cohort studies, including the Northwick Park Heart Study and the Caerphilly Prospective Study, did not show an association of platelet aggregability with cardiovascular events[3, 19].

Studies on the variability of platelet responsiveness to agonists have been mainly performed in the Caucasian population; studies in the Asian population are very limited. Since 1989, we have conducted the Suita Study, an epidemiological study of cerebrovascular and cardiovascular diseases, in a general urban population cohort in Japan[20-22]. The present study was undertaken to clarify the factors influencing the inter-individual variability of platelet responsiveness to agonists in a Japanese urban general population. This is a first step in unraveling systematically the complex interindividual variability of platelet responsiveness in our population.

Methods

Study Population

The study population of the Suita Study was based on samples randomly selected from 12,200 Japanese residents of Suita[20-22]. The participants had been visiting the National Cerebral and Cardiovascular Center every 2 years since 1989 for regular health checkups. Participants attended the National Cerebral and Cardiovascular Center from November, 2005 to December, 2007. A physician or nurse administered questionnaires covering medications, personal habits, and the personal history of cardiovascular diseases. Some cohort members of the study population were excluded from the study because they met one or more of the following criteria: past or present history of cardiovascular disease, failure to fast for at least 10 hours before venipuncture or missing data, age less than 39 years or more than 70 years, or use of any medications. After these exclusions, 937 individuals (men: 387, women: 550) remained in the study. Informed consent was obtained from all subjects. This study was approved by the Institutional Review Board of the National Cerebral and Cardiovascular Center.

Laboratory Measurements

Fasting (≥10 hours) blood samples for the platelet aggregation test were collected between 9 and 10 am from an antecubital vein through a needle into disposable, siliconized, evacuated plastic tubes containing 0.1 vol of 3.13% trisodium citrate, and blood collected in a second tube was used. The samples were centrifuged at 1,100 rpm for 10 minutes at room temperature within 1 hour of collection to obtain platelet-rich plasma. Platelet aggregation was measured using native platelet-rich plasma[23] by a single operator on a PA-200 platelet aggregometer (Kowa Company, Japan) using techniques based on the method of Born[24]. Incubation time was 5 minutes at 37℃, the stir bar speed was 1200 rpm, and sample run time was 7 minutes after addition of agonists. The agonists used were 1.7 μM ADP (Arkray Factory Inc., Japan) or 1.7 μg/mL equine-tendon-derived collagen (Arkray Factory)[25]. Percent platelet aggregation was expressed as the maximal percentage change in light transmission relative to that of platelet-poor plasma.

Glucose, total cholesterol, HDL cholesterol, and triglycerides were measured by enzymatic methods. Low-density lipoprotein (LDL) cholesterol was estimated using the Friedewald formula[26]. The LDL cholesterol /HDL cholesterol (L/H) ratio was obtained by dividing LDL cholesterol by HDL cholesterol. Non-HDL cholesterol was obtained by subtracting HDL cholesterol from total cholesterol. The subjects were classified as current smokers if they smoked at least one cigarette per day, and as non-smokers if they had never smoked or had stopped smoking. Similarly, subjects were classified as alcohol non-drinkers if they had never drunk or had drunk only in the past. Blood pressure (BP) was measured three times with subjects in a sitting position after 5 minutes of rest. Systolic BP (SBP) and diastolic BP (DBP) were taken to be the average of the second and third measurements recorded at least 1 minute apart by well-trained doctors. We measured height and weight in a fasting state. Body mass index was calculated as weight (kg) divided by the square of the height (m^2).

Statistical Analysis

For a comparison between gender groups, the

Table 1. Characteristics of study population by sex

	Men $n=387$	Women $n=550$	p value
Age, years	58.3 (7.1)	57.1 (7.1)	0.006
Systolic BP, mmHg	123.1 (17.3)	115.6 (17.0)	<0.001
Diastolic BP, mmHg	79.8 (11.2)	71.8 (10.6)	<0.001
Body mass index, kg/m^2	23.3 (2.6)	21.8 (2.9)	<0.001
Total cholesterol, mg/dL	200.6 (30.4)	218.8 (33.9)	<0.001
HDL cholesterol, mg/dL	57.1 (14.6)	67.1 (15.1)	<0.001
LDL cholesterol, mg/dL	120.4 (29.2)	134.4 (31.4)	<0.001
L/H ratio	2.23 (0.79)	2.11 (0.74)	0.015
non-HDL cholesterol, mg/dL	143.5 (31.4)	151.8 (34.9)	0.003
Platelet count, $\times 10^3/\mu$L	247 (62)	256 (60)	0.011
ADP-induced platelet aggregation, %	68.2 (12.0)	72.2 (10.6)	<0.001
Collagen-induced platelet aggregation, %	77.4 (9.1)	79.5 (8.4)	<0.001
Current smoking, %	33.7	20.7	<0.001
Current drinking, %	66.1	39.7	<0.001

Values are the means (standard deviation) or percent. BP, blood pressure; ADP, adenosine diphosphate; L/H ratio, LDL cholesterol/HDL cholesterol ratio.

