Table 3. Hazard ratios and 95% confidence intervals according to frequency of citrus fruit intake adjusted for potential confounders (women)

	Frequency of citrus fruit intake					*P* for trend
	Infrequent	1–2 times/month	1–2 times/week	3–4 times/week	Almost daily	
Person-years	5007	10617	22994	19623	13019	
Cardiovascular disease						
No. of cases	24	44	63	56	31	
HR1[a] (95% CI[b])	1.00	1.00 (0.61–1.64)	0.66 (0.41–1.06)	0.69 (0.43–1.11)	0.48 (0.28–0.83)	0.001
HR2[c] (95% CI)	1.00	0.83 (0.49–1.41)	0.68 (0.42–1.11)	0.75 (0.46–1.22)	0.51 (0.29–0.88)	0.02
All-stroke						
No. of cases	20	37	53	47	25	
HR1 (95% CI)	1.00	1.00 (0.58–1.72)	0.66 (0.39–1.10)	0.68 (0.40–1.14)	0.46 (0.25–0.83)	0.002
HR2 (95% CI)	1.00	0.84 (0.47–1.49)	0.67 (0.39–1.14)	0.73 (0.42–1.25)	0.47 (0.26–0.87)	0.02
Cerebral infarction						
No. of cases	10	23	29	30	11	
HR1 (95% CI)	1.00	1.29 (0.61–2.71)	0.77 (0.37–1.58)	0.94 (0.46–1.93)	0.43 (0.18–1.02)	0.02
HR2 (95% CI)	1.00	1.04 (0.47–2.33)	0.80 (0.37–1.73)	1.02 (0.48–2.20)	0.39 (0.15–1.00)	0.07
Hemorrhagic stroke						
No. of cases	10	14	23	17	14	
HR1 (95% CI)	1.00	0.72 (0.32–1.63)	0.54 (0.26–1.13)	0.45 (0.21–0.99)	0.49 (0.22–1.10)	0.05
HR2 (95% CI)	1.00	0.66 (0.29–1.52)	0.53 (0.25–1.13)	0.49 (0.22–1.08)	0.55 (0.24–1.23)	0.16
Myocardial infarction						
No. of cases	2	3	10	5	3	
HR1 (95% CI)	1.00	0.82 (0.14–4.93)	1.27 (0.28–5.84)	0.76 (0.15–4.00)	0.59 (0.10–3.54)	0.45
HR2 (95% CI)	1.00	0.83 (0.14–4.98)	1.47 (0.32–6.84)	0.84 (0.16–4.46)	0.67 (0.11–4.15)	0.57

[a]Hazard ratio adjusted for age and study area.
[b]Confidence interval.
[c]Hazard ratio adjusted for age, study area, body mass index, systolic blood pressure, total cholesterol concentration, physical activity index, smoking status, alcohol consumption, education level, and marital status.

protect against CVD or cerebral infarction and contribute to a marked reduction in incidence levels. As compared with the results from previous studies of the association of citrus fruit intake with the incidences of CVD[16,17] and cerebral infarction,[13] the reductions in these incidences associated with frequent citrus fruit intake were more marked in the present analysis. It is possible that the variation in results between studies was due to differences in the categories used to assess the frequency of citrus fruit intake. Nevertheless, our results are generally consistent with findings from prior studies. In addition, our finding of no significant association between the frequency of citrus fruit intake and hemorrhagic stroke incidence is in line with the report by Yokoyama et al.[14] Although a number of mechanisms by which fruit protects against CVD have been reported,[1–4] the results of the present study (ie, that citrus fruit intake is inversely associated with CVD, especially cerebral infarction, but not hemorrhagic stroke) support the hypothesis that antioxidants contained in citrus fruit have beneficial effects on atherosclerosis by inhibiting oxidant formation, reducing oxidized low-density lipoprotein, and repairing oxidant-induced injury.[27,28] In the present population, high fruit intake was not cross-sectionally associated with cholesterol concentration. Thus, whether high citrus fruit intake lowers serum cholesterol concentration is not clear from the present results. We recruited participants for mass screening in such a way as to ensure that serum cholesterol values would be distributed almost normally, as was also the case for BMI and blood pressure.

In the present study, the incidence of myocardial infarction was not reduced by frequent intake of citrus fruit. Studies in Western countries[15,29,30] have reported that there is an inverse relationship between the incidence of coronary heart disease and frequent consumption of fruit. In addition, Pereira et al[31] reported that consumption of dietary fiber from fruit was inversely associated with risk of coronary heart disease. In contrast, there was no reduction in the risk of coronary heart disease by fruit intake in the present study or in another study of Japanese.[11] Japanese have a lower incidence of myocardial infarction than individuals living in Western countries. This difference in disease structure, and the lower number cases, may have an effect on the results.

Our results show an inverse trend between CVD incidence and fruit intake among both men and women. There are few epidemiologic studies that show a protective effect of fruit intake against CVD risk in men,[6,9,18] although the effect in women has been more frequently documented.[10,13–15,17] It has been reported that men require much higher intakes of fruit to maintain serum vitamin C and carotenoid concentrations that are identical to those of women.[1,32] In addition, as compared with women, men have a substantially heavier burden of CVD risk factors, such as smoking, obesity, and low HDL concentration.[33] These observations may explain why there is a stronger inverse interaction between fruit intake and CVD risk among women than men. Our results indicate that fruit intake is protective in women and men, despite the potentially higher risk of CVD in the latter.

Current alcohol drinkers and cigarette smokers are exposed to reactive oxidants and free radicals due to their alcohol and cigarette consumption.[34,35] It has been extensively reported that serum vitamin C and carotenoid concentrations are low in alcohol drinkers and cigarette smokers.[1,14,32] Moreover, Sugiura et al[32] indicated that synergic depletion of serum carotenoid concentrations occurs as a result of alcohol consumption and cigarette smoking, after taking into account dietary carotenoid intake. We performed analyses on multiplicative interactions stratified by alcohol consumption and smoking status (data not shown). The results indicated a slight protective effect among men who were current drinkers and smokers, but the interaction was not statistically significant.

Fruit consumption and distribution vary by season. Satsuma mandarins (*mikans*), which account for more than half of citrus fruits distributed in Japan, are eaten mainly between October and March.[19] Overall, 87.4% of the present participants answered the FFQ between April and September, which is the period when there is less citrus fruit available. Therefore, this FFQ was less likely to have been affected by seasonal variation in citrus fruit intake. The inverse relationship between frequency of citrus fruit intake and CVD incidence did not differ when we analyzed the limited population that underwent baseline examination during the period from April through September (data not shown).

Our study has several potential limitations. First, we did not examine long-term dietary habits. The FFQ was conducted only once. The possibility that dietary habits changed during follow-up cannot be ruled out. Second, the present analysis is not quantitative, and the FFQ was self-reported; thus, the accuracy of the assessment of citrus fruit intake was limited. However, the reproducibility and validity of the FFQ were previously found to be acceptable.[22] We therefore consider the results of the present study to be reliable. Determining the amount of citrus fruit intake that is sufficient to protect individuals from CVD risk would be useful when providing advice on healthy dietary habits. Further studies are therefore warranted. Third, the potential effect of confounding variables, except those included in our analyses, cannot be totally excluded. In the present study, the participants who frequently consumed citrus fruit were older, had healthier overall behavior, and were more highly educated. These differences in baseline characteristics suggest the possibility that there were residual confounding variables that were incompletely measured. In addition to habitual variables, there are possibly dietary confounding variables, such as energy, saturated fat, fruit juice intake, and supplement use, about which, unfortunately, we have no information.

In conclusion, the present study indicates that frequent intake of citrus fruit may reduce CVD incidence—in particular, cerebral infarction—in both men and women. Citrus fruit consumption has diminished in recent years in Japan,[19] and this reduction in citrus fruit intake might partly contribute to the increasing number of CVD cases in Japan. Our results show that there are grounds for recommending that citrus fruit intake be increased in order to protect against CVD incidence.

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Conflicts of interest: None declared.

REFERENCES

1. Trout DL. Vitamin C and cardiovascular risk factors. Am J Clin Nutr. 1991;53(1 Suppl):322S–5S.
2. He FJ, MacGregor GA. Fortnightly review: Beneficial effects of potassium. BMJ. 2001;323:497–501.
3. Savige GS. Candidate foods in the Asia-Pacific region for cardiovascular protection: fish, fruit and vegetables. Asia Pac J Clin Nutr. 2001;10:134–7.
4. Chambers JC, Seddon MD, Shah S, Kooner JS. Homocysteine—a novel risk factor for vascular disease. J R Soc Med. 2001;94:10–3.
5. Gaziano JM, Manson JE, Branch LG, Colditz GA, Willett WC, Buring JE. A prospective study of consumption of carotenoids in fruits and vegetables and decreased cardiovascular mortality in the elderly. Ann Epidemiol. 1995;5:255–60.
6. Dauchet L, Ferrières J, Arveiler D, Yarnell JW, Gey F, Ducimetière P, et al. Frequency of fruit and vegetable consumption and coronary heart disease in France and Northern Ireland: the PRIME study. Br J Nutr. 2004;92:963–72.
7. Tucker KL, Hallfrisch J, Qiao N, Muller D, Andres R, Fleg JL; Baltimore Longitudinal Study of Aging. The combination of high fruit and vegetable and low saturated fat intakes is more protective against mortality in aging men than is either alone: the Baltimore Longitudinal Study of Aging. J Nutr. 2005;135:556–61.
8. Nöthlings U, Schulze MB, Weikert C, Boeing H, van der Schouw YT, Bamia C, et al. Intake of vegetables, legumes, and fruit, and risk for all-cause, cardiovascular, and cancer mortality in a European diabetic population. J Nutr. 2008;138:775–81.
9. Iso H, Kubota Y; Japan Collaborative Cohort Study for Evaluation of Cancer. Nutrition and disease in the Japan Collaborative Cohort Study for Evaluation of Cancer (JACC). Asian Pac J Cancer Prev. 2007;8 Suppl:35–80.
10. Nakamura K, Nagata C, Oba S, Takatsuka N, Shimizu H. Fruit and vegetable intake and mortality from cardiovascular disease are inversely associated in Japanese women but not in men. J Nutr. 2008;138:1129–34.
11. Nagura J, Iso H, Watanabe Y, Maruyama K, Date C, Toyoshima H, et al. Fruit, vegetable and bean intake and mortality from cardiovascular disease among Japanese men and women: the JACC Study. Br J Nutr. 2009;102:285–92.
12. Strandhagen E, Hansson PO, Bosaeus I, Isaksson B, Eriksson H. High fruit intake may reduce mortality among middle-aged and elderly men. The Study of Men Born in 1913. Eur J Clin Nutr. 2000;54:337–41.
13. Joshipura KJ, Ascherio A, Manson JE, Stampfer MJ, Rimm EB, Speizer FE, et al. Fruit and vegetable intake in relation to risk of