Mann-Whitney *U* test was used. The association between the platelet count or level of platelet aggregations and the analyzed parameters was assessed by Spearman correlation analysis. We used ANCOVA to investigate whether plasma levels of total cholesterol, LDL cholesterol and HDL cholesterol were positively and independently associated with the platelet count or level of platelet aggregation. We performed adjustments for age, body mass index, SBP, and lifestyle factors (current smoking and drinking) for each gender. Differences of $p<0.05$ were considered to be significant. All analyses were performed with SAS statistical software (release 8.2; SAS Institute Inc.).

Results

Characteristics of Populations

After exclusion of individuals with cardiovascular disease and medications, 937 individuals (men: 387, women: 550), aged from 40 to 69 years, were eligible (**Table 1**). Mean ages (standard deviations, SD) of men and women were 58.3 (7.1) and 57.1 (7.1), respectively. SBP, DBP, body mass index, and habits of smoking and drinking were higher in men than in women (**Table 1**). Total cholesterol, HDL cholesterol, LDL cholesterol, and non-HDL cholesterol were higher in women than in men. We calculated the L/H ratio as a new parameter of the lipid profile. This ratio was higher in men than in women. Platelet counts were higher in women than in men. Platelet aggregabilities induced by ADP and collagen were both higher in women than in men (**Table 1**).

Correlates of Platelet Counts and Platelet Aggregation in Response to ADP and Collagen with Age and Other Covariates

In men, the correlations between platelet count and ADP- and collagen-induced aggregations were $r_s=0.050$ and $r_s=0.022$, respectively. The correlation between ADP- and collagen-induced aggregations was $r_s=0.559$. In women, these correlations were $r_s=0.086$, $r_s=0.051$, and $r_s=0.590$, respectively.

Spearman's rank correlation coefficients of the platelet count, ADP- or collagen-induced aggregation with age and other factors are listed in **Table 2**. Platelet counts were negatively correlated with age in both sexes. Platelet counts were negatively correlated with HDL cholesterol and positively correlated with the L/H ratio in women. ADP- and collagen-induced aggregation was correlated with age in women. Collagen-induced aggregability was correlated with LDL cholesterol, the L/H ratio, and non-HDL cholesterol in women. Smoking was correlated with platelet counts and ADP- and collagen-induced aggregation in both sexes. Drinking was correlated with platelet counts in both sexes.

Age-Related Changes of Platelet Counts and Platelet Aggregation in Response to ADP and Collagen

Platelet counts and platelet aggregabilities induced by ADP and collagen are shown according to the decade of life and sex in **Table 3**. Platelet counts

Table 2. Correlation of platelet count and platelet aggregation with age and other covariates

	Men ($n=387$)			Women ($n=550$)		
	Platelet count	ADP-induced platelet aggregation	Collagen-induced platelet aggregation	Platelet count	ADP-induced platelet aggregation	Collagen-induced platelet aggregation
Age, years	−0.230**	−0.059	0.029	−0.227**	0.118**	0.143**
Systolic BP, mm Hg	−0.070	0.005	0.066	0.064	0.027	0.027
Diastolic BP, mm Hg	−0.045	−0.004	0.046	0.062	0.002	0.017
Body mass index, kg/m^2	−0.050	−0.062	−0.019	0.106*	0.058	0.034
Total cholesterol, mg/dL	0.092	0.044	0.033	0.013	0.050	0.137*
HDL cholesterol, mg/dL	−0.004	−0.057	−0.127*	−0.135**	−0.091*	−0.101*
LDL cholesterol, mg/dL	0.072	0.044	0.054	0.026	0.070	0.167**
L /H ratio	0.051	0.068	0.092	0.119**	0.101*	0.172**
non-HDL cholesterol, mg/dL	0.104*	0.047	0.076	0.054	0.087*	0.185**
Glucose, mg/dL	−0.038	−0.030	0.055	0.002	0.061	0.094*
Hemoglobin A1c,%	0.102*	−0.002	0.077	0.105*	0.055	0.047
Current smoking	0.145**	0.109*	0.146**	0.212**	0.237**	0.220**
Current drinking	0.191**	0.098	0.072	0.131**	0.105*	0.080

Data indicate Spearman's rank correlation coefficient. *$p<0.05$, **$p<0.01$, BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ADP, adenosine diphosphate; L/H ratio, LDL cholesterol/HDL cholesterol ratio.

Table 3. Age-related changes of platelet counts and ADP- and collagen-induced platelet aggregation by sex

	Age group, years	40-49	50-59	60-69
Men	Numbers of individuals	62	138	187
	Platelet count, $\times 10^3/\mu$L	261.0 (8.1)	256.0 (5.3)	235.9 (4.6)**
	ADP-induced platelet aggregation, %	68.0 (1.6)	69.2 (1.0)	67.6 (0.9)
	Collagen-induced platelet aggregation, %	76.3 (1.2)	77.1 (0.8)	78.0 (0.7)
Women	Numbers of individuals	99	234	217
	Platelet count, $\times 10^3/\mu$L	287.1 (5.9)	257.4 (3.8)**	241.4 (4.0)**
	ADP-induced platelet aggregation, %	71.9 (1.1)	71.8 (0.7)	73.0 (0.7)
	Collagen-induced platelet aggregation, %	77.7 (0.9)	79.5 (0.5)*	80.5 (0.6)**

*$p<0.05$, **$p<0.01$ compared with 40-49 age-group in the same sex. Values are the means (standard errors).

decreased in individuals aged 60-69 compared to aged 40-49 in both sexes. ADP-induced aggregability in individuals aged 60-69 was not different from aged 40-49 in both sexes; however, collagen-induced aggregability in individuals aged 50-59 and 60-69 was higher than aged 40-49 in women, but not men.

Multivariate Analysis of Lipid Levels According to the Quartile Rank of Platelet Counts or Platelet Aggregation

We divided platelet counts and platelet aggregability induced by ADP or collagen into quadripartite rank by sex and compared lipid levels among the quartiles after adjustment for age, SBP, body mass index, and lifestyle (current smoking and drinking).

In men, increased total cholesterol and an increased ratio of LDL cholesterol to HDL cholesterol were observed in the highest (Q4) platelet-count quartile (**Table 4**). Non-HDL cholesterol was associated with the platelet count (p for trend, 0.042). In women, the L/H ratio was associated with the platelet count (p for trend, 0.037).

In analysis of the quadripartite rank of ADP-induced platelet aggregability, a weak increment of HDL cholesterol in the Q2 rank was observed in women, but no other parameters showed significant differences among quartiles (**Table 5**).

In contrast to ADP-induced platelet aggregability, LDL cholesterol and non-HDL cholesterol and the L/H ratio in women were increased in the highest (Q4) quartile of collagen-induced platelet aggregability (**Table 6**). The L/H ratio and non-HDL choles-

Table 4. Lipid levels according to quadripartite rank of platelet counts by sex

	Rank	Q1	Q2	Q3	Q4	*p* for trend
Men	Platelet count, $\times 10^3/\mu L$	39-210	211-244	245-283	284-824	
	Total cholesterol, mg/dL	194.3 (3.2)	202.5 (3.1)	200.2 (3.1)	205.5 (3.1)**	0.085
	HDL cholesterol, mg/dL	57.3 (1.4)	56.6 (1.4)	58.4 (1.4)	56.3 (1.4)	0.679
	LDL cholesterol, mg/dL	115.0 (3.0)	122.7 (2.9)	120.0 (2.9)	124.0 (2.9)	0.155
	L/H ratio	2.13 (0.08)	2.30 (0.07)	2.16 (0.07)	2.35 (0.07)*	0.118
	non-HDL cholesterol, mg/dL	137.0 (3.2)	145.9 (3.1)	141.8 (3.1)	149.2 (3.1)	0.042
Women	Platelet count, $\times 10^3/\mu L$	75-210	211-244	245-283	284-569	
	Total cholesterol, mg/dL	218.0 (3.0)	217.1 (2.9)	217.4 (2.8)	222.5 (2.9)	0.514
	HDL cholesterol, mg/dL	69.3 (1.2)	67.6 (1.2)	65.4 (1.2)*	66.1 (1.2)	0.114
	LDL cholesterol, mg/dL	132.7 (2.7)	133.3 (2.6)	133.4 (2.6)	138.0 (2.6)	0.487
	L/H ratio	2.00 (0.06)	2.08 (0.06)	2.16 (0.06)	2.23 (0.06)*	0.037
	non-HDL cholesterol, mg/dL	148.6 (2.9)	149.5 (2.8)	152.1 (2.8)	156.4 (2.9)	0.231

Values are the means (standard errors) adjusted for age, systolic blood pressure, body mass index, and lifestyle factors (current smoking and drinking). *$p<0.05$, **$p<0.01$, compared with Q1. HDL, high-density lipoprotein; LDL, low-density lipoprotein; L/H ratio, LDL cholesterol/HDL cholesterol ratio.