ischemic stroke. JAMA. 1999;282:1233–9.
14. Yokoyama T, Date C, Kokubo Y, Yoshiike N, Matsumura Y, Tanaka H. Serum vitamin C concentration was inversely associated with subsequent 20-year incidence of stroke in a Japanese rural community. The Shibata study. Stroke. 2000;31:2287–94.
15. Liu S, Manson JE, Lee IM, Cole SR, Hennekens CH, Willett WC, et al. Fruit and vegetable intake and risk of cardiovascular disease: the Women's Health Study. Am J Clin Nutr. 2000;72:922–8.
16. Hung HC, Joshipura KJ, Jiang R, Hu FB, Hunter D, Smith-Warner SA, et al. Fruit and vegetable intake and risk of major chronic disease. J Natl Cancer Inst. 2004;96:1577–84.
17. Takachi R, Inoue M, Ishihara J, Kurahashi N, Iwasaki M, Sasazuki S, et al. Fruit and vegetable intake and risk of total cancer and cardiovascular disease: Japan Public Health Center-Based Prospective Study. Am J Epidemiol. 2008;167:59–70.
18. Sauvaget C, Nagano J, Allen N, Kodama K. Vegetable and fruit intake and stroke mortality in the Hiroshima/Nagasaki Life Span Study. Stroke. 2003;34:2355–60.
19. Statistics Datas [Internet]. Ministory of Agriculture, Forestory and Fisheries. Available from: http://www.maff.go.jp/j/tokei/index.html.
20. Ishikawa S, Gotoh T, Nago N, Kayaba K; Jichi Medical School (JMS) Cohort Study Group. The Jichi Medical School (JMS) Cohort Study: design, baseline data and standardized mortality ratios. J Epidemiol. 2002;12:408–17.
21. Kannel WB, Sorlie P. Some health benefits of physical activity. The Framingham Study. Arch Intern Med. 1979;139:857–61.
22. Date C, Fukui M, Yamamoto A, Wakai K, Ozeki A, Motohashi Y, et al. Reproducibility and validity of a self-administered food frequency questionnaire used in the JACC study. J Epidemiol. 2005;15 Suppl 1:S9–23.
23. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke. 1993;24:35–41.
24. The World Health Organization MONICA Project (monitoring trends and determinants in cardiovascular disease): a major international collaboration. WHO MONICA Project Principal Investigators. J Clin Epidemiol. 1988;41:105–14.
25. Yano M, Kato M, Ikoma Y, Kawasaki A, Fukuzawa Y, Sugiura M, et al. Quantitation of carotenoids in raw and processed fruits in Japan. Food Sci Technol Res. 2005;11:13–8.
26. Food Composition Database [Internet]. Ministory of Education, Culture, Sports, Science and Technology. Available from: http://fooddb.jp/.
27. Tribble DL. AHA Science Advisory. Antioxidant consumption and risk of coronary heart disease: emphasison vitamin C, vitamin E, and beta-carotene: A statement for healthcare professionals from the American Heart Association. Circulation. 1999;99:591–5.
28. Gaziano JM. Antioxidant vitamins and cardiovascular disease. Proc Assoc Am Physicians. 1999;111:2–9.
29. Steffen LM, Jacobs DR Jr, Stevens J, Shahar E, Carithers T, Folsom AR. Associations of whole-grain, refined-grain, and fruit and vegetable consumption with risks of all-cause mortality and incident coronary artery disease and ischemic stroke: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Clin Nutr. 2003;78:383–90.
30. Dauchet L, Amouyel P, Hercberg S, Dallongeville J. Fruit and vegetable consumption and risk of coronary heart disease: a meta-analysis of cohort studies. J Nutr. 2006;136:2588–93.
31. Pereira MA, O'Reilly E, Augustsson K, Fraser GE, Goldbourt U, Heitmann BL, et al. Dietary fiber and risk of coronary heart disease: a pooled analysis of cohort studies. Arch Intern Med. 2004;164:370–6.
32. Sugiura M, Nakamura M, Ogawa K, Ikoma Y, Matsumoto H, Ando F, et al. Synergistic interaction of cigarette smoking and alcohol drinking with serum carotenoid concentrations: findings from a middle-aged Japanese population. Br J Nutr. 2009;102:1211–9.
33. McCarthy JJ. Gene by sex interaction in the etiology of coronary heart disease and the preceding metabolic syndrome. Nutr Metab Cardiovasc Dis. 2007;17:153–61.
34. Koch OR, Pani G, Borrello S, Colavitti R, Cravero A, Farrè S, et al. Oxidative stress and antioxidant defenses in ethanol-induced cell injury. Mol Aspects Med. 2004;25:191–8.
35. Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury: Part II: animal and human studies. Circulation. 2003;108:2034–40.

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Original Article

Atrial Fibrillation Is a Major Risk Factor for Stroke, Especially in Women: The Jichi Medical School Cohort Study

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ABSTRACT

Background: Only a few population-based cohort studies have investigated the impact of atrial fibrillation (AF) on stroke in Japan.

Methods: A total of 10 929 participants (4147 men and 6782 women) were included in this population-based prospective cohort study. Baseline data, including electrocardiograms (ECGs) to ascertain AF status, were obtained from April 1992 through July 1995 in 12 areas in Japan. Cox proportional hazards models were used to analyze the association of AF with stroke.

Results: A total of 54 participants had AF (0.49%). The mean follow-up period was 10.7 years, during which 405 strokes were identified; 12 of these occurred in participants with AF. The crude incidence of stroke in participants with and without AF was 14.9 and 4.5 per 1000 person-years in men, respectively, and 39.3 and 2.7 per 1000 person-years in women. After adjusting for geographical area, sex, age, smoking status, drinking status, obesity, hypertension, dyslipidemia, and diabetes mellitus, the hazard ratios (95% confidence interval) of AF in all participants and in male and female participants were 4.11 (2.28–7.41), 2.12 (0.77–5.84), and 10.6 (5.01–22.4), respectively. The population attributable fraction (PAF) of stroke caused by AF was 2.2%; the PAFs were 1.0% and 3.6% in men and women, respectively.

Conclusions: The present Japanese population-based prospective cohort study showed that AF is a major risk factor for stroke, especially in women.

Key words: atrial fibrillation; stroke; women; cohort study

INTRODUCTION

Atrial fibrillation (AF) is the most common sustained arrhythmia and is a major risk factor for stroke.[1,2] The prevalence of AF is rising with the increasing age of many populations,[1–8] and it is more frequent in men than in women.[2,5–8] Studies have shown that the risk of stroke is 2 to 7 times higher in people with AF as compared with those without AF.[3,4,9–11] It has been suggested that the risk of stroke due to AF is higher in women than in men.[11–15] AF contributes to a number of medical, social, and economic problems by increasing the burdens on outpatient clinics, the extent of pharmacological treatment, admissions to hospital, and the incidence of disability due to cardiovascular diseases.[13,16]

In Japan, the percentage of the population in older age groups is increasing at the highest rate in the world. The estimated number of persons with AF is also rising rapidly in Japan.[5] However, there have only been a few Japanese population-based studies of the effect of AF on stroke.[17–20] Tanaka et al[17] and Tanizaki et al[19] conducted an epidemiologic study of cerebral infarction as a stroke subtype. Ohsawa et al[20] examined mortality risk, including stroke death, attributable to AF. In the Shibata study, Nakayama et al[18] classified stroke into 4 subtypes: hemorrhagic stroke, ischemic stroke, subarachnoid hemorrhage (SAH), and undetermined strokes. However, none of these studies found an effect of AF on hemorrhagic stroke or SAH. Nor did they address the differing effects of AF on stroke incidence in men and women. In this

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study, data from the JMS cohort were used to estimate hazard ratios (HRs) of stroke associated with AF, after adjusting for geographical area, sex, age, smoking status, drinking status, obesity, hypertension, dyslipidemia, and diabetes mellitus using Cox proportional hazards models. We also analyzed the effect of AF on all strokes, and on hemorrhagic stroke, ischemic stroke, and SAH, in both men and women. To estimate the proportion of strokes due to AF in this population, we calculated population attributable fractions (PAFs) of AF for all strokes and for stroke subtypes.