Table 5. Lipid levels according to quadripartite rank of ADP-induced platelet aggregation by sex

	Rank	Q1	Q2	Q3	Q4	*p* for trend
Men	ADP-induced platelet aggregation, %	23-62	63-71	72-77	78-93	
	Total cholesterol, mg/dL	200.6 (3.2)	198.3 (3.4)	202.3 (2.9)	200.8 (3.0)	0.859
	HDL cholesterol, mg/dL	57.3 (1.4)	57.6 (1.5)	57.8 (1.3)	55.9 (1.3)	0.758
	LDL cholesterol, mg/dL	119.1 (3.0)	117.8 (3.2)	123.4 (2.7)	120.4 (2.9)	0.577
	L/H ratio	2.18 (0.08)	2.19 (0.08)	2.25 (0.07)	2.29 (0.07)	0.669
	non-HDL cholesterol, mg/dL	143.3 (3.2)	140.7 (3.5)	144.5 (2.9)	144.8 (3.1)	0.818
Women	ADP-induced platelet aggregation, %	29-62	63-71	72-77	78-96	
	Total cholesterol, mg/dL	217.5 (3.1)	219.8 (2.9)	218.7 (2.7)	219.1 (2.9)	0.957
	HDL cholesterol, mg/dL	66.1 (1.3)	69.7 (1.2)*	67.1 (1.1)	65.4 (1.2)	0.072
	LDL cholesterol, mg/dL	134.0 (2.8)	133.0 (2.6)	135.3 (2.4)	134.9 (2.6)	0.927
	L/H ratio	2.12 (0.06)	2.04 (0.06)	2.13 (0.05)	2.18 (0.06)	0.377
	non-HDL cholesterol, mg/dL	151.3 (3.0)	150.1 (2.9)	151.5 (2.7)	153.7 (2.8)	0.841

Values are the means (standard errors) adjusted for age, systolic blood pressure, body mass index, and lifestyle factors (current smoking and drinking). *$p<0.05$, compared with Q1. HDL, high-density lipoprotein; LDL, low-density lipoprotein; L/H ratio, LDL cholesterol/HDL cholesterol ratio.

terol in women were associated with collagen-induced platelet aggregability (*p* for trend; 0.005 and 0.036, respectively).

Discussion

In the present study, we found gender differences in the platelet count and platelet aggregability and revealed the correlation of these parameters with some lipid levels. The interindividual variability of the platelet count and platelet responsiveness to ADP and collagen appeared to be partly explained by gender, age and lipid levels.

We found in the present study that women had higher platelet counts and platelet aggregability in response to ADP and collagen than men. These results were consistent with previous findings that women show higher platelet responsiveness to agonists in whole blood and platelet-rich plasma than men[16]. This gender difference in the platelet aggregability may be related to marked changes of the lipid profile in postmenopausal women. Both total cholesterol and

Table 6. Lipid levels according to quadripartite rank of collagen-induced platelet aggregation by sex

	Rank	Q1	Q2	Q3	Q4	*p* for trend
Men	Collagen-induced platelet aggregation, %	8-73	74-78	79-82	83-95	
	Total cholesterol, mg/dL	196.9 (3.4)	202.6 (3.2)	202.1 (3.0)	200.5 (2.9)	0.619
	HDL cholesterol, mg/dL	58.8 (1.5)	58.9 (1.4)	57.1 (1.3)	54.7 (1.2)*	0.088
	LDL cholesterol, mg/dL	115.6 (3.2)	122.6 (3.0)	121.2 (2.9)	121.4 (2.7)	0.400
	L/H ratio	2.11 (0.08)	2.22 (0.08)	2.25 (0.07)	2.32 (0.07)	0.296
	non-HDL cholesterol, mg/dL	138.1 (3.4)	143.7 (3.2)	145.0 (3.1)	145.8 (2.9)	0.342
Women	Collagen-induced platelet aggregation, %	7-73	74-78	79-82	83-97	
	Total cholesterol, mg/dL	212.7 (3.1)	218.9 (2.9)	221.2 (2.7)*	220.7 (2.8)	0.179
	HDL cholesterol, mg/dL	68.3 (1.3)	68.7 (1.2)	65.8 (1.1)	66.0 (1.2)	0.223
	LDL cholesterol, mg/dL	127.9 (2.9)	133.3 (2.6)	137.8 (2.4)*	136.5 (2.5)*	0.050
	L/H ratio	1.97 (0.06)	2.03 (0.06)	2.23 (0.05)*	2.20 (0.06)*	0.005
	non-HDL cholesterol, mg/dL	144.5 (3.1)	150.2 (2.9)	155.4 (2.6)**	154.7 (2.8)*	0.036

Values are the means (standard errors) adjusted for age, systolic blood pressure, body mass index, and lifestyle factors (current smoking and drinking). $^{*}p<0.05$, $^{**}p<0.01$, compared with Q1. HDL, high-density lipoprotein; LDL, low-density lipoprotein; L/H ratio, LDL cholesterol/HDL cholesterol ratio.

LDL cholesterol are markedly increased in postmenopausal women[20], and hypercholesterolemia is associated with hyperaggregability. In the present study, the mean age of women was 57.1 year old and thus most were postmenopausal. Furthermore, we found that platelet counts were negatively correlated with age and positively correlated with smoking in both men and women. We also found that smoking correlated with platelet aggregability which was not in agreement with the Framingham Heart Study[2]. The discrepancy in terms of smoking between the two studies is not clear; however, it might have been caused by the difference in the frequency of smokers.

Beside cellular interactions of platelets with other blood cells and vascular cells, interactions of platelets with lipoproteins seem to be quite important, and circulating lipoproteins in blood directly or indirectly influence platelet properties[27]. LDL is an atherogenic lipoprotein and increases platelet activation. Platelets are directly associated with LDL in blood[28]. LDL is modified to oxidative LDL. Oxidative LDL induces platelet activation followed by quick changes in shape and aggregation contributing to thrombus formation after plaque rupture. In contrast with LDL, HDL particles have several antiatherogenic activities, including anti-inflammatory, antithrombotic, antioxidative, and vasodilatory properties[29]. Lowering LDL cholesterol or raising HDL cholesterol therapy has well-established benefits in the primary and secondary prevention of atherothrombotic diseases[30-33]. Actually, platelet aggregation evaluated with a thrombus area on the aorta in an *ex vivo* superfusion chamber under 1,000 s^{-1} has been inversely correlated with HDL cholesterol levels[34]. Infusion of reconstituted HDL to humans showed a transient inhibition of platelet aggregation induced by arachidonic acid and collagen[35]. These findings also suggested that HDL has antiplatelet actions. In our study, HDL cholesterol was negatively associated with collagen-induced platelet aggregation. This negative association was also observed in the Framingham Heart Study[2].

In the present study, collagen-induced platelet aggregability was associated with the L/H ratio in women, even after adjustment for age, systolic blood pressure, body mass index, and current smoking and drinking. Recently, the L/H ratio has been considered to be a clinically useful marker, because it is more closely associated with the occurrence of cardiovascular events than the levels of LDL cholesterol or HDL cholesterol[36]. Therefore, our findings suggest that increased collagen-induced platelet aggregation in women is potentially associated with early atherosclerotic conditions.

There have been few studies on the prediction of cardiovascular events by platelet tests. Two small studies have suggested that platelet aggregability assessed by a light transmittance aggregometer could be predictive of cardiovascular events[37, 38]; however, the Northwick Park Heart Study, a large cohort study consisting of 740 men followed up for 10.1 years, found no association of ADP-induced aggregation with ischemic heart disease events[19]. In the Caerphilly Prospective Study, consisting of 2000 elderly men followed up for 10 years, the aggregative response to ADP in platelet-

rich plasma, that to ADP in whole blood measured using an impedance method, and platelet aggregation induced in whole blood by high-shear flow did not show an association with myocardial infarction[3].

In this study, we found that the platelet count and platelet aggregation are affected by factors such as gender, age, and lipid levels in the Japanese population. Furthermore, increased platelet aggregation by collagen in women is closely associated with the LDL-C/HDL-C ratio and LDL-C as risk factors for atherosclerotic disease. Therefore, this study offers modest support for the hypothesis that increased platelet aggregation by collagen even within the normal range might be associated with atherosclerosis in middle-aged women. However, future studies are necessary to establish whether platelet aggregation by collagen is a useful marker to predict coronary events and mortality. We are now following the occurrence of cardiovascular disease events in the Suita Study. Moreover, the response of platelets to agonist may have inter-individual variability within the population that is partly due to genetics. We are now genotyping the DNA polymorphisms of the study participants using a candidate gene approach.

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