METHODS

The Jichi Medical School (JMS) Cohort Study is a population-based prospective cohort study. Its primary objective was to clarify the relationship between potential risk factors and health outcomes such as stroke, cardiovascular disease, and cancer in 12 local communities across Japan. The baseline data of this cohort study were obtained between April 1992 and July 1995. A detailed description of standardized data collection at baseline has been previously published.[21,22] The study design and procedure were approved by the Ethical Committee at Jichi Medical University.

Participants

Invitations to this mass screening were issued by local government offices in each community, and personal invitations were also sent to all potential participants by mail. The age of the adults participating in the mass screening examinations was 40 to 69 years in 8 communities, 20 to 69 years in 1 community, and 35 years or older (ie, no upper age limit) in 1 community; there was no age limit in 2 communities. The overall participation rate for those invited to the mass screening examination program was 65.4%. Written informed consent to participate in the study was obtained individually from all respondents to the mass screening.

In total, 12490 people were available for participation. However, 95 declined follow-up, and 7 could not be contacted after collection of baseline data. We also excluded 109 individuals with a history of stroke, 1347 with missing electrocardiogram (ECG) information, and 3 with both a past history of stroke and missing ECG data. Thus, the final number of study participants was 10929 (4147 men and 6782 women).

Initial survey and definition of status

At the baseline examination, each participant filled out a questionnaire on their lifestyle and medical history. A series of physical examinations was performed, and a 12-lead ECG at rest was recorded. A diagnosis of AF was based on the independent determination of 2 cardiologists, who reviewed a single baseline ECG. In the event of a disagreement, the final decision was made after deliberation by the approval committee. Smoking status was classified as smoker or nonsmoker. Drinking status was classified as drinker and nondrinker. Body mass index (BMI) was calculated as weight in kilograms divided by the subject's height in meters squared (kg/m^2). Obesity was defined as a BMI of 25 kg/m^2 or higher. Blood pressure was measured once with a fully automated sphygmomanometer, the BP203RV-II (Nippon Colin, Komaki, Japan), that was placed on the right arm of a seated participant who had rested in a sitting position for at least 5 minutes before the measurement. Hypertension was defined as a systolic blood pressure of 140 mm Hg or higher, a diastolic blood pressure of 90 mm Hg or higher, or current use of antihypertensive agents. Dyslipidemia was defined as a total cholesterol level of 220 mg/dl or higher, high-density lipoprotein cholesterol lower than 40 mg/dl, or current use of medication for hyperlipidemia. Diabetes mellitus was defined as fasting blood glucose level of 126 mg/dl or higher, casual blood glucose of 200 mg/dl or higher, or current use of diabetes medication.

Follow-up study

After enrolling in the study, participants were asked at the annual mass screening whether they had developed stroke or cardiovascular disease. Participants who did not attend the annual mass screening examination were contacted by mail or phone. Medical records were checked, and if an incident case was found, we requested duplicate films from computer tomography or magnetic resonance for stroke, or ECGs for myocardial infarction. Data on participants who left the study due to moving out of the area were obtained annually from each municipal government.

Stroke and myocardial infarction were diagnosed as described previously.[21,22] A diagnosis committee—composed of 1 radiologist, 1 neurologist, and 2 cardiologists—diagnosed stroke and myocardial infarction independently from the data collection groups. Stroke was defined as a focal and nonconvulsive neurological deficit of sudden onset persisting at least 24 hours. Stroke subtypes were confirmed based on computed tomography or magnetic resonance imaging.[23] A diagnosis of myocardial infarction was made using the criteria from the World Health Organization's MONICA project.[24]

Statistical analysis

The baseline data were classified by AF status and sex. Ages were compared using the unpaired *t*-test. Continuous variables were expressed as age-adjusted means with 95% confidence intervals (CIs) and compared by analysis of covariance (ANCOVA). Data for proportions were expressed as percentages, which were compared using the chi-square test. Using the baseline data, the incidence of AF was calculated by sex and age group. The follow-up period was defined as the period from the date of baseline collection to either the date of incidence (stroke, myocardial infarction, or death), the end of follow-up in the respective area, or the date of moving out of

Table 1. Baseline data from JMS cohort study, by atrial fibrillation (AF) status and sex

	Without AF					With AF				
	Men		Women			Men		Women		
No.	4117		6758			30		24		
Characteristic	Mean	95% CI	Mean	95% CI	*P*	Mean	95% CI	Mean	95% CI	*P*
Age (yrs)	55.5	55.1–55.8	55.6	55.4–55.9	0.403	64.9***	60.8–69.1	66.8***	62.4–71.2	0.346
BMI	23.0	22.9–23.1	23.2	23.1–23.3	<0.001	24.0**	23.1–24.8	24.2	23.3–25.0	0.805
SBP (mm Hg)	134.0	133.3–134.6	130.7	130.2–131.2	<0.001	127.9	122.5–133.3	124.6	119.2–130.0	0.813
DBP (mm Hg)	80.2	79.8–80.6	77.3	77.0–77.6	<0.001	77.6	74.4–80.8	74.7	71.5–77.9	0.684
T-CHOL (mg/dl)	187.2	186.2–188.3	199.6	198.7–200.4	<0.001	180.9	171.8–189.9	193.2	184.1–202.3	0.135
TG (mg/dl)	128.7	126.3–131.1	110.5	108.6–112.4	<0.001	112.5	92.2–132.7	94.3	74.0–114.6	0.287
HDL-C (mg/dl)	48.8	48.3–49.2	52.4	52.1–52.7	<0.001	53.6	50.1–57.0	57.2*	53.8–60.7	0.322
BS (mg/dl)	107.6	106.8–108.5	102.1	101.5–102.8	<0.001	107.4	100.3–114.4	101.8	94.8–108.9	0.541
Smoking status (%)					<0.001					<0.001
Smoker	49.9		5.7			41.4		4.3		
Nonsmoker	50.1		94.3			58.6		95.7		
Drinking status (%)					<0.001					<0.001
Drinker	75.0		24.4			74.6		22.3		
Nondrinker	25.0		75.6			25.4		77.7		

Ages were compared using the unpaired *t* test.
On ANCOVA, continuous values except for age were adjusted using age 60 years.
The chi-square test was used for comparisons of proportions.
*$P < 0.05$ for comparison of participants with and without AF.
**$P < 0.01$ for comparison of participants with and without AF.
***$P < 0.001$ for comparison of participants with and without AF.
95% CI: 95% confidence interval, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, T-CHOL: total cholesterol, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, BS: blood sugar.

the study area. Incidence rates were calculated and expressed per 1000 person-years. Cumulative stroke incidence was estimated using the Kaplan-Meier method and *P*-values were calculated using the log-rank (Mantel–Cox) method. The Cox proportional hazards model was used to estimate HRs for stroke, adjusting for geographical area, age, sex, smoking status, drinking status, obesity, hypertension, dyslipidemia, diabetes mellitus and AF. Population attributable fraction (PAF) was calculated as Pe × (HR − 1)/HR, in which Pe is the proportion of stroke cases exposed to the risk factor for each type of stroke or for all stroke cases.[25,26] The analyses were performed using SPSS 16.0J for Windows and Microsoft Office Excel 2003.

RESULTS

The baseline characteristics of the participants are shown in Table 1. Although mean age was significantly higher in participants with AF than in those without AF, there was no significant difference in mean age with respect to AF status in men or women. BMI and high-density lipoprotein cholesterol were significantly higher in AF participants than in non-AF participants in men and women, respectively. In non-AF participants, systolic blood pressure, diastolic blood pressure, triglyceride, and blood sugar were significantly higher in men, whereas, BMI, total cholesterol, and high-density lipoprotein cholesterol were significantly higher in women. In both the AF and non-AF groups, significantly more men than women were smokers and drinkers.

Table 2. Prevalence of atrial fibrillation by age group and sex

Age, yrs	Men		Women		Total	
	AF(+)/*n*	%	AF(+)/*n*	%	AF(+)/*n*	%
–39	0/367	0	0/513	0	0/880	0
40–49	0/897	0	1/1376	0.07	1/2273	0
50–59	5/995	0.50	2/1974	0.10	7/2969	0.24
60–69	21/1651	1.27	11/2595	0.42	32/4246	0.75
70+	4/237	1.69	10/324	3.09	14/561	2.50
Total	30/4147	0.72	24/6782	0.35	54/10 929	0.49

AF: atrial fibrillation.

As shown in Table 2, there were 54 participants with AF (0.49%). The prevalence of AF increased with age in both men and women. In men, the prevalence of AF increased at age 50 to 59 years and progressively increased thereafter. In women, the prevalence of AF drastically increased and, at age 70 years or older, surpassed that in men. However, the proportion of men with AF (0.72%) was higher than in women (0.35%; $P = 0.008$).

The mean duration of follow-up was 10.7 years. During the follow-up period, we identified 405 strokes, including 91 hemorrhagic strokes, 262 ischemic strokes, 51 SAHs, and 1 unspecified stroke. Therefore, with hemorrhagic stroke as the reference (hemorrhagic stroke:ischemic stroke:SAH:

Table 3. Cox hazard ratios and 95% confidence intervals for overall stroke risk associated with various risk factors

Characteristic	HR	95% CI
Male sex	1.39	1.06–1.81
Age, per 10-year increment	1.09	1.07–1.10
Smoking	1.34	1.03–1.76
Drinking	1.03	0.81–1.31
Obesity	0.96	0.75–1.23
Hypertension	2.65	2.11–3.31
Dyslipidemia	1.08	0.87–1.35
Diabetes mellitus	2.07	1.40–3.06
AF	4.11	2.28–7.41

Cox hazard ratios were also adjusted by geographical area.
HR: hazard ratio, 95% CI: 95% confidence interval, AF: atrial fibrillation.

unspecified stroke), the ratio for each stroke subtype was 1:2.88:0.56:0.01.

The cumulative stroke incidences for participants with and without AF are shown in the Figure. Log-rank analysis revealed that the cumulative stroke incidence was higher in AF participants than in non-AF participants in both men ($P = 0.014$) and women ($P < 0.001$). Cox proportional hazards models were used to analyze the associations of all strokes with various risk factors (Table 3), including geographical area, sex, age, smoking status, drinking status, obesity, hypertension, dyslipidemia, diabetes mellitus, and AF. Stroke was associated with male sex, smoking status, hypertension, diabetes mellitus, and AF, which was the strongest risk factor for stroke (Table 3).

As shown in Table 4, there were 198 and 4 strokes in men without and with AF, respectively. The crude incidence rates were 4.5 and 14.9 per 1000 person-years; thus, the crude incidence rate in AF participants was about 3 times that of non-AF participants. In women, there were 195 and 8 strokes, corresponding to crude incidence rates of 2.7 and 39.3 per 1000 person-years, in non-AF and AF participants, respectively. The crude incidence rate in AF participants was about 15 times that of non-AF participants.

The HRs for stroke associated with AF were estimated using a Cox proportional hazards model (Table 4). AF increased the risk of stroke by factors of 2 and 11 in men and women, respectively. Thus, the effect of AF on stroke in women was about 5 times greater than in men. Strokes were divided by subtype into hemorrhagic stroke, ischemic stroke, and SAH (Table 4). In men, AF increased the risks of hemorrhagic stroke and ischemic stroke by factors of approximately 3 and 2, respectively. In women, AF increased the risks of hemorrhagic stroke, ischemic stroke, and SAH by factors of approximately 6, 13, and 9, respectively. The increases in overall stroke risk associated with AF was statistically significant in women, but not in men.

As shown in Table 4, with respect to PAF, AF contributed to only 1.4% of hemorrhagic stroke incidence. The PAF for ischemic stroke was 1.1% in men and 4.8% in women. For all strokes, the PAFs were 1.0% and 3.6% in men and women, respectively. Thus, the PAFs for ischemic stroke and all strokes in women were approximately 4 times those of men.

DISCUSSION

The JMS Cohort Study is a prospective population-based cohort study of risk factors for cardiovascular disease in Japan.[21,22] There are few population-based studies of the

Table 4. Number of incident strokes, crude incidence rates, multivariate-adjusted hazard ratios, and population attributable fractions for atrial fibrillation (AF) by stroke subtype

	No.	Crude incidence[a]	No.	Crude incidence[a]	HR (95% CI)[b]	PAF (%)
Total	Without AF (n = 10875)		With AF (n = 54)			
Hemorrhagic stroke	89	0.8	2	3.7	2.90 (0.69–12.2)	1.4
Ischemic stroke	253	2.2	9	16.7	4.51 (2.28–8.94)	2.7
SAH	50	0.4	1	1.9	4.09 (0.54–30.7)	1.5
All strokes	393	3.4	12	22.2	4.11 (2.28–7.41)	2.2
Men	Without AF (n = 4117)		With AF (n = 30)			
Hemorrhagic stroke	42	1.0	1	3.7	3.15 (0.40–25.0)	1.6
Ischemic stroke	143	3.3	3	11.1	2.16 (0.67–6.97)	1.1
SAH	13	0.3	0	0	0	—
All strokes	198	4.5	4	14.9	2.12 (0.77–5.84)	1.0
Women	Without AF (n = 6758)		With AF (n = 24)			
Hemorrhagic stroke	47	0.6	1	4.9	5.93 (0.77–45.6)	1.7
Ischemic stroke	110	1.5	6	29.5	13.2 (5.43–32.1)	4.8
SAH	37	0.5	1	4.9	8.69 (1.10–68.4)	2.3
All strokes	195	2.7	8	39.3	10.6 (5.01–22.4)	3.6

HR: hazard ratio, 95% CI: 95% confidence interval, PAF: population attributable fraction, SAH: subarachnoid hemorrhage.
[a]per 1000 person-years.
[b]HRs were calculated using a Cox proportional hazard model adjusted for geographical area, sex, age, smoking status, drinking status, obesity, hypertension, dyslipidemia, and diabetes mellitus.

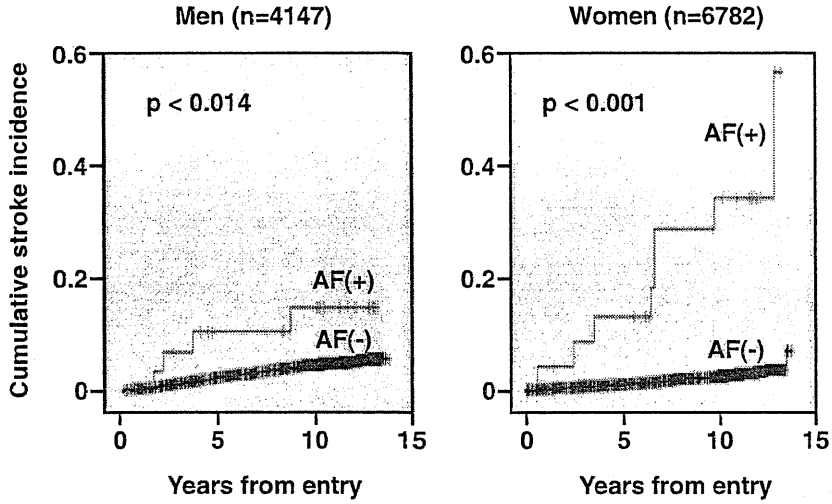

Figure 1. Cumulative stroke incidence by sex and atrial fibrillation (AF) status. *P* values were calculated using the log-rank (Mantel–Cox) method.

effect of AF on stroke in Japan. Among them, the number of participants in the present study is 1.3, 5, and 7 times those of the NIPPON DATA80,[20] Shibata Study,[17,18] and Hisayama Study,[19] respectively. The present study showed that the prevalence of AF was higher in men than in women and that the prevalence of AF increased with age in both men and women (Table 2). In addition, cumulative stroke incidences were significantly higher in AF participants than in non-AF participants in both men and women (Figure 1). Male sex, smoking status, hypertension, diabetes mellitus, and AF were shown to be major risk factors for stroke, after adjusting for geographical area, sex, age, smoking status, drinking status, obesity, hypertension, dyslipidemia, diabetes mellitus, and AF (Table 3), which was the strongest risk factor. AF also had the greatest effect on ischemic stroke and was a more unambiguous risk factor for stroke in women than in men (Table 4).

In the Japanese general population, the prevalence of AF was reported to range between 0.56% and 1.6%.[5–8] The prevalence of AF in Western countries is higher than in Japan,[1–4] and apparently increases with age.[1–8] AF is also more common in men than in women.[2,5–8] As shown in Table 2, the prevalence of AF in the present study was 0.49%, which was somewhat lower than in previous reports. This may be because the participants in the present study were healthier, as they were regular participants in annual mass screening examinations, and because we excluded participants aged 70 years or older in 9 of 12 areas. Thus, selection bias might have affected the results. However, our results confirmed that the prevalence of AF increases with age and that AF is more common in men than in women (Table 2).

In the JMS cohort study, the ratio of each stroke subtype (hemorrhagic stroke:ischemic stroke:SAH:unspecified stroke) was 1:2.88:0.56:0.01. In the Takasima Stroke Registry,[27] the equivalent ratio was 1:3.10:0.44:0.07, which is quite similar to our results. However, in the Framingham study,[14] the ratio of hemorrhagic stroke:ischemic stroke:SAH:others was 1:13.70:1.44:0.52. Therefore, the ratio of ischemic stroke was substantially higher in the United States than in Japan. Most studies in East Asia, including those in Japan, have suggested that the proportion of hemorrhagic stroke in those populations is significantly higher than in whites.[28]

In both men and women, cumulative stroke incidence was higher in participants with AF than in those without the condition (Figure 1). As shown in Table 3, AF was an independent risk factor for stroke after adjusting for geographical area, sex, age, smoking status, drinking status, obesity, hypertension, dyslipidemia, and diabetes mellitus; AF quadrupled the risk of stroke. Previous studies in Japan reported that AF increased the risk of stroke, including cerebral infarction and stroke death, by a factor of 3 to 7.[17–20] However, none of those studies investigated the effect of AF on stroke subtypes. In the present study, we classified stroke as hemorrhagic stroke, ischemic stroke, and SAH, and analyzed the influence of AF on each of these subtypes (Table 4). Although AF increased the risk for each type of stroke, the increase was not statistically significant for hemorrhagic stroke in either men or women, or for ischemic stroke and SAH in men. If a greater number of participants had been enrolled, the increase in risk might have been significant. Therefore, to clarify the effect of AF on hemorrhagic stroke and SAH, a longer observation period or a meta-analysis will be necessary.

The effect of AF on stroke was analyzed using Cox proportional hazards models (Table 4). The HRs for all types of stroke except hemorrhagic stroke were significant in women, but not in men. It is particularly noteworthy that the effect of AF on the risk of stroke in women was about 5

times that in men (Table 4). With respect to PAF, in women, the contribution of AF to ischemic stroke and overall stroke risk was approximately 4 times that in men (Table 4). In the present study, the highest PAF was only 4.8% because of the low prevalence of AF. However, the prevalence of AF increases with age,[1–8] and Japan is one of the most rapidly aging countries in the world.[29] Therefore, the PAF of AF is expected to increase, and AF will likely have a greater impact on Japanese society in the near future. Friberg et al[15] reported that AF is a much more significant risk factor for stroke in women than in men. Some reports also showed that women with AF were more likely to have a stroke than men with AF.[11–14] In addition, women with stroke were more likely to have AF than men with stroke.[30–32] However, it is not known why AF is associated with a higher risk of stroke in women.

This study has some limitations that warrant mention. As shown in Table 4, the HRs for all types of stroke were not significant in men, and the HR for hemorrhagic stroke was not significant in women. The number of participants with AF was only 54; therefore, the statistical power of the study may have been insufficient. Second, the prevalence of AF increases with age, but because participants aged 70 years or older were excluded in 9 of the 12 study areas, the number of such participants was small (Table 2). This might be a form of selection bias that led to the result indicating that the prevalence of AF at age 70 or older was higher in women than in men, which contradicts the results of previous reports. However, despite the lack of statistical power and possibility of selection bias, any distortion of relative risk estimates is likely to be small. A third limitation was that a diagnosis of AF was based on a single, baseline ECG recording. Therefore, AF could not differentiated as paroxysmal, persistent, or permanent. However, the risks for stroke and non-central nervous system embolism were reported to be similar in individuals with paroxysmal AF and those with sustained AF.[33] In the present study, participants with paroxysmal AF were probably not classified as having AF, and some participants probably developed AF during the follow-up period, so the effect of AF on stroke might have been underestimated. Another limitation of the study was that the anticoagulant agent warfarin apparently decreases the risk of stroke in patients with AF.[1,11,34] Anticoagulation therapy for stroke prevention in AF participants may increase hemorrhagic risk, thus possibly increasing the risk for hemorrhagic stroke or SAH. This study showed that AF may increase the risk of hemorrhagic stroke and SAH (Table 4). However, we did not collect data concerning the use of anticoagulant agents. Therefore, in future studies of AF and stroke, anticoagulant therapy must be evaluated as a confounding factor.

AF is a major risk factor for stroke, particularly in women, and its prevalence increases with age. The proportion of elderly people is increasing rapidly in Japan, and AF has recently been treated with anticoagulant agents to prevent cardiovascular disease. Therefore, the importance of AF as a medical, economic, and social issue is continuing to increase.

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REFERENCES

1. Feinberg WM, Blackshear JL, Laupacis A, Kronmal R, Hart RG. Prevalence, age distribution, and gender of patients with atrial fibrillation. Analysis and implications. Arch Intern Med. 1995;155:469–73.
2. Go AS, Hylek EM, Phillips KA, Chang Y, Henault LE, Selby JV, et al. Prevalence of diagnosed atrial fibrillation in adults: national implications for rhythm management and stroke prevention: the AnTicoagulation and Risk Factors in Atrial Fibrillation (ATRIA) Study. JAMA. 2001;285:2370–5.
3. Wolf PA, Abbott RD, Kannel WB. Atrial fibrillation: a major contributor to stroke in the elderly. The Framingham Study. Arch Intern Med. 1987;147:1561–4.
4. Krahn AD, Manfreda J, Tate RB, Mathewson FA, Cuddy TE. The natural history of atrial fibrillation: incidence, risk factors, and prognosis in the Manitoba Follow-Up Study. Am J Med. 1995;98:476–84.
5. Ohsawa M, Okayama A, Sakata K, Kato K, Itai K, Onoda T, et al. Rapid increase in estimated number of persons with atrial fibrillation in Japan: an analysis from national surveys on cardiovascular diseases in 1980, 1990 and 2000. J Epidemiol. 2005;15:194–6.
6. Iguchi Y, Kimura K, Aoki J, Kobayashi K, Terasawa Y, Sakai K, et al. Prevalence of atrial fibrillation in community-dwelling Japanese aged 40 years or older in Japan: analysis of 41,436 non-employee residents in Kurashiki-city. Circ J. 2008;72:909–13.
7. Ohsawa M, Itai K, Tanno K, Onoda T, Ogawa A, Nakamura M, et al. Cardiovascular risk factors in the Japanese northeastern rural population. Int J Cardiol. 2009;137:226–35.
8. Inoue H, Fujiki A, Origasa H, Ogawa S, Okumura K, Kubota I, et al. Prevalence of atrial fibrillation in the general population of Japan: an analysis based on periodic health examination. Int J Cardiol. 2009;137:102–7.
9. Wolf PA, Dawber TR, Thomas HE Jr, Kannel WB. Epidemiologic assessment of chronic atrial fibrillation and risk of stroke: the Framingham Study. Neurology. 1978;28:973–7.
10. Flegel KM, Shipley MJ, Rose G. Risk of stroke in non-rheumatic atrial fibrillation. Lancet. 1987;1(8532):526–9.
11. Wolf PA, Abbott RD, Kannel WB. Atrial fibrillation as an independent risk factor for stroke: the Framingham Study. Stroke. 1991;22:983–8.
12. Boysen G, Nyboe J, Appleyard M, Sorensen PS, Boas J, Somnier F, et al. Stroke incidence and risk factors for stroke in Copenhagen, Denmark. Stroke. 1988;19:1345–53.
13. Wolf PA, Mitchell JB, Baker CS, Kannel WB, D'Agostino RB. Impact of atrial fibrillation on mortality, stroke, and medical costs. Arch Intern Med. 1998;158:229–34.
14. Wang TJ, Massaro JM, Levy D, Vasan RS, Wolf PA, D'Agostino

RB, et al. A risk score for predicting stroke or death in individuals with new-onset atrial fibrillation in the community: the Framingham Heart Study. JAMA. 2003;290:1049–56.
15. Friberg J, Scharling H, Gadsbøll N, Truelsen T, Jensen GB; Copenhagen City Heart Study. Comparison of the impact of atrial fibrillation on the risk of stroke and cardiovascular death in women versus men (The Copenhagen City Heart Study). Am J Cardiol. 2004;94:889–94.
16. Ringborg A, Nieuwlaat R, Lindgren P, Jönsson B, Fidan D, Maggioni AP, et al. Costs of atrial fibrillation in five European countries: results from the Euro Heart Survey on atrial fibrillation. Europace. 2008;10:403–11.
17. Tanaka H, Hayashi M, Date C, Imai K, Asada M, Shoji H, et al. Epidemiologic studies of stroke in Shibata, a Japanese provincial city: preliminary report on risk factors for cerebral infarction. Stroke. 1985;16:773–80.
18. Nakayama T, Date C, Yokoyama T, Yoshiike N, Yamaguchi M, Tanaka H. A 15.5-year follow-up study of stroke in a Japanese provincial city. The Shibata Study. Stroke. 1997;28:45–52.
19. Tanizaki Y, Kiyohara Y, Kato I, Iwamoto H, Nakayama K, Shinohara N, et al. Incidence and risk factors for subtypes of cerebral infarction in a general population: the Hisayama study. Stroke. 2000;31:2616–22.
20. Ohsawa M, Okayama A, Okamura T, Itai K, Nakamura M, Tanno K, et al. Mortality risk attributable to atrial fibrillation in middle-aged and elderly people in the Japanese general population: nineteen-year follow-up in NIPPON DATA80. Circ J. 2007;71:814–9.
21. Ishikawa S, Gotoh T, Nago N, Kayaba K; Jichi Medical School (JMS) Cohort Study Group. The Jichi Medical School (JMS) Cohort Study: design, baseline data and standardized mortality ratios. J Epidemiol. 2002;12:408–17.
22. Ishikawa S, Kayaba K, Gotoh T, Nago N, Nakamura Y, Tsutsumi A, et al. Incidence of total stroke, stroke subtypes, and myocardial infarction in the Japanese population: the JMS Cohort Study. J Epidemiol. 2008;18:144–50.
23. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke. 1993;24:35–41.
24. The World Health Organization MONICA Project (monitoring trends and determinants in cardiovascular disease): a major international collaboration. WHO MONICA Project Principal Investigators. J Clin Epidemiol. 1988;41:105–14.
25. Nakayama T, Yokoyama T, Yoshiike N, Zaman MM, Date C, Tanaka H, et al. Population attributable fraction of stroke incidence in middle-aged and elderly people: contributions of hypertension, smoking and atrial fibirillation. Neuroepidemiology. 2000;19:217–26.
26. Rockhill B, Newman B, Weinberg C. Use and misuse of population attributable fractions. Am J Public Health. 1998;88:15–9.
27. Kita Y, Turin TC, Rumana N, Sugihara H, Morita Y, Hirose K, et al. Surveillance and measuring trends in Japan: The Takashima Stroke Registry (1988-present). Int J Stroke. 2007;2:129–32.
28. Sudlow CL, Warlow CP. Comparable studies of the incidence of stroke and its pathological types: results from an international collaboration. International Stroke Incidence Collaboration. Stroke. 1997;28:491–9.
29. Population Division, Department of Economic and Social Affairs, United Nations [Internet]. New York: World Population Ageing 2009 [updated 2009 Dec; cited 2010 Oct 12]. Available from: http://www.un.org/esa/population/publications/WPA2009/WPA2009_WorkingPaper.pdf.
30. Di Carlo A, Lamassa M, Baldereschi M, Pracucci G, Basile AM, Wolfe CD, et al. Sex differences in the clinical presentation, resource use, and 3-month outcome of acute stroke in Europe: data from a multicenter multinational hospital-based registry. Stroke. 2003;34:1114–9.
31. Roquer J, Campello AR, Gomis M. Sex differences in first-ever acute stroke. Stroke. 2003;34:1581–5.
32. Kapral MK, Fang J, Hill MD, Silver F, Richards J, Jaigobin C, et al. Sex differences in stroke care and outcomes: results from the Registry of the Canadian Stroke Network. Stroke. 2005;36:809–14.
33. Hohnloser SH, Pajitnev D, Pogue J, Healey JS, Pfeffer MA, Yusuf S, et al. Incidence of stroke in paroxysmal versus sustained atrial fibrillation in patients taking oral anticoagulation or combined antiplatelet therapy: an ACTIVE W Substudy. J Am Coll Cardiol. 2007;50:2156–61.
34. Hylek EM, Go AS, Chang Y, Jensvold NG, Henault LE, Selby JV, et al. Effect of intensity of oral anticoagulation on stroke severity and mortality in atrial fibrillation. N Engl J Med. 2003;349:1019–26.

Original Article

Plasma NOx Concentrations in Glucose Intolerance and Type 2 Diabetes

— A Case-control Study in a Vietnamese Population

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Aim: The Vietnamese develop type 2 diabetes (T2D) and metabolic syndrome (MS) at a lower BMI than other ethnicities. Thus, biomarkers that identify subjects at an increased risk of T2D independently of obesity are being sought. Recent studies show that circulating NO metabolites (NOx) are increased in T2D. We investigated whether plasma NOx levels predict insulin resistance and glucose intolerance before the development of T2D, independently of obesity.
Methods: The current study was derived from a population-based study in HCMC, Vietnam, which was designed to investigate the prevalence of MS and T2D in a population aged 30-69 years. Four hundred and twenty-two subjects were recruited from the study and were stratified into 4 age- and gender-matched groups according to a glucose tolerance test {normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and T2D}.
Results: Plasma NOx concentrations were significantly increased in T2D but not in IFG or IGT compared with NGT. Multiregression analysis showed that plasma NOx levels were inversely correlated with BMI in T2D whereas no association was found between plasma NOx levels and BMI in non-diabetic subjects. Moreover, there was no correlation between plasma NOx levels and homeostasis model assessment-insulin resistance (HOMA-IR) in both diabetic and non-diabetic subjects.
Conclusion: Plasma NOx levels did not predict glucose intolerance or insulin resistance before the development of T2D and the increase in plasma NOx levels in T2D was not caused by adiposity. Thus, plasma NOx is not a useful marker for the prediction of high-risk subjects for T2D among Vietnamese.

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Key words; Case-control study, Insulin resistance, Adiposity

Introduction

Obesity is a major predictor of type 2 diabetes (T2D); however, similar to other Asians, Vietnamese are known to develop metabolic syndrome and T2D at a lower body mass index (BMI) than Caucasians[1-3]. Moreover, abdominal obesity, dyslipidemia and hypertension despite having a normal BMI are more common among Vietnamese (unpublished observation), suggesting the widespread presence of insulin resistance without being overweight or obese. Therefore, it is hoped to identify circulating biomarkers that can predict high-risk subjects for developing T2D independently of obesity. Thus far, C-reactive protein

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(CRP) in association with TNF-α receptor 2 and IL-6 has been shown to predict T2D independently of BMI and other indices of obesity in some studies[4-8]; however, others have shown that the association between CRP and T2D is largely due to obesity[9-12].

Recently, several studies indicated that circulating levels of the metabolites of nitric oxide (NOx) are associated with T2D. A population-based study conducted by Zahedi *et al.* demonstrated that serum NOx concentrations were significantly elevated in subjects with metabolic syndrome and T2D compared to their corresponding controls[13]; however, there is a contradicting report showing that increased plasma NO levels were observed in T2D but not in non-diabetic subjects with the presence of insulin resistance[14]. Thus, although these studies unequivocally demonstrated that circulating NOx levels are increased in T2D subjects, it is still controversial whether circulating NOx levels can predict high-risk subjects before the development of T2D.

This study was undertaken to investigate whether plasma NOx levels predict high-risk subjects for T2D independently of obesity using a population in which obesity is rare and yet the incidence of T2D is markedly increasing.

Methods

Study design and measurements

The present study is derived from a population-based study in HCMC, Vietnam. Subjects aged 30-69 years attended a health check up and underwent anthropometric and clinical measurements (weight, height, waist circumference and blood pressure), sampling of venous blood at 5:00-7:00 a.m. after overnight fasting and 75-g oral glucose tolerance test (OGTT). Blood pressure was measured in the sitting position after 5 min of rest. BMI was determined as weight in kilograms divided by the square of the height in meters (kg/m^2). Fasting serum was separated from coagulated whole blood and insulin, high sensitive C-reactive protein (hsCRP), total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were measured by Diag Center International (Lab Group International-Division Vietnam) on the same day as blood sampling. The TG/HDL-C ratio was calculated as a surrogate marker for insulin resistance[15-16]. The homeostasis model of assessment of the insulin resistance (HOMA-IR) score was calculated as fasting insulin (μU/mL) multiplied by fasting glucose (mmol/L) divided by 22.5[17]. Plasma specimens were separated from non-coagulated whole blood and were frozen at −70℃ until NOx measurements.

75-g OGTT

Fasting and 2-h postload glucose levels of 75-g OGTT were measured. The criteria of the 1999 World Health Organization were used to stratify subjects into 4 groups: normal glucose tolerance (NGT); impaired fasting glucose (IFG); impaired glucose tolerance (IGT) and type 2 diabetes (T2D)[18]: for T2D, fasting ≥ 7.0 mmol/L (126 mg/dL), 2-h ≥ 11.1 mmol/L (200 mg/dL); for IGT, fasting <7.0 mmol/L (126 mg/dL) and 2-h ≥ 7.8 mmol/L (140 mg/dL) and < 11.1 mmol/L (200 mg/dL); and for IFG ≥ 6.1 mmol/L (110 mg/dL) and <7.0 mmol/L (<126 mg/dL) and if measured, 2-h <7.8 mmol/L (140 mg/dL). A total of 422 subjects were recruited from the list of the main study in such a way that each OGTT-stratified group was matched for gender and age group.

The study was approved by the Institutional Review Board of the Health Services of Ho Chi Minh City and all participants signed informed consent.

Measurements of plasma NOx

The concentrations of plasma $NO2^-$ (nitrite) and $NO3^-$ (nitrate), stable metabolites of NO, were measured by the Griess reaction using an HPLC-Griess system (NOx Analyzer ENO-10; EiCom Instrument, Kyoto, Japan) as previously described[19]. Briefly, 30 μL plasma is mixed with a equal volume of methanol and centrifuged at 15,000 rpm for 15 min to precipitate protein. Ten microliters each of supernatants are injected into the NOx Analyzer using an automatic injector (Gilson, Middleton, WN, USA). In the analyzer, nitrite and nitrate are separated on a reverse-phase separation column. Nitrite is then mixed with the Griess reagent to form a purple azo dye in the reaction coil, whose absorbance is measured at 540 nm by a flow-through spectrophotometer. The absorbance reaches a peak with a retention time of approximately 4.5 min. Nitrate is reduced to nitrite with a cadmium reduced copper column, which subsequently reacts with the Griess reagent. This peak arrives with a retention time of approximately 8 min. The area under the absorption curve is compared with that of a standard solution containing sodium nitrite and sodium nitrate (Wako Pure Chemical Industries Inc.) to determine plasma nitrite and nitrate concentrations. Plasma NOx concentration was obtained by summing nitrite and nitrate concentrations.

Statistical analysis

All statistical analyses were performed using SPSS for Windows, version 14.0 (SPSS, Chicago, IL). Cate-

Table 1. Characteristics of 4 OGTT-stratified groups

	NGT (n=120)	IFG (n=111)	IGT (n=101)	T2D (n=90)	All (n=422)
Gender, n (%)					
Men	57 (13.5)	51 (12.1)	47 (11.1)	47 (11.1)	201 (47.9)
Women	63 (14.9)	60 (14.2)	54 (12.8)	43 (10.2)	220 (52.1)
Age (yrs)	50.9±11.2	50.1±10.8	53.1±10.0	57.7±8.0***	52.7±10.6
BMI (kg/m^2)	22.7±2.9	23.4±3.5	24.2±4.1**	23.8±3.8*	23.5±3.6
Waist circumference (cm)	78.4±9.4	80.4±9.5	82.9±10.2**	83.1±9.2***	81.0±9.7
Systolic BP (mmHg)	118±18	123±22	127±20**	132±20***	125±20
Diastolic BP (mmHg)	73±10	75±12	78±11**	76±10*	75±11
Fasting glucose (mmol/L)	5.1 (0.4)	5.7 (0.3)	5.5 (0.7)	7.0 (2.7)	5.6 (0.8)
Fasting insulin (μU/mL)	6.4 (6.0)	7.6 (6.3)*	9.0 (8.4)***	10.2 (10.2)***	8.1 (7.3)
HOMA-IR	1.5 (1.4)	1.9 (1.6)*	2.3 (2.0)***	3.1 (2.8)***	2.0 (2.0)
Total cholesterol (mg/dL)	210±40	203±39	210±47	212±55	208±45
HDL-C (mg/dL)	55±14	54±15	52±12	49±13**	53±14
Non HDL-C (mg/dL)	155±41	149±40	157±46	163±54	156±45
Triglyceride (mg/dL)	137 (101)	138 (119)	168 (114)	204 (160)***	156 (128)
TG: HDL-C ratio	2.6 (2.8)	2.6 (3.8)	3.2 (3.3)	4.4 (4.1)***	3.1 (3.5)
hsCRP (mg/L)	1.0 (2.0)	1.0 (2.0)	2.0 (2.5)	2.0 (3.0)**	1.0 (2.0)
NOx (μmol/L)	26.4 (17.6)	26.4 (16.4)	23.5 (21.0)	35.7 (31.3)**	26.5 (21.5)

BMI, body mass index; BP: blood pressure; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model of assessment of insulin resistance; hsCRP, high sensitive C-reactive protein; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; NOx, nitrite and nitrate combined; OGTT: oral glucose tolerance test; T2D, type 2 diabetes; TG: triglyceride.
Average age, BMI, waist circumference, total cholesterol, HDL-C and non HDL-C are expressed as the mean±SD and differences among groups were examined by one-way ANOVA and t-test. Triglyceride, TG: HDL-C ratio, fasting glucose, fasting insulin, HOMA-IR, hsCRP and NOx are presented as the median followed by interquartile range in parentheses and differences among groups were examined by the non-parametric Mann-Whitney test. P values refer to differences as determined by t tests or the Mann-Whitney test. *P<0.05, **P<0.01, ***P<0.001 compared with NGT group.

gorical variables were presented as percentages with 95% confidence intervals, and differences between subgroups were examined using Pearson chi-squared tests. Normally distributed continuous variables were presented as the mean and standard deviation and differences among groups were examined by one-way ANOVA and t-test. Skewed continuous variables (TG, fasting glucose, fasting insulin, HOMA-IR, and NOx) were presented as the median and interquartile range and differences among groups were examined by the non-parametric Kruskal-Wallis and Mann-Whitney tests. Spearman correlation analysis was used to assess the univariate correlation between NOx and potential predictors. Skewed variables were logarithmically converted before correlation analysis. Gender, age and covariates that were positively correlated to NOx from univariate correlation analysis were selected for forward stepwise multiple linear regression analysis to determine factors independently associated with NOx. All statistical tests used a significance level of P<0.05.

Results

Characteristics of 4 OGTT-stratified groups

Characteristics of 4 OGTT-stratified groups are shown in **Table 1**. Four groups were similar in gender. Subjects with T2D were older than those with NGT and IFG (P<0.001). Both IGT and T2D groups had significantly greater BMI (P<0.01 and P<0.05, respectively) and waist circumference (P<0.01 and P<0.001, respectively) than NGT group. Both systolic and diastolic blood pressure were significantly higher in both IGT (P<0.01 for both systolic and diastolic blood pressure) and T2D (P<0.001 and P<0.05, respectively) groups than NGT group.

While no significant difference was found in the concentration of serum total cholesterol or non HDL-C among the 4 groups, T2D group had lower HDL-C (P<0.01) and higher serum triglyceride (P<0.001), resulting in a higher TG/HDL-C ratio (P<0.001) than NGT. This higher TG/HDL-C ratio was not observed in IFG or IGT group compared with NGT group.

Table 2. Spearman univariate correlations (r) between plasma NOx concentrations and demographic, anthropometric and biochemical variables in 4 OGTT-stratified groups

	NGT ($n=120$)	IFG ($n=111$)	IGT ($n=101$)	T2D ($n=90$)
Gender	-0.218^{*}	-0.240^{*}	-0.149	-0.176
Age	0.024	0.050	0.097	0.002
BMI	0.079	-0.038	-0.079	-0.247^{*}
Waist circumference	0.088	0.008	0.077	-0.183
Systolic blood pressure	0.079	0.141	0.018	-0.099
Diastolic blood pressure	0.018	0.150	0.008	-0.251^{*}
Fasting glucose	0.172	0.215^{*}	0.125	0.191
Fasting insulin	-0.076	-0.113	-0.050	-0.174
HOMA-IR	-0.057	-0.098	-0.019	-0.099
Total cholesterol	0.090	-0.101	-0.080	-0.071
Triglyceride	0.265^{**}	0.124	0.133	0.144
HDL-C	-0.141	-0.158	-0.239^{*}	-0.208^{*}
Non HDL-C	0.104	-0.056	-0.017	-0.018
TG: HDL-C ratio	0.258^{**}	0.133	0.159	0.198
hsCRP	-0.027	-0.155	-0.001	-0.016

BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model of assessment of insulin resistance; hsCRP, high sensitive C-reactive protein; NOx, nitrite and nitrate combined; TG: triglyceride.
r indicates Spearman correlation coefficient between plasma NOx concentration and each variable. $^{*}P<0.05$, $^{**}P<0.01$

Fasting serum insulin levels were higher in IFG, IGT and T2D groups ($P<0.05$, $P<0.001$ and $P<0.001$, respectively) compared with NGT group. HOMA-IR was significantly greater in IFG, IGT and T2D groups ($P<0.05$, $P<0.001$ and $P<0.001$, respectively) compared with NGT group.

Serum hsCRP and plasma NOx concentrations were higher in T2D ($P<0.01$) but not in IFG or IGT group compared with NGT group.

Univariate correlation analysis to determine factors associated with plasma NOx concentrations in 4 OGTT-stratified groups

To identify variables that are associated with plasma NOx concentrations, univariate correlation analysis was performed separately in each OGTT-stratified group. The Spearman correlation coefficients of variables in the association with plasma NOx concentration are shown in **Table 2**. Among subjects with T2D, BMI ($r=-0.247$, $P<0.05$), HDL-C ($r=-0.208$, $P<0.05$) and diastolic blood pressure ($r=-0.251$, $P<0.05$) were inversely correlated with plasma NOx concentrations whereas no significant association was found between plasma NOx concentrations and HOMA-IR.

Although plasma NOx levels were similar among 3 non-diabetic groups (NGT, IFG and IGT), factors associated with plasma NOx were different. Among NGT subjects, plasma NOx levels were positively correlated with male sex ($r=-0.218$, $P<0.05$), serum TG levels ($r=0.265$, $P<0.01$) and TG: HDL-C ratios ($r=0.258$, $P<0.01$). Among IFG subjects, plasma NOx levels were positively correlated with male sex ($r=-0.240$, $P<0.05$) and fasting glucose levels ($r=0.215$, $P<0.05$). Among IGT subjects, plasma NOx levels were inversely correlated with serum HDL-C levels ($r=-0.239$, $P<0.05$); however, no significant association was found between plasma NOx concentrations and HOMA-IR or fasting insulin when the analysis was performed on any OGTT-stratified group as well as on 3 non-diabetic groups combined (data not shown).

Multiple regression analysis to determine independent predictors of higher plasma NOx concentrations in 4 OGTT-stratified groups

Forward stepwise multiple linear regression analysis was performed to determine independent determinants of plasma NOx concentrations among 4 OGTT-stratified groups (**Table 3**). Gender, age and all other covariates that were found to positively correlate with plasma NOx concentrations by univariate correlation analysis were taken into multiple regression models. In T2D subjects, independent predictors of plasma NOx levels were diastolic blood pressure (B-coefficent $=-0.239$, $P=0.022$) and BMI (B-coefficent

Table 3. Predictors of log NOx determined by multiple regression analysis in 4 OGTT-stratified groups

	Predictors	B-coefficient	p	Adjusted R^2 value
NGT				0.093 ($P<0.001$)
	Log TG	0.317	<0.001	
	Variables entered in the model: gender, age, log triglyceride			
IFG				0.044 ($P=0.015$)
	Gender (1=men, 2=women)	−0.230	0.015	
	Variables entered in the model: gender, age, log fasting glucose			
IGT				0.049 ($P=0.015$)
	HDL-C	−0.241	0.015	
	Variables entered in the model: gender, age, HDL-C			
T2D				0.093 ($P=0.048$)
	Diastolic blood pressure	−0.239	0.022	
	BMI	−0.206	0.048	
	Variables entered in the model: gender, age, BMI, HDL-C, diastolic blood pressure			

BMI, body mass index; HDL-C, high-density lipoprotein cholesterol.
R^2 is the squared multiple correlation coefficient that indicates the coefficient of determination of the regression.

= −0.206, $P=0.048$) with low but significant adjusted R^2 of 0.093. The independent predictors of plasma NOx levels were different among 3 non-diabetic groups despite their similar NOx levels. In each group, a single determinant of plasma NOx concentrations was found with a quite low coefficient of determination of the regression (adjusted R^2 values varied from 0.044 to 0.093). Serum triglyceride levels were the determinant (B-coefficent=0.317, $P<0.001$) that accounted for 9.3% variations of plasma NOx concentrations in NGT subjects. Male gender was the determinant (B-coefficent = −0.230, $P=0.015$) that accounted for only 4.4% variations of plasma NOx levels in IFG subjects while serum HDL-C levels were the determinant (B-coefficent = −0.241, $P=0.015$) that accounted for only 4.9% variations of plasma NOx levels in IGT subjects.

Discussion

The current study clearly showed that plasma NOx levels were elevated in subjects with T2D compared with NGT among Vietnamese. This finding was in accordance with previous reports by others on Iranian, Taiwanese and Japanese populations[13, 14, 20]; however, plasma NOx levels in IFG and IGT were neither significantly increased nor decreased compared with NGT. Furthermore, no correlation was found between plasma NOx concentrations and HOMA-IR in both diabetic and non-diabetic subjects. Thus, plasma NOx levels predict neither insulin resistance nor glucose intolerance before the development of T2D.

Several experimental studies using mouse models have shown that adipose tissue is a potential source of NO[21-22] and the overproduction of NO by inducible NO synthase (iNOS) is involved in the development of insulin resistance in both genetic and diet-induced obesity[23-26]. However, the current study revealed that plasma NOx concentrations were not positively associated with obesity in T2D subjects but were instead inversely correlated with BMI, suggesting that the increase in plasma NOx levels in T2D was not caused by adiposity. Moreover, we found no association between plasma NOx levels and BMI or waist circumference in non-diabetic subjects where plasma NOx levels were not increased.

In the current study, we could not pinpoint why plasma NOx levels were increased in T2D, since the only variables that were significantly associated with higher plasma NOx levels in multiple regression analysis were lower BMI and lower diastolic blood pressure. The inverse association of diastolic blood pressure with plasma NOx levels was most likely due to the consequence of the effect of NO on blood pressure, since excess NO has been shown to decrease blood pressure in T2D[27]. There was a borderline pos-

itive correlation between fasting glucose and NOx concentrations ($r=0.191$, $P=0.071$) in T2D subjects (see **Table 2**); therefore, the inverse association between plasma NOx levels and BMI might indicate that higher plasma NOx levels in T2D are associated with more severe diabetes. In an animal model, the upregulation of iNOS mRNA was found in the pancreatic islets of Zucker diabetic rats, which further led to β cell destruction and impaired insulin secretion. Both nicotinamide and aminoguanidine, which lower NO production, ameliorated β cell destruction and hyperglycemia in this model[28]. The finding suggests that increased NO could exacerbate T2D by further decreasing insulin secretion. Thus, although plasma NOx did not predict high-risk subjects for T2D, it might predict the outcome of T2D.

Although plasma NOx levels in IFG and IGT were neither significantly increased nor decreased compared with those in NGT, predictors of plasma NOx levels were different among 3 non-diabetic groups. While the serum TG level was an independent predictor of the plasma NOx level in NGT, it was not in IGT; instead, the serum HDL-C level was an independent predictor in this group. We do not have enough evidence to explain exactly why serum TG and HDL-C levels were correlated with plasma NOx levels in NGT and IGT, respectively; however, it is clear that the association of plasma NOx levels with serum TG or HDL-C levels was not due to insulin resistance, since no correlation of plasma NOx levels with HOMA-IR was found in any of 3 non-diabetic groups. There was an association between plasma NOx levels and the TG: HDL-C ratio, which is considered to be a surrogate marker for insulin resistance, in NGT. However, the fact that plasma NOx levels did not correlate with HOMA-IR in NGT indicates that the association between plasma NOx levels and the TG: HDL-C ratio in this case was not due to insulin resistance. Although the exact mechanism regulating plasma NOx levels has not been well understood, plasma NOx levels can be affected by the expression levels and activity of eNOS and iNOS. The divergence in the predictors of plasma NOx levels in 3 non-diabetic groups suggests that the expression levels and activity of eNOS and iNOS may be different in the 3 non-diabetic groups even though plasma NOx levels were similar.

Conclusion

Plasma NOx concentrations were significantly increased in T2D but not in subjects with glucose intolerance before the development of T2D. Plasma NOx levels were not associated with adiposity or insulin resistance in both diabetic and non-diabetic subjects. Thus, plasma NOx is not a useful marker to predict high-risk subjects for T2D in the Vietnamese population.

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References

1) Nestel P, Lyu R, Low LP, Sheu WH, Nitiyanant W, Saito I, Tan CE: Metabolic syndrome: recent prevalence in East and Southeast Asian populations. Asia Pac J Clin Nutr, 2007; 16: 362-367
2) Zimmet P, Serrano RM: A new international diabetes federation worldwide definition of the metabolic syndrome: the rationale and the results. Rev Esp Cardiol, 2005; 58: 1371-1376
3) Le Nguyen TD, Tran TM, Kusama K, Ichikawa Y, Nguyen TK, Yamamoto S: Vietnamese type 2 diabetic subjects with normal BMI but high body fat. Diabetes Care, 2003; 26: 1946-1947
4) Hu FB, Meigs JB, Li TY, Rifai N, Manson JE: Inflammatory markers and risk of developing type 2 diabetes in women. Diabetes, 2004; 53: 693-700
5) Liu S, Tinker L, Song Y, Rifai N, Bonds DE, Cook NR, Heiss G, Howard BV, Hotamisligil GS, Hu FB, Kuller LH, Manson JE: A prospective study of inflammatory cytokines and diabetes mellitus in a multiethnic cohort of postmenopausal women. Arch Intern Med, 2007; 167: 1676-1685
6) Barzilay JI, Abraham L, Heckbert SR, Cushman M, Kuller LH, Resnick HE, Tracy RP: The relation of markers of inflammation to the development of glucose disorders in the elderly: the Cardiovascular Health Study. Diabetes, 2001; 50: 2384-2389
7) Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM:

C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA, 2001; 286: 327-334
8) Nakanishi S, Yamane K, Kamei N, Okubo M, Kohno N: Elevated C-reactive protein is a risk factor for the development of type 2 diabetes in Japanese Americans. Diabetes Care, 2003; 26: 2754-2757
9) Huffman FG, Whisner S, Zarini GG, Nath S: Waist circumference and BMI in relation to serum high sensitivity C-reactive protein (hs-CRP) in Cuban Americans with and without type 2 diabetes. Int J Environ Res Public Health, 2010; 7: 842-852
10) Laaksonen DE, Niskanen L, Nyyssonen K, Punnonen K, Tuomainen TP, Valkonen VP, Salonen R, Salonen JT: C-reactive protein and the development of the metabolic syndrome and diabetes in middle-aged men. Diabetologia, 2004; 47: 1403-1410
11) Thorand B, Lowel H, Schneider A, Kolb H, Meisinger C, Frohlich M, Koenig W: C-reactive protein as a predictor for incident diabetes mellitus among middle-aged men: results from the MONICA Augsburg cohort study, 1984-1998. Arch Intern Med, 2003; 163: 93-99
12) Spranger J, Kroke A, Mohlig M, Hoffmann K, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF: Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes, 2003; 52: 812-817
13) Zahedi AS, Ghasemi A, Azizi F: Serum nitric oxide metabolites in subjects with metabolic syndrome. Clin Biochem, 2008; 41: 1342-1347
14) Chien WY, Yang KD, Eng HL, Hu YH, Lee PY, Wang ST, Wang PW: Increased plasma concentration of nitric oxide in type 2 diabetes but not in nondiabetic individuals with insulin resistance. Diabetes Metab, 2005; 31: 63-68
15) Kannel WB, Vasan RS, Keyes MJ, Sullivan LM, Robins SJ: Usefulness of the triglyceride-high-density lipoprotein versus the cholesterol-high-density lipoprotein ratio for predicting insulin resistance and cardiometabolic risk (from the Framingham Offspring Cohort). Am J Cardiol, 2008; 101: 497-501
16) Kim-Dorner SJ, Deuster PA, Zeno SA, Remaley AT, Poth M: Should triglycerides and the triglycerides to high-density lipoprotein cholesterol ratio be used as surrogates for insulin resistance? Metabolism, 2010; 59: 299-304
17) Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia, 1985; 28: 412-419
18) Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med, 1998; 15: 539-553
19) Kurozumi R, Kojima S: Increase of intracellular glutathione by low-level NO mediated by transcription factor NF-kappaB in RAW 264.7 cells. Biochim Biophys Acta, 2005; 1744: 58-67
20) Maejima K, Nakano S, Himeno M, Tsuda S, Makiishi H, Ito T, Nakagawa A, Kigoshi T, Ishibashi T, Nishio M, Uchida K: Increased basal levels of plasma nitric oxide in Type 2 diabetic subjects. Relationship to microvascular complications. J Diabetes Complications, 2001; 15: 135-143
21) Elizalde M, Ryden M, van Harmelen V, Eneroth P, Gyllenhammar H, Holm C, Ramel S, Olund A, Arner P, Andersson K: Expression of nitric oxide synthases in subcutaneous adipose tissue of nonobese and obese humans. J Lipid Res, 2000; 41: 1244-1251
22) Ribiere C, Jaubert AM, Gaudiot N, Sabourault D, Marcus ML, Boucher JL, Denis-Henriot D, Giudicelli Y: White adipose tissue nitric oxide synthase: a potential source for NO production. Biochem Biophys Res Commun, 1996; 222: 706-712
23) Fujimoto M, Shimizu N, Kunii K, Martyn JA, Ueki K, Kaneki M: A role for iNOS in fasting hyperglycemia and impaired insulin signaling in the liver of obese diabetic mice. Diabetes, 2005; 54: 1340-1348
24) Morley JE, Flood JF: Effect of competitive antagonism of NO synthetase on weight and food intake in obese and diabetic mice. Am J Physiol, 1994; 266: R164- R168
25) Perreault M, Marette A: Targeted disruption of inducible nitric oxide synthase protects against obesity-linked insulin resistance in muscle. Nat Med, 2001; 7: 1138-1143
26) Tsuchiya K, Sakai H, Suzuki N, Iwashima F, Yoshimoto T, Shichiri M, Hirata Y: Chronic blockade of nitric oxide synthesis reduces adiposity and improves insulin resistance in high fat-induced obese mice. Endocrinology, 2007; 148: 4548-4556
27) Huynh NT, Tayek JA: Oral arginine reduces systemic blood pressure in type 2 diabetes: its potential role in nitric oxide generation. J Am Coll Nutr, 2002; 21: 422-427
28) Shimabukuro M, Ohneda M, Lee Y, Unger RH: Role of nitric oxide in obesity-induced beta cell disease. J Clin Invest, 1997; 100: 290-295